

FROM DISEASE TO DUST: IMPACTS OF BIOTIC & ABIOTIC STRESSORS  
ON THE MICROBIAL COMMUNITIES OF STONY CORALS  
AND REEF WATER IN THE FLORIDA KEYS

by

MAITE GHAZALEH BUCHER

(Under the Direction of Erin Lipp)

ABSTRACT

Coral reefs are vibrant biodiversity hotspots that provide socio-economic services to communities worldwide. Scleractinian, or stony, corals are essential foundation species that build the three-dimensional structures of coral reef habitats. The surface mucus layer of stony corals contains an immense diversity of microorganisms including *Vibrio*, one of the most diverse marine bacterial genera. *Vibrio* are ubiquitous in coastal environments and are well-known human pathogens. Opportunistic *Vibrio* are also commonly implicated in coral diseases. Globally, contemporary coral reefs continuously face pronounced changes in biotic and abiotic conditions. Biotic stressors, such as disease, threaten coral health. Abiotic stressors, such as episodic nutrient input, may alter coral resilience. The effect of changing environmental factors on coral microbiomes has only been studied in the last 15 years, and further research is needed to ascertain reef-associated microbiome responses and *Vibrio* dynamics when biotic and abiotic conditions are unfavorable. Therefore, this dissertation investigates how the bacterial microbiomes of coral and reef water in the Florida Keys, USA responds to biotic and/or abiotic stressors. First, by

confining spatiotemporal variability, data from a multi-species, multi-disease outbreak provides a unique opportunity to test trending frameworks used to describe coral disease dynamics. Results reveal commonalities and differences between coral microbiomes affected by the outbreak, suggesting that two seemingly competing frameworks are not mutually exclusive. Next, this dissertation describes the effect of trans-Atlantic Saharan dust deposition on coral-associated microbiomes and *Vibrio* bacteria in *Acropora palmata* and *Orbicella faveolata*. During a 26 d time series, dust deposition alters the composition and structure of bacterial communities and rapidly increases the abundance of *Vibrio*. These changes are ephemeral, and communities subsequently returned to baseline conditions. Lastly, reef water metatranscriptomes were analyzed from concurrently collected seawater samples. Results show differential gene expression in response to fluctuating dust aerosol optical thickness, dissolved iron concentration, and dissolved organic carbon concentration associated with trans-Atlantic Saharan dust deposition. In summary, the research presented here contributes to a foundational understanding of the interactions between the coral host, its associated microorganisms, and the environment.

INDEX WORDS: Coral reefs, Florida Keys, Seawater, Coral, *Acropora*, *Orbicella*, Microbial communities, Microbiome, Coral disease, Dysbiosis, Pathogenic bacteria, Vibrionaceae, *Vibrio*, Saharan, Sahel, Trans-Atlantic dust deposition, Anna Karenina principle, 16S rRNA gene sequencing, Metatranscriptomics, Bacterial gene expression, Time series, Host microbiome interaction

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B.S., University of North Carolina Chapel Hill, 2015

A Dissertation Submitted to the Graduate Faculty of The University of Georgia in Partial  
Fulfillment of the Requirements for the Degree

DOCTOR OF PHILOSOPHY

ATHENS, GEORGIA

2020

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## ACKNOWLEDGEMENTS

Deepest thanks to my advisor Dr. Erin Lipp for her continuous support, guidance, and understanding. Thank you very much to my committee, Drs. Travis Glenn, Mandy Joye, Liz Ottesen, and Jim Porter, for their encouragement, perspectives, and advice. Thank you to past and present Lipp Lab members, especially Dr. Keri Lydon, for cheerfully helping me. Sincere thanks to my friends and extended family for closely following and supporting my academic journey. Many thanks to Dr. Deborah McFarlane for her comforting humor and loyal companionship. I am also thankful for the mood-boosting, anxiety-reducing powers of my canine Otis McGillicutty. Heartfelt gratitude to my in-laws, Karl and Bonnie, for their steadfast support, and Kyle for his caring friendship. Wholehearted thanks to my late grandfather, Vô Tarcio, for enthusiastically introducing me to the marvels of the underwater world. I am eternally grateful for my siblings, Mariane, Dawud, Dahrel, Darion, Maryellen, and Dawson, for believing in me no matter what. An immense, loving thank you to my father, David Ghazaleh, for his commitment to “serve, protect, and provide” and inspiring me to always do my best. A truly heartfelt thank you to my mother, Andrea Ghazaleh, for her nurturing friendship, considerate advice, and powerful prayers. I endlessly thank my husband, Justin Bucher, for steadfastly supporting my personal and professional aspirations even under the most challenging of circumstances. His love, friendship, and encouragement made this dissertation achievable. My earnest gratitude to the Trinity, Our Lady, St. Joseph, St. Raphael, and Blessed Guadalupe Ortiz. *Nam et si ambulavero in medio umbræ mortis, non timebo mala, quoniam tu mecum es.*

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## CHAPTER 1

### INTRODUCTION

Scleractinian, or stony, corals are ecosystem engineers that directly and indirectly support one fourth to one third of all marine biodiversity by providing complex habitat structures (Mera & Bourne, 2018; Peixoto et al., 2017; Plaisance et al., 2011). Corals establish and maintain symbiotic relationships with microorganisms in the microhabitats within their outer mucosal layer, endodermal tissue, and calicoblastic tissue (Ainsworth et al., 2015; NOAA Coral Disease & Health Consortium, 2019; Egan & Gardiner, 2016). The metabolic and genetic diversity of microorganisms facilitates nutrient pathways between the coral host and its endosymbiotic *Symbiodinium* spp. (e.g., Davy et al., 2012; Hernandez-Agrede et al., 2016). Additionally, coral-associated microbial communities, also known as the coral microbiome, contribute to coral acclimation capacity and resilience (e.g., Egan & Gardiner, 2016; Mera & Bourne, 2018; Peixoto et al., 2017). The tripartite relationships between the coral, *Symbiodinium* spp., and associated microorganisms collectively make up the coral holobiont (Bourne et al., 2016; Mera & Bourne, 2018).

Worldwide, coral reefs are facing environmental degradation and overall decline due to climate change (e.g., Bindoff et al., 2019; Carpenter et al., 2008; van Oppen & Blackall, 2019; Woodley et al., 2016). Just in the last three decades, Caribbean reefs have lost 80% of coral cover due to rising sea temperatures, bleaching, and disease (Gardner et

al., 2003; NOAA Coral Reef Watch, 2017). More frequent and/or severe environmental disturbances attributed to climate change, such as marine heat waves, have serious implications for the future of coral reefs (e.g., Bourne et al., 2016; Gardner et al., 2003; Mera & Bourne, 2009). By 2100, coral reef ecosystems around the globe will experience a reasonably projected 1.5°C increase in global sea surface waters, severely compromising the health and resilience of reef-building corals (Ainsworth & Hoegh-Guldberg, 2009; Bindoff et al., 2019; IPCC, 2019; van Oppen & Blackall, 2019). In addition, corals that are already compromised by the effects of long-term climate change face daily biotic and abiotic perturbations that threaten the stability of the holobiont (Bindoff et al., 2019; Hayes, 2019; Hoegh-Guldberg et al., 2007; Robinson et al., 2019; Röthig et al., 2016). For example, *Vibrio* bacteria are ubiquitous in seawater and resident members of coral microbiomes (Kemp et al., 2018). However, *Vibrio* are also well-known human pathogens and commonly implicated in coral diseases (Sutherland et al., 2016). In optimal environmental conditions, *Vibrio* bacteria may bloom and/or become more virulent (Kemp et al., 2018; Shade et al., 2014; Sutherland et al., 2016). Such changes in environmental conditions may lead to widespread bleaching, disease, and mortality (Davy et al., 2012; Hernandez-Agreda et al., 2016; Webster & Reusch, 2017).

The microbial diversity within corals has the potential to increase resilience to disturbances and facilitate recovery (e.g., Ainsworth et al., 2009, Kim, 2016). Therefore, further research is critically needed to understand the interactions between the environment, coral hosts, and their associated microorganisms in order to predict the consequences of environmental stress on holobiont homeostasis.

## Dissertation Objectives

Globally, stony coral holobionts and surrounding reef water microbiomes contribute greatly to the health of coral reefs. From disease to dust, I elucidate the influence of biotic and abiotic stressors on the microbiomes associated with stony corals and reef water in the Florida Keys, FL, USA.

*Objective One (Chapter 3)* Spatial and temporal variability (within and between studies) hinders the ability to identify overarching patterns in coral disease dynamics. In August 2017, I captured a multi-species, multi-disease outbreak of coral disease. While unfortunate, this unique opportunity permitted that two coral disease hypotheses be tested with minimal, potentially confounding, spatiotemporal variability.

*Objective Two (Chapter 4)* Climate change compromises efficacy of host-microbe interactions, and natural events may become tipping points between stable and unstable states of the coral holobiont. During a 26 d time series, I describe the influence of natural, episodic trans-Atlantic Saharan dust deposition on the bacterial communities and *Vibrio* abundances associated with healthy *Acropora palmata* and *Orbicella faveolata* corals.

*Objective Three (Chapter 5)* The utilization of metagenomics tools enhances our understanding of the functional roles of marine microbial communities. During the same 26 d time series, I show differential gene expression in a reef water metatranscriptome in response to changes in environmental factors associated with Saharan dust deposition.

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## CHAPTER 2

### LITERATURE REVIEW

Coral reef ecosystems are ecologically significant biodiversity hotspots that make up an economically and culturally precious ecosystem (Bar-On et al., 2018; Bourne et al., 2016; van Oppen & Blackall, 2018). The ecosystem services provided by coral reefs are valued at hundreds of billions of dollars worldwide (Woodley et al., 2016). As foundation species, scleractinian corals produce a complex calcium carbonate skeleton that forms the three-dimensional habitat configuration of reefs hosting a vast number of marine organisms such as fish and crustaceans (Glasl et al., 2016; Mera & Bourne, 2018; Peixoto et al., 2017). At a microscale, corals act as energy carriers by releasing nutritious compounds consumed by zooplankton and phytoplankton (Fransolet et al., 2013; Webster & Reusch, 2017).

Microorganisms are key members of all ecosystems due to their indispensable contributions to biogeochemical and ecological processes, including chemical cycling, nutrient production, and contribution to organism health (Ainsworth & Hoegh-Guldberg, 2009; Herren & McMahon, 2017; Peixoto et al., 2017). Constituting an estimated 70% to 90% of living oceanic biomass, microorganisms have established symbiotic relationships with various marine organisms that utilize microbial genomic and metabolic diversity to improve host metabolism, fitness, and development (Aronson, 2006; Bar-On et al., 2018; Davy et al., 2012; Webster & Reusch, 2017). Therefore, host-microbe relationships are

both important and complex in marine ecosystems (Egan & Gardiner, 2016; Hernandez-Agreda et al., 2016; Mera & Bourne, 2018; Webster & Reusch, 2017). A subset of organisms in the Cnidaria phylum are symbiotic with intracellular dinoflagellate microalgae of the genus *Symbiodinium*, a relationship that has been retained since its evolution in the Triassic (Lehnert et al., 2012; Oakley et al., 2016). Marine invertebrate members of the Anthozoa class, including stony and soft corals, exchange carbon, nitrogen, phosphorus, trace metals, and vitamins with *Symbiodinium* spp. and the microbiome (Bourne et al., 2016; Oakley et al., 2016; Woodley et al., 2016). Dinoflagellates provide photosynthates, such as fixed organic carbon, that compose a majority, if not more than 100%, of corals' energy and nutrient requirements (Oakley et al., 2016). The microbiome also plays essential roles in the cycling and production of nitrogen, trace metals, and vitamins (Bourne et al., 2016). Together, the holobiont ensures the coral's survival and propagation in the oligotrophic waters of marine ecosystems (Bourne et al., 2009; Sweet et al., 2011; Thompson et al., 2015). Coral-associated microorganisms, composed of prokaryotes, viruses and fungi, are the least understood component of the holobiont. Research on viral and fungal interactions in corals is especially sparse due to technological limitations; however, the 'Omics Revolution has facilitated the study and understanding of coral-associated bacterial communities, particularly through 16S rRNA gene amplicon sequencing (Thompson et al., 2015).

Reef-building corals host one of the most diverse and variable microbiomes on the planet (Blackall et al., 2015; Egan & Gardiner, 2016; Hernandez-Agreda et al., 2016, 2017). Specifically, bacteria serve important functions in the holobiont such as nutrient translocation, homeostasis, evolution, and development (Mera & Bourne et al., 2018;

Sweet et al., 2011; Thompson et al., 2015; Webster et al., 2017). For example, members of the Gammaproteobacteria class contribute to essential sulfur cycling pathways and translocation of nutrients to other members of the holobiont (Bourne et al., 2016). *Endozoicomonas*, which has been suggested as a common taxon in healthy corals, contributes to the biological control of pathogens by producing antimicrobial compounds and potentially modulating coral immune responses (Meyer et al., 2014; Thompson et al., 2015). Quorum sensing may also influence bacterial colonization within the holobiont. For example, *Vibrio* capable of quorum sensing can overcome host defenses and contribute to tissue lesions (Mera & Bourne, 2018; Peixoto et al., 2017).

Distinct bacterial communities colonize the different spaces within the coral: the surface mucopolysaccharide layer, endodermal tissues, and skeleton (Ainsworth et al., 2015; Sweet et al., 2010). Understanding host-microbe interactions is especially difficult given that the microbial community exists interdependently, potentially hindering our ability to identify the roles of individual members of the coral microbial community (Ainsworth et al., 2009, 2020). Even in the human medical field, researchers struggle to describe complex host-symbiont interactions in mucosal membrane environments, such as the mouth, gut, and sinuses, since these habitats are areas of substantial overlap and interaction between the host and its microbial community (Chow et al., 2011; Lamont and Hajishengallis, 2015; Nelson et al., 2012). Over the last 15 years, a great effort has been made to describe the bacterial communities of corals. A majority of these studies have been observational or correlational studies on how the bacterial community of specific corals differ spatiotemporally or respond to environmental fluctuations (e.g., Ainsworth &

Hoegh-Guldberg, 2009; Bourne et al., 2009; Bruno et al., 2003; Kerswell & Jones, 2003; Thurber et al., 2009).

Further studies are needed to identify overarching trends in coral microbiomes (i.e., across multiple species and wide-ranging environmental conditions using increased replication) to better describe the symbiosis between coral and their resident bacteria. For example, Hernandez-Agrede et al. (2016) was the first study to group the coral microbial community into three distinct communities when given an adequate replication size of over 100 coral colonies and a broad range of environmental conditions. The study categorized the microbiome of *Pachyseris speciosa* into three distinct sub-communities: (i) a stable, ubiquitous microbiome consisting of very few host-selected bacteria, (ii) a spatially and/or regionally explicit microbiome fulfilling functional niches, and (iii) a highly variable microbiome that is responsive to transient biotic and abiotic environmental conditions.

Stable and niche-specific microbiomes may contribute to central, established metabolic pathways maintained across cnidarians (Hernandez-Agrede et al., 2017; Peixoto et al., 2017). For example, a stable microbiome consisting of few host-selected bacteria (termed “hub microbes”) has been observed colonizing hatching *Hydra* polyps and subsequently shaping adult *Hydra* microbiomes (Deines et al., 2017). Niche-specific microbiomes, shaped by regional geographic conditions, may enhance acclimatization of hosts without altering the host genome (Bourne et al., 2009). Given that microbes rapidly divide within the host, adaptive evolution could happen in the scale of weeks to months, much more rapidly than the host evolves (Bourne et al., 2009; Webster & Reusch, 2017). Members of the highly variable, responsive microbiome may include opportunistic microbes, such as *Vibrio* spp., that bloom after increased nutrient influx or during dysbiotic

coral states (Egan & Gardiner, 2016; Kemp et al., 2018; Sutherland et al., 2016; Westrich et al., 2016, 2018). Peixoto et al. (2017) calls for an effort to identify coral symbionts that possess nutritional and protective traits that improve coral fitness. However, to date, there are few studies that have directly addressed this objective (Ainsworth & Hoegh-Guldberg, 2009; Hernandez-Agreda et al., 2016).

### **Biotic Stressors Present in Coral Reefs**

Disease is a major driver of reef-building coral loss resulting in significant declines in coral cover, major shifts in coral community assemblages, and “flattening” of the three-dimensional reef habitat (e.g., Hayes, 2019; Bourne et al., 2009; Harvell et al., 1999; Woodley et al., 2016). The coral host is a part of an interdependent metaorganism, making it difficult to understand the diseased state of corals based solely on visual signs and outside of disease-relevant time scales (Bosch & McFall-Ngai, 2011; Sutherland et al., 2016). In the 1970s and 1980s, coral disease was described by gross signs and the range of hosts affected (Sutherland et al., 2016). Coral biologists monitored affected populations and classified candidate pathogens. By the mid-1990s, new coral diseases were described and efforts to identify causal agents through the fulfillment of Koch’s postulates were prioritized (Sutherland et al., 2016). As of the early 2000s, fulfillment of Koch’s postulates had been achieved for five of eighteen coral diseases (Sutherland et al., 2004). Current coral disease literature has identified only a few specific bacterial pathogens, leaving scientists with a long list of candidate but unconfirmed pathogens (Kemp et al., 2018).

The paucity of groundbreaking literature identifying coral pathogens may be attributed to the challenges scientists face when investigating disease. Different signs are

used to differentiate diseases that are caused by the same pathogen (different signs, same pathogen). In addition, the same signs on the same or multiple species may be caused by more than one pathogen (same signs, different pathogens). Not all diseases show signs that could be attributed to pathogenic disease rather than environmental stress, and further analyses are required for diagnosis (Burge et al., 2015). Lastly, established pathogens may no longer be found in all cases of the disease and aetiologies are changing over time (Sutherland et al., 2016). Due to these challenges, coral disease researchers are growing to realize the application of the “one pathogen, one disease” framework is limited, and new frameworks to describe and define coral disease are needed (Kemp et al., 2018; Mera & Bourne, 2018; Sweet & Bulling, 2017).

The literature on coral diseases can be generally grouped into two overarching frameworks used to define disease and/or describe disease development. In this dissertation, the two seemingly competing hypotheses are referred to as (a) the pathobiont disease hypothesis, and (b) the microbiome dysbiosis disease hypothesis. The pathobiont hypothesis favors grouping bacterial taxa by their association with health or disease status (e.g., Bourne et al., 2016; Herren et al., 2018; Sweet & Bulling, 2017; Sutherland et al., 2004; Sutherland et al., 2016; Vayssier-Taussat et al., 2014; Weil & Rogers, 2011). The dysbiotic disease hypothesis favors identifying broad microbial community patterns that reflect a transition from healthy to dysbiotic microbiome states that likely lead to disease (e.g., Beurmann et al., 2018; Bourne et al., 2009; Egan & Gardiner, 2016; Glasl et al., 2016; Hernandez-Agreda et al., 2016; Mao-Jones et al., 2010; Mera & Bourne, 2018; Zaneveld et al., 2017). When describing coral disease, it is becoming increasingly important to consider both the composition and structure of the coral and the classification of taxa as

health- or disease-associated (Gignoux-Wolfsohn & Vollmer, 2015; Kumar et al., 2003). Once the coral metaorganism experiences stress, a consequent change in the bacterial community may occur before visual signs of disease appear (Bourne et al., 2008; Lee et al., 2016; Pollock et al., 2019). For example, Rypien et al. (2010) observed increased competition among resident bacteria and an increased production of antibiotics in the microbial community of a healthy coral prior to invasion of pathogens. Research goals for the near future include identifying temporal patterns in both healthy and diseased corals to test if the bacterial community (i.e., as a bioindicator), rather than visual pathognomonic signs, can predict coral disease and/or stress prior to disease initiation and development (Blackall et al., 2020; Glasl et al., 2017; Peixoto et al., 2019; Pootakham et al., 2019; Shinn et al., 2015; Villela et al., 2019).

### **Abiotic Stressors Present in Coral Reefs**

Exposing corals to abiotic stressors, both anthropogenic and natural, has the potential to change coral physiology and shift coral microbial communities (Bruno et al., 2003; Mumby & Woesik, 2014; Richards & Beger, 2011; Sutherland et al., 2010). The major source of abiotic stress in coral reefs is elevated temperature; however, pH, salinity, and nutrient load also affect coral microbiomes and holobiont health (Hoegh-Guldberg et al., 2007; Robinson et al., 2019; Röthig et al., 2016).

Starting in 2014, a massive three-year global coral bleaching event set the record as the longest, most widespread bleaching event in history (Clarke & Belva, 2016). By 2015, 41% of global reefs were exposed to ocean temperatures 4°C above normal, and by 2016, 85-93% of individually surveyed reefs in the Great Barrier Reef had bleached

(Gardner et al., 2003; Hughes et al., 2017; NOAA Coral Reef Watch, 2017; NOAA News, 2015). Similar events have occurred only twice in recorded history (1998 & 2010) (Aronson et al., 2002; Goreau et al., 2000). A temperature change of only 1-2°C, caused by increasing atmospheric carbon dioxide from anthropogenic emissions, can cause mass bleaching events where the vital symbiosis between corals and endosymbiotic *Symbiodinium* breaks down, leading to inefficient nutrient recycling within the holobiont and an increased risk of coral mortality (Coles et al., 1976; Howells et al., 2013; Schoepf et al., 2019). Elevated seawater temperature may also cause some members of the coral-associated bacterial community to negatively impact coral health (Lee et al., 2016; Webster & Reusch, 2017). For example, bleaching events are linked to bacterial community shifts in corals that increase disease incidence and decrease coral resilience (Brandt & McManus, 2009; Garren et al., 2015; Lee et al., 2015). Rising seawater temperatures also coincide with an increase in the relative abundance of known bacterial pathogens in *Acropora muricata* and *Pocillopora damicornis* (Lee et al., 2015; Tout et al., 2015). One reason to explain this may be that heat stress “releases” some bacteria from antagonistic interactions that allow opportunist, potentially pathogenic bacteria to dominate the composition of the community (Rypien et al., 2010).

Ocean acidification is characterized by decreasing seawater pH and accompanying changes in seawater chemistry (Doney et al., 2009). Ocean seawater pH is expected to decrease 0.2 to 0.4 units by 2100, a change that has not been seen for the past 23 million years (IPCC, 2019; Rhein et al., 2013). Morrow et al. (2014) observed a decrease of functionally important *Endozoicomonas* in *Acropora millepora* exposed to naturally occurring carbon dioxide. Vibrionaceae and Alteromonadaceae increased in *Acropora*

*eurystoma* following experimental exposure to low pH (Meron et al., 2011). However, studies on the effect of ocean acidification on coral microbiomes are not necessarily conclusive. When exposed to elevated temperature and decreased pH, *Acropora millepora* and *Turbinaria reniformis* showed different microbial diversity trends, highlighting that dual abiotic stress may indeed alter microbial communities but genera-specific interactions may influence community responses (Grottoli et al., 2018; O'Brien et al., 2018).

While rising temperatures and acidification are wide-spread, well-studied abiotic factors, such as local stressors, also impact corals and their associated microbiomes (Woodley, 2016). Patch and in-shore reefs may experience extreme salinity fluctuations due to precipitation, freshwater runoff, and/or drought (Röthig et al., 2016). While short-term exposure to increased salinity did not shift the *Fungia granulosa* microbial community, long-term exposure to high salinity yielded to major restructuring of the microbiome (Röthig et al., 2016). Exposing the coral *Stylophora pistillata* to low salinity, or hypo-osmosis, caused a disruption between coral-algal symbiosis that lead to visible sublethal bleaching (Kerswell & Jones, 2003). Another local stressor, nutrient load, is an influencing factor in oligotrophic coral reefs (Atkinson, 2011; Gillis et al., 2014). For example, episodic trans-Atlantic Saharan dust pulses provide iron, a key micronutrient for bacteria, to the reefs in the Florida Keys via atmospheric deposition (Boyd & Ellwood, 2010; Mahowald et al., 2009). These events promote *Vibrio* bloom formation in reef surface waters (Westrich et al., 2016). While ubiquitous in the water column, *Vibrio* spp. are also members of the coral microbiome and have been proposed as opportunistic coral pathogens (Sutherland et al., 2016). Nutrient enrichment has been shown to increase non-specific microbial virulence genes in the coral mucus layer (Bruno et al., 2003; Thurber et

al., 2009). Additionally, *Acropora* spp. colonies exposed to nitrogen and phosphorus via fertilizer diffusion experienced microbial community shifts, including a significant increase of *Rickettsia*-like organisms which are potential disease agents (Jessen et al., 2013; Shaver et al., 2017; McDevitt-Irwin et al., 2017).

The governing abiotic factors that determine whether coral-associated bacteria have beneficial or deleterious effects on the holobiont is not well understood (Certner et al., 2015; Pantos et al., 2015). Scientists have looked to corals thriving on the periphery of their environmental tolerance to understand the mechanisms underlying resistance, acclimation, and adaptation abilities of corals in response to extreme abiotic stressors (Bijma et al., 2013; Camp et al., 2018; Perry & Larcombe, 2003; Schoepf et al., 2019). Still, further research is needed to identify the contributions of coral microbial communities to holobiont stability, particularly when corals experience unfavorable abiotic conditions.

## **Conclusion**

Corals are the most important foundation species in the world, and their populations are catastrophically declining (e.g., Gardner et al., 2003; Morgan et al., 2020; Toth et al., 2019). Not only are corals debilitated by long-term perturbation (i.e., climate change) and acute disturbances (i.e., pollution), they lack the time needed to recover between increasingly extreme biotic and abiotic events (Ainsworth et al., 2020; Cerutti et al., 2020; van Oppen & Blackall, 2019). While microbial diversity in corals has the potential to increase resilience to novel and recurring stressors, the impact of biotic and/or abiotic factors on the microbiomes of present-day, potentially compromised corals is not well understood (Bar-On et al., 2018; Bourne et al., 2016; van Oppen & Blackall, 2018).

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## CHAPTER 3

### CONFINED SPATIOTEMPORAL SAMPLING OF CORALS DURING A MULTI-SPECIES, MULTI-DISEASE OUTBREAK REVEALS DIFFERENT BUT COMPLEMENTARY MICROBIAL COMMUNITY RESPONSES <sup>1</sup>

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<sup>1</sup> Bucher, M. G., Westrich, J. R., Lydon, K. A., & Lipp, E. K. To be submitted to *OCEANS Special Issue: The Future of Coral reefs*. International Society of Microbial Ecology (ISME).

## **Abstract**

Disease is a major driver of reef-building coral loss. Currently, the literature on coral disease can be generally grouped into two overarching frameworks. The first framework, defined here as the pathobiont disease hypothesis, favors grouping bacterial taxa by their association with health or disease. The second framework, defined here as the microbiome dysbiosis hypothesis, favors identifying broad bacterial community transitions from healthy to dysbiotic states. Opportunities to test these hypotheses have been limited due to inadequate sampling across several coral species and disease types in the absence of spatial and temporal variability. In this study, coral mucus samples were collected within 96 h during an active multi-species, multi-disease outbreak in two adjacent Middle Keys Reefs (Florida, USA), effectively limiting potential confounding spatiotemporal variability during collection. Here we show evidence of two seemingly competing coral disease hypotheses that are not mutually exclusive. Surface mucus layers from 102 colonies were sampled and processed for 16S rRNA gene amplicon sequencing and analysis. Coral bacterial communities represented ten species and four diseases. Core community analysis grouped nine amplicon sequence variants (ASVs) as common across healthy corals (regardless of species) and six ASVs as common across diseased corals (regardless of species or disease type), suggesting that specific bacterial taxa are consistently observed according to health status. Additionally, beta-diversities across all healthy corals were similar (regardless of species), and beta-diversities of diseased corals across all species (regardless of disease type) were distinctly dissimilar. These results reconcile two apparently contradicting hypotheses used to describe coral disease and suggest that both taxa- and community-level patterns should be considered when defining coral disease.

## **Introduction**

Coral reefs are culturally, economically, and environmentally valuable ecosystems; however, coral reefs face a multitude of threats from nutrient pollution, overfishing, recreational diving, and climate change (Woodley et al., 2016). Disease is also a major cause of decreasing coral cover worldwide, but particularly in the Caribbean where 85% of all species are affected by disease and the number of reefs exhibiting disease has dramatically increased (Aeby et al., 2019; AIMS Long-term Reef Monitoring Program, 2017; Gardner et al., 2003; Global Coral Reef Monitoring Network, 2014; Porter et al., 2013). Additionally, rates of disease-related coral loss are predicted to increase every year (Green & Bruckner, 2000; Harvell et al., 1999, 2002; Muller et al., 2018; Precht et al., 2016; Rosenberg et al., 2002). For example, in 2014 the first case of stony coral tissue loss disease (SCTLD) was observed in the Florida Keys Reef Tract (FKRT), the third largest barrier reef system in the world, and as of 2019, 49% of stony corals were experiencing the disease with 22 of the 45 coral species in the FKRT affected (Meyer et al., 2019; Muller et al., 2018; Precht et al., 2016).

Despite a growing body of research to document, describe, and understand disease and disease processes in corals, significant challenges remain in our understanding of mechanisms underlying many coral diseases (Mera & Bourne, 2018). The literature on coral diseases can be generally grouped into two overarching frameworks used to define disease and/or describe disease development. In this dissertation, the two seemingly competing hypotheses are referred to as (a) the pathobiont disease hypothesis, and (b) the microbiome dysbiosis disease hypothesis. The pathobiont hypothesis favors grouping bacterial taxa by their association with health or disease status (e.g., Ainsworth et al., 2015;

Bourne et al., 2016; Castañeda-Chávez et al., 2018; Chow et al., 2011; Gavish et al., 2018; Gignoux-Wolfsohn & Vollmer, 2015; Herren et al., 2018; Hugget & Apprill, 2018; Hussien et al., 2019; Insafitri et al., 2020; Kellogg, 2019; Kelly et al., 2019; Kim, 2016; Klinges et al., 2019; Peixoto et al., 2017; Sutherland et al., 2004; Sutherland et al., 2016; Sweet & Bulling, 2017; Vayssier-Taussat et al., 2014; Weil & Rogers, 2011). For example, Ushijima et al. (2014) identify a *Vibrio coralliilyticus* strain as an etiological agent of acute white syndrome. *Vibrionaceae*, *Alteromonadaceae*, and *Rhodobacteraceae* have been routinely associated with coral diseases, such as stony coral tissue loss, white band, and white pox (Kim 2016; Meyer et al., 2019; Kemp et al., 2018; Sutherland et al., 2016). The pathobiont disease framework also applies to identifying specific bacteria associated with coral health. For example, studies have attempted to group members of the coral microbiome as either “beneficial microorganisms for corals” (Peixoto et al., 2017). *Endozoicomonas*, *Actinomycelates*, and *Burkholderiales* have been consistently associated with healthy corals including *Porites astreoides*, *Acropora millepora*, *Acropora granulosa*, *Leptoseris* spp., and *Montipora capitata* (Ainsworth et al., 2015; Hernandez-Agreda et al., 2016; Meyer et al., 2014). However, identifying and confirming the role of bacterial taxa in order to categorize them is very difficult (Webster & Reusch, 2017). Some bacteria, such as *Vibrio*, are present in corals exhibiting different signs of disease (same pathogen, different signs), but corals exhibiting the same signs of disease, such as brown band disease, may have different bacteria present that could be candidate pathogens (different pathogen, same signs) (Jompa & Tahir, 2019; Sutherland et al., 2016). Additionally, candidate pathogens that were confirmed as causative agents of disease in the past, such as

*Serratia marcescens*, have not been found in later surveillance studies of the disease (Sutherland et al., 2010; Sutherland et al., 2016).

The microbiome dysbiosis disease hypothesis favors identifying broad microbial community patterns that reflect a transition from healthy to dysbiotic microbiome states that lead to disease (e.g., Ainsworth et al., 2009; Bass et al., 2019; Beurmann et al., 2018; Bourne et al., 2009; Egan & Gardiner, 2019; Glasl et al., 2016; Hernandez-Agreda et al., 2016; Herren & McMahon, 2018; Mao-Jones et al., 2010; Mera & Bourne, 2018; McDevitt-Irwin et al., 2019; Pollock et al., 2019; Sweet & Bulling, 2017; Sweet et al., 2019; Vayssier-Taussat, 2014; Zaneveld et al., 2017). For example, describes healthy host-associated microbiomes as similar in composition and structure while perturbed microbiomes, including those affected by disease, have dysbiotic microbiomes (Zaneveld et al., 2017). Dysbiosis is defined as a state of imbalance between the host and its associated microorganisms (such as members of the coral holobiont) due to disrupted host-microbe and microbe-microbe interactions (Petersen & Round, 2014; Stecher et al., 2013). Multiple mechanisms may contribute to microbial dysbiosis including changes in microbial diversity, loss of beneficial taxa, and colonization of opportunistic heterotrophs (Kemp et al., 2018; Petersen & Round, 2014; Stecher et al., 2013). This framework posits that beta-diversity dissimilarity and dispersion measures quantify the state of the microbiome in transition from health to dysbiosis. Similarly, cohesion, or connectivity, is proposed as a means of describing the stability of a microbiome (McMahon & Herren, 2018). Using Bray-Curtis dissimilarity, cohesion aims to quantify the degree of community connectivity and has been suggested to predict instability of microbiomes that are negatively influenced by their environments. Egan & Gardiner (2016) highlights how disease in marine systems

could be a result of dysbiotic host-associated microbial communities and followed by an increase of opportunistic or polymicrobial infections.

Opportunities to compare these two coral disease hypotheses (including resistance to, initiation of, and response to disease) in a single system such as the coral holobiont have been limited by significant challenges. Often, studies are influenced by uncontrollable and sometimes confounding variables, such as limitations in time and space during sample collection. Transporting samples can also influence the microbial composition of the sample in a matter of hours, compromising sample integrity (Mera & Bourne, 2018). These logistical challenges make it difficult to adequately survey healthy and diseased corals across different species and disease types at sufficient scales to describe bacterial community dynamics. Additionally, the microbiome associated with reef-building corals is one of the most diverse and variable microbiomes studied to date, making it challenging to recognize and consistently identify patterns across disease types and coral species (Blackall et al., 2015; Egan & Gardiner, 2016; Hernandez-Agreda et al., 2016, 2017).

Perhaps the most limiting of factors is the spatiotemporal differences present within and between studies that hinders our ability to identify overarching trends in coral microbiomes before, during, and after disease (Bourne et al., 2009; Hernandez-Agreda et al., 2016; Hugger & Apprill, 2018; Röthig et al., 2020; Sweet & Bulling, 2017; Webster & Reusch, 2017). Current studies on coral disease often sample from different time periods (common when focusing on a specific disease) or from different locales (common when focusing on a specific species) (Ainsworth et al., 2009; Bass et al., 2019; Mera & Bourne et al., 2018; Peixoto et al., 2017; Sweet & Bulling, 2017). In these cases, differences in coral microbial communities between healthy and diseased colonies may be undetectable

due to variability imposed by geographic distance between study areas and/or the timing of sample collection (Pearman et al., 2019). In order to test hypotheses of the aforementioned coral disease frameworks as accurate, microbial communities must be analyzed across species and diseases without potential spatiotemporal confounding factors.

In this study, we captured an active multi-species, multi-disease outbreak in the Middle Florida Keys, FL, USA from 27 August to 28 August 2017. This unfortunate outbreak event provided a unique opportunity to test current coral disease frameworks over a defined space and time. Within two days, we collected samples from adjacent reefs where multiple species were affected by several disease types, effectively confining spatiotemporal variability during sample collection. We describe commonalities and differences in coral bacterial microbiomes to show that two apparently contradicting coral disease frameworks (pathobiont disease hypothesis vs. microbiome dysbiosis hypothesis) are complementary and not mutually exclusive.

## **Methods**

### *Field sample collection*

From 27 to 28 August of 2017, a total of 113 coral surface mucus layer samples were collected from two reefs in the Middle Florida Keys, FL, USA (less than 13 km apart). Reef 1 (not named) was located at 24.824, -80.688 and Tennessee Reef was located at 24.745, -80.780. Coral surface mucus layer was collected *in situ* by SCUBA diving using sterile, needleless 10 mL syringes (Monoject by Covidien, Dublin, Ireland). Sampled coral colonies were characterized as healthy (i.e., the entire colony showed no visual signs of disease) or diseased (i.e., the colony showed visual signs of disease on part of the colony).

Disease types were identified by their gross appearance *in situ*. Of note, white plague corals were characterized by white tissue lesions starting at the base of the colony, moving inward (unlike stony coral tissue loss that is characterized by loss of tissue with a tendency of lesions to begin at any position on the coral colony, often the top, and expanding outwards) (Aeby et al., 2019; Meyer et al., 2019; Work & Aeby, 2006, 2011).

Coral mucus samples collected from healthy colonies were characterized as “healthy,” and mucus samples collected from disease colonies were characterized as either “diseased” (i.e., collected at the disease lesion) or “apparently healthy” (i.e., collected from visually healthy sections of a diseased colony approx. 5-10 cm away from the disease front depending on colony size). All 10 mL of mucus was passed through a sterile Sterivex 0.22 µm pore-size cartridge filter (MilliporeSigma, MA, USA) on the boat less than 2 h from collection. Sterivex filters were pumped dry with an extra 10 mL of air using a needleless syringe, capped, and placed immediately on ice. Upon returning to the Keys Marine Laboratory (Long Key, FL, USA), sterivex filters were stored at -80°C within 5 h of collection until processing.

#### *DNA extraction, library preparation and sequencing*

DNA from coral mucus was extracted from the Sterivex cartridge filters using a phenol-chloroform with salt precipitation extraction protocol described in Lydon et al. (2017). The V4 hypervariable region of the 16S rRNA gene was amplified by polymerase chain reaction (PCR) (Caporaso et al., 2011). Each 10 µL reaction contained 2 µL of 1x Q5 Buffer (New England BioLabs (NEB), Ipswich, MA, USA), 0.2 µL of 10 mM dNTP mix (NEB, Ipswich, MA, USA), 0.1 µL of Q5 Hot Start High-Fidelity DNA polymerase (NEB,

Ipswich, MA, USA), 4.7  $\mu$ L of PCR-grade molecular water, 0.5  $\mu$ L of 10  $\mu$ M 515F primer (V4 hypervariable region; Caporaso et al., 2011), 0.5  $\mu$ L of 10  $\mu$ M 806R primer (V4 hypervariable region; Caporaso et al., 2011), and 2  $\mu$ L of extracted DNA. Two technical replicates per biological sample were prepared to account for PCR variability. PCR conditions were 98°C for 30 sec for initial denaturation, 25 cycles of 10 sec at 98°C, 30 sec at 52°C, 30 sec at 72°C, and a final extension step lasting 2 min at 72°C. In a second reaction, samples were barcoded using custom barcode sequences (Tinker & Ottesen, 2016). Technical replicates were combined before a second round of amplification. Each 30  $\mu$ L reaction contained 11.1  $\mu$ L of PCR-grade molecular water, 6.0  $\mu$ L of 5x Q5 Buffer (NEB, Ipswich, MA, USA), 0.6  $\mu$ L of 10 mM dNTP mix (NEB, Ipswich, MA, USA), 0.3  $\mu$ L of Q5 Hot Start High-Fidelity DNA polymerase (NEB), 1.5  $\mu$ L of 10  $\mu$ M custom forward barcoded adaptors, 1.5  $\mu$ L of 10  $\mu$ M custom reverse barcoded adaptors, and 9  $\mu$ L of reaction 1 pooled PCR technical replicate product. PCR conditions were 30 sec at 98°C, 4 cycles of 10 sec at 98°C, 30 sec at 52°C, 30 sec at 72°C, followed by 6 cycles of 10 sec at 98°C, 1 min at 72°C, followed by a final extension step at 72°C for 2 min.

Amplified and barcoded PCR products were purified using 1:1 volume SPRI magnetic beads (Sera-Mag SpeedBeads, GE, UK), diluted in 10 mM Tris, and quantified on a Qubit dsDNA HS Assay Kit (Thermo Scientific, DE, USA) (Rohland & Reich, 2012). Samples were then diluted to 10 nM in 25  $\mu$ L of 10 mM Tris HCl and sent to the Georgia Genomics and Bioinformatics Center (GGBC, GA, USA) for quality testing on a Fragment Analyzer (Advanced Analytical Technologies, now known as Agilent Technologies, IA, USA). Libraries were pooled in equimolar concentrations and sequenced on an Illumina MiSeq (V2 chemistry) to obtain PE250 reads (Illumina, Inc., CA, USA).

### *Sequence processing and microbial community analysis*

All sequence data processing and microbial community analyses were conducted in R Studio (R version 3.5.0; R Core Development Team, 2018). Sample sequences were processed and quality filtered using the DADA2 package version 1.8.0 (Callahan et al., 2016). The DADA2 1.8 Tutorial was followed to produce amplicon sequence variants (ASVs), a higher-resolution analogue of operational taxonomic units (OTUs) (Callahan et al., 2016, 2017). Sequences were trimmed and filtered in DADA2 using standard default values. A parametric error model was used to calculate error rates using a minimum of  $5 \times 10^8$  total bases (nbases=5e8). Multithreading was enabled (multithread=TRUE) to allow multiple error rate estimations to be tested simultaneously until convergence. Samples were dereplicated to produce unique sequences with a corresponding absolute abundance. While DADA2 does not throw away singletons, it does not infer ASVs based on a single read by default. Therefore, true sequence variants were inferred using a core sample inference algorithm with the option to pool samples (pool=TRUE) to resolve low-abundance, rare amplicon sequence variants including singletons within samples that are present in more than a single sample. Paired ends were then aligned using the denoised forward and reverse reads and merged to construct a contig sequence that overlapped by at least 12 bases and were identical to each other in the overlap region. An ASV table was created and chimeric sequences were removed using default DADA2 parameters.

Taxonomy was assigned using a native implementation of naïve Bayesian classifier using the RDP (version 16) DADA2 training set with species level assignments based on exact matching (Cole et al., 2014). The Greengenes database was not used for taxonomy assignment given it has been shown to mis-assign taxa associated with *Vibrio* and

Vibrionales (Lydon & Lipp, 2018). The DADA2 ASV table and taxonomic assignments were imported into the phyloseq package in R (McMurdie & Holmes, 2013), and taxa that were identified as Chloroplast or Mitochondria were removed.

Relative abundances of ASVs per sample were calculated and plotted using the phyloseq package (McMurdie & Holmes, 2013). Alpha-diversity measures were calculated using Observed measure to represent richness and Chao1 to represent richness accounting for rare ASVs. Observed and Chao1 measurements were calculated in phyloseq, and plotted using the `plot_richness()` function (McMurdie & Holmes, 2013). Alpha-diversity measures were tested for normality using the Shapiro-Wilk test of normality (Shapiro et al., 1972), and means tested for significant differences between all healthy, apparently healthy and diseased samples combined, between diseased samples of different disease types and species combinations, between healthy samples of different species, between apparently healthy samples of different disease types and species, and between healthy and diseased samples for each combination of species and disease type using ANOVA and followed by Tukey's honestly significant difference (Tukey-HSD) tests accounting for multiple comparisons (Abdi & Williams, 2010; Gelman, 2005).

Beta-diversity was measured using weighted Bray-Curtis dissimilarity and analyzed in groups by coral health status (healthy or diseased regardless of species or disease type), species (for mucus from healthy colonies), and species-disease combinations (for disease lesion samples). Dissimilarities were compared using a permutational multivariate analysis of variance (PERMANOVA) using the `adonis()` function followed by a pairwise PERMANOVA test using the `pairwise.perm.manova()` function in the RVAideMemoire package (Anderson et al., 2014; Hervé, 2018). Principal coordinate

analysis (PCoA) based on weighted Bray-Curtis dissimilarity was used to visualize beta-diversity of raw abundance of taxa using the `ordinate()` and `plot_ordination()` functions in the `phyloseq` package (McMurdie & Holmes, 2013). Beta-diversity variability was also measured using dispersion (the distance between a sample's median dissimilarity across replicates and the grouping variable's centroid, or median dissimilarity between samples categorized together). Beta-variability was analyzed the PERMDISP2 procedure by testing the homogeneity of multivariate dispersions using the `betadisp()` function from the `vegan` package (Anderson, 2006a; Anderson, 2006b; Oksanen et al., 2018). A separate permutation test for pairwise multivariate group dispersions was conducted with the `permutest.betadisp()` function from the `vegan` package (Oksanen et al., 2018). Beta-diversity dispersions were plotted using the `boxplot()` function (R Core Development Team, 2018). Notably, beta-diversity between white plague lesions and paired healthy samples of the same species was analyzed separately because of the large representation of this disease in the dataset. The remaining coral samples collected from dark spots disease, wasting disease, and yellow band disease lesions were combined due to insufficient replication of each disease type and the likelihood of sampling bias that could conceal overarching patterns in microbiome dynamics (Hernandez-Agreda et al., 2017; Zaneveld et al., 2017). The beta-diversity between disease types (combined) and healthy counterpart samples of the same species were analyzed as described above.

Samples were also imported into the AMPVIS2 package (version 2.3.14; Andersen et al., 2018) to identify the core ASVs associated with healthy samples regardless of species ("H CORE"), disease samples regardless of disease type ("DZ CORE"), and both healthy and disease samples regardless of species or disease type ("H & DZ CORE"). The core

communities were identified through the `amp_venn()` function which included two user-defined thresholds. The first threshold was an ASV abundance cutoff which we set to zero so that all ASVs would be considered. The second threshold was an ASV frequency cutoff to filter out ASVs that fall below a defined frequency percentage (i.e., persistence) across all samples. In the literature, coral microbiome core taxa have been defined as groups with a (usually arbitrary) persistence between 30-100% (e.g., Ainsworth et al., 2015; Glasl et al., 2016; Hernandez-Agreda et al., 2016, 2017; Sweet & Bulling, 2017). Hernandez-Agreda et al. (2017) suggests that defining a persistence level should reflect technical limitations of the study such as replication size, sample types (i.e., multiple species vs. single species of coral), and sequencing effort. Given that this study has a high sample size (n=102), controlled spatial and temporal variability, high sequencing effort (Illumina MiSeq), but a wide range of sample types (four diseases and ten species), the frequency cutoff was set at 50%. Lastly, the relative abundances of ASVs per sample were calculated using the `phyloseq` package, and microbial community composition bar plots were generated using the `ggplot2` package (McMurdie & Holmes, 2013; Wickham, 2016).

## **Results**

### *Sample collection*

After quality control, a total of 102 coral mucus samples (out of 113 collected samples) were used for microbial community analysis. A total of 32 mucus samples were collected from ten healthy coral species representing nine genera, 33 disease lesions samples were collected from colonies with one of four diseases types, and 37 apparently healthy mucus samples were collected from apparently healthy sections of an otherwise diseased colony

**(Table 3.1).** Sampled disease types included dark spots disease (DS; n=8), wasting disease (WD; n=3), white plague disease (WP; n=18), and yellow band disease (YB; n=4).

#### *Sequence processing and microbial community analysis*

After sequence processing via DADA2, the average number of reads per sample was 24,823 with a range of 1,529 to 37,2391. On average, 67% of each sample's sequences were retained after quality filtering. A total 8,625 ASVs were represented in the final dataset. Resulting sequences were submitted to NCBI and can be found under project PRJNA516715.

Alpha-diversity measures increased from healthy to diseased states but were not significantly different due to high variability. Alpha-diversity (Observed) for samples collected from healthy colonies averaged 171 ( $\pm$  196 SD; range: 21 to 945) across all species, 215 ( $\pm$  303 SD; range: 35 to 1451) for samples from apparently healthy colonies, and 321 ( $\pm$  499 SD; range: 32 to 2872) for samples from disease lesions. Chao1 measures for alpha-diversity averaged 230 ( $\pm$  250 SD; range: 30 to 1386) for samples collected from healthy colonies, 286 ( $\pm$  370 SD; range: 59 to 1697) for apparently healthy mucus from diseased colonies, and 384 ( $\pm$  523 SD; range: 64 to 3026) for disease lesion samples. Alpha-diversity measures were statistically similar between mucus from healthy colonies and disease lesions from colonies identified by species-disease combinations (data not shown). Furthermore, alpha-diversity measures were similar between samples from healthy colonies regardless of coral species and between samples from apparently healthy mucus on diseased colonies regardless of species or disease type (p-value<0.05; data not shown). Between individual disease types, alpha-diversity was significantly different. Alpha-

diversity measures were statistically lower for YB lesions compared to both DS lesions (Observed  $p$ -value=0.022; Chao1  $p$ -value=0.015) and WP lesions (Observed  $p$ -value=0.022; Chao1  $p$ -value=0.015), regardless of coral species. Alpha-diversity (Observed) averaged 83 ( $\pm$  54 SD; range: 32 to 155) for YB lesions, 699 ( $\pm$  929 SD; range: 121 to 2872) for DS lesions, 198 ( $\pm$  97 SD; range: 53 to 436) for WP lesions, and 578 ( $\pm$  630 SD; range: 58 to 1279) for WD lesions. Chao1 alpha-diversity measures averaged 121 ( $\pm$  48 SD; range: 64 to 188) for YB lesions, 772 ( $\pm$  978 SD; range: 149 to 3026) for DS lesions, 261 ( $\pm$  106 SD; range: 76 to 481) for WP lesions, and 641 ( $\pm$  652 SD; range: 128 to 1375) for WD lesions. No other significant changes in alpha-diversity were observed between disease lesions by disease type.

Beta-diversity dissimilarity and dispersion were statistically similar between all species of healthy corals ( $p$ -value=0.03; dispersion  $p$ -value=0.03; **Figure 3.1**). For disease lesions (identified by species-disease combination), dissimilarity and dispersion varied significantly when independently compared by all species ( $p$ -value=0.001; dispersion  $p$ -value=0.039; **Table S3.1**) and all disease types ( $p$ -value=0.001; dispersion  $p$ -value=0.002; **Table S3.2**). Additionally, beta-diversity of disease lesions varied between species and disease type together ( $p$ -value=0.001; **Table S3.3**). To further test beta-diversity, Bray-Curtis dissimilarity and variability were analyzed for all healthy colonies and all diseased lesions together, regardless of species or disease type. Samples clustered by status (healthy vs. diseased) regardless of species or disease type ( $p$ -value=0.001; **Figure 3.2**). Dispersion was greater in disease lesions compared to healthy mucus, irrespective of species or disease type ( $p$ -value<0.001; **Figure 3.3**). All samples from white plague lesions, along with paired healthy samples representing the same species were analyzed separately due to the large

representation of white plague disease in the dataset. WP lesions clustered separately from healthy mucus regardless of species ( $p$ -value=0.001; **Figure 3.2**); likewise, dispersion was greater in WP lesions compared to healthy mucus counterparts ( $p$ -value=0.004; **Figure 3.3**). The remaining samples collected from YB, DS, and WD lesions (YB-DS-WD dataset) were combined for analysis due to the insufficient representation of each disease and the likelihood of sampling bias that could conceal overarching patterns in microbiome dynamics (Zaneveld et al., 2017). DS, WD, and YB lesions clustered separately from healthy counterpart mucus representing the same species ( $p$ =0.027; **Figure 3.2**). While dispersion was greater in the YB-DS-WD lesions compared to paired healthy mucus, the difference was not statistically significant (**Figure 3.3**).

Core community analysis categorized a total 4,849 ASVs present in mucus collected from healthy colonies and those from disease lesions into three categories: (1) H & DZ CORE or taxa associated with both healthy and diseased corals, (2) DZ CORE or disease-associated taxa, and (3) H CORE or taxa associated with healthy corals. A total of 27 unique ASVs were categorized as members of the H & DZ CORE, and included ASVs from the following orders: Pirellulales ( $n$ =2), SAR11 ( $n$ =1), Puniceispirillales ( $n$ =1), Phycisphaerales ( $n$ =1), Rhodospirillales ( $n$ =1), Flavobacteriales ( $n$ =9), Rhodobacterales ( $n$ =1), Cellvibrionales ( $n$ =1), Synechococcales ( $n$ =9), and Actinomarinales ( $n$ =1) (**Figure 3.4; Table S3.4**). DZ CORE consisted of six unique ASVs from the following orders: SAR11 ( $n$ =2; Clade Ia genus), Flavobacteriales ( $n$ =2; including NS4 genus and NS9 family), one Rhodobacterales (*Ruegeria* genus), and one Vibrionales (*Vibrio* genus) (**Figure 3.4; Table S3.4**). The H CORE community of health-associated taxa comprised of nine ASVs in the following orders: Sphingobacteriales ( $n$ =3; including NS11-12

family), a SAR11 (Clade Ia genus), Flavobacteriales (n=6; including NS5 genus, NS7 family, and an unidentified Cryomorphaceae), and a Cytophagales (*Marinoscillum* genus) (**Figure 3.4; Table S3.4**). A total of 4,807 ASVs were not assigned as core taxa. These ASVs represented 61.1% of all ASVs considered in core community analysis.

## Discussion

Major losses of living coral cover in the Caribbean and Florida Keys can be attributed to disease (e.g., Aeby et al., 2019; AIMS Long-term Reef Monitoring Program, 2017; Bruckner 2019). Currently, two coral disease frameworks are described in the literature. The first framework, defined here as the pathobiont disease hypothesis, characterizes coral disease, or conversely corals in a healthy state, by association with specific bacterial taxa (e.g., Ainsworth et al., 2015; Bourne et al., 2016; Castañeda-Chávez et al., 2018; Chow et al., 2011; Gavish et al., 2018). The second framework, defined here as the microbiome dysbiosis disease hypothesis, identifies broad microbial community patterns that reflect a transition from healthy to dysbiotic microbiome states that influence disease (e.g., Ainsworth et al., 2009; Bass et al., 2019; Beurmann et al., 2018; Zaneveld et al., 2017). While, these hypotheses are not necessarily mutually exclusive, it can be challenging to compare disease dynamics across disease types and/or coral species in large part due to *in situ* sampling limitations.

In most cases, only a single disease may occur on a particular reef at a time or only a particular species may be affected at a time (e.g., Ainsworth et al., 2015; Hernandez-Agreda et al., 2016; Meyer et al., 2014, 2019). Often, efforts to compare commonalities in microbial dynamics between species or disease types are constrained to samples collected

at different times and/or different locations according to the health status of the coral reef ecosystem of interest (Bass et al., 2019; Neave et al., 2016; Webster & Reusch, 2017). The timing and location of sample collection could potentially outweigh effects of disease itself or confound the patterns observed in the microbiome samples (e.g., McDevitt-Irwin et al., 2017; Röthig et al., 2016; Woodley, 2016). Confining the extent of spatiotemporal variability during sample collection requires sampling from multiple disease types across one species or genus of coral, sampling from multiple species exhibiting the same disease, sampling over a short time period or a single location, or sampling a multi-disease, multi-species outbreak on a single reef. Initiatives that closely monitor coral reef ecosystems and maintain databases (i.e., Florida Fish and Wildlife Conservation (FWC) Coral Reef Evaluation and Monitoring Project (CREMP), or the Australian Institute of Marine Science (AIMS) Long-term Reef Monitoring Program) could assist researchers plan when and where to collect samples.

This study was able to capture such an event on 27 August to 28 August 2017 and aimed to test the accuracy of frameworks present in the literature that are used to describe coral disease (Mera & Bourne et al., 2018; Peixoto et al., 2017; Sweet & Bulling, 2017). Within 96 h, we collected coral mucus samples from adjacent reefs in the Middle Florida Keys, FL, USA without the potential for confounding factors such as variability in time and space during sample collection. Additionally, the representation of species and disease types in our dataset allowed us to capture commonalities and differences in the bacterial communities and of specific bacterial taxa present in the surface mucus layer of healthy and diseased corals.

### *Pathobiont disease hypothesis*

The pathobiont disease hypothesis favors grouping bacterial taxa by their association with health or disease status. In this study, 27 ASVs were present in H & DZ CORE at a persistence of at least 50% across species and disease types. For example, Cyanobacteria, which comprised 21% of ASVs in H & DZ CORE, is a dominant member of coral microbiomes playing an important part in diurnal nitrogen fixation and other functions that maintain host homeostasis (Bernasconi et al., 2019; Beurmann et al., 2018; Lesser et al., 2007; van Oppen & Blackall, 2019).

The DZ CORE comprised of six ASVs, regardless of disease type. Rhodobacteraceae have been associated in diseased coral, including stony coral tissue loss disease, black band disease, and white band disease white plague disease type II (Gignoux-Wolfsohn & Vollmer, 2015; Meyer et al., 2019; Roitman, 2018). Rhodobacteraceae have also been suggested as an indicator species for thermal stress in coral (Pootakham et al., 2019). Specifically, an ASV identified as *Rugeria* is present in DZ CORE and associated with disease in *Pocillopora damicornis* (Rosado et al., 2018). *Vibrio* are well known for their association with several coral diseases, including white syndromes and yellow band disease (e.g., Cervino et al., 2008; Ushijima et al., 2014). *V. alginolyticus* has been observed as one of few dominant members of stressed coral microbiomes while several other unidentified *Vibrio* species have been implicated in coral disease (Blackhall, 2013; Castañeda-Chávez et al., 2018; Certner et al., 2015; Gignoux-Wolfsohn & Vollmer, 2015; Hussein et al., 2019; Kemp et al., 2018; Sweet et al., 2017). Our finding of these common disease-associated ASVs does not necessarily indicate their role in disease initiation (i.e., frank pathogens), as suggested in Sweet & Bulling (2017). However, the commonality of

these ASVs across coral species and disease types suggests they could contribute to disease development as opportunistic pathogens and/or secondary invaders, inhibiting coral recovery (Ainsworth et al., 2009; Egan & Gardiner, 2016; Mera & Bourne, 2018; Sweet & Bulling, 2017). The chemical makeup of coral mucus under stress (such as in white band diseased *Acropora* and *Platygyra* corals) could attract these specific bacteria to vulnerable hosts (Ochsenkühn et al., 2018).

The H CORE comprised of nine ASVs. Members of Bacteroidetes and Proteobacteria in H CORE have similar persistence in *Pachyseris speciosa*, *Porites astreoides*, *Siderastrea siderea*, and *Orbicella annularis* (Frade et al., 2016; Hernandez-Agreda et al., 2016; Meyer et al., 2019). Bacteroidetes is particularly ubiquitous in healthy coral and have been observed in greater abundance in non-outbreak coral samples compared to white plague disease coral samples (Beurmann et al., 2018; van Oppen & Blackall, 2019). Cryomorphaceae has been observed in healthy coral samples of *Acropora digitifera*, a member of the same genus as the elkhorn and staghorn corals (*A. palmata* and *A. cervicornis*, respectively) sampled in this study (Bernasconi et al., 2019). However, other studies (e.g., Meyer et al., 2019) have found Cryomorphaceae associated with diseased rather than healthy corals, including coral species sampled in this study. The order Cytophagales has been recently identified as a member of the coral microbiome and shown to be opportunistic pathogens (Reyes et al., 2018; Sweet & Bulling, 2017). Because Cytophagales may be considered conditionally rare taxa for its ability to rapidly respond to environmental changes, increased abundance of these bacteria in coral could suggest the health of the coral host has been compromised (Gignoux-Wolfson & Vollmer, 2015; Shade et al., 2014).

Only approximately 40% of coral-associated ASVs in this dataset were associated with a core community. Our results are supported by studies that have found very low numbers (i.e., less than 15 taxa) bacterial taxa that are consistently present across all species and/or disease types (Ainsworth et al., 2015; Herren & McMahon, 2018; Kellogg, 2019; Meyer et al., 2019; Ziegler et al., 2019). The majority of taxa that were not characterized as H CORE, DZ CORE, or H & DZ CORE (considered as “non-core” in this study), may be species-specific and therefore did not meet the core community analysis criteria of a 50% persistence across the dataset (Hernandez-Agreda et al., 2016; Meyer et al., 2019). It is common that a large portion of the microbiome is dominated by low-abundance bacteria, and that these members identify as highly variable taxa in an exposed mucus layer that are responsive to transient biotic and abiotic environmental conditions (Certner et al., 2015; Hernandez-Agreda et al., 2016; Peixoto et al., 2017; Voolstra & Ziegler, 2020). Non-core members may even alter the coral’s mucus metabolite makeup to promote diversity and functional redundancy which could strengthen coral resilience (Pollock et al., 2019; Rolig et al., 2015; Ochsenkühn et al., 2018).

Grouping bacterial taxa by their association with coral health or disease may prove useful in identifying groups of taxa that may be used as biological indicators of coral holobiont instability, especially if they are consistently found across studies regardless of sample collection locale, timing, species, and/or disease type (Blackall et al., 2020; Glasl et al., 2017; Peixoto et al., 2019; Pootakham et al., 2019; Villela et al., 2019). Alternatively, bacterial taxa associated with coral holobiont stability may be used as marine probiotics to modulate coral microbiomes during unfavorable environmental conditions (Peixoto et al., 2017; Rosado et al., 2019).

### *Microbiome dysbiosis disease hypothesis*

The microbiome dysbiosis disease hypothesis favors identifying broad microbial community patterns that reflect a transition from healthy to dysbiotic microbiome states that lead to disease. In this study, we observed that the mucosal microbiome of healthy corals exhibited similar community composition and structure, regardless of coral species. This supports the results of Hernandez-Agreda et al. (2018) that showed taxonomic redundancy and consistent whole community structure across the microbiomes of three healthy corals. Under favorable conditions animal hosts (including Cnidaria) implement high microbiome selectivity and maintain low Bray-Curtis dissimilarity by actively restricting the diversity of bacterial symbionts in the community (Pearman et al., 2019; Ziegler et al., 2019). Corals with selective microbiomes have depicted beta-diversity similarity and strong community connectivity, or cohesion, between resident bacterial taxa (Herren & McMahon, 2018; Zaneveld et al., 2017; Ziegler et al., 2019). Strong cohesion in a bacterial community may mitigate the effects of disturbance (internal and/or external) that may be triggers for disease (Banerjee et al., 2018; Egan & Gardiner, 2016; Herren & McMahon, 2018; Peixoto et al., 2017).

While our study collected samples within 96 h under stable environmental conditions, we show that when visualized in PCoA plots, healthy mucus samples across ten coral species overlapped more often than diseased samples. Additionally, our healthy samples did not clearly show an altered degree of dispersion, supporting the hypothesis that microbiome connectivity potentially contributes to increased resilience and decreased susceptibility to environmental perturbations (Reshef et al., 2006; Röthig et al., 2020; Sweet et al., 2020; Voolstra & Ziegler 2020; Zaneveld et al., 2017; Ziegler et al., 2019).

However, Hernandez-Agreda et al. (2016) highlight that more research is needed to determine the bacterial associations present in healthy samples and how they might contribute to microbiome stability.

In contrast, our data shows that disease lesions, regardless of species or disease type exhibit higher microbial beta-diversity variability compared to healthy mucus samples. The Anna Karenina principle (AKP) states that biotically and/or abiotically stressed microbiomes exhibit greater beta-diversity dispersion than healthy or control equivalents. The microbial communities of animals become dysbiotic when perturbation is introduced, and this change is can be visualized through increasing inter-individual variation (Zaneveld et al., 2017). The principle has already been observed in corals such as *O. faveolata*, *P. astreoides*, *A. hemprichii*, *M. annularis*, and *O. annularis* when exposed to pollution and eutrophication (Klaus et al., 2007; Roitman, 2018). Additionally, corals competing with the macroalgae *Dictyota* exhibit greater variability of their microbial community (Thurber et al., 2012). Several other studies support the AKP by observing an increased following disturbance (e.g., McDevitt-Irwin et al., 2017, 2019; Rocca et al., 2019; Roitman, 2018; Rosado et al., 2018; Voolstra & Ziegler, 2020; Wang et al., 2018; Zaneveld et al., 2016).

The AKP also explains that increased beta-diversity variability is associated with disrupted microbiomes increasingly drifting apart. Instead of predictably shifting to a dysbiotic state, AKP suggests that disturbed microbial communities are “dysbiotic in their own way.” When visualized in PCoA plots by species-disease combinations, our samples generally clustered by their unique species and disease type combinations with little overlap or similarity in community composition. When visualized in PCoA plots solely by health status (healthy vs. diseased) regardless of species or disease type, we did not observe

distinct clustering of disease lesions and healthy mucus that suggested a predictable transition from healthy to dysbiotic states. These observations support the AKP in that our disease lesion samples had bacterial communities that were uniquely dysbiotic and dissimilar.

Considering the coral innate immune system may explain why perturbed microbiomes become dysbiotic in a stochastic manner. Corals regulate their microbiomes through an innate immune system that produces antimicrobial peptides and contain pattern recognition receptors, mechanisms that are common in hosts with innate immune systems, including all species of the coral model *Hydra* (Bosch, 2014; Eberl, 2010; Palmer, 2018). This may explain why healthy corals (presumably not stressed by other environmental factors) maintain similar microbiomes. Under stress (including biotic stress such as disease) coral-microbe interactions collapse and the coral's ability to regulate its symbionts is compromised (Weis et al., 2008). At this state, opportunistic pathogens and secondary invaders infiltrate the coral (Egan & Gardiner, 2016; Sweet & Bulling, 2017). The regulation of the coral microbial composition may now be directly dependent on the host's species-specific or individual-specific physiological trade-offs in effort to regain homeostasis (Certner et al., 2015; Ochsenkühn et al., 2018; Brown & Bythell, 2005; Glasl et al., 2016; Palmer, 2018; Wright et al., 2017). Additionally, as the coral holobiont loses stability, the composition of bacteria is further diversified within microscale habitats of the host (Hernandez-Agreda et al., 2016). These subsequent and/or simultaneous activities within the holobiont may fuel dysbiosis and explain why disturbed microbiomes are “dysbiotic in their own way” (Zaneveld et al., 2017).

### *Seemingly competing but not mutually exclusive*

Due to the complexity of coral host habitats and coral reef ecosystems, it is essential for researchers to define and understand microbial community dynamics that play a role in disease resilience, particularly in a rapidly changing environment (Bourne et al., 2009; Tracy et al., 2019). Opportunities to adequately compare commonalities across coral diseases and species are confounded by local scale differences (in space or time) that overshadow the effects of disease (Hernandez-Agreda et al., 2016, 2017; Neave et al., 2016; Webster & Reusch, 2017). In this study, our ability to control for temporal and spatial variability and collect a variety of samples from several species and disease types, allowed us to reconcile the pathobiont and the microbiome dysbiosis coral disease hypotheses. We provide evidence that grouping bacterial taxa and identifying microbial community patterns are both likely play a role in the initiation and development of coral disease. Our results associate specific ASVs with diseased states and at the same time identified patterns of stochastic microbial community dysbiosis. The concept of cohesion may help explain our observations. With a loss of cohesion in the holobiont (where increased beta-diversity is associated with the loss of community connectivity), the coral's ability to regulate the composition of bacterial taxa is compromised, possibly opening the door to opportunistic pathogens and secondary infections (Banerjee et al., 2018; Egan & Gardiner, 2016; Herren & McMahon, 2018; Peixoto et al., 2017).

### **Conclusion**

To understand the aetiologies of coral diseases, systematic descriptions at both the gross and cellular scale are required first steps to identifying disease causation. By observing

bacterial community dynamics at a fine-scale (i.e., at the level of specific core taxa) and a grand-scale (i.e., at the level of microbial communities), our results provide evidence that two seemingly competing frameworks for describing coral disease are not mutually exclusive. Future research should investigate the mechanisms that facilitate the convergence of these two frameworks, such as specific host-microbe and/or microbe-microbe interactions. Additionally, research is needed to test if these complementary frameworks uphold in other microhabitats of the coral, such as the tissue layers where essential endosymbiotic *Symbiodinium* spp. reside.

### **Acknowledgements**

This work was supported by NSF Biological Oceanography award OCE 1357423 (EKL and JRW) and NSF Graduate Research Fellowship Program (GRFP) 1842396 (MGB). Coral surface mucus layer and seawater samples were obtained under permit FKNMS-2016-046. We would like to thank Joshua Farmer and Tom Bartlett for boating, diving, and sample collection assistance. Thank you, Dr. Cindy Lewis, for coral disease identification guidance and Keys Marine Laboratory, FL, USA for your hospitality.

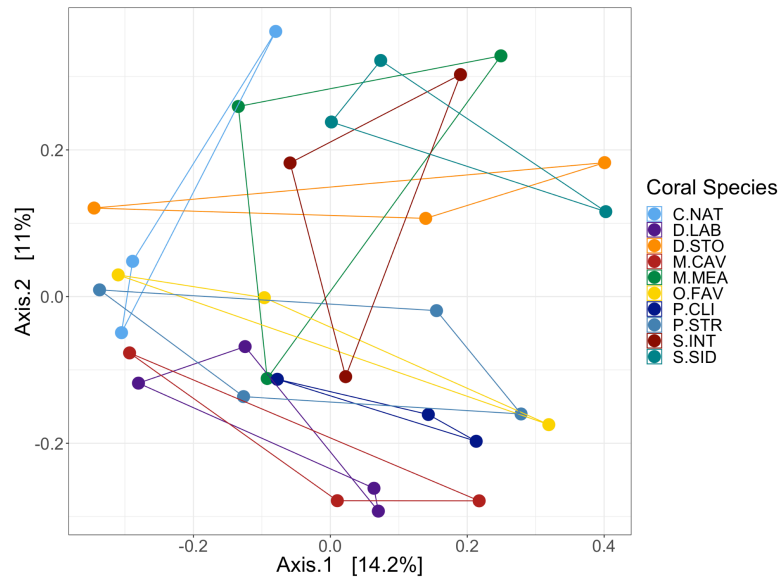
**Table**

**Table 3.1. Sample inventory.** A total of 102 coral surface mucus samples represent ten species and four disease types. Replicates (n) of healthy mucus (status: H) were collected from separate colonies of each coral species. Replicates of disease lesions (status: white plague/WP, yellow band/YB, wasting disease/WD, or dark spots/DS) were collected from separate colonies with the same disease type. Apparently healthy mucus (status: APPH) were collected as matched pairs of diseased colonies with the same disease type but in an area where mucus did not show signs of disease. Correct identification of two colonies (denoted with †) or three colonies (denoted with ‡) not guaranteed due to disease extent, diving conditions, and/or photo resolution.

| Species                                       | Abbreviation | Status  | n              |
|-----------------------------------------------|--------------|---------|----------------|
| <i>Colpophyllia natans</i>                    | C.NAT        | H       | 3              |
| <i>Colpophyllia natans</i>                    | C.NAT        | WP APPH | 2              |
| <i>Dichocoenia stokesi</i>                    | D.STO        | H       | 3              |
| <i>Dichocoenia stokesi</i>                    | D.STO        | WP      | 3              |
| <i>Dichocoenia stokesi</i>                    | D.STO        | WP APPH | 3              |
| <i>Diploria labyrinthiformis</i>              | D.LAB        | H       | 4              |
| <i>Diploria labyrinthiformis</i>              | D.LAB        | WP      | 3              |
| <i>Diploria labyrinthiformis</i>              | D.LAB        | WP APPH | 4              |
| <i>Meandrina meandrites</i>                   | M.MEA        | H       | 3              |
| <i>Meandrina meandrites</i>                   | M.MEA        | WP      | 3              |
| <i>Meandrina meandrites</i>                   | M.MEA        | WP APPH | 3              |
| <i>Montastrea cavernosa</i>                   | M.CAV        | H       | 3              |
| <i>Montastrea cavernosa</i>                   | M.CAV        | YB      | 4              |
| <i>Montastrea cavernosa</i>                   | M.CAV        | YB APPH | 4              |
| <i>Orbicella faveolata</i>                    | O.FAV        | H       | 3              |
| <i>Orbicella faveolata</i>                    | O.FAV        | WP APPH | 1              |
| <i>Orbicella faveolata</i>                    | O.FAV        | YB APPH | 1              |
| <i>Pseudodiploria clivosa</i>                 | P.CLI        | H       | 3              |
| <i>Pseudodiploria clivosa</i>                 | P.CLI        | WP APPH | 1              |
| <i>Pseudodiploria strigosa</i>                | P.STR        | H       | 4              |
| <i>Pseudodiploria strigosa</i>                | P.STR        | WP      | 4              |
| <i>Pseudodiploria strigosa</i>                | P.STR        | WP APPH | 4              |
| <i>Siderastrea siderea</i>                    | S.SID        | H       | 3              |
| <i>Siderastrea siderea</i>                    | S.SID        | DS      | 5              |
| <i>Siderastrea siderea</i>                    | S.SID        | DS APPH | 4              |
| <i>Siderastrea siderea</i>                    | S.SID        | WD      | 3              |
| <i>Siderastrea siderea</i>                    | S.SID        | WD APPH | 3              |
| <i>Siderastrea siderea</i>                    | S.SID        | WP      | 2              |
| <i>Siderastrea siderea</i>                    | S.SID        | WP APPH | 2              |
| <i>Stephanocoenia intersepta</i>              | S.INT        | H       | 3 <sup>†</sup> |
| <i>Stephanocoenia intersepta</i> <sup>†</sup> | S.INT        | DS      | 3 <sup>†</sup> |
| <i>Stephanocoenia intersepta</i> <sup>†</sup> | S.INT        | DS APPH | 2              |
| <i>Stephanocoenia intersepta</i> <sup>†</sup> | S.INT        | WP      | 3 <sup>‡</sup> |
| <i>Stephanocoenia intersepta</i> <sup>†</sup> | S.INT        | WP APPH | 3 <sup>‡</sup> |

## Figures

(A)



(B)

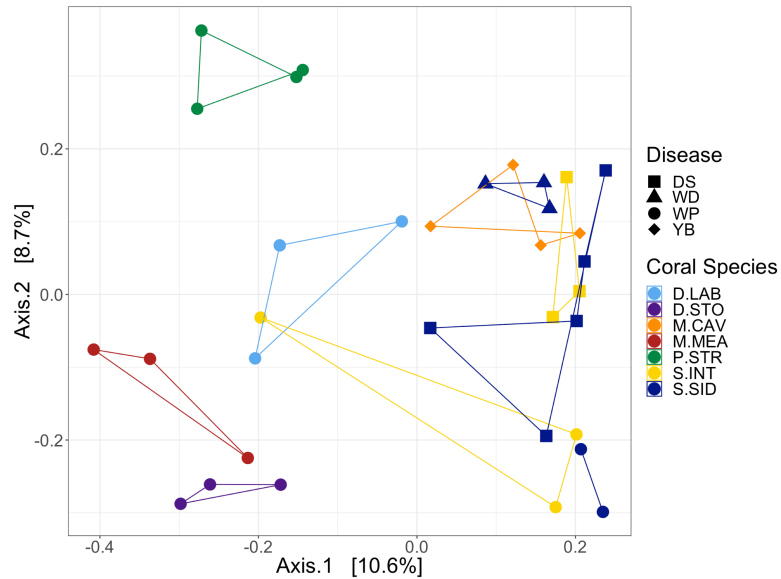
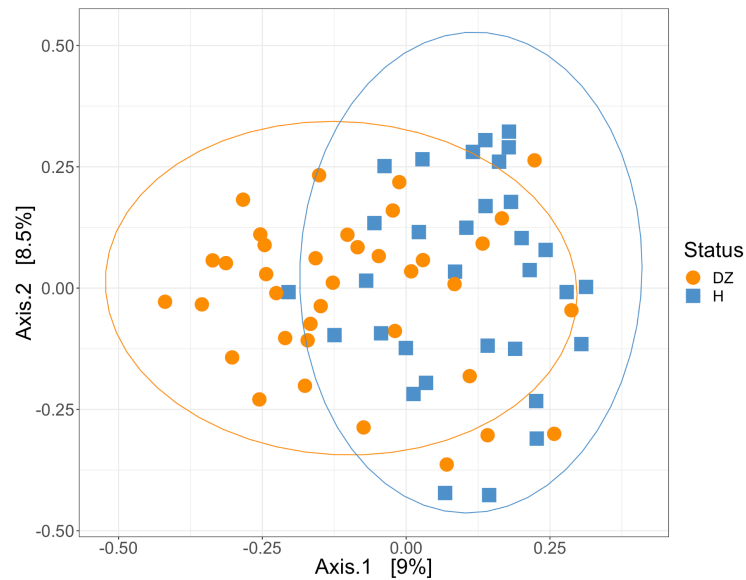


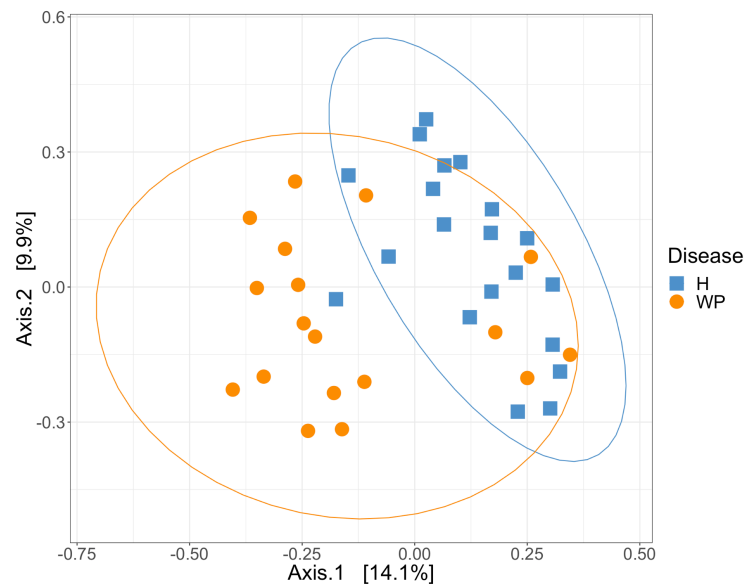
**Figure 3.1. Beta-diversity of microbial communities from healthy mucus and disease lesions by species and disease type.** Principal coordinates analysis (PCoA) plot shows weighted Bray-Curtis dissimilarity. **(A)** Healthy coral mucus microbiomes by species. **(B)** Diseased coral mucus microbiomes by species-disease combinations. C.NAT (*C. natans*), D.LAB (*D. labyrinthiformis*), D.STO (*D. stokesi*), M.CAV (*M. cavernosa*), M.MEA (*M. meandrites*), O.FAV (*O. faveolata*), P.CLI (*P. clivosa*), P.STR (*P. strigosa*), S.INT (*S. intersepta*), S.SID (*S. siderea*), DS (dark spots), WD (wasting disease), WP (white plague), and YB (yellow band).

**Figure 3.2. Beta-diversity of microbial communities of healthy mucus vs. disease lesions.** Principal coordinates analysis (PCoA) plot shows weighted Bray-Curtis dissimilarity. **(A)** All healthy mucus and all disease lesion samples, regardless of species or disease type. **(B)** WP disease lesions, regardless of species, and respective healthy mucus of the same species. **(C)** Combined DS, WD, and YB disease lesions and respective healthy mucus of the same species. H (healthy mucus), DZ (disease lesion), WP (white plague lesions), DS (dark spots lesions), WD (wasting disease lesions), YB (yellow band lesions).

**(A)**



**(B)**



(C)

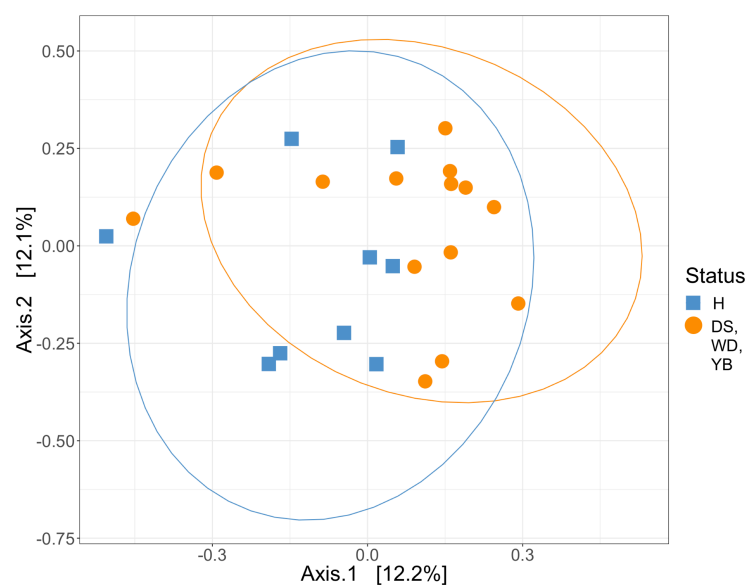
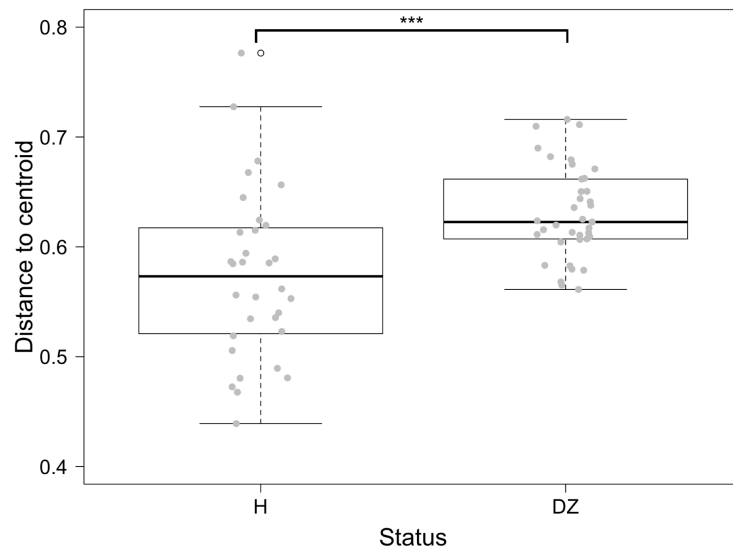
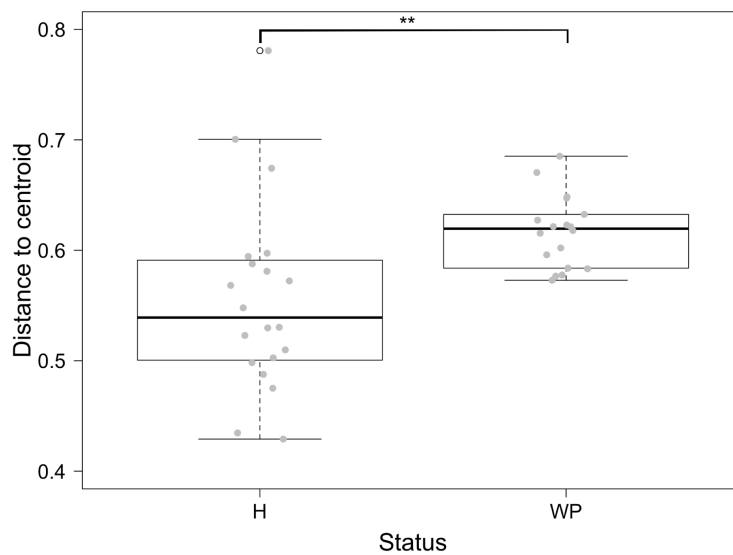


**Figure 3.3. Beta-diversity variability of microbial communities of healthy mucus vs. disease lesions.** Dispersion compared **(A)** between healthy mucus and disease lesions, regardless of species or disease type, **(B)** between WP disease lesions (regardless of species) and respective healthy mucus of the same species, and **(C)** between combined DS, WD, and YB disease lesions and respective healthy samples of the same species. H (all healthy mucus), DZ (all disease lesions), WP (white plague lesions), DS (dark spots lesions), WD (wasting disease lesions), YB (yellow band lesions). Statistical significance: ‘\*\*\*’ p-value<0.001, ‘\*\*’ p-value<0.01, ‘.’ p-value<0.1.

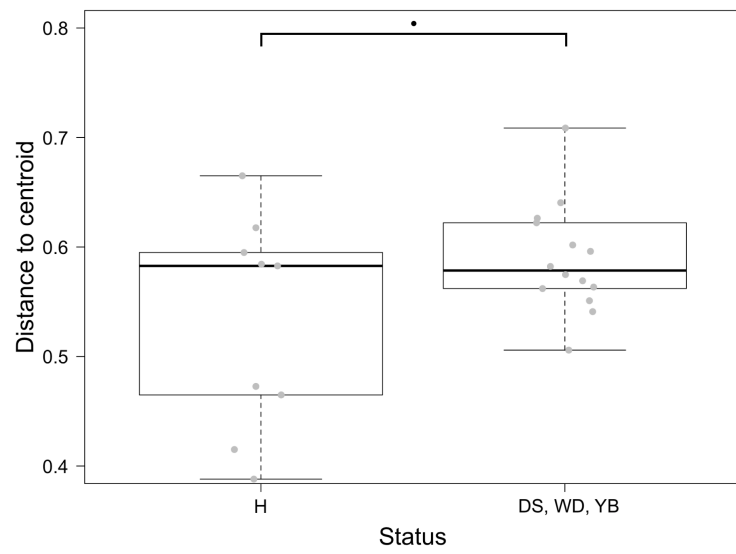
**(A)**



**(B)**



(C)



| DZ CORE<br>(6 ASVs, 1.5%) |                  |  |
|---------------------------|------------------|--|
| FAMILY                    | GENUS            |  |
| Clade_I                   | Clade_Ia         |  |
| Clade_I                   | Clade_Ia         |  |
| Flavobacteriaceae         | NS4_marine_group |  |
| Rhodobacteriaceae         | Ruegeria         |  |
| NS9_marine_group          |                  |  |
| Vibrionaceae              | Vibrio           |  |

| H & DZ CORE<br>(27 ASVs, 34.2%) |                         |  |
|---------------------------------|-------------------------|--|
| FAMILY                          | GENUS                   |  |
| Pirellulaceae                   | NA                      |  |
| Pirellulaceae                   | Rhodopirellula          |  |
| Clade_II                        | NA                      |  |
| SAR116_clade                    | NA                      |  |
| Phycisphaeraceae                | Urania-1B-19            |  |
| AEGEAN-                         | marine_sediment_group   |  |
| 169_marine_group                | NA                      |  |
| Flavobacteriaceae               | NS4_marine_group        |  |
| Flavobacteriaceae               | NS5_marine_group        |  |
| Flavobacteriaceae               | NS5_marine_group        |  |
| Flavobacteriaceae               | NS4_marine_group        |  |
| Flavobacteriaceae               | NS4_marine_group        |  |
| Flavobacteriaceae               | NS2b_marine_group       |  |
| Flavobacteriaceae               | NS5_marine_group        |  |
| Flavobacteriaceae               | NS4_marine_group        |  |
| Rhodobacteriaceae               | HIMB11                  |  |
| Flavobacteriaceae               | NA                      |  |
| Halieaceae                      | OM60(NOR5)_clade        |  |
| Cyanobiaceae                    | Synecococcus CC9902     |  |
| Cyanobiaceae                    | Synecococcus CC9902     |  |
| Cyanobiaceae                    | Synecococcus CC9902     |  |
| Cyanobiaceae                    | Synecococcus CC9902     |  |
| Cyanobiaceae                    | Synecococcus CC9902     |  |
| Cyanobiaceae                    | Synecococcus CC9902     |  |
| Cyanobiaceae                    | Cyanobium_PCC-6307      |  |
| Cyanobiaceae                    | Synecococcus CC9902     |  |
| Cyanobiaceae                    | Prochlorococcus MIT9313 |  |
| Actinomarinaceae                | Candidatus_Actinomarina |  |

| H CORE<br>(9 ASVs, 3.3%) |                  |  |
|--------------------------|------------------|--|
| FAMILY                   | GENUS            |  |
| NS11-12_marine_group     | NA               |  |
| Clade_I                  | Clade_Ia         |  |
| Flavobacteriaceae        | NS5_marine_group |  |
| Flavobacteriaceae        | NS5_marine_group |  |
| Flavobacteriaceae        | NS5_marine_group |  |
| Flavobacteriaceae        | NS5_marine_group |  |
| Cryomorphaceae           | NA               |  |
| NS7_marine_group         | NA               |  |
| Cyclobacteriaceae        | Marinoscillum    |  |

**Figure 3.4. Core community analysis.** A total of 27 core taxa were identified across healthy mucus and disease lesions (H & DZ CORE), nine core taxa for only healthy mucus (H CORE), and six core taxa for only disease lesions (DZ CORE). Percent abundances represent the total number of ASVs within each core community divided by the total of all ASVs considered in the core community analysis. ASVs that did not meet the 50% persistence, and therefore were not assigned as core taxa, included 4,807 ASVs and totaled 61.1% (data not shown).

### Supplementary Tables

**Table S3.1.** Beta-diversity dissimilarity and variability statistics for healthy mucus microbial communities. **(A)** Pairwise dissimilarity p-values by coral species, regardless of disease type. **(B)** Pairwise dispersion p-values by coral species, regardless of disease type. Statistical significance based on p-values<0.05 are bolded. D.LAB (*D. labyrinthiformis*), D.STO (*D. stokesi*), M.CAV (*M. cavernosa*), M.MEA (*M. meandrites*), P.STR (*P. strigosa*), S.INT (*S. intersepta*), S.SID (*S. siderea*).

**(A)**

|       | D.LAB        | D.STO        | M.CAV        | M.MEA        | P.STR        | S.INT        |
|-------|--------------|--------------|--------------|--------------|--------------|--------------|
| D.STO | 0.100        | NA           | NA           | NA           | NA           | NA           |
| M.CAV | <b>0.031</b> | <b>0.032</b> | NA           | NA           | NA           | NA           |
| M.MEA | 0.100        | 0.100        | <b>0.028</b> | NA           | NA           | NA           |
| P.STR | <b>0.037</b> | <b>0.033</b> | 0.037        | <b>0.025</b> | NA           | NA           |
| S.INT | <b>0.014</b> | <b>0.013</b> | <b>0.006</b> | <b>0.014</b> | <b>0.006</b> | NA           |
| S.SID | <b>0.005</b> | <b>0.004</b> | <b>0.004</b> | <b>0.006</b> | <b>0.002</b> | <b>0.013</b> |

**(B)**

|       | D.LAB        | D.STO        | M.CAV | M.MEA        | P.STR        | S.INT |
|-------|--------------|--------------|-------|--------------|--------------|-------|
| D.STO | 0.362        | NA           | NA    | NA           | NA           | NA    |
| M.CAV | 0.341        | 0.119        | NA    | NA           | NA           | NA    |
| M.MEA | 0.416        | 0.107        | 0.774 | NA           | NA           | NA    |
| P.STR | 0.854        | 0.532        | 0.595 | 0.761        | NA           | NA    |
| S.INT | 0.309        | 0.114        | 0.776 | 0.635        | 0.462        | NA    |
| S.SID | <b>0.006</b> | <b>0.001</b> | 0.078 | <b>0.047</b> | <b>0.038</b> | 0.223 |

**Table S3.2.** Beta-diversity dissimilarity and variability statistics for disease lesion microbial communities. **(A)** Pairwise dissimilarity p-values by disease type, regardless of coral species. **(B)** Pairwise dispersion p-values by disease type, regardless of coral species. Statistical significance based on p-values<0.05 are bolded. DS (dark spots), WD (wasting disease), WP (white plague), YB (yellow band).

**(A)**

|           | <b>DS</b>    | <b>WD</b>    | <b>WP</b>    |
|-----------|--------------|--------------|--------------|
| <b>WD</b> | 0.129        | NA           | NA           |
| <b>WP</b> | <b>0.002</b> | <b>0.005</b> | NA           |
| <b>YB</b> | <b>0.017</b> | <b>0.036</b> | <b>0.002</b> |

**(B)**

|           | <b>DS</b>    | <b>WD</b>    | <b>WP</b>    |
|-----------|--------------|--------------|--------------|
| <b>WD</b> | 0.191        | NA           | NA           |
| <b>WP</b> | <b>0.009</b> | <b>0.001</b> | NA           |
| <b>YB</b> | 0.270        | 0.756        | <b>0.001</b> |

**Table S3.3.** Beta-diversity dissimilarity and variability statistics for the microbial communities of samples grouped by species and disease type. **(A)** Pairwise dissimilarity p-values by species-disease type combinations. **(B)** Pairwise dispersion p-values by species-disease type combinations. Statistical significance based on p-values<0.05 are bolded. D.LAB (*D. labyrinthiformis*), D.STO (*D. stokesi*), M.CAV (*M. cavernosa*), M.MEA (*M. meandrites*), P.STR (*P. strigosa*), S.INT (*S. intersepta*), S.SID (*S. siderea*), DS (dark spots), WD (wasting disease), WP (white plague), YB (yellow band).

**(A)**

|             | D.LAB<br>WP  | D.STO<br>WP  | M.CAV<br>YB  | M.MEA<br>WP  | P.STR<br>WP  | S.INT<br>DS  | S.INT<br>WP | S.SID<br>DS | S.SID<br>WD |
|-------------|--------------|--------------|--------------|--------------|--------------|--------------|-------------|-------------|-------------|
| D.STO<br>WP | 0.100        | NA           | NA           | NA           | NA           | NA           | NA          | NA          | NA          |
| M.CAV<br>YB | <b>0.032</b> | <b>0.026</b> | NA           | NA           | NA           | NA           | NA          | NA          | NA          |
| M.MEA<br>WP | 0.100        | 0.100        | <b>0.029</b> | NA           | NA           | NA           | NA          | NA          | NA          |
| P.STR<br>WP | <b>0.028</b> | <b>0.034</b> | <b>0.034</b> | <b>0.029</b> | NA           | NA           | NA          | NA          | NA          |
| S.INT<br>DS | 0.100        | 0.100        | <b>0.032</b> | 0.100        | <b>0.029</b> | NA           | NA          | NA          | NA          |
| S.INT<br>WP | 0.100        | 0.100        | <b>0.028</b> | 0.200        | <b>0.035</b> | 0.100        | NA          | NA          | NA          |
| S.SID<br>DS | <b>0.017</b> | <b>0.016</b> | 0.065        | <b>0.012</b> | <b>0.018</b> | <b>0.027</b> | 0.115       | NA          | NA          |
| S.SID<br>WD | 0.100        | 0.100        | <b>0.027</b> | 0.100        | <b>0.030</b> | 0.100        | 0.100       | 0.357       | NA          |
| S.SID<br>WP | 0.100        | 0.100        | 0.067        | 0.100        | 0.067        | 0.100        | 0.700       | 0.248       | 0.100       |

**(B)**

|             | D.LAB<br>WP | D.STO<br>WP  | M.CAV<br>YB  | M.MEA<br>WP  | P.STR<br>WP | S.INT<br>DS  | S.INT<br>WP | S.SID<br>DS  | S.SID<br>WD |
|-------------|-------------|--------------|--------------|--------------|-------------|--------------|-------------|--------------|-------------|
| D.STO<br>WP | 0.352       | NA           | NA           | NA           | NA          | NA           | NA          | NA           | NA          |
| M.CAV<br>YB | 0.330       | 0.113        | NA           | NA           | NA          | NA           | NA          | NA           | NA          |
| M.MEA<br>WP | 0.430       | 0.111        | 0.784        | NA           | NA          | NA           | NA          | NA           | NA          |
| P.STR<br>WP | 0.867       | 0.484        | 0.574        | 0.752        | NA          | NA           | NA          | NA           | NA          |
| S.INT<br>DS | 0.181       | 0.577        | <b>0.048</b> | <b>0.031</b> | 0.357       | NA           | NA          | NA           | NA          |
| S.INT<br>WP | 0.676       | 0.381        | 0.853        | 0.994        | 0.820       | 0.273        | NA          | NA           | NA          |
| S.SID<br>DS | 0.062       | <b>0.009</b> | 0.369        | 0.193        | 0.206       | <b>0.004</b> | 0.408       | NA           | NA          |
| S.SID<br>WD | 0.560       | 0.236        | 0.723        | 0.939        | 0.803       | 0.114        | 0.968       | 0.246        | NA          |
| S.SID<br>WP | 0.064       | 0.107        | <b>0.035</b> | <b>0.019</b> | 0.202       | <b>0.048</b> | 0.209       | <b>0.003</b> | 0.093       |

**Table S3.4.** Detailed taxonomy of unique ASVs assigned to the core community of only disease lesions regardless of species or disease type (DZ CORE; “DZ”), only healthy mucus regardless of species (H CORE; “H”), and healthy mucus and disease lesions regardless of species or disease type (H & DZ CORE; “HDZ”). Marine group abbreviated as mar. grp.

| Core | Class               | Order              | Family            | Genus                   |
|------|---------------------|--------------------|-------------------|-------------------------|
| DZ   | Bacteroidia         | Flavobacteriales   | Flavobacteriaceae | NS4 mar. grp.           |
| DZ   | Bacteroidia         | Flavobacteriales   | NS9 mar. grp.     | NA                      |
| DZ   | Alphaproteobacteria | Rhodobacterales    | Rhodobacteraceae  | Ruegeria                |
| DZ   | Alphaproteobacteria | SAR11 clade        | Clade I           | Clade Ia                |
| DZ   | Alphaproteobacteria | SAR11 clade        | Clade I           | Clade Ia                |
| DZ   | Gammaproteobacteria | Vibrionales        | Vibrionaceae      | Vibrio                  |
| H    | Bacteroidia         | Cytophagales       | Cyclobacteriaceae | Marinoscillum           |
| H    | Bacteroidia         | Flavobacteriales   | Cryomorphaceae    | NA                      |
| H    | Bacteroidia         | Flavobacteriales   | Flavobacteriaceae | NS5 mar. grp.           |
| H    | Bacteroidia         | Flavobacteriales   | Flavobacteriaceae | NS5 mar. grp.           |
| H    | Bacteroidia         | Flavobacteriales   | Flavobacteriaceae | NS5 mar. grp.           |
| H    | Bacteroidia         | Flavobacteriales   | Flavobacteriaceae | NS5 mar. grp.           |
| H    | Bacteroidia         | Flavobacteriales   | NS7 mar. grp.     | NA                      |
| H    | Bacteroidia         | Sphingobacteriales | NS11-12 mar.grp.  | NA                      |
| H    | Alphaproteobacteria | SAR11 clade        | Clade I           | Clade Ia                |
| HDZ  | Acidimicrobiia      | Actinomarinales    | Actinomarinaceae  | Candidatus Actinomarina |
| HDZ  | Bacteroidia         | Flavobacteriales   | Flavobacteriaceae | NA                      |
| HDZ  | Bacteroidia         | Flavobacteriales   | Flavobacteriaceae | NS2b mar. grp.          |
| HDZ  | Bacteroidia         | Flavobacteriales   | Flavobacteriaceae | NS4 mar. grp.           |
| HDZ  | Bacteroidia         | Flavobacteriales   | Flavobacteriaceae | NS4 mar. grp.           |
| HDZ  | Bacteroidia         | Flavobacteriales   | Flavobacteriaceae | NS4 mar. grp.           |
| HDZ  | Bacteroidia         | Flavobacteriales   | Flavobacteriaceae | NS4 mar. grp.           |
| HDZ  | Bacteroidia         | Flavobacteriales   | Flavobacteriaceae | NS5 mar. grp.           |
| HDZ  | Bacteroidia         | Flavobacteriales   | Flavobacteriaceae | NS5 mar. grp.           |
| HDZ  | Bacteroidia         | Flavobacteriales   | Flavobacteriaceae | NS5 mar. grp.           |
| HDZ  | Oxyphotobacteria    | Synechococcales    | Cyanobiaceae      | Cyanobium PCC-6307      |
| HDZ  | Oxyphotobacteria    | Synechococcales    | Cyanobiaceae      | Prochlorococcus MIT9313 |
| HDZ  | Oxyphotobacteria    | Synechococcales    | Cyanobiaceae      | Synechococcus CC9902    |
| HDZ  | Oxyphotobacteria    | Synechococcales    | Cyanobiaceae      | Synechococcus CC9902    |
| HDZ  | Oxyphotobacteria    | Synechococcales    | Cyanobiaceae      | Synechococcus CC9902    |
| HDZ  | Oxyphotobacteria    | Synechococcales    | Cyanobiaceae      | Synechococcus CC9902    |
| HDZ  | Oxyphotobacteria    | Synechococcales    | Cyanobiaceae      | Synechococcus CC9902    |
| HDZ  | Oxyphotobacteria    | Synechococcales    | Cyanobiaceae      | Synechococcus CC9902    |
| HDZ  | Oxyphotobacteria    | Synechococcales    | Cyanobiaceae      | Synechococcus CC9902    |
| HDZ  | Phycisphaerae       | Phycisphaerales    | Phycisphaeraceae  | Urania-1B-19            |
| HDZ  | Planctomycetacia    | Pirellulales       | Pirellulaceae     | NA                      |
| HDZ  | Planctomycetacia    | Pirellulales       | Pirellulaceae     | Rhodopirellula          |
| HDZ  | Alphaproteobacteria | Puniceispirillales | SAR116 clade      | NA                      |
| HDZ  | Alphaproteobacteria | Rhodobacterales    | Rhodobacteraceae  | HIMB11                  |
| HDZ  | Alphaproteobacteria | Rhodospirillales   | AEGEAN-169        | NA                      |
| HDZ  | Alphaproteobacteria | SAR11 clade        | Clade II          | NA                      |
| HDZ  | Gammaproteobacteria | Cellvibrionales    | Haliaceae         | OM60 (NOR5) clade       |

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## CHAPTER 4

### IMPACTS OF TRANS-ATLANTIC SAHARAN DUST DEPOSITION ON CORAL-ASSOCIATED *VIBRIO* DYNAMICS AND MICROBIAL COMMUNITY STRUCTURE<sup>2</sup>

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<sup>2</sup> Bucher, M. G., Westrich, J. R., Lydon, K. A., Ebling, A. M., Landing, W. M., & Lipp, E. K. To be submitted to *Frontiers in Marine Science*.

## Abstract

Aerosolized particles from episodic dust events provide a natural source of nutrients to the tropical and subtropical North Atlantic Oceans. Studies investigating surface water response to dust deposition suggest that microbial communities are altered from hours to days after deposition. Specifically, the deposition of dust in the Caribbean has been suggested as one driver of *Vibrio* bacterial blooms. *Vibrio* are ubiquitous opportunistic heterotrophs in coastal environments such as coral reefs that support up to one-third of marine life. While *Vibrio* are residents in reef-building coral microbiomes, several species of *Vibrio* are commonly associated with coral disease as direct or secondary pathogens. The extent to which coral-associated *Vibrio* respond to dust deposition is not known; likewise, there is a paucity of knowledge on the effects of dust deposition on coral microbiomes. Here we describe daily shifts in *Vibrio* populations in surface mucus among healthy colonies of *Acropora palmata* (APAL) and *Orbicella faveolata* (OFAV). We also characterize bacterial community composition and structure during the Saharan dust season in the Florida Keys, FL, USA. In both coral species, mucus-associated *Vibrio* increased significantly between days of low and heavy dust influence, shifting from 133 CFU mL<sup>-1</sup> to 380 CFU mL<sup>-1</sup> (APAL) and 72 CFU mL<sup>-1</sup> to 152 CFU mL<sup>-1</sup> (OFAV). Relative abundance of *Vibrio* also increased from 0.02% to 0.052% (APAL) and 0.002% to 0.006% (OFAV) due to dust deposition. *Vibrio* blooms were ephemeral. Additionally, both corals exhibited increased microbiome beta-diversity during days of heavy dust influence. While this study did not observe coral disease, corals that are increasingly compromised by climate change may be exposed to opportunistic infections during temporary blooms in *Vibrio* and/or dust-induced microbiome restructuring.

## Introduction

Desert dust aerosols provide nutrients and essential trace metals to the global ocean via atmospheric deposition (Duarte et al., 2006; Mahowald et al., 2008). The Sahara-Sahel provides nearly one billion metric tons of dust to the atmosphere where dust can persist for several days in transit (Prospero et al., 2010). Dust originating from northern Africa travels along easterly trade winds to the Caribbean, tropical and subtropical Atlantic, and Gulf of Mexico (including along Florida and the Florida Keys) (Boyd & Ellwood, 2010; Mahowald et al., 2009; Trapp et al., 2010). In the Northern Hemisphere, dust events occur as multiple episodic events (3 to 5 d) annually between June and September (Perry et al., 1997; Prospero et al., 2007, 2010).

Deposited Saharan dust is an important source of carbon, nitrogen, and phosphorus (Duarte et al., 2006; Ebling & Landing, 2017; Mahowald et al., 2008). In addition, it is an episodic source of critical trace metals such as iron, which are limiting in marine waters (Lenes et al., 2001). Iron is an essential micronutrient facilitating metabolic processes including aerobic respiration, photosynthesis, and nitrogen fixation (Langlois et al., 2012; Prospero et al., 2010; Strzepek et al., 2005; Sunda et al., 2012; Yong et al., 2014). Therefore, these episodic pulses of deposited dust can affect biological responses in surface waters including in both autotrophic (e.g., Borchardt et al., 2020; Maranen et al., 2010; Sunda et al., 2012) and heterotrophic microbial communities (e.g., Mahowald et al., 2009; Pulido Villena et al., 2014; Westrich et al., 2016, 2018). Changes in surface water microbiomes in the Florida Keys can begin within a matter of hours to days after dust deposition, especially among opportunistic (conditionally rare) heterotrophic bacteria (Borchardt et al., 2020; Mahowald et al., 2009; Pulido Villena et al., 2014; Westrich et al.,

2016, 2018). In particular, taxa from the *Vibrio* genus have been noted to increase in both relative and absolute abundance within a few hours of dust arrival (Westrich et al., 2016). Like other conditionally rare taxa, these bacteria are relatively large, have a wide metabolic repertoire, and typically persist at low concentrations (Grimes et al., 2009; Takemura et al., 2014; Thompson & Polz, 2006; Yooseph et al., 2010). However, with the addition of new substrates and nutrients, they have an extremely fast growth rate giving them a temporary competitive advantage to rapidly increase in abundance (Grimes et al., 2009; Shade et al., 2014; Yooseph et al., 2010; Westrich et al., 2016, 2018). *Vibrio* experience an up to a 30-fold increase in abundance 24 h after Saharan dust deposition in Looe Key Reef, FL, USA (Westrich et al., 2016). *Vibrio* produce and capture siderophores, enhancing their ability to quickly acquire episodically bioavailable iron (Payne et al., 2016).

*Vibrios* are also common members of the coral surface mucus microbial community in low abundances but are more widely known as opportunistic pathogens associated with coral stress and disease (Bourne et al., 2009; Castañeda-Chávez et al., 2018; Egan & Gardiner, 2016; Gomez-Gil et al., 2014; Hugget & Apprill, 2018; Kemp et al., 2018; Sutherland et al., 2016). Stony corals such as *Acropora palmata* and *Orbicella faveolata* are ecosystem engineers that build the three-dimensional habitat of coral reefs (e.g., D'Antonio et al., 2016; Edmunds, 2015; Mera & Bourne, 2018; Peixoto et al., 2017; Plaisance et al., 2011). Due to anthropogenic and natural stressors, including rising sea temperatures, land-based sources of pollution, and rising incidence of disease, have all resulted in severe declines in coral cover and resilience (Aronson, 2006; Aronson & Percht, 2001; Bruckner & Bruckner, 2006; Kuffner & Toth, 2016; Toth et al., 2019; Woodley, 2016). With increasing biotic and abiotic environmental stress, corals become increasingly

susceptible to disease and less resilient to environmental change (Ainsworth et al., 2009; Bass et al., 2019; Glasl et al., 2016; Harvell et al., 1999; Hugget & Apprill, 2019; van Oppen & Blackall, 2019). This raises the question of whether coral reef ecosystems will persist as the global climate continues to alter natural environmental conditions (Bourne et al., 2009; Harvell et al., 1999; Lee et al., 2015; Perry et al., 2013; Rypien et al., 2009; Toth et al., 2015). Research on whether episodic environmental events, particularly dust deposition, could contribute to changes in the coral microbial community is scarce (Bourne et al., 2009; Pearman et al., 2019; Toth et al., 2015).

While dust events in marine ecosystems are not novel, the combined effects of lower coral resilience (i.e., due to temperature) with an environmental event-induced shift in the microbial community could facilitate disease initiation or act as a tipping point for coral health (Egan & Gardiner, 2016; Sweet & Bulling, 2017). Dust events could temporarily alter coral surface communities with direct introduction of novel microbes from atmospheric deposition (Archer et al., 2020). Alternately, heavy deposition events could spur the growth of certain taxa (i.e., *Vibrio*) with the introduction of micro and macro nutrients, similar to that observed in surface water (e.g., Borchardt et al. 2020). *Vibrio* in particular have been associated with both nutrient input from Saharan dust deposition and implicated as either frank or opportunistic pathogens of coral diseases (e.g., Bourne et al., 2009; Egan & Gardiner, 2016; Kemp et al., 2018; Sutherland et al., 2016).

While the effect of dust deposition on seawater-associated bacteria has been studied, the extent to which these events affect the microbial communities of benthic organisms, such as corals, is not known. This study analyzes a 26 d time series of healthy coral mucus sample collections in Looe Key Reef, FL, USA in 2016. We investigate the

response of microbial communities in the surface mucus layer of reef-building *Acropora palmata* and *Orbicella faveolata* in response to Saharan dust events. Specifically, we assess *Vibrio* abundance dynamics in response to dust deposition events.

## Methods

### *Saharan dust event tracking and aerosol analysis*

A global, three-dimensional aerosol mass transport model, Navy Aerosol Analysis and Prediction System (NAAPS; <http://www.nrlmry.navy.mil/aerosol/>), was used to track daily dust-specific aerosol optical thickness (Rubin et al., 2016; Westphal et al., 1987, 1988, 2009). NAAPS is particularly effective at modeling and forecasting dust transport from large deserts through tracking dust aerosol optical thickness (dust AOT), a unitless measure of total dust concentrations over a 1 by 1 degree grid across 24 vertical atmospheric levels ([https://www.nrlmry.navy.mil/aerosol\\_web/Docs/globaler\\_model.html](https://www.nrlmry.navy.mil/aerosol_web/Docs/globaler_model.html)). From 12 July to 06 August 2016, the daily average dust AOT from 18:00 h to 18:00 h UTC was recorded. The source of aerosols was determined using the Hybrid Single-Particle Lagrangian Integrated Trajectory (HYSPLIT) Model based on 14 d backward trajectories of Global Data Assimilation Systems (GDAS) meteorological data (Stein et al. 2015).

### *Field collections*

Coral surface mucus layer samples were collected daily from Looe Key Reef in the Florida Keys National Marine Sanctuary (FKNMS), FL, USA (24.545, -81.409) from 12 July to 06 August 2016 (26 d sampling period). Due to unfavorable weather conditions, no samples were collected on 17-20 July or 02 August 2016. Prior to coral mucus sampling, 15 colonies

of *Acropora palmata* and 15 of *Orbicella faveolata* were identified and tagged using yellow, numbered cattle ID ear tags. Only corals that showed no signs of disease were included. Coral colonies were haphazardly selected for daily mucus sample collection. For each coral species, three out of 15 tagged colonies were sampled to avoid confounding factors that may be caused by consecutive daily sampling of the same colony.

Following the protocol described by Kemp et al. (2015), coral surface mucus layers were collected with minimal agitation using sterile, needleless 12 mL syringes (Monoject by Covidien, Dublin, Ireland) and transferred to sterile 15 mL centrifuge tubes (Thermo Fisher Scientific, MA, USA) on the boat immediately after collection. Within 5 min of transfer, 100  $\mu$ L aliquots of sample were spread in triplicate onto *Vibrio*-selective agar plates (thiosulfate-citrate-bile-salts-sucrose, TCBS; Oxoid, Thermo Fisher Scientific, MA, USA) and stored in the shade for ~ 2 h until return to Mote Marine Laboratory's Elizabeth Moore International Center for Coral Reef Research & Restoration (IC2R3; Summerland Key, FL, USA) for overnight incubation. When weather conditions were favorable to prepare samples for flow cytometry on-board immediately following collection, 4 mL aliquots of coral mucus were transferred into 5 mL cryovials (Thermo Fisher Scientific, MA, USA) within 20 min of sample collection, fixed with formaldehyde (4% v/v final concentration) in triplicates, and stored in a cooler filled with ice with any remaining mucus sample volume until return to IC2R3 (Bettarel et al., 2016). Surface water samples were collected concurrently for microbial and chemical analyses, and these data are reported in Borchardt et al. (2020).

Within 2-3 h of collection, all samples were transported back to IC2R3. TCBS plates were placed inside a 30°C incubator and fixed mucus samples were stored at -80°C

until processing. Within 1 h of return to the IC2R3, remaining mucus sample volumes that were stored on ice were aliquoted (2 mL per sample in triplicates) into sterile 2 mL microcentrifuge tubes (Thermo Fisher Scientific, MA, USA). Samples were centrifuged at 20,000 x g for 20 min at 4°C. Supernatant fluid was pipetted, removed, and discarded. The remaining dried pellets were stored at -80°C until DNA extraction.

### *Vibrio analyses*

Within 24 h of incubation, TCBS plates were removed from the incubator, and all yellow and green colonies were counted and reported as colony forming units (CFU mL<sup>-1</sup>). Total culturable *Vibrio* concentrations were determined for *A. palmata* and *O. faveolata* surface mucus samples (APAL and OFAV, respectively) based on the average of three technical replicates per sample. Based on 16S rRNA gene amplicon sequence data (described under *Community sequence processing and analysis*), the average relative abundance of bacterial taxa identified as members of the *Vibrio* genus level was determined for each APAL and OFAV sample. The distributions of APAL-associated and OFAV-associated total culturable *Vibrio*, APAL-associated and OFAV-associated *Vibrio* relative abundances, and dust AOT (recorded every 6 h) were tested for normality using the Shapiro-Wilk test for normality (Shapiro et al., 1972). APAL total culturable *Vibrio*, APAL *Vibrio* relative abundance, OFAV total cultural *Vibrio*, OFAV *Vibrio* relative abundance, and dust AOT data were not normally distributed (all p-values<0.0001). APAL total culturable *Vibrio* and OFAV total cultural *Vibrio* were log transformed. APAL log transformed total culturable *Vibrio* data were still not normally distributed (p-value<0.0001), but OFAV log transformed total culturable *Vibrio* data were normally distributed (p-value=0.39).

A Spearman's rank correlation test was performed using the `cor.test()` function to determine the association between dust AOT (lagged forward 24 h due to dust deposition delay; Borchardt et al., 2020) and each of the following datasets: APAL log transformed total culturable *Vibrio*, APAL *Vibrio* relative abundance, and OFAV *Vibrio* relative abundance (Hollander & Wolfe, 1973; R Development Core Team, 2018). In addition, the Kruskal-Wallis rank sum test for non-parametric data was performed to compare dust influence (heavy- vs. low-influence) to the following data: APAL log transformed total culturable *Vibrio*, APAL *Vibrio* relative abundance, and OFAV *Vibrio* relative abundance (Breslow, 1970; R Core Development Team, 2018). To determine the association between OFAV log transformed total culturable *Vibrio* and dust AOT, a Spearman's rank correlation test was performed since dust AOT was not normally distributed (Hollander & Wolfe, 1973; R Development Core Team, 2018). In addition, an analysis of variance (ANOVA) for parametric data was used to compare OFAV log transformed total culturable *Vibrio* by dust influence (heavy- vs. low-influence) (Chambers et al., 1992). APAL-associated and OFAV-associated log transformed total culturable *Vibrio* were plotted with dust AOT measures (recorded every 6 h) using the `ggplot2` package in R Studio (R version 3.5.0; R Development Core Team, 2018; Wickham, 2016). For both APAL and OFAV, daily *Vibrio* relative abundances were also plotted with dust AOT measures (recorded every 6 h).

#### *Flow cytometry analysis for total bacterial biomass*

Fixed and stored samples from *A. palmata* and *O. faveolata* mucus were thawed and stained with SYTO62, a red-fluorescent dye (Invitrogen, Carlsbad, CA, USA; Ex/Em: 649/680 nm), following coral-specific flow cytometry methods described in Bettarel et al. (2016).

Briefly, 2  $\mu$ L of 10  $\mu$ M SYTO62 stock was mixed with 998  $\mu$ L of mucus sample and incubated for 3 min before cell quantification. Samples were analyzed at the University of Georgia Center for Tropical and Emerging Diseases (CTEGD) Cytometry Shared Resource Laboratory with a CytoFLEX flow cytometer (Beckman Coulter, CA, USA). A range of 630 to 660 nm was set to eliminate background noise, and cells were counted for 1 min for *O. faveolata* samples and 3 min for *A. palmata* based on preliminary cell count data.

The bacterial cell concentrations (cells mL<sup>-1</sup>) for each coral species was tested for normality using the Shapiro-Wilk test (Shapiro et al., 1972). APAL bacterial cell concentrations were normally distributed (p-value=0.36), and dust AOT values associated with APAL-specific sampling dates (see *Total bacterial concentration*) were also normally distributed (p-value=0.12). Therefore, the association between APAL bacterial biomass and dust AOT were determined using Pearson's correlation coefficient using the `cor.test()` function in R (Hollander & Wolfe, 1973; R Development Core Team, 2018). APAL bacterial cell concentrations were compared between heavy- and low-influence dates using analysis of variance (ANOVA) for parametric data (Chambers et al., 1992).

OFAV bacterial cell concentrations were not normally distributed (p-value=0.002), and dust AOT values for OFAV-specific sampling dates (see *Total bacterial concentration*) were normally distributed (p-value=0.19). Because OFAV data distribution was not normal, the association between OFAV bacterial cell concentrations and dust AOT was determined using Spearman's rank correlation coefficient using the `cor.test()` function in R (Hollander & Wolfe, 1973; R Development Core Team, 2018). OFAV bacterial biomass was compared between heavy- and low-influence dates using Kruskal-Wallis rank sum test for non-parametric data (Breslow, 1970; R Core Development Team, 2018).

### *DNA extraction, library preparation and sequencing*

DNA was extracted from dried and frozen pellets using the QIAGEN DNeasy PowerSoil Kit (Cat No./ID: 12888, QIAGEN, Inc., Germantown, MD, USA). PowerBeads and extraction buffer were transferred into 2 mL microcentrifuge tubes containing dried pellets and gently mixed by vortex before proceeding to the second step of the PowerSoil kit protocol. After following the PowerSoil kit protocol, extracted DNA was quantified using a NanoDrop 1000 (Thermo Scientific, DE, USA). Due to low total DNA concentrations, samples were not diluted prior to amplification. The V4 hypervariable region of the 16S rRNA gene was amplified via polymerase chain reaction (PCR) (Caporaso et al., 2011). Each 10  $\mu$ L reaction contained 2  $\mu$ L of 1x Q5 Buffer (New England BioLabs (NEB), Ipswich, MA, USA), 0.5  $\mu$ L of 10  $\mu$ M 515F primer (V4 hypervariable region; Caporaso et al., 2011), 0.5  $\mu$ L of 10  $\mu$ M 806R primer (V4 hypervariable region; Caporaso et al., 2011), 0.2  $\mu$ L of 10 mM dNTP mix (NEB, Ipswich, MA, USA), 0.1  $\mu$ L of Q5 Hot Start High-Fidelity DNA polymerase (NEB, Ipswich, MA, USA), 4.7  $\mu$ L of PCR-grade molecular water, and 2  $\mu$ L of extracted DNA. Two technical replicates per biological sample were prepared to account for PCR variability. PCR conditions were 98°C for 30 sec for initial denaturation, 25 cycles of 10 sec at 98°C, 30 sec at 52°C, 30 sec at 72°C, and a final extension step lasting 2 min at 72°C. In a second reaction, samples were barcoded using custom barcode sequences (Tinker & Ottesen, 2016). Technical replicates were combined before a second round of amplification. Each 30  $\mu$ L reaction contained 11.1  $\mu$ L of PCR-grade molecular water, 6.0  $\mu$ L of 5x Q5 Buffer (NEB, Ipswich, MA, USA), 0.6  $\mu$ L of 10 mM dNTP mix (NEB, Ipswich, MA, USA), 0.3  $\mu$ L of Q5 Hot Start High-Fidelity DNA polymerase (NEB, Ipswich, MA, USA), 1.5  $\mu$ L of 10  $\mu$ M custom forward barcoded

adaptors, 1.5 µL of 10 µM custom reverse barcoded adaptors, and 9 µL of reaction 1 pooled PCR technical replicate product. PCR conditions were 30 sec at 98°C, 4 cycles of 10 sec at 98°C, 30 sec at 52°C, 30 sec at 72°C, followed by 6 cycles of 10 sec at 98°C, 1 min at 72°C, followed by a final extension step at 72°C for 2 min.

Amplified and barcoded PCR products were diluted to 10 nM in 25 µL of 10 mM Tris HCl and sent to the Georgia Genomics and Bioinformatics Center (GGBC, GA, USA). Samples were tested for quality using a Fragment Analyzer (Advanced Analytical Technologies/Agilent Technologies, IA, USA) and pooled in equimolar concentrations. Pooled samples were sequenced using V2 chemistry on an Illumina MiSeq to obtain PE250 reads (Illumina, Inc., CA, USA).

#### *Community sequence processing and analysis*

16S rRNA gene amplicon sequence processing and analysis was conducted in R Studio (R version 3.5.0; R Development Core Team, 2018). The DADA2 package (version 1.8.0) and tutorial documentation (version 1.8) was used to produce amplicon sequence variants (ASVs), a higher-resolution analogue of operational taxonomic units (OTUs) (Callahan et al., 2016, 2017). Sequences were trimmed and filtered in DADA2 using standard default parameters. A parametric error model was used to calculate error rates using a minimum of  $5 \times 10^8$  total bases (nbases=5e8). Multithreading was enabled (multithread=TRUE) to allow multiple error rate estimations to be tested simultaneously until convergence. Samples were dereplicated to produce unique sequences with a corresponding absolute abundance. While DADA2 does not throw away singletons, it does not infer ASVs based on a single read by default. Therefore, true sequence variants were inferred using a core

sample inference algorithm with the option to pool samples (pool=TRUE) to resolve low-abundance, rare amplicon sequence variants including singletons within samples that are present in more than a single sample. Paired ends were then aligned using the denoised forward and reverse reads and merged to construct a contig sequence that overlapped identically by at least 12 bases. An ASV table was created and chimeric sequences were removed using default DADA2 parameters. Taxonomy was assigned with a native implementation of naïve Bayesian classifier using the RDP DADA2 training set (version 16) with species level assignments based on exact matching (Cole et al., 2014). The Greengenes database was not used for taxonomy assignment given it has been shown to mis-assign taxa associated with *Vibrio* and Vibrionales (Lydon & Lipp, 2018). The ASV table and taxonomic assignments were imported into the phyloseq package in R, and taxa that identified as Chloroplast or Mitochondria were removed (McMurdie & Holmes, 2013).

Alpha-diversity measures were calculated using the estimate\_richness() function in phyloseq using raw abundance of bacterial taxa (McMurdie & Holmes, 2013). Observed (richness) and Chao1 (richness and evenness) measures were calculated. Alpha-diversity measures were tested for normality using the Shapiro-Wilk test and determined to follow a normal distribution (Observed p-value=0.37; Chao1 p-value=0.12) (Shapiro et al., 1972). Alpha-diversity was compared between coral species and determined to statistically vary between APAL and OFAV. Because diversity varied by coral species, additional analysis to determine differences between heavy-influence and low-influenced dust days were conducted separately for each coral species. Diversity estimates were compared between categories and between consecutive days using analysis of variance (ANOVA) followed

by pairwise Tukey's Honestly Significant Difference (Tukey-HSD) test accounting for multiple comparisons (Abdi & Williams, 2010; Gelman, 2005).

Beta-diversity was measured using weighted Bray-Curtis dissimilarity and analyzed by species, by dust influence, and between consecutive days using a permutational multivariate analysis of variance (PERMANOVA) using the `adonis()` function followed by a pairwise PERMANOVA test using the `pairwise.perm.manova()` function in the `RVAideMemoire` package (Anderson et al., 2014; Hervé, 2018). Principal coordinate analysis (PCoA) based on weighted Bray-Curtis dissimilarity and non-metric multidimensional scaling analysis (NMDS) based on unweighted Bray-Curtis dissimilarity were used to visualize beta-diversity of raw abundance of taxa using the `ordinate()` and `plot_ordination()` functions in the `phyloseq` package (McMurdie & Holmes, 2013). Beta-diversity variability was also measured by dispersion (the distance between a sample's median dissimilarity across replicates and the grouping variable's centroid, or median dissimilarity between samples categorized together). Beta-variability was compared by species, by dust influence, and between consecutive days. Dispersion was analyzed the PERMDISP2 procedure by testing the homogeneity of multivariate dispersions using the `betadisp()` function from the `vegan` package (Anderson, 2006a; Anderson, 2006b; Oksanen et al., 2018). A separate permutation test for pairwise multivariate group dispersions was conducted with the `permutest.betadisp()` function from the `vegan` package (Oksanen et al., 2018). Beta-diversity dispersions were plotted using the `boxplot()` function (R Core Development Team, 2018). Relative abundances of ASVs per sample were calculated using the `phyloseq` package, and microbial community composition bar plots were generated using the `ggplot2` package (McMurdie & Holmes, 2013; Wickham, 2016).

To examine the 26 d time series of bacterial abundances from coral surface mucus samples in response to Saharan dust influence, the web application “Temporal Insights into Microbial Ecology (TIME)” was used (Baksi et al., 2018; <https://web.rniapps.net/time>). A taxonomy hierarchy tab separated file and a metadata file for each coral species was prepared using *A. palmata* and *O. faveolata* phyloseq data according to instructions in the TIME user manual. The rarefaction depth was set to 10,000 reads, and sequencing data was normalized using relative abundance. First, TIME workflow 4 was used to identify significant variations in the relative abundance of bacterial orders between two extended time ranges, for example a series of dates experiencing heavy-influence and a series of dates experiencing low-influence from dust deposition (Baksi et al., 2018). Next, TIME workflow 1 was used to identify abundance-based variations in bacterial orders over time. Taxa were identified as “core” (present at all time points and all samples), “persistent” (present across extended periods of time, but not all), or “transient” (appearing and disappearing frequently over time). Default parameters were used to identify core, persistent, and transient taxa. Core taxa are defined as present in 100% of samples (all time points), persistent taxa are defined as present at >20% prevalence across an extended period of time and observed consecutively across 90% of samples, and transient taxa are identified as prevalent in >60% of time points and observed consecutively across 75% of samples.

## **Results**

### *Environmental conditions and sample collections*

Dust aerosol optical thickness (dust AOT) was obtained from NAAPS every 6 h over a 26 d time series. Over the time series, dust AOT averaged 0.0745 ( $\pm$  0.036 SD; range: 0.02 to

0.17). Daily dust AOT was calculated as the average dust AOT levels over 24 h (18:00 to 18:00 h UTC) as described in Borchardt et al. (2020) (**Table 4.1**). Days were categorized as heavily influenced by dust deposition (samples categorized as heavy-influence) if dust AOT was greater than 0.075 (Seim et al., in prep) (**Table 4.1**). Samples collected on days when dust AOT was less than 0.075 were categorized as low-influence. Samples were not collected on 17-20 July 2016 or 02 August 2016 due to adverse weather conditions. NOAA HYSPLIT Model backward trajectories data confirmed that during the 26 d sampling period, 19 d followed expected trajectories of air masses from western Africa into the lower Florida Keys, FL, USA (e.g., **Figure S4.1**), indicating that in addition to the 13 d defined as heavy-influence days, most days had some level of dust intrusion. A total of 63 *A. palmata* (APAL) and 62 *O. faveolata* (OFAV) surface mucus samples were collected and utilized for quantification of total cultural *Vibrio* during the 26 d time series (**Table 4.1**).

#### *Vibrio dynamics*

Total culturable *Vibrio* did not statistically vary between *A. palmata* and *O. faveolata*. APAL-associated total culturable *Vibrio* averaged 261 ( $\pm$  851 SD; range: 3 to 6270) CFU mL<sup>-1</sup>, and OFAV-associated total culturable *Vibrio* averaged 115 ( $\pm$  169 SD; range: 10 to 1220) CFU mL<sup>-1</sup>. Over the 26 d time series, dust AOT was significantly associated with concentrations of both total culturable *Vibrio* in APAL mucus samples (Spearman's  $p$ -value=0.046;  $\rho$ =0.25) and total culturable *Vibrio* in OFAV mucus samples (Spearman's  $p$ -value=0.001;  $\rho$ =0.398) (**Figure 4.1**).

APAL and OFAV total culturable *Vibrio* concentrations were significantly higher in heavy-influence dust days compared to low-influence days (data not shown; APAL  $p$ -

value=0.03; OFAV p-value=0.002). APAL total cultural *Vibrio* averaged 378 ( $\pm$  1121 SD; range: 23 to 6270) CFU mL<sup>-1</sup> on heavy-influence days and 133 ( $\pm$  357 SD; range: 3 to 2007; n=30) CFU mL<sup>-1</sup> on low-influence days. OFAV total cultural *Vibrio* averaged 152 ( $\pm$  211 SD; range: 100 to 1220) CFU mL<sup>-1</sup> on heavy-influence days and 72 ( $\pm$  86 SD; range: 53 to 480) CFU mL<sup>-1</sup> on low-influence days.

*Vibrio* relative abundances did not statistically vary between *A. palmata* and *O. faveolata*. The relative abundances of ASVs identified as *Vibrio* at the genus level in each mucus sample were averaged for each coral replicate prior to analysis. APAL-associated *Vibrio* relative abundances averaged 3.77% ( $\pm$  6.81% SD; range: 0.10 to 37.37%), and OFAV-associated *Vibrio* relative abundances averaged 0.42% ( $\pm$  0.93% SD; range: 0 to 6.23%). Dust AOT was significantly correlated with APAL-associated *Vibrio* relative abundances (Spearman's p-value=0.0186;  $\rho$ =0.301) but not OFAV-associated *Vibrio* relative abundances (**Figure 4.1**). Relative abundances of APAL-associated *Vibrio*, but not OFAV-associated *Vibrio*, were greater during heavy-influence days compared to low-influence days (data not shown; p-value=0.047). *Vibrio* relative abundances in APAL mucus averaged 5.25% ( $\pm$  8.60% SD; range: 0.010 to 37.37%) on heavy-influence days and 2.02% ( $\pm$  3.08% SD; range: 0.26 to 15.24%) on low-influence days. *Vibrio* relative abundance in OFAV mucus averaged 0.58 % ( $\pm$  1.20 % SD; range: 0.007 to 6.23%) on heavy-influence days and 0.25% ( $\pm$  0.49% SD; range: 0 to 2.84%) on low-influence days.

#### *Total bacterial concentration*

Mucus samples from *A. palmata* (n=21) and *O. faveolata* (n=26) were fixed for flow cytometry analysis on 13, 21, 24-26, and 28-29 July 2016. Additional *A. palmata* samples

were fixed on 03-04 August 2016, and additional *O. faveolata* samples on 30 July and 01 August 2016. Bacterial concentrations for APAL averaged  $9.10 \times 10^6$  ( $\pm 5.92 \times 10^6$  SD; range:  $0.25 \times 10^6$  to  $19.1 \times 10^6$ ) cells mL<sup>-1</sup>, and for OFAV, bacterial concentrations averaged  $1.51 \times 10^7$  ( $\pm 1.07 \times 10^7$  SD; range:  $0.15 \times 10^7$  to  $4.71 \times 10^7$ ) cells mL<sup>-1</sup>.

APAL bacterial concentrations did not correlate with dust AOT, but bacterial biomass was lower in heavy-influence days (n=13;  $6.56 \times 10^6 \pm 4.58 \times 10^6$  cells mL<sup>-1</sup>) compared to low-influence days (n=8;  $13.22 \times 10^6 \pm 5.71 \times 10^6$  cells mL<sup>-1</sup>) (p-value=0.008; **Figure 4.2**). OFAV bacterial concentrations were significantly correlated with dust AOT (Spearman's p-value=0.0007;  $\rho=0.62$ ), and bacterial concentrations were higher during heavy-influence dust days (n=17;  $1.81 \times 10^7 \pm 1.19 \times 10^7$  cells mL<sup>-1</sup>) compared to low-influence days (n=9;  $0.94 \times 10^7 \pm 0.47 \times 10^7$  cells mL<sup>-1</sup>) (p-value=0.038; **Figure 4.2**).

#### *Coral surface mucus layer microbial community*

After quality filtering via DADA2, the average number of reads per sample was 26,151 for *A. palmata* (range: 4,929 to 93,541) and 30,414 for *O. faveolata* samples (range: 6,464 to 62,372; **Table 4.2**). On average, 57% of reads from individual *A. palmata* samples and 68% of reads from individual *O. faveolata* samples were retained after quality filtering. A total 11,782 ASVs were represented in the final dataset across all samples and are summarized by dust influence and by species across dates in **Figure S4.2**. Resulting sequences were submitted to NCBI and can be found under project PRJNA521399.

Alpha-diversity measures (Observed and Chao1; data not shown) for *A. palmata* (n=61) averaged 579 ( $\pm 177$  SD; range: 161 to 1,102) and 691 ( $\pm 204$  SD; range: 232 to 1260), respectively. Alpha-diversity measures (Observed and Chao1) for *O. faveolata*

(n=65) averaged 458 ( $\pm$  132 SD; range: 155 to 775) and 524 ( $\pm$  152 SD; range: 209 to 922), respectively. Observed and Chao1 alpha-diversity measures were significantly greater in *A. palmata* compared to *O. faveolata* (Observed and Chao1 p-values<0.0001). However, Observed and Chao1 measures for either species did not vary between heavy-influence dust days compared to low-influence days.

Across the time series, beta-diversity dissimilarity was significantly different between *A. palmata* and *O. faveolata* (p-value=0.001) resulting in clustering by coral species (**Figure 4.3**). For both species, beta-diversity did not change between consecutive dates. Beta-diversity differences between heavy-influence and low-influence days showed distinct clustering within each species (APAL: p-value=0.006; OFAV: p-value=0.026; **Figure 4.4**). *A. palmata* beta-diversity variability (dispersion) was significantly greater during heavy-influence days (n=33; average=0.45) compared to low-influence days (n=28; average=0.39; p-value=0.033). *O. faveolata* dispersions did not vary between heavy-influence and low-influence days. For both species, beta-diversity variability did not change between consecutive dates.

TIME workflow 4 analyses revealed eight bacterial taxa in *A. palmata* mucus and ten bacterial taxa in *O. faveolata* mucus that significantly increased or decreased based on dust AOT variability (**Table 4.3**). Ten taxa across both coral species increased significantly in low-influence dust days while eight taxa across both coral species increased significantly in heavy-influence days. Desulfobacterales and Bacteroidales showed increased abundance during low-influence days in both species. Rhodocyclales, SAR11, and Acholeplasmatales showed increased abundance during heavy-influence days in both species. In addition, TIME workflow 1 analyses revealed four core orders (Chromatiales, Desulfovibrionales,

Cyanobacteria Family\_IV, and Hydrogenophilales) unique to *A. palmata* and five core orders (Campylobacterales, Chlamydiales, Enterobacteriales, Erysipelotrichales, and Fusobacteriales) unique to *O. faveolata* (**Table 4.4**). Twelve orders were identified as transient (disappearing and appearing over time based on heavy- vs. low-influence days), suggesting that their abundance may be correlated with dust AOT variability.

## Discussion

Aerosolized particles from the Saharan-Sahel desert provide a natural source of nutrients and trace metals to the tropical and subtropical North Atlantics through episodic dust deposition events. The deposition of dust aerosols in the Caribbean has been shown as one driver of *Vibrio* bloom formation (Westrich et al., 2016). *Vibrio* are heterotrophic bacteria ubiquitous in the marine environment and commonly associated with coral stress and disease in Caribbean reef-building corals. Borchardt et al. (2020) (a concurrent study) observed successional patterns in the surface water bacterial community in response to Saharan dust deposition (and association with temporal variability of dissolved and particulate iron concentrations). However, the extent to which these responses affect the bacterial communities associated with benthic organisms is not well understood. Here we show the extent to which coral-associated *Vibrio* respond to dust deposition and the patterns of coral-associated bacterial microbiomes in response to episodic nutrient influx.

During a 26 d time series in the Florida Keys, FL, USA, we collected samples during heavy-influenced dust days and days when dust deposition was low. The concentration of dust AOT throughout the time series was variable, and the majority of heavy-influence dust days happened late in the time series beginning on 26 July 2016. This

pattern may be explained by an active tropical wave that formed along the coast of west Africa on 26 July 2016 and later became Hurricane Earl on 2 August, landing on Belize on 4 August (Ramos, 2016; Rivera-Acevedo, 2016).

We observed significant changes in coral-associated *Vibrio* concentrations associated with dust deposition. When dates were grouped by dust influence (low vs. heavy), total culturable *Vibrio* were significantly higher on heavy-influence days compared to low-influence days for both *A. palmata* and *O. faveolata* bacterial microbiomes. The relative abundance of *Vibrio* in *A. palmata* was also statistically higher on heavy-influence days. As conditionally rare taxa (CRT), *Vibrio* may outcompete other heterotrophs in the coral bacterial community and bloom in surface mucus when dust deposition is significant in the water column (Borchardt et al., 2020; Shade et al., 2014; Westrich et al., 2016, 2018).

*Vibrio* are iron scavengers with diverse metabolic capabilities that contribute to rapid iron uptake (Grimes et al., 2009; Payne et al., 2016; Yooseph et al., 2010; Westrich et al., 2016, 2018). Concentrations of *Vibrio* reported as total culturable *Vibrio* and *Vibrio* relative abundance for both coral species were statistically correlated with dust AOT. Hunter & Cervone (2017) used remote sensing and in-situ geophysical data to analyze host-environment interactions in the Caribbean. The analysis exposed statistical significance African dust's impact on the prevalence of coral disease in the Caribbean. Additionally, the correlation between dust deposition and coral disease was influenced by other environmental parameters, especially sea surface temperature (Hunter & Cervone, 2017). Kemp et al. (2018) recorded trends in *Vibrio* abundance in *A. palmata* corals in the Florida Keys during a significant dust event on 25 July 2013. The study observed an increase of *Vibrio* in apparently healthy mucus of otherwise diseased colonies at an abundance of

roughly 25% of the microbial community (nearly equating levels observed in white pox diseased mucus). In addition, short-term nutritional enrichments of mucus released from bleaching corals suggested heterotrophic microbial growth (Wright et al., 2019).

Beta-diversity measures between heavy-influence and low-influence days was statistically significant for both *A. palmata* and *O. faveolata*, suggesting that dust-associated *Vibrio* blooms that may affect the connectivity between coral-associated taxa and perhaps decrease coral microbiome stability (Herren & McMahon, 2018). For already compromised coral hosts influenced by simultaneous biotic and abiotic stressors, nutrient enrichment via dust deposition followed by non-specific heterotrophic blooms in coral mucus is likely to contribute to coral microbiome dysbiosis and the development of disease (Bourne et al., 2009; Egan & Gardiner, 2016; Hernandez-Agreda et al., 2016; Kim, 2016; Kemp et al., 2018). Environmentally induced bacterial blooms might promote horizontal gene transfer and/or the activation of virulence genes within the SML that affects the coral host's resilience to other stressors, such as macroalgae competition or local pollution (Stecher et al., 2013; Thurber et al., 2009, 2012; Webster & Reusch, 2017). Climate change also impacts a variety of environmental factors that, in the ocean, most often occur simultaneously (Mera & Bourne, 2018). The environmental variability that Florida Keys corals face on a daily basis may influence their disease resistance and potentially increase disease susceptibility (e.g., Mera & Bourne, 2018; Meyer et al., 2019; Rocca et al., 2019; Sweet & Bulling, 2017). Environmental disturbance such as nutrient enrichment has also been implicated in changing host physiology which could alter the entire coral microbiome, even if only in the short-term (Ainsworth et al., 2009; Mera & Bourne, 2017).

The bloom of *Vibrio* bacteria associated with heavy-influence days was ephemeral. Within 3 d after dust deposition, *Vibrio* levels returned to baseline levels. Bacterial blooms are exposed to predation. *Bdellovibrionales* have been observed to prey on *Vibrio corallyticus* and *Vibrio harveyi* (Welsh et al., 2015). *Bdellovibrionales* are members of the coral microbiome in very low concentrations but drive significant changes in bacterial mortality (Welsh et al., 2015). Coral-associated viruses are also members of the coral microbiome and play important functions associated with both bacterial members and symbiotic *Symbiodinium* in coral microbiomes (Thurber & Correa, 2011). A virulent bacteriophage was identified by Sun et al. (2019) and observed to infect a coral derived *Vibrio* strain. Several *Vibrio* phages have also been identified as potential mitigators of coral disease due to their effectiveness in eliminating pathogenic *Vibrio* (Cohen et al., 2013; Jacquemot et al., 2018). Both coral-associated *Bdellovibrionales* and viruses are likely to influence the ephemeral nature of *Vibrio* blooms by contributing to “boom and bust” cycles particularly promoted by nutrient enrichment such as Saharan dust deposition in coral microbiomes (Bourne et al., 2009; Westrich et al., 2018).

Beta-diversity each coral was greater during heavy-influence days compared to low-influence days. Changes in beta-diversity has been associated with instability after disturbance in aquatic ecosystems and are suggested to influence microbial community composition (Banerjee et al., 2018; Herren & McMahon, 2018; Peixoto et al., 2017; Thurber et al., 2019). Pollock et al. (2019) observed a decrease in beta-diversity of coral bacterial communities in response to disease. Corals also exhibit altered community structure that is aligned with spatial and temporal environmental differences.

Additionally, beta-diversity dispersion (measured as the average distance to mean) was significantly greater during heavy-influence days compared to low-influence days for *A. palmata*. Environmental stressors and environmental gradients are known to affect coral microbial beta-diversity variability (e.g., Rocca et al., 2019). The microbial communities associated with animals may become dysbiotic when perturbation is introduced, and this change is can be visualized through increasing inter-individual variation, such as the increased dispersion observed in *A. palmata* (Roitman, 2018; Wang et al., 2018; Zaneveld et al., 2016, 2017). It is possible that *A. palmata* exhibits greater microbiome flexibility through the gain or loss of taxa that potentially promote rapid adaptation to dust influx and that is reflected in beta-diversity variability (Voolstra & Ziegler, 2020).

In contrast, we did not observe changes in dispersion in *O. faveolata*. Low microbiome flexibility of coral microbiomes presumably supports stable coral-microbe and microbe-microbe interactions that maintain essential holobiont functions (Voolstra & Ziegler, 2020). Stable alpha-diversities after perturbation, such as that observed in *O. faveolata*, have been suggested to contribute to high connectivity between members of the microbiome and generally contribute to host stability during stress (Reshef et al., 2006; Röthig et al., 2020). For example, Ziegler et al. (2019) shows that environmental changes promote a highly flexible microbial community in *Acropora hemprichii* whereas the microbiome of *Pocillopora verrucosa* showed less flexibility under the same conditions, suggesting that changes in community structure to abiotic stress is host-specific.

*O. faveolata* bacterial biomass, unlike *A. palmata* bacterial biomass, was significantly associated with dust AOT suggests that different coral species modulate their bacterial communities differently. The surface mucus layer is the first line of defense

against biotic and abiotic disturbances and activates signaling pathways to the clonal host (Certner et al., 2015; Ochsenkühn et al., 2018; Sweet et al., 2010). Corals may have the ability to actively structure their microbiomes by controlling nutrient availability or releasing antimicrobials, thus restructuring their microbiome to its baseline condition (Brown & Bythell, 2005; van Oppen & Blackall, 2019).

The differences in beta-diversity observed in response to dust deposition was statistically different between the two coral species. This could be attributed to the differences in mucus sugar content that alter microbiome structure both between species and under stress (Klaus et al., 2007; Lee et al., 2016). A coral's SML harbors a diverse prokaryotic community, releases metabolites, and constantly produces glycoproteins, polysaccharides, and lipids that serve multiple important functions (i.e., UV protection, pathogen barrier) and may be species-specific (Bourne et al., 2016; Brown & Bythell, 2005; Gavish et al., 2018; Glasl et al., 2016). Ochsenkühn et al. (2018) show that *Acropora* and *Platygyra* corals have unique chemical signatures that consist of chemical-attractants and antibacterial compounds that potentially modulate surface mucus microbial structure. We support that species-specific responses may dictate microbial community composition and structure. For example, we show that while the bacterial biomass of *O. faveolata* significantly increased during heavy-influence days, the bacterial biomass of *A. palmata* significantly decreased during low-dust days.

This study also identified the bacterial orders present that changed significantly in abundance between heavy-influence and low-influence days. Certain sub-groups of host-associated bacteria are driven by perturbation and dysbiosis, and therefore associated with disrupted states of the microbiome (van Oppen & Blackall, 2019; Zaneveld et al., 2017).

Additionally, it has been proposed that under favorable conditions (i.e., low-influence dust days), “keystone guilds” may arise consisting of low diversities of bacterial taxa with similar functions to maintain the stability of the host microbiome (Banerjee et al., 2018). In contrast, under unfavorable conditions (i.e., heavy-dust influence days), host microbiomes exhibit disorganization and high dissimilarity, allowing a more diverse bacterial community (Herren & McMahon, 2018; Ziegler et al., 2019).

Twelve orders were identified as transient (disappearing and appearing over time based on heavy- vs. low-influence days), suggesting that their abundance may be correlated with dust AOT variability. Transient bacteria likely comprised of low-abundance taxa that identify as highly variable taxa in an exposed mucus layer and are responsive to sudden biotic and abiotic environmental conditions (Certner et al., 2015; Hernandez-Agreda et al., 2016; Peixoto et al., 2017; Voolstra & Ziegler, 2020). Differences in the composition of core, persistent, or transient taxa in *A. palmata* and *O. faveolata* could be a function of host-regulated bacterial communities that influence homeostasis and/or adaptation to rapid environmental changes in a host-specific manner (Voolstra & Ziegler, 2020; Ziegler et al., 2019). Our results show low numbers of core and persistent bacteria consistently present between *A. palmata* and *O. faveolata*, supporting other studies that have found core taxa to be a small percentage of coral microbiome members (Ainsworth et al., 2015; Herren & McMahon, 2018; Kellogg, 2019; Meyer et al., 2019; Ziegler et al., 2019). The presence of a taxon can have a stabilizing effect by influencing host immunity or contributing to microbiome modulation through quorum sensing (Voolstra & Ziegler 2020).

*Vibrio*, which are marine in origin, are not typically detected in dust aerosols (Griffin, 2007). Seim et al. (in prep) studied the microbial community associated with

aerosol samples concurrently collected during our 26 d time series. While one *Vibrio* operational taxonomic unit (OTU) was found in aerosol filters, *Vibrio* ubiquitously present in marine water have a greater chance of being aerosolized by wave action (Seim et al., in prep). Another study concurrently collected water column samples and observed a bloom of Vibrionales in response to dust; however, this OTU was not matched to the single OTU found in aerosol filters collected in a concurrent study (Borchardt et al., 2020).

### **Conclusion**

In this study, we provide evidence that Saharan dust deposition alters *Vibrio* dynamics in coral surface mucus layers and associated bacterial communities. While *Vibrio* abundances increase after dust deposition, the rapid bloom is ephemeral, and *Vibrio* concentrations return to baseline abundances within days. Additionally, while species-specific differences in the response of microbial diversity and associated taxa were observed between *A. palmata* and *O. faveolata*, both species maintained relatively stable microbiomes throughout the 26 d time series. Future research goals should include how microbiome modulation is controlled by coral hosts and/or environmental conditions. Further research is also needed to identify the functional potential of bacterial taxa identified as core, persistent, and transient in this study. The influence of natural, episodic events contribute to the long-term health of corals, particularly hosts with compromised health, and may impact ecosystem health and viability.

### **Acknowledgements**

This work was supported by NSF Biological Oceanography award OCE 1357423 (EKL and JRW), NSF Graduate Research Fellowship Program (GRFP) 1842396 (MGB), and OCE 1357140 (AME and WML). Coral mucus and seawater samples were obtained under permit FKNMS-2016-046. Field-work would not have been possible without Captain James “Buddy” Williams’ boating/SCUBA expertise and enthusiasm for science. Thank you to Julie Nelson at the University of Georgia Cytometry Shared Resource Laboratory for flow cytometry processing assistance.

## Tables

**Table 4.1. Sample collection summary.** Dust aerosol optical thickness (dust AOT) was obtained every 6 h over a 26 d time series. Daily dust AOT was calculated as the average dust AOT levels over 24 h (18:00 to 18:00 h UTC; Borchardt et al., 2020). Days were categorized as heavy-influence if dust AOT > 0.075 (n=13; Category; highlighted orange). Each day of the time series, three out of 15 tagged colonies each of *A. palmata* colonies (APAL Colony ID) and *O. faveolata* colonies (OFAV Colony ID) were haphazardly selected and their mucus sampled. Samples were not collected on 17-20 July and 02 August 2016 due to unfavorable conditions.

| Date    | Category        | Dust AOT | OFAV Colony ID | APAL Colony ID |
|---------|-----------------|----------|----------------|----------------|
| 7/12/16 | Low-influence   | 0.0625   | 41, 42, 40     | 65, 70, 64     |
| 7/13/16 | Heavy-influence | 0.0975   | 43, 44, 53     | 57, 60, 19     |
| 7/14/16 | Heavy-influence | 0.0800   | 56, 55, 54     | 65, 64, 71     |
| 7/15/16 | Low-influence   | 0.0250   | 50, 52, 51     | 68, 66, 59     |
| 7/16/16 | Low-influence   | 0.0250   | 40, 41, 42     | 72, 73, 64     |
| 7/17/16 | Low-influence   | 0.0325   | -              | -              |
| 7/18/16 | Low-influence   | 0.0375   | -              | -              |
| 7/19/16 | Heavy-influence | 0.0800   | -              | -              |
| 7/20/16 | Low-influence   | 0.0475   | -              | -              |
| 7/21/16 | Low-influence   | 0.0700   | 53, 43, 44     | 74, 18, 24     |
| 7/22/16 | Low-influence   | 0.0400   | 56, 55, 54     | 62, 65, 60     |
| 7/23/16 | Low-influence   | 0.0375   | 50, 51, 52     | 30, 71, 64     |
| 7/24/16 | Low-influence   | 0.0450   | 40, 41, 42     | 58, 70, 21     |
| 7/25/16 | Low-influence   | 0.0625   | 43, 44, 50     | 59, 68, 57     |
| 7/26/16 | Heavy-influence | 0.0800   | 51, 52, 53     | 24, 18, 65     |
| 7/27/16 | Low-influence   | 0.0500   | 51, 52, 53     | 65, 60, 73     |
| 7/28/16 | Heavy-influence | 0.1425   | 54, 55, 56     | 58, 30, 21     |
| 7/29/16 | Heavy-influence | 0.1225   | 40, 41, 42     | 64, 71, 62     |
| 7/30/16 | Heavy-influence | 0.1125   | 43, 44, 52     | 62, 68, 19     |
| 7/31/16 | Heavy-influence | 0.0750   | 50, 54, 55     | 72, 74, 66     |
| 8/1/16  | Heavy-influence | 0.1000   | 40, 41, 42     | 64, 68, 71     |
| 8/2/16  | Heavy-influence | 0.1025   | -              | -              |
| 8/3/16  | Heavy-influence | 0.0925   | 50, 56, 54     | 70, 60, 57     |
| 8/4/16  | Heavy-influence | 0.1525   | 55, 50, 40     | 30, 18, 24     |
| 8/5/16  | Heavy-influence | 0.0925   | 41, 42, 43     | 59, 58, 73     |
| 8/6/16  | Low-influence   | 0.0650   | 56, 55, 54     | 72, 66, 74     |

**Table 4.2. Summary of 16S rRNA gene amplicon (V4 region) sequence processing.** Sequences were quality filtered and processed through DADA2. Samples were categorized by coral species and dust influence (Sample). Table includes the number of samples collected per sample (n), average number of reads per sample type that passed quality control filtering (Average Reads), average percent of reads per sample type that passed quality control filtering (Average Percent), minimum number of reads per sample type after quality control (Min), and maximum number of reads per sample type after quality control (Max).

| Sample               | n  | Average Reads | Average Percent | Min   | Max    |
|----------------------|----|---------------|-----------------|-------|--------|
| APAL                 | 61 | 26,151        | 57.27%          | 4,929 | 93,541 |
| OFAV                 | 65 | 30,414        | 68.22%          | 6,464 | 62,372 |
| Heavy-influence      | 66 | 29,649        | 65.40%          | 7,486 | 93,541 |
| Low-influence        | 60 | 26,920        | 60.19%          | 4,929 | 62,126 |
| APAL Heavy-influence | 33 | 28,441        | 60.47%          | 7,486 | 93,541 |
| APAL Low-influence   | 28 | 23,451        | 53.50%          | 4,929 | 52,667 |
| OFAV Heavy-influence | 33 | 30,857        | 70.33%          | 9,940 | 62,372 |
| OFAV Low-influence   | 32 | 29,956        | 66.04%          | 6,464 | 62,126 |

**Table 4.3. Significant changes in bacterial abundance between heavy- and low-influence dust deposition days.** Bacterial Orders identified in *A. palmata* (APAL) and *O. faveolata* (OFAV) characterized by their increased abundance in heavy-influence or low-influence days through TIME Workflow 4. “Log Mean Abundance Ratio” represents the log of the mean of the order during heavy-influence divided by the mean of the order during low-influence. Log mean abundance ratios that are positive represent taxa that were more abundant in heavy-influence dust days. The “Mean” abundance of each order was calculated for both heavy- and low-influence dust days. P-values were calculated using Wilcoxon Rank Sum Test.

| Species | Order                   | Log Mean Abundance Ratio | Heavy-influence Mean | Low-influence Mean | P-value |
|---------|-------------------------|--------------------------|----------------------|--------------------|---------|
| APAL    | Desulfobacterales       | -3.096                   | 0.012                | 0.263              | 0.013   |
| APAL    | Kordiimonadales         | -2.183                   | 0.001                | 0.011              | 0.037   |
| APAL    | Bacteroidales           | -2.067                   | 0.078                | 0.620              | 0.028   |
| APAL    | Cyanobacteria Family IV | -0.765                   | 0.058                | 0.125              | 0.015   |
| APAL    | Rhodocyclales           | 0.740                    | 0.121                | 0.058              | 0.028   |
| APAL    | SAR11                   | 1.087                    | 10.795               | 3.641              | 0.021   |
| APAL    | Xanthomonadales         | 1.675                    | 0.079                | 0.015              | 0.037   |
| APAL    | Acholeplasmatales       | 1.690                    | 0.063                | 0.012              | 0.008   |
| OFAV    | Desulfovibrionales      | -1.965                   | 0.006                | 0.041              | 0.011   |
| OFAV    | Clostridiales           | -1.075                   | 0.074                | 0.216              | 0.004   |
| OFAV    | Rhizobiales             | -0.931                   | 0.072                | 0.182              | 0.008   |
| OFAV    | Desulfobacterales       | -0.839                   | 0.018                | 0.042              | 0.049   |
| OFAV    | Bacteroidales           | -0.822                   | 0.069                | 0.157              | 0.021   |
| OFAV    | Chromatiales            | -0.780                   | 0.050                | 0.109              | 0.008   |
| OFAV    | Puniceicoccales         | 0.732                    | 0.416                | 0.200              | 0.037   |
| OFAV    | Rhodocyclales           | 0.995                    | 0.076                | 0.028              | 0.011   |
| OFAV    | Acholeplasmatales       | 1.451                    | 0.050                | 0.012              | 0.011   |
| OFAV    | SAR11                   | 2.381                    | 1.948                | 0.180              | 0.003   |

**Table 4.4. Species-specific core, persistent, and transient taxa.** Summary of TIME workflow 1: identification of core, persistent, and transient bacterial taxa based on their relative abundances over the 26 d time series in *A. palmata* (APAL; n=61) and *O. faveolata* (OFAV; n=65). Core taxa were identified as present at all time points and all samples, persistent taxa were identified as present for extended periods of time, and transient taxa appeared and disappeared over time (suggesting association with dust AOT variability).

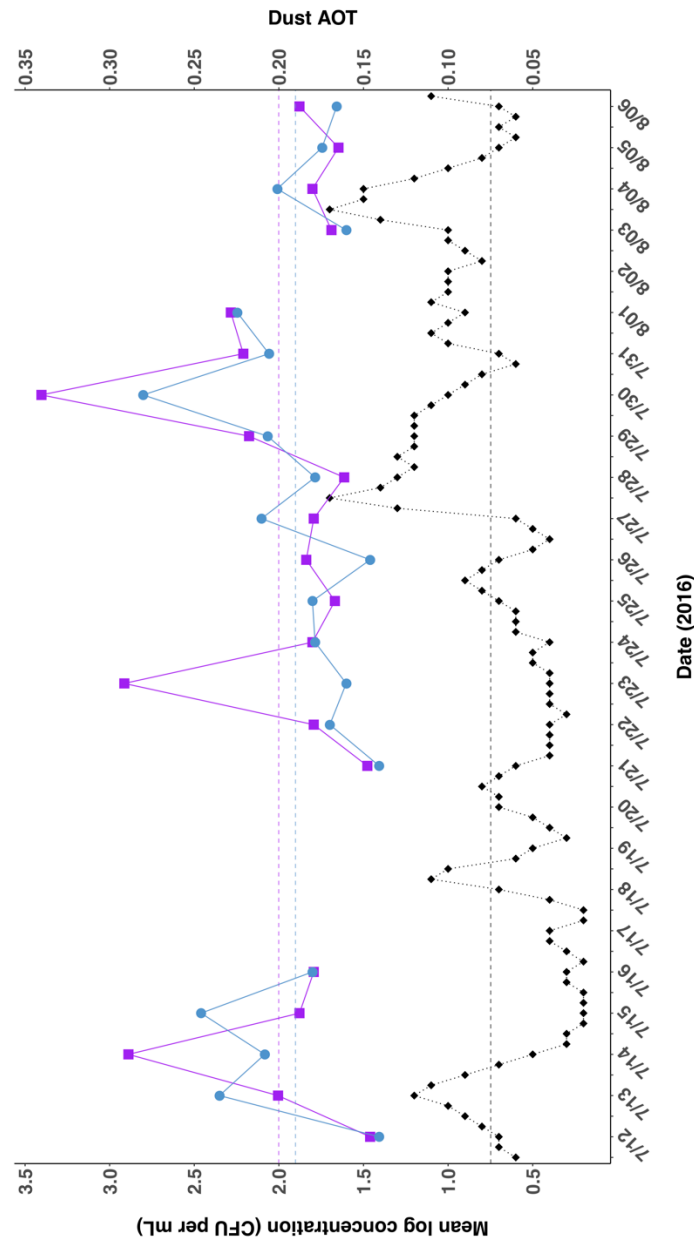
| Order                              | Species     | Type       |
|------------------------------------|-------------|------------|
| Chromatiales                       | APAL        | Core       |
| Desulfovibrionales                 | APAL        | Core       |
| Family_IV (Cyanobacteria class)    | APAL        | Core       |
| Hydrogenophilales                  | APAL        | Core       |
| Campylobacteriales                 | APAL        | Persistent |
| Fusobacteriales                    | APAL        | Persistent |
| Chlamydiales                       | APAL        | Transient  |
| Enterobacteriales                  | APAL        | Transient  |
| Erysipelotrichales                 | APAL        | Transient  |
| Family_XIII (Cyanobacteria class)  | APAL        | Transient  |
| Kiloniellales                      | APAL        | Transient  |
| Kordiimonadales                    | APAL        | Transient  |
| Lentisphaerales                    | APAL        | Transient  |
| Xanthomonadales                    | APAL        | Transient  |
| Acidimicrobiales                   | APAL & OFAV | Core       |
| Actinomycetales                    | APAL & OFAV | Core       |
| Alteromonadales                    | APAL & OFAV | Core       |
| Bacillales                         | APAL & OFAV | Core       |
| Bacteroidales                      | APAL & OFAV | Core       |
| Bdellovibrionales                  | APAL & OFAV | Core       |
| Burkholderiales                    | APAL & OFAV | Core       |
| Caulobacteriales                   | APAL & OFAV | Core       |
| Clostridiales                      | APAL & OFAV | Core       |
| Cytophagales                       | APAL & OFAV | Core       |
| Desulfobacteriales                 | APAL & OFAV | Core       |
| Family_II (Cyanobacteria class)    | APAL & OFAV | Core       |
| Flavobacteriales                   | APAL & OFAV | Core       |
| Gammaproteobacteria_incertae_sedis | APAL & OFAV | Core       |
| Lactobacillales                    | APAL & OFAV | Core       |
| Legionellales                      | APAL & OFAV | Core       |
| Myxococcales                       | APAL & OFAV | Core       |
| Oceanospirillales                  | APAL & OFAV | Core       |
| Planctomycetales                   | APAL & OFAV | Core       |
| Pseudomonadales                    | APAL & OFAV | Core       |
| Puniceicoccales                    | APAL & OFAV | Core       |
| Rhizobiales                        | APAL & OFAV | Core       |
| Rhodobacteriales                   | APAL & OFAV | Core       |
| Rhodocyclales                      | APAL & OFAV | Core       |
| Rhodospirillales                   | APAL & OFAV | Core       |

| <b>Order (cont.)</b>            | <b>Species (cont.)</b> | <b>Type (cont.)</b> |
|---------------------------------|------------------------|---------------------|
| SAR11                           | APAL & OFAV            | Core                |
| Sphingobacteriales              | APAL & OFAV            | Core                |
| Sphingomonadales                | APAL & OFAV            | Core                |
| Verrucomicrobiales              | APAL & OFAV            | Core                |
| Vibrionales                     | APAL & OFAV            | Core                |
| Methylophilales                 | APAL & OFAV            | Transient           |
| Neisseriales                    | APAL & OFAV            | Transient           |
| Pasteurellales                  | APAL & OFAV            | Transient           |
| Spirochaetales                  | APAL & OFAV            | Transient           |
| Campylobacteriales              | OFAV                   | Core                |
| Chlamydiales                    | OFAV                   | Core                |
| Enterobacteriales               | OFAV                   | Core                |
| Erysipelotrichales              | OFAV                   | Core                |
| Fusobacteriales                 | OFAV                   | Core                |
| Chromatiales                    | OFAV                   | Persistent          |
| Family IV (Cyanobacteria class) | OFAV                   | Persistent          |
| Anaerolineales                  | OFAV                   | Transient           |
| Arenicellales                   | OFAV                   | Transient           |
| Deinococcales                   | OFAV                   | Transient           |
| Desulfarculales                 | OFAV                   | Transient           |
| Desulfovibrionales              | OFAV                   | Transient           |
| Hydrogenophilales               | OFAV                   | Transient           |
| Parvularculales                 | OFAV                   | Transient           |
| Selenomonadales                 | OFAV                   | Transient           |

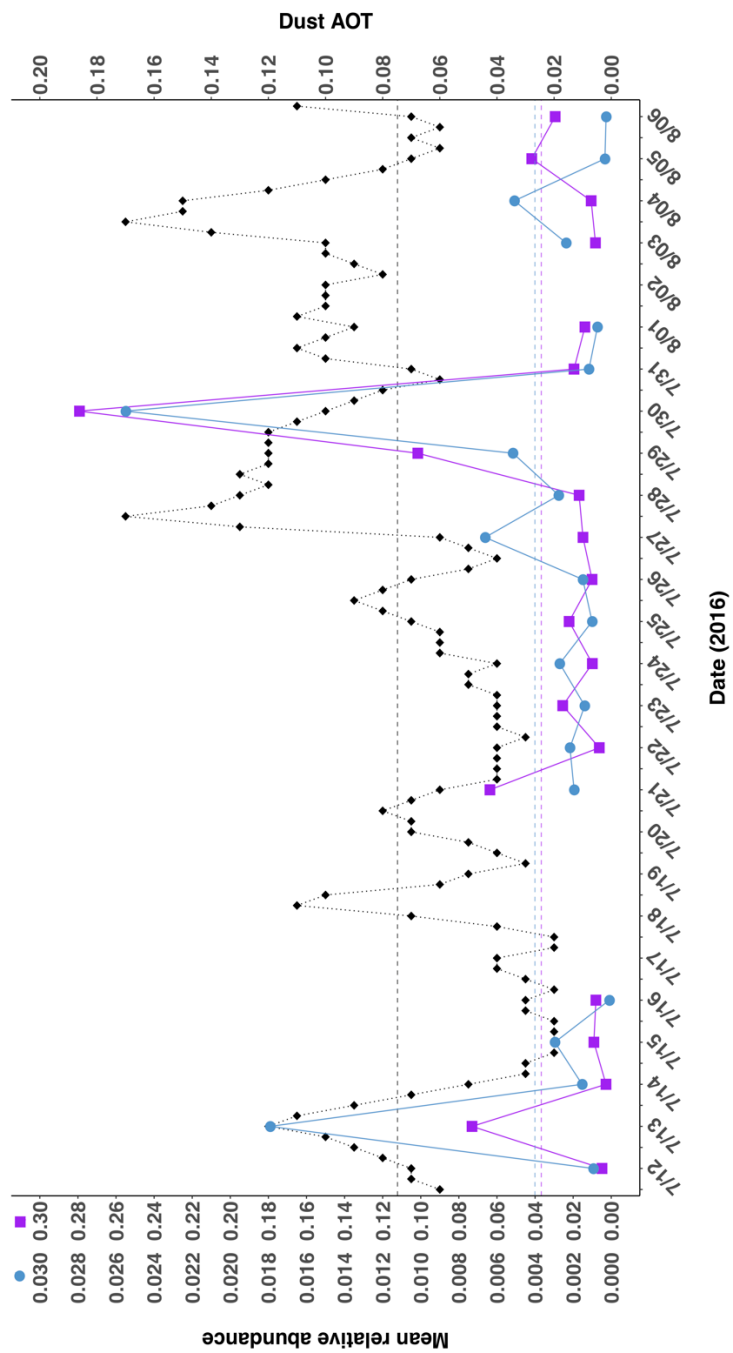
## Figures

**Figure 4.1. Coral-associated *Vibrio* and dust AOT over time.** Black diamond points denote dust AOT, purple square points denote *A. palmata* samples, and blue square points denote *O. faveolata* samples. Daily dust AOT (n=104) was recorded every 6 h. **(A)** *Vibrio* mean log concentration (daily average of approx. three replicates) for APAL (n=63) and OFAV (n=62). **(B)** *Vibrio* mean relative abundance (daily average of approx. three replicates) for APAL (n=61) and OFAV (n=65). Dashed horizontal lines of respective point colors denote the mean over the 26 d time series: dust AOT (mean=0.075), APAL (mean=2.001 CFU mL<sup>-1</sup>; mean=0.037), OFAV (mean=1.902 CFU mL<sup>-1</sup>; mean=0.004).

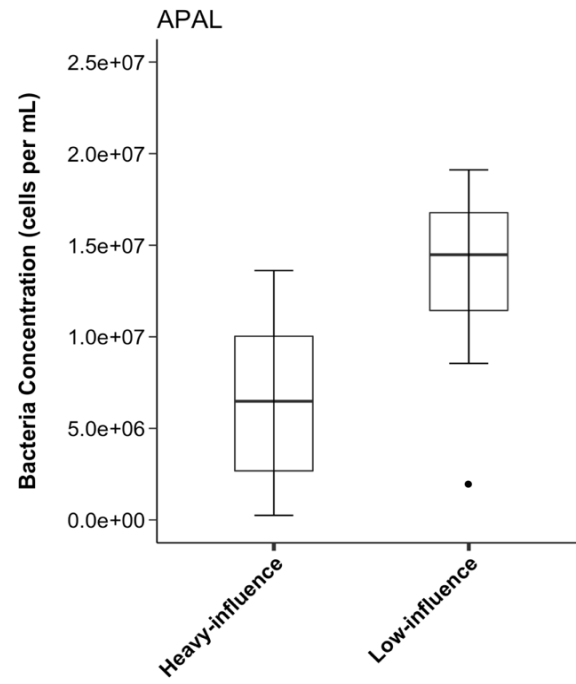
**(A)**



(B)



(A)



(B)

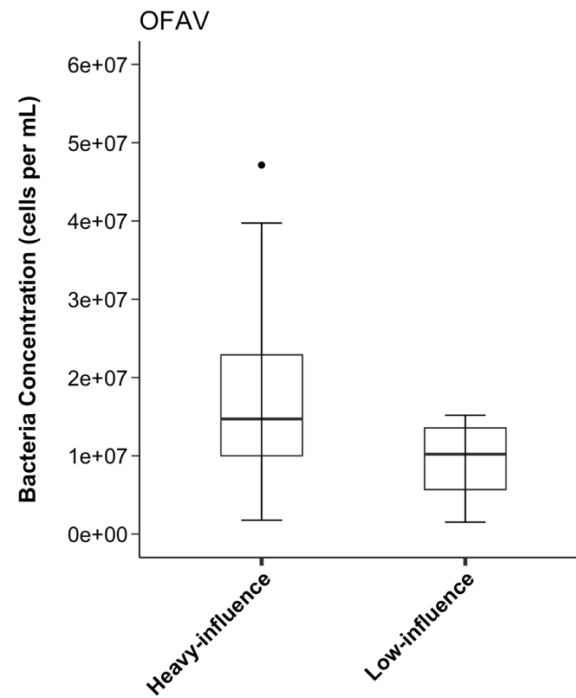
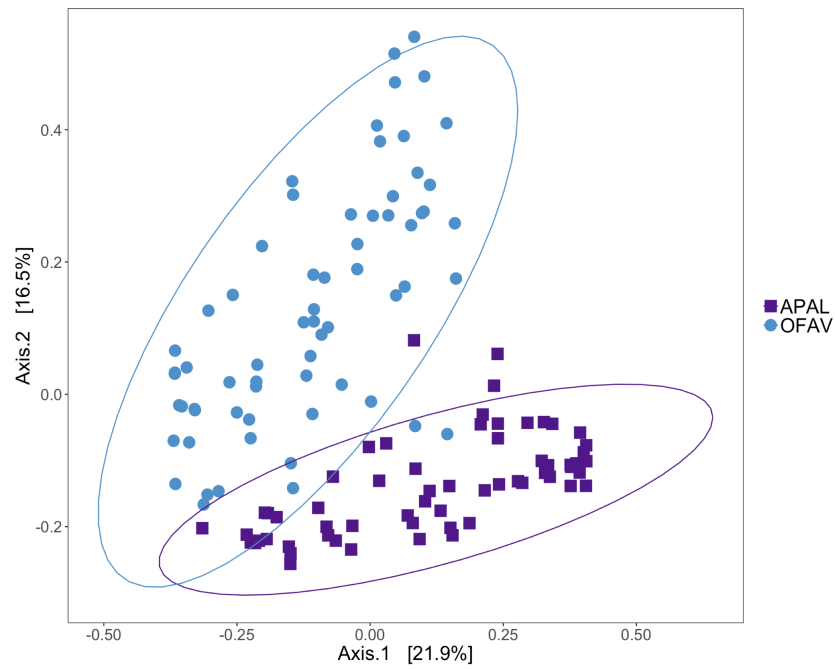


**Figure 4.2. Bacterial biomass by dust influence.** Bacterial concentrations of (A) *A. palmata* (APAL; n=21) and (B) *O. faveolata* (OFAV; n=26) between heavy- vs. low-influence days.

(A)



(B)

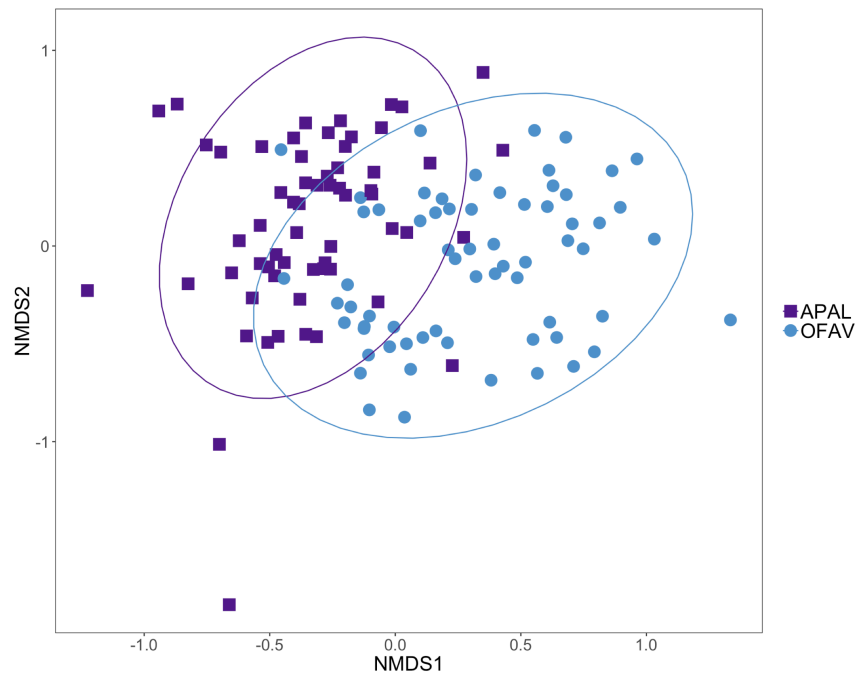
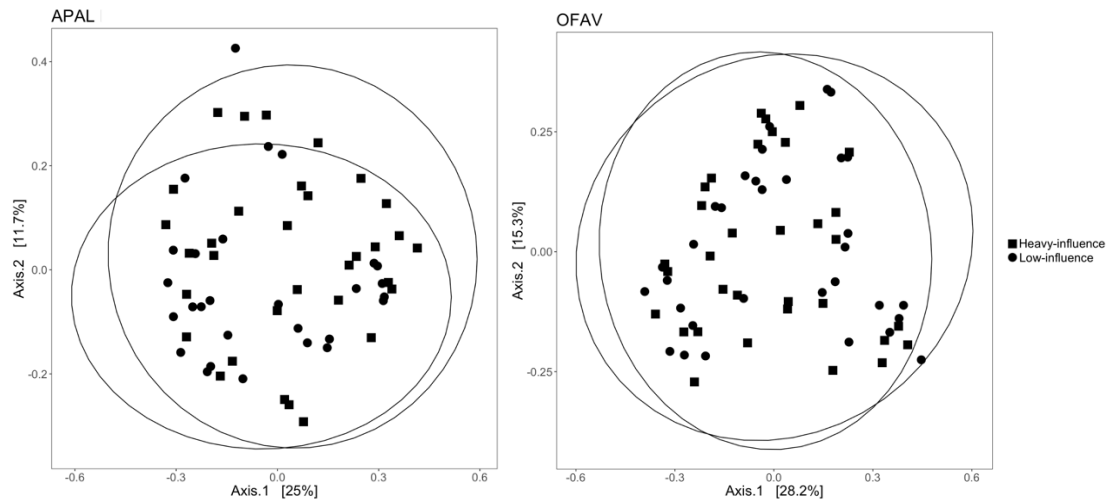


**Figure 4.3. Beta-diversity plotted by coral species. (A)** Principal coordinate analysis (PCoA) and **(B)** non-metric multidimensional scaling analysis (NMDS) for *A. palmata* (APAL; n=61) and *O. faveolata* (OFAV; n=65) bacterial communities. Ellipses represent a confidence level of 0.95.

(A)



(B)

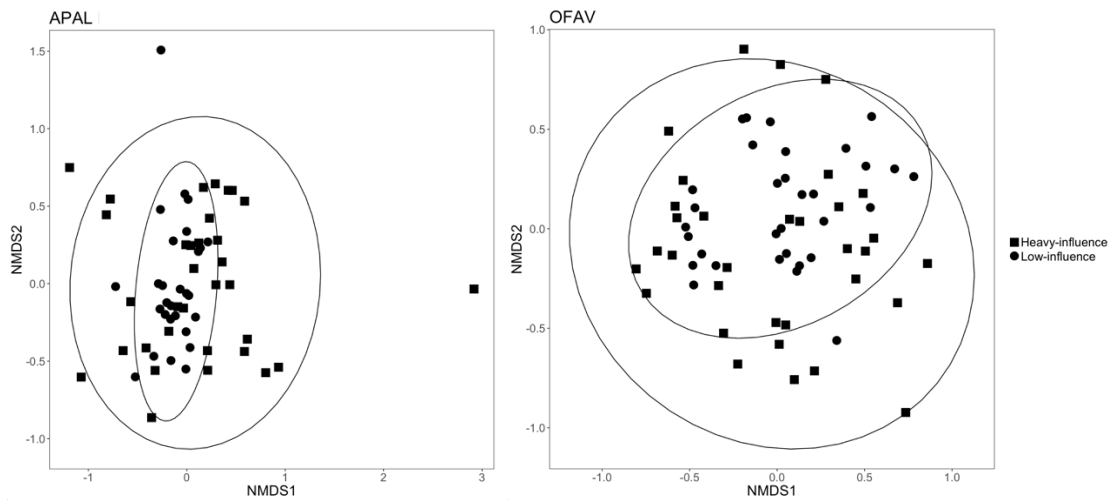


**Figure 4.4. Beta-diversity of microbial communities for each coral species by dust influence. (A) PCoA and (B) NMDS plots for *A. palmata* (APAL) and *O. faveolata* (OFAV) characterized by heavy-influence (APAL n=33; OFAV n=33) or low-influence (APAL n=28; OFAV n=32) days. Ellipses represent a confidence level of 0.95.**

## Supplementary Figures

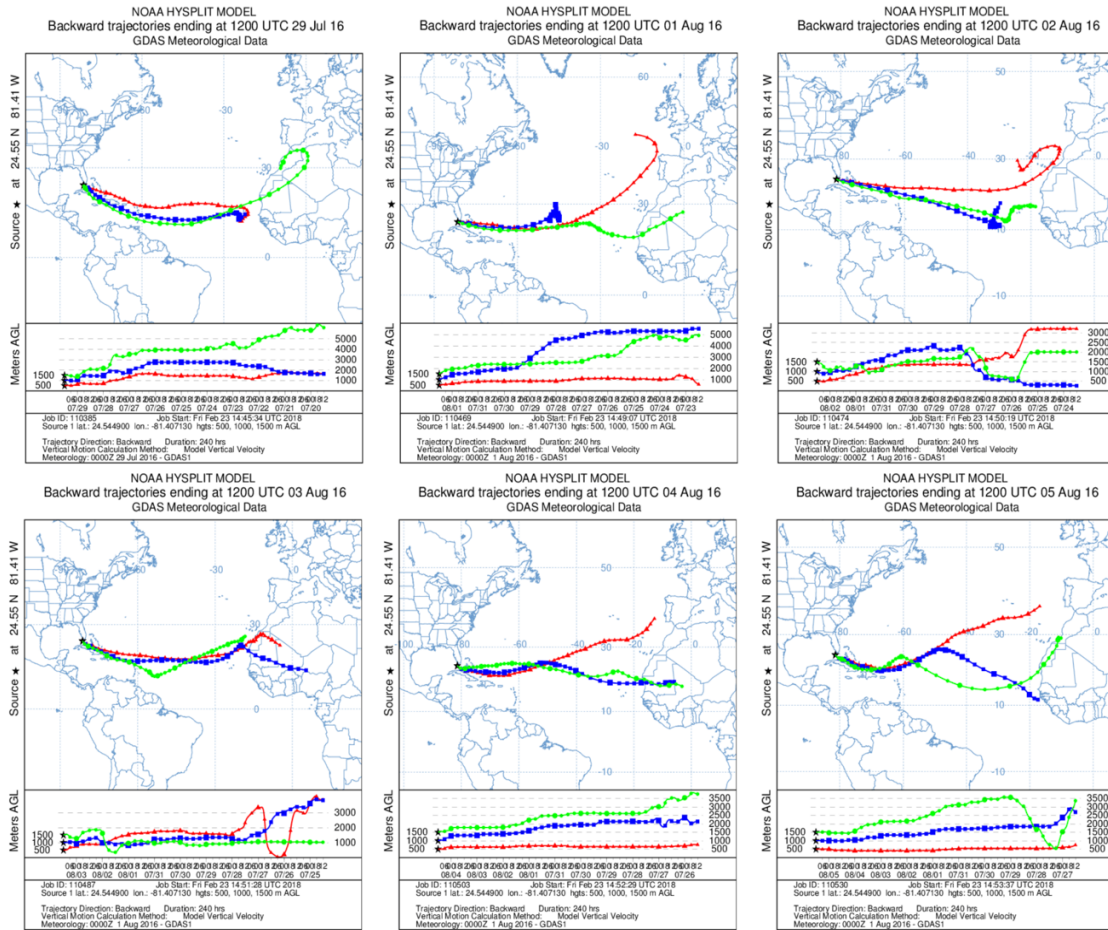
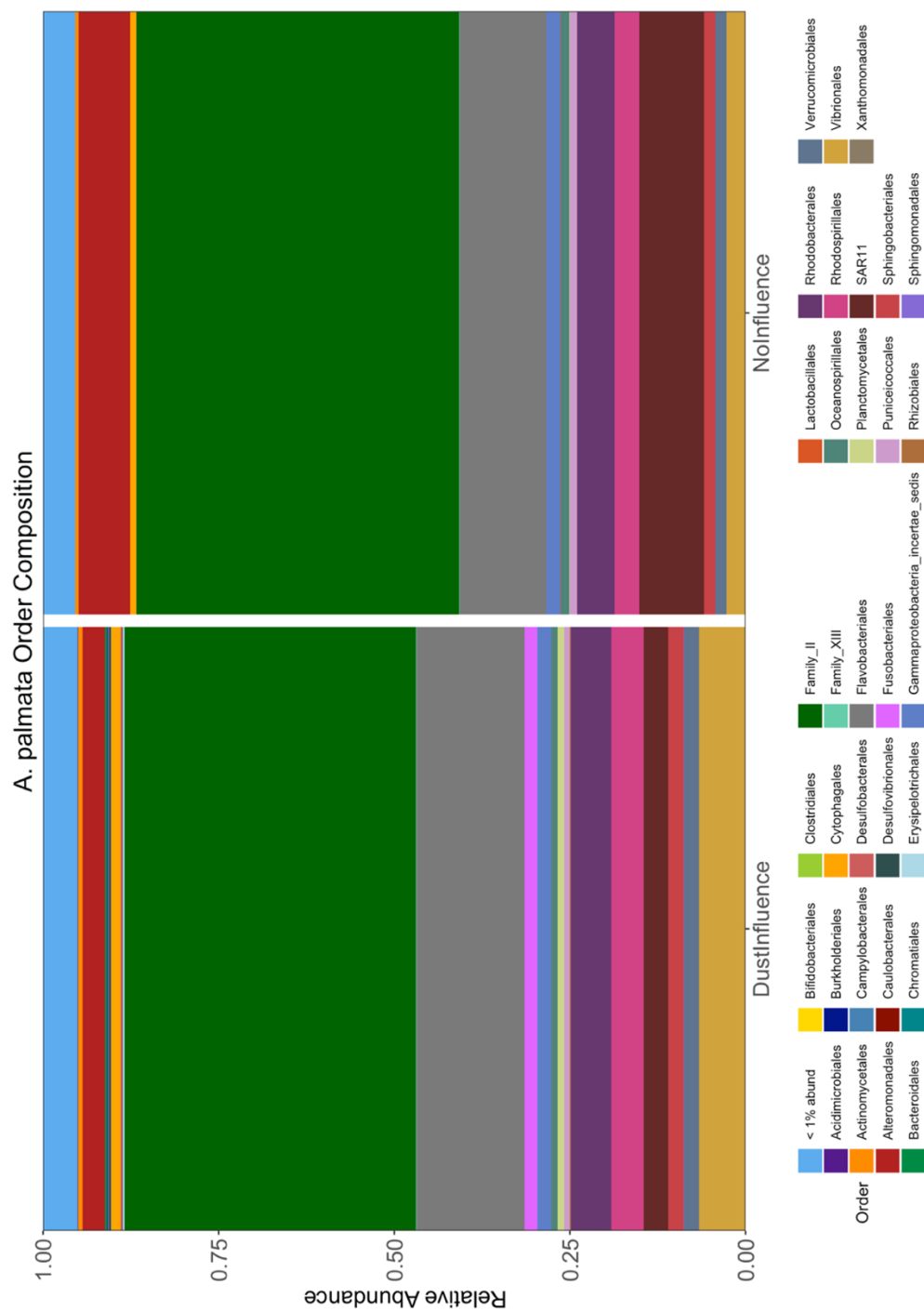


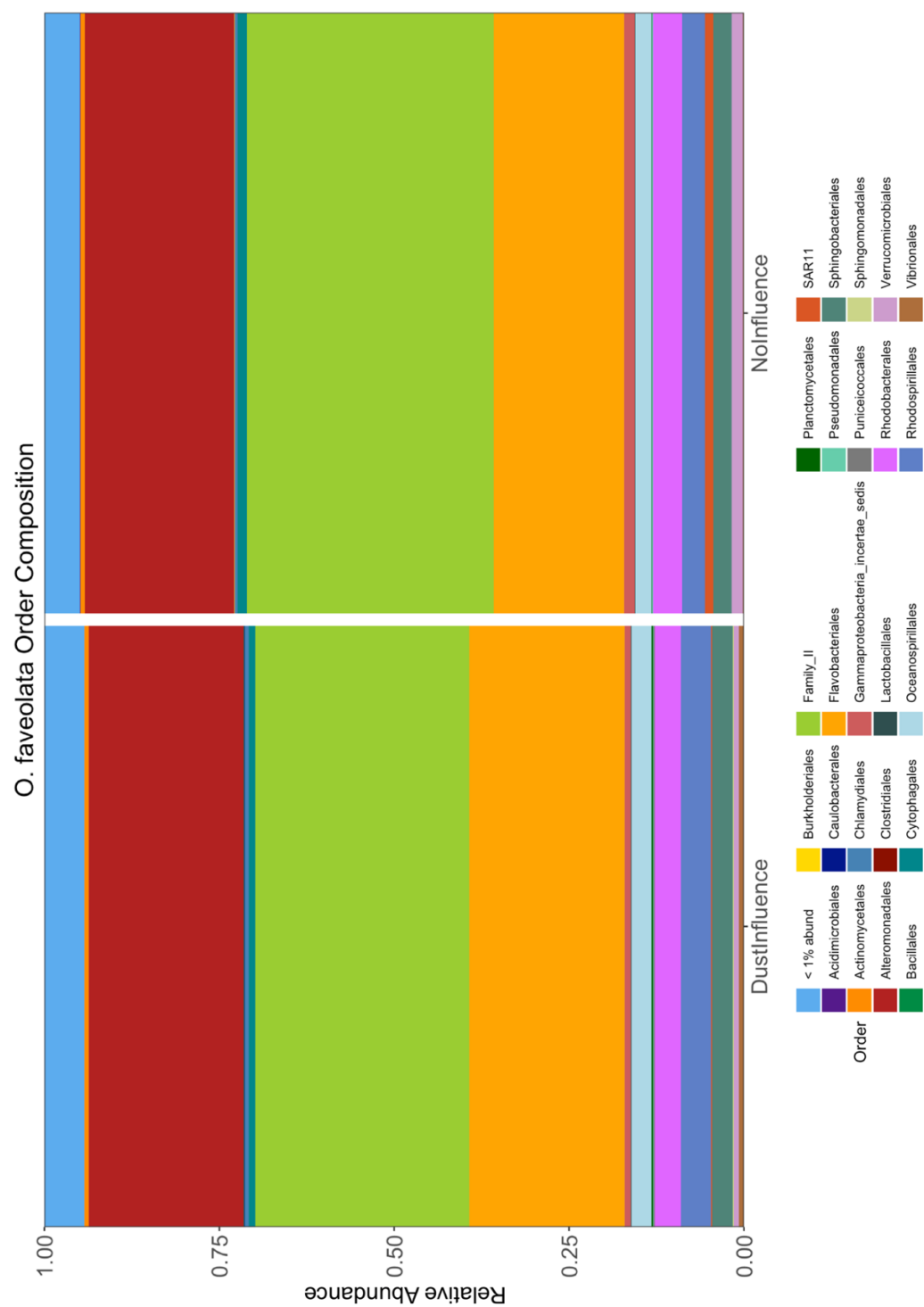
**Figure S4.1.** NOAA HYSPLIT Model Backward trajectories from GDAS meteorological data featuring the following 2016 dates: 29 July, 01 August, 02 August, 03 August, 04 August, and 05 August. Trajectories were computed using meteorological forecast data sets from North American Mesoscale (NAM; 12 km)/Global Forecast System (GFS) (red triangle), Rapid Update Cycle/GFS (blue squares), and MM5 (45 km)/GFS (green circles).

**Figure S4.2.** Relative abundance of bacterial orders representing > 1% of sequences in any one sample of **(A)** *A. palmata* (APAL) by dust influence (low-influence n=28; heavy-influence n=33), **(B)** *O. faveolata* (OFAV) by dust influence (low-influence n=32; heavy-influence n=33), **(C)** APAL over time (n=61), and **(D)** OFAV over time (n=65).

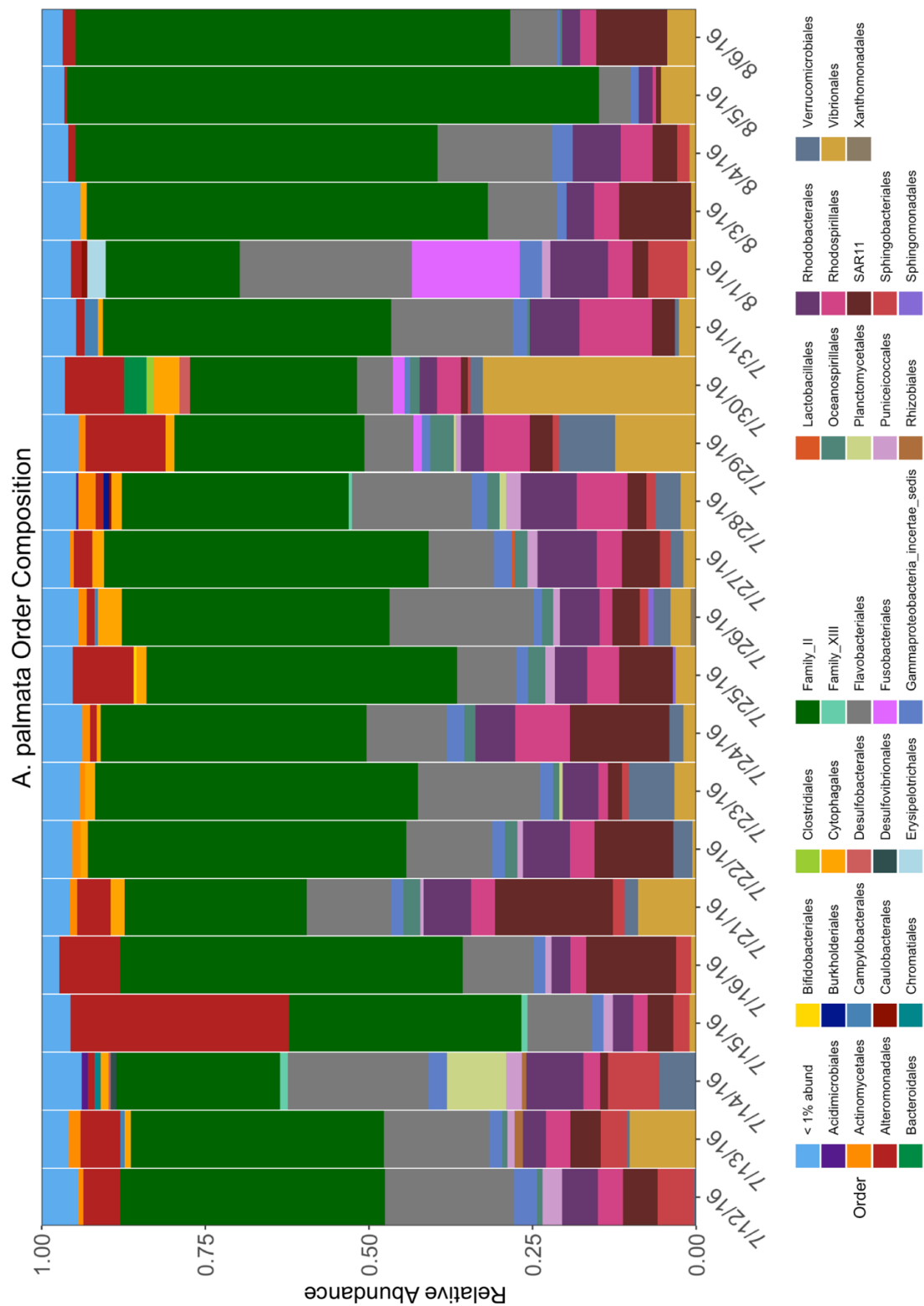
**(A)**



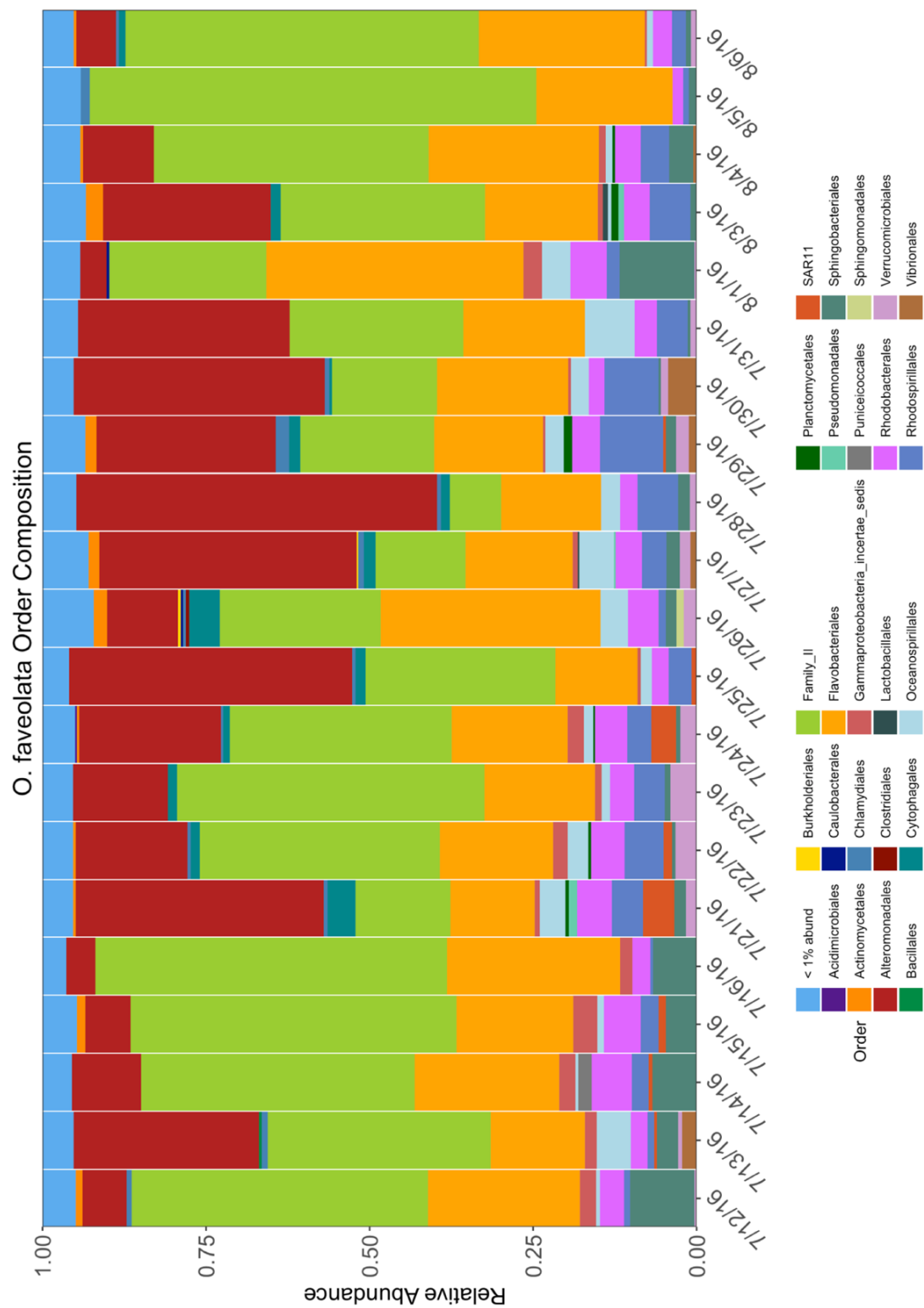
(B)



(C)



(D)



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## CHAPTER 5

### EFFECTS OF SAHARAN DUST DEPOSITION ON THE SURFACE WATER TRANSCRIPTOME IN LOOE KEY REEF, FLORIDA, USA <sup>3</sup>

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<sup>3</sup> Bucher, M. G., Alabady, M. S., Norfolk, W. A., Tinker, K. A., Borchardt, T. D., Lipp, E. K., & Ottesen, E. A. To be submitted to *Limnology and Oceanography*.

## **Abstract**

Aerosolized particles from episodic dust storm events that travel from the Sahara-Sahel and into the Caribbean carry nutrients such as iron and phosphorous. Current research shows that microbial community composition in marine surface waters shift with the arrival of dust, resulting in short term blooms of specific taxa before returning to baseline conditions. Investigating bacterial transcriptional patterns could reveal how dust events shape the community and their response to dust influx. Here we investigate the effects of Saharan dust deposition on the surface water transcriptome during a 26 d time series during the summer of 2016. Dust aerosol optical thickness levels along with concentrations of dissolved iron and dissolved organic carbon were associated with significant changes in gene expression in the microbial community. Ten ortholog clusters were down-regulated and eight were up-regulated in response to dissolved organic carbon concentrations, including functions such as carbohydrate metabolism and genetic information processing. Six ortholog clusters were down-regulated and four were up-regulated in response to dissolved iron concentrations, including functions such as signal transduction mechanisms and carbohydrate metabolism. Five ortholog clusters were down-regulated and one was up-regulated in response to dissolved iron, including functions such as genetic information processing, replication, and repair. Additionally, dissolved organic carbon significantly affected the overall transcriptional patterns over the 26 d time series. In summary, the impact of Saharan dust influx on the bacterial transcriptome was characterized by rapid changes in gene expression.

## Introduction

Aerosolized dust from the Sahara-Sahel delivers nutrients and essential trace metals to marine ecosystems through episodic dust deposition events (Moulin et al., 1997; Moxim et al., 2011; Tucker & Nicholson, 1999). Saharan dust suspended in the atmosphere can persist for several days in transit prior to dust deposition in downwind ecosystems (Prospero et al., 2010). Desert dust aerosols travel along easterly trade winds and into the Caribbean, tropical and subtropical Atlantic and Gulf of Mexico, including eastern Florida and the Florida Keys (Boyd & Ellwood, 2010; Mahowald et al., 2009; Trapp et al., 2010). In these regions, multiple dust deposition events occur annually between June and September and last 3 to 5 d (Perry et al., 1997; Prospero et al., 2007, 2010).

The exchange of nutrients and trace metals between the ocean and the atmosphere fuels biogeochemical cycling within surface water bacterial communities (Falkowski et al. 2008). Dust deposition events fertilize marine systems with essential nutrients, such as nitrogen, phosphorus, and carbon (Ebling & Landing, 2015; Morel et al., 2006; Pullido-Villena et al., 2008). Importantly, episodic Saharan dust deposition delivers critical trace metals that are key limiting nutrient in oligotrophic ocean systems (Lenes et al., 2001; Mills et al., 2004; Moxim et al., 2011). Iron is a limiting factor in metabolic processes (i.e., aerobic respiration, photosynthesis, and nitrogen fixation) and can affect biological responses of autotrophic and heterotrophic microorganisms (e.g., Emerson et al., 2016; Hill et al., 2010; Langlois et al., 2012; Maranen et al., 2010; Mahowald et al., 2009; Sunda et al., 2012). For example, autotrophic primary production by cyanobacteria (*Trichodesmium*) was induced by aeolian derived iron in marine surface water and released dissolved organic nitrogen into the water column (Lenes et al. 2001). In particular,

heterotrophic taxa from the *Vibrio* genus have been noted to increase in both relative and absolute abundance within a few hours of dust arrival in the Florida Keys, USA (Westrich et al., 2016). Heterotrophic bacteria, like *Vibrio*, contribute to carbon, nitrogen, and phosphorus cycling in marine surface waters (Fourquez et al., 2014; Yong et al., 2014).

Dust deposition can also affect marine microbial communities (e.g., Borchardt et al., 2020; Davey et al., 2008; Laghdass et al., 2011; Pulido-Villena et al., 2008; Reche et al., 2009). Following experimental Saharan dust addition, a shift in the eastern Mediterranean microbial community composition was observed within 3 h after exposure followed by increased activity of phosphorus cycling (Guo et al., 2016). In addition, iron had a greater effect on phytoplankton community composition and diversity compared to other trace metals (Sunda et al., 2012). Therefore, the study of microbial community composition in the context of episodic nutrient and trace metal enrichment is an important component in understanding marine microbial ecology (Brown et al. 2014; Duarte et al., 2006).

Bacterial community responses to dust have been well-studied in marine waters close to desert sources including the subtropical and tropical northeast Atlantic (e.g., Davey et al., 2008; Duarte et al., 2016; Hill et al., 2010; Mills et al., 2004) and Mediterranean Sea (e.g., Feingersch et al., 2010; Guo et al., 2016; Herut et al., 2006; Laghdass et al., 2011; Pitta et al., 2017; Pulido-Villena et al., 2008). In these settings, large quantities of dust reach surface waters more frequently and potentially greater intensity. Dust aerosols that travel wider distances (i.e., trans-Atlantic dust) experience significant atmospheric processing resulting in changes to nutrient and/or trace metal bioavailability, particle size and overall volume of deposition (Mahowald et al., 2009; Moxim et al., 2011; Prospero et

al., 2007, 2010). While these far downwind deposition events still elicit significant changes in microbial communities (e.g., Borchardt et al., 2020; Westrich et al., 2016, 2018), further research is needed to describe the functional changes in these responses. Understanding transcriptional shifts can provide insight into the mechanisms behind surface water microbial community shifts in response to trans-Atlantic Saharan dust transport in western subtropical Caribbean surface waters (Berger, 1989; Fasham et al., 2001; Karl et al., 2002; Longhurst, 1998).

Here we investigate the effects of Saharan dust influx on the metatranscriptome of surface water communities in the Florida Keys National Marine Sanctuary (near the Looe Key Reef, Florida Keys, USA) during a 26 d time series. A concurrent study by Borchardt et al. (2020) of microbial community composition during the time series showed that Saharan dust deposition initiates a successional pattern in the response of specific members of the microbial community including early increases in the relative abundance of picoeukaryotes and series of heterotrophic bacteria (Borchardt et al., 2020). Prior studies also noted ephemeral blooms of key bacterial taxa (e.g., *Vibrio*) following dust influx before returning to baseline levels (Westrich et al., 2016, 2018). Our goal for this study was to characterize the microbial community transcriptional response that accompany these taxonomic shifts.

## **Methods**

### *Sample and environmental data collection*

Seawater samples (1 L) were collected in acid-washed polypropylene bottles at < 0.5 m depth at Looe Key Reef in the Florida Keys National Marine Sanctuary (FKNMS), FL,

USA (24.545, -81.409) over a 26 d sampling period from 12 July to 06 August 2016. Due to unfavorable weather conditions, no samples were collected on 17-20 July or 02 August 2016. Surface water samples were collected between 14:00 and 18:30 UTC and vacuum-filtered approximately 1 h after collection using a Masterflex E/S Portable Sampler Pump (115 VAC) with L/S 15 Masterflex tubing (Cole-Parmer, IL, USA). Seawater samples were filtered through a Durapore 5.0  $\mu\text{m}$  pore-size, polyvinylidene difluoride (PVDF), 47 mm diameter membrane prefilter followed by a Durapore 0.22  $\mu\text{m}$  pore-size, polycarbonate, 25 mm diameter membrane filter (EDM Millipore, Darmstadt, Germany). The 0.22  $\mu\text{m}$  sample filters were immediately stored in 300  $\mu\text{L}$  of RNAlater in a 1.5 mL microcentrifuge tube (Sigma-Aldrich, Darmstadt, Germany), and placed on ice until return to the laboratory where they were stored at  $-80^{\circ}\text{C}$  until RNA extraction.

Concurrent surface water samples were collected and assayed for total iron (*in situ* tFe;  $\text{nmol L}^{-1}$ ), dissolved iron (*in situ* dFe;  $\text{nmol L}^{-1}$ ) and dissolved organic carbon (DOC;  $\mu\text{mol L}^{-1}$ ) as described in Borchardt et al. (2020). Aerosol samples were collected from 15 July to 06 August 2016, assayed for total iron (aerosol Fe;  $\text{nmol m}^{-3}$ ), and used to calculate the daily dust flux (dust flux;  $\mu\text{g m}^{-2} \text{d}^{-1}$ ) as described in Borchardt et al. (2020). The Navy Aerosol Analysis and Prediction System (NAAPS; <http://www.nrlmry.navy.mil/aerosol/>) was used to model daily dust-specific aerosol optical thickness (dust AOT), a unitless vertical integral of total dust-specific concentrations (Borchardt et al., 2020; Lynch et al., 2016; Westrich et al., 2018). As described in Borchardt et al. (2020), the average dust AOT from 18:00 to 18:00 h UTC was calculated for each day to align with aerosol collection periods.

### *Sample RNA extraction*

Preserved, frozen filters were thawed on ice. RNAlater was pipetted from sample microcentrifuge tubes and discarded. Total RNA was extracted from filters using a modified version of the EZNA HP Total RNA Kit (Omega Bio-Tek, GA, USA). Briefly, 200  $\mu$ L of PBS was added to each sample and centrifuged at 5,000 x g at room temperature for 5 min to rinse away remaining RNAlater. Supernatant was removed, and filters were resuspended in 100  $\mu$ L of lysozyme/TE buffer. RNase inhibitor (2  $\mu$ L) was added to each sample, vortexed for 30 s, and incubated at 30°C for 10 min at 300 rpm/10 x g. Next, 700  $\mu$ L of GTC lysis buffer (20  $\mu$ L B-mercapoethanol per 1 mL of GTC lysis buffer) was added to each sample and mixed by pipetting. About 25-35 mg of 0.5 mm RNase-free glass beads were added to each sample, vortexed for 5 min, centrifuged for 5 min at 13,000 x g, and the resulting lysate was transferred to a DNA clearance column in a collection tube. From this step, the EZNA HP Total RNA Kit protocol (December 2010 version) was followed beginning at step 5. The EZNA MicroElute RNA Clean Up Kit (Omega Bio-tek, GA, USA; Jul 2014) was used immediately after total RNA extraction, starting at step 17. Cleaned samples were eluted in 15  $\mu$ L of DEPC-treated water, and 1  $\mu$ L was used to quantify total RNA using NanoDrop Lite (Thermo Scientific, DE, USA). An Agilent 2000 Bioanalyzer was used to assess RNA quality and concentration. Due to very low RNA concentrations, rRNA subtraction was not performed.

### *Sample cDNA synthesis, library preparation, and sequencing*

The ScriptSeq v2 RNA-seq Library Preparation Kit (Illumina Epicentre, CA, USA) was used for cDNA synthesis. Briefly, RNA was fragmented for 5 min at 85°C in a

thermocycler then placed on ice. cDNA was synthesized by adding 4  $\mu$ L of ScriptSeq cDNA Synthesis Master Mix to each sample, incubated at 25°C for 5 min followed by 42°C for 20 min. Reactions were cooled to 37°C before 1  $\mu$ L of finishing solution was added to each reaction. Samples were then incubated at 37°C for 10 min, 95°C for 3 min, and cooled to 25°C. A volume of 8  $\mu$ L of Terminal Tagging Master Mix was added to each reaction before incubation at 25°C for 15 min, 95°C for 3 min and cooling to 4°C. Agencourt AMPure XP 1.8X system (Beckman Coulter, CA, USA) was used to purify cDNA as per ScriptSeq v2 RNA-Seq Library Preparation Kit instructions. Samples were eluted in 24.5  $\mu$ L of nuclease free water, and 22.5  $\mu$ L of sample was amplified with a forward PCR primer (provided by kit), unique ScriptSeq Index PCR Primers, FailSafe PCR PreMix E, and FailSafe PCR enzyme (1.25 U) for 15 cycles set at 95°C for 30 s, 55°C for 30 s, 68°C for 3 min and 68°C for 7 min. RNA-Seq libraries were then purified using a 0.9X AMPure XP purification scheme as per ScriptSeq v2 RNA-Seq Library Preparation Kit instructions using 45  $\mu$ L of beads. Samples were run through an Agilent 2100 Bioanalyzer to assess quality and concentration and then submitted to the Georgia Genomics & Bioinformatics Center (GGBC, GA, USA) for sequencing via NextSeq PE150 (Illumina, Inc., CA, USA). Samples that returned with extremely low sequence quantities underwent an additional cDNA synthesis and cleaning procedure (as described above) from the original RNA extracts followed by another sequencing run.

#### *Metatranscriptome sequence processing*

The metatranscriptome reads for each sample from all sequencing runs were combined in one file keeping R1 and R2 reads in separate files. The analysis was performed in the

following steps: quality control and read filtration, removal of rRNA/tRNA reads, identifying mRNA reads, and mapping reads to reference database. We conducted the analysis using the metatrans pipeline (Martinez et al., 2016). The first step in the analysis included quality control of the sequencing reads. Both low quality and short reads as well as Illumina adapter traces were removed. Both Kraken (Wood et al., 2014) and FastQC (Andrews, 2010) tools were used in the quality control step. The second step included the removal of rRNA/tRNA reads using SortMeRNA (Kopylova et al., 2012) and a reference database comprising all prokaryotic tRNA and SILVA 16S, 18S, 23S, 28S, SSU, LSU sequences (Quast et al., 2012). The remaining non-rRNA/tRNA reads were subjected to FragGeneScan (Rho et al., 2010) to identify putative gene reads. Gene reads that passed quality filtering were mapped to the M5nr-20130801 database (Wilke et al., 2012) using Diamond (Buchfink et al., 2015).

#### *Metatranscriptome analysis*

Transcription patterns were analyzed across seven variables: *in situ* total Fe, *in situ* dissolved Fe, dissolved organic carbon, aerosol Fe, total dust flux, dust AOT, and dust influence category (heavy-influence vs. low-influence). There were insufficient replicates to compare day-to-day change; therefore, categorical analyses were required. For each variable (except dust influence), measurements were split into quartiles (lowest concentration quartile referred to as CAT1 and highest concentration quartile referred to as CAT4). We defined dust influence days as heavy-influence using a threshold of NAAPS dust AOT greater than 0.075 (Seim et al., in prep).

Statistical analyses and data visualization were performed in R Studio (R version 3.5.0; R Core Development Team, 2018). Differential expression analysis was conducted using the DESeq2 package in R Studio (Love & Anders, 2014). Differential expression was tested across *in situ* tFe, *in situ* dFe, DOC, aerosol Fe, total dust flux, and dust AOT grouped by quartiles (CAT1 to CAT4) as well as between heavy-influence and low-influence days. In order to visualize metatranscriptome DESeq2 results in R Studio, the ortholog cluster counts for each sample in the entire dataset was divided by the total number of protein-annotated reads for that date (R Core Development Team, 2018). This normalization procedure did not include ortholog clusters identified as “unknown.” Heatmaps were generated using the heatmap.2() function (gplots package) with the parameter scale=“row” to scale data by ortholog cluster across sampling dates according to a row-centered Z-score (values in each row, excluding NAs, were subtracted by the row mean) divided by the row’s standard deviation (Warnes et al., 2015). A dendrogram was also created using the heatmap.2() function parameter Rowv=TRUE (Warnes et al., 2015). Boxplots for individual ortholog clusters by category was plotted using the ggplot2 package (Wickham, 2016). Significant differences in ortholog cluster expression was determined using the compare\_means() function which uses Student’s t-Test or Wilcoxon Rank Sum and Signed Rank test to compare the means between sample categories (De Winter, 2013; Wilcoxon et al., 1970).

Beta-diversity analyses were conducted for DOC, DOC (lagged 24 h; as described in Borchardt et al., 2020), *in situ* dFe, *in situ* dFe (lagged 24 h), as well as between heavy-influence and low-influence days. Beta-diversity was visualized using non-metric multidimensional scaling analysis (NMDS) using non-parametric rank-order correlation to

avoid bias due to variability in ortholog cluster abundances. The metaMDS() function from the vegan package was utilized with the parameter autotransform=FALSE to calculate multidimensional scaling scores using monoMDS(), and NMDS plots were visualized using the ggplot2 package (Borchardt et al., 2020; Oksanen et al., 2018; Wickham, 2016). Unweighted Bray-Curtis dissimilarity was calculated using the vegdist() function from the vegan package (Oksanen et al., 2018). Beta-diversity dissimilarity was analyzed using a permutational multivariate analysis of variance (PERMANOVA) using the adonis() function followed by a pairwise PERMANOVA test using the pairwise.perm.manova() function from the RVAideMemoire package (Anderson et al., 2014; Hervé, 2018). Beta-diversity variability was measured using dispersion (the distance between a sample's dissimilarity and the grouping variable's centroid, the median dissimilarities between samples). Dispersions were analyzed using the PERMDISP2 procedure by testing the homogeneity of multivariate dispersions using the betadisp() function from the vegan package (Anderson, 2006a; Anderson, 2006b; Oksanen et al., 2018). A separate permutation test for pairwise multivariate group dispersions was conducted by the permutest.betadisp() function from the vegan package (Oksanen et al., 2018).

## Results

The average number of mRNA sequence reads that passed quality filtering parameters was 2,990,105 reads per sample with a range of 654,475 to 12,490,781 (n=21; **Table S5.1**). The percentage of mRNA reads varied between samples, ranging from 5% to 57%. The percent of reads identified as non-mRNA averaged 64%, and the average of unknown reads was 25%. A total of 12,081 ortholog clusters were observed in the metatranscriptome of Looe

Key surface water over the 26 d time series. To reach the number of replicates needed for differential expression analysis, quartiles were calculated for each environmental variable and samples were grouped based on associated daily concentrations of *in situ* tFe, *in situ* dFe, DOC, aerosol Fe, total dust flux, and dust AOT grouped by quartiles (CAT1 to CAT4; **Table 5.1**) as well as heavy-influence and low-influence days grouped by a dust AOT threshold of greater than 0.075 (Seim et al., in prep). A detailed summary of the concentrations of each environmental variable is available in Borchardt et al. (2020).

DESeq2 analyses revealed three environmental variables had a significant effect on the differential expression of ortholog clusters: *in situ* dFe, DOC, and dust AOT (**Table 5.2, Figure 5.1**). *In situ* dFe concentrations across the 26 d time series (n=19) averaged 2.25 nmol L<sup>-1</sup> ( $\pm$  0.89 SD; range: 0.81 to 3.74 nmol L<sup>-1</sup>). The first *in situ* dFe quartile ranged from 0.81 to 1.55 nmol L<sup>-1</sup> (CAT1; n=5), the second ranged from 1.62 to 2.12 nmol L<sup>-1</sup> (CAT2; n=5), the third ranged from 2.18 to 2.56 nmol L<sup>-1</sup> (CAT3; n=4), and the fourth ranged from 3.14 to 3.74 nmol L<sup>-1</sup> (CAT4; n=5).

DOC concentrations (n=20) averaged 87.7  $\mu$ m L<sup>-1</sup> ( $\pm$  16.6 SD; range: 69 to 126.7). The first DOC quartile ranged from 69 to 77.4  $\mu$ m L<sup>-1</sup> (CAT1; n=6), the second ranged from 78.3 to 84.5  $\mu$ m L<sup>-1</sup> (CAT2; n=4), the third ranged from 85.3 to 89.3  $\mu$ m L<sup>-1</sup> (CAT3; n=5), and the fourth ranged from 92 to 126.7  $\mu$ m L<sup>-1</sup> (CAT4; n=5).

Dust AOT (n=21) averaged 0.0784 ( $\pm$  0.04 SD; range: 0.025 to 0.1525). The first dust AOT quartile ranged from 0.025 to 0.0475 (CAT1; n=6), the second ranged from 0.05 to 0.075 (CAT2; n=5), the third ranged from 0.08 to 0.0975 (f; n=5), and the fourth ranged from 0.1 to 0.1525 (CAT4; n=5).

*Differential expression: In situ dFe*

Ten ortholog clusters varied between categories of *in situ* dFe (**Figures 5.2, 5.3**). Three ortholog clusters (NOG315155, NOG331261, NOG330822) showed general upregulation on 23 July, 25 July, and 27 July, and out of these three ortholog clusters, two (NOG315155, NOG331261) were significantly upregulated in *in situ* dFe CAT1 compared to CAT4 (p-value=0.016 and 0.008, respectively). Four ortholog clusters (NOG243116, NOG296499, COG0071, NOG288790) were generally upregulated with increasing *in situ* dFe. NOG243116 exhibited greater gene expression in CAT4 compared to CAT1 (p-value=0.016) and CAT2 (p-value=0.036). Ortholog clusters that varied by *in situ* dFe included orthologs identified with broad functions such as stress response, genetic information processing, signal transduction mechanisms, replication and repair, and carbohydrate transport and metabolism (**Figure 5.3**).

*Differential expression: DOC*

Eighteen ortholog clusters varied between categories of dissolved organic carbon (**Figures 5.4, 5.5**). Nine ortholog clusters (COG1544, COG1825, COG2190, COG4166, COG4864, NOG256212, NOG25698, NOG260736, NOG331239) showed a general decrease in expression with increasing DOC category. We observed upregulation of these ortholog clusters in the lower CAT1 and CAT2 on 24 July, 28 July, and 29 July. Six out of the nine orthologs had significantly lower expression in CAT4 than CAT1 (COG2190 p-value=0.040, COG4166 p-value=0.030, NOG256212 p-value=0.017, NOG25698 p-value=0.017, NOG260736 p-value=0.006, NOG331239 p-value=0.017). Additionally, six ortholog clusters (COG0071, COG1592, COG1629, COG4772, NOG120039, NOG71724)

were generally upregulated on 12 July and 01 August, which are both CAT4 days. Out of those six orthologs, all but one (COG0071), was expressed more in CAT4 than CAT1. Similarly, NOG243116 and NOG280772 exhibited greater expression on CAT4 days 14 July and 01 August and were significantly upregulated in CAT4 compared to CAT1 (p-values=0.008 and 0.004, respectively). Lastly, COG0225 was significantly more expressed in CAT1 compared to CAT4 (p-value=0.004). Ortholog clusters that varied by DOC included orthologs identified with broad functions replication and repair, signal transduction mechanisms, defense mechanisms, genetic information processing, and energy production and conversion (**Figure 5.5**).

*Differential expression: dust AOT*

Six ortholog clusters varied between categories of dust AOT (**Figures 5.6, 5.7**). COG0081 and COG0093 showed general upregulation on 15 July and 01 August, with significant downregulation in CAT4 compared to CAT1 (p-value=0.032 and 0.016, respectively), CAT2 (p-value=0.029 and 0.029, respectively), and CAT3 (p-value=0.029 and 0.029, respectively). Similarly, NOG250938 showed a general increase in expression on 15 July, 16 July and 05 July, with significant downregulation in CAT4 compared to CAT1 (p-value=0.032). COG3637 showed an alternating trend in expression from 27 July to 05 August, with CAT2 and CAT4 showing significantly higher expression when compared to CAT1 (p-value=0.030 and 0.015, respectively). Ortholog clusters that varied by dust AOT included orthologs identified with broad functions such as carbohydrate transport and metabolism, defense mechanisms, replication and repair, and genetic information processing (**Figure 5.7**).

### *Bray-Curtis dissimilarity analysis*

Unweighted Bray-Curtis dissimilarity (representing all ortholog clusters in the dataset) for each sample did not significantly change by all but one environmental variable. Daily DOC concentration affected the metatranscriptome beta-diversity (p-value=0.02; **Figure 5.8**). Similarly, beta-diversity dispersions did not vary by any of the tested environmental variables, even when the environmental variable concentrations were lagged by 24 h to account for potential dust deposition delays and lagged microbial response (as described in Westrich et al. (2018) and Borchardt et al. (2020)).

## **Discussion**

In this study, we investigated the effects of Saharan dust nutrient deposition on the metatranscriptome of Looe Key Reef, FL, US surface water microbial communities in a 26 d time series from 12 July to 06 August 2016. We tested six environmental variables that fluctuated across the 26 d field study against the expression of Looe Key ortholog clusters. We primarily observed specific expressional patterns of ortholog clusters unique to the concentration levels (characterized into four categories) of three environmental parameters: *in situ* dFe, DOC, and dust AOT. In general, these changes in gene expression are likely natural microbial responses to the sudden availability of nutrients that are limited in the SE Atlantic/Caribbean and have been shown to drive metabolic activity (Davey et al., 2008; Guieu et al., 2014; Guo et al., 2016; Tan et al., 2019).

NADH dehydrogenase activity (NOG315155) has been observed as part of the production of ubiquinone in the transport chain in the plasma membranes of prokaryotes (Gaudet et al., 2011; Morgat et al., 2019). Some *Proteobacteria* have developed plasticity

in producing ubiquinone in oxygen-dependent and oxygen-independent pathways given the gradient of environmental oxygen levels (Pelosi et al., 2019). In Borchardt et al. (2020), *Proteobacteria* abundance was higher on 21 July and 29 July when *in situ* dFe concentrations were above average. Given ubiquinone is used in the aerobic respiratory chain, *Proteobacteria* potentially decreased aerobic respiration when a decrease in oxygen may have followed a bloom in *Proteobacteria* due to dust deposition metabolic activity. This could suggest a reason why NADH dehydrogenase activity was significantly upregulated when *in situ* dFe (bioavailable iron) concentrations are lower and oxygen levels are higher. For example, iron-oxidizing bacteria metabolize  $\text{Fe}^{2+}$  better in low oxygen conditions where there is less oxygen to oxidize the iron and make it unusable to the iron-oxidizing bacteria (Emerson, 2016).

While not statistically significant, a potential porin (NOG330822) showed generally higher expression when *in situ* dFe levels were lower. In Gram-negative bacteria, a homologue of porin is present in the trans-outer-membrane channel of certain (Tanoue et al., 1995). Due to porin's availability as a source of protein during low nutrient conditions, bacteria without protective lipid bi-layers, such as *Synechococcus*, are exposed to bottom-up controls of their population "protease attack" of their membranes and must upregulate porin production (Miyoshi & Suzuki et al., 2004). After dust nutrient influx, Borchardt et al. (2020) observed increasing abundances of *Synechococcus*, and we observed decreasing defensive porin expression, suggesting that the influx of dust alleviates stress from oligotrophic conditions (Tanoue et al., 1995).

Similarly, during CAT1 when the bacterial community is stressed due to low nutrient conditions (i.e., lower concentrations of both *in situ* dFe and DOC), bacteria may

be competing for nutrients and proteins meanwhile expressing the general stress protein 17M (NOG25698). It has been observed that once nutrients are back in the system (such as when conditions shift to CAT4 for *in situ* dFe and DOC), new sources of carbon decrease the expression of the general stress protein 17M by cyanobacteria like *Synechecoccus* and picocyanobacteria such as *Prochlorococcus* (Long et al., 2019). Borchardt et al. (2020) may have observed this dynamic reflected in the dip in concentration of *Prochlorococcus* prior to dust arrival and increased concentrations of *Synechecoccus* after dust deposition.

The production of PHUM524800 (NOG243116), identified from a *Wolbachia* strain collected from *Pediculus humanus*, was significantly upregulated at the highest level (CAT4) of *in situ* dFe and DOC compared to CAT1 and CAT2 (Baldo et al., 2005; Wilke et al., 2012). *Wolbachia* is a member of the Alphaproteobacteria subclass, and while the function of the protein is unknown, the abundance of *Alphaproteobacteria* represented > 5% of microbial communities for both surface water incubation experiments and time series analysis completed by Borchardt et al. (2020). Additionally, *Alphaproteobacteria* are one of three primary phyla in airborne Asian (Kosa) dust, suggesting members of *Alphaproteobacteria* that produce PHUM524800 may survive the trans-Atlantic atmospheric journey to the Caribbean before deposition (Maki et al., 2014). *Sphingomonas* spp. have been observed as to metabolize carbon more efficiently than competing bacteria on plants leaves, using this function as niche defense (Innerebner et al., 2011).

Two ortholog clusters related to the processing of iron were upregulated in CAT4 DOC conditions: COG1629 (outer membrane Fe transport/receptor) and COG4772 (outer membrane receptor for Fe<sup>3+</sup>-dicitrate. These two functions are associated with energy-independent systems on the outer membranes of bacteria used for iron uptake (Andrews et

al., 2003). For some bacteria, such as *E. coli*, dicitrate can be used as a source of carbon and energy, however, citrate can also be used as an ion chelator and bind to  $\text{Fe}^{3+}$  to acquire this essential nutrient of life that controls pathogenesis, immune defense, signal transduction and more (Pintor et al., 1994). For example, *Neisseria* spp., mostly known for causing life-threatening sepsis in humans, also uses dicitrate as “shuttle” for iron which can increase its ability to live in symbiosis with marine mammals such as dolphins or live as free living species in habitats such as dust in the sub-Saharan Desert (Liu et al., 2015; Pintor et al., 1994). Interestingly, during the dry seasons, scientists have used wind speeds and dust load in the Saharan Desert and Sahel region to predict meningitis outbreaks in Africa (García-Pando et al., 2014).

During CAT1 and CAT2, when DOC concentrations are low, microbial communities are working harder to find and metabolize carbon for energy. We observed the upregulation of phosphotransferase system IIA components (COG2190) and ABC-type oligopeptide transporters (COG4166) that work to bind to and transport carbohydrates (Jeckelmann et al., 2019; Rahman et al., 2019). Similarly, when DOC concentrations are low, the expression of ankyrin repeat genes (NOG260736) is more active in CAT1 compared to CAT4. In corals, ankyrin repeat genes are associated with the immune system and is particularly active when trace metals are scarce (Li et al., 2020). Likewise, peptide methionine sulfoxide reductase (COG0225) production is greater in DOC CAT1 and CAT2 compared to CAT3 and CAT4 to produce a rescue protein that prevents damage from oxidative stress (Hsu et al., 2012).

Alternatively, rubrerythrin protein genes (COG1592) and Ragb/Susd domain protein genes (NOG120039) are upregulated in DOC CAT4 compared to CAT1.

Rubrerhythrin is a non-heme iron protein that has potential function as an enzyme that utilizes O<sub>2</sub> and Fe<sup>2+</sup> in a non-toxic pathway for bacteria such as *Desulfovibrio vulgaris* that are anaerobic sulfate-reducing bacteria (Bonomi et al., 1996). This feature is particularly important for *Desulfovibrio* and related heterotrophic diazotrophs including *Pseudomonas*, which have enhanced capabilities to be transported by dust aerosol across the Atlantic (Bonomi et al., 1996; Kakikawa et al., 2008). The Ragb/Susd domain gene function has been identified in *Flavobacteria* and observed to facilitate the taxa's attachment and growth on organic matter (Dong et al., 2014). Importantly, a member of Flavobacteriaceae was the single most abundant member of the Looe Key, Florida, USA surface water microbial community three days after a major Saharan dust event (Borchardt et al., 2020).

Dust deposition in the marine surface waters has been shown to stimulate biological responses, presumably from the deposition of a combination of iron, nitrate, phosphorus, and carbon to ocean ecosystems (Eblings & Landing, 2015; Borchardt et al., 2019; Westrich et al., 2016; Westrich et al., 2018). This is supported by studies that investigated the response of bacterial communities in the subtropical and tropical northeast Atlantic (e.g., Davey et al., 2008; Duarte et al., 2016; Hill et al., 2010; Mills et al., 2004) and Mediterranean Sea (e.g., Feingersch et al., 2010; Guo et al., 2016; Herut et al., 2006; Laghdass et al., 2011; Pitta et al., 2017; Pulido-Villena et al., 2008) where dust deposition is perhaps more frequent and more intense due to the proximity of desert dust sources includes. While trans-Atlantic dust experiences longer atmospheric suspension times, episodic Saharan dust still delivers critical trace metals that are key limiting nutrient in marine systems (Lenes et al., 2001; Mills et al., 2004; Moxim et al., 2011). For example, iron is a limiting factor that can affect biological responses of autotrophic and heterotrophic

microorganisms (e.g., Emerson et al., 2016; Hill et al., 2010; Langlois et al., 2012; Maranen et al., 2010; Mahowald et al., 2009; Sunda et al., 2012). In particular, heterotrophic taxa from the *Vibrio* genus have been noted to increase in both relative and absolute abundance within a few hours of trans-Atlantic dust arrival and were associated with increased concentrations of iron (Westrich et al., 2016). Along with picocyanobacteria and heterotrophic bacteria, *Chaetoceros* have been significantly positively associated with increased DOC and dissolved iron in the water column of the concurrent study (Borchardt et al., 2020). These abundant diatoms are known to bloom in marine waters and may be primary contributors to the cycling of DOC (Booth et al., 2002).

## Conclusion

Our study identifies several ortholog clusters that vary due to nutrient input, particularly DOC, from Saharan dust deposition events. For example, in the North Atlantic Ocean, Saharan dust was linked in increases of carbon cycling. Therefore, we support the observations of changing microbial community composition and structure in a concurrent study that showed strong correlations between beta-diversity and DOC. While we were limited in the number of samples collected and the high variability of mRNA reads between samples, our study makes progress in understanding microbiome dynamics at a temporal scale (26 d time series) that could reveal temporal-specific patterns and may explain successional patterns of specific taxa in response to dust deposition (such as those observed in Borchardt et al., 2020). Future studies should implement long-term, field-based sampling for transcriptomic and metabolomic analyses that will further elucidate how dust-derived nutrients are being utilized in marine surface water. Identifying functional responses

associated with marine microbiomes experiencing episodic nutrient deposition could have wide implications to the cycling of nutrients (especially carbon) in marine ecosystems, particularly if natural, episodic events change in frequency and intensity under future climate change conditions.

### **Acknowledgements**

This work was supported by NSF Biological Oceanography award OCE 1357423 (EKL and EAO) and NSF Graduate Research Fellowship Program (GRFP) 1842396 (MGB). Field-work would not have been possible without Captain James “Buddy” Williams’ expertise and enthusiasm for science.

## Tables

**Table 5.1. Environmental variables.** Three out of six environmental variables yielded significant differential expression results. Quartiles were calculated for each variable and singleton surface water samples (n=21) were grouped into categories based on associated daily concentrations. Surface water dissolved Fe (*in situ* dFe; n=19), dissolved organic carbon (DOC; n=20), dust aerosol optical thickness (dust AOT; n=21), category (CAT).

| Date   | Aerosol Fe | Aerosol Fe CAT | Total Dust Flux | Total Dust Flux CAT | Dust AOT | Dust AOT CAT | <i>In situ</i> tFe | <i>In situ</i> tFe CAT | <i>In situ</i> dFe | <i>In situ</i> dFe CAT | DOC   | DOC CAT |
|--------|------------|----------------|-----------------|---------------------|----------|--------------|--------------------|------------------------|--------------------|------------------------|-------|---------|
| Jul 12 | NA         | NA             | NA              | NA                  | 0.063    | CAT2         | 5.47               | CAT1                   | 3.74               | CAT4                   | 124.2 | CAT4    |
| Jul 13 | NA         | NA             | NA              | NA                  | 0.098    | CAT3         | 7.68               | CAT2                   | 2.56               | CAT3                   | 89.3  | CAT3    |
| Jul 14 | NA         | NA             | NA              | NA                  | 0.080    | CAT3         | 10.10              | CAT2                   | 3.67               | CAT4                   | 126.7 | CAT4    |
| Jul 15 | NA         | NA             | 0.258           | CAT1                | 0.025    | CAT1         | 14.20              | CAT4                   | 2.51               | CAT3                   | 93.5  | CAT4    |
| Jul 16 | 0.16       | CAT1           | 0.678           | CAT1                | 0.025    | CAT1         | 25.90              | CAT4                   | NA                 | NA                     | 92.0  | CAT4    |
| Jul 20 | 3.03       | CAT2           | 2.373           | CAT2                | 0.048    | CAT1         | NA                 | NA                     | NA                 | NA                     | NA    | NA      |
| Jul 22 | 1.50       | CAT1           | 1.336           | CAT1                | 0.040    | CAT1         | 10.30              | CAT3                   | 1.47               | CAT1                   | 85.4  | CAT3    |
| Jul 23 | 0.97       | CAT1           | 1.971           | CAT1                | 0.038    | CAT1         | 3.15               | CAT1                   | 1.48               | CAT1                   | 74.1  | CAT1    |
| Jul 24 | 1.39       | CAT1           | 3.022           | CAT2                | 0.045    | CAT1         | 3.79               | CAT1                   | 1.65               | CAT2                   | 77.4  | CAT1    |
| Jul 25 | 2.21       | CAT2           | 7.944           | CAT3                | 0.063    | CAT2         | 5.96               | CAT2                   | 0.81               | CAT1                   | 69.0  | CAT1    |
| Jul 26 | 5.93       | CAT3           | 2.234           | CAT1                | 0.080    | CAT3         | 3.61               | CAT1                   | 1.55               | CAT1                   | 74.6  | CAT1    |
| Jul 27 | 1.85       | CAT1           | 4.347           | CAT2                | 0.050    | CAT2         | 13.70              | CAT4                   | 1.85               | CAT2                   | 84.5  | CAT2    |
| Jul 28 | 3.26       | CAT2           | 16.060          | CAT4                | 0.143    | CAT4         | 6.97               | CAT2                   | 2.22               | CAT3                   | 80.0  | CAT2    |
| Jul 29 | 12.42      | CAT4           | 17.484          | CAT4                | 0.123    | CAT4         | 3.69               | CAT1                   | 1.31               | CAT1                   | 70.0  | CAT1    |
| Jul 30 | 13.52      | CAT4           | 4.769           | CAT3                | 0.113    | CAT4         | 33.00              | CAT4                   | 2.18               | CAT3                   | 78.3  | CAT2    |
| Jul 31 | 3.49       | CAT3           | 14.792          | CAT4                | 0.075    | CAT2         | 13.50              | CAT3                   | 3.70               | CAT4                   | 78.3  | CAT2    |
| Aug 01 | 11.62      | CAT4           | 7.810           | CAT3                | 0.100    | CAT4         | 17.80              | CAT4                   | 3.39               | CAT4                   | 116.5 | CAT4    |
| Aug 03 | 5.69       | CAT3           | 15.621          | CAT4                | 0.093    | CAT3         | 9.07               | CAT2                   | 1.62               | CAT2                   | 77.4  | CAT1    |
| Aug 04 | 12.88      | CAT4           | 6.717           | CAT3                | 0.153    | CAT4         | 11.00              | CAT3                   | 3.14               | CAT4                   | 89.1  | CAT3    |
| Aug 05 | 5.56       | CAT3           | 3.665           | CAT2                | 0.093    | CAT3         | 10.10              | CAT2                   | 1.77               | CAT2                   | 89.3  | CAT3    |
| Aug 06 | 3.10       | CAT2           | NA              | NA                  | 0.065    | CAT2         | 10.10              | CAT2                   | 2.12               | CAT2                   | 85.3  | CAT3    |

**Table 5.2. Significant differential gene expression.** Significant differential gene expression categorized by environmental variable (*in situ* dFe, DOC, and dust AOT) and compared between categories (1 through 4). P-values were calculated using Student's t-Test or Wilcoxon Rank Sum and Signed Rank test depending on data normality as determined by Shapiro-Wilk normality tests.

| Variable           | Ortholog Cluster | Category      |               |               |               |               |               |
|--------------------|------------------|---------------|---------------|---------------|---------------|---------------|---------------|
|                    |                  | 1 vs. 2       | 1 vs. 3       | 1 vs. 4       | 2 vs. 3       | 2 vs. 4       | 3 vs. 4       |
| <i>In situ</i> dFe | COG0071          | > 0.05        | > 0.05        | > 0.05        | > 0.05        | > 0.05        | > 0.05        |
|                    | COG1393          | <b>0.0317</b> | > 0.05        | <b>0.0079</b> | > 0.05        | > 0.05        | > 0.05        |
|                    | COG4166          | > 0.05        | > 0.05        | <b>0.0079</b> | > 0.05        | > 0.05        | > 0.05        |
|                    | NOG243116        | > 0.05        | <b>0.016</b>  | <b>0.016</b>  | > 0.05        | <b>0.036</b>  | > 0.05        |
|                    | NOG25698         | > 0.05        | > 0.05        | <b>0.0075</b> | > 0.05        | > 0.05        | > 0.05        |
|                    | NOG288790        | > 0.05        | > 0.05        | > 0.05        | > 0.05        | > 0.05        | > 0.05        |
|                    | NOG296499        | > 0.05        | <b>0.037</b>  | > 0.05        | > 0.05        | > 0.05        | > 0.05        |
|                    | NOG315155        | > 0.05        | > 0.05        | <b>0.016</b>  | > 0.05        | > 0.05        | <b>0.016</b>  |
|                    | NOG330822        | > 0.05        | > 0.05        | > 0.05        | > 0.05        | <b>0.032</b>  | <b>0.032</b>  |
|                    | NOG331261        | > 0.05        | > 0.05        | <b>0.0079</b> | > 0.05        | <b>0.0159</b> | > 0.05        |
| DOC                | COG0071          | > 0.05        | > 0.05        | > 0.05        | > 0.05        | > 0.05        | > 0.05        |
|                    | COG0225          | > 0.05        | <b>0.0173</b> | <b>0.0043</b> | <b>0.0317</b> | <b>0.0159</b> | > 0.05        |
|                    | COG1544          | > 0.05        | > 0.05        | > 0.05        | > 0.05        | > 0.05        | > 0.05        |
|                    | COG1592          | > 0.05        | > 0.05        | <b>0.0087</b> | > 0.05        | <b>0.0159</b> | <b>0.0159</b> |
|                    | COG1629          | > 0.05        | > 0.05        | <b>0.0043</b> | > 0.05        | <b>0.0159</b> | <b>0.0079</b> |
|                    | COG1825          | > 0.05        | > 0.05        | > 0.05        | > 0.05        | > 0.05        | > 0.05        |
|                    | COG2190          | > 0.05        | > 0.05        | <b>0.04</b>   | > 0.05        | > 0.05        | > 0.05        |
|                    | COG4166          | > 0.05        | <b>0.0087</b> | <b>0.0303</b> | > 0.05        | > 0.05        | > 0.05        |
|                    | COG4772          | > 0.05        | <b>0.035</b>  | <b>0.008</b>  | > 0.05        | > 0.05        | > 0.05        |
|                    | COG4864          | > 0.05        | > 0.05        | > 0.05        | > 0.05        | <b>0.019</b>  | > 0.05        |
|                    | NOG120039        | <b>0.0209</b> | <b>0.0067</b> | <b>0.0067</b> | > 0.05        | <b>0.0159</b> | <b>0.0079</b> |
|                    | NOG243116        | <b>0.0247</b> | > 0.05        | <b>0.008</b>  | <b>0.0317</b> | > 0.05        | <b>0.0079</b> |
|                    | NOG256212        | > 0.05        | <b>0.017</b>  | <b>0.017</b>  | <b>0.042</b>  | <b>0.042</b>  | > 0.05        |
|                    | NOG25698         | > 0.05        | <b>0.028</b>  | <b>0.017</b>  | > 0.05        | <b>0.042</b>  | > 0.05        |
|                    | NOG260736        | > 0.05        | <b>0.0055</b> | <b>0.0055</b> | <b>0.0416</b> | <b>0.0416</b> | > 0.05        |
|                    | NOG280772        | > 0.05        | > 0.05        | <b>0.0043</b> | > 0.05        | <b>0.0159</b> | <b>0.0159</b> |
|                    | NOG331239        | > 0.05        | <b>0.028</b>  | <b>0.017</b>  | > 0.05        | <b>0.011</b>  | > 0.05        |
|                    | NOG71724         | > 0.05        | <b>0.008</b>  | <b>0.008</b>  | > 0.05        | <b>0.016</b>  | <b>0.032</b>  |
| Dust AOT           | COG0081          | > 0.05        | > 0.05        | <b>0.032</b>  | > 0.05        | <b>0.029</b>  | <b>0.029</b>  |
|                    | COG0093          | > 0.05        | > 0.05        | <b>0.016</b>  | > 0.05        | <b>0.029</b>  | <b>0.029</b>  |
|                    | COG1850          | > 0.05        | > 0.05        | > 0.05        | > 0.05        | > 0.05        | <b>0.029</b>  |
|                    | COG3637          | <b>0.03</b>   | > 0.05        | <b>0.015</b>  | > 0.05        | > 0.05        | > 0.05        |
|                    | NOG250938        | > 0.05        | > 0.05        | <b>0.032</b>  | > 0.05        | > 0.05        | > 0.05        |
|                    | NOG84501         | > 0.05        | > 0.05        | <b>0.018</b>  | > 0.05        | > 0.05        | <b>0.027</b>  |

## Figures

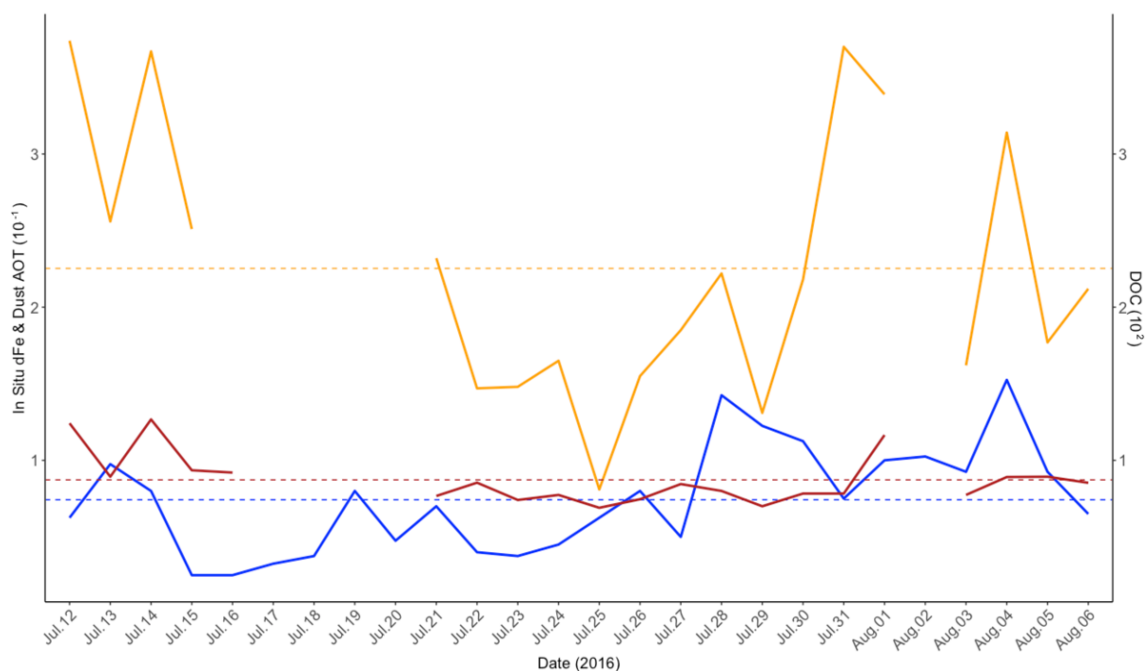


**Figure 5.1. Environmental variables across the time series.** Concentrations of surface water dissolved iron (orange line; *in situ* dFe; nmol L<sup>-1</sup>), dissolved organic carbon (red line; DOC; μm L<sup>-1</sup>), and dust aerosol optical thickness (blue line; dust AOT). Dashed lines represent each variable's mean across the 26 d collection period (*in situ* dFe mean=2.25 nmol L<sup>-1</sup>; DOC mean=0.87 μm L<sup>-1</sup>; dust AOT=0.74).

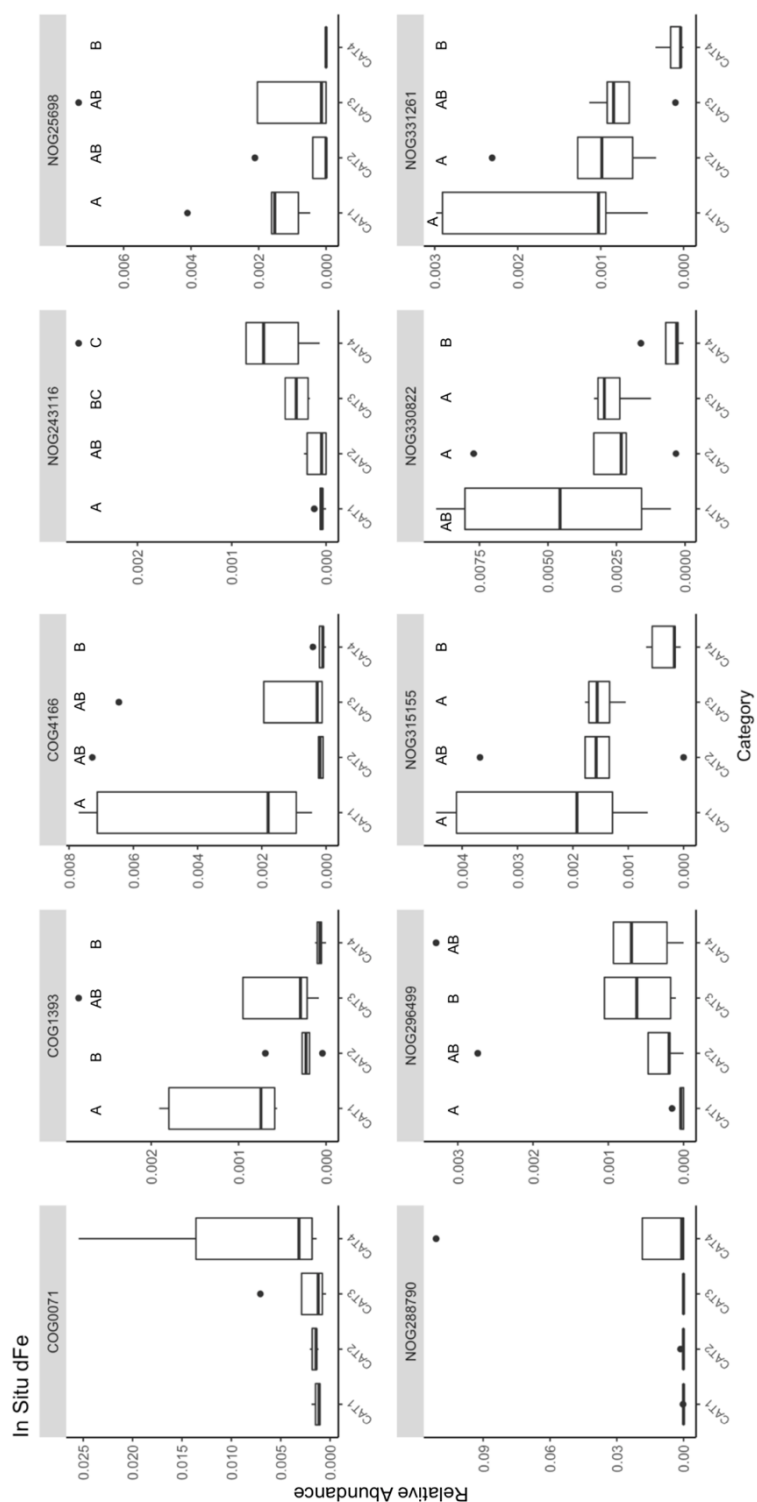


**Figure 5.2. *In situ* dFe differential expression by categories.** Relative abundance of ortholog clusters that vary by surface water dissolved iron (*in situ* dFe) categories. Letters denote statistical significance between categories within each plot. P-values are provided in **Table 5.2**.

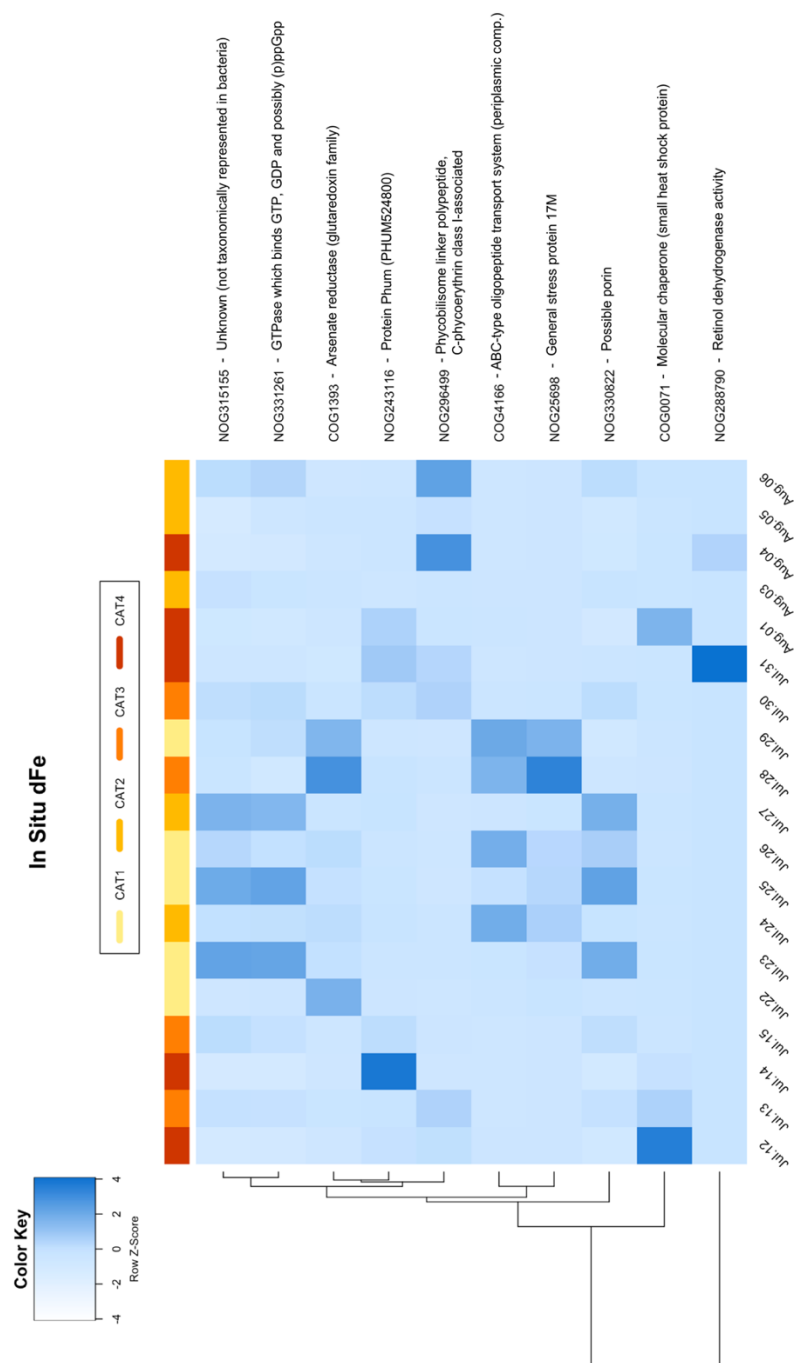


**Figure 5.3. *In situ* dFe differential expression by date.** Heatmap representing the relative abundance of ortholog clusters (rows) across the 26 d sampling period (columns). Featured ortholog clusters are those that varied significantly between two or more *in situ* dFe categories (CAT1 through CAT4). Functions are annotated to the right of ortholog cluster identification numbers. Z-score represents row-based scaling. Dendrogram represents similarities between ortholog clusters in response to *in situ* dFe categories.

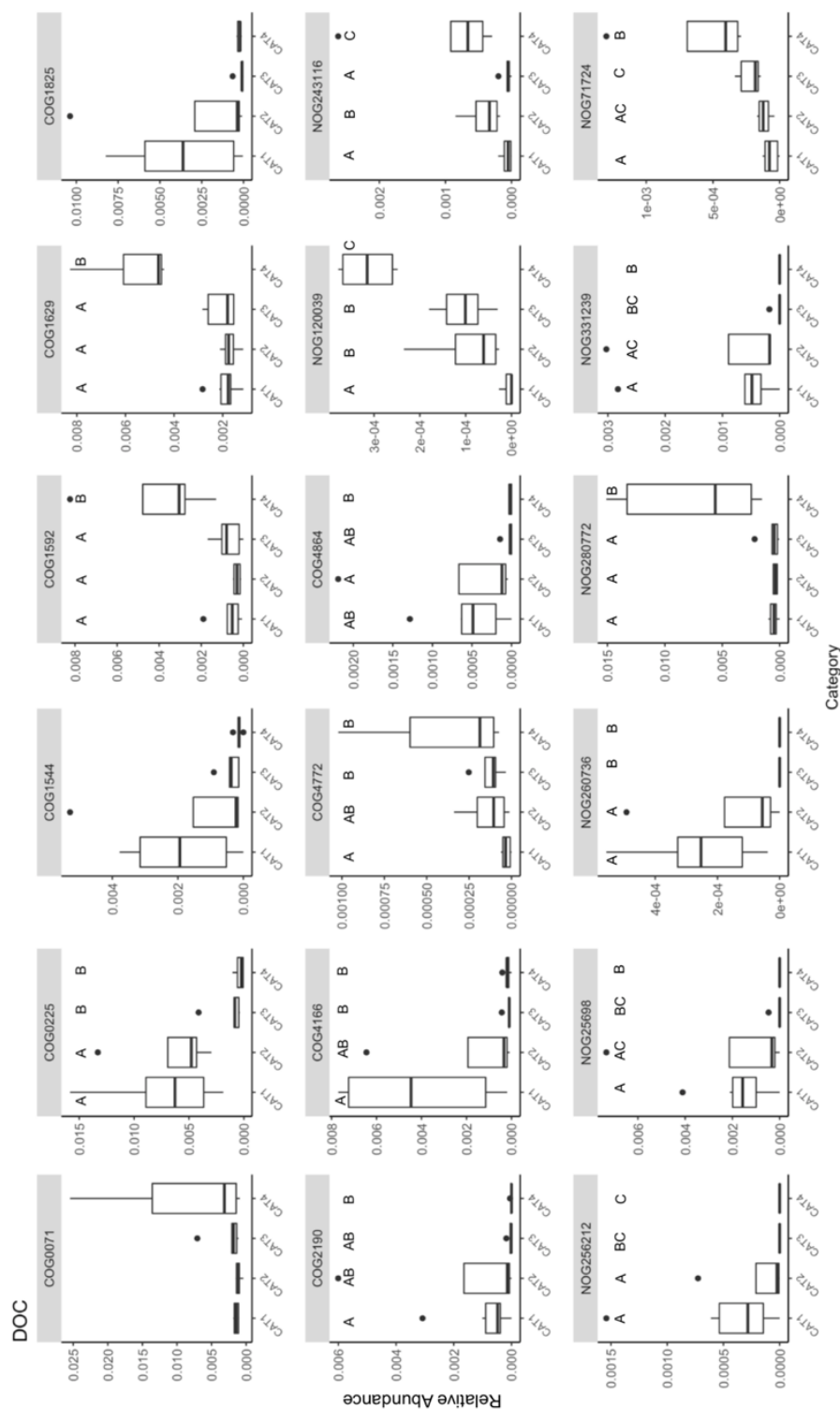


**Figure 5.4. DOC differential expression by categories.** Relative abundance of ortholog clusters that vary by dissolved organic carbon (DOC) categories. Letters denote statistical significance between categories within each plot. P-values are provided in **Table 5.2**.

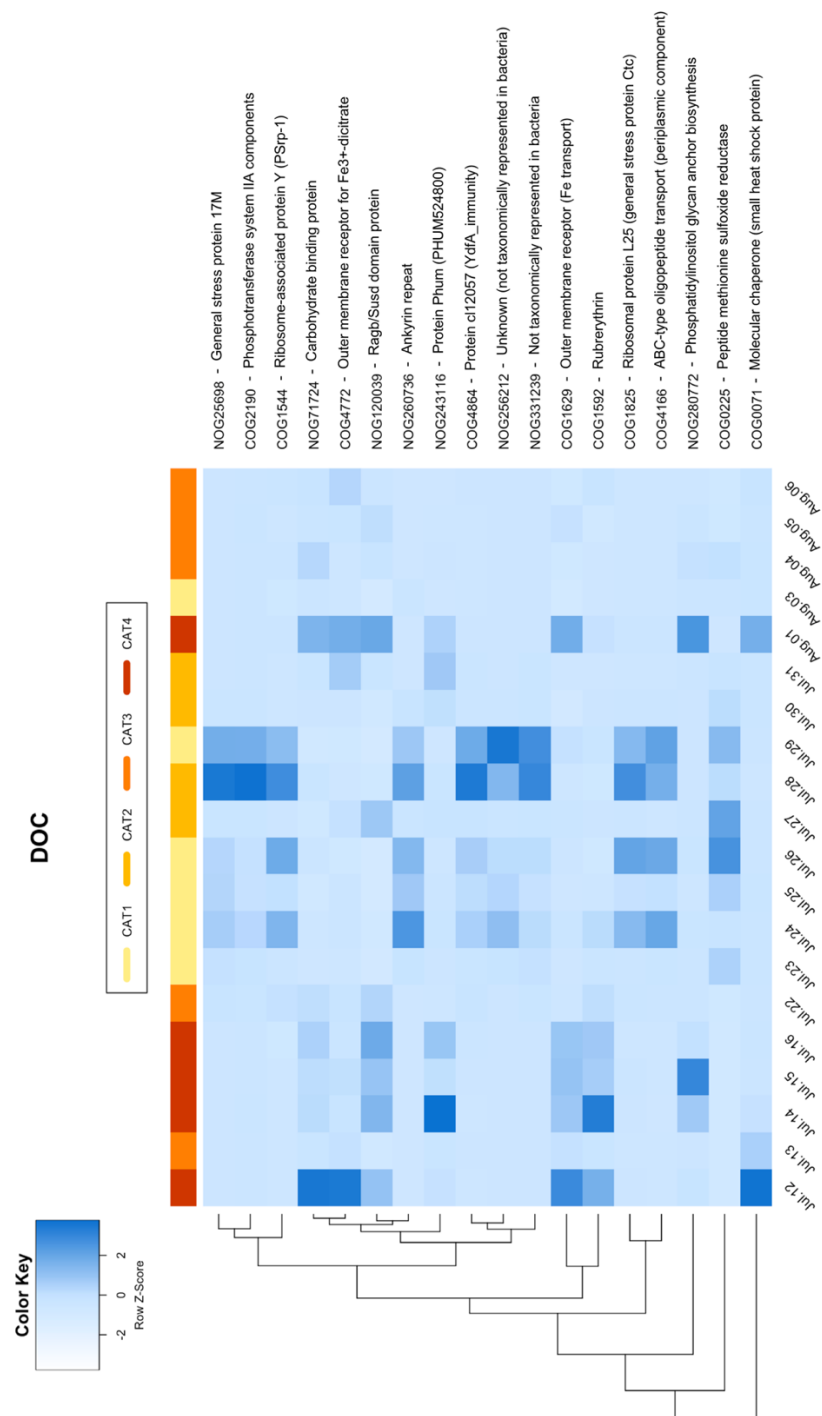


**Figure 5.5. DOC differential expression by date.** Heatmap representing the relative abundance of ortholog clusters (rows) across the 26 d sampling period (columns). Featured ortholog clusters are those that varied significantly between two or more dissolved organic carbon (DOC) categories (CAT1 through CAT4). Functions are annotated to the right of ortholog cluster identification numbers. Z-score represents row-based scaling. Dendrogram represents similarities between ortholog clusters in response to DOC categories.

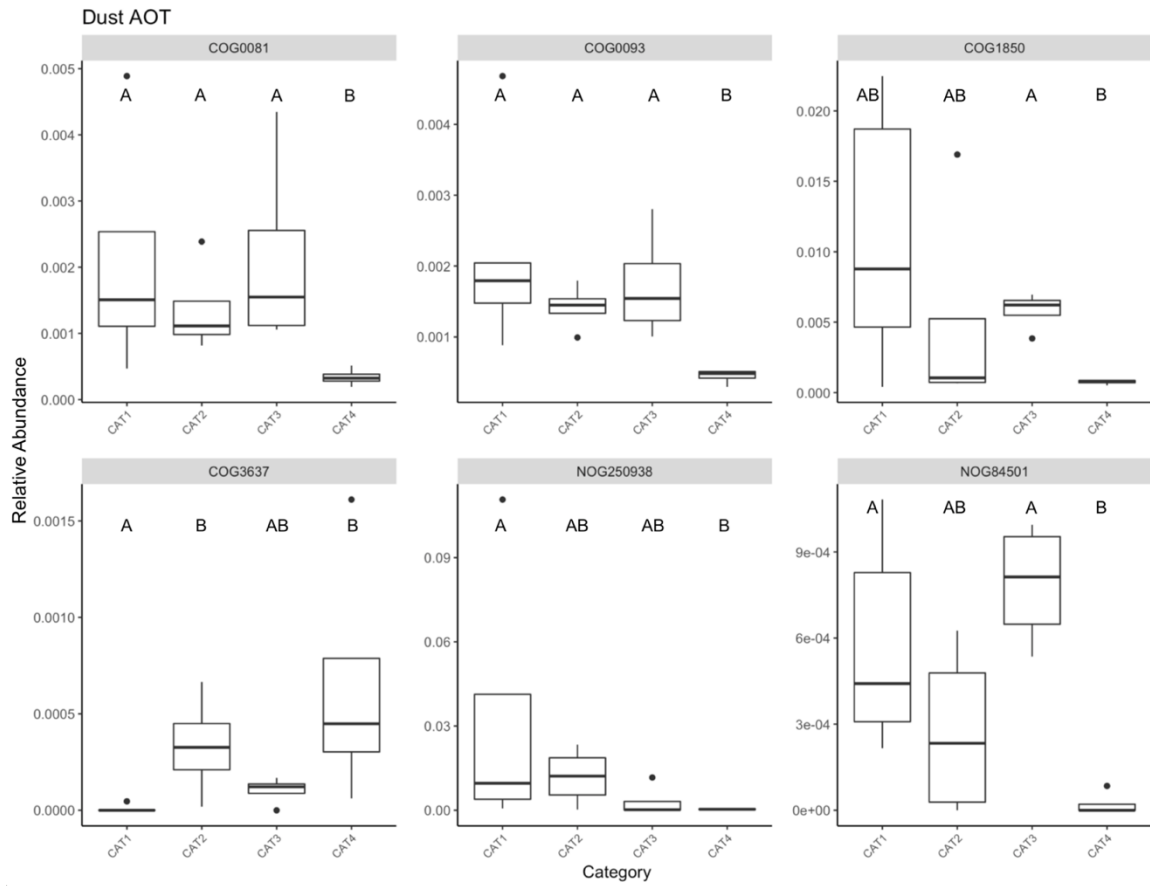


**Figure 5.6. Dust AOT differential expression by categories.** Relative abundance of ortholog clusters that vary across dust aerosol optical thickness (dust AOT) categories. Letters denote statistical significance between categories within each plot. P-values are provided in **Table 5.2**.

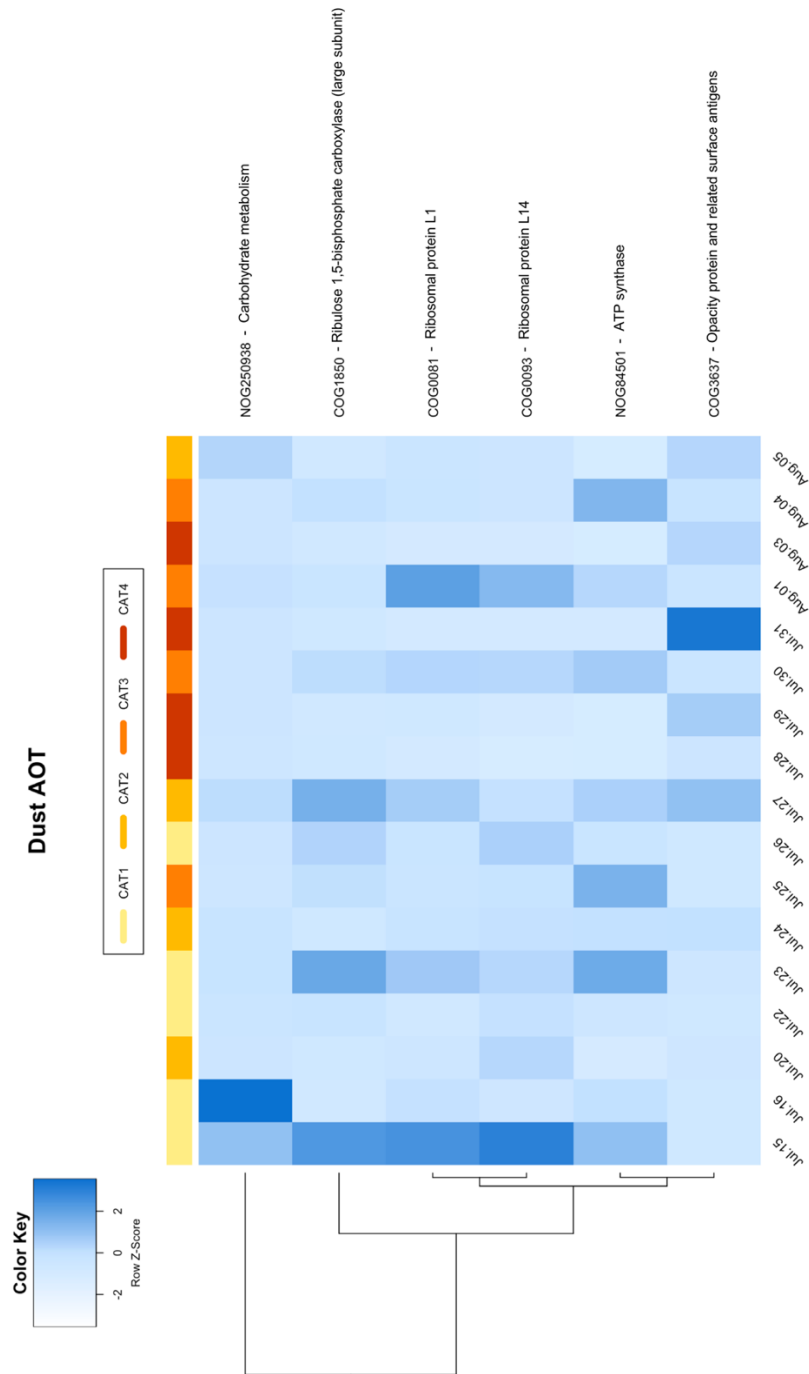


**Figure 5.7. Dust AOT differential expression by date.** Heatmap representing the relative abundance of ortholog clusters (rows) across the 26 d sampling period (columns). Featured ortholog clusters are those that varied significantly by dust AOT categories (CAT1 through CAT4). Functions are annotated to the right of ortholog cluster identification numbers. Z-score represents row-based scaling. Dendrogram represents similarities between ortholog clusters in response to dust AOT categories.

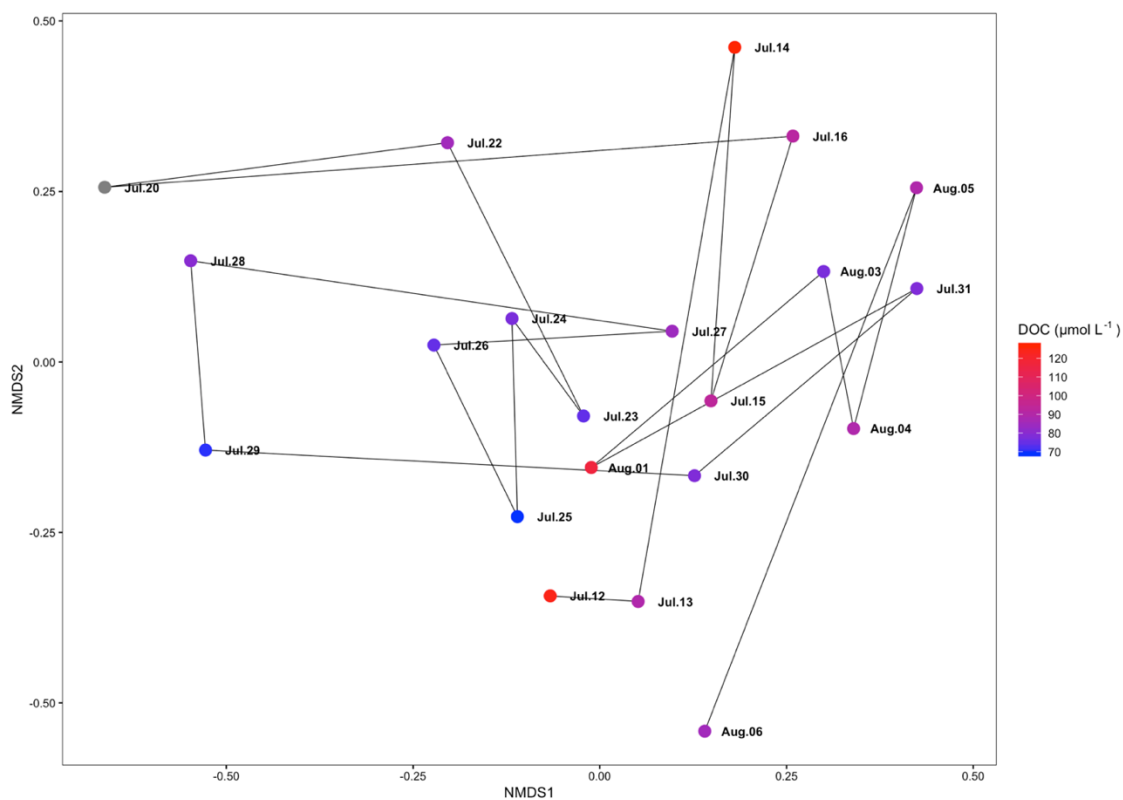


**Figure 5.8. NMDS for time series samples by date and DOC.** Non-metric multidimensional scaling (NMDS) plot of all ortholog clusters by date, connected chronologically by a black line. Point color represents DOC concentration ( $\mu\text{mol L}^{-1}$ ) for each day.

### Supplementary Table

**Table S5.1.** Sequencing summary for singleton samples collected over 26 d. cDNA was sequenced via NextSeq PE150. Columns represent total raw paired reads, total reads that passed filtering (PF Reads) and reads that were identified as mRNA, non-mRNA (including Silva rRNA reads characterized as 16S/18S/SSUref or 23S/28S/LSURef, Rfam reads, and tRNA reads), and unknown. Percentages were calculated by dividing raw reads by total PF reads (% of Raw) and dividing each of mRNA, non-mRNA, and unknown reads by total PF Reads.

| Sample | Raw Reads   |            | PF Reads |            | PF Reads |            |         |            |     | % of PF |
|--------|-------------|------------|----------|------------|----------|------------|---------|------------|-----|---------|
|        | Total       | Total      | % of Raw | mRNA       | % of PF  | non-mRNA   | % of PF | Unknown    |     |         |
| Jul.12 | 91,847,806  | 67,585,802 | 74%      | 5,024,352  | 7%       | 45,594,434 | 67%     | 16,967,016 | 25% |         |
| Jul.13 | 77,402,264  | 74,785,240 | 97%      | 5,782,714  | 8%       | 51,561,776 | 69%     | 17,440,750 | 23% |         |
| Jul.14 | 9,461,848   | 9,195,474  | 97%      | 857,300    | 9%       | 7,401,094  | 80%     | 937,080    | 10% |         |
| Jul.15 | 17,510,384  | 17,221,437 | 98%      | 1,551,497  | 9%       | 13,912,914 | 81%     | 1,757,026  | 10% |         |
| Jul.16 | 21,102,562  | 19,497,449 | 92%      | 2,024,082  | 10%      | 11,584,883 | 59%     | 5,888,484  | 30% |         |
| Jul.20 | 20,726,799  | 19,666,273 | 95%      | 2,131,912  | 11%      | 15,012,125 | 76%     | 2,522,236  | 13% |         |
| Jul.22 | 17,625,167  | 16,398,375 | 93%      | 1,008,443  | 6%       | 10,386,087 | 63%     | 5,003,845  | 31% |         |
| Jul.23 | 17,777,047  | 17,209,741 | 97%      | 1,606,153  | 9%       | 13,481,622 | 78%     | 2,121,966  | 12% |         |
| Jul.24 | 52,937,074  | 47,823,337 | 90%      | 7,391,689  | 15%      | 29,897,215 | 63%     | 10,534,433 | 22% |         |
| Jul.25 | 8,478,941   | 7,439,682  | 88%      | 654,475    | 9%       | 4,115,360  | 55%     | 2,669,847  | 36% |         |
| Jul.26 | 42,568,585  | 39,726,229 | 93%      | 2,299,779  | 6%       | 21,365,554 | 54%     | 16,060,896 | 40% |         |
| Jul.27 | 13,367,178  | 12,313,097 | 92%      | 1,119,257  | 9%       | 9,484,419  | 77%     | 1,709,421  | 14% |         |
| Jul.28 | 55,865,893  | 49,770,942 | 89%      | 2,453,754  | 5%       | 19,034,615 | 38%     | 28,282,573 | 57% |         |
| Jul.29 | 99,434,358  | 11,527,272 | 12%      | 6,557,055  | 57%      | 968,237    | 8%      | 4,001,980  | 35% |         |
| Jul.30 | 219,283,652 | 84,130,614 | 38%      | 12,490,781 | 15%      | 41,266,777 | 49%     | 30,373,056 | 36% |         |
| Jul.31 | 11,822,250  | 10,446,797 | 88%      | 1,048,493  | 10%      | 8,130,179  | 78%     | 1,268,125  | 12% |         |
| Aug.01 | 14,652,989  | 14,363,793 | 98%      | 1,042,356  | 7%       | 11,638,522 | 81%     | 1,682,915  | 12% |         |
| Aug.03 | 25,395,116  | 23,734,754 | 93%      | 1,857,751  | 8%       | 12,751,690 | 54%     | 9,125,313  | 38% |         |
| Aug.04 | 21,328,220  | 20,495,612 | 96%      | 1,776,793  | 9%       | 11,438,070 | 56%     | 7,280,749  | 36% |         |
| Aug.05 | 29,738,190  | 25,621,082 | 86%      | 2,136,485  | 8%       | 19,507,994 | 76%     | 3,976,603  | 16% |         |
| Aug.06 | 29,294,156  | 29,071,018 | 99%      | 1,977,088  | 7%       | 22,792,427 | 78%     | 4,301,503  | 15% |         |

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## CHAPTER 6

### CONCLUSION

Scleractinian corals build the complex, three-dimensional structure that supports the most biodiverse ecosystems on the planet. Vibrant and brightly colored, coral reefs are home to an immense assortment of marine animals and plants. Coral reefs also support the welfare of millions of people by providing sustenance, income, medicine, and protection.

Scleractinian corals establish symbiotic relationships with a myriad of microorganisms that contribute to coral resilience to environmental disturbances. From disease to dust, this dissertation described the impact of biotic and abiotic factors on essential microorganisms living in protective coral surface mucus and present in surrounding reef waters. In Chapter 3, confined spatiotemporal sampling of corals offered a chance to test trending coral disease frameworks. Studying microbial communities at the fine-scale (i.e., specific bacterial taxa) and at the grand-scale (i.e., whole bacterial communities) reconciled two seemingly competing frameworks and revealed commonalities and differences between the bacterial communities of multiple coral species affected by multiple disease types. Chapter 4 investigated how natural, episodic pulses of nutrients from trans-Atlantic Saharan dust affects coral-associated bacterial communities in the Florida Keys. The results in this chapter showed that dust deposition temporarily increased the abundance of *Vibrio* bacteria in corals. Notably, *Vibrio* are potentially pathogenic and frequently

associated with coral disease. Dust deposition also contributed to short-term changes in the microbial communities of threatened *A. palmata* and *O. faveolata* corals. Chapter 5 described the response of reef water microbial gene expression to Saharan dust deposition. Studying microbial transcription patterns revealed that dust aerosol optical thickness, dissolved iron, and dissolved organic carbon from dust alter microbial gene expression.

In summary, the research presented in this dissertation described the interaction between the environment, corals, and their associated microorganisms. Healthy coral reefs support a healthy planet. Yet, corals currently face worldwide decline and degradation due to environmental disturbances that have serious implications for the future of coral reefs. Understanding how environmental factors affect the dynamics of vital microbial communities associated with coral reefs enhances our ability to save and protect coral reef ecosystems.

## ADDENDUM A

### ADDENDUM TO CHAPTER 3: THE CORAL MICROBIOME STABILITY RATIO

## **Introduction**

The coral surface mucus layer is in constant interaction with the surrounding water column and its associates, and it is the coral's first line of defense against environmental disturbances (Certner et al., 2015; Ochsenkühn et al., 2018; Sweet et al., 2010; Bourne et al., 2016; Brown & Bythell, 2005; Gavish et al., 2018; Glasl et al., 2016). Therefore, understanding the microbial dynamics of coral health states aids in the understanding of whole ecosystem health. To understand the different health states (healthy, apparently healthy, and diseased) of the coral holobiont relies on our understanding bacterial taxa that are associated with holobiont health and dysbiosis (Bass et al., 2019; Neave et al., 2016; Webster & Reusch, 2017). Peixoto et al. (2017) calls for an effort to identify bacterial coral symbionts possessing nutritional and protective traits that improve coral fitness. However, to date, there are few studies that have directly addressed this objective (Ainsworth & Hoegh-Guldberg, 2009; Hernandez-Agreda et al., 2016; Mera & Bourne, 2018). In this addendum, we attempt to quantify the coral health states based on bacterial taxa that are associated with healthy, diseased, and healthy & diseased core microbial communities (see Chapter 3 for more information).

## **Methods**

Briefly, the microbial community of coral mucus samples from a multi-species, multi-disease outbreak was analyzed using the AMPVIS2 package (version 2.3.14; Andersen et al., 2018) to identify the core bacterial taxa associated with healthy samples regardless of species ("H CORE") or disease samples regardless of disease type ("DZ CORE"). A coral microbiome stability (CMS) ratio was calculated for healthy, apparently healthy, and

disease mucus samples using the ASVs in H CORE and DZ CORE. The CMS ratio equals the log of the average relative abundances of healthy-associated taxa divided by the average relative abundances of disease-associated taxa. Please refer to Chapter 3 for details on how specific bacterial taxa were associated with H CORE or DZ CORE. A Kruskal-Wallis rank sum test for non-parametric data followed by a pairwise Wilcoxon Rank Sum and Signed Rank test were performed to compare the CMS ratio between healthy, apparently healthy, and disease samples (Breslow, 1970; De Winter, 2013; R Core Development Team, 2018; Wilcoxon et al., 1970).

## **Results**

The CMS ratio decreased significantly from a median of 0.7414 ( $\pm$  0.4695 SE; n=32) in healthy mucus to a median of -0.2645 ( $\pm$  0.3898 SE; n=37) in apparently healthy mucus on a diseased colony (p-value=0.012) (**Figure A3.1**). The ratio further decreased in disease lesions to a median of -0.8323 ( $\pm$  0.4076 SE; n=37) (p-value<0.01 and 0.019 between disease lesions and healthy or apparently healthy mucus, respectively). The CMS ratio trended similarly across the entire dataset, the WP dataset, and the YB-DS-WD dataset (data not shown; see Chapter 3 for details).

## **Discussion & Conclusion**

Grouping bacterial taxa by their association with healthy vs. diseased corals may prove useful in identifying groups of taxa that could be used as biological indicators especially if they are consistently found across studies, geographic locale, time, and species (Blackall et al., 2020; Glasl et al., 2017; Peixoto et al., 2019; Pootakham et al., 2019; Villela et al.,

2019). By quantifying coral health via a CMS ratio, we postulate that individual taxonomic groups can generally indicate coral holobiont stability. In support of this framework, we show that our samples, collected in a manner that controlled for confounding spatial and temporal variability, specify several ASVs that are particularly associated with different health states. This effort to quantitatively capture microbiome health states has been successful in the medical field. Meuric et al. (2017) utilized a dysbiosis ratio based on the relative abundances of genera associated with healthy subgingival samples and genera associated with chronic periodontitis to distinguish between clinically healthy and diseased oral samples.

Here, the CMS ratio allowed us to quantify an in-between state of apparently healthy mucus samples from otherwise diseased colonies that could represent a transitional, or temporarily disturbed, state. While the CMS ratio may be useful as a clue to the health status of a coral prior the development of visual signs of disease, the ratio is specific to the coral species and diseases sampled in this study. The CMS ratio could be utilized in other coral reefs if matched healthy and disease samples from that system identify common core taxa associated with particular health states. Further research is needed to identify ubiquitous coral-associated bacterial taxa that are represented in many reef systems to determine if these potential microbiological indicators, such as the CMS ratio, may improve our ability to predict coral health status (Blackall et al., 2020; Glasl et al., 2017; Peixoto et al., 2019; Pootakham et al., 2019; Villela et al., 2019). For references cited in this addendum, please refer to the Chapter 3 References section.

**Figure**

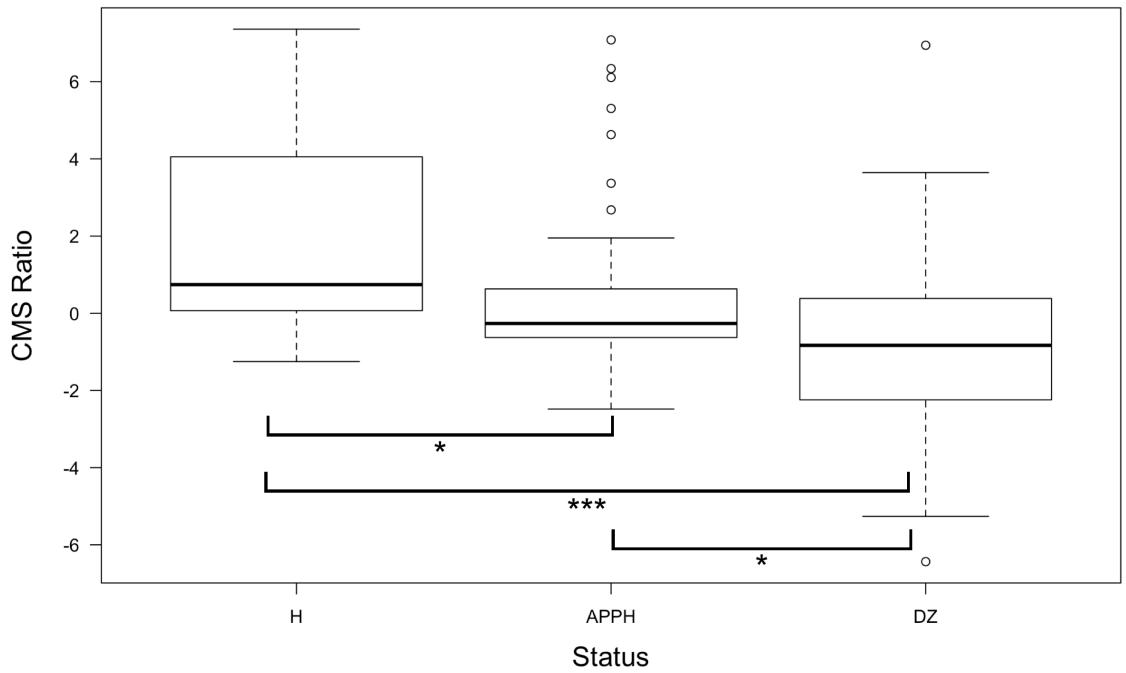


**Figure A3.1. Coral microbiome stability (CMS) ratio.** A CMS ratio used the relative abundance of health-associated and disease-associated core community taxa within the same healthy (H), apparently healthy (APPH), or diseased (DZ) sample. The ratio was calculated by taking a log-transformation of the average relative abundance of H-associated ASVs divided by the average relative abundance of DZ-associated ASVs. Statistical significance: ‘\*\*\*’ p-value<0.001, ‘\*’ p-value<0.05.

## ADDENDUM B

### ADDENDUM TO CHAPTER 4: CONTROLLED EXPERIMENTAL EXPOSURE TO SAHARAN DUST AEROSOLS ON *ACROPORA CERVICORNIS*

## Introduction

Deposited Saharan dust is an important source of carbon, nitrogen, and phosphorus (Duarte et al., 2006; Mahowald et al., 2008). In addition, Saharan dust is an episodic source of critical trace metals such as iron (Lenes et al., 2001). Iron (Fe) is a key, limiting micronutrient for microorganisms that facilitates many metabolic processes including aerobic respiration, photosynthesis and nitrogen fixation (Langlois et al., 2012; Strzepek et al., 2005; Sunda et al., 2012; Yong et al., 2014). Because *Vibrio* has been implicated in dust events, are members of the coral microbial community, and have been suggested as pathogens for coral diseases, there is a growing need to investigate coral microbial community dynamics, particularly *Vibrio* dynamics, associated with episodic dust influx (Bourne et al., 2009; Patterson et al., 2002; Ritchie et al., 2006; Tracy et al., 2019). Here we experimentally investigate the response of reef-building *Acropora cervicornis* microbiomes, specifically *Vibrio*, in response to Saharan dust aerosol nutrients.

## Methods

### *Experiment design*

*Acropora cervicornis* fragments from three clonal variants were collected from the IC2R3 coral nursery off-shore of Summerland Key, FL, USA in August 2017. Three fragments (one from each clonal variant) were placed in each of ten 10 L aquaria. Fragments were hung on a 4 to 5 in fishing line approximately 3 in from the bottom of the tank. Fishing lines were hung from plastic covers atop each tank. Tanks were placed inside an outdoor seawater raceway and mesh covers were used to cover the basin and simulate underwater light scatter. Five aquaria were assigned as controls, and five received a dust-dosing

treatment. Nitrocellulose filter disks (47 mm; Whatman 41, Kent, UK) used to collect aerosol particles in 2016 were stored at -80°C, thawed, and cut into four using flame sterilized scissors. One-fourth of a filter from the highest dust influx event day (29 July 2016) was placed in each dust-dosing treatment tank. One fourth of a sterile control filter was also placed in each control aquaria.

#### *Experiment sample collection*

Each day for five days (24 h time points), three coral samples were collected from each of one control and one treatment tank, and the tank was forfeited. Coral mucus was collected using a sterile, needleless 12 mL syringe (Monoject by Covidien, Dublin, Ireland) with minimal agitation, and tank water was collected in sterile 50 mL centrifuge tubes. Immediately after collection, 100 µL of mucus and 100 µL of tank water spread plated onto TCBS plates in triplicate for quantification of total culturable *Vibrio*. Plates were incubated at 30°C and enumerated within 24 h. Three replicates of 2 mL of both mucus and water were collected in separate 2 mL microcentrifuge tubes and centrifuged at 20,000 x g for 20 min. Supernatant fluid was discarded and dried pellets were stored at -80°C. DNA extraction, library preparation, sequencing, microbial community analyses, and *Vibrio* analyses were performed as outlined in the Chapter 4 Methods section.

## **Results**

#### *Coral surface mucus layer and tank water microbial communities*

After quality filtering via DADA2, the average number of reads per sample was 29,873 across all *A. cervicornis* fragments (range: 5,287 to 187,424) and 40,151 for tank water

(range: 75 to 256,256; **Table A.1**). On average, 59% of reads from mucus of individual *A. cervicornis* fragments and 62% of reads from individual tank water samples were retained after quality filtering. A total 5,003 ASVs were represented in the final dataset across all samples (**Figure A.1**). Resulting sequences were submitted to NCBI and can be found under project PRJNA521399.

Shannon alpha-diversity indices for *A. cervicornis* fragments were lower in T0-control vs. T1-control (p-value<0.0001) and greater in T1-control vs. T2-control samples (p-value<0.0001), T3-control vs. T4-control (p-value=0.001), T1-dust vs. T2-dust (p-value=0.009), and T3-dust vs. T4-dust (p-value=0.002). Observed and Chao1 alpha-diversity did not vary cross timepoint-treatment combinations. Alpha-diversity for tank water samples did not vary by timepoint or treatment. Beta-diversity calculated using Bray-Curtis dissimilarity between treatment-timepoint combinations varied for *A. cervicornis* experimental coral (p-value=0.003) but not for tank water (**Figure A.2**).

The relative abundance of ASVs grouped by taxa changed significantly across timepoint-treatment combinations. Eleven ASVs representing Caulobacterales, Rhodobacterales, Flavobacteriales, Vibrionales, Oceanospirillales, Alteromonadales, and Rhodospirillales varied significantly between control and dust treatments for each timepoint (**Figure A.3**). A total of 6 ASVs representing Cryomorphaceae, Alteromonadaceae, Rhodospirillaceae, and Rhodobacteraceae had greater abundances in control treatments, and a total of 8 ASVs representing *Halomonadaceae*, *Cryomorphaceae*, *Rhodobacteraceae*, *Vibrionaceae*, *Hyphomonadaceae*, and *Flavobacteriaceae* had higher relative abundance in dust treatments (**Figure A.3**).

### *Vibrio* dynamics

*A. cervicornis*-associated total culturable *Vibrio* in control tanks averaged 49 ( $\pm$  90 SD) CFU mL<sup>-1</sup> with a range of 0 to 367 CFU mL<sup>-1</sup>. *A. cervicornis*-associated total culturable *Vibrio* in dust dosed tanks averaged 611 ( $\pm$  1,139 SD) CFU mL<sup>-1</sup> with a range of 0 to 4,276 CFU mL<sup>-1</sup>. *A. cervicornis*-associated *Vibrio* relative abundance based on ASVs in control tanks averaged 0.36% ( $\pm$  0.50 SD) with a range of 0 to 1.4%. *A. cervicornis*-associated *Vibrio* relative abundance in dust dosed tanks averaged 1.2% ( $\pm$  1.5 SD) with a range of 0.01 to 5.2%. Tank water total culturable *Vibrio* in control tanks averaged 55 ( $\pm$  53 SD) CFU mL<sup>-1</sup> with a range of 3 to 220 CFU mL<sup>-1</sup>. Tank water total culturable *Vibrio* in dust dosed tanks averaged 233 ( $\pm$  275 SD) CFU mL<sup>-1</sup> with a range of 3 to 710 CFU mL<sup>-1</sup>. Tank water *Vibrio* relative abundance in control tanks averaged 1.1% ( $\pm$  1.6 SD) with a range of 0 to 5.3%. Tank water *Vibrio* relative abundance in dust tanks averaged 0.93% ( $\pm$  0.80 SD) with a range of 0.06 to 2.1%. Neither total culturable *Vibrio* nor *Vibrio* relative abundance in experimental coral varied significantly by timepoint in either treatment (**Figure A.4**). Because only one water sample was collected per experimental tank to avoid pseudoreplication, we did not have enough replicates to analyze total culturable *Vibrio* and *Vibrio* relative abundance between timepoints by treatment (**Figure A.5**).

Regardless of timepoint, coral-associated total culturable *Vibrio* was greater in the dust-amended treatment compared to the control treatment (p-value=0.030; **Figure A.6**). Coral-associated *Vibrio* relative abundance did not change between dust and control treatments (**Figure A.7**). Neither total culturable *Vibrio* or *Vibrio* relative abundance changed in abundance between treatments (including all timepoints) for tank water (**Figure A.7**). When each timepoint was analyzed separately, coral-associated total culturable

*Vibrio* and *Vibrio* relative abundance were greater in the T4 dust treatment compared to T4 control treatment (p-value=0.002; p-value=0.044, respectively; **Figure A.8**). Because only one water sample was collected per experimental tank to avoid pseudoreplication, we did not have enough replicates to test total culturable *Vibrio* and *Vibrio* relative abundance across timepoint-treatment combinations for tank water samples (**Figure A.9**).

## Discussion & Conclusion

The deposition of dust aerosols in the Caribbean has been shown as one driver of *Vibrio* bloom formation (Westrich et al., 2016). *Vibrio* are heterotrophic bacteria ubiquitous in the marine environment and commonly associated with coral stress and disease in Caribbean reef-building corals. However, the extent to which these responses affect the bacterial communities associated with benthic organisms is not well understood. Here we show the experimental response of reef-building *Acropora cervicornis* microbiomes, specifically *Vibrio*, to Saharan dust nutrient influx

Our main finding was that regardless of experimental time point, total culturable *Vibrio* increased in abundance in dust-amended treatments. As conditionally rare taxa (CRT), *Vibrio* may outcompete other heterotrophs in the coral bacterial community and bloom in surface mucus when dust deposition is significant in the water column (Borchardt et al., 2020; Shade et al., 2014; Westrich et al., 2016, 2018). Additionally, we found that at timepoint 4 both total culturable *Vibrio* and *Vibrio* relative abundances increased in dust-amended treatments compared to controls. *Vibrio* are iron scavengers with diverse metabolic capabilities that contribute to rapid iron uptake (Grimes et al., 2009; Payne et al., 2016; Yooseph et al., 2010). *Vibrio* response to nutrient influx may be rapid in reef

waters (~ 24 h after dust deposition) (Westrich et al., 2016, 2018), but coral-associated *Vibrio* respond at later timepoints (i.e. 96 h after dust deposition). While *Vibrio* concentrations are not typically affected by bottle effects, we observed a tank effect on beta-diversity of *A. cervicornis* microbial communities during the dust dosing experiment. Therefore, we do not recommend long-term coral microbiome experiments that confine corals and restrict water flow. For references cited in this addendum, please refer to the Chapter 4 References section.

### **Acknowledgements**

This work was supported by NSF Biological Oceanography award OCE 1357423 (EKL and JRW) and NSF Graduate Research Fellowship Program (GRFP) 1842396 (MGB). Field-work would not have been possible without equipment and expertise from Keys Marine Laboratory.

**Table**

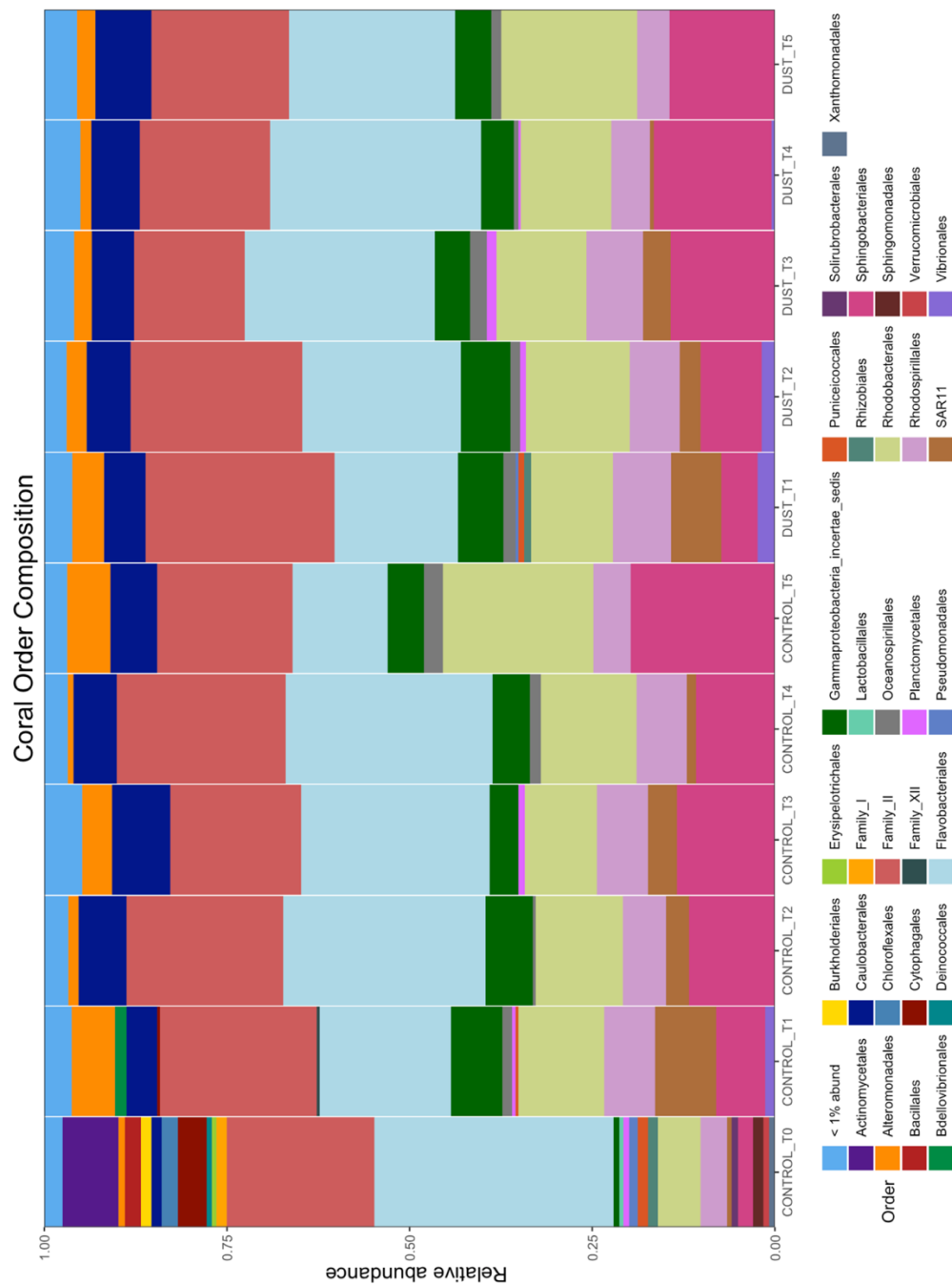
**Table A.1.** Summary of sequencing parameters after 16S rRNA gene amplicon sequencing of the V4 region for experimental coral and tank water samples. Data represent quality filtered sequences processed using DADA2. Table columns: samples collected (n), average reads across sample type that passed quality control filtering (Average Reads), average percent of reads across sample type that passed quality control filtering (Average Percent), minimum number of reads across sample type (Min), maximum number of reads across sample type (Max).

| Sample Type             | n  | Average Reads | Average Percent | Min    | Max     |
|-------------------------|----|---------------|-----------------|--------|---------|
| Coral                   | 30 | 29,873        | 59.37%          | 5,287  | 187,424 |
| Water                   | 21 | 40,151        | 62.19%          | 75     | 256,256 |
| Coral Dust Treatment    | 14 | 25,318        | 58.46%          | 7,259  | 49,666  |
| Coral Control Treatment | 16 | 33,858        | 60.16%          | 5,287  | 187,424 |
| Water Dust Treatment    | 5  | 24,915        | 60.60%          | 13,387 | 37,152  |
| Water Control Treatment | 16 | 44,912        | 62.69%          | 75     | 256,256 |
| Coral Timepoint 0       | 2  | 9,161         | 68.82%          | 5,287  | 13,035  |
| Coral Timepoint 1       | 6  | 26,940        | 60.85%          | 7,259  | 51,306  |
| Coral Timepoint 2       | 6  | 52,768        | 59.92%          | 10,079 | 187,424 |
| Coral Timepoint 3       | 4  | 22,629        | 58.56%          | 16,688 | 32,523  |
| Coral Timepoint 4       | 6  | 24,704        | 56.13%          | 8,838  | 43,178  |
| Coral Timepoint 5       | 6  | 26,810        | 57.96%          | 12,988 | 49,666  |
| Water Timepoint 0       | 11 | 57,475        | 68.27%          | 10,484 | 256,256 |
| Water Timepoint 1       | 2  | 14,242        | 49.92%          | 75     | 28,409  |
| Water Timepoint 2       | 2  | 14,336        | 60.96%          | 13,387 | 15,284  |
| Water Timepoint 3       | 2  | 27,475        | 53.74%          | 17,797 | 37,152  |
| Water Timepoint 4       | 2  | 25,572        | 52.13%          | 13,991 | 37,152  |
| Water Timepoint 5       | 2  | 27,065        | 56.44%          | 14,910 | 39,220  |

## Figures

**Figure A.1.** Relative abundance of bacterial Orders in **(A)** *A. cervicornis* and **(B)** tank water during a 5 d controlled dust dosing experiment. Orders representing > 1% of sequences in any one sample are shown.

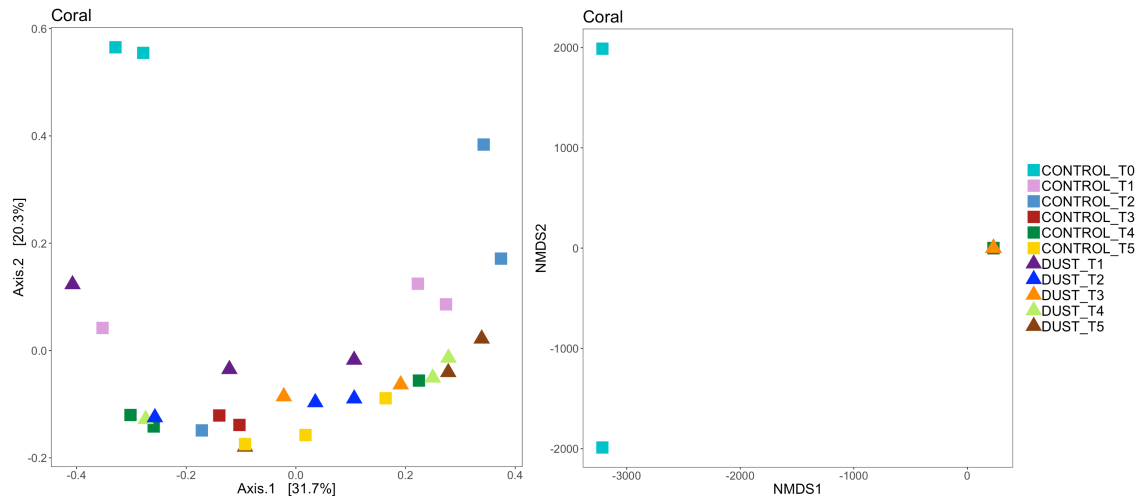
**(A)**



(B)



(A)



(B)

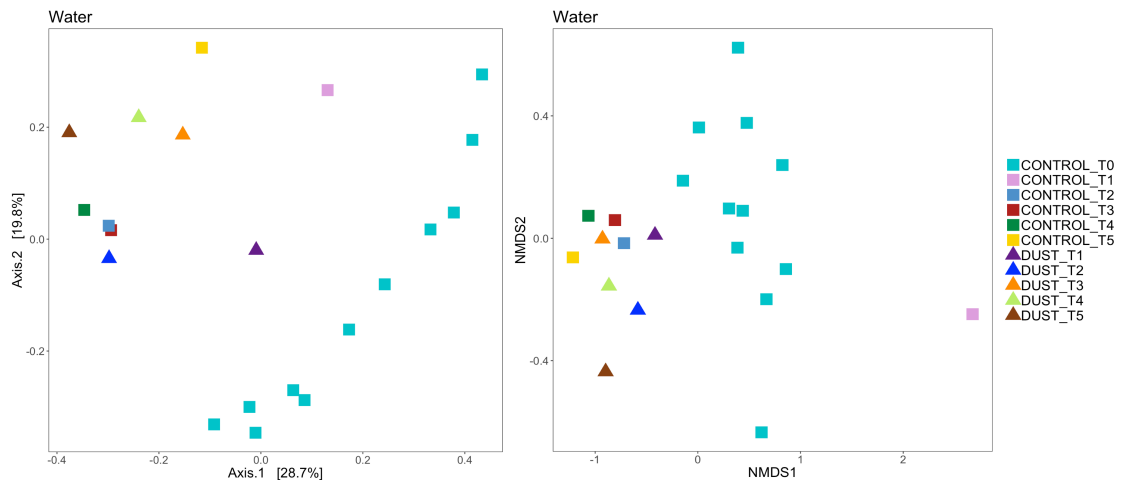


**Figure A.2.** Beta-diversity principal coordinate analysis (left) and non-metric multidimensional scaling analysis (right) calculated using Bray-Curtis dissimilarity for (A) *A. cervicornis* experimental coral and (B) tank water by treatment and across timepoints.

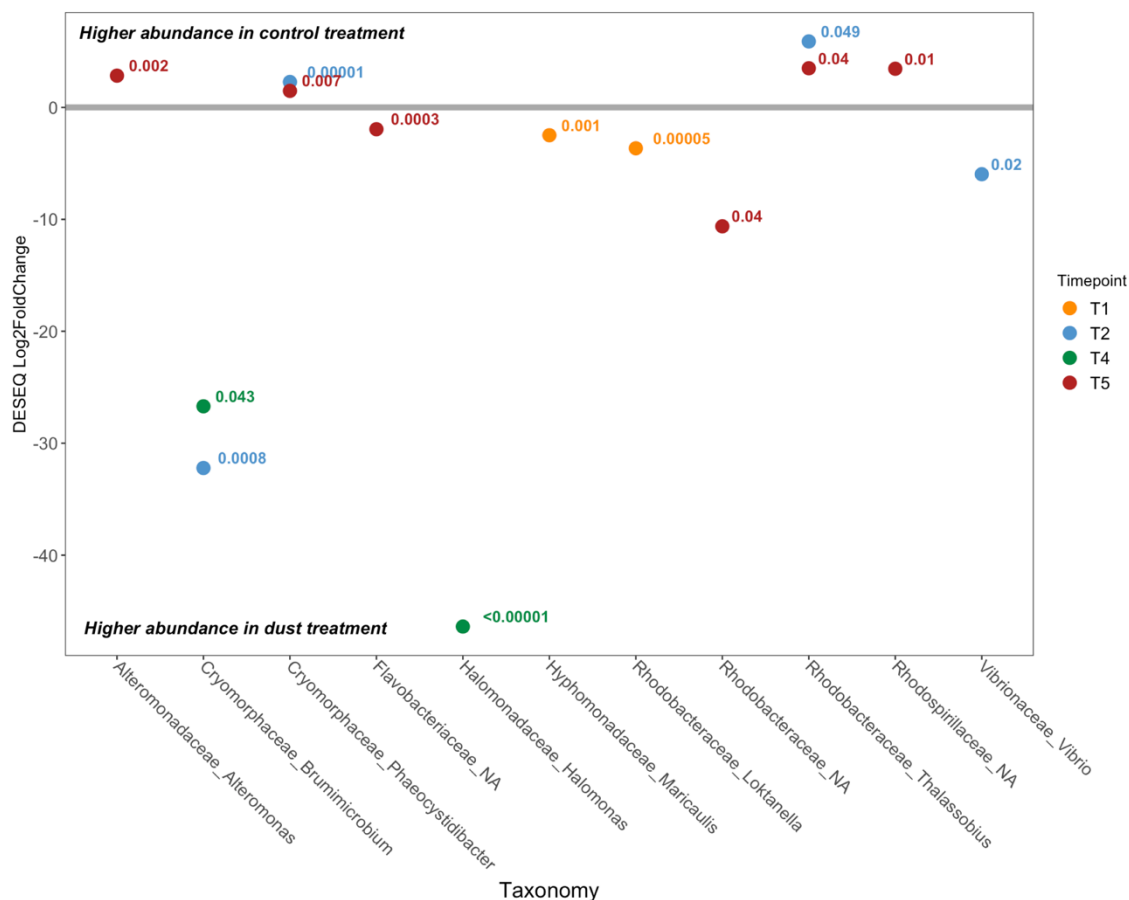
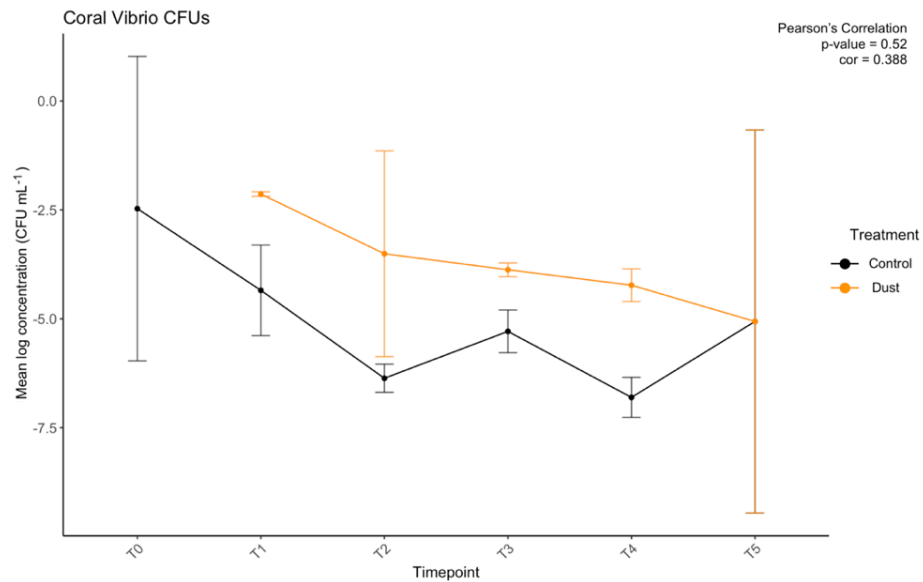


**Figure A.3.** Differential abundance analysis between experimental treatments showed specific ASVs (dots) identified by taxonomy (“Family\_Genus” x-axis) that changed significantly in abundance between control and dust-amended treatments by timepoint. Different colors annotate different timepoints. DESeq2 analysis “Log2FoldChange” above zero (gray horizontal line) indicate ASVs with higher abundance in control treatment, and “Log2FoldChange” below zero indicate ASVs with higher abundance in dust treatments. Point text indicates DESeq2 analysis adjusted p-values. A total of eleven ASVs represent the following orders: Caulobacterales, Rhodobacterales, Flavobacteriales, Vibrionales, Oceanospirillales, Alteromonadales, and Rhodospirillales.

(A)



(B)

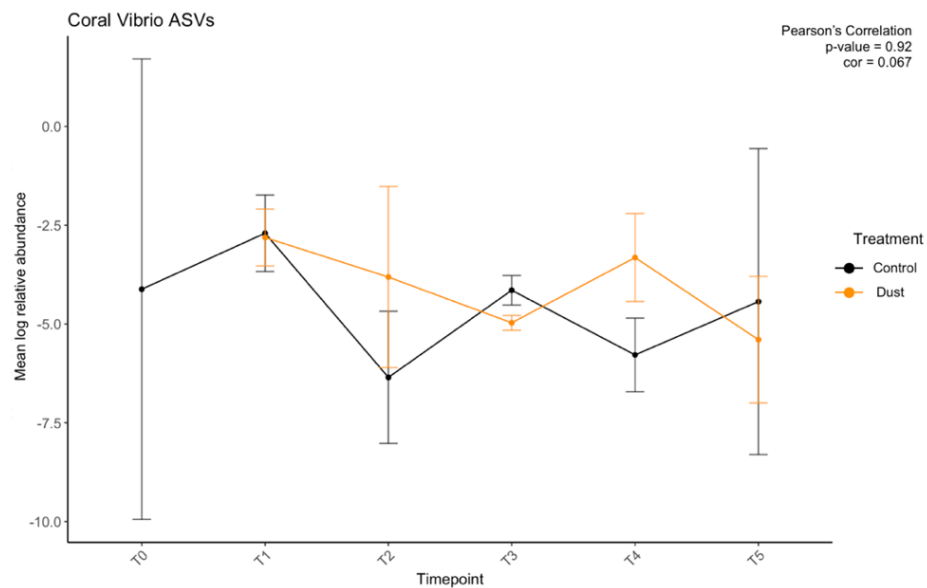
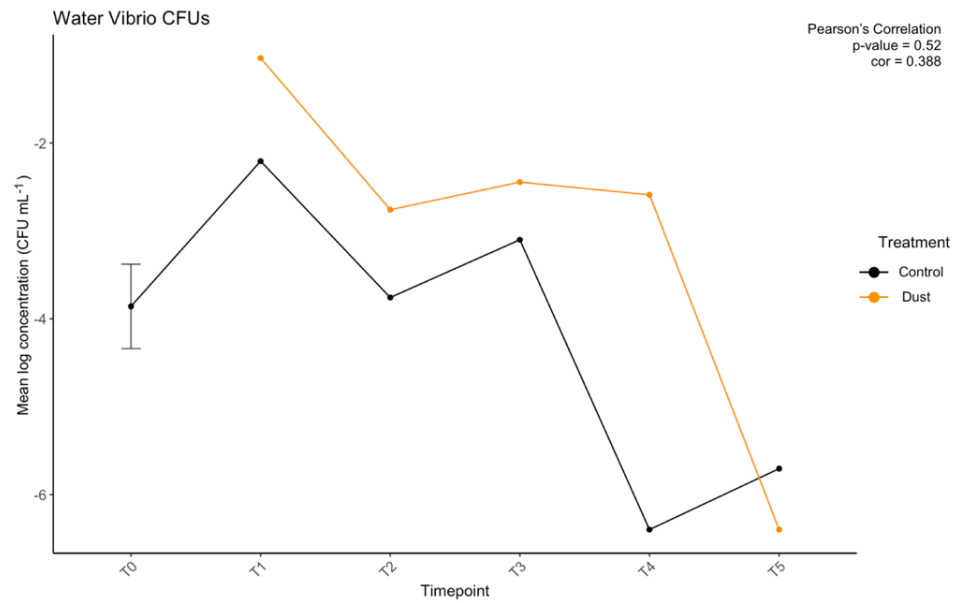


**Figure A.4.** *Vibrio* concentrations across five experimental timepoints for *A. cervicornis*. *Vibrio* abundance in control (black line) and dust-amended (orange line) treatments. (A) Total cultural *Vibrio* mean log CFU mL<sup>-1</sup> and (B) mean log relative abundance. Error bars represent standard deviations from the mean. Pearson's correlation results are denoted in the top right-hand corner to assess the correlation between *Vibrio* concentrations between treatments.

(A)



(B)

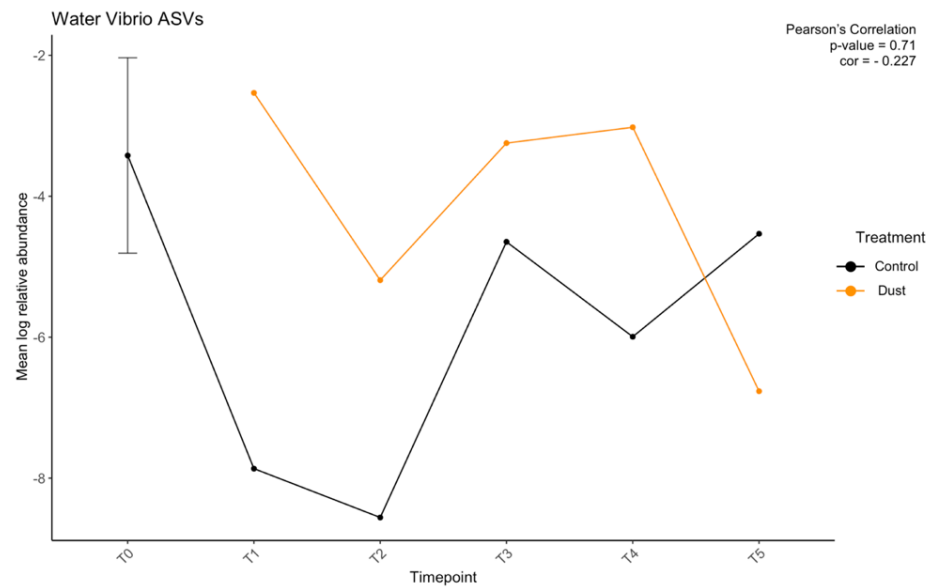


**Figure A.5.** Control (black solid line) and dust-amended (orange solid line) treatment *Vibrio* concentrations across five experimental timepoints for (A) dust-amended tank water total culturable *Vibrio* mean log concentration (CFU mL<sup>-1</sup>) and (B) *Vibrio* mean log relative abundance. Error bars represent standard deviations from the mean. Pearson's correlation results are denoted in the top right-hand corner to assess the correlation between *Vibrio* concentrations between treatments.

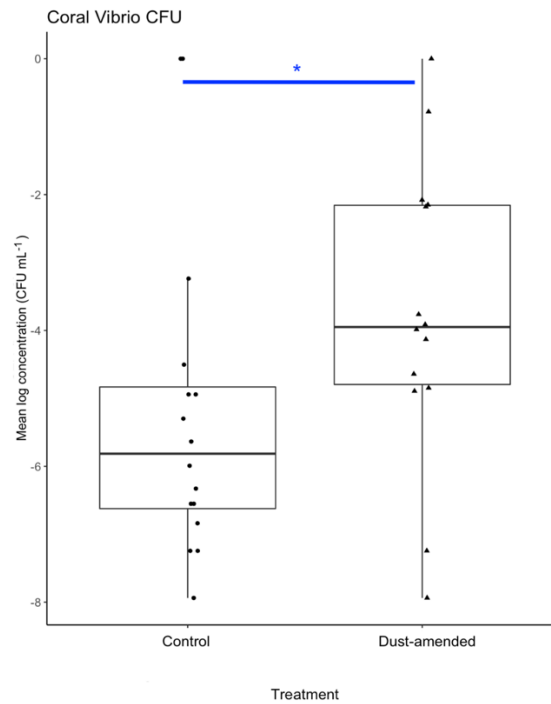


**Figure A.6.** *A. cervicornis*-associated total culturable *Vibrio* log mean concentration (CFU mL<sup>-1</sup>) between control and dust-amended treatments. All timepoints are represented in each treatment: control (n=16) and dust-amended (n=14).

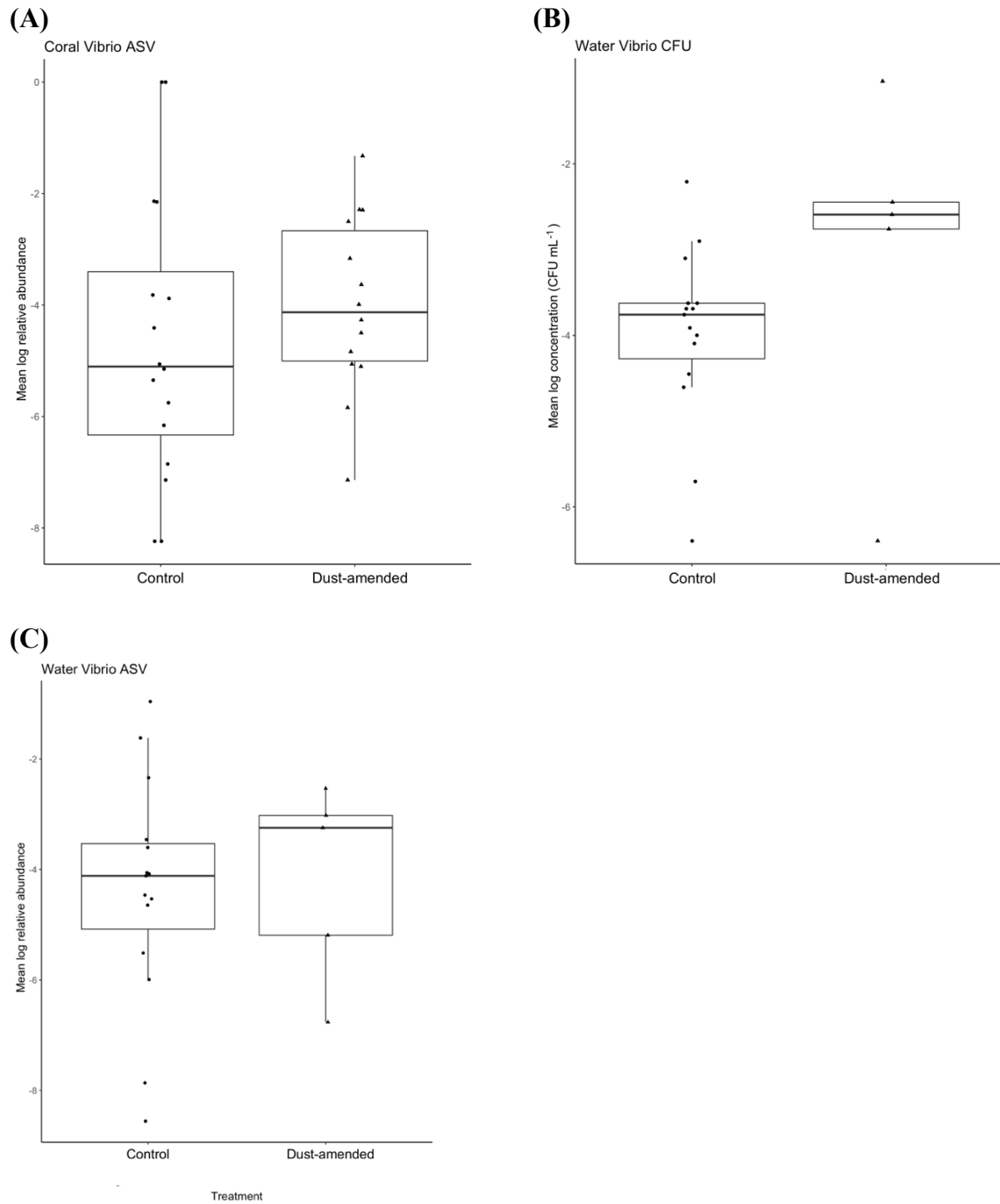
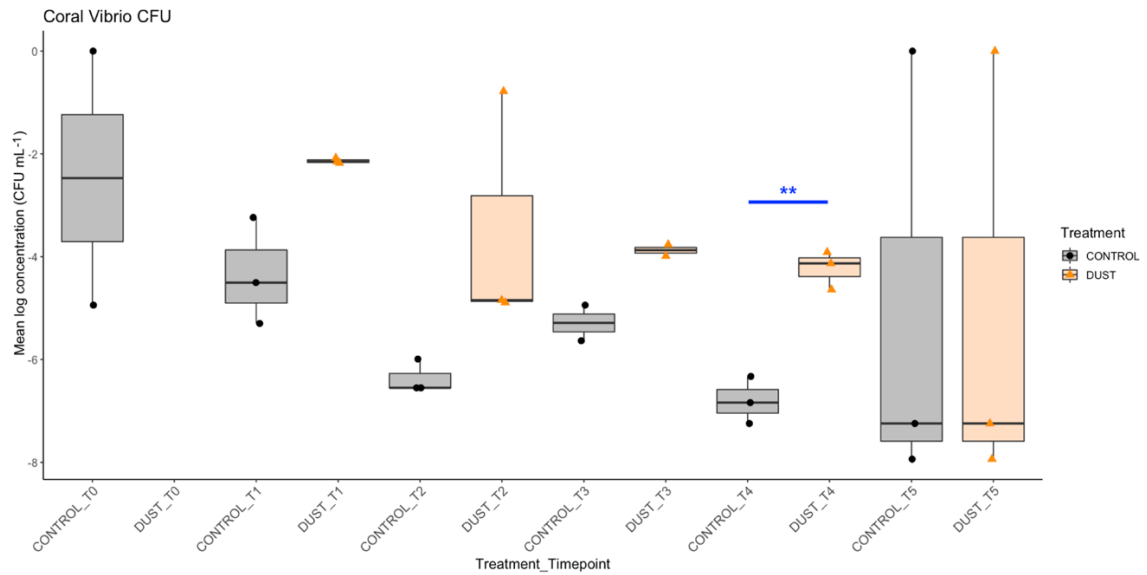


**Figure A.7.** (A) Coral-associated *Vibrio* mean log relative abundance (ASV; control n=16 and dust-amended n=14), (B) tank water-associated *Vibrio* mean log concentration (CFU mL<sup>-1</sup>; control=15 and dust-amended=5), and (C) tank water-associated *Vibrio* mean log relative abundance (ASV; control=16 and dust-amended=5) between control and dust-amended treatments (including all timepoints). Pairwise comparisons of *Vibrio* concentrations between treatments were not statistically significant.

(A)



(B)

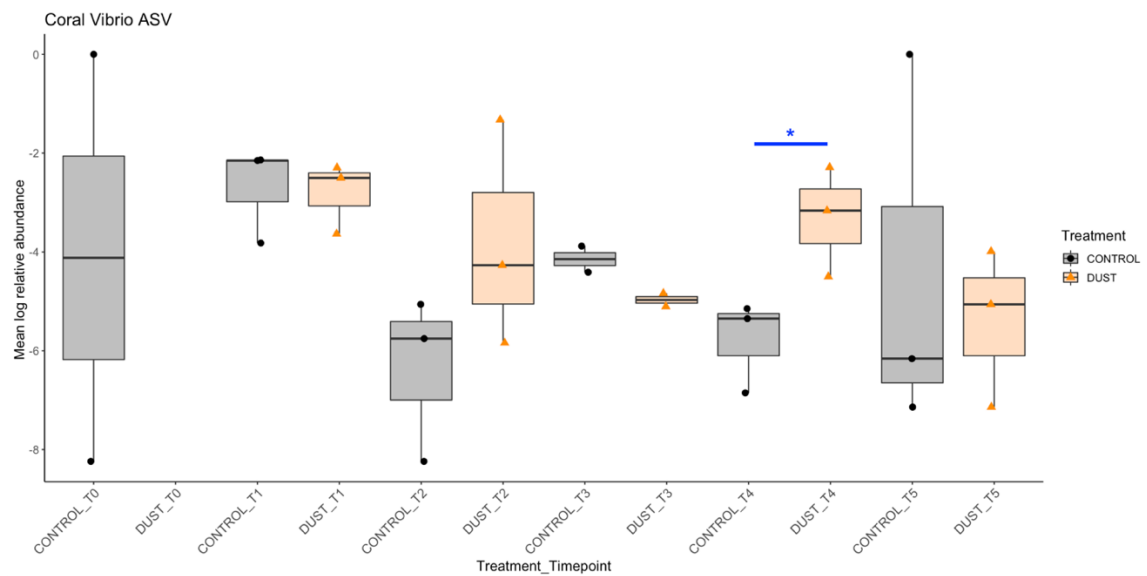
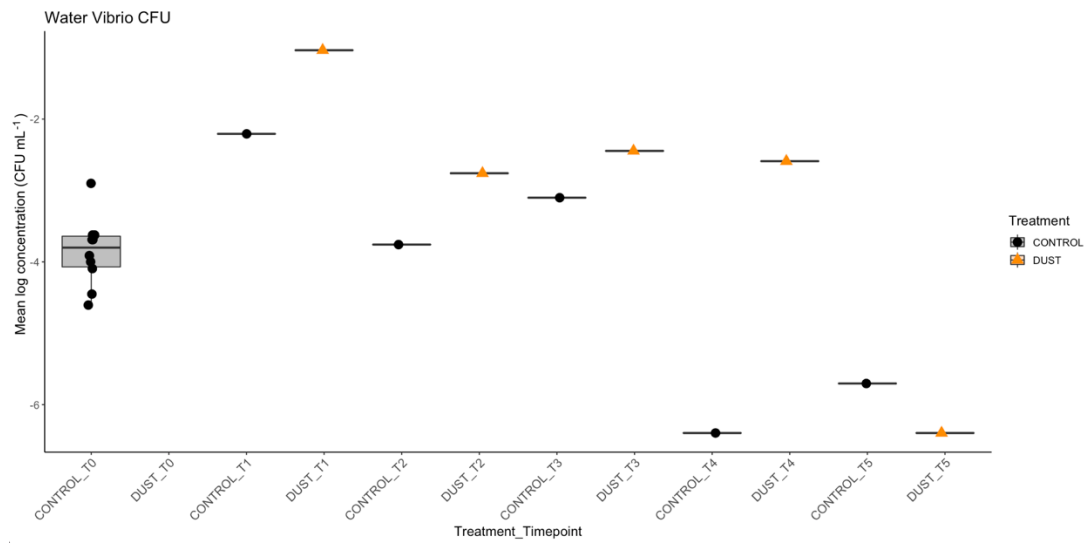


**Figure A.8.** (A) Total culturable *Vibrio* log mean concentration (CFU mL<sup>-1</sup>), and (B) *Vibrio* mean log relative abundance for control and dust-dosed corals across timepoints. Gray boxplots denote control treatments, and orange boxplots denote dust-amended treatments. Statistical significance: “\*\*” p-value<0.01, “\*” p-value<0.05.

(A)



(B)

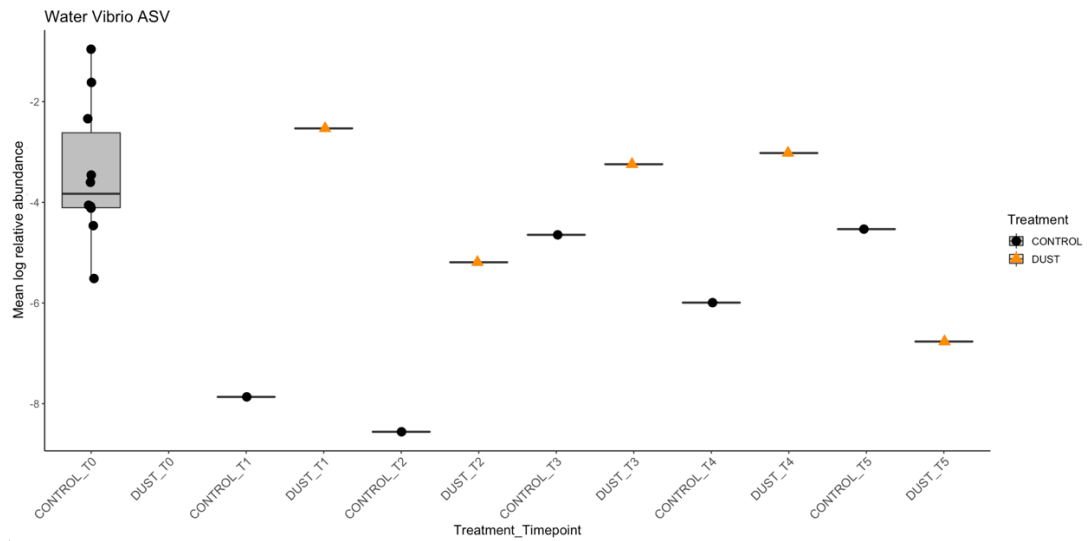


**Figure A.9.** (A) Total culturable *Vibrio* log mean concentration (CFU mL<sup>-1</sup>), and (B) *Vibrio* mean log relative abundance for control and dust-dosed tank water across timepoints. Gray boxplots denote control treatments, and orange boxplots denote dust-amended treatments.