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INVESTIGATION OF SPATIAL AND SEASONAL MICROBIOME DYNAMICS AND ASSOCIATED ANTIBACTERIAL ACTIVITY IN SCLERACTINIAN CORAL *PLATYGYRA DAEDALEA* IN THE ARABIAN GULF

BY

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ABSTRACT

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Title: Investigation of Spatial and Seasonal Microbiome Dynamics and associated Antibacterial Activity in Scleractinian Coral *Platygyra daedalea* in the Arabian Gulf

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Corals have critical interactions with their associated microbes, which play an integral part in maintaining coral health and stability. Environmental factors can influence the microbial community of the coral resulting in dysbiosis which often correlates with the appearance of disease. However, the foundational understanding of variations in coral microbiomes in response to environmental factors across spatial and temporal scales is limited. In this study, 16s rRNA sequencing was utilized to study the spatial and seasonal dynamics of the microbiome of the coral *Platygyra daedalea* in Qatari reefs. *P. daedalea* microbiomes displayed significant variability in diversity and abundance among the 5 study sites. On the other hand, although there were apparent variations in microbiome composition between summer and winter, there was no evidence to support these variations were seasonal, indicating that *P. daedalea* microbiome is mainly driven by reef location. Analysis of the free-living microbial communities identify potential microbial taxa as indicators of environmental perturbations that can influence coral health. Fifty coral associated bacteria were isolated from *P. daedalea* comprising of 13 families belonging to Proteobacteria, Firmicutes and Bacteroidetes. Investigation of the antimicrobial activity of coral associated bacteria emphasized that microbial interactions regulate microbiome community structure. *Pseudovibrio denitrificans* N40 exhibited strong activity against 40% of the cultured coral isolates.

DEDICATION

For my loving family

For coral reefs of the world

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDCIES

Chapter 1 : INTRODUCTION

Coral reefs are one of the most diverse ecosystems on earth, they support more species per unit area than any other marine ecosystem (Reaka-Kudla, 1997). Although reefs cover less than 1% of the sea floor, they are home to 25% of all marine species, and are considered an important source of food and income for coastal communities worldwide (Costanza et al., 1997). Latest global estimate of total net benefit of reef ecosystem services is measured to be $29.8 billion per year (Samonte-Tan, 2008). Aside from the evident economic value of coral reefs in tourism and fisheries, reefs play an important role in nutrient recycling as well as buffering wave action in order to protect shorelines and reduce erosion (Bourne & Webster, 2013; Costanza et al., 2014). The physical structure of reefs is attributed to reef-building corals, they excrete calcium carbonate exoskeletons at their base to form coral colonies. Reef building corals also provide organisms inhabiting the reef with food and shelter (Beltran & Camacho, 2018). According to Hernández-Delgado et al. (2018), coral reef health is influenced by a number of factors, including ecosystem processes, ecosystem structure, connectivity, among others. The health and function of corals is supported by a healthy microbiome (Glasl et al., 2016). Environmental factors can influence the microbial community of the coral resulting in dysbiosis which often correlates with the appearance of disease (Krediet et al., 2013). However, the foundational understanding of variations in coral microbiome in response to environmental factors across spatial and temporal scales is limited (Dunphy et al., 2019; M. J. Sweet & Bulling, 2017). Understanding processes that contribute to microbiome stability, such as production of antimicrobial compounds, is also important but understudied (Bourne & Webster, 2013). The microbiomes of corals found within the Arabian Gulf are yet to be explored despite it representing one of the harshest and most unique marine environments in the world (Naser, 2014). Therefore, this study aims to provide a baseline for understanding microbiome composition, processes (antibiotic production) that contribute to bacterial

community structure and the spatial and seasonal dynamics of microbiome composition on a widespread coral species in Qatari waters.

Chapter 2 : REVIEW OF THE LITERATURE

Coral Biology

Corals are benthic marine invertebrates belonging to Kingdom Animalia, Phylum Cnidaria, Class Anthozoa (Beltran & Camacho, 2018). They are composed of many individual sac-like polyps that usually grow interconnectedly to form colonies. Each polyp is composed of a central mouth which takes in food and expels waste, a stomach containing digestive filaments and tentacles that host nematocysts cells which capture food and are used as a defense mechanism for corals (Porter & Tougas, 2001; Veron, 2011). Corals reproduce both sexually and asexually. Sexual reproduction of corals occurs through external (broadcast spawners) or internal fertilization (brooders) of sperms and eggs which results in a genetically diverse planula larvae that ultimately settle and mature into primary polyps. On the other hand, asexual reproduction occurs through budding or fragmentation and it produces genetic replicate of adult corals (Richmond & Hunter, 1990). Corals can be hermatypic (hard) which are the reef building corals or ahermatypic such as soft corals, black corals and gorgonians (Pilcher, 2001). Hard corals (Order Scleractinia) build reefs by secreting calcium carbonate. The shape of the skeleton at the polyp level is species specific, however, the overall morphology of the colony is partially influenced by environmental factors (Pilcher, 2001; Porter & Tougas, 2001).

Platygyra is a genus of scleractinian corals initially described as *Maeandra* by German zoologist Ehrenberg in 1834. Although originally placed within the reef coral Family Faviidae, it was revised and placed with the reef coral family Merulinidae (Budd et al., 2012; Hoeksema, 2021). Colonies of *Platygyra* are generally massive or plate-like, characterized by a surface that resembles a mammalian brain, hence the common name “brain coral”. They have long meandroid valleys which vary in size. For instance, the valleys of *Platygyra daedalea* are around 2 to 3 cm long and 0.5 to 0.6 cm wide and can be monocentric or continuous. Figure 2.1 shows Qatari *Platygyra daedalea* at one of our study sites. These corals typically grow up to 1 m in diameter or larger and have been documented in all depths (Carpenter & Niem, 1998;

Miller, 1994). *Platygyra* occurs in a variety of reef environments and has been found to be widespread in the Indo-Pacific region, namely, East Coast of Africa, Australia, South China Sea the Red Sea, Gulf of Aden, and the Arabian Gulf. The conservation status of *Platygyra daedalea* as assessed by the International Union for Conservation of Nature (IUCN) in 2008 is of "least concern" (Turak et al., 2014).

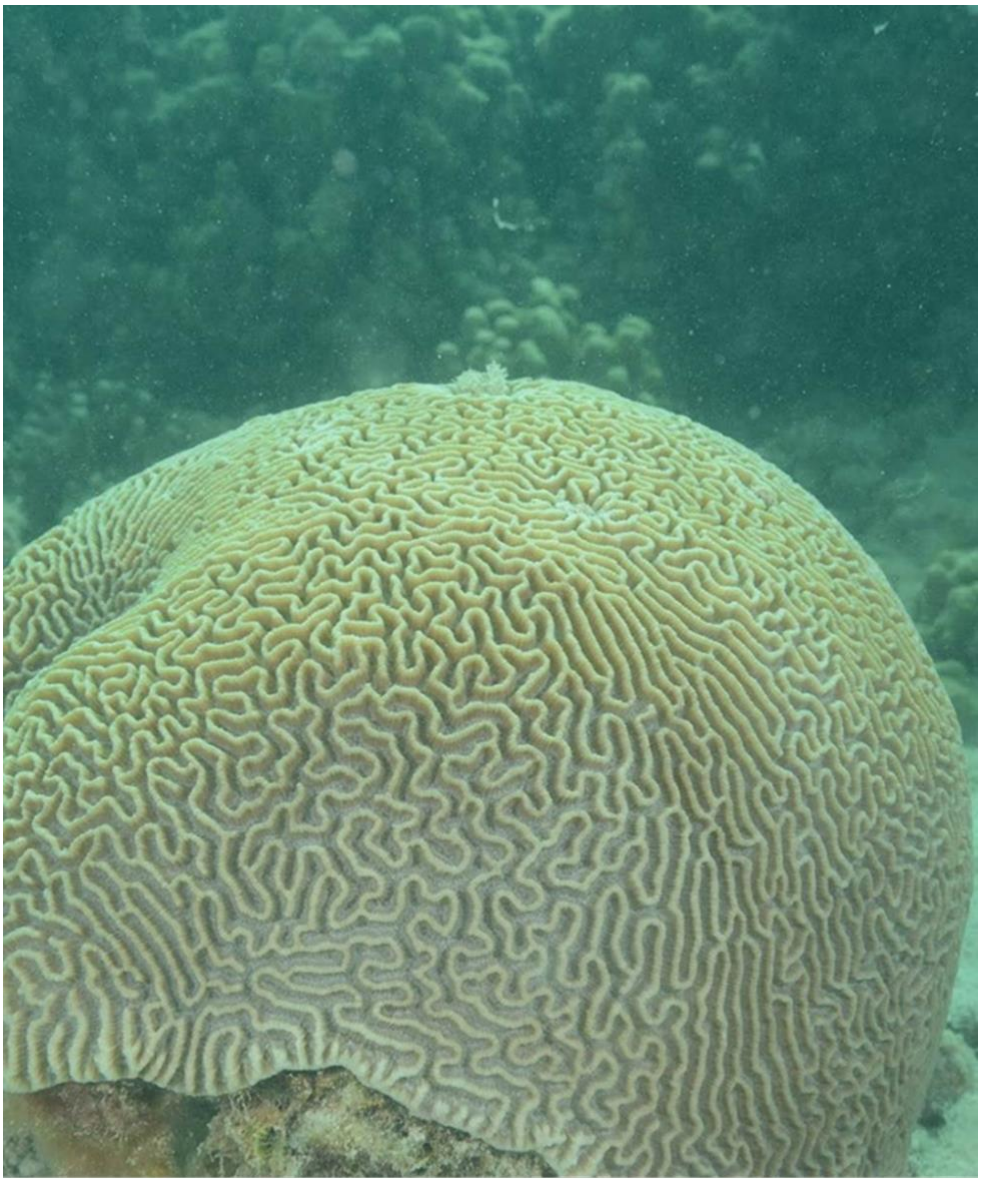


Figure 2.1: Coral *Platygyra daedalea* photographed at one our study sites in Qatar.

The Coral Microbiome

Similar to humans, plants and other animals, corals are holobionts or meta-organisms, which means they are comprised of the macroscopic coral host and its synergistic interdependence with a consortium of microorganisms including microalgae, bacteria, fungi, archaea and viruses (Blackall et al., 2015; Bosch & McFall-Ngai, 2011). Dinoflagellates, commonly known as zooxanthellae, are among the most widely studied and understood coral endosymbionts. Zooxanthellae provide coral polyps with organic material as food and, in turn, the coral provides the microalgae with nutrients mainly nitrogen and sulfur (Stambler, 2010). On the other hand, the role and function of the remaining members of the coral microbiome remains largely unknown, due, in part, to the high variability found in community associations (Hernandez-Agreda et al., 2018). One of the most troublesome communities in coral research are bacterial communities. In addition to bacteria being the most abundant life form on earth, the diversity of this domain has shown to be positively correlated with environmental heterogeneity, leading to challenges in understanding the host microbe interaction in corals (Curd et al., 2018; Rohwer et al., 2002). Coral microbial communities signify complex study systems in the natural environment due to the diversity of host, the microbe and the marine environment (Hernandez-Agreda et al., 2018). However, it is well established that bacteria along with other microbiome components play an integral functional role in respect to its specialized microhabitat within the coral (mucus, tissue, gastric cavity and skeleton) (Bourne et al., 2016). The coral mucus or the surface mucopolysaccharide layer (SML) supports 10^6–10^8 microbial cells per milliliter (Bourne et al., 2016). Mucus-associated microbes provide nutritional advantages, as well as, protection from UV light, protection from pathogens by producing antimicrobial agents and many more (Bourne et al., 2016). The coral tissue microbial community represent a more specific symbiotic association than mucus and despite the methodological limitations investigating coral tissue community, it has been suggested that coral tissue generally has a lower microbial abundance than the mucus layer (Bourne et al.,

2016). Krediet et al. (2013) demonstrated a link between nitrogen acquisition and tissue microbiota, suggesting that tissue microbiomes might have a role in nitrogen fixation and Pollock et al. (2018) suggests that the tissue microbiome is more host dependent than the mucus layer microbiome. Similarly, the microbiome of the gastric cavity is responsible for nutrient recycling and catabolism of ingested organic matter (Bourne et al., 2016). It is characterized by low oxygen concentrations and high inorganic nutrients which would normally result in a different microbial community, however, corals are known to transfer some of the mucus to the gastric cavity which could help maintain the symbiotic associations (Bourne et al., 2016). Lastly, the coral skeleton represents the largest microhabitat, and it presents a unique environment for a number of endolithic microbes which perform an important role in coral fitness. The skeletal microbiome aids coral in achieving homeostasis especially after dysbiosis by inducing tissue recolonization (Ricci et al., 2019). Figure 2.2 describes the coral microhabitats inhabited by the microbiome. These microhabitats represent distinct niches for microbes, hence influencing the composition, richness and structure. According to Blackall et al. (2015), Gammaproteobacteria and Alphaproteobacteria are the dominant bacterial classes in corals. The gammaproteobacterial genus *Endozoicomonas* is the most abundant genera across corals, potentially exhibiting an important ecological role. Among Alphaproteobacteria, the family *Rhodobacteraceae* are highly abundant and discussed in the literature likely due to their relation to the white plague disease. Actinobacteria, Bacteriodetes, Cyanobacteria and Firmicutes are also highly abundant bacterial phyla in corals (Hernandez-Agreda et al., 2017) Although the information on the bacterial communities of *Platygyra daedalea* is limited, Damjanovic et al. (2020) reported high abundance of Alphaproteobacteria families such as Rhodospirillaceae, Rhodobacteraceae, Bradyrhizobiaceae as well as Bacteriodetes and Endozoicomonas.

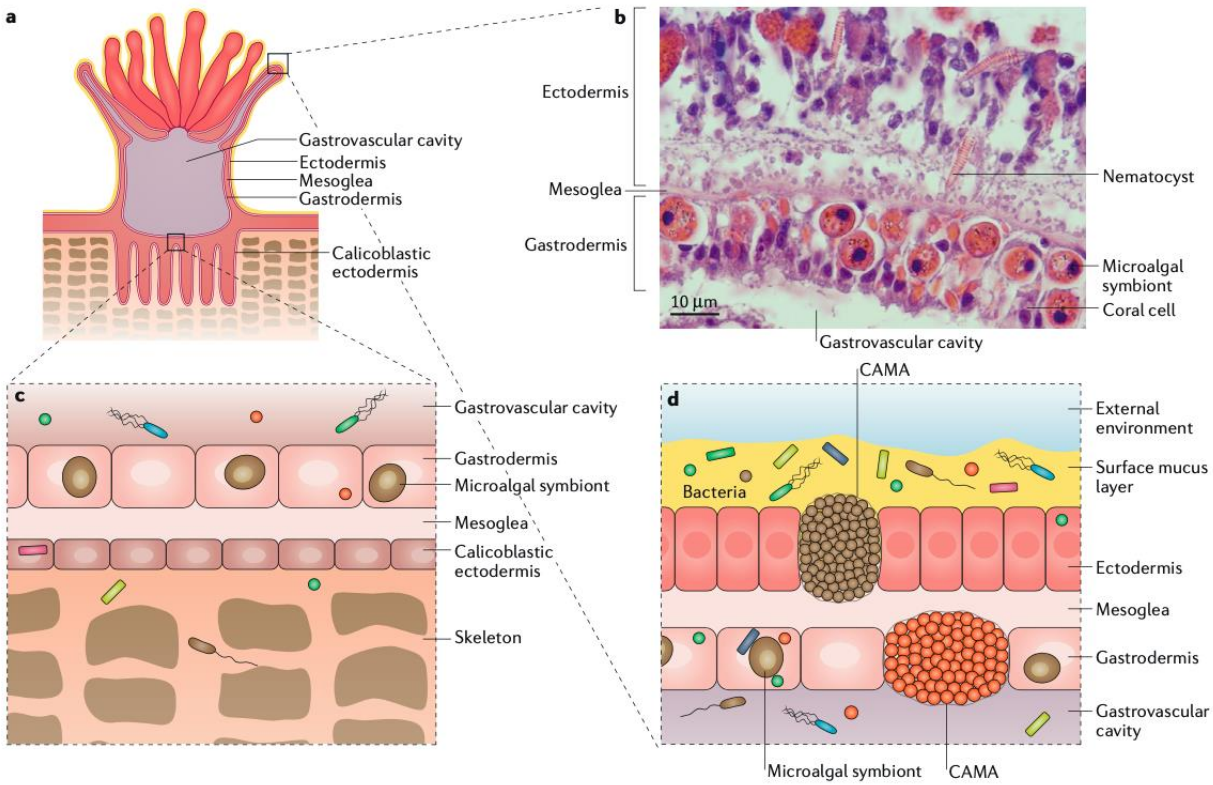


Figure 2.2: the coral microhabitats inhabited by the microbiome. a) represents the anatomy of the coral, b,c and d show the structure and specific habitats of bacteria in different compartments of the coral (Oppen et al., 2019).

The Core Coral Microbiome

In recent years, studies have found that most hosts species share a set of core microbial genes that were common across the entire species (Risely, 2020). Many studies have applied the concept of core microbiome to taxonomically defined microbial communities in order to identify prevalent microbial communities in various host populations (Amato et al., 2015; Antwis et al., 2019; Ainsworth et al., 2015). The persistence of associations between the host and these microbial communities is considered a criteria to identify microbes with crucial function to the host (Hernandez-Agreda et al., 2017).

The core microbiome is being studied through a more inclusive lens, as evidence of rare taxa having vital importance to the microbial ecosystem irrespective of their abundance is increasing. Five types of core microbiome have been discussed in the literature, common core, temporal core, ecological core, functional core and host-adapted core (Risely, 2020). The common core identifies the most prevalent taxa among the host population. Although it does not infer function, it provides an understanding of the structure of the microbiome as well as link it to microbial ecology. The temporal core describes temporally stable taxa or taxa with predictable fluctuations within host population, for instance, fluctuations in relation to season or life stages (Risely, 2020). The ecological core identifies keystone taxa in shaping the ecological structure of the microbiome, it can be widely important in mediating host and community functions such as pathogen resistance and nutrient recycling, respectively. This is different from the functional core where this core represents the microbial taxa that perform essential biological functions, that include biochemical, physiological, and ecological host services. Lastly the host-adapted core identifies microbial taxa that have coevolved with the host and is linked to increased fitness in the host (Risely, 2020).

According to Hernandez-Agreda et al. (2017), the core microbiome concept is fairly young in the coral world. As of 2017, the core microbiome of 16 coral species have been explored where the persistence of core annotations ranged from 100% to 30% in different next

generation sequencing platforms. Ainsworth et al. (2015) were able to identify 7 core bacterial phylotypes (mainly belonging to *Actinobacteria* and *Ralstonia*) that are universal for the coral microbiome across geographical locations. Two of which were localized with endosymbiotic algae and endosymbiotic algae host cells inferring potential role in coral-dinoflagellate symbiosis (Ainsworth et al., 2015). Another meta-analysis that included soft and stony deep-sea corals was able to identify 23 core operational taxonomic units (OTUs) corresponding to bacterial families functional in the nitrogen cycle such as *Bradyrhizobiaceae*, *Methylobacteriaceae* and *Pirellulaceae*. Many of the sequences identified were conserved in both soft and hard corals proposing conservation across the class of Anthozoa (Kellogg, 2019). In a study characterizing the healthy microbiome of 100 coral samples (6 species), it was evident that there was remarkable bacterial taxa similarity between all coral samples. Numerous OTUs from Endozoicimonaceae, Campylobacteraceae and *Vibrio* were abundant across corals hosts, however these microbiomes still display a level of host specificity (Chu & Vollmer, 2016). Hernandez-Agreda et al. (2016) proposed that a better way to characterize the coral microbiome is not only to identify its core microbiome but to study three distinct subcommunities, the ubiquitous core microbiome, an individually explicit core microbiome (resident microbiome) and a highly changeable microbiome that is responsive to environmental influences (environmental responsive community). Hernandez-Agreda et al. (2018) highlighted the individual microbiome in each coral, the study shows that around 3% of the phylotypes of each studied coral is its "resident microbiome" and around 96% is its "environmentally responsive community", while the core microbiome was only 0.1% of the phylotypes.

Co-evolution of the Coral Holobiont

The concept of the holobiont has had wide implications on the understanding of the evolution of the host in parallel to its microbiome (Limborg & Heeb, 2018). Coevolution which is the reciprocal evolution on lineages in response to another lineage does not only occur in holobionts, it is widely observed in predator-prey relationships and host-parasite relationships. However, microbiome-host interactions are complex mutualistic associations, this complexity resulted in difficulties understanding these associations leaving many gaps in the literature (O'Brien et al., 2019). According to Rosenberg et al. (2007), differentiations of both the host and microbiome genomes are attributed to interactions over evolutionary timescales, this process is coined as hologenome evolution (Thompson et al., 2015).

Mutualistic coevolution is linked to several key concepts such obligate symbiosis, vertical inheritance and metabolic partnerships (O'Brien et al., 2019). Traits of symbiosis can assist in identifying coevolved endosymbionts. For example, the genome sizes of bacteria demonstrate the evolutionary dependence of bacteria and host, the smallest genome sizes are those of obligate endosymbionts compared to facultative endosymbionts and free-living bacteria. This is due to elimination of redundant genes during adaptation to the host environment (O'Brien et al., 2019; Thompson et al., 2015). Secondly, vertical inheritance where bacteria is acquired vertically from parent to offspring within host population demonstrates a likelihood of occurrence of coevolution. This can also result in loss of genes due to adaptation to the host (O'Brien et al., 2019). Therefore, the genome of vertically transmitted coral endosymbionts are likely to be smaller comparatively to other facultative and free-living mutualistic bacteria (Thompson et al., 2015). In addition, the localization of symbionts in specialized compartments within a host is also a candidate for coevolved relationships. Lastly, metabolic complementation is another feature of coevolution. Indeed, the mutualistic nutritional relationships corals have with *Symbiodinium* is well established (Muscatine, 1990). Moreover, relationships between bacteria and coral and bacteria and

Symbiodinium are likely to include metabolic complementation where the microbe produces gene products that are essential in the survival of the host and vice versa (Gerardo, 2013). These genes are potentially acquired by horizontal gene transfer (HGT). Studies highlight this potential as the bacterial shikimic acid pathway (bacterial metabolic pathways responsible for biosynthesis of aromatic amino acids) has been observed in corals (Shinzato et al., 2011; Shoguchi et al., 2013). An important concept when discussing coevolution is the concept of phylosymbiosis which occurs when the composition of the microbiome reflects evolutionary history of the host. In other words, the phylogenetic signals of the host are retained within its microbiome (O'Brien et al., 2019, 2020). Pollock et al., (2018) were able to demonstrate phylosymbiosis in the tissue and skeleton of corals. Furthermore, it was evident that the microbial abundance of some members of the microbiome was influenced by the host-microbiome cophylogeny. Another study showed agreement to previous findings and displayed patterns of phylosymbiosis in marine invertebrates. However, the authors hypothesized that these patterns can also be caused by the host filtering processes of horizontally acquired microbes (O'Brien et al., 2021).

Microbiome and Coral Health

Coral reefs are homes to millions of marine species and are considered an important source of food and income for coastal communities worldwide (Costanza et al., 1997). Corals - similar to all other animals - face the threat of disease. Therefore, it is necessary to protect these valuable ecosystems through, among others approaches, understanding the dynamics of coral health and disease. The field of coral health and disease is an evolving field, encompassing a little over five decades of progress so far. Disease prevalence, environmental and spatial influences are of the most commonly discussed topics within the literature but a lot remains to be discovered given the diversity of corals and the nature of disease emergence (Montilla et al., 2019). Coral health in relation to its whole microbiome is a progressing new area of study but according to Bourne et al. (2016), the lack of dataset and baseline studies on

the microbiome dynamics of corals is obstructing the process of understanding the role of the environment in coral health and stability. The coral microbiome has an essential part in maintaining holobiont homeostasis, it creates a network of links that include carbon, nitrogen, sulfur cycling, and production of antimicrobial compounds (Bourne et al., 2016). For example, diazotrophs which are nitrogen-fixing microbes form species-specific interactions with corals by providing limiting fixed nitrogen to the coral and it algal symbiont *Symbiodinium*. Similarly, Ritchie (2006) illustrated that 20% of the culturable coral isolates exhibited antimicrobial activity against other isolates and pathogens. Moreover, microbes residing in the coral mucus present a commensal community which prevents colonization of coral by outcompeting pathogens and by stimulating the corals immune response (Glasl et al., 2016).

It is evident that the coral microbiome plays a role at all the life stages of coral development, as well as coral settlement, metamorphosis and colony formation (Thompson et al., 2015). Both vertical (from parent to offspring) and horizontal (from the environment) transmission of symbionts have been observed in corals (Oppen et al., 2019). A study investigating the transmission mode of bacteria in coral *Pocillopora acuta* demonstrated the influence of both host factors and the environment on the microbiome of brooding corals (Menéndez et al., 2020). Similarly, Bernasconi et al. (2019) show that mixed mode transmission is evident in broadcast spawner *Acropora digitifera.* These studies highlight the dynamic nature that describes the bacterial assemblages of coral early life stages and the phase of selection of the consortium, also known is winnowing. These winnowing processes clarify the role of microbiome in development of the coral. According to Nyholm & McFall-Ngai (2004), winnowing ensures for the maximum benefit for the physiological requirements of the host life stages and current environmental conditions through microbial selective mechanisms which are controlled by the hots and it symbionts. Bernasconi et al. (2019) give an example for relative abundance changes in response to metabolic requirements of the developmental stage

of corals. Day 4 larvae and new settlers report Clostridiaceae which contribute to carbon and nitrogen availability, in contrast, in juvenile and spat phases a high abundance of sulfur degrading bacteria Desulfovibrionaceae and Desulfobacteraceae as well as antifungal metabolites producer *Janthinobacterium* was observed (Bernasconi et al., 2019).

Diseases have been identified as major contributor for coral decline worldwide (Bourne et al., 2009). Although host-microbe relationships can be beneficial such as symbiotic, mutualistic and commensal interactions, some interactions can be harmful such as parasitic, pathogenic and dysbiotic interactions (Hernandez-Agreda et al., 2017; Prokšová et al., 2020). Many bacteria have been associated with signs of disease and lesions in corals. However, confirming causation has been difficult. For example, in a study analyzing the white pox disease in *Acropora palmata* corals, pathogen *Serratia marcescens* was detected in 27% of the corals with white pox disease lesions (Joyner et al., 2015). Although, *S. marcescens* is known as the causative agent of *acroporid serratiosis* when isolated form coral with white pox lesions, it could not be cultured from diseased *Acropora palmata* instead it was cultured from other healthy coral species (Joyner et al., 2015). This observation suggests that other pathogens or stressors might be contributing to white pox disease. Similarly, *Vibrio shiloi* was believed to be associated with bleaching of the Mediterranean coral *Oculina patagonica*, instead it was an opportunistic colonization induced by environmental conditions (Ainsworth et al., 2008; Rosenberg et al., 2007). Comparisons between healthy and diseased corals demonstrated that disturbances to the structure of the microbiome can result in infections and signs of disease (Mhuantong et al., 2019). The link between coral disease and environmental stressors is well established (Harvell et al., 2007). For example, Aeby et al. (2020) examined coral health in a gradient of environmental conditions within the Arabian Peninsula, specifically, The Arabian Gulf, Oman Sea and the Strait of Hormoz. The Arabian gulf, displaying the harshest conditions, had evidently the highest disease prevalence compared to the other regions. Accordingly, the

majority of variations in disease prevalence were associated with thermal stress (Aeby et al., 2020). Furthermore, stress resulting from environmental changes can cause disruption to what is referred to as the "Beneficial Microorganisms for Corals" (BMC). The BMC refers to a number of specific microbiome elements which promote coral health. Imbalances in BMC composition leads to loss of nutritional pathways and loss of antimicrobials eventually leading to coral disease (Peixoto et al., 2017). The concept of BMC along with the concept of the pathobiome proposed by Sweet & Bulling (2017) both show that the concept of one pathogen equals one disease has its limits, instead a number of pathogenic microbes interact with each other and the community. The pathobiome is defined mainly as the consortium of microbes which have a direct role in causing disease. It can be expanded to include microbes indirectly affecting abundance of disease mitigating microbes. One study examining the microbiome and pathobiome of the Mediterranean coral, *Cladocora caespitosa*, showed that the pathobiome composition was in fact affected by geographic location and human influences. Rubio-Portillo et al. (2018) found that the pathobiome of locations with a higher human footprint was entirely composed of V*ibrio* species including known pathogens, *Vibrio coralliilyticus* and *V. mediterranei.* Interestingly, one study displayed the effect of human footprint on the microbiome of *Pocillopora verrucosa* and *Turbinaria peltata* in the South China Sea. *Sphingomonas* which has been linked to hydrocarbon degradation was found in abundance in the core microbiome of both coral species (Chen et al., 2021).

In a recent study, the applicability of free living microbial community around corals as an indicator for the environmental state has been investigated (Glasl et al., 2019). Glasl et al. (2019) argued that the high sensitivity and predictability of the free-living microbes would deem them a great candidate for rapid and sensitive identification of the early signs of declining reef health. In fact, 56% of the variation observed on the composition of the free-living microbiome were attributed to environmental changes. The use of such sensitive indicators

could support management and restoration strategies as well as enhance long term monitoring of reef ecosystems (Glasl et al., 2019).

Environmental, Spatial and Temporal Variability in the Coral Microbiome

The structure and stability of coral microbiomes is shaped by both, the coral host and the environment, and the microbiomes of some coral species show more robustness to environmental perturbations than others (Dunphy et al., 2019). Environmental changes across spatial gradients are governed by a variety of interacting physical, chemical and biological processes that can influence corals and coral reefs (Adjeroud et al., 2019). Environmental forcings can be biotic or abiotic factors. Microbiome composition, abundance and richness has shown to shift in response to changes in environmental factors such as temperature, pH, light, nutrients and dissolved organic carbon (Sweet & Bulling, 2017). Many studies suggest that the microbial community dissimilarity increases with increased geographical distance. Hence, the more the distance between coral hosts is, the more their microbiomes will differ. (Dunphy et al., 2019; Hernandez-Agreda et al., 2016; Kelly et al., 2014). Additionally, one study demonstrated coral-associated bacterial communities can differ over small geographical scales. Wainwright et al. (2019) suggest that small scale environmental influences such as surface current and wind direction can have a strong influence on the coral microbiome even over small spatial gradients. Variability in coral microbiomes has also been shown to occur at different geographical scales depending on the coral species including different reefs in the same location and species in the same reef at different depths (Glasl et al., 2017; Morrow et al., 2012; Ziegler et al., 2019).

In addition, shifts in the coral microbiome are also correlated to biological events such as disease, reproduction or macroalgal competition (Sweet & Bulling, 2017). One study investigating the interplay between the coral microbiome and white syndrome disease event found that there was a significant reduction in bacterial diversity months preceding the first visible signs of disease (Pollock et al., 2019). This highlights the important role microbiome

diversity plays in stabilizing microbial communities that govern coral health. Similarly, Ng et al. (2015), demonstrated that the composition of the coral microbial communities were significantly different between healthy corals and corals with growth anomalies. Diseased corals were associated with higher abundances of *Acidobacteria* and *Gemmatimonadetes*, and a lower abundance of *Spirochaetes* (Ng et al., 2015). Furthermore, interstitial associates may also influence the coral microbiome as hundreds of invertebrate species live on and within coral colonies. Some coral associated invertebrates have been shown to aid in coral recovery by debriding and consuming diseased tissue. On the other hand, coral associated invertebrates can also vector pathogens and allow for bacterial colonization by creating wounds (Ainsworth et al., 2020). Although the effect of the macrobiome is seldom discussed, Ainsworth et al. (2020) brought attention to the importance of integrating the interstitial associates in the concept of meta-organism to better understand coral health and utilize it for coral restoration.

Seasonal fluctuations along with tide related shifts in coral microbiome have also been observed in recent studies (Ziegler et al., 2019). Sharp et al. (2017) presented that the temperate coral *Astrangia poculata* was strongly influenced by seasonal changes as a re-structuring of the composition of the microbiome, observed upon transition from winter to spring. Sharp et al. (2017) hypothesize that in the cold months, the microbiome becomes destabilized and fall into a state of quiescence, then it re-structures again as temperatures rise and activity resumes. Likewise, Yu et al. (2021) came to a similar conclusion studying coral *Acropora pruinosa*, alpha diversity and bacterial richness shifted according to season and a recombination of the bacterial structure was observed seasonally. On the contrary, some studies address the need for longer surveys to infer seasonal related shifts. These studies state that coral microbiomes vary depending on host and reef and are dynamic through time, however, not reflective of seasonality (Epstein et al., 2019; Yang et al., 2017). Short temporal scales such as tidal events have been documented to generate shifts in coral microbial communities. Sweet et al. (2017),

explained that the timing of these shifts is age related where older coral colonies displayed delayed response compared to younger colonies. The physical changes in the environment related to tidal events may induce production of mucus or oxidative stress that can result in microbiome composition shifts (Sweet et al., 2017). A recent study has investigated the changes in the microbiome over the diel cycle (Caughman et al., 2021). Interestingly, the study found that coral microbiomes are highly variable over the day–night cycle implicating that the environmentally responsive community may constitute the majority of the coral microbiome and that corals are very sensitive to the time of sampling (Caughman et al., 2021).

Coral reefs are facing significant challenges from anthropogenic stressors, including climate change, water pollution overfishing and physical destruction. Human influence has been shown to affect the microbiome of corals (Vanwonterghem & Webster, 2020). Climate change is a driver for many other phenomena that destabilize the microbiome, resulting in a state of dysbiosis, which can develop into a state of disease, bleaching, and mortality. Increased diversity, variability in the community and abundance of potentially pathogenic taxa have all been linked to thermal stress events (Maher et al., 2020). Many studies also report rising ocean temperatures and ocean acidification result in microbiome shifts from putatively beneficial bacterial taxa, such as *Endozoicomonas* to pathogenic and opportunistic taxa such as *Vibrionaceae* (Vanwonterghem & Webster, 2020). Shifts in microbial community due to bleaching events have also been documented where a *Vibrio*-dominated community was observed prior to visual signs of bleaching (Bourne et al., 2008). Moreover, water pollution has been linked to changes in the coral microbiome, microbiome taxa in polluted sites can be more pathogenic. Indeed, addition of nutrients drastically influences ecosystem functioning in oligotrophic environments, therefore, sewage and runoff can alter microbial communities and result in disease (McDevitt-Irwin et al., 2017). Recently, anthropogenetic activity related bacteria such as *Escherichia coli* and *Sphingomonas* have been observed in the core

microbiome of corals which indicated that anthropogenic activity has affected coral habitats (Chen et al., 2021). Overfishing can have an indirect effect on the coral microbiome. As the herbivorous fish population decreases, the algal population increases resulting in a shift in the water column microbial community composition, increasing algal interaction and possibly triggering disease in corals (McDevitt-Irwin et al., 2017; Morrow et al., 2013; Nugues et al., 2004).

Antimicrobials and their Role in Maintaining Microbiome Structure

Throughout this review, the role of environmental factors in shaping microbial communities has been discussed intensively, however the role of ecological processes within the microbiome has not been elaborated. Ecological processes are also important in shaping the structure and diversity of microbial communities, ecological interactions of community members, their evolutionary history, and immigration from adjacent ecosystems all linked to the composition of the microbial community, however, their level of importance is determined by the ecosystem in question (Faust et al., 2018). It is important to note that structure and function are inextricably linked, it is widely believed that the composition of microbial communities determines and/or influences the function of the community (Nemergut et al., 2013). Many secondary metabolites are an essential aspect of microbial interaction, they usually perform specific functions within the ecosystem. Quorum sensing is a widely investigated microbial interaction mechanism that is mediated by signaling molecules (Braga et al., 2016; Krediet et al., 2013). This cooperative interaction is responsible for forming biofilms that are highly organized and diverse, capable of protecting the microbial community within the biofilm from environmental challenges (Li & Tian, 2016). Microbes also structure and regulate their community by producing antimicrobial compounds. The antimicrobial activity of coral associated microbes has been documented a number of times, many reports demonstrate the ability of coral probiotics and extracts to inhibit coral pathogens as well as show potential for useful human drugs (Damjanovic et al., 2019; Kelman et al., 2006). Kvennefors et al., (2012) suggest that a combination of host-derived and microbial interactions maintain the stability of coral microbial communities. The coral bacteria isolated in this study were able to inhibit a wide range of coral associated bacteria (Kvennefors et al., 2012). Interestingly, bacteria associated with healthy corals were inhibiting putative coral pathogens or bacteria closely related to it. on the other hand, bacteria associated with diseased corals had potent antimicrobial activity against a wide range of bacteria, suggesting that it holds a

competitive advantage inhibiting probiotic bacteria (Kvennefors et al., 2012). Some coral associated bacteria exhibiting antimicrobial activity have been shown to belong to *Bacillus, Vibrio, Micrococcus, Pseudoalteromonas, Arthrobacter,* and *Pseudovibrio* (Budiani, 2008). Ritchie, (2006) illustrated that 20% of the culturable coral isolates exhibited antimicrobial activity against other isolates and pathogens. Moreover, Pereira et al., (2017), found that 83% of all coral mucus isolates had antimicrobial activity against *Serratia marcescens*, an opportunistic coral pathogen. Some efforts have been made to investigate the compound responsible for the antimicrobial activity of coral associated bacteria. Raina et al., (2016) were able to isolate and identify tropodithietic acid a sulfur-containing compound from *Pseudovibrio* sp. P12, a common coral-associated bacterium. Cultured bacteria with antimicrobial activity were found to exhibit genes for polyketide synthase and nonribosomal peptide synthetase which highlights the isolates' potential of producing antimicrobials (Kuek et al., 2015). According to Peixoto et al., (2017) the production of antimicrobial compounds can be a BMC mechanism, this is important as BMCs can be used for developing environmental probiotics. The concept of marine probiotics includes the manipulation of coral through inoculation with a beneficial consortium of bacteria (BMC) aiming to allow coral to adapt rapidly to fluctuating environmental conditions (Blackall et al., 2020). Rosado et al., (2019) were the first to demonstrate the success and promise of coral probiotics for mitigating coral bleaching. This study observed significant reduction in bleaching metrics in treated corals in comparison with control group indicating that the coral microbiome can be manipulated to minimize the effects of bleaching and possibly other stressors (Rosado et al., 2019). Therefore, it is vital to culture, identify and assess coral isolates for their potential activities including antimicrobial activity. This can open doors for promoting coral resilience and provide novel strategies for reef restoration efforts (Sweet et al., 2021).

Corals in The Arabian Gulf and Qatar

Corals in the Arabian Gulf live under very unique conditions, it is considered the world's hottest sea during the summer and it is displays remarkable fluctuations between summer and winter (Naser, 2014; Riegl & Purkis, 2012). Since the Gulf is a shallow basin with high evaporation rates, it is exposed to temperature, and salinity extremes (Naser, 2014). In spite of that, corals of the Arabian Gulf display remarkable resilience facing one of the most hostile climatic conditions and seasonal variability (Riegl & Purkis, 2012). Corals reefs of the Arabian gulf are comprised of 66 reef-building coral species and host up to 200 species of reef fishes. (Berumen et al., 2019; DiBattista et al., 2016; Pilcher, 2001). Corals occur virtually across the entire Arabian Gulf with best development in offshore shoals particularly in Saudi Arabia, UAE, and Qatar (Riegl & Purkis, 2012). Corals are present in the northern and eastern coasts of Qatar and in territorial waters on the offshore islands, including Halul Island (Wilkinson, 2004). Aside from the harsh climatic conditions, corals face a number of anthropogenic threats such as coastal and industrial development, land reclamation, trawling, dredging and thermal pollution from cooling water discharge (Wilson et al., 2002). Climate change has is also impacting reef ecosystem through disturbances and bleaching. Bleaching event such as 2012 and 2017 events are projected to drive *Acropora* populations into extinction which can lead in disturbances in community structure (Riegl et al., 2018). Corals in Qatar are exposed to these conditions, but unfortunately, they are considered the least studied coral communities in the region. According to Burt et al., (2016), Qatari coral reef research represents less than 3% of the coral reef research in the Arabian Gulf.

Coral microbiome research in the Arabian Gulf is also extremely limited. One study examined the microbial communities in corals along the Red Sea and Arabian Gulf sides of Saudi Arabia (Hadaidi et al., 2017). Bacterial functional profiling revealed that abundance of bacteria associated with nitrogen recycling has shifted. Unlike the Red Sea which is limited in nitrogen, the Arabian Gulf coral microbiome lacks the need for efficient ammonia oxidizers

since nitrogen is readily available (Hadaidi et al., 2017). Coral microbiome research in Qatar is lacking, a great deal remains undiscovered and its crucial to understand how the symbiotic interactions are affected, focusing on different scales and dynamic observation of the microbiome.

Studying the Coral Microbiome: Research Methods

Methods of studying microbiomes do not vary much depending on the host, therefore the overall sequence of methods used to study the human microbiome and the coral microbiome can be very similar. However, these methods can vary depending on the research question, whether the question asked is structural or functional and which components of the microbiome are to be investigated (Walker-Daniels, 2021). The general sequence of steps is sample preparation, DNA extraction, target gene amplification and purification, sequencing, and bioinformatics. After sampling, coral samples can usually be preserved in a chemical preservative such as DMSO or flash frozen with liquid nitrogen and kept in - 80˚C. Bead beating and crushing using a mortar and pestle have shown to be effective methods in preparing the sample for DNA extraction (Hernandez-Agreda, Leggat, & Ainsworth, 2018). Many method have been used to extract DNA form corals including CTAB (Baquiran et al., 2020), MoBio PowerPlant (Sunagawa et al., 2010) PowerSoil (Beurmann et al., 2018) UltraClean Tissue and Cells (Weber et al., 2017) DNA extraction kits and Qiagen DNeasy Powersoil kit (Meyer et al., 2019). Many DNA extraction kit have shown success in capturing coral associated bacteria (Weber et al., 2017). For structural microbiome studies, the standard approach is analyzing marker genes such as 16S rRNA gene for bacteria and archaea and 18S rRNA gene for eukaryotes. 16S rRNA sequencing is very popular as it uses universal primers that target different regions of the 16S rRNA gene (Méndez-García et al., 2018). Examples used in coral microbiome studies include the 515F and 806R covering the V4 region (Meyer et al., 2019) and 784F and 1061R covering the V5-V6 region (Epstein et al., 2019) Internal transcribed spacer (ITS) sequencing is a method that can give a better strain resolution and has

been used to study the coral microbiome as well (Epstein et al., 2019). On the other hand, functional microbiome studies require whole genome shotgun sequencing. Studying the whole genome provides a more complete picture of the community structure and function but it can be very resource intensive (Walker-Daniels, 2021). Many sequencing platforms have been used to sequence the microbiome of corals, most notably sanger sequencing (ABI) (Morrow et al., 2013) 454 sequencing (Glasl et al., 2017; Yang et al., 2017), PacBio (Pootakham et al., 2017) and Illumina Miseq sequencing (Maher et al., 2020; Pollock et al., 2018). Illumina sequencing has dominated other sequencing platforms due to its cost-effectiveness and high throughput nature (Walker-Daniels, 2021). To analyze the data generated by sequencers, bioinformatics tools such as QIIME2, Mothur, Usearch, Blast and Ion ReporterTM are used. These tools which are commonly used in combination provide taxonomic information about the microbiome such as OTUs and Amplicon Sequence Variant (ASVs) as well as abundance, diversity, and phylogenetic analysis (Walker-Daniels, 2021).

The general aim of this study is to investigate the stability of the coral microbiome in space and time by comparing the composition, structure, and dynamics of coral microbiome in different geographical locations in Qatar and through different seasons.

The specific objectives of this research are:

- Characterize the composition, structure, and dynamics of the microbiome of the scleractinian coral species *Platygyra daedalea* in Qatari waters.
- Investigate spatial and seasonal variability of the coral microbiome and its relationship with environmental factors.
- Determine the applicability of the free-living microbial community around the coral as an indicator of environmental changes.
- Examine the antimicrobial activity of isolated bacteria from target coral species.

Chapter 3 : RESEARCH METHODOLOGY

This chapter provides the detailed description of the methodological approach developed to answer the research questions of this thesis to investigate the stability of the coral microbiome in space and time. Two main approaches are described below. The first approach which utilized Metagenomic techniques, was used to characterize the microbiome of the scleractinian coral species *Platygyra daedalea* in Qatari waters, investigate its seasonal variability and determine the applicability of the free-living microbial community around the coral as an indicator of environmental changes. The second approach focuses on the singular question of examining the antimicrobial activity of cultured isolates through identification and in vitro assays. Figure 3.1 describes the sequence of general steps used in each approach. The Approach adopted was developed after thorough review of literature and trial and error. The layout of this chapter is divided into 2 sections, Microbiome Analysis and Antimicrobial Activity Assessment.

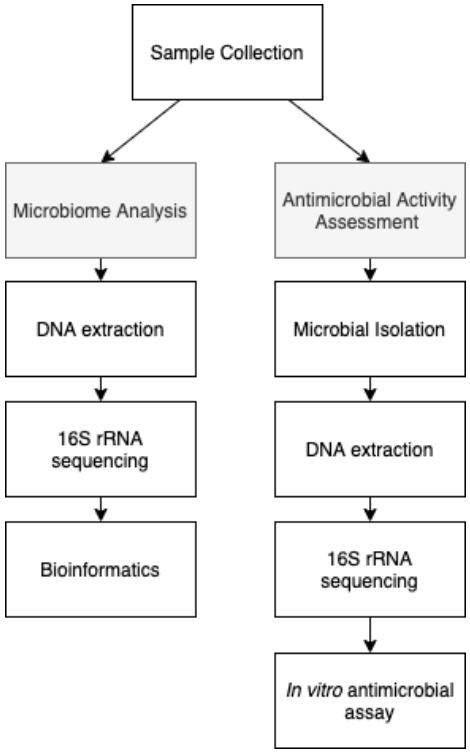


Figure 3.1: Flowchart of the methodological approach adopted.

Study Sites

Site Information

The study was conducted in the peninsular State of Qatar which is located between 25° 17' 9.9816" N and 51° 32' 5.3412" E. Qatar borders the Arabian Gulf on the north, east and southeast and the Gulf of Bahrain on the west (Soliman et al., 2014). The study sites were selected after screening for locations with an abundance of our species of interest, *Platygyra daedalea*. Five sites were later identified, Balhambar, Umm Al Shaef, Maydan Mahzam, Bulhanin 4 and Fasht East Halul. Balhambar and Umm Al Shaef are located in northeast of Qatar while Maydan Mahzam, Bulhanin 4 and Fasht East Halul are located in the east of Qatar as displayed in Figure 3.2. The coordinates of the sites and their depths are shown in Table 3.1.

Figure 3.2: Map showing the location of the 5 study sites selected for this study.

Platygyra daedalea was selected for this study due to several factors. Firstly, it was noted after screening of the sites that *Platygyra daedalea* is widespread and that colonies of *P.*

daedalea found in Qatar are considerably large in size. This ensures that the sample size needed can be attained and the colonies can withstand seasonal resampling without affecting the health of the colonies. Although the microbiomes of corals found within the Arabian Gulf are yet to be explored, *P.daedalea* corals specifically are understudied in the literature as a whole, therefore this species is the ideal choice for this study.

Table 3.1: Sampling sites characteristics

Site	Region	Latitude (N)	Longitude (E)	Depth (m)
Balhambar	North east of Qatar	25.9699	51.8777	14
Umm Al Shaef	Northeast of Qatar	26.1500	51.7006	12
Maydan Mahzam	East of Qatar	25.5118	52.5172	15.2
Bul Hanine 4	East of Doha near an oil field	25.5242	52.8089	15
Fasht East Halul	East of Qatar	25.7135	52.6396	18

Colony Tagging and Line Intercept Transect

This study has both spatial and seasonal dimensions, therefore measures where set in place in order to locate the coral colonies sampled in the summer to be resampled in the winter. The sampling protocol consisted of setting up two 30 meter transects per site; each transect was marked using green color sequentially numbered cow tags that were secured at every 5 meters along the transect. The depth, GPS coordinates and cow tag numbers were recorded in order to be able to locate the transects in the upcoming sampling events. After securing the transects, 10 colonies were tagged per site. Once located, colonies were tagged by screwing orange colored cow tags on the closest substrate to the colony and not on the colony. Information such as distance of colony along the transect, distance from the transect, direction (left or right) from transect and maximum diameter of the colony were recorded to facilitate relocating the colonies in future sampling dives.

Along each transect, line intercept method was used to estimate the benthic cover of coral and other organisms such as sponge, algae and cyanobacteria. The method included recording the distance of substrates intercepted by the measuring tape from on point to another (e.g., sand, coral, algae, etc.).

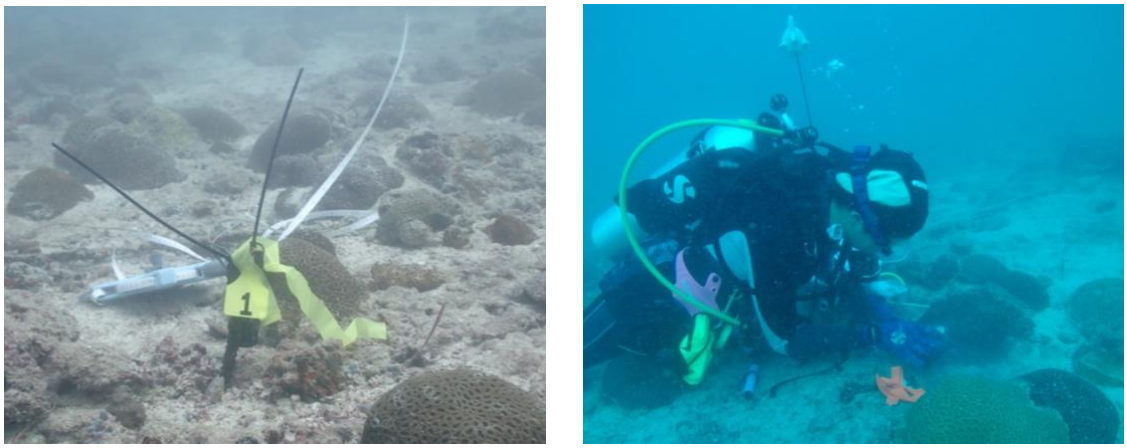


Figure 3.3: Aspects of the coral transect set up and coral tagging process.

Microbiome Analysis

Sample Collection

Coral Samples

A total of 91 brown color morph *P. daedalea* were collected from all of the five sites during the summer sampling period in August/September and Winter sampling period in March. In the winter, some tags were lost, therefore some colonies where not located and subsequently not re sampled in the winter. Table 3.2 shows the dates of sampling and the number of samples per site per season.

Table 3.2: Sampling dates and number of coral samples per site per season.

Site	**Summer sampling**		**Winter sampling**	
	Sampling Date	Number of Samples	Sampling Date	Number of Samples
Balhambar	19/8/20	10	18/3/21	8
UmmAl Shaef	23/8/20	10	18/3/21	9
Maydan Mahzam	27/8/20	10	1/3/21	8
Bul Hanine 4	3/9/20	10	16/3/21	7
Fasht East Halul	4/9/20	10	4/3/21 & 16/3/21	9

Using an underwater hammer drill (NEMO, U.S.A), small fragment of the tagged corals were sampled and put in numbered sterile whirlpak bags at depth and the colony tag was recorded. After sampling was done, marine epoxy (AVES, U.S.A) was used to seal the location of the sampling. Colonies were photographed and the temperature was recorded at the time of sampling, a temperature logger that records hourly reading was also deployed at each site. Once on the boat, all coral fragments were loosely rinsed with sterile filtered seawater (filtered using 0.22 µm filters and autoclaved prior to sampling trip) and placed in a new sterile whirl-pak bags. Coral samples were transported on ice and stored at -80˚C in the lab awaiting DNA

extraction.

Water Samples

Water samples were collected for two different purposes (n=17). Firstly, reef water samples were collected to identify the water microbes at coral sampling sites and understand the free-living microbial community around the coral. One litre of seawater was collected at each site at the reef depth for each season. The second water sampling protocol was utilized to study the free-living microbial community in close proximity around corals. One litre sample were collected from very close proximity to the coral which was also sampled. A total of 7 water samples were collected from Maydan Mahzam, the colony tag and bottle number were recorded to identify colony samples for comparison. All water samples were stored in ice along with the coral samples. Once in the lab, the seawater was immediately filtered using 0.22µm Sterivex filter (Milipore, U.S), filters were stored at -80˚C until DNA extraction.

DNA Extraction

Coral DNA Extraction

Extraction of metagenomic DNA from corals was initiated by breaking fragments in equal halves, one half was returned to -80˚C and the other was retained for further processing. Excess skeleton was removed using sterile bone cutter and obtained fragments for extraction were between 1-3 grams of tissue, skeleton, and frozen mucus. Coral pieces were then crushed using sterile mortar and pestle and liquid nitrogen until a fine paste was obtained. The paste was kept in labeled Eppendorf tubes and stored in -20˚C awaiting DNA extraction. The Qiagen DNeasy PowerSoil Extraction kit (Germany) was used for DNA extraction as per manufacturer's instruction with a few modifications. The DNeasy PowerSoil kit is composed of 5 basic steps which are lysis, inhibitor removal, column binding, washing and elution. The Lysis step utilizes beads and lysis buffer to ensure cell disruption, this step was modified by adding a tissue lyzing (RETSCH MM400, Germany) sub step at 30 Hz for 2 min to enhance

lysis in replacement of vortexing for 10 minutes as the protocol suggests. The elution step was also modified to elute in 50 µL instead of 100 µL to obtain higher concentration of DNA.

Water DNA Extraction

The water was previously filtered using 0.22 µm Sterivex filters (Milipore, U.S). To acquire the filter paper from inside the plastic casing, pincers were used to break it open and sterile forceps were used to peel the filter from the plastic support. The extraction was done using the DNeasy PowerWater DNA Extraction Kit (Germany) following manufacturer's instruction with a few modifications. Similar to the PowerSoil Kit, the PowerWater has the same 5 basic steps, lysis, inhibitor removal, column binding, washing and elution. The lysis step was also optimized by adding an incubation for 30 min at 65˚C , followed by 10 min tissue lyzing at 30 Hz in replacement of 5 min vortexing and followed by additional incubation for 30 min at 65˚C in order to enhance lysis. The elution step was similarly optimized by eluting in 50 µL instead of 100 µL.

Amplification of 16S rRNA Gene

Polymerase Chain Reaction (PCR) was utilized to amplify the V4 region (Amplicon size ~300 bp) of the 16S rRNA gene. As described by Meyer et al. (2019) the V4 region of the 16s rRNA gene was amplified using the pair of primers 515F and 806RB (Invitrogen, Thermo Fisher Scientific, U.S.A). The forward primer 515F sequence was extracted from Parada et al. (2016), while, the reverse 806RB primer sequence was extracted form Apprill et al. (2015).

Forward Primer: 515F (515F-C); (5′-GTGCCAGCMGCCGCGGTAA) (Parada et al., 2016)

Reverse Primer: 806RB; (5'-GGA CTA CNV GGG TWT CTA AT-3') (Apprill et al., 2015).

The Platinum SuperFi II Green PCR Master Mix (Invitrogen, Thermo Fisher Scientific, U.S.A.) was used in a total reaction volume of 50 µl as described in Table 3.3. The thermal cycling conditions for the PCR reaction was performed according to thermocycler protocol (Apprill et al., 2015) as described in Table 3.4. To assess the success of the PCR product, 5 µl

were loaded in 1% agarose gel at 100V for 30 min. Ladder used was 100bp-10kb PCRBIO Ladder I (Biosystems, U.S.A)

Table 3.3: Components of the PCR reaction mixture

Component	Coral sample Volume (µl)	Water sample Volume (µl)	Final Concentration
Water(nuclease-free)	15	5	
2X Platinum SuperFi PCR Master Mix	25	25	1X
Forward Primer (10 µM)	2.5	2.5	0.5 µM
Reverse Primer (10 µM)	2.5	2.5	0.5 µM
Template DNA	5	15	varies
Total volume	50 µL/Reaction	50 µL/Reaction	

Table 3.4: Thermocycler conditions.

PCR Step	Temperature	Time, 96-well	Repeat cycle
Initial Denaturation	94 °C	3 min	1
Denaturation	94 °C	45 s	x35
Annealing	50 °C	60 s	
Extension	72 °C	90 s	
Final Extension	72 °C	10 min	1
Hold	4 °C	hold	

Purification of the PCR product

PCR product was purified from a 1.5% agarose gel ran at 50V for 70 min in autoclaved 0.5X TAE buffer. To avoid contamination, the PCR product was loaded in alternating wells, leaving one well empty in between each sample and all equipment were sterilized after every use. Bands were compared to the 100bp-10kb PCRBIO Ladder I (Biosystems, U.S.A) and the appropriate size band was excised using a sterile scalpel under minimal UV exposure. The 300

bp band was excised using a sterile scalpel under minimal UV exposure. The purification kit used was the Pure Link Quick Gel Extraction Kit (Thermo Fisher Scientific, U.S.A) following manufacturer's instruction with a few modifications. The weights of the excised bands were determined in order to add 3 volumes of the gel solubilization buffer per sample. The incubation temperature for gel solubilization was optimized to be 60˚C and absolute ethanol was used in replacement of isopropanol to enhance the DNA yield. The remaining column binding, washing and elution steps were performed according to the kits protocol.

DNA Quantity and Quality control

The DNA quality and quantity were checked by reading the whole absorption spectrum (220–750 nm) and calculating DNA concentration and absorbance ratio at both 260/280 and 230/260 using NanoDrop 2000 spectrophotometer (Thermo Scientific, USA) nm. To confirm the success of the purification step, a verification gel was also run at 100V for 10 min using 1% agarose gel. Table 3.5 summarizes gel electrophoresis conditions based on purpose.

Table 3.5: Gel electrophoresis conditions used in this study based on purpose.

Gel Purpose	Gel Percentage	Voltage	Time	Loading Volume
Standard Gel: Checking Extraction and PCR product	1%	100V	30 min	5 µl
Purification Gel: provides maximum band separation (in the presence of several bands).	1.5%	50V	60-70 min	30 µl
Verification Gel: for quick verification of the success of the purification	1%	100V	10 min	1-2 µl

Illumina MiSeq Sequencing

The clean PCR product was submitted for sequencing at Weill Cornell Medicine – Qatar. In brief, NGS libraries were made using the NEBNext Ultra II DNA Library Prep Kit for Illumina (New England BioLabs, USA) using the manufacturers recommendation. Amplicons were each end-repaired, indexed-adaptor ligated, amplified for 5 cycles, and cleaned. The resulting Illumina NGS libraries were then quality, and quantity checked, normalized, and pooled. The pooled NGS libraries were paired end sequenced using the Illumina MiSeq V2 reagent kit (2x150) with ~5% PhiX control. The pooled libraires were then demultiplexed and used for downstream bioinformatics analysis.

Bioinformatics and Data Analysis

QIIME 2 pipeline version 2021.8 was used to preform microbiome bioinformatics (Bolyen et al., 2019). Demultiplexed sequence data were quality filtered using q2-demux plugin. Adapters were trimmed using q2-cutadapt plugin (Martin, 2011) and sequence data was denoised using DADA2 (q2-dada2 plugin) (Callahan et al., 2016). Taxonomy was assigned to ASVs using the q2-feature-classifier against pre-trained classifier Greengenes 13_8 99% OTUs from 515F/806R region (Bokulich et al., 2018). q2-taxa was used to generate taxa bar plots. Mafft alignment and fasttree2 were used to construct a phylogenetic tree (via q2-alignment and q2-phylogeny) (Katoh et al., 2002; Price et al., 2010). Samples were rarified (sampling depth =1612) and Plugin q2- diversity was used to estimate alpha and beta diversity metrics. Robust Aitchison PCA was used to analyze compositional beta diversity using plugin q2-deicode (Martino et al., 2019). Differential abundance analysis (ANCOM) was done using plugin q2-composition (Mandal et al., 2015). Venn diagrams were created using http://bioinformatics.psb.ugent.be/webtools/Venn/. The surface area of coral colonies was measured using ImageJ image processing program using polygon feature (Schneider et al., 2012).

Antimicrobial Activity Assessment

Sampling

The objective of this section is to examine the antimicrobial activity of cultured isolates form *P. daedalea.* Five coral samples were collected form 3 different site during the summer. Sampling dates, sampling sites and time until processing are all shown (or described) in Table 3.6. Directly after collection, all samples were rinsed with sterile filtered seawater, placed in sterile Whirl-Pak bags and kept in ice on the boat. Upon arrival at the laboratory, samples were placed in an aquarium filled with sterile seawater maintained at 28˚C, water motion was maintained through an air pump and the salinity was maintained by covering the aquarium with a lid. Coral fragments were kept at a distance from the heater, air pump and from each other.

Table 3.6: Details of the coral samples collected for antimicrobial activity assessment.

Label	Collection Date	Collection Site	No. of hours in Aquaria
4L	27/8/20	Maydan Mahzam	Processed Immediately
4L2	3/9/20	Bulhanin 4	8 hours
5L	3/9/20	Bulhanin 4	8 hours
6L	4/9/20	Fasht East Halul	Processed Immediately
7L	4/9/20	Fasht East Halul	Processed Immediately

Bacterial Isolation

To culture bacteria, the coral samples were crushed using sterile mortar and pestle in 10 ml sterile seawater. The supernatant from the crushate was diluted serially (10^{-0} to 10^{-6}) in sterile seawater and plated in triplicate on 2 types of media, a general marine media, Glycerol Artificial Seawater (GASWA), and a *Vibrio* selective media Thiosulfate Citrate Bile Sucrose (TCBS). The composition of media used for coral microbial culturing is described below:

- Glycerol Artificial Seawater (GASW) Agar: 20.8 g/L Sodium Chloride NaCl (VWR, UK), 0.56 g/L Potasium Chloride KCl (BDH, UK), 4.8 g/L Magnesium Sulfate Heptahydrate MgSO4•7H2O (VWR, UK), 4.0 g/L Magnesium Chloride

Hexahydrate MgCl2•6H2O (GBioscience, USA), 0.01 g/L Potassium Phosphate Dibasic K2HPO4 (Sigma, Germany), 0.001 g/L FeSO4•7H2O (FUJIFILM Wako, USA), 2.0 g/L Aquarium salt (Coral Reefs, Qatar), 0.48 g/L Tris Base C4H11NO3 (Promega,USA), 4.0 g/L Tryptone (Biochem Chemopharma, France), 2.0 g/L Yeast Extract (FUJIFILM Wako, USA), 2 ml/L Glycerol (Promega, USA) and 15 g/L Agar (FUJIFILM Wako, USA) dissolved in distilled water and autoclaved at 121℃, 15 psi for 15 min.

- Thiosulfate Citrate Bile Sucrose (TCBS) Agar: 10 g/L Peptone, 5 g/L Yeast Extract, 10 g/L Sodium Sitrate, 10 g/L Sodium Thiosulfate, 1 g/L Iron (III) Citrate, 10 g/L Sodium Chloride, 8 g/L Dried Bovine Bile, 20 g/L Sucrose, 0.04 g/L Bromothymol Blue, 0.04 g/L Thymol blue, 15 g/L Agar dissolved in autoclaved (121℃, 15 psi for 15 min) 10 g/L NaCl solution. Media was prepared following manufacturer's instruction (Liofilchem, Italy).

The total bacterial load and percentage of *Vibrio* bacteria in coral samples were determined by counting colony forming units (CFUs). Bacteria were sorted according to their morphological characteristics and isolated by streaking in media plates until pure colonies were obtained. Five additional random bacterial colonies from each sample were also streaked to isolate pure strain and all bacteria was preserved in Microbank bacterial preservation cryovial system (Pro Lab Diagnostics, U.S.A).

Bacterial Identification Using 16S rRNA Gene Amplification

To extract DNA from the pure coral isolates, the Genomic Bacteria DNA Purification Kit (GENAXXON bioscience, Germany) was used. Prior to extraction, gram staining was performed for all isolates to determine whether a lysozyme step was needed. All bacteria were extracted from an overnight culture of a single colony suspended in 3 ml of GASW media in 15 ml tubes. For gram positive bacteria, isolates were treated with 40 µl of 100 mg/mL Lysozyme. The remaining steps of the protocol which included RNA and Protein removal, column binding, washing and elution were performed as per manufacturers' specifications. The quality of the DNA was assess using 1% agarose gel electrophoresis at 100V for 30 min. PCR and purification of DNA of all isolates was done following the methods described in sections 3.3.3 and 3.3.4. Purified PCR products were prepared in replicates (1 forward primer and 1 reverse primer) and then sent for sanger sequencing (Genetic Analyzer) at Weill Cornell Medicine-Qatar. Forward and reverse sequence reads were aligned using ClustalW and BLAST, compared to each other and cleaned using 4Peaks (V 1.8) and BioEdit (7.1) to generate consensus DNA sequence. Using BLAST, all sequences were identified by searching identical or similar sequences available in NCBI Genbank database and the most similar outcome was selected by comparing Percent Identity and other parameters like Total Score, E-value, Percent Query Coverage. Phylogenetic trees were constructed using maximum likelihood tree using MegaX (0.1)

In vitro Antimicrobial Assay

The potential of coral bacterial isolate to inhibit the growth of each other was screened using a cross-streaking method (Kvennefors et al., 2012) on Glycerol Artificial Seawater (GASWA) agar plates (150 mm x 15 mm). Prior to inoculation the plates were mapped the specification of each bacterium were indicated on each plate using a ruler and a marker. Specifications of the length, thickness and distance between test and target bacteria are shown in figure 3.5. To prepare the 'test isolate' 0.5 McF was prepared from a 24 h culture and 20 µl was deposited and streaked in the middle line of the plate. Plates of test isolate were incubated at 28˚C for 96 h. To prepare the 'target isolate' 0.5 McF of a 24 h culture was prepared and diluted 1:10 (10^{-1}). Diluted culture was used to cross streak against the 'test isolate' previously grown in the plate. The plates were then incubated at 28C˚ for 48h. Inhibition zones were measured from the edge of the vertical streak of the 'test isolate' to the first colony of 'target isolate' cross-streak.

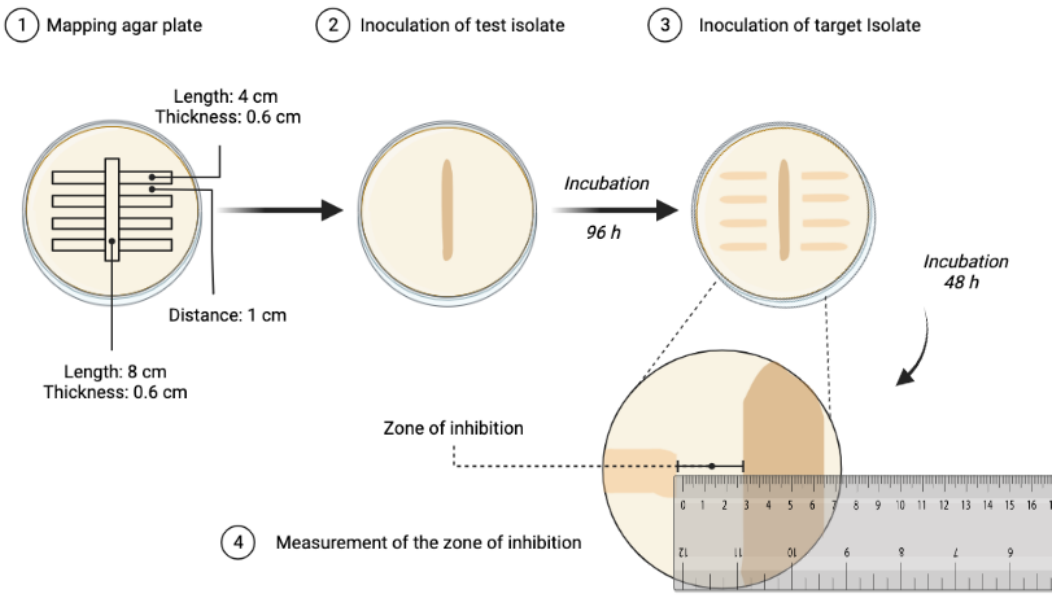


Figure 3.4: Cross streak method for testing antimicrobial activity.

Chapter 4 : RESULTS

Environmental Characteristics and Colony Condition

Colonies tagged and sampled appeared visually healthy throughout the duration of this study. Several biological and environmental variables were recorded at each site including reef type, reef depth, temperature, colony diameter, coral cover, algae cover and invertebrate cover. The reef type for Balhambar (BH), Umm Al Shaef (UMA) and Maydam Mahzam (MM) was flat, compared to Bulhanin 4 (BUL4) which was a Porites reef and Fasht East Halul (FEH) which had moderate relief. The depth of sites varied, the deepest site being Fasht East Halul at 18 m, followed by Maydan Mahzam, Bulhanin 4 and Balhambar at 15.2, 15 and 14 m respectively. The shallowest site was Umm Al Shaef at 12 m. The hourly temperature was recorded throughout summer and winter sampling events from August to March. Figure 4.1 and Table 4.1 shows the daily average temperature of study sites. Fasht East Halul was not included as temperature logger was lost. The temperature recorded for each site during the sampling event for the summer was 33˚C at Balhambar and Fasht East Halul while it was 34 ˚C at Umm Al Shaef, Maydan Mahzam and Bulhanin 4. On the other hand, the temperature recorded for each site during the sampling event for the winter was 22˚C at Balhambar, Bulhanin 4 and Fasht East Halul , 23 ˚C at Umm Al Shaef and 21˚C at Maydan Mahzam.

Table 4.1: Lowest and highest average daily temperature and temperature during sampling events.

Site	Lowest Temperature Recorded (˚C)	Highest Temperature Recorded (˚C)	Temperature on summer sampling day (˚C)	Temperature on winter sampling day (˚C)
Balhambar	20	34	33	22
Bulhanin 4	22	34	34	22
Fahset East Halul	21	34	34	22
Maydam Mahzam	22	34	34	21
Umm Al Shaef	20	35	34	23

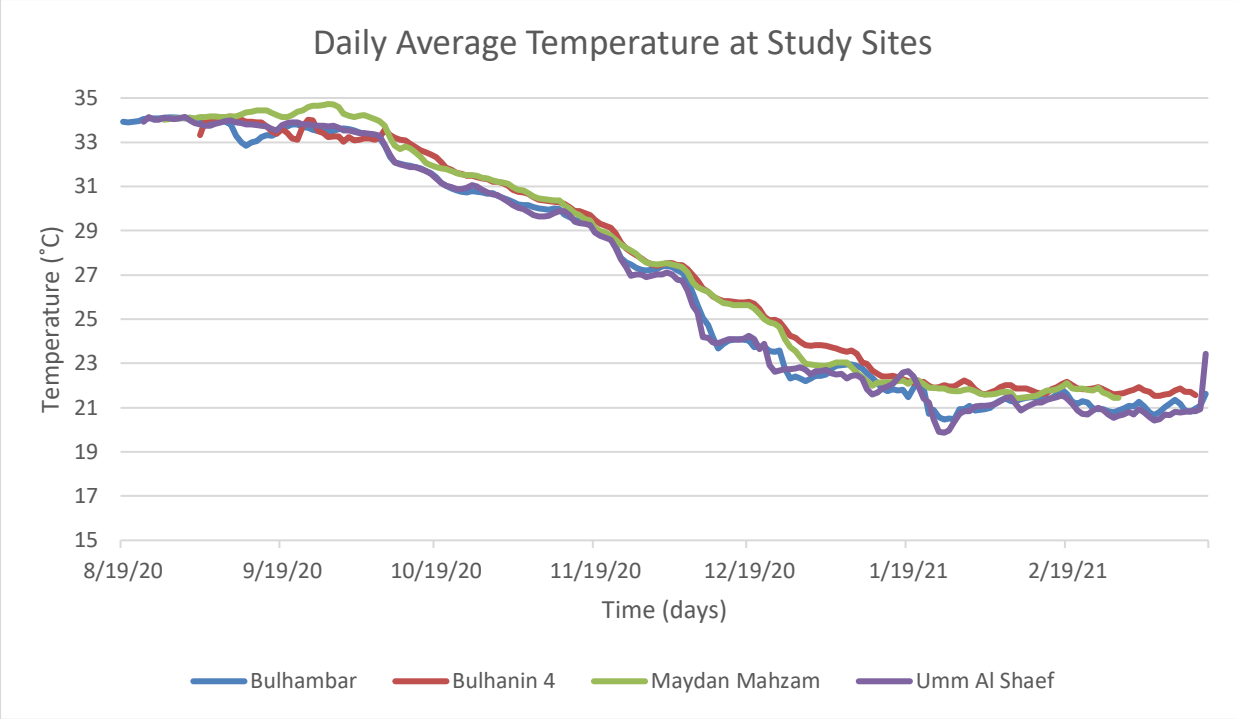


Figure 4.1: Daily average temperature of study sites.

Measurements of colony diameter were used to calculate the surface area of each tagged colony. The surface area of colonies ranged between 193 cm^2 and 26536 cm^2 with an average colony size of 3383 $\pm$ 4351 cm^2. Coral colonies in Umm Al Shaef, Balhambar and Fasht East Haluol were large compared to colonies in Maydan Mahzam and Bulhanin 4 which were considerably smaller as described in Table 4.2. The highest variability in colony size was observed at Umm Al Shaef.

Table 4.2: Sizes of tagged *P. daedalea* colonies used for microbiome analysis

Site	**Minimum Surface Area (cm^2)**	**Maximum Surface Area (cm^2)**	**Average Surface Area $\pm$ SD (cm^2)**
Balhambar	979.71	10505.48	5393.78±2849.99
Bulhanin 4	192.90	2172.54	830.15±775.07
Fasht East Halul	1036.40	7384.58	4390.97±1864.73
Maydan Mahzam	342.70	2650.46	821.42±701.06
Umm Al Shaef	746.50	26536.11	5474.97±8086.13

Figure 4.2 describes the substrate characteristics of the study sites as percentage coverage of benthic groups. The hard coral cover ranged between 14% and 38% with Umm Al Shaef having the lowest hard coral cover and Bulhanin 4 having the highest hard coral cover. Algae cover was high at all sites ranging between 21% and 35%. Similar to algae cover, the highest crustose coralline algae cover was observed in Fasht East Halul (3.23%) followed by Bulhanin 4 (3.15%) and Maydam Mahzam (2.33%). Umm Al Shaef and Balhambar had crustose coralline algae cover lower that 1%. Sponge cover was highest at Umm Al Shaef (6.4%) and lowest at Fasht East Halul (0.33%). Coral rubble was observed in 3 sites, Maydam Mahzam, Bulhanin 4 and Fasht East Halul with the highest cover at Maydam Mahzam. Cyanobacteria, soft corals, and other invertebrates such as hydroids, zoanthids, bryozoans and ascidians were also observed at some sites, however they were not in high abundance.

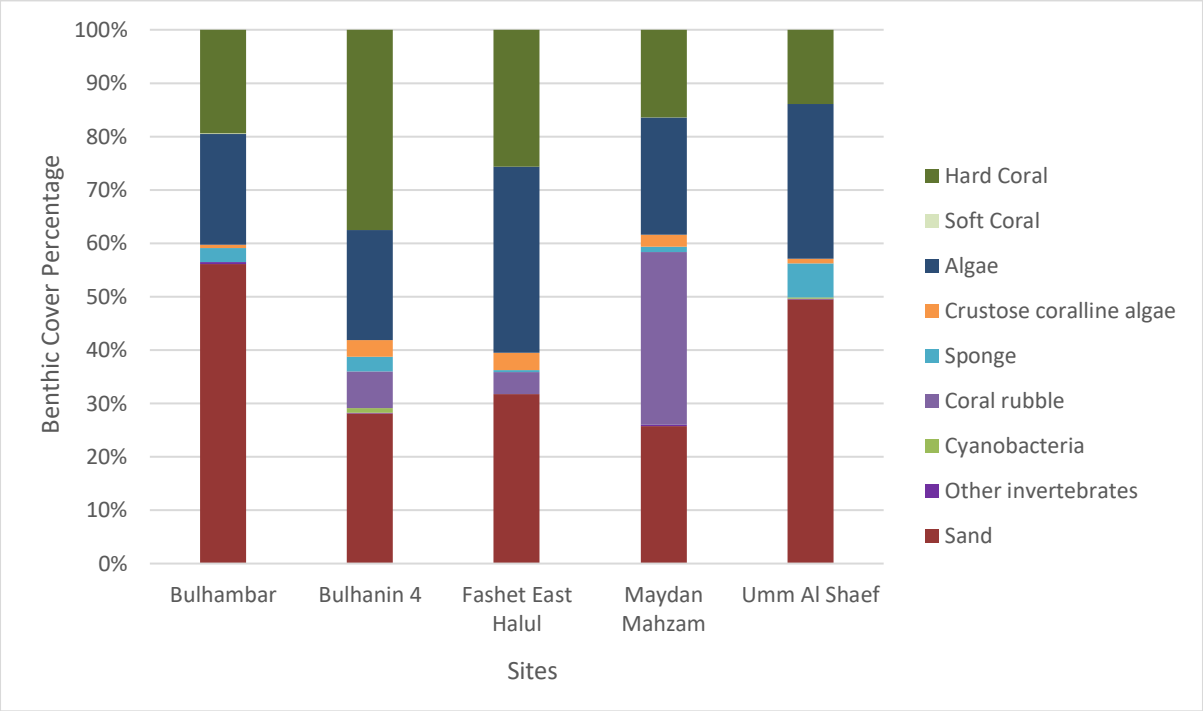


Figure 4.2: Substrate characteristics of sites used for repeated microbiome sampling collected underwater using line intercept method.

Bacterial Community Characterization

A total of 108 samples were processed including 91 coral samples and 17 water samples. Sequencing produced 6,517,960 raw reads across all samples, after merging, denoising, chimera filtering and removing mitochondria and chloroplast, the amplicon sequence variant's (ASVs) obtained totaled 16,666 reads. The minimum frequency of ASVs in a sample was 1612 while the maximum frequency of ASVs in a sample was 81,142. The median ASV frequency of samples was 22,004. Rarefaction curves for all samples plateaued, indicating that sequencing captured adequate ASV diversity. Coral samples (n=91) contained 15,833 ASVs, the minimum frequency of ASVs in a sample was 1612 while the maximum frequency of ASVs in a sample was 57,438. The median ASV frequency of samples was 20,298. After collapsing ASVs to genus level, 779 unique genus-level taxa were obtained. The core microbiome[1] of *P. daedalea* (here described as genus-level taxa present in 80% of the samples) made up about 5% of all taxa and consisted of 39 genera belonging to 2 kingdoms and 13 phyla. *P. daedalea* core microbiome is composed of archaeal phyla Parvarchaeota and Crenarchaeota (classes: Thaumarchaeota; Cenarchaeaceae and Parvarchaea) and the bacterial phyla Proteobacteria (families: Rhodospirillaceae, Rhodobacteraceae, Phyllobacteriaceae, Hyphomicrobiaceae and Piscirickettsiaceae), Actinobacteria (class: Acidimicrobiia), Bacteroidetes (families: Flammeovirgaceae, Flavobacteriaceae, Amoebophilaceae), Chloroflexi (classes: SAR202, Chloroflexi, TK17 and Anaerolineae), Planctomycetes (families: Pirellulaceae, Phycisphaeraceae and Planctomycetaceae) , Spirochaetes (family: Spirochaetaceae), Chlamydiae, Firmicutes (family: Clostridiaceae), Cyanobacteria (family: Pseudanabaenaceae), Gemmatimonadetes and SBR1093 (now recognized as Dadabacteria).

[1] Taxa comprising the core microbiome are available in Appendix A

***P. daedalea* Microbiome over Space (Site Comparisons)**

Alpha diversity was significantly higher for site BUL4 compared to all other sites (Kruskal Wallis, $p < 0.04$)[2] while there were no significant differences in the other four sites. Figure 4.3 describes Shannon index as a measure for alpha diversity. The evenness in all sites was high, the average Pielou's evenness index is equal to 0.8 ± 0.1. BUL4 evenness was significantly higher than other sites except when compared to MM (Kruskal Wallis, $p < 0.01$). Faith phylogenetic diversity index did not show any significant differences between the five sites.

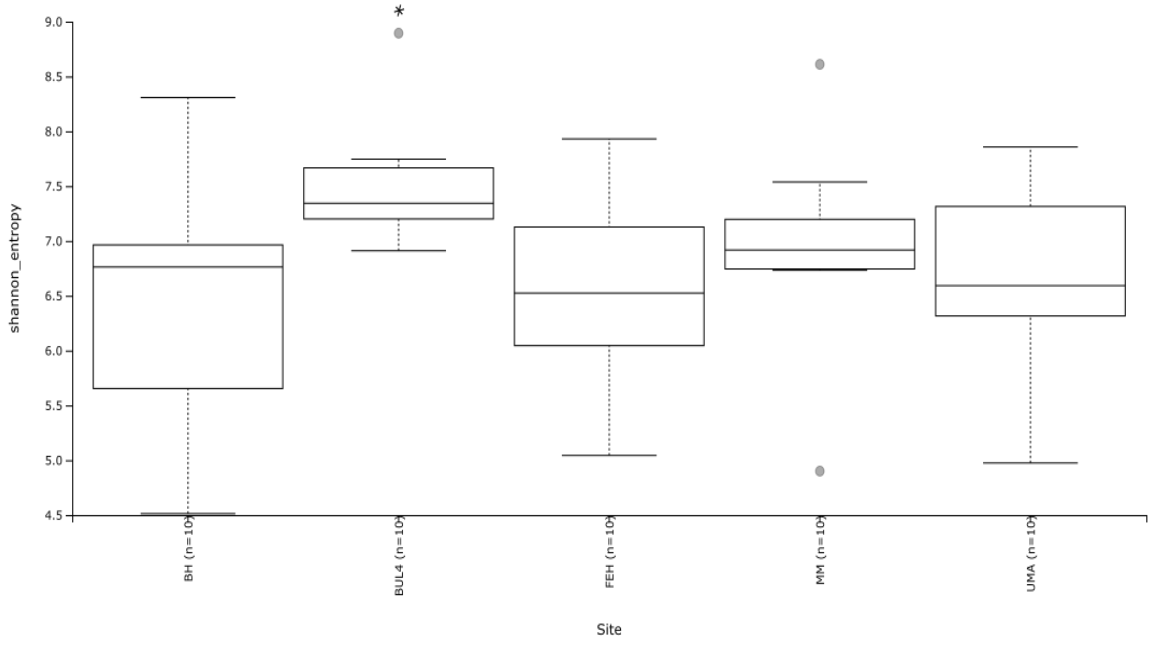


Figure 4.3: Spatial variation of mean values of the Shannon H indices at the the 5 study sites BH (n=10), UMA (n=10), MM (n=10), BUL4 (n=10), FEH (n=10). The star denote significant differences as determined by Kruskal Wallis ($p < 0.04$).

Beta diversity significantly varied according to site (PERMANOVA, $p < 0.001$)[3]. FEH was significantly different from all sites (PERMANOVA pairwise, $p < 0.01$). BH and UMA

[2] All Kruskal Wallis tests of are included in Appendix B.
[3] All PERMANOVA tests are included in Appendix C.

were not significantly different, however each was significantly different in comparison to the 4 other sites (PERMANOVA pairwise, $p < 0.01$). Similarly, BUL4 and MM were not significantly different, however each was significantly different in comparison to the 4 other sites (PERMANOVA pairwise, $p < 0.005$). Robust Aitchison PCA was used to understand the compositional beta diversity, Figure 4.4 shows evident clustering in agreement with the distance analysis. The scatter plot represents the individual samples while the arrows represent log ratio between features that strongly influence the principal component axis. The taxa that strongly influences the axis are *Nitrosopumilus* sp., Flammeovirgaceae spp, unidentified Pseudanabaenaceae species , *Sporotomaculum* sp., unidentified Halanaerobiaceae species, unidentified Alphaproteobacteria species and Piscirickettsiaceae species.

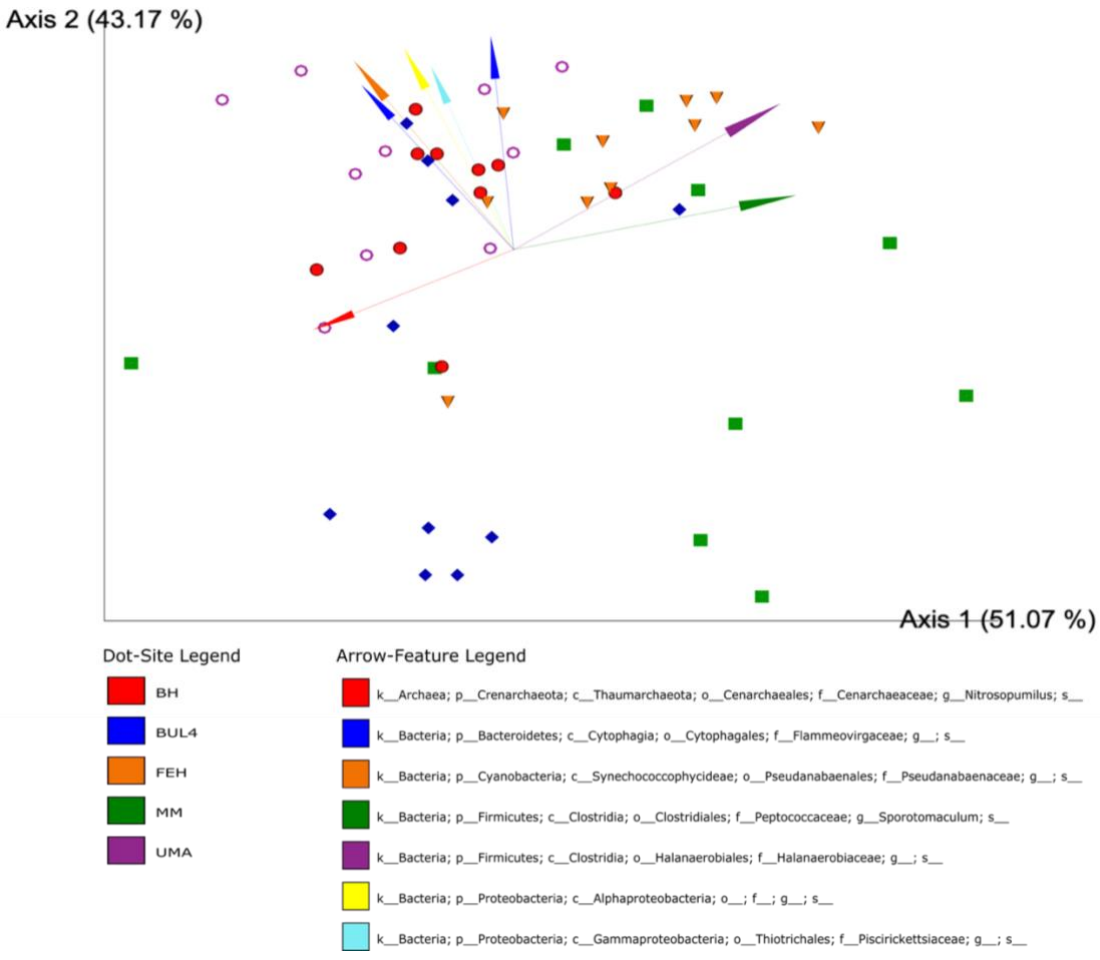


Figure 4.4: Principal component analysis (PCA) showing clustering of the microbiome of *P. daedalea* according to site. Dot-Site legend shows the 5 study sites BH (n=10), UMA (n=10), MM (n=10), BUL4 (n=10), FEH (n=10). Arrow-Feature legend displays the most influential taxa on clustering. In Arrow-Feature legend, in the case of no species level available, presence of (s) indicates that it was classified to species level, however, there was no close match in the database (Greengenes), absence of (s) indicates that it was classified to higher level taxonomy

Analysis of the core features of sites revealed that BH had 64 core taxa (present in 80% of all samples), UMA had 91, MM 82, BUL4 85 and FEH had73 core taxa. Thirty of the core taxa were shared between all five sites. Figure 4.5 is a Venn diagram showing the shared core taxa between the sites. BH, UMA and BUL4 all showed 12 unique taxa only present in the core microbiome of each of these sites, while MM and BH showed 4 and 2 unique core taxa respectively.

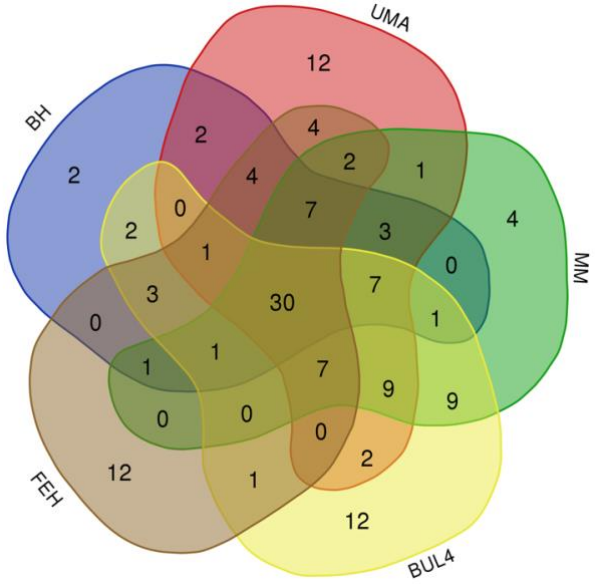


Figure 4.5: Venn diagram displaying the number of shared and unique core taxa between sites.

The differential abundance using ANCOM[4] revealed that 5 taxa were differentially abundant among the different sites as specified in Figure 4.6. Two cyanobacteria taxa; Pseudanabaenaceae (genus, W=669) and YS2 (genus, W=652) were differentially abundant across all sites. Gammaproteobacterial genus *Marinobacter* (W=635) was differentially abundant across all sites but was not present in BUL4. Two alphaproteobacterial genera were also differentially abundant, *Erythrobacter* (W=625) showed differential abundance in all sites except MM where it was not present while *Labrenzia* (W=619) showed differential abundance between MM, BUL4 and FEH.

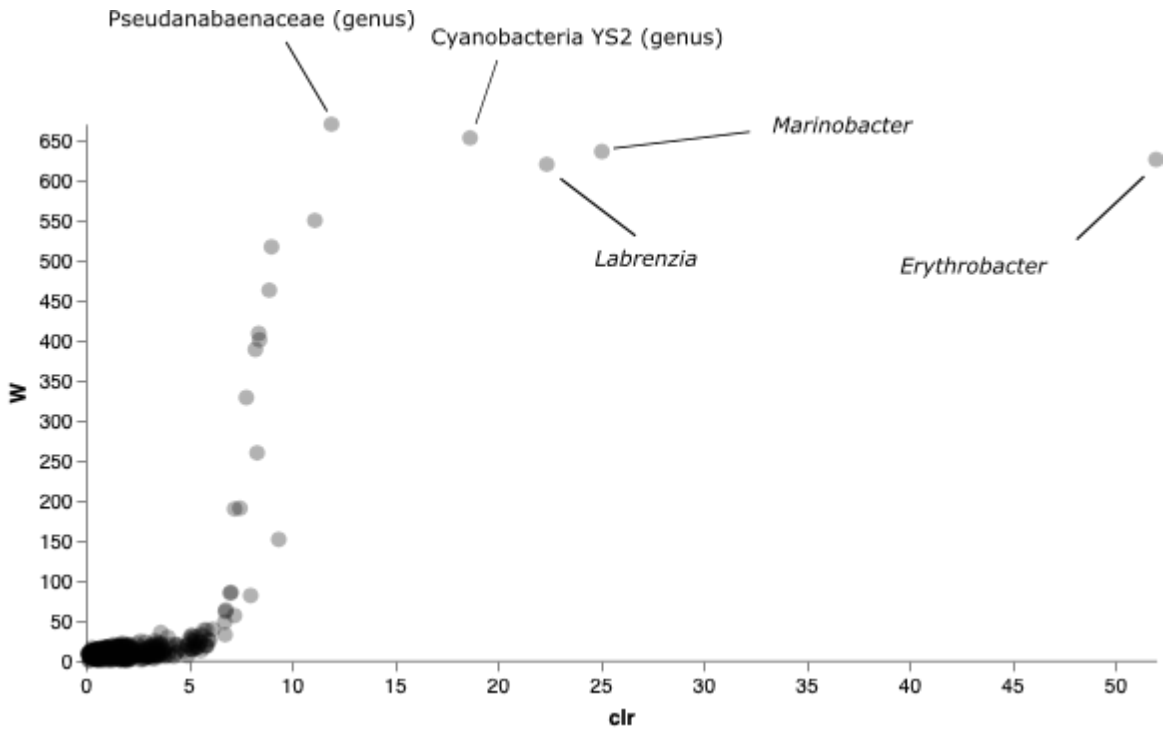
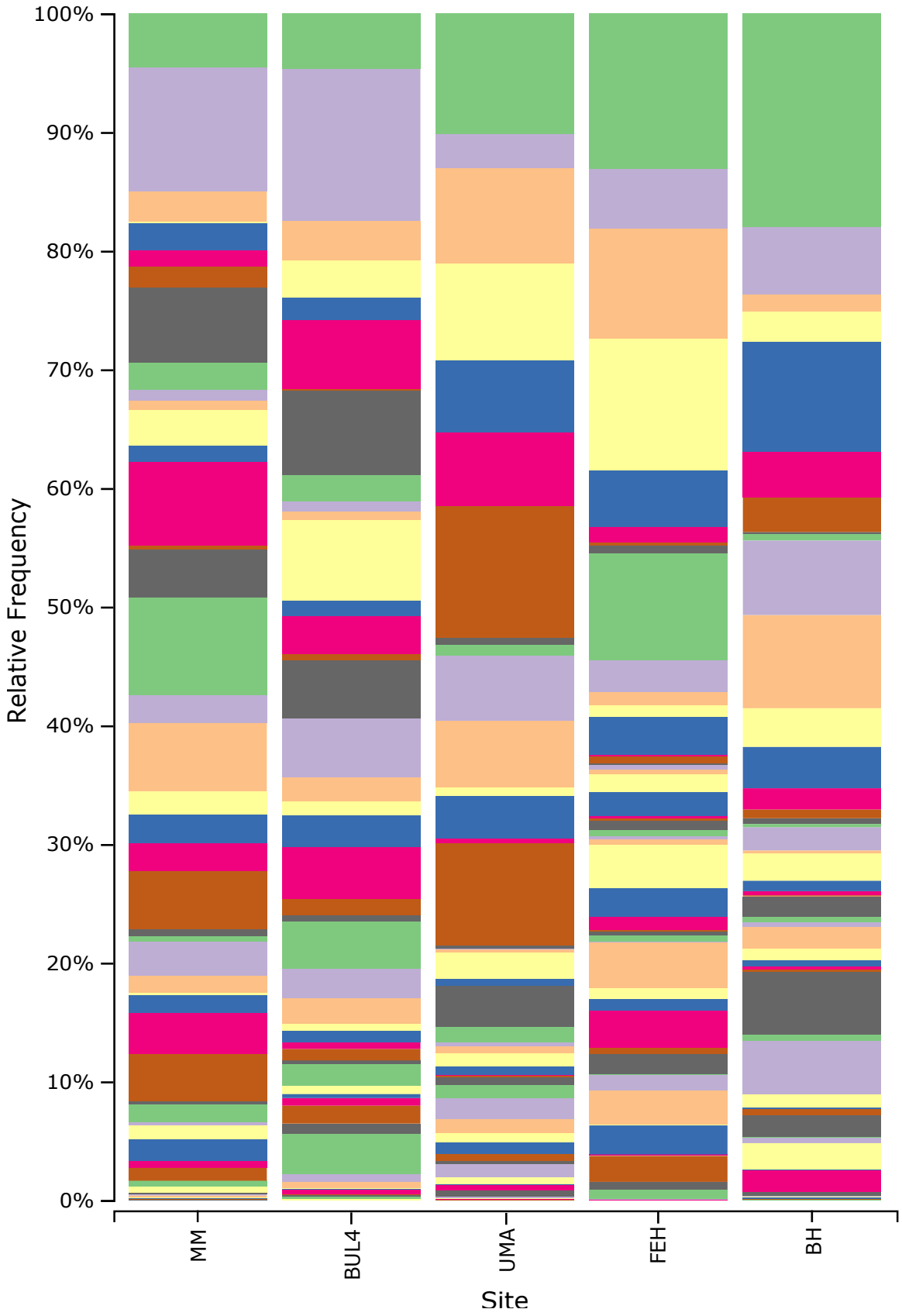


Figure 4.6: Volcano plot of ANCOM differential abundance between the 5 sites (n=50). The X-axis is Centered Log Ratio (CLR) represent log-fold change relative to the average microbe using mean difference in abundance of a given genera between sites. The Y-axis W value represents the number of times of the null hypothesis was rejected for a given genera. The null

[4] All ANCOM differential abundance tests are included in Appendix D

hypothesis states that the average abundance of a given genera in a site is equal to that in the other site. Genera where null hypothesis was rejected >95% is indicated and labeled.

The frequency of taxa between the 5 sites is displayed in the taxa bar plot in Figure 4.7. It is evident there are compositional changes between the sites, most notably, BH und BUL4 showed lower relative frequency of Alphaproteobacteria (genus) compared to the other sites. On the other hand, these two sites showed higher relative frequency of *Nitrosopumilus* compared to the 3 remaining sites. Pseudanabaenaceae (genus) showed a higher relative frequency in UMA and FEH while it showed very minute relative frequency in MM. Flammeovirgaceae (genus) displayed its highest relative frequency in BH, while it reported its lowest in BUL4. In addition, *Pseudoalteromonas* had great relative frequency in UMA compared to its frequency in BUL4 and FEH. BH and UMA demonstrated low relative frequency of Amoebophilaceae (genus) compared to other sites, especially FEH. *Roseivivax* was only evident in BH in low frequency and at MM in high relative frequency making up almost 10% of all ASV frequencies. *Pseudovibrio* displayed high relative frequency in UMA and minor frequencies at other sites. *Prosthecochloris* was only evident in UMA and FEH.


100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Relative Frequency
MM
BUL4
UMA
FEH
BH
Site

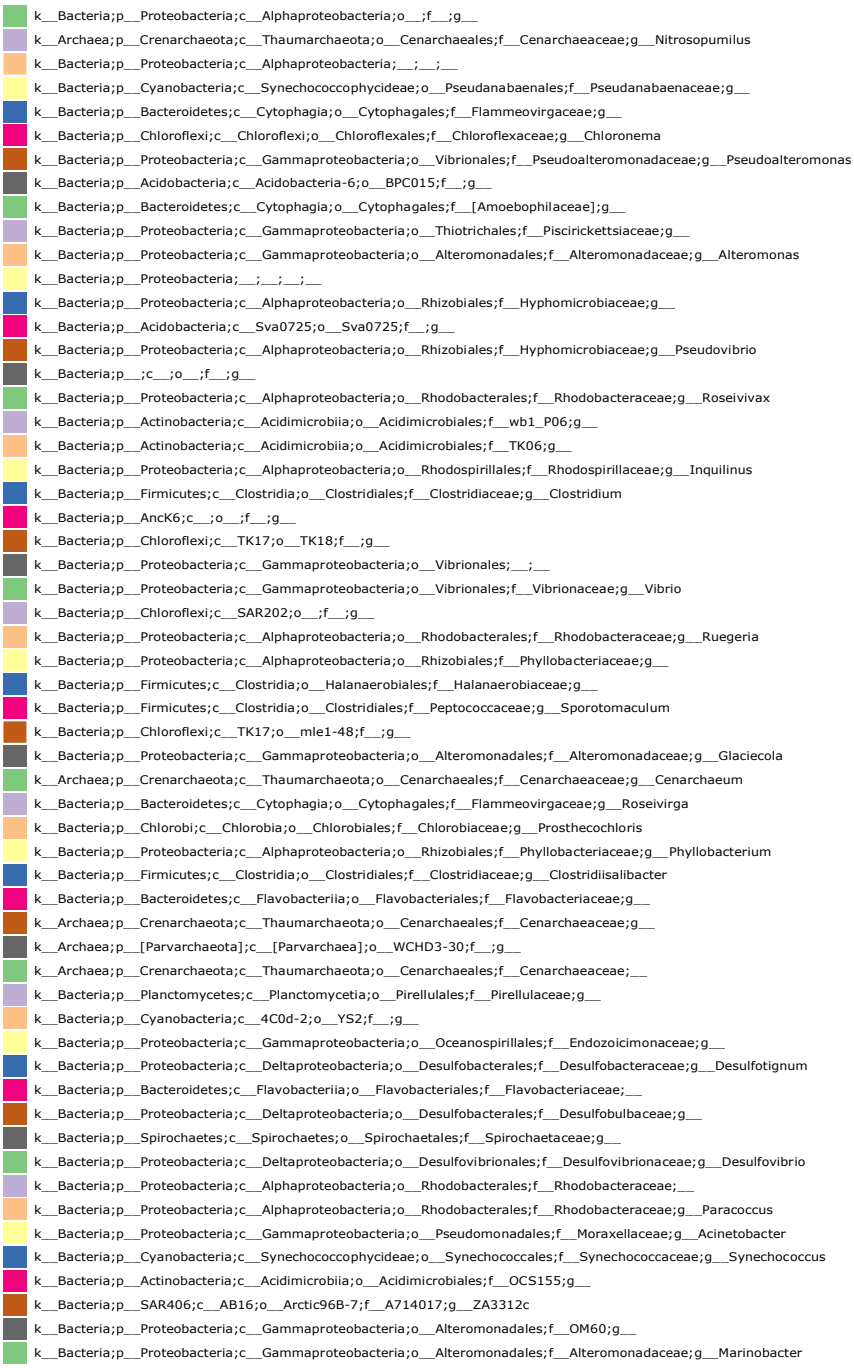
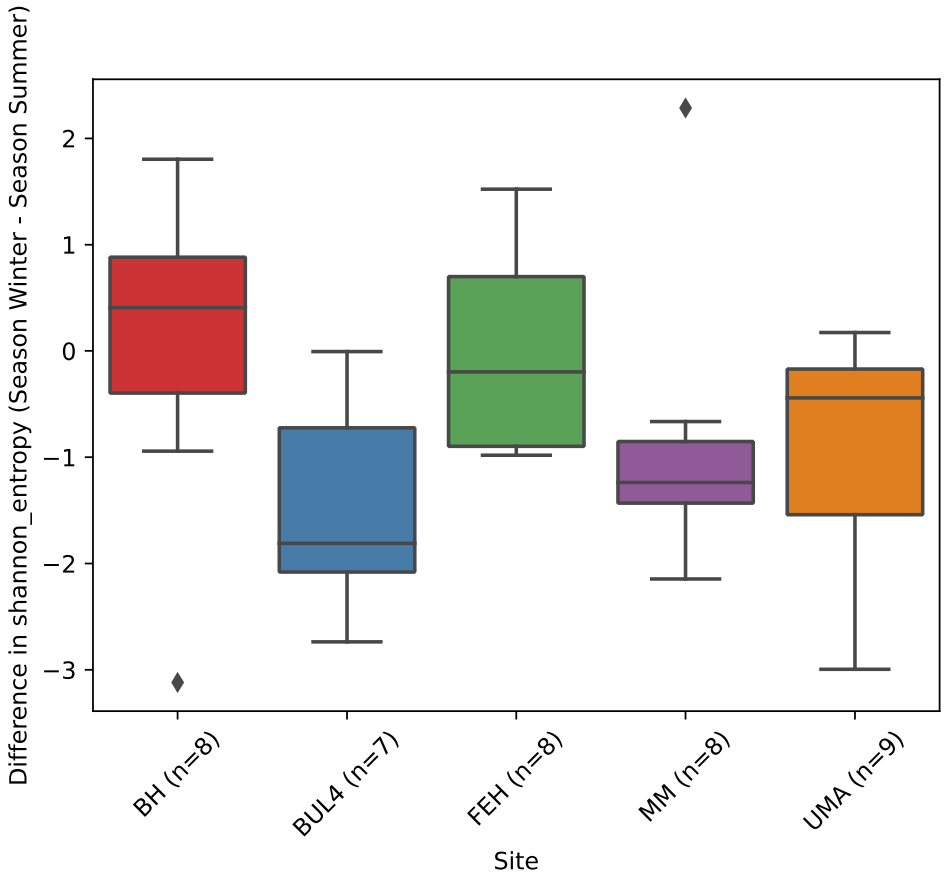


Figure 4.7: The relative frequency of the coral microbiome compared between the 5 study sites BH (n=10), UMA (n=10), MM (n=10), BUL4 (n=10), FEH (n=10). The taxonomy was collapsed to genus level. In the legend, where there was no genus level available, presence of (g) indicates that it was classified to genus level, however, there was no close match in the database (Greengenes), absence of (g) indicates that it was classified to higher level taxonomy. Features of a ASV frequencies below 5000 were removed to allow for better comprehension of taxa bar plot.

P. daedalea Microbiome over Time

Coral colonies were tagged and sampled in the summer and resampled in the winter therefore seasonal differences were analyzed as pairs using the paired difference Wilcoxon signed-rank test.[5] BUL4 and UMA both had significant differences ($p < 0.01$) between the summer and winter displaying lower alpha diversity in the winter as shown in Figure 4.8. Pairwise analysis of the differences between the sites using Mann-Whitney U test[6] revealed that FEH was significantly different from BUL4 ($p < 0.04$) and MM ($p < 0.04$). Evenness and Faith phylogenetic diversity showed no significant difference between seasons. Beta diversity between seasons did not show any statistical significance (Kruskal Wallis, $p > 0.05$) as shown in Figure 4.9.



[5] All Wilcoxon signed-rank tests are included in Appendix E.
[6] All Mann-Whitney U tests are included in Appendix F.

Figure 4.8: Seasonal variation of alpha diversity between summer and winter paired difference grouped by site. Significant differences were determined by Mann-Whitney U test ($p < 0.04$).

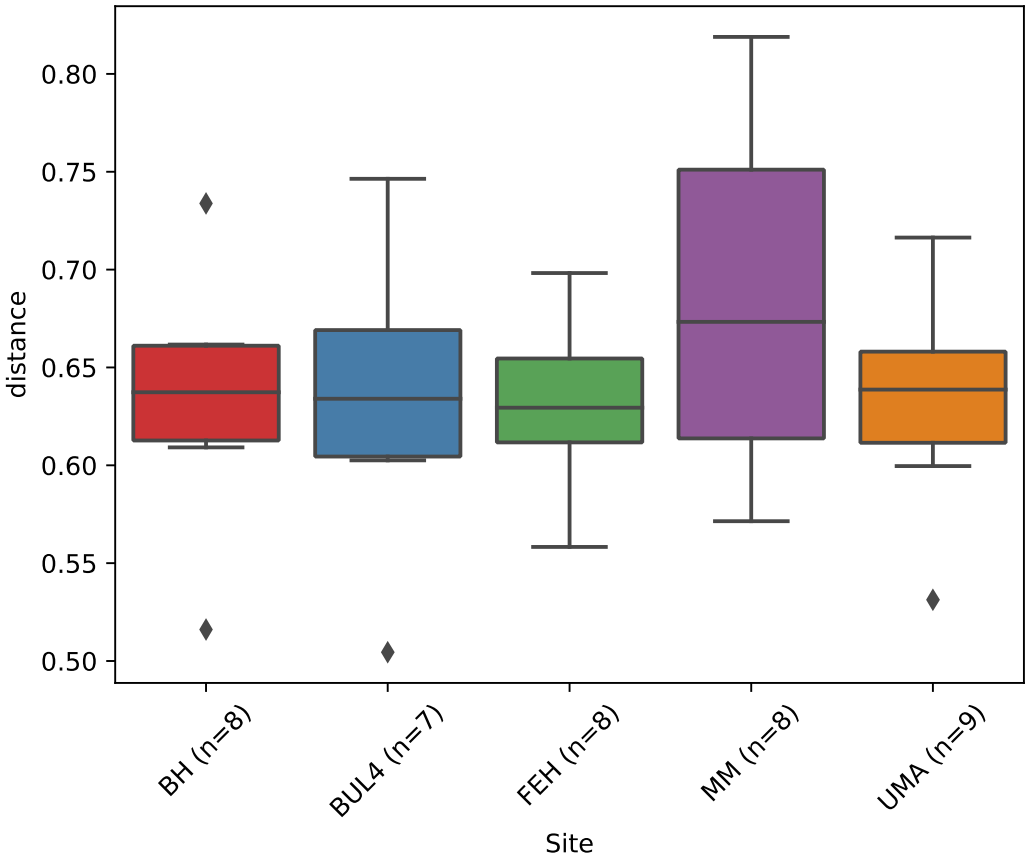


Figure 4.9: Seasonal variation of beta diversity between summer and winter paired difference grouped by site. Significant differences were determined by Mann-Whitney U test.

Principal component analysis of the compositional beta diversity agrees with the distance analysis, no clear or minimal clustering is evident as shown in Figure 4.10. The taxa responsible for clustering are *Nitrosopumilus*, Piscirickettsiaceae (species), *Pseudoalteromonas*, Flammeovirgaceae spp, Alphaproteobacteria and Amoebophilaceae (species). As shown in Figure 4.9.

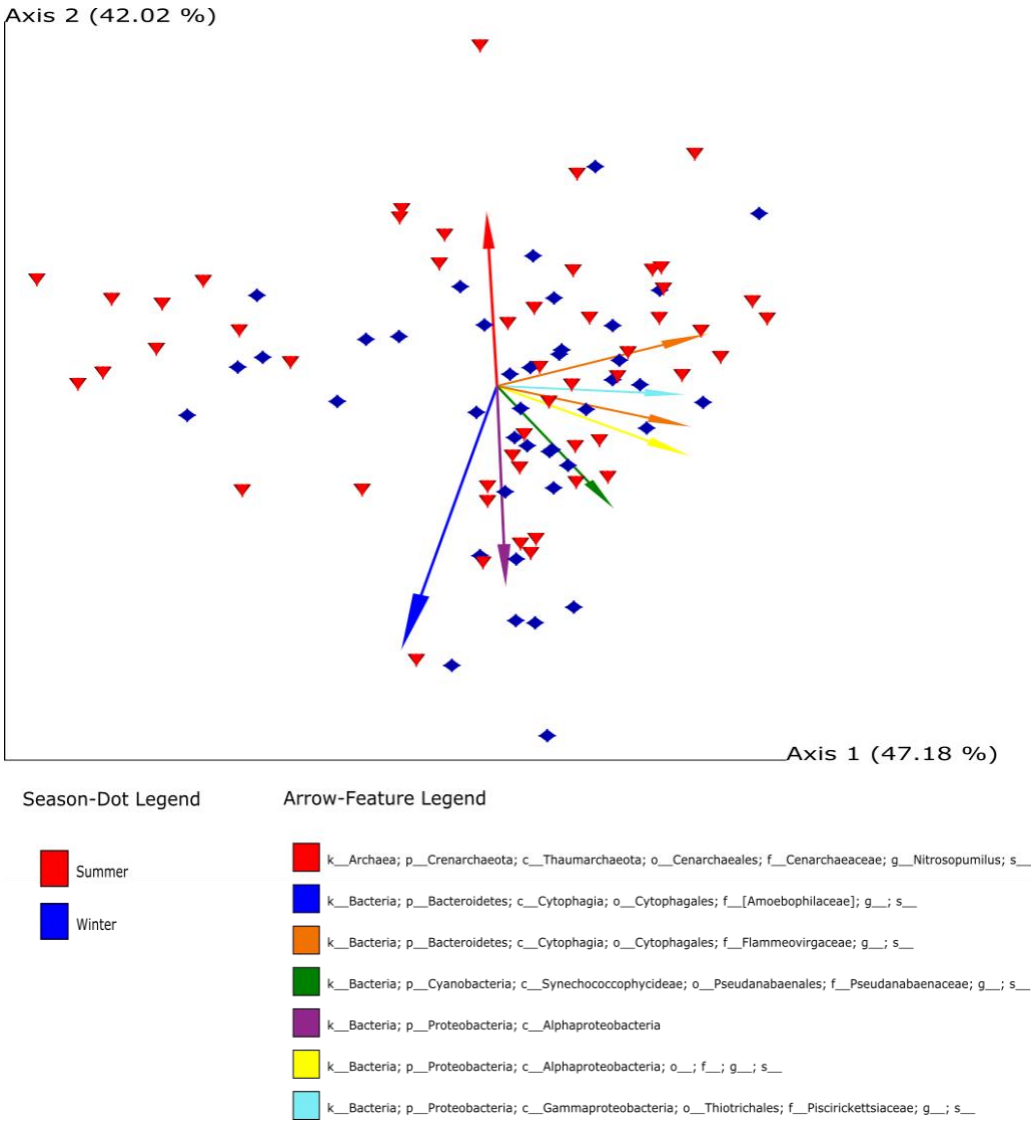


Figure 4.10: Principal component analysis (PCA) showing clustering of the microbiome of *P. daedalea* according to season. Dot-Season legend shows the 2 seasons, Summer (n=50) and Winter (n=41). Arrow-Feature legend displays the most influential taxa on clustering. In Arrow-Feature legend, in the case of no species level available, presence of (s) indicates that it was classified to species level, however, there was no close match in the database (Greengenes), absence of (s) indicates that it was classified to higher level taxonomy.

Analysis of the core features of sites revealed that Summer had 57 core taxa, 25 were unique to summer while Winter showed 36 core taxa where 4 were unique to winter. Thirty-two of the core taxa were shared between the 2 seasons. Figure 4.11 is a Venn diagram showing the shared core taxa between the seasons

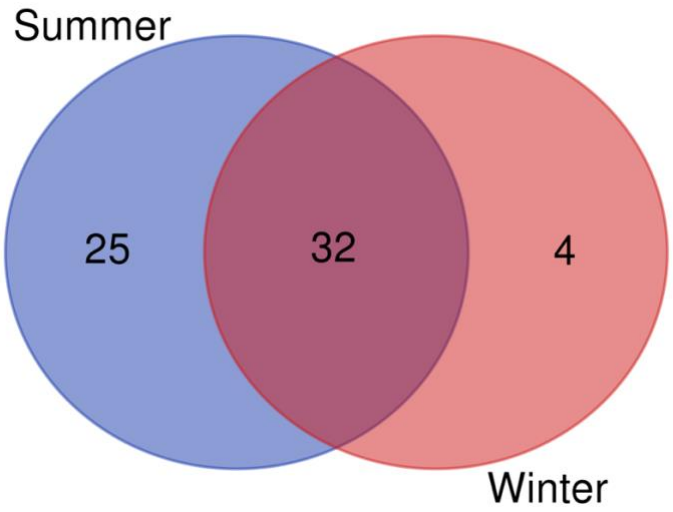


Figure 4.11: Venn diagram displaying the number of shared and unique core taxa between seasons.

. The differential abundance using ANCOM revealed that 4 taxa were differentially abundant among the seasons. *Erythrobacter* (W= 774), *Loktanella* (W= 773), *Marinobacter* (W= 742) were differentially abundant in the winter while *Henriciella* (W= 774) was differentially present in the winter and not present in the summer. Figure 4.12 is a volcano plot shown the highly differentiated taxa.

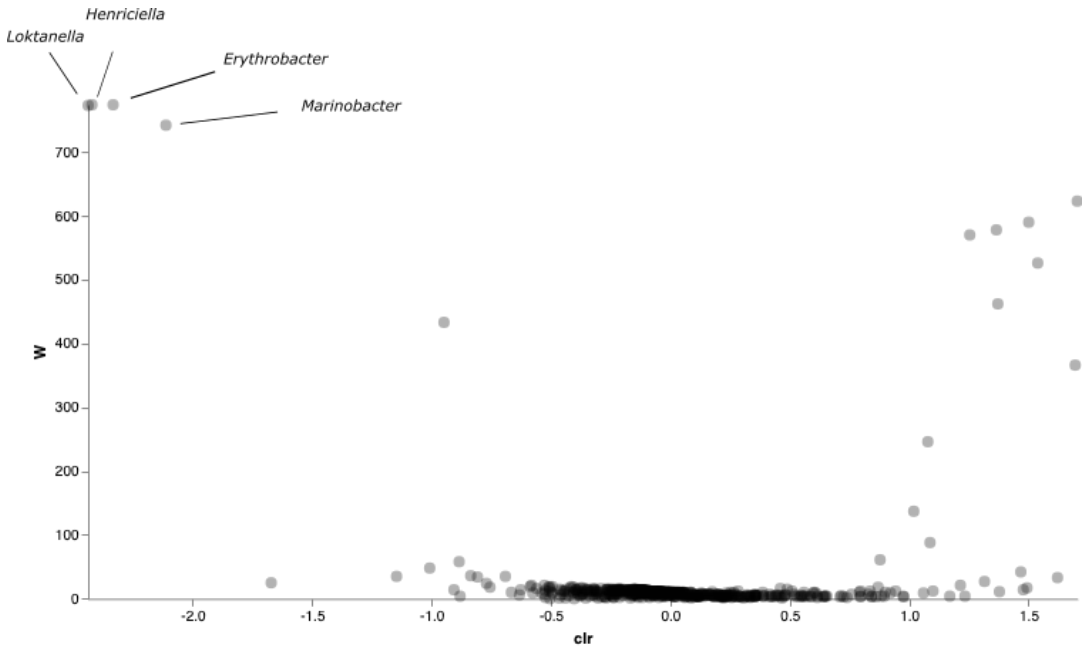
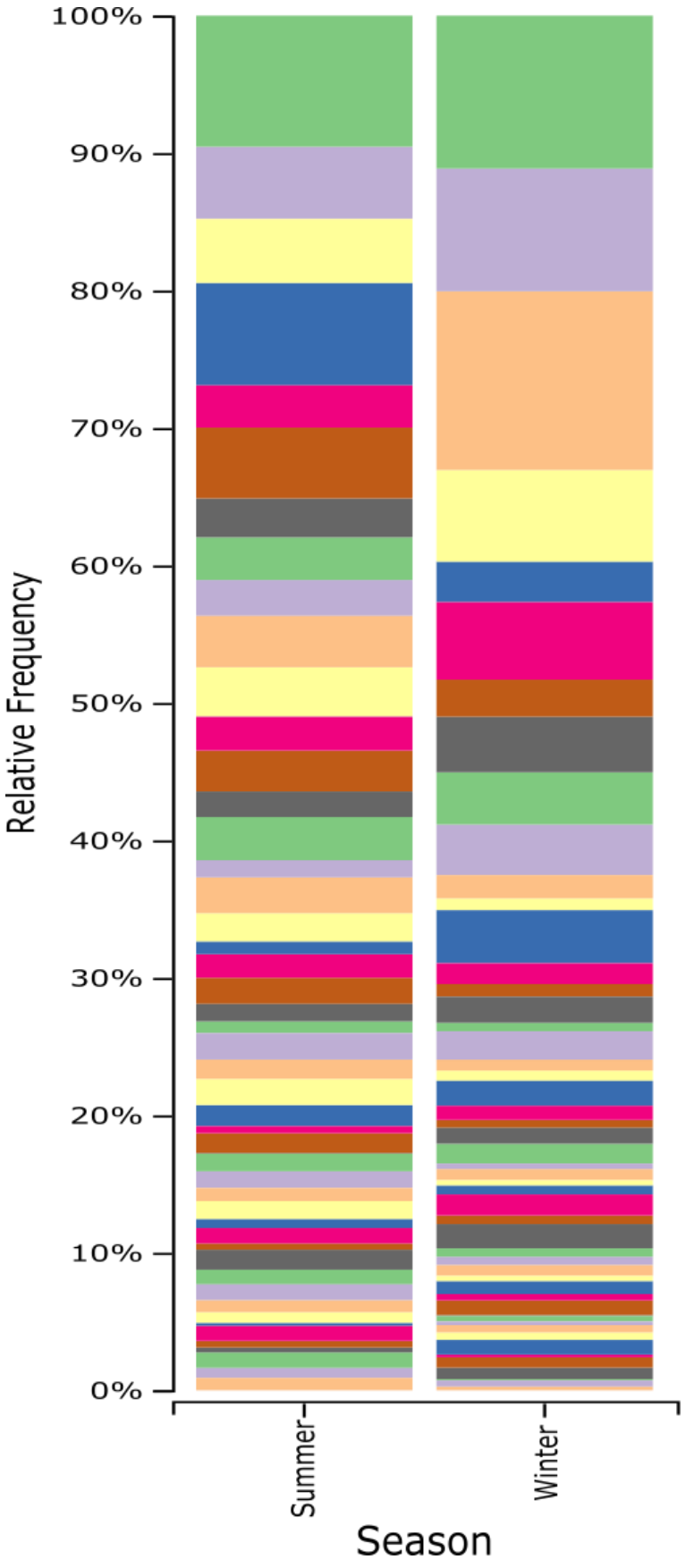


Figure 4.12: Volcano plot of ANCOM differential abundance between the 2 seasons (n=91). The X-axis is Centered Log Ratio (CLR) represent log-fold change relative to the average microbe using mean difference in abundance of a given genera between sites. The Y-axis W value represents the number of times of the null hypothesis was rejected for a given genera. The null hypothesis states that the average abundance of a given genera in a site is equal to that in the other site. Genera where null hypothesis was rejected >95% is indicated and labeled.

The frequency of taxa between summer and winter is displayed in the taxa bar plot in Figure 4.13. It is evident there are compositional changes between the sites, most notably, *Acinetobacter, Marinobacter, Desulfotignum, Paracoccus, Desulfovibrio,* Piscirickettsiaceae (genus) and Spirochaetaceae (genus) all had higher relative frequencies in the winter. On the other hand, archaeal genus *Nitrosopumilus* and bacterial *Pseudoalteromonas, Roseivivax, Chloronema,* Pseudanabaenaceae (genus) Acidobacteria (genus), Chloroflexi (genus), *Glaciecola* and *Clostridiisalibacter* showed higher relative frequencies in the summer compared to the winter.


100%
90%
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20%
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Relative Frequency
Summer
Winter
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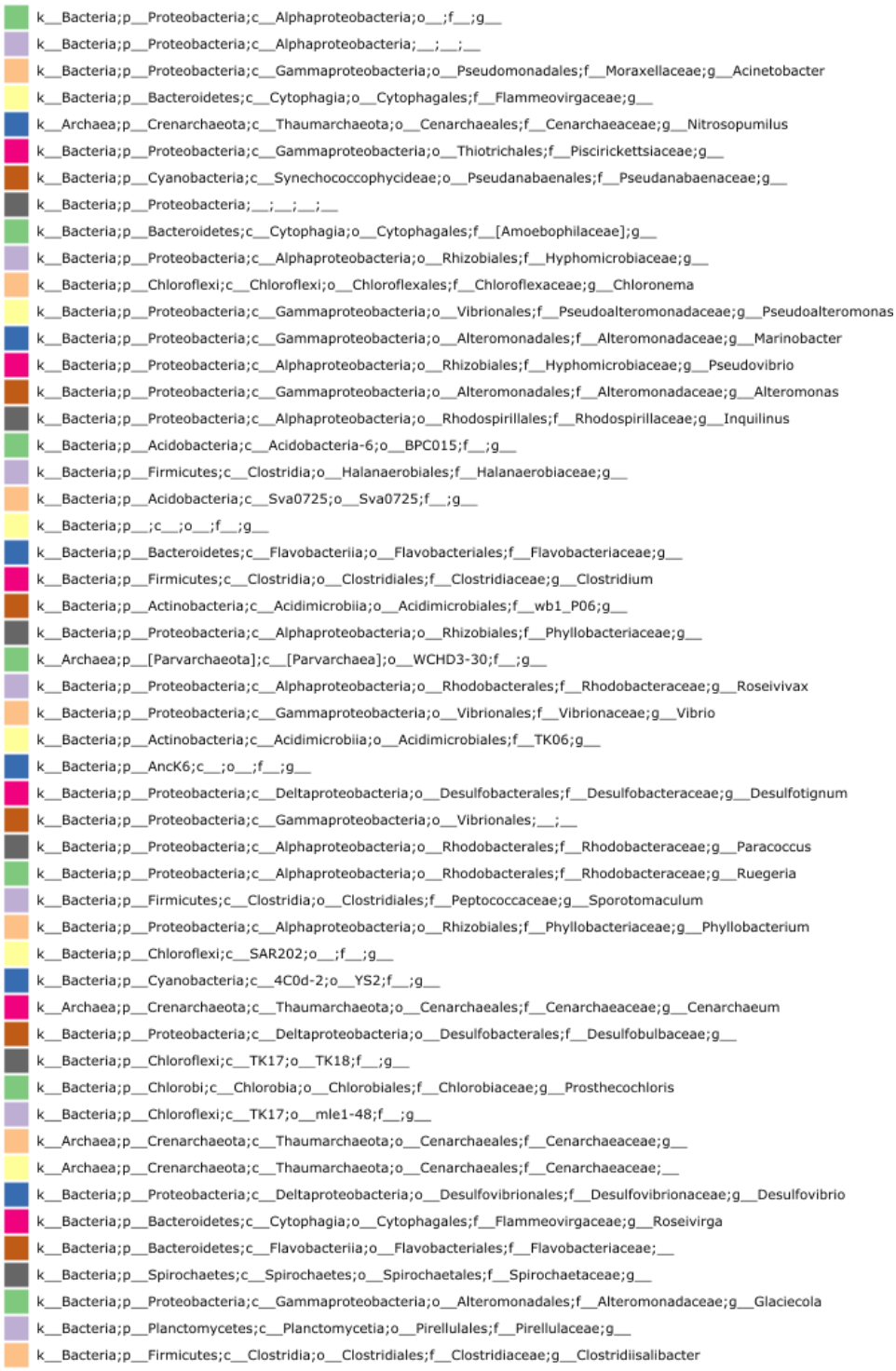


Figure 4.13: The relative frequency of the coral microbiome compared between the 2 seasons Summer (n=50) and Winter (n=41). The taxonomy was collapsed to genus level. In legend, in the case of no genus level available, presence of (g) indicates that it was classified to genus level, however, there was no close match in the database (Greengenes), absence of (g) indicates that it was classified to higher level taxonomy. Features of a frequency below 5000 were removed to allow for better comprehension of taxa bar plot.

Free-living Community around the Coral as an Indicator for Environmental Changes

Comparison of Microbial Communities between Coral and Coral Adjacent Water

Alpha diversity was measured as paired difference in Shannon entropy between coral and coral adjacent water using Wilcoxon signed rank test. There were statistically significant difference in alpha diversity ($p < 0.01$) where coral colonies had a higher diversity compared to their adjacent seawater. Beta diversity also demonstrated high significant difference between coral and coral adjacent water (PERMANOVA, $p < 0.001$). Principal component analysis (PCA) showed differentiation between coral and coral adjacent water and between individual corals. There is some level of clustering coral and their respective water. Species that were relevant in this clustering are *Pseudovibrio denitrificans*, Acidimicrbiales (species), Alteromonas (species) and *Vibrio* (species) as displayed in Figure 4.14.

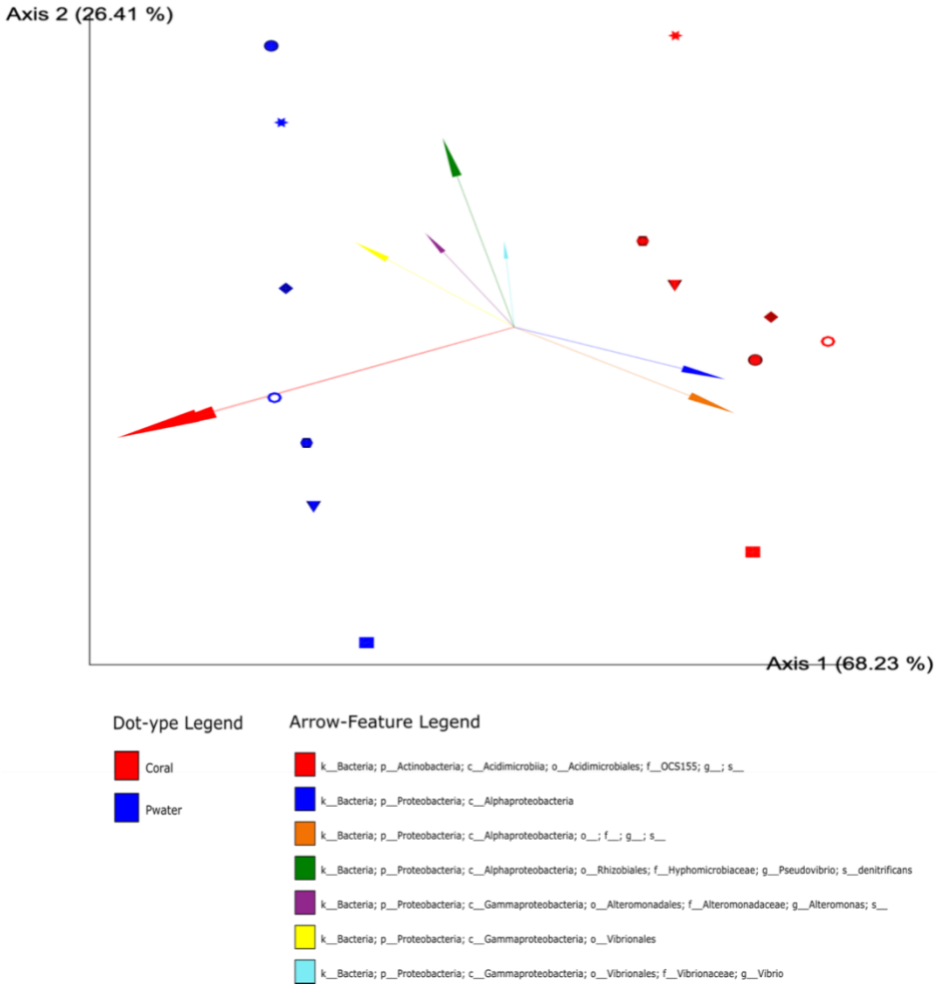


Figure 4.14: Principal component analysis (PCA) shows comparison of the microbial communities between coral and coral adjacent water. Dot-Sample Type legend shows the 2 sample types, coral (n=7) and water (n=7). Arrow-Feature legend displays the most influential taxa on clustering. In Arrow-Feature legend, in the case of no species level available, presence of (s) indicates that it was classified to species level, however, there was no close match in the database (Greengenes), absence of (s) indicates that it was classified to higher level taxonomy.

Core feature analysis showed that coral adjacent water had 40 core feature 28 of which were unique while corals had 31 core features, 19 of which were unique. Coral and coral adjacent water shared 12 taxa as displayed in Figure 4.15. ANCOM differential analysis in Figure 4.16 revealed that Actinobacteria OCS155 (genus, W=392), SAR406 ZA3312c (genus, W=379) and Gammaproteobacterial Candidatus Portiera (W=367) are differentially abundant in water, while Gammaproteobacterial OM60 (genus, W=385) Synechococcus (W=382), Octadecabacter (W=359) and Thiohalorhabdaceae (genus, W=355) are differentially present in water and not in coral.

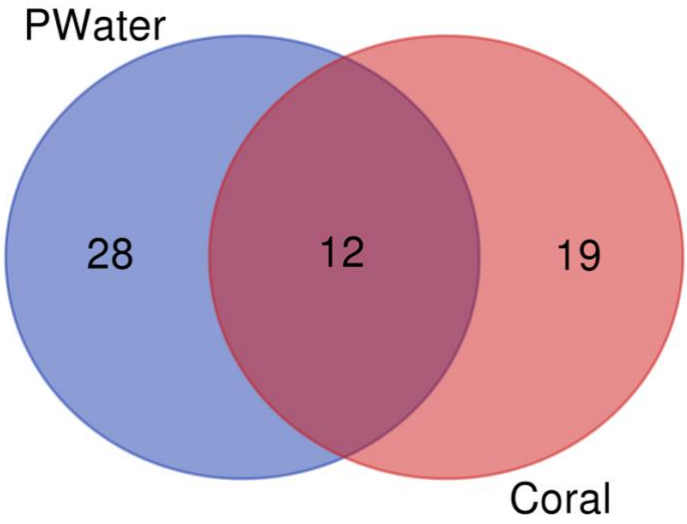


Figure 4.15: Venn diagram displaying the number of shared and unique core taxa between coral and coral adjacent water (Pwater).

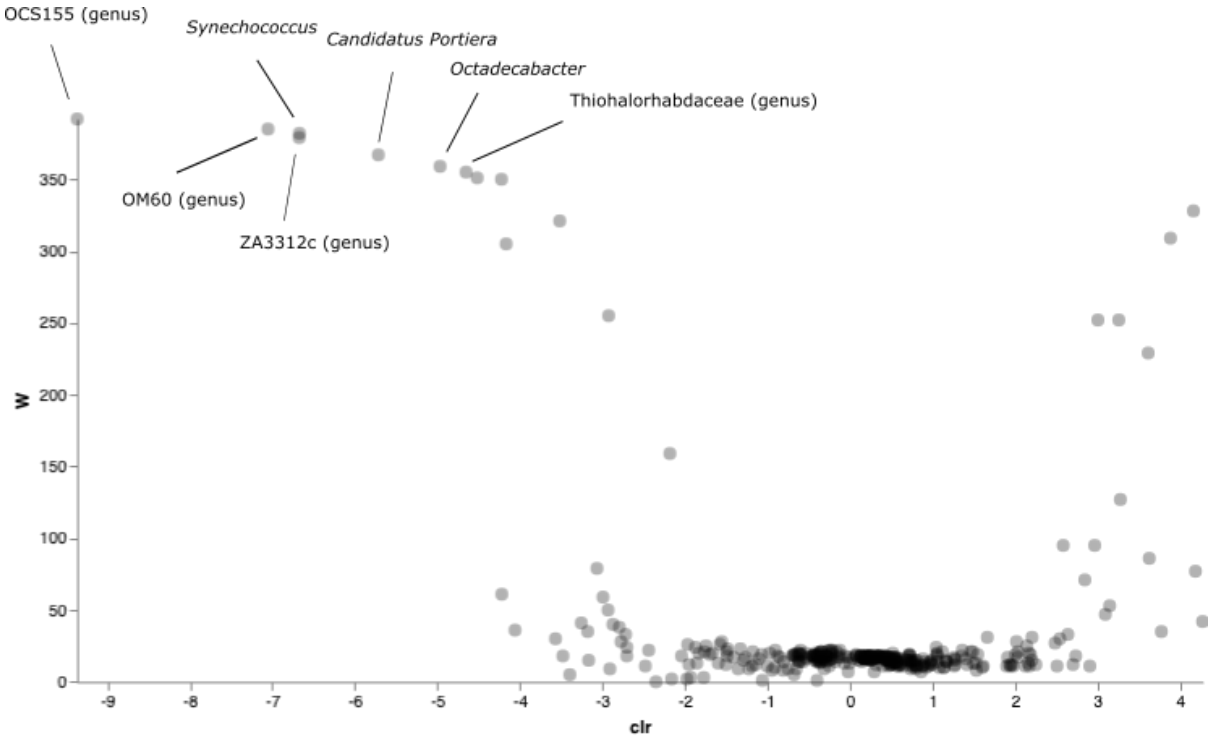


Figure 4.16: Volcano plot of ANCOM differential abundance between the 2 sample types (n=14). The X-axis is Centered Log Ratio (CLR) represent log-fold change relative to the average microbe using mean difference in abundance of a given genera between sites. The Y-axis W value represents the number of times of the null hypothesis was rejected for a given genera. The null hypothesis states that the average abundance of a given genera in a site is equal to that in the other site. Genera where null hypothesis was rejected >95% is indicated and labeled. (genus) next to family name indicates classification to to species level, however, there was no close match in the database (Greengenes).

Comparison of Microbial Communities between General Reef Water and Coral Adjacent Water

Analysis of Alpha diversity between general reef water and coral adjacent water showed that Shannon index, Evenness and Faith phylogenetic diversity were all non-significant (Kruskal Wallis $p > 0.05$). Beta diversity demonstrated significant difference between general reef water and coral adjacent water (PERMANOVA, $p < 0.002$). Principal component analysis in Figure 4.17 showed clustering of each type of water sample, Acidimicrbiales (species), Flavobacteriaceae (species) *Pseudovibrio denitrificans*, Candidatus Portiera (species) resulted in most of this clustering. ANCOM differential abundance showed no differentially abundant taxa.

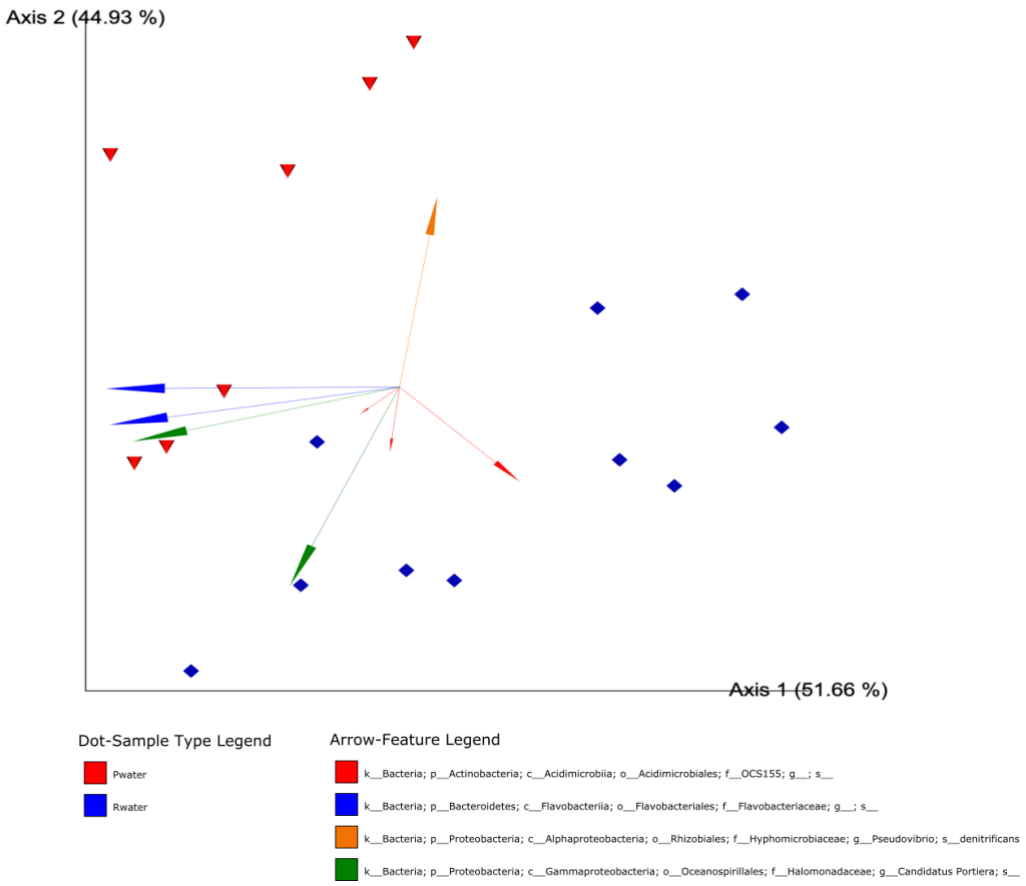
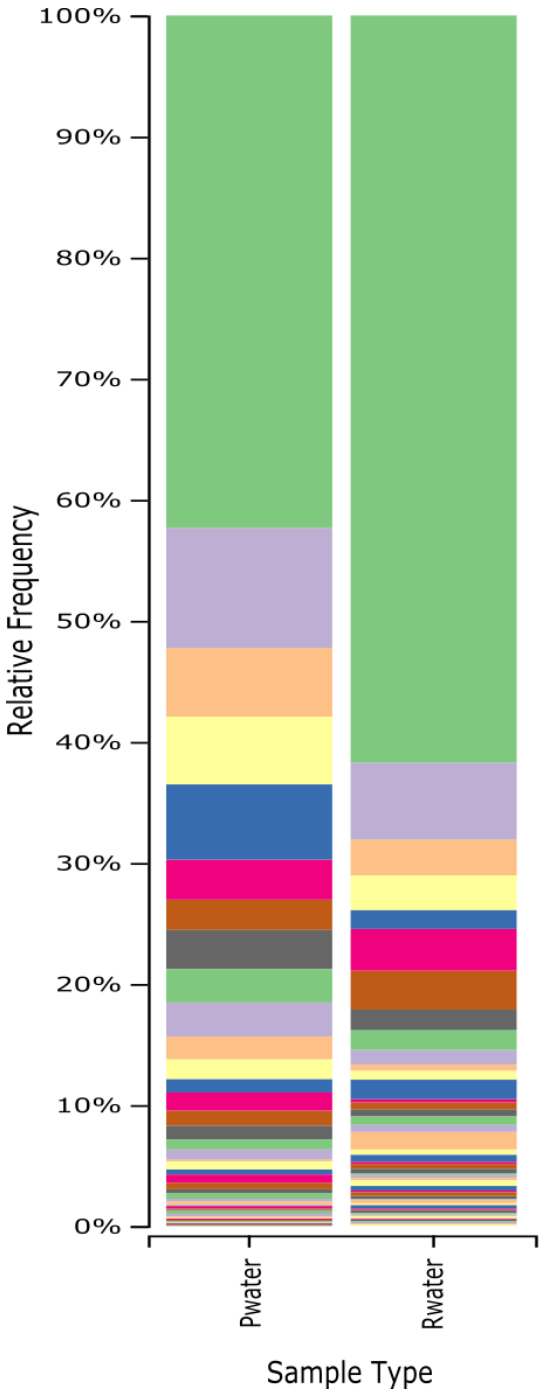


Figure 4.17: Principal component analysis (PCA) shows comparison of the microbial communities between coral adjacent water (Pwater) and general reef water (Rwater). Dot-

Sample Type legend shows the 2 sample water types, general reef water (Rwater, n=10) and coral adjacent water (Pwater, n=7). Arrow-Feature legend displays the most influential taxa on clustering. In Arrow-Feature legend, in the case of no species level available, presence of (s) indicates that it was classified to species level, however, there was no close match in the database (Greengenes), absence of (s) indicates that it was classified to higher level taxonomy.

Figure 4.18 is a taxa bar plot showing the relative frequency of microbial taxa between general reef water (Rwater) and coral adjacent water (Pwater). Acidimicrobiales OCS155 (genus) represented the majority of both sampling distances, however, it was more relatively frequent in Rwater. Flavobacteriaceae (genus), *Alteromonas, Vibrio, Pseudovibrio, Pseudoalteromonas, Roseivirga, Tenacibaculum, Shimia, Amphritea, Pseudoruegeria* and Methylophilaceae (genus) all showed higher relative frequency in PWater in comparison with Rwater. *Staphylococcus, Streptococcus, Loktanella, Corynebacterium, Acinetobacter, Pelagibacter, Propionibacterium, Prochlorococcus,* Gemellaceae, Pasteurellaceae and Tissierellaceae displayed higher relative frequency in Rwater compared to Pwater.


100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Relative Frequency
Pwater
Rwater
Sample Type

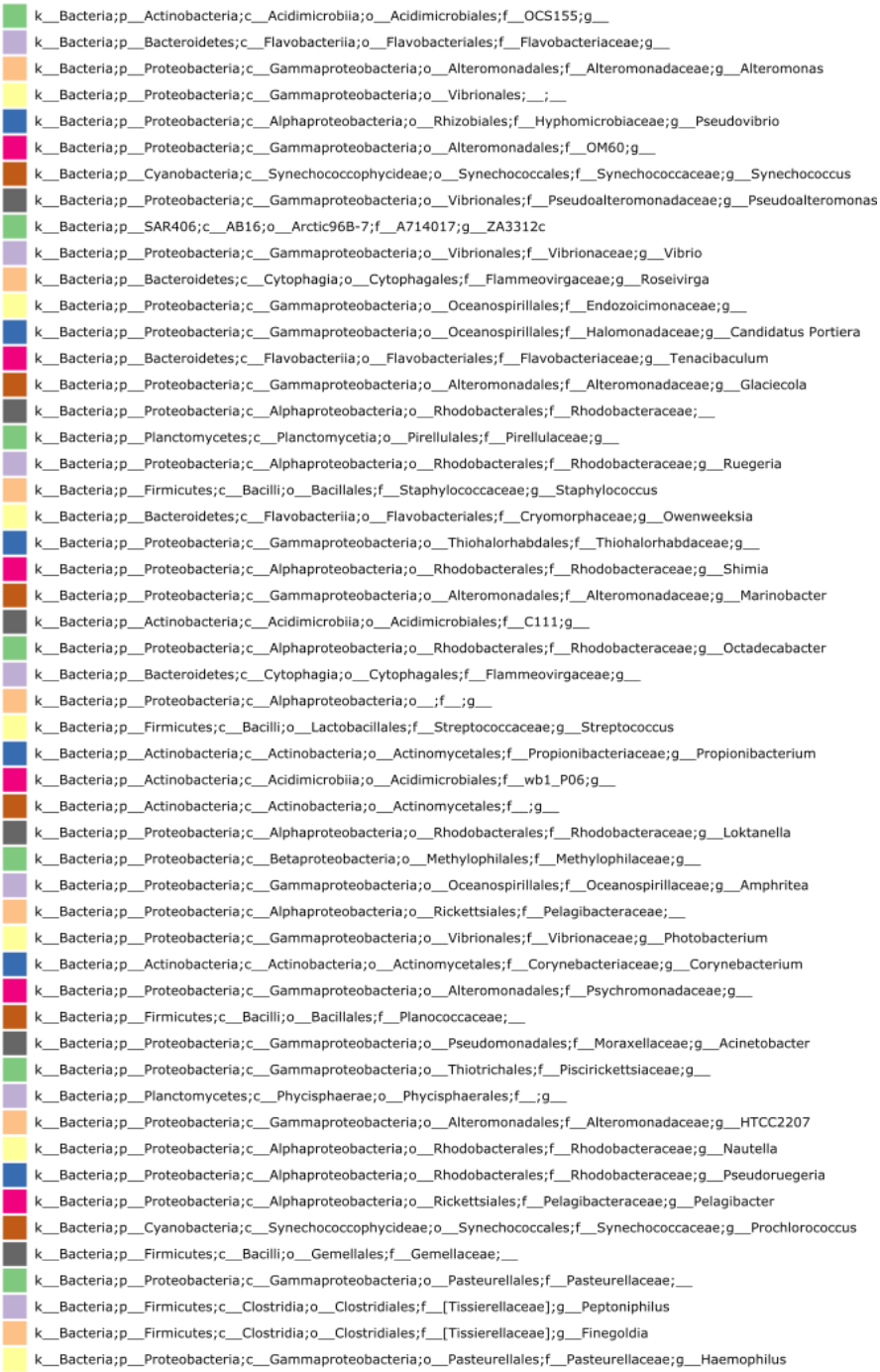


Figure 4.18: The relative frequency of the coral microbiome compared between the 2 water types, general reef water (Rwater, n=10) and coral adjacent water (Pwater, n=7). The taxonomy was collapsed to genus level. In legend, in the case of no genus level available, presence of (g) indicates that it was classified to genus level, however, there was no close match in the database (Greengenes), absence of (g) indicates that it was classified to higher level taxonomy. Features of a frequency below 5000 were removed to allow for better comprehension of taxa bar plot.

Culturing the Coral Microbiome

Bacterial Isolation and Identification

Five *P. daedalea* coral fragments were plated on GASWA and TCBs to determine CFU/g and *Vibrio* percentage. Densities of culturable bacteria averaged $2.59 \times 10^5 \pm 3.61 \times 10^5$ CFU/g and 2.62% of all cultured bacteria belonged to *Vibrio*. Table 4.3 shows the sampling site, time samples spent in aquaria until processing, sample weight, average CFU/g and vibrio percentage of all five coral samples.

Table 4.3: Colony and sample information, average CFU/g and *vibrio* percentage of coral samples.

Coral Fragment	Collection Site	No. of hours in Aquaria	Weight (g)	Average CFU/g	% Vibrio
4L	MM	None	8.8	2.23E+04 ± 2.63E+03	0.61
4L2	BUL4	8 hours	13.8	3.53E+05 ± 5.09E+04	1.77
5L	BUL4	8 hours	8.2	8.54E+05 ± 1.76E+05	5.62
6L	FEH	None	17.8	6.07E+04 ± 1.49E+04	1.48
7L	FEH	None	7.2	5.65E+03 ± 8.49E+02	3.61
Average				**2.59E+05 ± 3.61E+05**	**2.62**

Fifty bacterial strains were isolated from *P. daedalea*. Identification of isolates using 16S rRNA sequencing was successful. Subsequent BLAST analysis revealed that isolates belonged to 13 families (3 phyla: Proteobacteria, Firmicutes and Bacteroidetes). *Vibrionaceae* and *Alteromonadaceae* dominated among all other families followed by *Pseudoalteromonadaceae*, *Rhodobacteraceae* and *Stappiaceae*. Table 4.4 shows all isolates their assigned phylogenetic grouping, query cover, percent identity and accession number of closest blast match. Phylogenetic analysis of the isolates is shown in Figure 4.19.

Table 4.4: BLAST identification of coral bacteria isolated in this study.

Isolate	Assigned Phylogenetic grouping	Query Cover	Per. Identity	Accesion Number

N2	*Pseudoalteromonas* sp	99%	100.00%	NR_114190.1
N3	*Aestuariibacter aggregatus strain WH169*	100%	98.09%	NR_116838.1
N4	*Thalassotalea euphylliae strain Eup-16*	100%	100.00%	NR_153727.1
N5	*Alteromonas* sp	100%	100.00%	NR_114053.1
N7	*Vibrio* sp	100%	100.00%	NR_113786.1
N8	*Amphritea spongicola strain MEBiC05461*	100%	100.00%	NR_135881.1
N9	*Rhodobacteraceae*	100%	99.12%	NR_163664.1
N10	*Vibrio* sp	100%	100.00%	NR_118258.1
N14	*Vibrio* sp	100%	99.53%	NR_113786.1
N15	*Pseudoalteromonas* sp	99%	100.00%	NR_114237.1
N16	*Fabibacter halotolerans strain UST030701-097*	99%	100.00%	NR_043530.1
N17	*Alteromonas* sp	99%	100.00%	NR_114053.1
N18	*Alteromonas* sp	100%	99.52%	NR_114053.1
N19	*Alteromonas* sp	99%	100.00%	NR_043100.1
N20	*Alteromonas* sp	99%	100.00%	NR_114053.1
N22	*Rhodobacteraceae*	98%	100.00%	NR_114024.1
N23	*Marinobacter hydrocarbonoclasticus ATCC 49840*	100%	99.12%	NR_074619.1
N24	*Shimia marina strain CL-TA03*	100%	100.00%	NR_043300.1
N25	*Owenweeksia hongkongensis DSM 17368 strain UST20020801*	100%	93.61%	NR_040990.1
N27	*Corallincola platygyrae strain JLT2006*	100%	99.54%	NR_149805.1
N28	*Pseudoalteromonas* sp	100%	99.53%	NR_113971.1
N30	*Photobacterium* sp	100%	100.00%	NR_114269.1
N32	*Vibrio* sp	100%	97.36%	NR_164874.1
N33	*Corallincola platygyrae strain JLT2006*	95%	99.63%	NR_149805.1
N34	*Alteromonas* sp	100%	99.15%	NR_114053.1
N35	*Vibrio* sp	99%	100.00%	NR_113786.1
N36	*Alteromonas* sp	100%	100.00%	NR_159349.1
N37	*Vibrio* sp	100%	100.00%	NR_118258.1
N38	*Alteromonas* sp	100%	100.00%	NR_114053.1
N39	*Sporosarcina soli strain I80*	99%	100.00%	NR_043527.1
N40	*Pseudovibrio denitrificans strain NBRC 100825*	100%	100.00%	NR_113946.1
N51	*Hyphomonas pacifica strain MCCC 1A04387*	95%	99.63%	NR_169342.1
N52	*Hyphomonas pacifica strain MCCC 1A04387*	100%	99.17%	NR_169342.1
N54	*Ruegeria profundi strain ZGT108*	100%	99.56%	NR_159175.1
N55	*Alteromonas* sp	100%	100.00%	NR_159349.1
N56	*Vibrio* sp	100%	100.00%	NR_118258.1
N57	*Alteromonas* sp	100%	97.56%	NR_114053.1
N58	*Vibrio* sp	100%	100.00%	NR_113786.1
N59	*Fabibacter halotolerans strain UST030701-097*	100%	100.00%	NR_043530.1

N60	*Vibrio* sp	99%	100.00%	NR_113786.1
N61	*Vibrio* sp	97%	94.92%	NR_113786.1
N63	*Ruegeria profundi strain ZGT108*	100%	98.75%	NR_159175.1
N65	*Vibrio* sp	100%	100.00%	NR_118258.1
N67	*Vibrio* sp	99%	100.00%	NR_113786.1
N70	*Pseudoalteromonas* sp	100%	100.00%	NR_114188.1
N71	*Vibrio* sp	100%	100.00%	NR_118258.1
N72	*Pseudovibrio denitrificans strain NBRC 100825*	100%	100.00%	NR_113946.1
N73	*Pseudovibrio denitrificans strain NBRC 100825*	100%	100.00%	NR_113946.1
N74	*Vibrio* sp	100%	99.56%	NR_113786.1
N75	*Pseudovibrio denitrificans strain NBRC 100825*	100%	100.00%	NR_113946.1

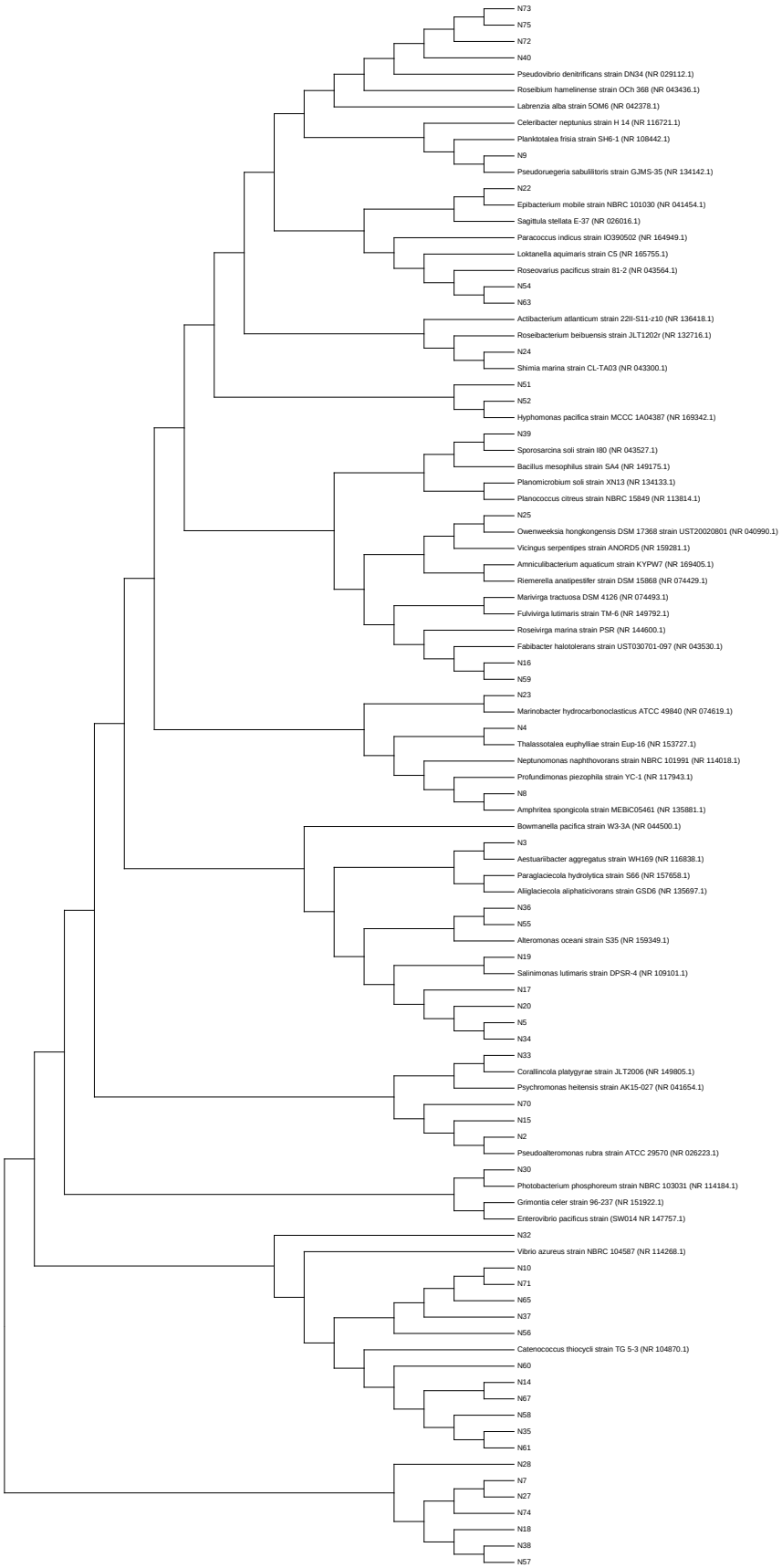


Figure 4.19: Phylogenetic tree of coral isolates and their closest matches on BLAST.

Regulation of Coral Bacterial Communities through Antimicrobial Activity

To understand the role of coral bacteria antimicrobials in regulating community structure, the cross-streak method was used to test coral bacteria's ability to inhibit one another. Twenty-one representative bacteria from each group were selected to test for antimicrobial activity and total of 270 tests were performed of which 14 showed positive results[7]. The results show that only 2 coral isolates demonstrated antimicrobial activity against the remaining coral isolates that makes up around 10% of all tested isolates. N22 which was identified as *Rhodobacteraceae* sp. showed activity against 6 isolates. The highest inhibition was observed against N25 (*Owenweeksia hongkongensis*) with an inhibition zone of 6 mm followed by N30 (*Photobacterium* spp) with an inhibition zone of 5 mm. N33 (*Corallincola platygyrae*) and N63 (*Ruegeria profundi*) showed 4 mm inhibition while N15 (*Pseudoalteromonas spp*) was inhibited by 2 mm. N70 (*Pseudoalteromonas spp*) was inhibited the least with 1 mm inhibition zone. N40 which was identified as *Pseudovibrio denitrificans* displayed activity against 8 isolates N30 (*Photobacterium* spp) was the most sensitive to N40 antimicrobial activity with an inhibition zone of 17 mm, followed by N25 (*Owenweeksia hongkongensis*) with an inhibition zone of 10 mm. N33 (*Corallincola platygyrae*) and N63 (*Ruegeria profundi*) showed 5 mm inhibition while N15 (*Pseudoalteromonas spp*) was inhibited by 4 mm. N7 (*Vibrio spp*) and N70 (*Pseudoalteromonas spp*) were the least sensitive with 3 mm inhibition zone Figure 4.20 shows the inhibition zones of N40 and N22 against N30 as an example.

[7] All antimicrobial activity tests and recorded inhibition zone are in Appendix H

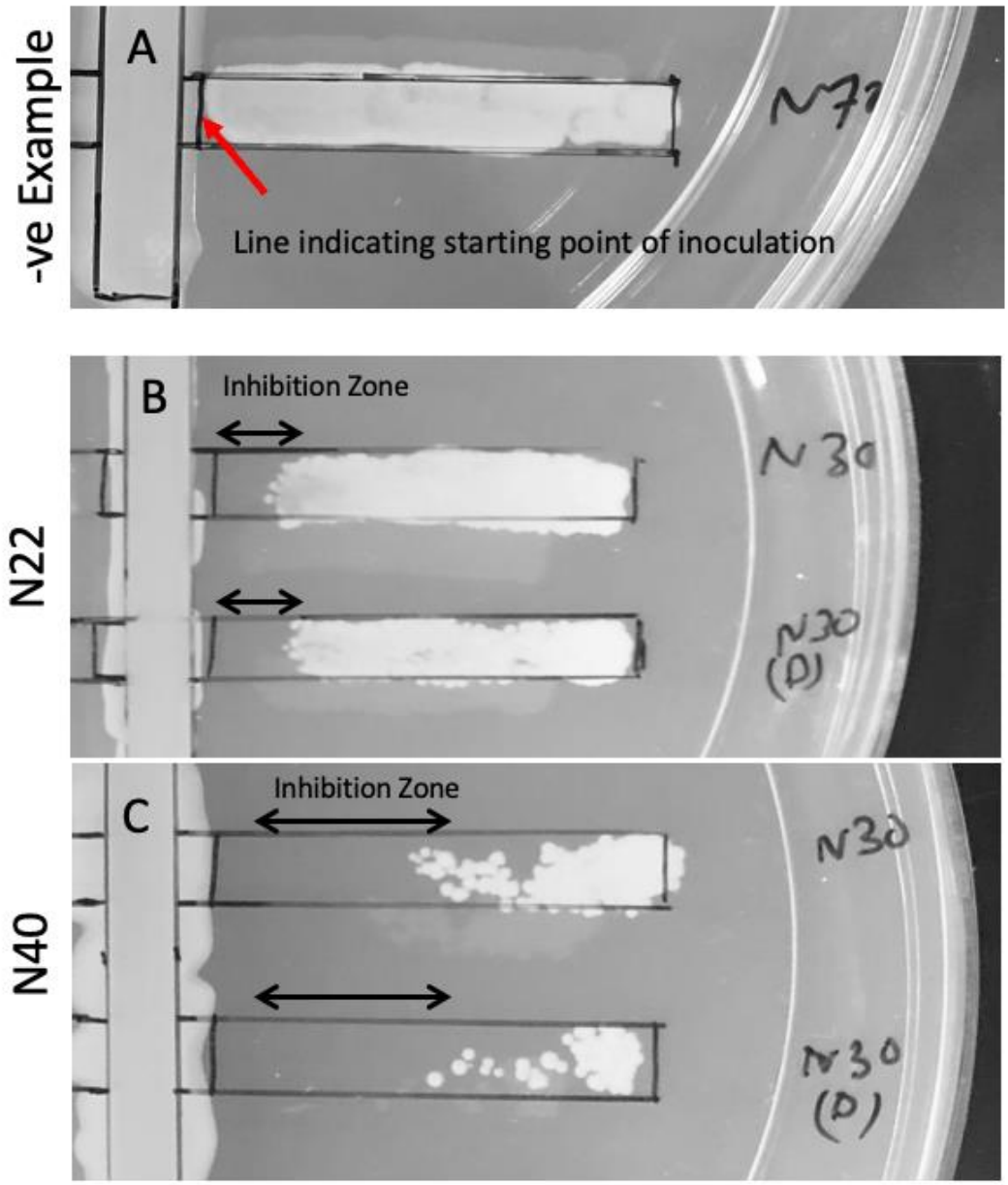


Figure 4.20: Representative culture plates showing cross streak method for testing antimicrobial activity of cultured isolates from corals against each other. The figure shows an example of no inhibition and the inhibition of N22 and N40 against N30 in duplicates

Chapter 5 : DISCUSSION

A recent field survey of Arabian Gulf corals revealed that *Platygyra* corals in this region are facing the threat of disease. According to Aeby et al., (2020) , tissue loss, growth anomalies and black band disease have all been reported in *Platygyra* and are associated with organic pollution and thermal stress. The coral microbiome plays an integral role in maintaining coral health, our lack of understanding of the microbiome of corals in the Arabian Gulf is obstructing efforts of disease mitigation and threating coral survival. This study is a pioneering effort in understanding and characterizing the microbiome of *Platygyra* corals in Qatar.

The core microbiome of *P. daedalea* made up about 5% of all taxa and consisted of 39 genera belonging to 2 kingdoms and 13 phyla. *P. daedalea* core microbiome is composed of archaeal phyla Parvarchaeota and Crenarchaeota and the bacterial phyla Proteobacteria, Actinobacteria, Bacteroidetes, Chloroflexi, Planctomycetes, Spirochaetes, Chlamydiae, Firmicutes, Gemmatimonadetes and SBR1093 (Dadabacteria). Members of Proteobacteria have been documented as coral symbionts which play an integral role in coral fitness, including energy production and sulfate and nitrate reduction (D. Bourne et al., 2016; Lawler et al., 2016). Crenarchaeota has been reported to be important in nitrogen cycling and ammonia removal while, Planctomycetes and Spirochaetes, have been documented in carbon fixation and organic carbon degradation (Lawler et al., 2016; Weidler et al., 2008). Planctomycetes members are also thought to contribute by removing metabolic wastes of the coral host (Lawler et al., 2016). Actinobacteria has been shown to produce antimicrobial agents (Mahmoud & Kalendar, 2016). Bacteroidetes have been linked to larval settlement in marine invertebrates while Firmicutes, Cyanobacteria, and Chloroflexi have been associated with healthy corals (M. Li et al., 2021; Liang et al., 2017)

The literature is lacking studies focusing on the characterization of *P. daedalea* microbiome and the investigation of core microbiome of *Platygyra* species in general.

Damjanovic et al. (2020) reported high abundance of Alphaproteobacteria families in *P. daedalea* such as Rhodospirillaceae, Rhodobacteraceae, which agrees with our findings. Ochsenkühn et al. (2018) explored some aspects of an unidentified *Platygyra* species, the focus of this study is different, however, it demonstrates the presence of Rhodobacteraceae, Flavobacteriaceae, Alphaproteobacteria and Bacteroidetes in all *Platygyra* samples which is consistent with our findings as these taxa were present in *P. daedalea* core microbiome. There is also a lack of microbiome studies in the Arabian Gulf, there is one study that examined the microbial communities in healthy and bleached in corals along the Red Sea and Arabian Gulf sides of Saudi Arabia (Hadaidi et al., 2017). In Arabian Gulf, healthy *Porites lobata* samples of that study, OCS155, Dermabacteraceae Rhodobacteraceae Halomonadaceae Flavobacteriaceae Pseudomonadaceae and Rhodospirillaceae were highly abundant. Flavobacteriaceae, Rhodobacteraceae and Rhodospirillaceae are similarly core bacterial phylotypes in Qatari *P. daedalea*. One study investigated the core microbiome in 6 coral species in the Red Sea and reported Pseudoalteromonas, Alteromonas and *Vibrio* as core phylotypes (Osman et al., 2020). These do not necessarily agree with our findings however, there is one Gammaproteobacteria phylotype that was not assigned further classification; therefore, they cannot be ruled out. Regarding, the remaining core phylotypes, not much overlap was present, however Flavobacteriaceae which is a core in our study was highly abundant in Osman et al., (2020) study. Ainsworth et al. (2015) studied the core microbiome of *Acropora granulosa* in the Northern Coral Sea, Australia, they were able to identify 7 core bacterial phylotypes that are universal for the *A. granulosa* microbiome across geographical locations. Some of the most abundant phylotypes were *Actinobacteria, Altermonadales* and *Burkholderiales,* although present in the microbiome, they are not within the core microbiome of our study species, *P. daedalea*. However, Cyanobacteria, Bacteroidetes, Chloroflexi, Planctomycetes, and Spirochaetes were all shared cores. Another meta-analysis that included

soft and stony deep-sea corals was able to identify 23 core operational taxonomic units (OTUs) corresponding to bacterial families functional in the nitrogen cycle such as *Pirellulaceae*, which is also a core phylotype in our study species. Aside from that, not many were evident to be shared cores. Many studies point to Endozoicimonaceae as a universal recurrent phylotype in coral microbiome, for, example, in a study characterizing the healthy microbiome of 100 coral samples (6 species), although they displayed a level of host specificity, it was evident that there was remarkable bacterial taxa similarity between all coral samples. This study showed Numerous OTUs from Endozoicimonaceae, Campylobacteraceae and *Vibrio* were abundant across corals hosts (Chu & Vollmer, 2016). Interestingly, Endozoicimonaceae showed very low frequencies in Qatari *P. daedalea*. Campylobacteraceae were also low in abundance while *Vibrio* had higher abundance but were not classified as a core phylotype. Through comparing with the literature, it is evident that there are many unique and interesting core phylotypes in *P. daedalea* from Qatar.

P. daedalea Microbiome over Space

In this study, spatial variations were observed in the microbiome of *P. daedalea* between the different sampling sites. The findings support that coral microbiome has a location specific component. Alpha diversity was higher in BUL4 indicating that this site has a higher variety of taxa compared to the other sites. Microbiome Diversity significantly varied according to sites, FEH was significantly different from all sites, it was the deepest site (18 m). BH and UMA were not significantly different, however each was significantly different in comparison to the 4 other sites. These 2 sites are the closest to each other, and both located in the northeast of Qatar. Similarly, BUL4 and MM were not significantly different, however each was significantly different in comparison to the 4 other sites, this could also be due being located near one another and sharing a similar depth. The Robust Aitchison PCA analysis gives us a deeper insight into this differentiation by looking at the key player taxa, it is shown that Halanaerobiaceae (species) is strongly influencing the clustering of FEH, this anaerobic family of bacteria has been linked to disease in coral (Closek et al., 2014). The magnitude of this phylotype suggest that oxygen limiting conditions my lead to development of disease or that biotic factors are influencing the coral microbiome. Similarily, Flammeovirgaceae spp, which is linked to degradation of petroleum based plastics (Pinnell & Turner, 2019) and Piscirickettsiaceae which dominated the spat and juvenile core microbiome of *A. digitifera* in Western Australia (Bernasconi et al., 2019) are strongly influencing UMA and BH. Furthermore, *Nitrosopumilus sp.* which is a symbiotic archaeon to sponges (Chekidhenkuzhiyil et al., 2021) was also influencing UMA; interestingly, UMA had the highest sponge cover in comparison with the other sites indicating that it may be environmentally acquired from sponges. *Sporotomaculum sp.* showed to affecting MM where one of the earliest oil rigs in Qatar is located. This bacterium is linked to wastewater coming from petrochemical industries (Qiu et al., 2003). These findings demonstrate the how biotic and abiotic factors to as well as anthropogenic activities occurring at the regional scale can substantially influence the coral

microbiome composition. In term of abundance, some phylotypes were differentially abundant in some sites and not others. Five taxa were differentially abundant among the different sites. Two cyanobacteria taxa; Pseudanabaenaceae (genus) and YS2 (genus) were differentially abundant across all sites. Pseudanabaenaceae (genus) was highest at UMA and lowest at MM, While YS2 which is associated with feces and the gut of humans and animals (Lin et al., 2013; Zeng et al., 2015) was highest at FEH and lowest at MM. Gammaproteobacterial genus *Marinobacter* was highest in UMA and FEH while it was not present in BUL4. This genus is detected in newly released coral larvae (Sharp et al., 2012), along with presence of Piscirickettsiaceae as stated earlier, UMA is likely to have higher larvae abundance where conditions are allowing for coral reproduction (another example of biotic influences). *Erythrobacter* which is associated with nutrient rich waters and *Labrenzia* which are known coral associated bacteria (Lu et al., 2017; Raj Sharma et al., 2019; Setiyono et al., 2019) were highly differentiated in FEH. Moreover, many of these examples relate to the variability of the Arabian Gulf, and water quality which was shown to influence the coral microbiome (Morrow et al., 2012). The Gulf war is also a factor which affected water quality leaving a significant amount of pollutants (Qafisheh et al., 2020). The finding of this study are in agreement with previous research that the coral microbiome is responsive to environmental changes and is driven by reef location (Chen et al., 2021; Hernandez-Agreda et al., 2016).

P. daedalea Microbiome over Time

While there was variation in some sites specifically, UMA and BUL4 where the diversity in summer was significantly higher than winter, there no consistent evidence to support that these variations have seasonal basis. The overall communities were relatively similar between summer and winter with observed changes in relative abundances. These differences are likely influenced by more than seasonal temperature variations alone. For example, Costa et al., (2006), showed that nutrient concentration in water is influenced by both seasonal and anthropogenic activity which in turn can influence coral reefs. This example supports that two time points are influenced by many factors; therefore, it is important to host long-term microbiome studies to discover persistent cyclic patterns due to annual changes in environmental conditions. These findings are consistent with Epstein et al., (2019) and Yang et al., (2017) who showed a number of significant correlation that were underwhelming to infer that the coral microbiome is seasonally variable. This is not to say that these correlations are not important, they signify that a small number of co-occurrences are indeed persistent through time. For example, *Erythrobacter, Loktanella,* and *Marinobacter* were differentially abundant in the winter. *Loktanella* has shown to be a cold adapted genus and is known to be important in the development of coastal seasonal microbiota mats (Cardoso et al., 2019). *Henriciella* was differentially present in the winter and not present in the summer, the family of this bacteria Hyphomonadaceae is known to increase in response to coral exudates in the seawater (Walsh et al., 2017). This does not only show that the microbiome composition changes through time but also their interactions with other microbial members of the environment. These genera can play a role as indicator taxa and may have functional specificity in response to the environmental conditions related to season. To some extent, Sharp et al., (2017) and Yu et al., (2021) hypothesis in the cold months, the microbiome becomes destabilized and falls into a state of quiescence, then it re-activates again as temperatures rise is also valid in this case as the same pattern of abundance shifts are observed. *Acinetobacter, Marinobacter, Paracoccus,*

Desulfovibrio, Piscirickettsiaceae (genus) and Spirochaetaceae (genus) all had higher relative frequencies in the winter, all of which are reported to live in or tolerate cold temperatures (Flynn et al., 2019; Niederberger et al., 2009; Rutkiewicz-Krotewicz et al., 2016; Ryzhmanova et al., 2019; Sun et al., 2020) Not to mention, analysis of the core features of season and summer revealed that the number of unique phylotypes are less in the winter, this supports Sharp et al., (2017) conclusions on dormancy.

Free-living Community around the Coral as an Indicator for Environmental Changes

A method of effectively monitoring habitat conditions and environmental changes is using indicator taxa. Genera showing differential abundance between coral and coral adjacent water as well compositional beta diversity analysis (PCA) can all be evaluated to be indicator taxa for environmental changes. There is a total 12 taxa which are as follows: *Pseudovibrio denitrificans*, Acidimicrbiales (species), Alteromonas (species), *Vibrio* (species), OCS155 (genus), SAR406 ZA3312c (genus), Candidatus Portiera (genus), OM60 (genus) Synechococcus, *Octadecabacter* and Thiohalorhabdaceae. Similarly, compositional beta diversity analysis (PCA) between general reef water and coral adjacent water showed strong differentiation based three taxa, Flavobacteriaceae (species) *Pseudovibrio denitrificans*, Candidatus Portiera (species). Lastly, other bacteria that displayed difference in relative abundance between the 2 water types include *Prochlorococcus*, *Streptococcus and Staphylococcus.* Some of our taxa above have been discussed recently as indicator taxa in the Great Barrier Reef. OCS155, Flavobacteriaceae, Synechococcaceae and Rhodobacteraceae (our genus is *Octadecabacter*), OM60 have been linked to increased nutrients load. Rhodobacteraceae specifically have been associated with poor coral health. On the other, Prochlorococcaceae were linked to decreased nutrient loads (Frade et al., 2020). In general, cyanobacterial families such as Synechococcus and Prochlorococcaceae can be used to monitor eutrophication. Interestingly, SAR406 (Marinimicrobia) ZA3312c has been associated with

oxygen limiting zone and anoxic conditions (Bertagnolli et al., 2017). It potentially could also be used to monitor eutrophication. Thiohalorhabdaceae is also a potential indicator as it has been correlated with nutrient and temperature changes (Valdespino-Castillo et al., 2021). Not to mention, opportunistic taxa such as *Vibrio* can be used as an indicator for coral disease and degradation. Candidatus Portiera is associated with whiteflies (Jiang et al., 2013), understanding it presence in water can give an indication of possible human activity namely agricultural activities. Fecal *Streptococcus* and *Staphylococcus* has been used as indicator water quality (Gerba & Pepper, 2019; Gilmore, 2013). As covered here, many of the bacteria identified are or can be potential indicators of changing environments as well as predict water quality. Integrating microbial monitoring through the use of microbial indicators in long-term monitoring of reefs can allow for an early diagnosis of environmental condition changes and possible disease outbreaks.

Culturing and Identification of Coral Bacteria

According to Wada et al., (2019), very few studies have determined accurately the bacterial densities associated with corals. However, Chiu et al., (2012) show that CFU per core in *Platygyra* ranged from 4050 to 50 980. This study also shows that the bacterial densities were not responsive to changes in environmental conditions. The average bacterial densities in our study were consistent with the literature. *Vibrio* count, in our *P. daedalea* samples were also in agreement with the literature. Rubio-Portillo et al., (2018) reported counts between 1.38×10^4 and 9.80×10^4 in healthy corals. Not mention, the average *Vibrio* percentage was around 2.6% (± 2.0) which is similar to the health coral average of 2.4% reported by Ushijima et al., (2012).

In recent years, coral-associated microbes' studies have been using sequencing techniques which is integral in identifying coral associated bacteria and how they contribute to coral health metabolically and functionally, especially since many bacteria are unculturable. However,

culture dependent techniques provide valuable insights that are unachievable using metagenomic techniques alone. Here we isolated coral associated bacteria that belonged to 13 families (3 phyla: Proteobacteria, Firmicutes and Bacteroidetes). *Vibrionaceae* and *Alteromonadaceae* dominated among all other families followed by *Pseudoalteromonadaceae*, *Rhodobacteraceae* and *Stappiaceae.* A recent meta-analysis assembling coral isolates from published studies and unpublished collections were able to combine 3,055 isolates (Sweet et al., 2021). Our findings share the same bacterial families discussed in this meta-analysis. Our study focused on studying the antimicrobial activity of the isolates, which along with other activities is essential in coral probiotic research. Therefore, it is vital to culture, identify and assess coral isolates for their potential activities including antimicrobial activity. This can open doors for promoting coral resilience and provide novel strategies for reef restoration efforts.

Regulation of Coral Bacterial Communities through Antimicrobial Activity

The antimicrobial activity of coral associated microbes has been documented a number of times, many reports demonstrate the ability of coral probiotics and extracts to inhibit coral pathogens (Damjanovic et al., 2019; Kelman et al., 2006). Some coral-associated bacteria exhibiting antimicrobial activity have been shown to belong to *Bacillus, Vibrio, Micrococcus, Pseudoalteromonas, Arthrobacter,* and *Pseudovibrio* (Budiani, 2008). Ritchie, (2006) illustrated that 20% of the culturable coral isolates from *Acropora palmata* exhibited antimicrobial activity against other isolates and pathogens. The outcomes of our study, show that around 10% of all *P. daedalea* culturable coral isolates exhibited antimicrobial activity against other isolates. This could also mean that *P. daedalea* utilizes other mechanisms along with antimicrobial activity for regulation of its microbiome. As Wilkins et al., (2019) discussed, microbes secrete agents that include not only bactericidal antibiotics but also compounds that disrupt bacterial quorum sensing and inhibit biofilm from forming. Ochsenkühn et al., (2018), have documented compounds associated with biofilm disruption in

diseased *Platygyra* corals. Nonetheless, both mechanisms are used by corals to regulate its structure and fight disease.

Pseudovibrio denitrificans N40 showed activity against 40% of tested coral bacteria. metagenomic analysis revealed that *P. denitrificans* was present in 61% of all samples with a frequency of occurrence of 1.5%. This genus has been associated with sponge, tunicate and it has shown to have antimicrobial activity. Multiple studies agree with our findings (Esteves et al., 2017; Sertan-de Guzman et al., 2007). *P. denitrificans* has recently been used as a probiotic for shrimp aquaculture (Domínguez-Borbor et al., 2019). It has shown to be effective in the control of *Vibrio* species, this is promising for potential use of *P. denitrificans* as coral probiotic. Rhodobacteraceae sp. N22 was not identified to species level however it showed activity against 30% of all tested isolates. Rhodobacteraceae species have been documented to exhibit antimicrobial activity (Penesyan et al., 2009).

Chapter 6 : CONCLUSION AND RECOMMENDATIONS

This research was the first to characterize the microbiome of the Qatari reef coral, *P. daedalea*, making it the first coral microbiome to be analyzed in Qatar. The core microbiome of *P. daedalea* made up about 5% of all taxa and consisted of 39 genera belonging to 2 kingdoms and 12 phyla. *P. daedalea* core microbiome is composed of archaeal phyla Parvarchaeota and Crenarchaeota and the bacterial phyla Proteobacteria, Actinobacteria, Bacteroidetes, Chloroflexi, Planctomycetes, Spirochaetes, Chlamydiae, Firmicutes, Gemmatimonadetes and SBR1093 (Dadabacteria). The core microbiome members are linked to nutrient cycling, carbon fixation, production of antimicrobial compounds, removal of metabolic waste and aid in larval settlement. We were also pioneering in exploring the coral microbiome in response to spatial and seasonal variations in of the world's harshest marine environment, the Arabian Gulf. We clearly illustrate that the coral microbiome is highly influenced by spatial variation. Variable taxa present at each site demonstrate how biotic and abiotic factors, as well as anthropogenic activities occurring at the regional scale, can substantially influence the coral microbiome composition. While spatial variations were highly influential on the microbiome of Qatari *P. daedalea*, our findings suggest that seasonal variability is also linked to site variability. The microbiome diversity were significantly different for 2 of the 5 sites sites with observed changes in relative abundances. These differences are likely influenced by more than seasonal temperature variations alone. Our findings suggest that the coral microbiome is rather responsive to environmental changes and is driven by reef location. This study also investigated water microbial taxa we found and nominated multiple potential indicator taxa that can be used for monitoring of coral reef health, as well as an early diagnosis of environmental conditions of water quality forcings that are linked to disease dysbiosis and disease. Namely OCS155, Flavobacteriaceae, Synechococcaceae, *Octadecabacter,* OM60, Prochlorococcaceae, Thiohalorhabdaceae and

Marinimicrobia ZA3312c can be used as indicators of water quality in relation to nutrient load and potential eutrophication, the latter specifically for predicting anoxic conditions. In addition, opportunistic taxa such as vibrio can potentially be used as an indicators for coral disease and degradation. Moreover, we instigated the first coral associated bacteria isolation and identification effort in Qatar and established a coral microbial collection of a total of 50 cultured isolates. Our collection of coral-associated bacteria belonged to 13 families (3 phyla: Proteobacteria, Firmicutes and Bacteroidetes). *Vibrionaceae* and *Alteromonadaceae* dominated among all other families followed by *Pseudoalteromonadaceae*, *Rhodobacteraceae* and *Stappiaceae.* The isolates were investigated for antimicrobial activity against one another to understand which members of the microbiome were influential in structuring the community through production of antimicrobial agents. We concluded that *P. denitrificans* N40 and Rhodobacteraceae sp. N22 exhibited regulation potential, inhibiting 40% and 30% of all tested isolated respectively.

Our research approach was effective in answering all our research questions, while it would be beneficial to study the different microhabitats of the coral host, to further understand the roles, functions, and variation of the microbiome, it was outside the scope of this study. Therefore, future studies should address investigating the microhabitats of the coral in parallel with studying the microbiome of other coral species in Qatar. Furthermore, long-term studies should be implemented to better understand the temporal variations in the microbiome and observing any inter-annual patterns. Coral monitoring efforts should include microbiome analysis as it can provide valuable insights in the status of reef health. Specifically, indicator taxa should be targeted to potentially predict and mitigate dysbiosis and signs of disease. Coral isolates should be further investigated and screened for use in coral probiotics which is a promising technique in coral disease mitigation and recovery also prevention or recovery from bleaching.

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APPENDICES

Appendix A: Core Microbiome of Qatari *P. daedalea* across sites and seasons.

Feature	Kingdom	Phylum	Class	Order	Family	Genus	98%
1	Bacteria	Proteobacteria	Alphaproteobacteriao				5034.8
2	Bacteria	Proteobacteria	Gammaproteobacteriao	Thiotrichales	Piscirickettsiaceae		2592.8
3	Bacteria	Bacteroidetes	Cytophagiao	Cytophagales	Flammeovirgaceae		2786.2
4	Bacteria	Proteobacteria	Alphaproteobacteria				4565
5	Archaea	Crenarchaeota	Thaumarchaeotao	Cenarchaeales	Cenarchaeaceae	Nitrosopumilus	4100.8
6	Bacteria	Planctomycetes	Planctomycetiao	Pirellulales	Pirellulaceae		2495.6
7	Bacteria	Proteobacteria	Alphaproteobacteriao	Rhizobiales	Hyphomicrobiaceae		1512.4
8	Bacteria	Proteobacteria	Alphaproteobacteriao	Rhodospirillales	Rhodospirillaceae		1435
9	Archaea	[Parvarchaeota]	[Parvarchaea]o	WCHD3-30			1767.8
10	Bacteria	Proteobacteria	Alphaproteobacteriao	Rhodospirillales	Rhodospirillaceae	Inquilinus	1178.6
11	Bacteria	Proteobacteria	Alphaproteobacteriao	Rhizobiales	Phyllobacteriaceae		1112.2
12	Bacteria	Actinobacteria	Acidimicrobiiao	Acidimicrobiales	koll13		1012.4
13	Archaea	Crenarchaeota	Thaumarchaeotao	Cenarchaeales	Cenarchaeaceae		1708.8
14	Bacteria	Actinobacteria	Acidimicrobiiao	Acidimicrobiales			1427.8
15	Bacteria	Chloroflexi	SAR202o				5447.8
16	Bacteria	Proteobacteria					2858.2
17	Bacteria	Planctomycetes	Phycisphaeraeo	Phycisphaerales			777
18	Bacteria	Proteobacteria	Gammaproteobacteriao	Chromatiales			711.8
19	Bacteria	Bacteroidetes	Cytophagiao	Cytophagales	[Amoebophilaceae]		2337.2
20	Bacteria	Firmicutes	Clostridiao	Clostridiales	Clostridiaceae	Clostridium	2228.6
21	Bacteria	Planctomycetes	Phycisphaeraeo	Phycisphaerales	Phycisphaeraceae		704.2
22	Bacteria	Bacteroidetes	Flavobacteriiao	Flavobacteriales	Flavobacteriaceae		1050.6
23	Bacteria	Spirochaetes	Spirochaeteso	Spirochaetales	Spirochaetaceae		1664.2
24	Bacteria	Proteobacteria	Alphaproteobacteriao	Rhodobacterales	Rhodobacteraceae		1283.4
25	Bacteria						1658
26	Bacteria	Chloroflexi	Chloroflexio	Chloroflexales	Chloroflexaceae	Chloronema	1308.2
27	Bacteria	Proteobacteria	Alphaproteobacteriao	Rhizobiales	Phyllobacteriaceae	Phyllobacterium	414.6
28	Bacteria	Cyanobacteria	Synechococcophycideaeo	Pseudanabaenales	Pseudanabaenaceae		2350.2
29	Bacteria	Bacteroidetes	Flavobacteriiao	Flavobacteriales	Flavobacteriaceae		789.4

30	Bacteria	Gemmatimonadetes	Gemm-2o				2708
31	Bacteria	Chloroflexi	TK17o				1150.4
32	Bacteria	Chloroflexi	Anaerolineaeo	GCA004			642
33	Bacteria	Proteobacteria	Alphaproteobacteriao	BD7-3			602.6
34	Bacteria	Planctomycetes	Planctomycetiao	Planctomycetales	Planctomycetaceae	Planctomyces	265.4
35	Bacteria						372
36	Archaea	Crenarchaeota	Thaumarchaeotao	Cenarchaeales	Cenarchaeaceae		1104.4
37	Bacteria	Proteobacteria	Gammaproteobacteria				225.4
38	Bacteria	Chlamydiae	Chlamydiiao	Chlamydiales			498.2
39	Bacteria	SBR1093	VHS-B5-50o				95.4

Appendix B: Pairwise Kruskal Wallis Tests

1. Pairwise Kruskal Wallis for Shannon index of the study sites.

Group 1	Group 2	H	p-value	q-value
BH (n=10)	BUL4 (n=10)	6.605714285714290	0.010165201891956300	0.07780734004296990
BH (n=10)	FEH (n=10)	0.005714285714276460	0.939742989577122	0.939742989577122
BH (n=10)	MM (n=10)	1.1199999999999900	0.2899184539425690	0.48319742323761500
BH (n=10)	UMA (n=10)	0.05142857142857340	0.8205958397554380	0.9117731552838200
BUL4 (n=10)	FEH (n=10)	5.8514285714285700	0.015564411386633800	0.07780734004296990
BUL4 (n=10)	MM (n=10)	5.142857142857140	0.02334220201289100	0.07780734004296990
BUL4 (n=10)	UMA (n=10)	4.165714285714290	0.04125001659393970	0.10312504148484900
FEH (n=10)	MM (n=10)	1.1199999999999900	0.2899184539425690	0.48319742323761500
FEH (n=10)	UMA (n=10)	0.2057142857142790	0.6501474440948590	0.8126843051185740
MM (n=10)	UMA (n=10)	0.6914285714285670	0.40567889528505500	0.5795412789786500

2. Pairwise Kruskal Wallis for Pielou evenness of the study sites.

Group 1	Group 2	H	p-value	q-value
BH (n=10)	BUL4 (n=10)	6.605714285714290	0.010165201891956300	0.050826009459781300
BH (n=10)	FEH (n=10)	0.1428571428571390	0.7054569861112770	0.7054569861112770
BH (n=10)	MM (n=10)	1.2857142857142800	0.25683925795785500	0.4280654299297580
BH (n=10)	UMA (n=10)	0.1428571428571390	0.7054569861112770	0.7054569861112770
BUL4 (n=10)	FEH (n=10)	8.251428571428580	0.004071994217732750	0.04071994217732750
BUL4 (n=10)	MM (n=10)	3.5714285714285700	0.058781721355358900	0.14695430338839700
BUL4 (n=10)	UMA (n=10)	4.480000000000000	0.03429372103649280	0.11431240345497600
FEH (n=10)	MM (n=10)	0.6914285714285670	0.40567889528505500	0.5795412789786500
FEH (n=10)	UMA (n=10)	0.4628571428571390	0.49629170223109500	0.6203646277888680
MM (n=10)	UMA (n=10)	1.2857142857142800	0.25683925795785500	0.4280654299297580

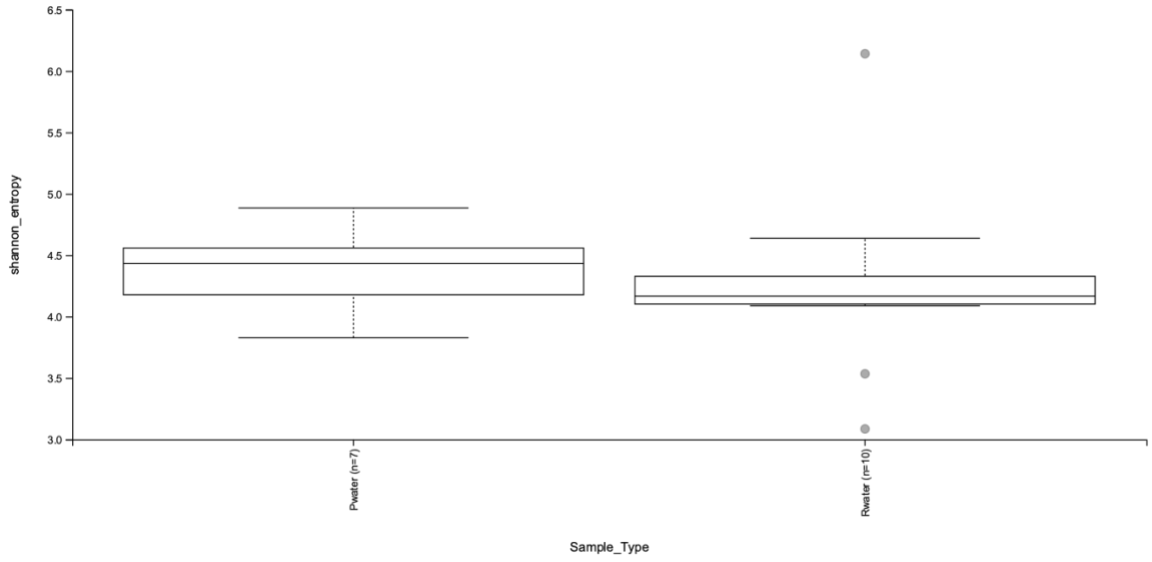
3. Pairwise Kruskal Wallis for Faith phylogenetic diversity index of the study sites.

Group 1	Group 2	H	p-value	q-value
BH (n=10)	BUL4 (n=10)	0.5714285714285690	0.4496917979688920	0.7790709543016020
BH (n=10)	FEH (n=10)	0.4628571428571390	0.49629170223109500	0.7790709543016020
BH (n=10)	MM (n=10)	0.9657142857142920	0.32575135447871200	0.7790709543016020
BH (n=10)	UMA (n=10)	0.3657142857142900	0.5453496680111210	0.7790709543016020
BUL4 (n=10)	FEH (n=10)	1.462857142857130	0.22647606604348500	0.7549202201449490
BUL4 (n=10)	MM (n=10)	0.02285714285714850	0.8798291600118150	0.939742989577122
BUL4 (n=10)	UMA (n=10)	0.005714285714276460	0.939742989577122	0.939742989577122
FEH (n=10)	MM (n=10)	2.0628571428571400	0.15092695006671200	0.7546347503335620
FEH (n=10)	UMA (n=10)	2.2857142857142800	0.13057001811573700	0.7546347503335620
MM (n=10)	UMA (n=10)	0.1428571428571390	0.7054569861112770	0.8818212326390970

4. Pairwise Kruskal Wallis for Shannon index of general reef water and coral adjacent water.

Group 1	**Group 2**	**H**	**p-value**	**q-value**
Pwater (n=7)	Rwater (n=10)	0.7714285714285720	0.37977547484094900	0.37977547484094900

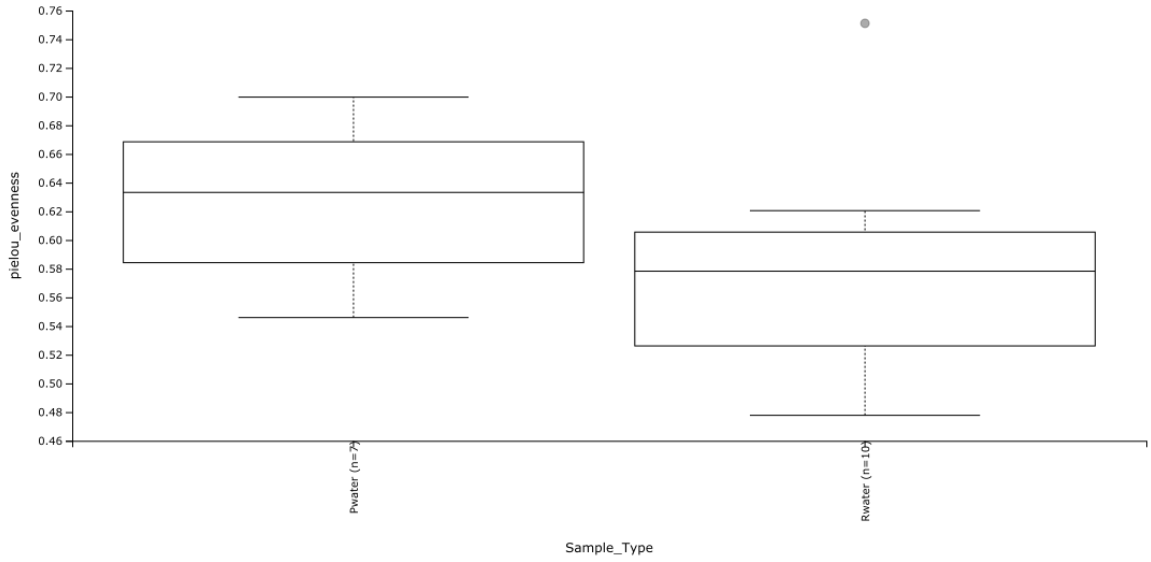
5. Mean values of the Shannon H indices of general reef water (Rwater) and coral adjacent water (Pwater)



6. Pairwise Kruskal Wallis for Pielou index of general reef water and coral adjacent water.

Group 1	Group 2	H	p-value	q-value
Pwater (n=7)	Rwater (n=10)	2.4380952380952400	0.11841994270812500	0.11841994270812500

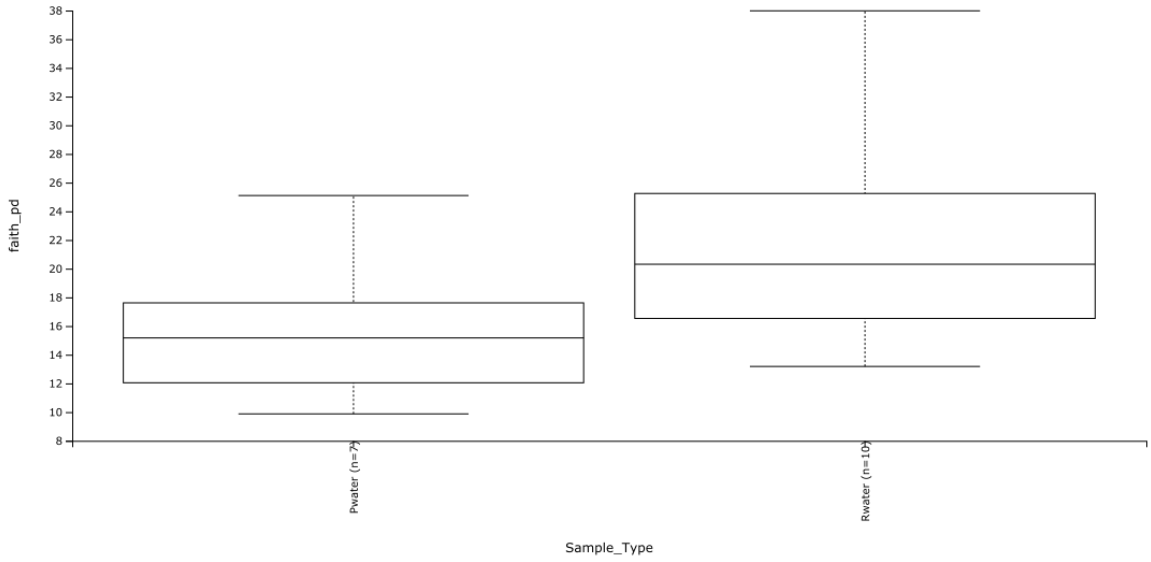
7. Mean values of the Pielou evenness indices of general reef water (Rwater) and coral adjacent water (Pwater)



8. Pairwise Kruskal Wallis for Faith phylogenetic diversity index of general reef water and coral adjacent water.

Group 1	**Group 2**	**H**	**p-value**	**q-value**
Pwater (n=7)	Rwater (n=10)	3.085714285714290	0.07898257926378250	0.07898257926378250

9. Mean values of the Faith phylogenetic diversity indices of general reef water (Rwater) and coral adjacent water (Pwater)



Appendix C: Pairwise PERMANOVA test

1. Unweighted Unifrac pairwise PERMANOVA test between the study sites.

Group 1	Group 2	Sample size	Permutations	pseudo-F	p-value	q-value
BH	BUL4	20	999	2.489483887475070	0.002	0.005
BH	FEH	20	999	1.8139434615201800	0.01	0.012500000000000000
BH	MM	20	999	2.0549330642049500	0.003	0.006
BH	UMA	20	999	1.1948392684994400	0.117	0.117
BUL4	FEH	20	999	3.2482472051167000	0.002	0.005
BUL4	MM	20	999	1.3210146241274900	0.096	0.10666666666666700
BUL4	UMA	20	999	2.3756322787654700	0.002	0.005
FEH	MM	20	999	3.0244077660721500	0.001	0.005
FEH	UMA	20	999	2.0187713125844000	0.005	0.0071428571428571400
MM	UMA	20	999	2.0424334805695600	0.005	0.0071428571428571400

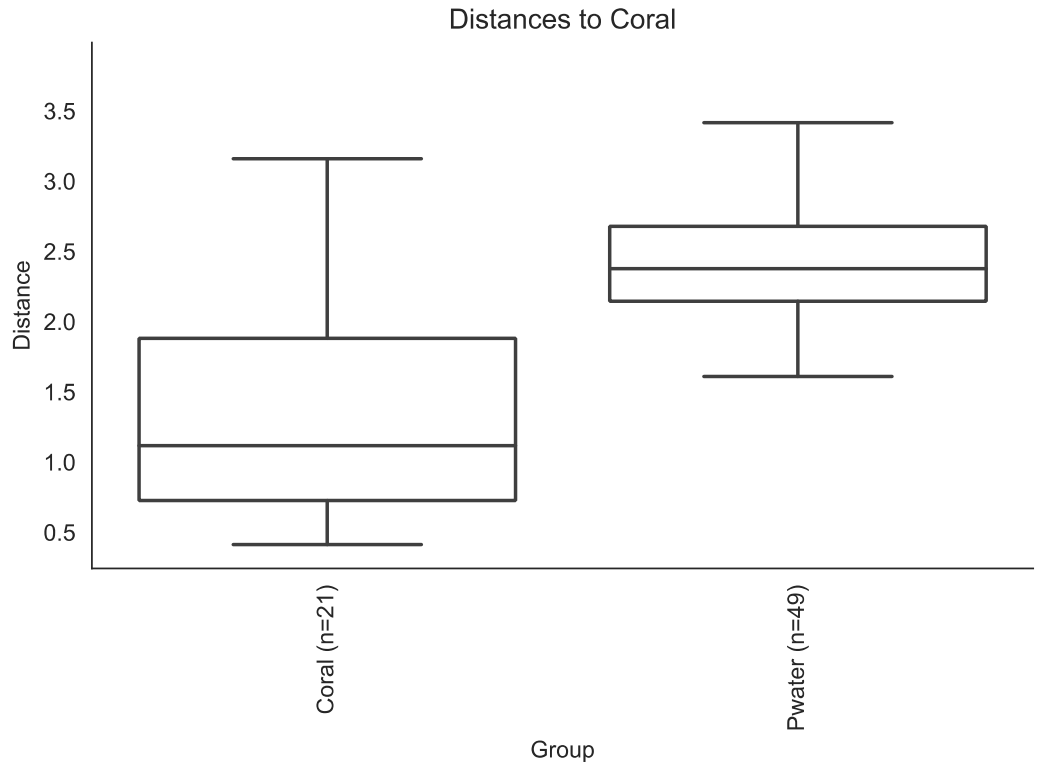
2. Unweighted Unifrac pairwise PERMANOVA test between general reef water and coral adjacent water.

Group 1	Group 2	Sample size	Permutations	pseudo-F	p-value	q-value
Pwater	Rwater	17	999	2.4387225781572100	0.002	0.002

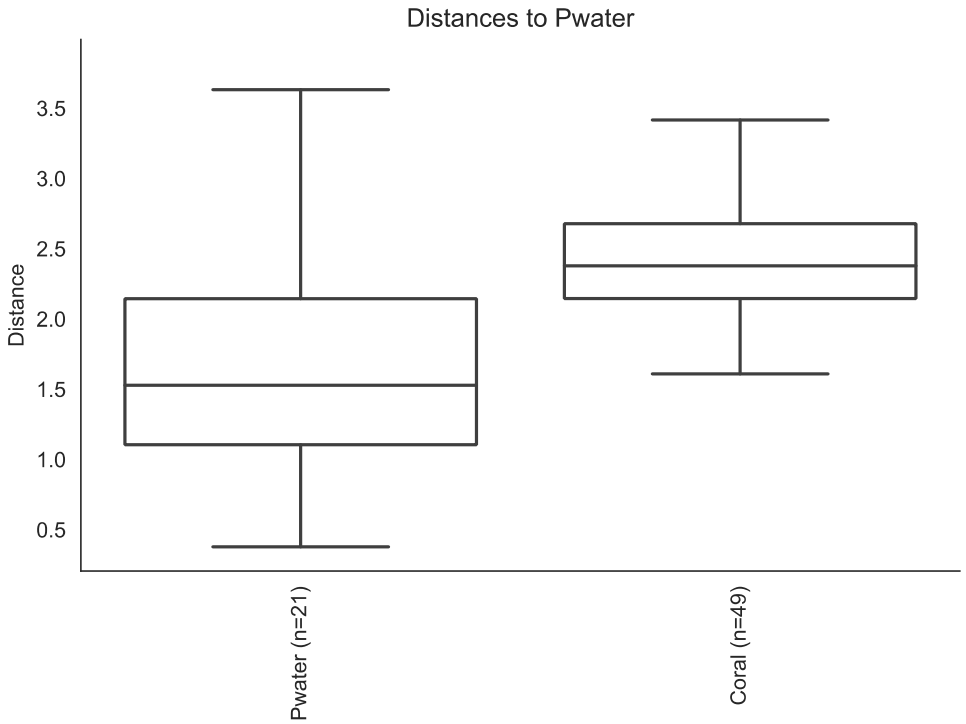
3. Aitchison distance PERMANOVA test between coral and coral adjacent water.

Group 1	Group 2	Sample size	Permutations	pseudo-F	p-value
Coral	Pwater	14	999	9.729029	0.001

4. Boxplot of Aitchison distance between coral and coral adjacent water (distances to Coral)



5. Boxplot of Aitchison distance between coral and coral adjacent water (distances to Pwater)



Appendix D: ANCOM Differential Abundance

1. ANCOM differential Abundance (%) between study sites at the 100th percentile. Values equal to 1 are pseduocounts, they should be considered as 0.

Percentile	**100**	**100**	**100**	**100**	**100**
Group	**BH**	**BUL4**	**FEH**	**MM**	**UMA**
k__Bacteria;p__Cyanobacteria;c__Synechococcophycideae;o__Pseudanabaenales;f__Pseudanabaenaceae;g__	672	2112	2936	154	4553
k__Bacteria;p__Cyanobacteria;c__4C0d-2;o__YS2;f__;g__	26	446	1271	4	13
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Alteromonadales;f__Alteromonadaceae;g__Marinobacter	15	1	104	26	136
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Sphingomonadales;f__Erythrobacteraceae;g__Erythrobacter	11	6	87	1	6
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Hyphomicrobiaceae;g__Labrenzia	1	29	143	34	1

2. ANCOM differential Abundance (%) between seaons at the 100th percentile. Values equal to 1 are pseduocounts, they should be considered as 0.

Percentile	100	100
Group	**Summer**	**Winter**
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhodobacterales;f__Hyphomonadaceae;g__Henriciella	1	146
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Sphingomonadales;f__Erythrobacteraceae;g__Erythrobacter	87	370
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhodobacterales;f__Rhodobacteraceae;g__Loktanella	213	1596
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Alteromonadales;f__Alteromonadaceae;g__Marinobacter	136	8304

3. ANCOM differential Abundance (%) between seaons at the 100th percentile. Values equal to 1 are pseduocounts, they should be considered as 0.

Percentile	**100**	**100**
Group	**Coral**	**Pwater**
k__Bacteria;p__Actinobacteria;c__Acidimicrobiia;o__Acidimicrobiales;f__OCS155;g__	17	31361
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Alteromonadales;f__OM60;g__	1	2833
k__Bacteria;p__Cyanobacteria;c__Synechococcophycideae;o__Synechococcales;f__Synechococcaceae;g__Synechococcus	1	2784
k__Bacteria;p__SAR406;c__AB16;o__Arctic96B-7;f__A714017;g__ZA3312c	3	2636
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Oceanospirillales;f__Halomonadaceae;g__Candidatus Portiera	4	1183
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhodobacterales;f__Rhodobacteraceae;g__Octadecabacter	1	473
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Thiohalorhabdales;f__Thiohalorhabdaceae;g__	1	471

Appendix E: Pairwise Wilcoxon Signed Rank Test

1. Pairwise Wilcoxon signed-rank test for paired differences in Shannon entropy between summer and winter for each site .

Group	W (wilcoxon signed-rank test)	P-value	FDR P-value
BH	15.0	0.7421875	0.927734375
BUL4	0.0	0.015625	0.048828125
FEH	18.0	1.0	1.0
MM	8.0	0.1953125	0.32552083333333300
UMA	3.0	0.01953125	0.048828125

2. Pairwise Wilcoxon signed-rank test for paired differences in Shannon entropy between coral and coral adjacent water.

Group	W (wilcoxon signed-rank test)	P-value	FDR P-value
all subjects	0.0	0.015625	0.015625

Appendix F: Pairwise Mann-Whitney U test

1. Pairwise Mann-Whitney U test for seasonal difference between study sites. (alpha diversity)

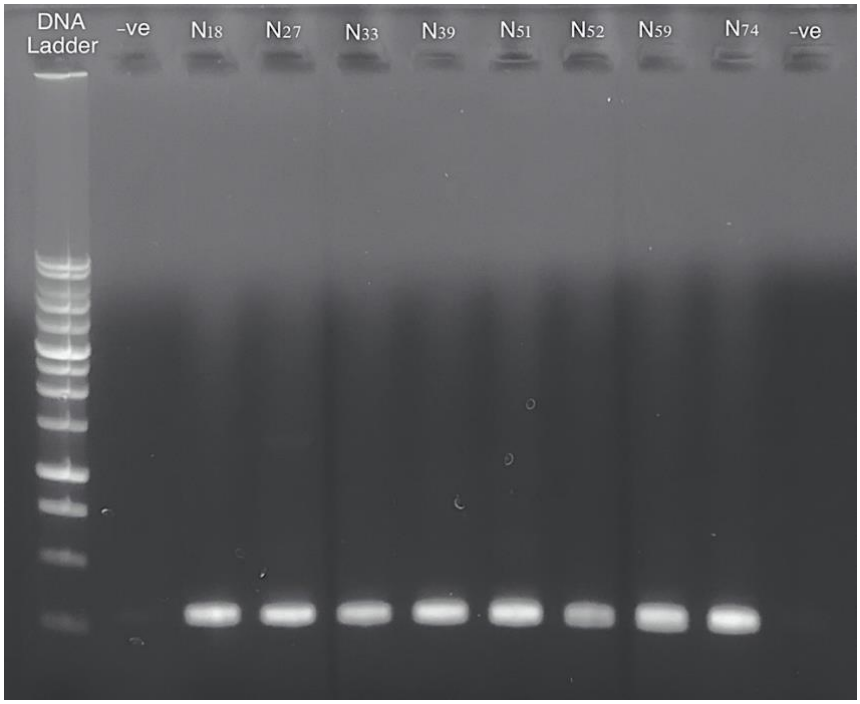
Group A	Group B	Mann-Whitney U	P-value	FDR P-value
BH	BUL4	45.0	0.054079254079254100	0.18026418026418000
BH	FEH	36.0	0.7209013209013210	0.7209013209013210
BH	MM	47.0	0.13038073038073000	0.26076146076146100
BH	UMA	54.0	0.09271904566022210	0.2317976141505550
FEH	BUL4	46.0	0.04009324009324010	0.18026418026418000
MM	BUL4	34.0	0.5358197358197360	0.6697746697746700
MM	FEH	13.0	0.04988344988344990	0.18026418026418000
UMA	BUL4	41.0	0.35104895104895100	0.5014985014985020
UMA	FEH	21.0	0.16717400246812000	0.2786233374468670
UMA	MM	42.0	0.6058412176059240	0.673156908451026

2. Pairwise Mann-Whitney U test for seasonal difference between study sites. (beta diversity)

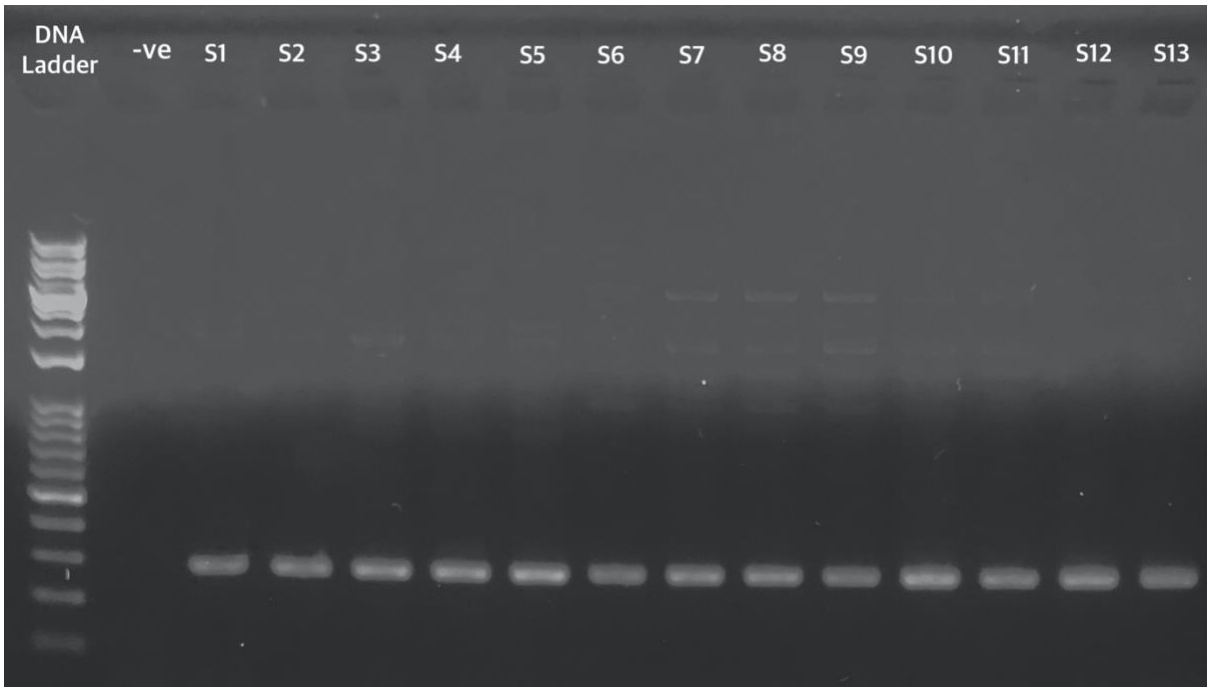
Group A	Group B	Mann-Whitney U	P-value	FDR P-value
BH	BUL4	45.0	0.054079254079254100	0.18026418026418000
BH	FEH	36.0	0.7209013209013210	0.7209013209013210
BH	MM	47.0	0.13038073038073000	0.26076146076146100
BH	UMA	54.0	0.09271904566022210	0.2317976141505550
FEH	BUL4	46.0	0.04009324009324010	0.18026418026418000
MM	BUL4	34.0	0.5358197358197360	0.6697746697746700
MM	FEH	13.0	0.04988344988344990	0.18026418026418000
UMA	BUL4	41.0	0.35104895104895100	0.5014985014985020
UMA	FEH	21.0	0.16717400246812000	0.2786233374468670
UMA	MM	42.0	0.6058412176059240	0.673156908451026

Appendix G DNA Quality and Quantity

1. Example of successful amplification of 16s rRNA gene for a representative set of coral bacterial isolates



2. Example of successful amplification of 16s rRNA gene for metagenomic analysis of coral fragments



3. DNA concentration of all purified sample sent for sequencing using Nanodrop

Sample	Concentration ng/microliter
1	8.7
2	12.2
3	6.6
4	8.4
5	13.1
6	2.3
7	8.6
8	5.3
9	4.8
10	5.8
11	24.3
12	15.7
13	20.2
14	7.8
15	9.1
16	4.4
17	10.9
18	22.2
19	7.3
20	6.4
21	13.3
22	7.4
23	12.7
24	6.1
25	9.7
26	7.8
27	13.5
28	9.7
29	16.6
30	8.5
41	4.2
42	4.2
43	8.1
44	9.2
45	11.6
46	12.1
47	11.7
48	7.7
49	6.6
50	11.4
51	13.4
52	7.5
53	15.7
54	10.8
55	13

56	9.8
57	13.1
58	16.6
59	17.8
60	11.3
M21	9.9
M22	5.1
M23	9.5
M24	3
M25	5.8
M26	2
M27	7.5
M28	3.7
SBH1	9.6
SUMA2	7.3
SMM3	12.9
SBUL4	14.3
SFEH5	17.3
WMM3	12.6
P1	12.7
P2	9.6
P3	11.8
P4	19.3
P5	15.2
P6	17.3
P7	13.6
M1	7.4
M2	12.5
M3	12.2
M4	12.7
M5	12.5
M6	11.7
M7	17.9
M9	14
M10	14.5
M11	6.7
M12	3.9
M13	6
M14	10.4
M15	7.4
M17	1.2
M18	6.5
M31	8.3
M32	2
M33	6.4
M34	11.6
M35	5.1
M36	4.1

M37	5.7
M38	6.2
M41	5.9
M42	6.2
M43	3.8
M44	5.3
M45	4.4
M46	7
M47	6.2
M48	4.7
M49	8.3
WBH	1.2
WUMA	5.5
WBUL4	1.7
WFEH	4.3

Appendix H: Inhibition zones of coral bacterial isolates against each other

	N4	N5	N7	N8	N9	N10	N14	N15	N20	N22	N23	N24	N25	N30	N33	N39	N40	N51	N59	N63	N70
N4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
N5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
N7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
N8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
N9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
N10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
N14	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
N15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
N20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
N22	0	0	0	0	0	0	0	0.2	0	0	0	0	0.6	0.5	0.4	0	0	0	0	0.4	0.1
N23	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
N24	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
N25	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
N30	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
N33	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
N39	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
N40	0	0	0.3	0	0	0	0	0.4	0	0	0	0	1	1.7	0.5	0	0	0.3	0	0.5	0.3
N51	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
N59	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
N63	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
N70	0	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0

* Red labeled were not tested