TRAIT-MEDIATED EFFECTS AND THE EXTENDED PHENOTYPE: COMMUNITY INTERACTIONS ON CORAL REEFS

by

ANYA LEARD BROWN

(Under the Direction of CRAIG W. OSENBERG)

ABSTRACT

Interaction modifiers can induce changes in (a) physio-chemical conditions and (b) phenotypes of individuals, including host-associated microbial communities, thus influencing species interactions. Among the increasingly best-studied holobionts (hosts + microbes) are corals.

Corals experience a myriad of stressors, including interactions with algae, which can decrease coral growth and survival. A hypothesized mechanism underlying the deleterious effects of algae on corals involves the release of dissolved organic carbon (DOC), which stimulates microbial growth leading to changes in microbial communities/hypoxia.

The microbial effects of algae on corals are context-dependent, and change depending on the physical environment (*e.g.*, water flow) and the presence of other species. One such species is the sessile vermetid gastropod, *Cereasignum maximum*, which uses its mucus net for food capture. When a mucus net covers a coral in contact with algae, the net can exacerbate the negative effects of algae on corals by 1) decreasing water flow, 2) synergistically decreasing coral growth, and/or 3) leading to changes in the coral's microbial communities, favoring

potentially pathogenic groups and/or enhancing stress by changing the concentration of dissolved materials.

Using a series of field surveys, lab flume studies, and field experiments, I found that mucus nets reduce water flow and lower oxygen concentrations at the surface of corals. Algal presence led to the greatest changes in microbial communities. Vermetids and algae decrease different aspects of coral growth.

I hypothesized that the absence of a strong response of corals to vermetids arose because corals may have acclimatized to the presence of vermetids. To test this hypothesis I conducted a reciprocal transplant study of corals with and without previous exposure to vermetids. I found significant effects of prior exposure of vermetids on coral traits, and plasticity of the microbiome, but no evidence of a weakened vermetid effect. Interestingly, I also found genetic differences between coral on reefs with vs. without vermetids, and suggest vermetids may be part of a coral's extended phenotype.

My dissertation demonstrated interaction modifications and trait-mediated effects of vermetids and algae on corals, and showed novel, but likely general mechanisms for these effects involving changes in the coral's microbial community.

INDEX WORDS: Coral reefs, *Ceraesignum maximum,* algae, ecology, microbiome, extended phenotype, interaction modifications, trait-mediated interactions

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DEDICATION

To my family and their unwavering support.

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CHAPTER 1

INTRODUCTION

Pairwise interactions, especially consumer-resource and competitive interactions, inform much of ecology. Yet, rarely do pairs of species occur in isolation. As a result, species dynamics are affected by a suite of interactions, including indirect effects arising from interactions among three or more species. Indirect effects can challenge ecologists as they can make the outcome of interactions difficult to predict, and lead to ecological surprises. Some indirect effects are easily predicted: they are the sum of the direct interactions (i.e., density-mediated indirect effects: Vandermeer 1969). However, there also are less predictable indirect effects, called interaction modifications (also known as, higher order interactions or trait-mediated indirect effects) in which the strength or outcome of a direct interaction between two species is modified by a third species (Vandermeer 1969, Wootton 1994, Werner & Peacor 2003).

Interaction modifications can arise when a third species alters the ecological context in which a pairwise interaction occurs. For example, canopy trees occlude light to understory habitats, and this alteration of the physical environment influences the strength of competition between understory plants (Pagès et al. 2003). Similarly, the litter of *Pinus ponderosa* (pine) changes the soil chemical environment, thereby modifying the strength of competition between a grass and a shrub (Metlen et al. 2013, Metland and Callaway 2015). Often, interaction modifiers can induce a phenotypic (i.e., trait) shift in one of the species involved in a pairwise interaction: e.g., wood frog larvae outcompete leopard frog larvae in the absence of predators, however, in

the presence of predators, predator chemical cues induce morphological changes in the tadpoles causing leopard frog larvae to outperform wood frogs (Relyea 2000).

Indeed, many interaction modifications arise via changes in the phenotype of one or more of the interacting species, either through phenotypic plasticity (i.e., changes in traits due solely to variation in the environment) or evolutionary change (i.e., changes in the genetic composition of a population). However, "phenotype" can include more than the classic traits (morphological, physiological and behavioral) of an organism: it also can include characteristics beyond the traditionally classified individual (i.e., its extended phenotype: *sensu* Dawkins 1976). The extended phenotype includes the effects that genes or a genotype can have on a community or an ecosystem (i.e., a community phenotype): reviewed in Whitham et al. (2006). For example, *Populus* tree diversity is positively correlated with arthropod community diversity, as different tree genotypes support different arthropod communities (Wimp et al. 2005, Whitham et al. 2006). The hypothesis is that the underlying genetic variation in the host plant leads to differences in arthropod colonization. Thus, the arthropod communities are an extended phenotype of the trees (Whitham et al. 2006).

The concept of an extended phenotype has also been expanded to holobionts, which consist of a host and its microbial associates. Most living organisms are inhabited by a wide diversity of microbes (e.g., bacteria, Achaea and eukaryotes, like fungi), which can enhance nutrient cycling, competitive ability, and protection against pathogens (Hartnett et al. 1993, Wahl 2008, Nasrolahi et al. 2012). Distinct microbial communities are found in specific host locations (Wahl 2008, Roughgarden 2017), and some hosts need microbial associates to perform specific functions (such as metabolism of hard to digest sugars in humans, Sekirov et al. 2010). Thus, the fitness of a host is influenced not only by its own traits, but also by the traits of the microbes that

it harbors. As a result, the phenotype of a holobiont is thus defined by the traits of the host and its symbionts (Mindell 1992, Rohwer et al. 2002, Zilber-Rosenberg & Rosenberg 2008). Microbial traits and host traits can both respond to environmental stimuli, yielding plastic responses in both the microbes and host (Carrier & Reitzel 2018). Additionally, the traits of symbionts can affect host behavior, growth, or mortality, which can alter its interactions with other species (Ezenwa et al. 2012). Similarly, interactions with other species can lead to changes in the host, which can then alter the microbial constituents of the holobiont.

Host-associated microbial communities can change quickly in response to the environment (Armstrong et al. 2001, van der Heijden et al. 2008, Augustin et al. 2009, Nasrolahi et al. 2012, Goh et al. 2013, Wagner et al. 2014). For example, acquisition of microalgae, *Symbiodinium* from clade D allows corals to withstand bleaching compared to associations with other clades of *Symbiodinium* (Jones et al. 2008). Thus, on ecological timescales, when including microbes as a phenotypic trait, species may show phenotypic plasticity (i.e., changes in microbial communities in response to environmental stimuli) in response to direct and indirect effects. Therefore, even when the host cannot change genetically (e.g., via selection) or phenotypically (via plasticity), its microbial assemblage can change, thus altering its "extended" phenotype. Here, I consider microbes as traits (adapted from "interactors", sensu Dawkins 1976 and Roughgarden 2017), which can allow the holobiont to acclimatize (e.g., show adaptive phenotypic plasticity) to biological and/or physical conditions. Rapid change in the holobiont may occur through ecological dynamics of the microbial community (causing shifts in the representation of different microbes), or evolutionary dynamics of the constituent microbes (causing changes in the functional traits of a given group: Bordenstein & Theis 2015). In either case, the genotype of the holobiont changes, in response to external stimuli on much shorter time

scales than required for a change in the genotype of the host (Zilber-Rosenberg & Rosenberg 2008, Rosenberg et al. 2018).

In summary, pairwise interactions exist on a backdrop of biological and physical contexts. Pairwise interactions are modified by other species, both through changes in the physical environment and through changes in the phenotypes of the interacting species. For holobionts, these phenotypic changes can include shifts in associated microbial communities. Because microbial communities can change on short time scales, indirect effects that are mediated through symbionts can result in rapid changes that can facilitate (or impede) resilience of hosts to new environments.

For my dissertation, I used a coral reef ecosystem to determine the consequences of direct and indirect effects on the ecology of corals. In particular, I examined how interactions between corals and algae are modified by a third group, vermetid gastropods. Furthermore, I determined whether the interactions among corals, algae and vermetids could be understood by studying the dynamics of the microbial community that occupies the coral holobiont.

Study System - Coral reefs

Coral reefs are the most diverse marine ecosystem in the world. Scleractinian (stony) corals create the reef structure, which houses the diverse assemblages characteristic of coral reef ecosystems. Coral reefs also influence important local, regional, and global elemental cycles (Odum & Odum 1955, Moberg & Folke 1999), and provide important ecosystem services to coastal human communities (e.g., via storm protection, and food production, Moberg & Folke 1999). Most corals are colonial organisms, and are engaged in complex symbioses that make up the coral holobiont. For example, most corals contain single-celled algae in the genus

Symbiodinium that provide photosynthate in exchange for nitrogen (Davy et al. 2012). Coral-microalgal symbioses are already considered part of the coral's extended phenotype (Parkinson & Baums 2014), as different strains of *Symbiodinium* can lead to functional differences between corals of the same genotype. The coral-microalgal symbiosis has been well studied, and has been suggested to be adaptive: i.e., in which changes in associations between symbionts and hosts confer benefits to the holobiont (e.g., the adaptive bleaching hypothesis, Buddemeier et al. 1993, Brown 1997, Baker et al. 2004, Howells et al. 2011).

Corals also host a diversity of other microbes (including bacteria, archaea, fungi, and viruses, Davy et al. 2012) that are involved in nutrient cycling (Wegley et al. 2007) and pathogen resistance (Rypien et al. 2010, Garren & Azam 2011). Previous studies show that microbial communities change in response to abiotic conditions (e.g., temperature, pCO_2 or water flow, Webster et al. 2012, Morrow et al. 2014, Lee et al. 2017) and biotic conditions (e.g., competition and predation: Barott et al. 2012a, Shaver et al. 2017). Additionally, microbial associations can either be harmful (e.g., lead to disease) or beneficial, as they are also associated with the development of resistance to disease (Reshef et al. 2013, Glasl et al. 2016, Peixoto et al. 2017).

Nearly all coral reefs are in danger of shifting from species-rich, coral-dominated communities to species-poor, macroalgal-dominated communities (Pandolfi et al. 2005). Climate change, overfishing, storms, disease, and predator outbreaks all contribute to the death of corals, which opens up free space on reefs and allows for colonization and growth of benthic algae (Hughes et al. 2007). Because algae can outcompete coral (River & Edmunds 2001, Bellwood et al. 2004, Nugues et al. 2004, Smith et al. 2006, Vermeij 2006, Box & Mumby 2007, Vermeij et al. 2009, Rasher 2010, Rasher & Hay 2010), increases in algal cover are likely to further exacerbate the decline of coral reefs.

Algae can negatively affect corals through their effects on coral microbiomes. For example, algae leak dissolved organic carbon, which provides substrate for microbes that live on corals and in the water column (Bell 1983, Kline et al. 2006, Nelson et al. 2011). This can lead to 1) shifts of coral microbial communities towards more heterotrophic or disease-related groups (Vega Thurber et al. 2009, Barott et al. 2012b, Vega Thurber et al. 2012) and/or 2) hypoxia due to the increased respiration of heterotrophic microbes (Kline et al. 2006, Smith et al. 2006, Barott et al. 2009). Water flow, and factors that affect flow, can further modify these effects (Wangpraseurt et al. 2012, Brown & Carpenter 2013).

Ceraesignum maximum

Corals and algae are not the only sessile species on reefs. Among other sessile species are vermetid gastropods, which are common throughout most of the world's oceans (Hadfield et al. 1972, Safriel 1975). These organisms form worm-like, loosely coiled tubes that are cemented to the reef matrix (Safriel 1975). Many of these snails are suspension feeders that feed by excreting a mucus net from their pedal gland (Morton 1950), allowing the net to collect particles, and then retracting and eating the net and its contents (Kappner et al. 2000). Among the vermetid gastropods, the largest and one of the most common, is *Ceraesignum maximum* (G.B. Sowerby I, 1825), formerly in the genus *Dendropoma* (see inset).

On coral reefs, *C. maximum* causes changes in coral morphology (Colgan 1985, Zvuloni et al. 2008, Shima et al. 2010, 2015) and reductions in coral growth and survival (Shima et al. 2010, 2013). These negative effects likely involve the snail's mucus nets, which contain bio-active compounds (Klöppel et al. 2013), although the precise mechanism underlying the

deleterious effect of vermetids on corals is not yet known. Although the nets do not reduce light levels (Brown and Osenberg 2018), they appear to impair the photosynthetic ability of *Symbiondinium* (Shima et al. 2015). They may also impede heterotrophic feeding by preventing particle capture, alter coral microbiomes, and/or decrease water flow at coral surfaces which could trap wastes or create other harmful conditions at coral surfaces (***Chapter 2)***. Additionally, the presence of vermetid mucus nets decreases herbivory (Tootell & Steele 2014), potentially facilitating macroalgal take-over of reefs. Larvae of *C. maximum* cannot settle to live coral, and they likely depend on disturbances that create open space to colonize new habitat (Phillips et al. 2014). Vermetids are more abundant in sites with higher proportions of dead coral (Shima et al. 2010), likely due to both their settlement requirements and their deleterious effects on corals.

Given the similarity in mechanisms that potentially underlie algal-coral and vermetid-coral interactions (e.g., allelopathy, and/or microbially-mediated mechanisms McCook 2001, Smith et al. 2006), **it is likely that vermetids and algae, when they co-occur, may act in concert and exacerbate deleterious effects on corals.** For example, vermetid nets may impede water flow at the coral surface. Thus, any dissolved chemicals in the water (e.g., DOC from nearby algae) may remain at coral surfaces for prolonged periods of time potentially leading to more pronounced changes in the coral-associated microbial community, which could lead to stronger deleterious effects (**Chapter 2, Chapter 3**). Therefore, I hypothesize that vermetids will exacerbate the effects of algae (**Chapter 4**), which can have downstream negative effects, and lead to a deceleration of reef recovery or acceleration of reef degradation in areas where algae and vermetids co-occur.

Some studies (e.g., Shima et al. 2010) have shown large effects of vermetids on coral growth (>50% reduction in growth), while other studies have shown negligible effects (Zill et al.

2017). For some of those studies that showed large effects (e.g., Shima et al. 2010), corals were collected as juveniles from an environment lacking vermetids; thus, these juveniles most likely had not been growing in close proximity to vermetids (and were likely "naive"). The prior experience of corals used in most experiments is not known. I hypothesize that variation in the effects of vermetids on corals is due to variation in the prior exposure of corals to vermetids: i.e., that vermetid exposure led to increased tolerance via acclimatization (i.e., adaptive plastic responses) of the coral and/or its associated microbial community (Reshef et al. 2006, Todd 2008). Interestingly, at my study sites in Mo'orea, French Polynesia, there are reefs that have vermetids and reefs that do not have vermetids on them (Shima et al. 2010), providing a powerful opportunity to test this hypothesis. Alternatively, the presence of some reefs with vermetids and some reefs without vermetids may be due to the underlying genetic differences among corals, which could influence whether or not vermetids could successfully colonize a reef. I explored how prior exposure of corals to vermetids affects the traits of corals and their microbes, and determined if there was evidence of coral acclimatization to vermetid interactions and/or if there was genetic variation among corals associated with the presence vs. absence of vermetids (**Chapter 5**).

The goals of my dissertation were to (1) use laboratory studies and field surveys to evaluate the potential effects of vermetids on water flow, dissolved oxygen dynamics, and microbial communities (**Chapters 2 and 3**); (2) experimentally quantify the direct and indirect effects (i.e., interaction modifications) of the vermetid*, C. maximum*, on corals, algae, and coral-algal interactions (**Chapter 4**); and (3) test if prior exposure of corals to vermetids leads to greater tolerance of corals to vermetids and evaluate if this plastic response may be due to shifts in the microbiome (**Chapter 5**).

CHAPTER 2

VERMETID GASTROPODS MODIFY PHYSICAL AND CHEMICAL CONDITIONS ABOVE CORAL-ALGAL INTERACTIONS[1]

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Abstract

Interaction modifications can arise when a third species alters the physical and chemical environment within which two other species interact. On coral reefs, corals and algae commonly interact amid a suite of other species that may modify their interaction. Massive *Porites* coral and algal turfs often are covered by mucus nets cast by the vermetid gastropod, *Ceraesignum maximum*. Previously, vermetid mucus nets have been shown to have deleterious effects on corals. Here, we hypothesized that vermetids not only have direct effects on coral, but they also change the local physical and chemical environment establishing the potential for interaction modifications by intensifying the effects of algae on corals. To test this, we examined the effect of vermetids on physical and chemical aspects of the environments. We quantified light penetration, water flow, diffusive boundary layer (DBL) thickness, and oxygen concentrations in the presence and absence of vermetid nets. Vermetid nets did not affect light levels. Because we observed reduced water flow and increased DBL thickness in the presence of nets, we also expected to observe high oxygen concentration over coral surfaces. Instead, we observed no difference in oxygen concentrations in the presence of mucus nets. To explain the lower than expected oxygen concentrations, we hypothesize that nets decreased photosynthesis and/or increased respiration of corals and algae and their associated microbiota. This is the first study to explore mechanisms underlying the deleterious effects of vermetids on corals, and shows that vermetid mucus nets may modify coral-algal interactions by intensifying physical and chemical conditions.

Introduction

Interaction modifications (or higher order interactions: Vandermeer 1969) arise when a third species alters the strength and/or direction of a pairwise interaction. For example, the presence of a non-lethal predator can decrease the intensity of competition between a potential prey and its competitor if the predator reduces the feeding activity of its prey (Peacor and Werner 2001). Predators also can induce changes in prey morphology, thus modifying the prey's interactions with its resources, competitors, or other predators (e.g., Relyea and Yurewicz 2002). In other cases, the strength of a predator-prey interaction can be modified by a biogenic habitat, which can alter the search ability of the predator. For example, the presence of large seaweeds can increase survival of mussels by decreasing their visibility to their crab predators (Bertness et al. 1999).

Interaction modifications can also arise when a species ameliorates harsh environmental conditions. For example, in the stress gradient hypothesis, one species modifies the physical environment, and thereby alleviates stress in another species, which may, therefore, affect interactions with other species in fundamentally different ways (Callaway 1997). The presence of a third species may also confer other changes in the physical environment. For example, increased shading by canopy trees intensifies competition among understory plants (Pagès et al. 2003). Similarly, the litter of *Pinus ponderosa* (pine) changes the soil chemical environment, thereby modifying the strength of competition between a grass and a shrub (Metlen et al. 2013, Metland and Callaway 2015). The importance of environmental modification by a third species and its effect on interactions between two other species emphasizes how species-driven changes to the physical and chemical environment can lead to complex ecological effects.

The influence of the physical and chemical environment on species interactions likely occurs in a diversity of systems. Here, we examine effects in a coral reef, where previous work has demonstrated that coral growth and survival is sensitive to changes in the physical-chemical environment. For example, reduced light availability can inhibit photosynthesis of the coral's symbiotic algae, *Symbiodinium*, and may lead to decreased coral growth (Chalker and Taylor 1975, Marubini et al. 2001). Reduced water flow increases boundary layers, and thicker boundary layers can lead to the build-up of harmful metabolic by-products and thus can decrease coral growth (Dennison and Barnes 1988, Kühl et al. 1995, Finelli et al. 2007).

Corals also are affected by interactions with other species. For example, algae are fast growing sessile competitors that can overgrow or shade corals (Box and Mumby 2007), transfer harmful microbes to corals (Nugues et al. 2004, Barott et al. 2011, 2012), or exude allelopathic chemicals that harm corals (Rasher and Hay 2010). Algae can also facilitate the growth of heterotrophic bacteria via the production of bio-available dissolved organic carbon (DOC, Kline et al. 2006, Smith et al. 2006, Nelson et al. 2013). Increased microbial growth leads to higher microbial respiration, which can result in hypoxic conditions at coral surfaces when corals are in close proximity to algae (Smith et al. 2006). Furthermore, algae likely mediate their deleterious effects, in part, by altering water flow. For example, algal turf (small, <5mm in height, multispecific filaments) can reduce water flow just above the coral surface, leading to greater retention of solutes (Brown and Carpenter 2015), thicker boundary layers (Wangpraseurt 2012, Brown and Carpenter 2013, Stocking et al. 2016), and increased concentrations of bacteria (Brown and Carpenter 2015). Importantly, the strength of the interaction between coral and algae (Wangpraseurt et al. 2012, Brown and Carpenter 2013, Jorissen et al. 2016) is affected by water flow. For example, reduced water flow increases exposure of corals to metabolic waste

products released by the algae, which can enhance deleterious effects and produce hypoxia or hyperoxia (Hauri et al. 2010, Brown and Carpenter 2013, 2015, Haas et al. 2013a).

Competition between coral and algae, and the importance of physical factors (such as light and water flow), set the stage for other species to affect corals if they alter the physical environment. For example, sessile vermetid gastropods may alter the ecological context in which corals and algae interact by changing light and/or water flow. The largest vermetid, *Ceraesignum* (formerly *Dendropoma*) *maximum*, inhabits shallow coral reefs in the Pacific and Indian Oceans and the Red Sea. This worm-like snail casts a mucus net over the substrate to capture small particles, including plankton. Frequently, their nets cover corals, and have deleterious effects on adult coral growth, survival, and photophysiology (Shima et al. 2010, 2013, 2015). The putative mechanisms underlying this negative effect likely involve the mucus net, although the mechanism(s) has not yet been identified. Here we hypothesize that the net alters the physical and chemical conditions around corals, leading not only to potential direct effects on corals but also to modification of coral-algal interactions. We therefore predict that vermetid nets: 1) reduce light availability, and 2) decrease water flow. 3) We also predict that vermetid nets will have greater effects on physical conditions in the presence of turf algae because vermetids will reduce water flow and thus exacerbate effects of algal turf on the microenvironment. In particular, we expect that vermetid nets alter the physical and chemical conditions on coral surfaces by increasing the thickness of the diffusive boundary layer (DBL) over corals, which should lead to enhanced hyperoxic conditions at the coral surface in the light due to the production of oxygen by turf algae (and similarly enhanced hypoxia in the dark due to respiration, although in our study we focused on daytime responses).

Methods

We conducted our studies in Mo'orea, French Polynesia at the UC Berkeley Richard B. Gump Marine Station, where massive *Porites* corals dominate the shallow back reefs and frequently interact with a myriad of algal species, especially algal turf (Brown and Carpenter 2015). The vermetid gastropod, *C. maximum*, is common in shallow water where *Porites* and turf algae also are common (Shima et al. 2010; but see Brown et al. 2016). To feed, this vermetid casts a mucus net, which collects particles in the water column before the net is retracted and the net and its contents are consumed by the snail (Kappner et al. 2000). Thus, microsites in which massive *Porites* and algal turfs interact are frequently covered by vermetid mucus nets (Appendix 2A Fig 1). Our studies were designed to evaluate how vermetids modify the physical microenvironment (with respect to light, flow, and boundary layers) and alter the context in which corals and turf algae interact.

Light Levels

To determine how vermetids influence the light environment, we used DEFI-L 2pi light meters to measure light levels in the presence and absence of mucus nets. We drilled holes onto dead areas of coral colonies where vermetids were present and placed a light sensor into the drilled hole, which served to hold the sensor upright, with the sensor surface just protruding above the top of the reef. The sensor was approximately flush with the coral surface and therefore measured light levels a coral would experience. We placed a second sensor 2-5m away from the primary sensor, in an area without vermetids nearby, and at a comparable water depth. The second sensor was secured in a weighted frame to ensure that it was upright and stable. Sensors recorded light levels (μmol photon m^{-2} s^{-1}) every second. To determine when the primary sensor was covered by vermetid mucus nets, we placed a GoPro camera in front of the primary

sensor and photographed the sensor every 30 seconds. At the end of a four-hour period, the sensors and camera were collected. This procedure was repeated on 6 days in 2013: June 7, 9, 10, 26, 30 and July 17. Light sensors were randomly assigned to a different treatment and new location each day.

Using the time series for each deployment, light levels were averaged every 30s to align with the photographic time series. Each photograph from the primary sensor was then scored as either having a net present or not. We then calculated the log-ratio (ln(Primary sensor/Second sensor)) to control for ambient fluctuations in light levels, and averaged these log-ratios during all times when the nets were present and during all times when they were absent. Thus, each deployment (i.e., a temporal block) generated two log-ratios (i.e., differences on a log-scale). If vermetids reduced light levels, then the log-ratio between the two sensors should have been reduced (e.g., become more negative) when nets were present. These data were analyzed using a paired t-test (n=6 pairs) to quantify the effect of vermetids on light levels and test the null hypothesis that the presence of nets did not affect light levels. Additional information, including justification for this design and additional analyses, are included in the electronic supplementary materials.

Retention Time of Water

We tested if the presence of vermetid nets and algae influenced the retention of water near corals. Changes in retention times of fluorescein dye have previously been used as a proxy for changes in water flow and diffusive boundary layers (Brown and Carpenter 2015). We recorded the retention time of fluorescein dye in the presence vs. absence of a vermetid net in microsites at which only coral was present or in sites at which algal turf also was present. After identifying an area of coral (or coral and algal turf) covered by a mucus net, we inserted 2 ml of fluorescein dye

(40 mg ml^{-1}) underneath the mucus net using a 5 ml syringe with a needle attached, and recorded (to the nearest second) the elapsed time until the dye had visually dissipated from a 1x1 cm^2 area. The net was then removed and, after 10 s, another 2 ml of dye was released in the same spot and the dissipation time re-measured. To check our methods, we also assessed dissipation using a fluorometer. All methods were identical to the previous approach, except that we sampled the water with a clean syringe 2 s after releasing the dye and determined the residual concentration of dye using a fluorometer. We analyzed the data using a 2-way ANOVA (net presence vs. absence crossed with algae presence vs. absence) with a random effect for site (since the same site was measured in the presence and absence of a net) using the lme4 package with Satterthwaite approximation for degrees of freedom (Bates et al. 2014, Kuznetsova 2016).

Oxygen Concentration Profiles

We quantified oxygen concentrations under two flow regimes (Low flow at 7.7 cm s^{-1} and high flow at 14.5 cm s^{-1}) using a laboratory flume, and used these data to estimate diffusive boundary layer (DBL) thickness at microsites above coral, above algae, and above the coral where it was directly interacting with algae (hereafter the "coral-algal interface"). We conducted our study with field-collected cores, the tops of which were comprised by half living massive *Porites* coral and half algal turf. We identified interfaces in the field and used a pneumatic drill with a hole saw attachment (6.35 cm diameter, 3.81 cm cutting depth; McMaster Carr) to remove the core. Cores were brought back to the lab and placed in aquaria with constant flow of seawater until they were used in the flume (always within a week of collection). On the day that a core was to be placed in the flume, we first took it into the field and draped a vermetid mucus net over the top to simulate the typical field condition in the presence of vermetids. The core (with the overlaying net) was enclosed in a small plastic container, brought back to the lab and returned to

the seawater table until placed in the flume 1-4 hours later. A core (with an intact net) was placed in the center of a ramp in the working section (160 × 10 × 12 cm) of the flume to ensure smooth flows, and then acclimated to the flume for 5 minutes at low flow (7.7 cm s^{-1}) in the light (800 μmol photon m^{-2} s^{-1}; Hubbell 1000 W metal halide light). Although this handling and transport may stress corals and algae and therefore affect the absolute oxygen concentrations we measured, we expected the relative differences among the treatments to reflect relative responses measured under more natural conditions.

We measured oxygen concentration profiles using a PreSens needle microsensor oxygen probe (diameter: <50 μm) attached to a PreSens Microx TX3 system (PreSens Precision Sensing GmbH). The probe was attached to a micromanipulator, which allowed fine-scale, precise motion to measure oxygen concentration profiles. The probe was lowered through the net to the coral surface. From the surface (distance = 0 μm), the probe was then raised in incremental steps of 100 μm until reaching a height of 2500 μm. At each step (every 100 μm) the probe was paused for 1 s and the oxygen concentration recorded. See Brown and Carpenter (2013) for more information about probe calibration, flume measurements, and description of the flume set up.

Oxygen profiles were obtained directly above the live coral (n=6-7), above the coral-algal interface (n=8), and above the algal turf (n=6-8). The net was then removed and the profiles obtained in all three locations again. All six profiles were obtained under low (7.7 cm s^{-1}) and high (14.5 cm s^{-1}) flow for each coral core with the net present, and again after nets were removed. Light levels were saturating (Carpenter 1985, Chalker 1981) and similar to those experienced in the field. Flow speeds also were chosen based on conditions experienced in the field (Brown and Carpenter 2015) and were calibrated in the flume with an Acoustic Doppler Velocimeter (Nortek AS Vectrino).

The thickness of the diffusive boundary layer (DBL) was determined by the height (above the substrate) at which oxygen concentrations reached 99% of the bulk oxygen concentration (Jorgensen & Revsbech 1985) based upon graphical representations of the profiles (concentrations by distance), and finding the distance at which oxygen concentrations reach 99% of the bulk oxygen concentration (see Kühl et al. 1995, Brown and Carpenter 2013). Oxygen profiles are provided in the electronic supplementary materials. Surface oxygen concentrations (i.e., measurements at distance 0) were compared to determine if mucus nets exacerbated hyperoxic conditions at the coral surface, indicative of retention of oxygen due to the presence of a mucus net.

DBL thicknesses and surface oxygen concentrations were compared using mixed model ANOVA, in which core was treated as a random effect. All data analyses were conducted using the programming language R (R Core Team 2015, version 3.2.3); ANOVA was performed with the lme4 package (Bates et al. 2014) with the degrees of freedom approximated using the Sattherwaite approximation (Kuznetsova et al. 2016). Tukey HSD post hoc tests were completed on the model using the lsmeans and multcompView packages in R (Graves et al. 2015, Lenth 2016).

Results

Light Levels

Light levels at the field sites were typically between 600 and 850 μmol photons m^{-2} s^{-1}, although one day (9-June-2013) had consistent cloud cover so light levels averaged only 182-245 μmol photons m^{-2} s^{-1} and the sensor located away from vermetids measured consistently higher light levels. The average log ratio between the two sensors was typically small and near 0 (0.035 ±

0.005, mean ± 95% Confidence Interval), and ratio did not vary consistently due to the presence versus absence of a vermetid net (difference in log ratio = 0.0081 ± 0.0393, mean ± 95% Confidence Interval; $t_5 = 0.529$, p=0.620, Appendix 2A Table 1, Fig. 2.1), which resulted in a less than 1% decrease in light. The most extreme difference in light levels (30-June-2013) corresponded to only a 6% reduction in light in the presence of nets. On five of the six dates, light levels were likely saturating (Chalker et al. 1981, Marubini et al. 2001). We therefore concluded that vermetid nets do not appreciably affect corals via reductions in light levels.

Retention time of water

Fluorescein dye was retained for significantly longer in the presence of vermetid mucus nets compared to when the nets were absent ($F_{1,32} = 13.79$, p=0.0008, Fig.2.2a). Additionally, we observed longer retention times when algae were present ($F_{1,32}$=6.9, p=0.0131, Fig. 2.2a). On average, dye was retained nearly twice as long in the presence of nets (1.7x for the coral alone, and 1.9x when corals were interacting with algae: see Appendix 2A. Table 2). Results using a fluorometer to measure fluorescein concentrations after 2s revealed similar patterns (Fig. 2.2b).

Oxygen concentration profiles

There were significant complex interactions among the factors (surface x net presence and a nearly significant three-way interaction), in part because there was little effect of flow or net presence on DBL thickness over algal turf (Table 2.1, Fig. 2.3). Over the coral and coral-algal interface, in general, we observed thicker boundary layers in the presence of mucus nets (Table 2.1, Fig. 2.3), although because of the complex interactions, we cautiously interpret this main effect. Increasing flow had no effect on DBL thickness over the coral-algal interface (or over algae), although higher flow did reduce the DBL thickness over coral when mucus nets were

present (Fig. 2.3a,b; Table 2.1). Thus, it appears that flow reduced DBL thickness except in the presence of algae (i.e., algae alone or at the coral-algal interface) and nets, suggesting that nets reduce the mixing effects of flow over complex 3-dimensional surfaces (e.g., algal turf) but not over relatively simple surfaces (e.g., coral).

All surface oxygen concentrations were hyperoxic, as expected given that profiles were obtained in the light when algal turf and *Symbiodinium* would be photosynthesizing. High flow decreased surface oxygen concentrations (as expected from greater mixing, Fig. 2.4). However, there was a nearly significant complex three-way interaction between flow, substrate and nets (Table 2.2). For coral surfaces, in low flow, surface oxygen concentrations were elevated in the presence of nets, but there were no differences between low and high flow in the absence of a net (Fig. 2.4a). At the coral-algal interface, the presence of a net had little effect on surface oxygen concentration (Fig. 2.4b, Table 2.2). For algal surfaces, nets appeared to depress surface oxygen concentrations especially compared to low flow conditions (Fig. 2.4c, Table 2.2).

Discussion

Our study is one of the first to evaluate possible mechanisms that underlie the deleterious effect of vermetids on corals (as documented by Shima et al. 2010, Stier et al. 2010, and Shima et al. 2013). Vermetids change the physical and chemical microenvironment below their mucus nets by reducing water flow, increasing retention times, and increasing boundary layer thickness, but not by reducing light levels. These effects, however, depended on the substrate over which the measurements were made. When corals and algae abut, the presence of vermetid mucus nets increase retention times. However, the effects of mucus nets and flow were dependent on surface type. Notably, when algae were present near corals, increased water flow did not

decrease DBL thickness, indicating that the presence of algae and mucus nets combine to create conditions that lower flow. As a result, algae and nets create more homogeneous conditions near coral surfaces that are independent of the overlying water flow regime.

These modified physical and chemical conditions induced by vermetids may intensify the interactions between coral and algae. For example, Smith et al. (2006) hypothesized that algae indirectly affect coral by releasing DOC and increasing heterotrophic microbial growth, leading to hypoxic conditions and coral mortality. Others have suggested that this process depends on water flow, and can only occur in low flow conditions (Wangprasseurt 2012, Brown and Carpenter 2013, Haas 2013a, Jorissen 2016). Additionally, long retention times, lowered flow, and/or thicker diffusive boundary layers can lead to the build-up of noxious conditions, for example, by maintaining harmful waste products near the surface of corals (Hauri et al. 2010). Thus, the presence of mucus nets, by lowering flow and increasing boundary layer thickness, can exacerbate the mechanisms underlying coral-algal competition. This is especially evident at the surface of coral-algal interactions, where increasing water flow, which usually decreases boundary layer thickness, has little effect on DBL thickness when nets are present (Fig. 2.3).

Interestingly, we did not observe an accumulation of oxygen concentration in the presence of mucus nets (Fig. 2.4). This result is curious, as previous work has shown a positive relationship between diffusive boundary layer thickness and oxygen concentrations at coral and algal surfaces during daylight (Brown and Carpenter 2013, Jorissen et al. 2016). The lack of increase in surface oxygen concentrations in the presence of nets and algae suggests that nets not only increased the DBL but also led to lower net production of oxygen (although the overall condition remained hyperoxic). We propose and discuss two hypotheses: 1) lowered

photosynthesis in the presence of vermetid mucus nets; 2) increased oxygen consumption (i.e., respiration), potentially by the microbial community.

If photosynthesis of *Symbiodinium* and/or algal turf was reduced by the presence of nets, oxygen production would have been reduced, which may have prevented the accumulation of oxygen despite increased DBL thickness. This reduction in photosynthesis must involve a mechanism other than light (Fig. 2.1) since vermetid nets did not appreciably reduce light intensity. For algal turf, the physical barrier the net creates may lead to difficulty acquiring DIC (dissolved inorganic carbon) needed to maintain high rates of photosynthesis (Carpenter and Williams 2007). Corals may also exhibit reduced photosynthetic efficiency under low flow (Finelli et al. 2007; Mass et al. 2010), a condition created by vermetids (Fig. 2.2), possibly because hyperoxic conditions can damage *Symbiodinium* photosynthetic apparatuses (Mass et al. 2010). Effects on surface oxygen concentrations of algal turfs are likely to be even more pronounced than effects on *Symbiodinium* (as observed: see Fig. 2.4). For example, algal turfs in low flows, are mass-transfer limited, but generally have high rates of photosynthesis (0.3 to 3.2 μ mol O_2 cm^{-2} h^{-1}, Carpenter and Williams 2007) compared to photosynthetic rates of *Symbiodinium*/massive *Porites* (0.21 μ mol O_2 cm^{-2} h^{-1}; Anthony et al. 2008). Thus, a reduction in the photosynthetic rate of algal turfs would likely have a more demonstrable effect on oxygen concentrations than a reduction in photosynthesis by *Symbiodinium*.

Alternatively, the presence of the nets may lead to an increase in respiration: e.g., by increasing the activity of heterotrophic microbes or through photorespiration of *Symbiondinium* (Mass and Genin 2010). Heterotrophic microbes can reduce oxygen concentrations at the interface between corals and algae (Smith et al. 2006, Barott et al. 2011, Jorrisen et al. 2016, but see Brown and Carpenter 2013), putatively due to excess DOC produced by leaky algae (Kline et

al. 2006). For example, in no flow, the interface between coral (*Favia* sp.) and algae (*Chaetomorpha* sp.) can approach hypoxia due to microbial respiration, even in the light (Haas et al. 2013a). Evidence for increased microbial activity due to algal byproducts has been found in Moorea, French Polynesia where DOC produced by algal turf leads to increased growth and respiration of heterotrophic microbes (Haas et al. 2013b). If algal-derived DOC or coral metabolic byproducts used by heterotrophic microbes are trapped under the net, microbial activity might increase, leading to increased respiration, and thus preventing the accumulation of oxygen when nets are present. Additionally, mucosal products of corals and other mucus-producing organisms have been previously found to fuel microbial growth (Wild et al. 2004, Wild et al. 2010). Similarly, it is possible that the mucus net from vermetids may provide a food source for the heterotrophic microbial communities on corals, leading to increased respiration in the presence of mucus nets. However, vermetid nets also contain bioactive compounds, which may have antibacterial properties (Kloppel et al. 2013), suggesting that nets could instead reduce microbial growth and respiration.

These hypotheses are not mutually exclusive, and may act in concert to influence the chemical conditions around coral-algal interactions when vermetid nets are present. Indeed, previous studies have shown that vermetids decrease the photosynthetic efficiency of corals (Shima et al. 2015), indicating vermetids may reduce coral photosynthesis. Alternatively, mucus nets, by affecting coral microbial communities, indirectly could affect the efficiency of photosynthesis (e.g., chemical effects of algae on corals decrease photosynthetic efficiency, Rasher and Hay 2010). Thus, nets, by lowering flow, preventing mixing and exacerbating deleterious chemical conditions, may lead to a combination of decreased photosynthesis and

increased respiration, which would depress oxygen concentration below that expected from thicker DBLs.

Although our short-term, mechanistic studies do not directly quantify the effects of vermetids on coral-algal competition, many past studies demonstrate that the effects on the physical environment will alter the interaction between corals and algae. For example, reduced flow (and increased DBL thickness) intensifies the deleterious effects of algae on corals (Brown and Carpenter 2015, Gowan et al. 2014). Because coral-algal dynamics are critical to the understanding of coral reef resilience, we suggest that vermetid gastropods, through their modification of the environment, could play an important role in coral reef community dynamics in areas where corals, algae and vermetids co-occur (e.g., South Pacific and Red Sea). Previous studies have demonstrated the dominant role that higher order interactions can have on species interactions (Werner and Peacor 2003, Schmitz et al. 2004). Often, interaction modifications are characterized by phenotypic or behavioral responses to the presence of another organisms (e.g., a predator: Werner and Peacor 2003), or by the effects of another organism on the physical structure of the environment (Bertness et al. 1999, Pagès et al. 2003). Our study is the first to demonstrate how a gastropod (in this case, *C. maximum*) acts as an interaction modifier by changing the physical and chemical environment in which competitors, corals and algae, interact.

Table 2.1: Results of mixed model ANOVA on the thickness of the diffusive boundary layer (DBL). Surface refers to the location of the profile (over the coral, coral-algal interface, or algae); flow is either low or high; and net presence is either present or absent. Significant terms ($P<0.05$) are indicated in bold and nearly significant terms are italicized ($0.05<P<0.10$).

	Numerator DF	Denominator DF	F	P
Net presence	**1**	**76.57**	**14.82**	**0.0002**
Flow	**1**	**77.16**	**11.59**	**0.001**
Surface	**2**	**82.47**	**3.46**	**0.036**
Net presence x Flow	1	76.57	0.54	0.466
Net presence x Surface	**2**	**76.57**	**5.52**	**0.006**
Flow x Surface	2	78.64	0.33	0.716
Net presence x Flow x Surface	*2*	*76.57*	*2.81*	*0.066*

Table 2.2. Results of mixed model ANOVA on surface oxygen concentration in the light. Surface refers to the location of the profile (over the coral, coral-algal interface, or algae); flow is either low or high; and net presence is either present or absent. Significant terms ($P<0.05$) are indicated in bold and nearly significant terms are italicized ($0.05<P<0.10$).

	Numerator DF	Denominator DF	F-value	p-value
Net presence	1	74.657	0.105	0.7463
Flow	**1**	**75.147**	**26.19**	**2.3×10^{-6}**
Surface	**2**	**81.15**	**29.05**	**3.06×10^{-10}**
Net presence x Flow	1	74.657	0.071	0.7901
Net presence x Surface	*2*	*74.657*	*2.463*	*0.0921*
Flow x Surface	2	76.202	0.823	0.4428
Net presence x Flow x Surface	*2*	74.657	*2.836*	*0.0650*

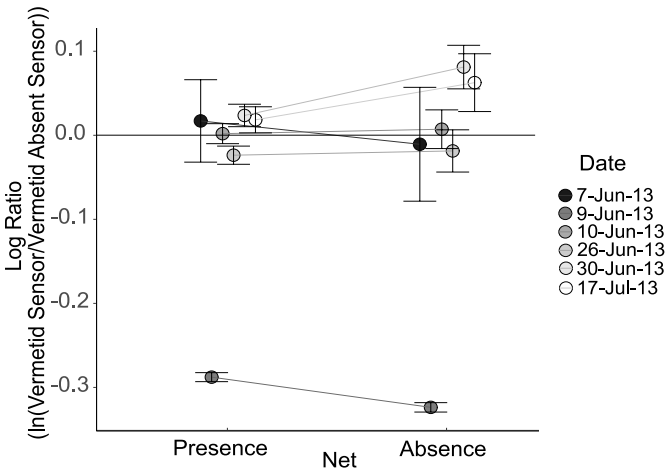


Figure 2.1. Mean log-ratio (± 95% Confidence Intervals) in light levels between sensors that were either close to (primary sensor), or isolated from (secondary), vermetids during periods when the sensor close to vermetids was covered by a mucus net (net present) or not covered by a mucus net (net absent) for each date sampled (circles represent the different dates: June 7, 9, 10, 26, 30 and July 17). The second sensor provided a standard to adjust for temporal fluctuations in light intensity. Mean log-ratio and confidence intervals are based upon observations of light levels (taken every second but averaged over 30s periods) on the sensors near vs. isolated from vermetids. Departures of the log-ratio from 0 in the absence of the net simply indicate that one sensor was in a location or orientation that received slightly more (or less) light on average. If vermetids reduced light levels, then the log-ratio should be reduced (e.g., become more negative) when nets were deployed (i.e., the mean log-ratio when nets were present should consistently lie below the log-ratios when nets were absent)

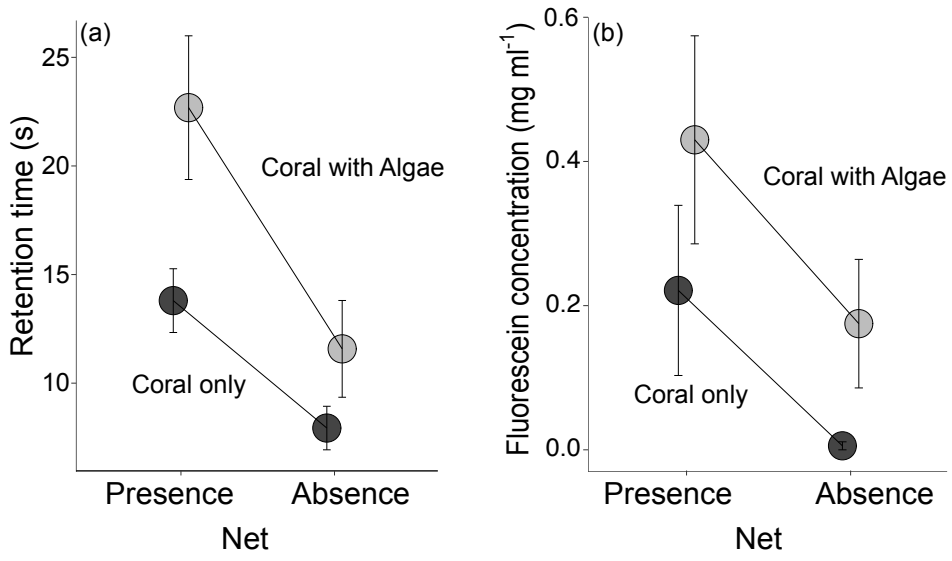


Figure 2.2. Mean (± SE; n=15 for algae absent and 19 for algae present) a) retention time of fluorescein dye, and b) fluorescein dye concentrations (after 2 s), over corals when algae are absent and when algae are present at the coral-algal interface, and when vermetid snail mucus nets are absent (no net) or present (net). Both algal presence and mucus nets led to significant increases in fluorescein dye retention times (P=0.0131 and P=0.0008, respectively). Nets also led to higher concentrations of fluorescein dye (P=0.045)

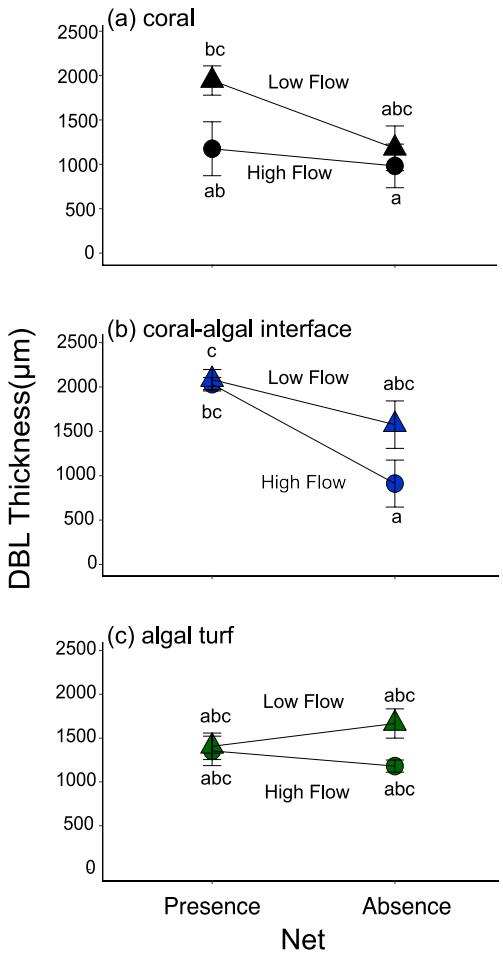


Figure 2.3. Mean (±1 SE; n ranges from 6-8) diffusive boundary layer thickness (DBL) over: a) coral, b) the coral-algal interface, and c) algae, at two flow speeds (low: triangles; and high: circles) in the presence and absence of a vermetid net. DBLs were thicker in the presence of nets over coral and the coral-algal interface, but not over the algae. Under most conditions, increased flow reduced thickness of the DBL, except over the coral-algal interface and over algae, in the presence of nets. See Table 1 for statistical analyses. Letters refer to Tukey HSD post hoc analyses, where the same letters indicate no significant difference where groups with different letters indicate significant differences at a $P < 0.05$

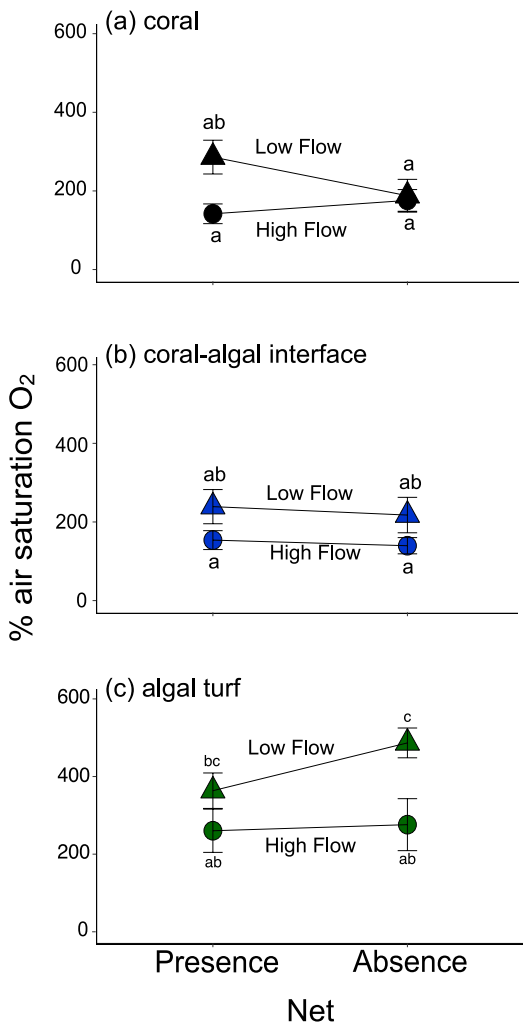


Figure 2.4. Mean (±1 SE; n ranges from 6-8) surface oxygen concentrations in the light over (a) coral, (b) the coral-algal interface, and (c) the algae, at two flow speeds (low: triangles; high: circles). The effects of mucus are influenced by the flow regime and the surface type (Table 2). Letters refer to Tukey HSD post hoc analyses, where different letters indicate significant differences at a $P < 0.05$

2. Appendix

2A Table 1: Results of Mixed Effects Model evaluating the effect of net cover on the log-ratio of light levels with random effects of date and time. Results were analyzed using the lme4 packages (Bates et al. 2015). This test ignores temporal autocorrelation in the time series and thus likely inflates the error degrees of freedom. We prefer the analysis provided in the main text; however, we include this analysis for completeness. Because neither analysis provides evidence for a consistent effect of vermetid nets on light, we conclude that our interpretation that effects of vermetids on light are small (or non-existent) is robust to the specific approach take to analyze the data.

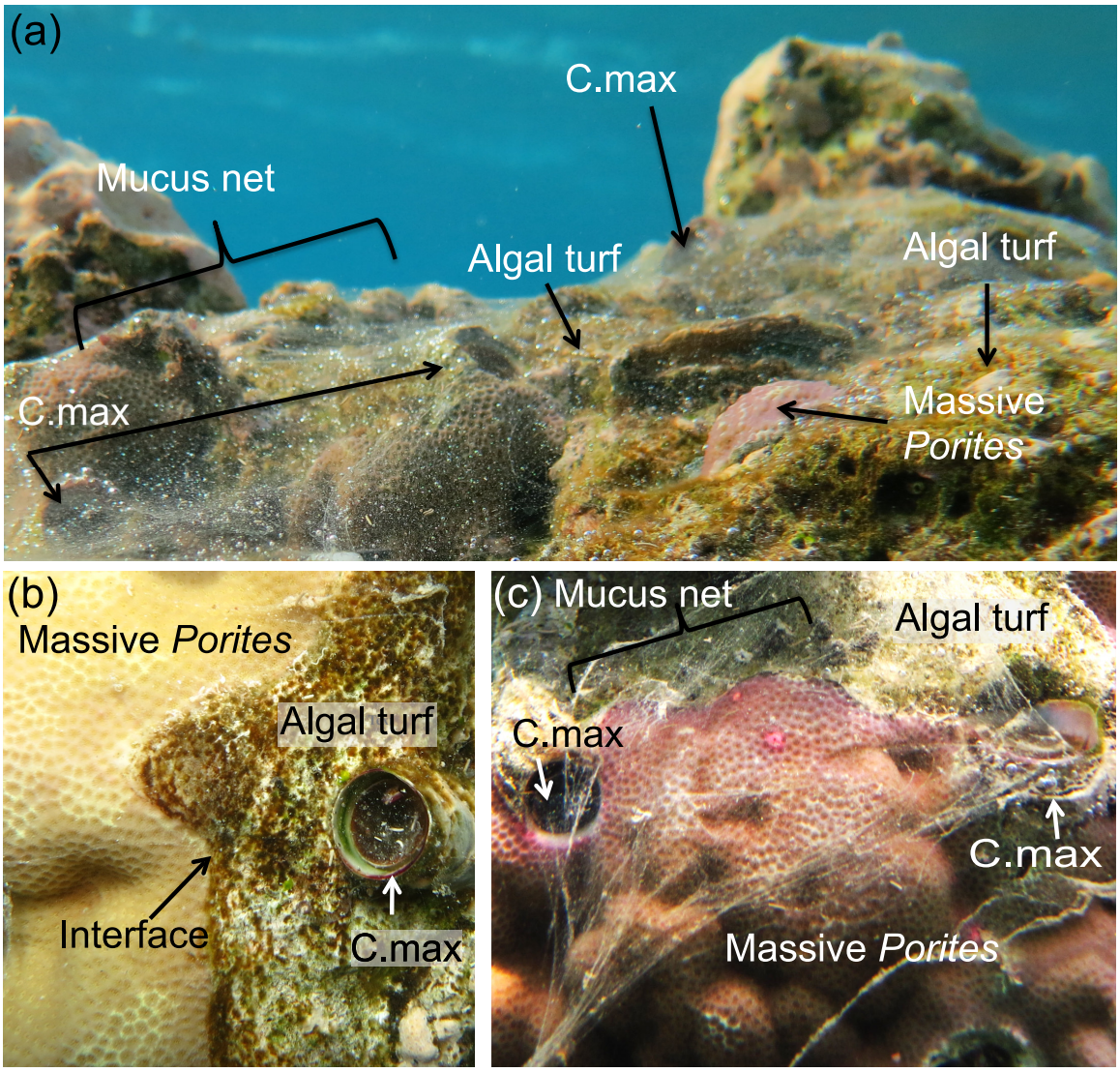
	Estimate	SE	df	t value	P
(Intercept)	-0.034	0.049	6	-0.69	0.52
Net Cover	-0.01	0.006	3180	-1.48	0.14

2A Table 2: Mean ± SE (sample size) of retention times of fluorescein dye over corals and the coral-algal interfaces.

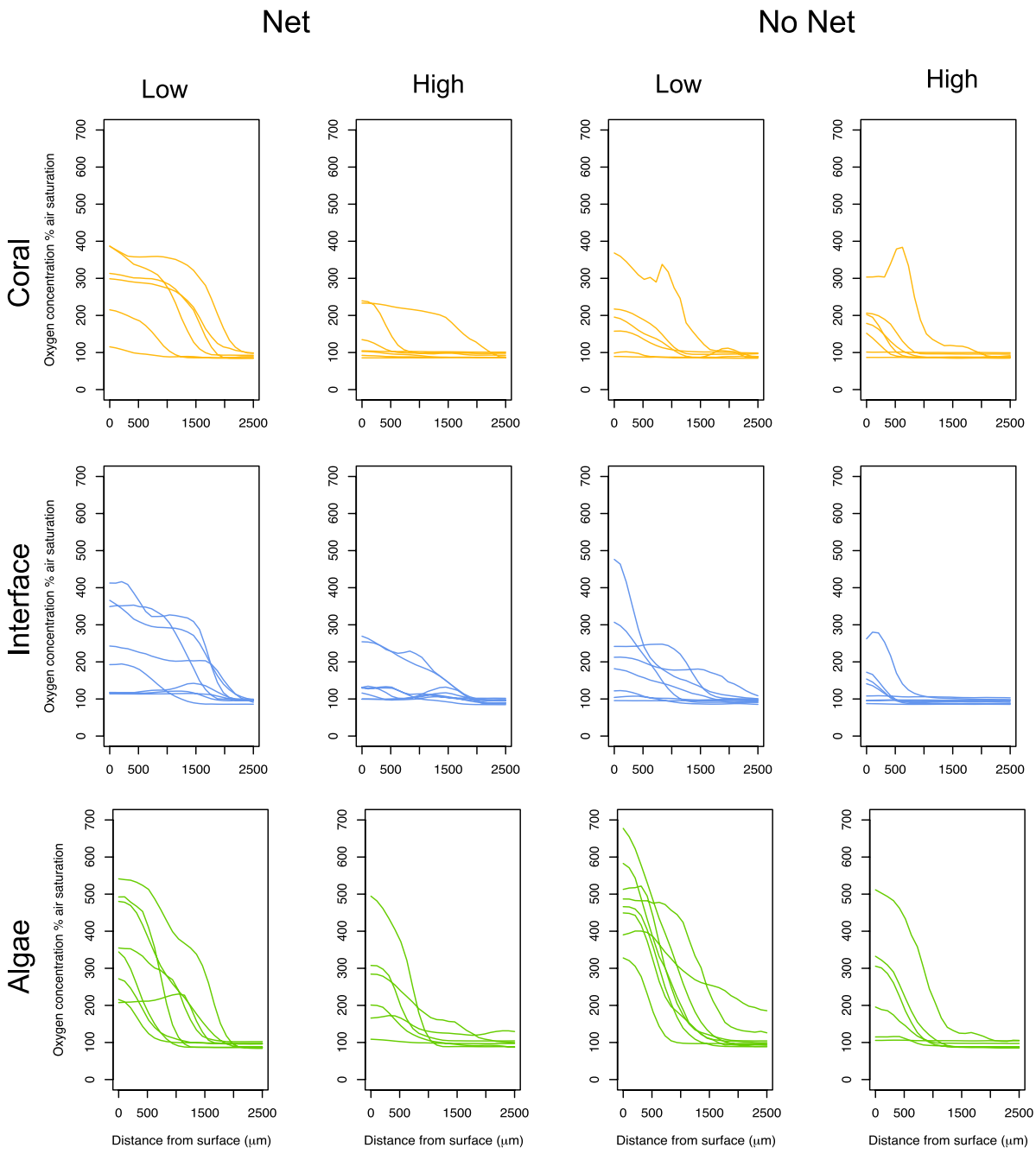
	Retention time (s)		
Surface	Net Present	Net Absent	Net Present – Net Absent
Coral	13.8±1.5 (15)	7.9±3.9(15)	5.9±1.2 (15)
Interface	22.7±14.4 (19)	11.6±9.7 (19)	11.1±4.7 (19)

2A Table 3: Mean±SE (sample size) thickness of the diffusive boundary layer (DBL) over the coral, the coral-algal interface, and over the algae (algal turf).

Surface	Flow	Net (μm)	No Net (μm)
Coral	Low	1944.4±165.1 (6)	1180.6± 251.8 (6)
	High	1175.6± 303.9 (7)	982.1±245.7 (7)
Interface	Low	2083.3±113.1 (8)	1575.5±267.4 (8)
	High	1966.1±69.4 (8)	494.8±167.9 (8)
Algae	Low	1406.2±151.2 (8)	1666.6±167.0 (8)
	High	1354.1±168.0 (6)	1180.5±69.4 (6)



2A. Figure 1. (a) Photograph of the top of a coral bommie, showing the vermetid (*C. maximum*: C.max), live coral (massive *Porites*), algal turf, and mucus nets (the opaque, spider-web like material covering the algae and coral with bubbles forming underneath the net). (b) Image of massive *Porites* coral, algal turf and the interface between them, near a vermetid that has not yet exuded a net. (c) Image of massive *Porites* coral, algal turf and vermetids with mucus nets deployed (the nets are the opaque spider-web like material).



2A. Figure 2. Raw data for oxygen concentration profiles in the light. Measurements were taken in 100µm steps from the surface of the coral, the interface or the algal turf. Rows give results for different surfaces (Coral in orange; coral-algal interface in blue; algae in green). Columns give

results in the presence vs. absence of nets and under low vs. high water flow. DBL thickness and initial oxygen concentrations were extracted from these data.

CHAPTER 3

ALGAE DICTATE MULTIPLE STRESSOR EFFECTS ON CORAL MICROBIOMES[2]

[2] To be submitted to *Coral Reefs*, and authored by A.L. Brown, E. K. Lipp, C.W. Osenberg.

Abstract

Most studies of stressors focus on the response of traditionally classified organisms (e.g., via effects on growth, mortality or physiology); however, most organisms have microbial associates that may mediate the response of the host to the stressor. Additionally, organisms rarely experience one stressor alone, but instead are bombarded with multiple, potentially interacting stressors. I evaluated how coral microbiomes responded to two two biotic stressors: the vermetid gastropod, *Ceraesignum maximum*, and algal turfs, both of which have been previously shown to decrease coral growth, survival and photophysiology. I collected coral mucus in the presence vs. absence of both algae and vermetids and then sequenced the 16S rRNA gene to characterize the coral surface microbial communities. The presence of algae had much greater effects than vermetids on coral microbial community alpha diversity and community composition, which is likely driven by the increases in rare members of the community. Algae led to lower variation across communities, which I hypothesized was due to the way in which algae changed the physical environment when they were present. In contrast, vermetids had only small effects on microbial communities, although other studies demonstrate that vermetids have deleterious effects on coral growth. I suggest that algae primarily affect corals through their effects on microbes whereas vermetids primarily affect the host directly; these complementary effects may limit the extent to which stressors can interact.

Introduction

Ecological systems are affected by multiple stressors that can have negative effects on individuals and populations. These factors can be abiotic (e.g., extreme temperatures) as well as biotic (e.g., disease or predation). Rarely do stressors occur alone. More frequently, species

experience multiple stressors concurrently (Lenihan 1999, Boone et al. 2007, Buck et al. 2011). When stressors combine, their effects can be additive (in which the effects of the stressors are the sum of their effects alone), antagonistic or synergistic (in which effects are smaller or larger, respectively, than the effects predicted under the assumption of additivity; Crain et al. 2008, Darling and Côté 2008). Often, the effects of stressors on individuals are measured by differences in physiology, growth or survival. For example, pollutants and predators combine synergistically to increase mortality of tadpoles (Relyea & Mills 2001). However, nearly all organisms are associated with microbial communities (Wahl et al. 2012), which also respond to environmental pressures. Studies of holobionts (the microbial communities plus host tissues, Mindell 1992, Rohwer et al. 2001) are beginning to explore how stressors affect host-associated microbial communities.

Changes in host-associated microbial (here I focus on bacterial) communities are often associated with stress. For example, increases in temperature can lead to changes in the diversity (i.e., the number of unique groups present) of the microbiome of sessile species (Vega Thurber et al. 2009), and changes in nutrient regimes (e.g., diet) can lead to compositional shifts in the human gut microbiome (Turnbaugh et al. 2009). Often, however, many studies of microbiomes have only looked at single stressors and we do not understand how combinations of stressors can influence microbial communities, especially in critical ecosystems like coral reefs.

Coral reefs are highly diverse ecosystems that experience local and global stressors, like temperature increases, nutrient pollution and disease. These stressors affect the health, survival and growth of corals, and are likely mediated through effects on their microbial communities. Corals are holobionts that are composed of host tissue plus a diverse array of microbial associates, including bacteria, viruses, and fungi (Rohwer et al. 2001, Peixoto et al. 2017).

Hereafter, I focus on prokaryotic microbes (i.e., bacteria). Coral bacterial communities can be found in coral tissues (Sweet et al. 2011, Davy et al. 2012), in their gastrovascular canal (Sweet et al. 2010, Davy et al. 2012), as well as on their surface mucus layer (Rohwer et al. 2001, Sweet et al. 2011, Davy et al. 2012). Although not well described, these bacterial communities are associated with nutrient cycling (Davy et al. 2012) and defense (Davy et al. 2012, Reshef et al. 2006, Peixoto et al. 2017). Indeed, the surface mucus layer of corals is considered the coral's first line of defense, as it is the first area of a coral that comes in contact with the environment.

Single stressors, like increased temperature and DOC and reduced pH, are all associated with changes in coral surface microbial communities (i.e., in alpha diversity, beta-diversity/composition; Vega Thurber 2009, McDevitt-Irwin et al. 2017). Recently, combinations of abiotic stressors (increased temperatures and ocean acidification) and biotic stressors (nutrient increases and predation) have been shown to change coral microbial communities (Webster et al. 2016, Shaver et al. 2017). However, few studies explore the potential effects of multiple biotic stressors on coral microbial communities.

Macroalgae (hereafter referred to as algae) decrease coral growth (Jompa & McCook 2003), increase coral bleaching (Rasher & Hay 2010), and decrease coral survival (Kuffner et al. 2006). Furthermore, algae has been implicated in changes in coral microbial communities near the point of contact i.e., the interface of coral and algae (Barott et al. 2011, 2012). Algal presence leads to increases in alpha diversity (Vega Thurber et al. 2012) and compositional changes, including increasing the relative abundance of heterotrophic bacterial groups and groups that are implicated in coral diseases (Nugues et al. 2004, Vega Thurber et al. 2012, Sweet et al. 2013). Algae also lead to increases in beta-diversity: higher variation across coral microbial communities (Zaneveld et al. 2016).

The mechanism underlying effects of algae on coral is that algal-derived photosynthate (dissolved organic carbon, DOC) fuels microbial growth or changes in microbial communities, and leads to hypoxic conditions on coral surfaces via increased microbial respiration, which could result in lower coral cover through coral death (Kline et al. 2006, Smith et al. 2006, Barott et al. 2011). Among the algal groups that are known to have the greatest effect on microbial communities are multiphyletic, filamentous algal turfs, due to their high production of DOC (Nelson et al. 2013). However, this hypothesized pathway is only possible when water flow is low and diffusive boundary layers (regions of molecular transport) are thick enough to create conditions that would allow for the retention of DOC or low O_2 (Wangpraseurt et al. 2012, Brown & Carpenter 2013, Haas et al. 2013, Jorrisen 2016).

Vermetid gastropods, especially the largest species, *Ceraesignum maximum*, are another group known to induce deleterious effects on corals, including decreased growth (Shima et al. 2010, 2013), decreased survival (Shima et al. 2010), and decreased photosynthetic yield (Shima et al. 2015). Vermetids are sessile gastropods that feed by casting a mucus net that covers the nearby benthos and collects particles from the water column. The putative mechanism underlying these effects of vermetids on corals is their mucus net. However, the effects of vermetids on microbes are unknown.

Recently, it has been shown that vermetids can also create thicker boundary layers around corals (Brown & Osenberg 2018; Chapter 2), especially when algae are present. As a result, I expected that vermetid mucus nets would create low-flow conditions that would facilitate the growth of microbes in ways that were comparable to effects of algae on coral microbes. Thus, I expected that 1) effects of vermetids on microbial communities would be similar to effects of algae; and 2) the combined presence of vermetids and algae would lead to synergistic changes in

coral microbiomes. Specifically, I asked if there is evidence of multiple stressor effects on coral microbial communities via changes in alpha diversity (rarefied and Chao1 richness measures, and Shannon Diversity), beta diversity (i.e., within group variance) and compositional changes that lead to increases in heterotrophic groups (i.e., potential pathogens like *Vibrios*).

Methods

System

Samples were collected on the north shore shallow back reef environment of Mo'orea, French Polynesia in the austral winter 31 July 2014 (S17° 28.466' W149° 47.313'). *Ceraesignum maximum* in Mo'orea reach high abundances (up to 30 snails m^{-2}, Shima et al. 2010, but see Brown et al. 2016). Vermetids can only settle to areas that have been previously disturbed, as they cannot settle to living coral (Phillips et al. 2014). Thus they are frequently located on bommies (i.e., reefs) composed of a mosaic of living coral and other benthic components, like algal turfs. Algal turf is a functional group made up of a collection of filaments that belong to a multiphyletic functional group (Steneck & Dethier 1994). Algal turf is frequently in contact with massive *Porites* corals in the back reef of Mo'orea (Brown & Carpenter 2014). Massive *Porites* is a species complex of visually indistinguishable mounding corals that are 1-2m in height. The taxonomy of the massive *Porites* species complex is ambiguous (Forsman et al. 2009, 2015), but are frequently assigned to *Porites lobata*, *Porites lutea*, and *Porites austrialiensis*. Vermetids are frequently located on bommies that were once living massive *Porites*, but are typically a mosaic of live coral, dead coral, algae and other sessile groups. As a result, vermetid nets often overlay interactions between massive *Porites* and algal turf.

Sampling

I sampled coral mucus from 10 massive *Porites* bommies, all of which also had the vermetid, *C. maximum*, as well as algal turf. On each reef, I placed two, 5 x 5 cm quadrats around the interface between the massive *Porites* colony and the algal turf. One quadrat was placed near a *C. maximum* (i.e., vermetid present treatment), and the other was placed ≥20 cm away from the nearest vermetid (i.e., vermetid absent treatment). Twenty centimeters is the maximum reach of the net of a vermetid (Allen-Jacobson 2018). Coral-algal turf interfaces were ~7 cm in length. Ten milliliter syringes were used to agitate coral surfaces and then collect mucus from two locations within each quadrat: 1) from the coral that was within 2 cm of the coral-algal interface; and 2) from the coral 5 cm away from the interface (Fig 3.1). Previous work has shown that the microbiome ≥5 cm away from the coral-algal interface is devoid of any effect of the algae (Barott et al. 2011, but see Pratte et al. 2017, where they found a small effect of algae 5 cm away from the interface). Samples were transported on ice to the lab. Mucus was allowed to settle to the bottom of the syringe, and then ejected into a 1.5 mL micro-centrifuge tube and spun down to a pellet in a centrifuge at 10,000 RPM, for five minutes. In addition to the coral mucus samples, we also collected water samples (n = 8) and sediment samples (n = 4). Water was collected with a 10 ml syringe mid water column at haphazard locations along the reef; 2 ml of each water sample was frozen. Sediments were collected haphazardly in Whirlpaks ©. In the lab, approximately 0.5 g of sediment were inserted into 1.5 ml microcentrifuge tubes. Coral mucus, water, and sediment samples were frozen in a -80°C freezer and later transported on dry ice to the University of Georgia, where samples were immediately placed in a -20°C freezer until extracted.

Extractions

All samples were extracted using methods outlined in Boström et al. (2004) with minor modifications. I included a bead-beating step, in which I initially added 0.04g glass beads (Omega Biotech), and then after adding lysozyme (concentration: 1mg ml^{-1}), vortexed the samples for 10 minutes at full speed with a vortex adapter (© Mobio). At the end of the extraction, pellets were eluted in 25 µl of Qiagen Elution buffer. To remove PCR inhibitors, I added equal volumes of magnetic beads in PEG solution. PEG coats the beads and "grabs" DNA (Rudi et al. 1997). Following two wash steps in 200 µl of 80% ETOH, a final volume of 25 µl of Omega Elution buffer was added to elute the DNA and beads.

Sequencing

Extracted DNA was sent to a commercial laboratory for sequencing (www.mrdnalab.com, Shallowater, TX, USA) on an Illumina MiSeq platform. The V4 region of the 16S rRNA gene was sequenced using the 515F/806R primer pair with the barcode on the forward primer. The company performed PCRs on the samples. They used a 30 cycle PCR using the HotStarTaq Plus Master Mix Kit (Qiagen, USA) under the following conditions: 94°C for 3 minutes, 28 cycles of 94°C for 30 seconds, 53°C for 40 s and 72°C for 1 min, and a final elongation step at 72°C for 5 minutes. Samples were purified using calibrated Ampure XP beads. The PCR products then were used to prepare the DNA library using the Illumina TruSeq DNA library preparation protocol. Raw sequence data were returned to UGA for analysis.

Bioinformatics and Analysis

I assembled data using the QIIME pipeline (Caporaso et al. 2010, 2011). I used SeqPrep to assemble the forward and reverse reads. Chimeras (incorrectly merged sequences) were identified using usearch61 (Edgar 2010) and the Greengenes database (Feb 2011, DeSantis et al.

2006), and subsequently removed. OTUs (operational taxonomic units) were assembled using open reference frame OTU picking, which matches sequences to the Greengenes database at 97% sequence similarity (McDonald et al. 2012, Werner et al. 2012), and clusters the remaining OTUs de novo (uclust, Caporaso et al. 2010, Edgar 2010). Following OTU classification, non-bacterial, mitochondrial and chloroplast sequences were removed, and data were compiled into a biom table that was imported into R (version 3.3.2, R Core Team, 2016) for further analyses.

Microbial sequence data were analyzed using the phyloseq (McMurdie and Holmes 2013) and vegan packages (Oksanen et al. 2017). For alpha diversity analyses, samples were rarefied to an even sampling depth and then richness (number of unique OTUs), rarefied richness, Chao1 richness, and Shannon diversity were calculated using the phyloseq package. These analyses were performed with and without the water and sediment samples. Treatment effects on rarefied richness, Chao1 richness and Shannon diversity were determined using a linear model with vermetid presence/absence crossed with algal presence/absence, and reef as a random effect. Water and sediment were excluded from this analysis, but included in a graphical presentation: see Appendix 3A.Figure 1.

For compositional and beta diversity comparisons, I summarized the data (not rarefied) at the family level and used the number of sequences per sample to estimate relative abundances. I examined differences in beta diversity (among samples within a treatment) and evaluated variation across treatments using multivariate homogeneity of group dispersions (betadisper the equivalent of PERMADISP, in the vegan package in R, Oksanen et al. 2017) based on Bray-Curtis dissimilarity matrices. These values represent distances from each sample to its group's centroid.

I visualized the relative abundances of OTUs at the family level using non-multidimensional scaling of Bray-Curtis dissimilarity matrices, in which each sample was represented as a point in the nMDS space. I conducted two visualizations: one with and one without the water and sediment samples. The data from the crossed design (Algae x Vermetid) were analyzed for differences in composition with PERMANOVA using the adonis function in the vegan package in R (Oksanen et al. 2017)

Using the families that significantly contributed to differences observed in the nMDS plot (significance based on 999 permutations), I compared the relative abundance differences of each family separately using the crossed design (Algae x Vermetid). Lastly, because the genus *Vibrio* includes coral pathogens (Vezzulli et al. 2010, Peixoto et al. 2017, Kemp et al. 2017), I also compared the relative abundance of OTUs that were assigned to the genus *Vibrio*.

For all linear models, data were analyzed using a linear mixed effects model from the lme4 (Bates et al. 2014) and lmerTest packages (Kuznetsova et al. 2016) with Satterthwaite approximation for degrees of freedom. Fixed effects were Vermetids, Algae and Vermetids x Algae. I treated bommies from which corals microbes were sampled as a random effect (random intercept).

Results

After quality filtering, I had a total of 4,777,381 sequences across all samples. The average number of sequences per sample was 78,317 ± 44,176 (±sd; n=52 samples). After rarefying (without water and sediments), each sample contained 11,629 sequences. Rarefied OTU richness (alpha diversity) increased approximately 2-fold in the presence of algae ($p<0.001$) but did not change significantly in the presence of vermetids ($F_{1,30} = 2.18$ $p=0.15$). There was no interaction ($F_{1,30} = 0.08$, $p=0.77$, Fig 3.2a) suggesting the absence of a synergistic

or antagonistic effect between algae and vermetids. Shannon diversity index, which takes into account evenness as well as richness, showed similar results (Fig 3.2c, Shannon: algae: $F_{1,30}$ = 42.4 , p <0.001; vermetids: $F_{1,30}$ = 1.7, p = 0.2; interaction: $F_{1,30}$=0.09, p = 0.76). Similarly, Chao1 richness, which estimates total richness by considering the number of rare species showed an effect of algae (Fig 3.2b, Chao1: algae: $F_{1,30}$ = 38.15, p < 0.001), a weak (but not significant) effect of vermetids, (vermetids: $F_{1,30}$ = 3.0, p = 0.09), and no interaction ($F_{1,30}$ = 0.06, p = 0.81). Sediment and water samples had lower rarefied richness, Chao1 richness, and Shannon diversity (See Appendix 3AFigure 1).

Beta diversity (compositional variation among corals within the same treatment) was high for all coral treatments (Appendix 3AFigure 2), and variance was lower when algae were present (Fig 3.3, Algae: $F_{1,40}$ = 13.25, p = 0.0008, Vermetid: $F_{1,40}$ = 0.67, p = 0.42, Algae x Vermetid: $F_{1,40}$ = 1.12, p = 0.30). Although this result indicates heterogeneity in multivariate dispersion, I continued to analyze the data with a PERMANOVA, because the balanced design should be robust to departures from multivariate homogeneity (Anderson and Walsh 2013). Microbial composition changed due to algae (Table 3.1; Figure 3.4), but not vermetids (Table 3.1); again, there was no significant interaction between algae and vermetids (Table 3.1).

The composition of the coral microbiome varied among treatments. Sediment and water samples separated from the coral samples in the nMDS plots (Appendix 3AFigure 2), which indicate that the samples I took on coral surfaces reflected the microbiomes of the corals, not contamination from the sediment or water. I observed 44 common families (present in more than 3% of a sample) out of 254 total families detected in the coral mucus samples (Figure 3.5). Endozoicimonaceae, Phyllobacteriaceae, Comamonadaceae, Verrucomicrobiaceae, and Rhodobacteraceae were common, making up 25-80% of all sequences in a sample. Treatments

with algae had a greater number of rare families (rare defined as groups present in <3% relative abundance). There were several families that led to significant separation of treatments in nMDS space, and likely led to the significant effect of algae on community composition. Common marine bacteria that are not well described (e.g. Planctomycetaceae, Figure 3.6f) were 2x more abundant on corals near algae, compared to corals not near algae. Heterotrophic, potentially pathogenic bacterial groups (i.e., Flavobacteriaceae, Bdellovibrionaceae, Piscirickettsiaceae, Clostridiaceae, Legionellaceae and Rhodobacteraceae: Figures 3.6b-h) also were more abundant in the presence of algae. Two of these families, Legionellaceae and Rhodobacteraceae (Figures 3.6g,h), also showed a slight increase in the presence of vermetids. Fusobacteriaceae, which contain pathogenic taxa as well as taxa that require lower oxygen concentrations (Olsen 2014, Staley and Whitman 2010), increased the most when both algae and vermetids are present (Fig. 3.6i). Endozoicimonaceae, a family that includes potential coral symbionts (Neave et al. 2016), were 3x more abundant in the absence of algae (Figures 3.6a, $p < 0.001$), but showed no effect of vermetids, nor an interaction between algal and vermetid presence.

The relative abundance of *Vibrios* did not differ significantly among the treatments (Figure 3.7, Algae: $F_{1,27} = 0.95$, $p=0.34$; Vermetid: $F_{1,27} = 0.002$, $p =0.96$; interaction: $F_{1,27} = 1.25$, $p = 0.27$),.

Discussion

Frequently, multiple stressor studies have focused on the growth, survival and other physiological aspects of an organism's phenotype. Here, I tested the effects of two stressors, algae and vermetids, which have previously shown negative effects on corals when tested alone. I observed one stressor had a greater effect on corals than the other: algae had an overwhelmingly larger effect on coral microbial communities compared to vermetid mucus nets.

The combined effect of both stressors primarily reflected the effects of algae; there was no indication of a synergism or antagonism.

Algae increased alpha diversity (rarefied richness), Chao1 richness, and Shannon diversity in the coral microbial community Figure 3.2), which was likely caused by an increase in rare taxa (Figure 3.5). The increase in taxa may be the result of increased availability of substrate (i.e., dissolved organic carbon) produced by algal turf and retained near the coral surface by the complex topography created by turf filaments (Carpenter & Williams 1993, Wangpraseurt et al. 2012). Algal turfs are among the most prolific producers of DOC, and can increase diffusive boundary layer thickness (Carpenter & Williams 1993, Brown & Carpenter 2013), especially when filaments are ungrazed (Carpenter and Williams 1993, Jorrisen et al. 2016). These conditions are known to encourage high microbial growth (Kline et al. 2006, Haas et al. 2011). Previous studies that examined changes in microbial communities on *Montastrea* and *Porites* corals also have shown increased bacterial alpha diversity near algae (Barott et al. 2011, Pratte et al. 2017). The increases in rare microbial members likely contribute to the separation in communities near vs. far from algae that was observed in the nMDS and the PERMANOVA results (Figure 3.5).

In many ecosystems increased alpha diversity is hypothesized to increase ecosystem function (Tilman et al. 2014); however, in coral microbial systems, increased diversity is often associated with disruption in the normal functioning of an organism, and has often been associated with disease (Mera & Bourne 2017) and stress (McDevitt-Irwin et al. 2017). Thus, it is likely that the increased microbial diversity reflects an instability in host-associated (e.g., coral) microbial communities, which facilitates the invasion of deleterious microbes. Indeed, algae and, to a lesser extent, vermetids, lead to similar communities that are composed of

microbial families that are associated with pathogens and/or can withstand low oxygen conditions (Figure 3.6).

Increased beta-diversity is also associated with the presence of stressors (McDevitt-Irwin et al. 2017, Pratte et al. 2017) and hypothesized to indicate instability (Zaneveld et al. 2016, 2017) in host-microbe relationships. In contrast to this expectation, I observed a decrease in beta diversity (at the family level) in the presence of algae (Figure 3.4). This pattern may be driven by a homogenization of the physio-chemical conditions created at the coral-algal interface (i.e., low flow, retention of chemical conditions; Brown & Carpenter 2013, Brown & Osenberg 2018). Perhaps these conditions (i.e., high retention of DOC) are more similar across coral-algal interactions than are the conditions across coral surfaces in the absence of algae.

Few microbial groups were affected by vermetids. However, Fusobacteriaceae relative abundance changed most drastically when both vermetids and algae were present (significant interaction, Figure 3.6i). Not only does this family of bacteria contain pathogens, its members are associated with low-oxygen environments (Olsen 2014, Staley and Whitman 2010). Thus, the environmental conditions that algae and vermetids create when both are present (i.e., low flow, maintained lower oxygen, Brown and Osenberg 2018, Haas et al. 2013) may allow for the increase in this family. However, other groups that are common in low to no oxygen environments increase just due to algal presence (e.g., Clostridiaceae), indicating that algal presence alone may lead to sustained low oxygen conditions (Haas et al. 2013).

Several of the families that show higher relative abundances in the presence of algae and to a more limited extent, vermetids, commonly increase in the presence of stressors (i.e., Planctomycetaceae, Flavobacteriaceae), likely due to the increased substrate available for consumption (i.e., algal derived DOC). Although the genus *Vibrio* is associated with stress states,

and several coral diseases have been associated with bacteria in the genus *Vibrio* (Mera & Bourne 2017, Kemp et al. 2018), there were no significant differences in *Vibrio* relative abundance across treatments. Yet, I did observe increases in families that are associated with other pathogens (Piscirickettsiaceae, Bdellovibrionaceae, Legionellaceae, Rhodobacteraceae). The increase in Bdellovibrionaceae is especially interesting, as this group contains predatory bacteria that attack other bacteria, which may include other potential pathogens or beneficial microbes of the coral mucus (Martin 2002). This group has the potential to actively reduce other microbial groups, including potential coral symbionts.

Endozoicimonaceae is a family that contains potential microbial symbionts that are associated with pathogen-resistance (i.e., acts as a probiotic, Morrow et al. 2012, Bourne et al. 2013, Meyer et al. 2014, Neave et al. 2016, Peixoto et al. 2017). I observed reduced abundance of this family in the presence of algae, which may indicate that coral health was compromised at the point of contact with algae. Additionally, low Endozoicimonaceae relative abundance may be evidence of algae disrupting beneficial symbioses of corals and their microbial partners, allowing for an increase in the pathogenic and opportunistic groups observed.

Because mucus nets can decrease water flow, and trap conditions near coral surfaces (Brown & Osenberg 2018), I hypothesized that this should lead to conditions ideal for microbially-mediated mechanisms of competition between corals and algae. I observed limited evidence of vermetids exacerbating algal effects: algal presence primarily drove the patterns on coral microbial communities through the proliferation of heterotrophic and pathogenic bacteria and reduction in symbiotic microbes. Vermetids show a slight increase only in Chao1 richness (although $p = 0.09$), which takes into account missed singletons. The relative abundance of OTUs belonging to Rhodobacteraeae and Legionellaceae families show increases due to the

presence of vermetids as well as algae, indicating additive effects of vermetids. Fusobacteriaceae is the only family that shows evidence of a synergistic effect of algae and vermetids on relative abundance. Thus, there is weak evidence for multiple stressor effects on the coral microbiome.

Variable mucus net cover may drive the weak vermetid effect: it is unknown if mucus nets need to reach a certain density over a coral-algal interaction in order to manifest retention effects. For example, previous studies show that increasing densities of vermetids lead to increased net cover over corals (Allen-Jacobson 2018), and coral lateral extension is most affected by vermetids when net coverage is high (Allen-Jacobson 2018). Additionally, it is possible that the time scale at which mucus nets lead to retention of solutes are not sufficiently long enough to create long-lasting changes in most microbial community members beyond which are created by algae alone. Mucus nets are cast every 30 minutes to an hour and then pulled back in and consumed by the snail (Kappner et al. 2000). Thus, members may not change, but microbial metabolism may be affected during the periods in which nets are covering interactions, which was not tested here (i.e., a switch towards increased respiration when the nets are present compared to a switch in dominant community members as suggested by Chapter 2). Alternatively, all of the reefs that were sampled had vermetids on them; hence, even if the presence of vermetids does affect microbial communities, then it is possible that all of the samples were affected by vermetid presence. Future studies should be carried out on reefs completely devoid of vermetids versus with vermetids present to further evaluate the role of vermetids on coral microbial communities (see Chapter 5).

Testing for the role of multiple stressors on reefs is an important task, as coral reefs are experiencing a multitude of new stressors that can result in both lethal and sub-lethal effects on corals (Harborne et al. 2017). Here I show evidence that although both algae and vermetids can

influence corals in isolation on different aspects of the coral holobiont, I only saw effects driven by algae on the microbial community. Thus, stressors do not act on all aspects of an organism. This may lead to downstream positive effects, in which at least parts of an organism are resilient to the effects of a stressor. For corals, uneven effects of sub-lethal stressors across the whole organism may lead to a greater propensity for recovery and resilience of corals, and the reefs that depend on them.

Table 3.1: PERMANOVA results based on the Bray-Curtis dissimilarity index. Terms in bold indicate significant differences in communities due to treatment.

	df	Sum of Squares	Mean Squares	F. Model	R^2	P
Algae	**1**	**1.91**	**1.91**	**9.16**	**0.20**	**0.01**
Vermetids	1	0.13	0.13	0.63	0.01	0.62
Algae x Vermetids	1	0.06	0.05	0.26	0.01	0.96
Residuals	36	7.49	0.21		0.78	
Total	39	9.58			1	

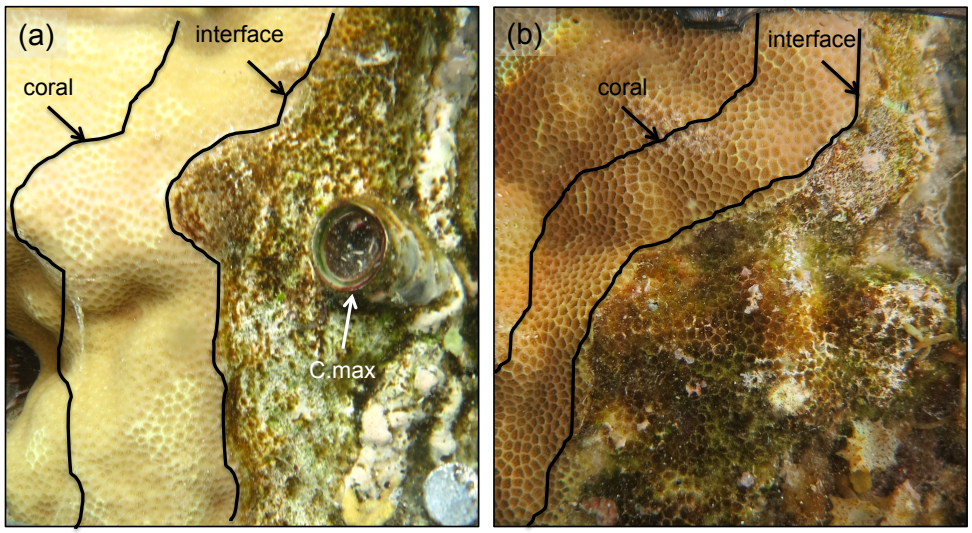


Figure 3.1: Images of (a) coral-algal interactions in the presence of vermetids (+V), and when (b) vermetids are absent (-V). Line marked “interface” shows sampling for the + Algae samples, and line marked “coral” shows sampling for the –Algae samples.

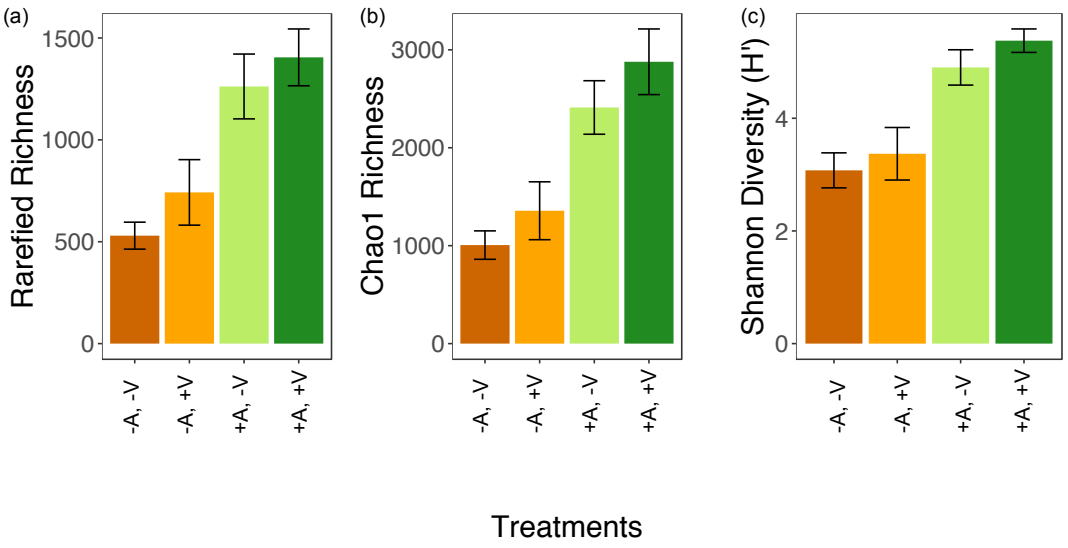


Figure 3.2: (a) Observed rarefied OTU richness. (b) Chao1 richness, (c) Shannon diversity (H') for each treatment combination (means ± SE; n=10). For each panel, algae had a significant effect on alpha diversity measures, whereas the effects of vermetids and the interaction between algae and vermetids were not significant.

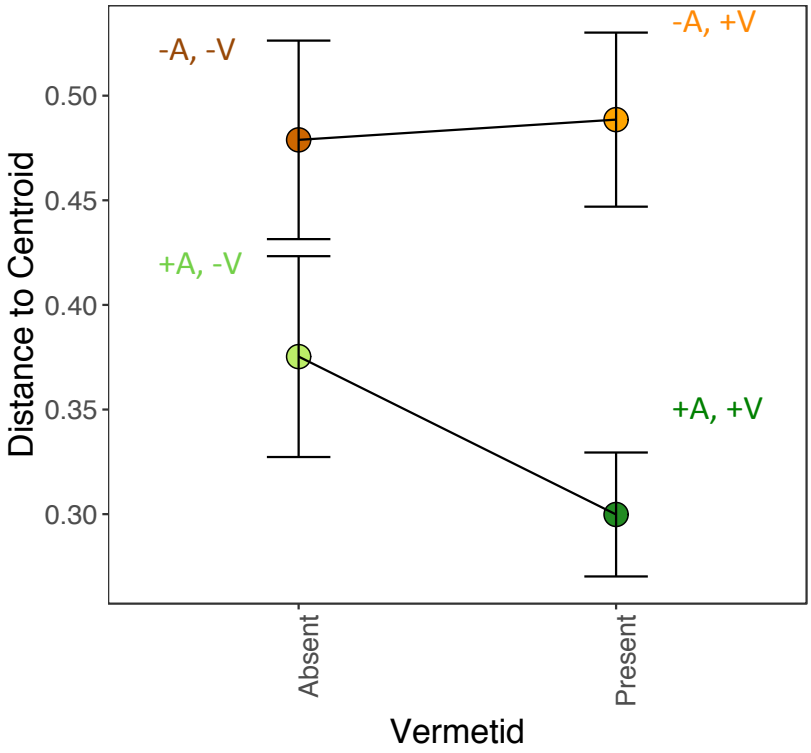


Figure 3.3: Beta diversity (means ± SE), measured as dispersion within a treatment (i.e., distance to centroid). Values are based on Bray-Curtis distance matrices of data summarized by family. Green circles (light and dark) indicate the presence of algae, and orange circles (light and dark) indicate the absence of algae. Algae significantly reduced dispersion ($p < 0.001$), but there were no significant effects of vermetids or a significant interaction.

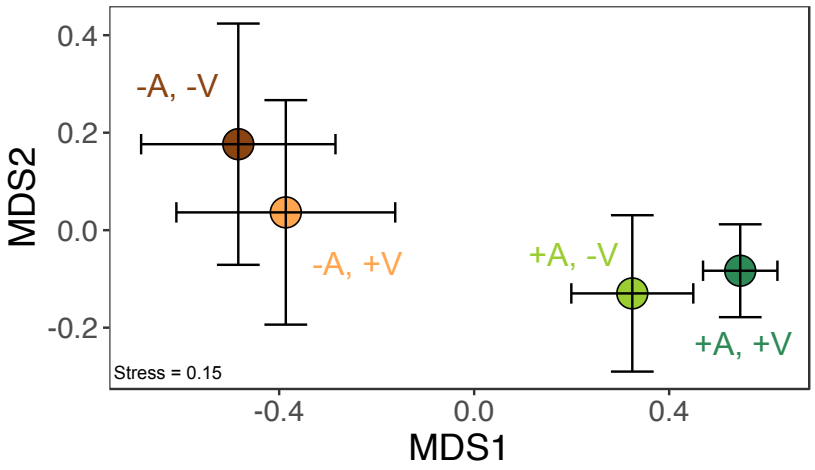


Figure 3.4: Non-multidimensional scaling plot, where each dot represents the mean ± se of each treatments' microbial community. Points that are further away indicate communities are more different from each other. Data are based on Bray-Curtis dissimilarity matrices of sample data summarized at the family level. Stress value indicates the fit is acceptable (Legendre and Legendre 1998). Dark green dots indicate both algae (A) and vermetids (V) present (+A, +V), light green indicates Algae present (+A, -V), light orange indicates vermetids present (-A, +V) and dark orange indicates coral only (-A, -V).

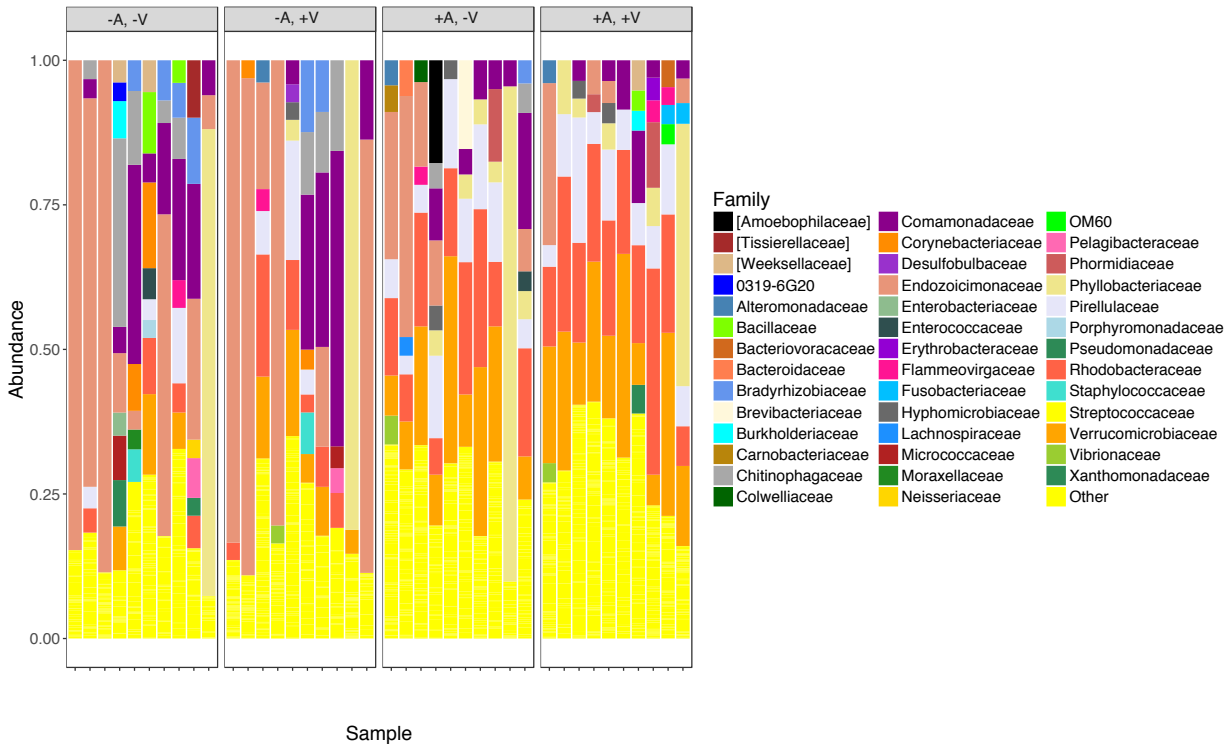


Figure 3.5: Relative abundance of OTUs belonging to different microbial families in each of the treatments. Bars represent a separate coral sample. Each color represents a different family. "Other" indicate OTUs from families present in < 3% of samples.

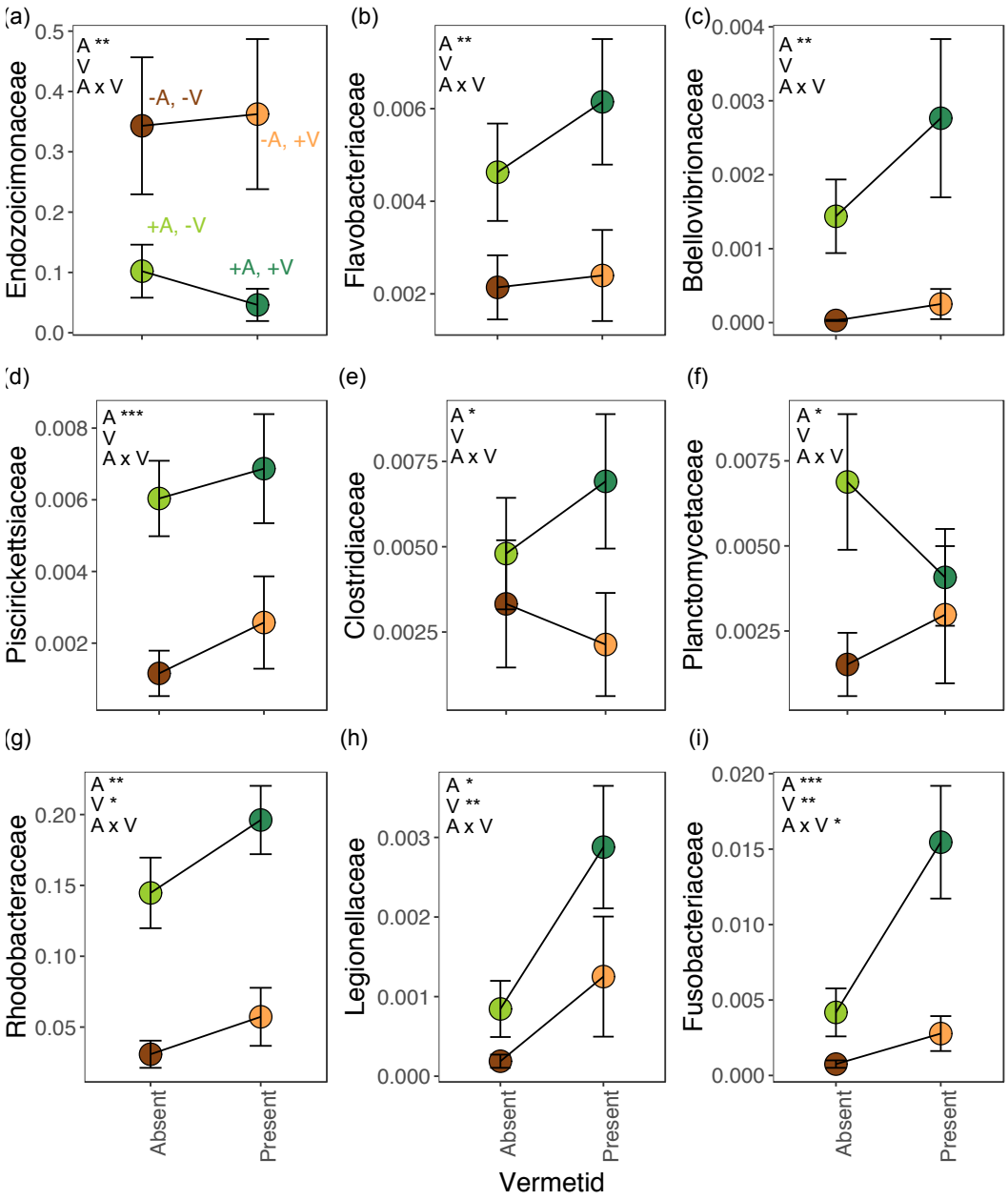


Figure 3.6: Mean (± SE) relative abundance for families that significantly contribute to compositional differences in nMDS space (significance determined by permutation test, 1000 permutations, Oksanen et al. 2017). Green (light and dark) indicates algal presence, and Orange (dark and light) indicates algal absence. Asterisks indicate significant main or interactive effects (* = $0.01<p\leq0.05$, ** $0.001<p<0.01$, *** $0<p<0.001$).

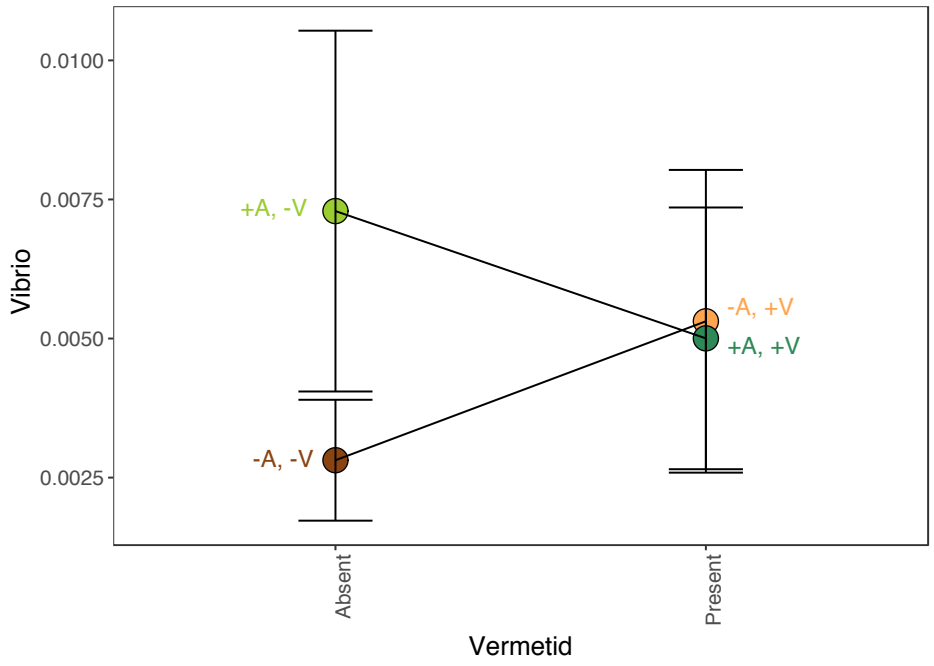
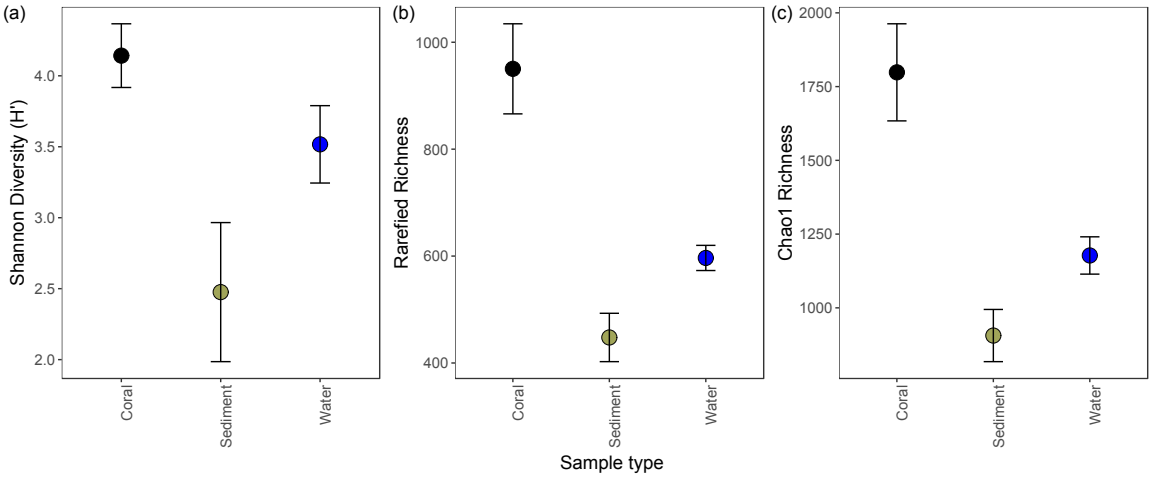
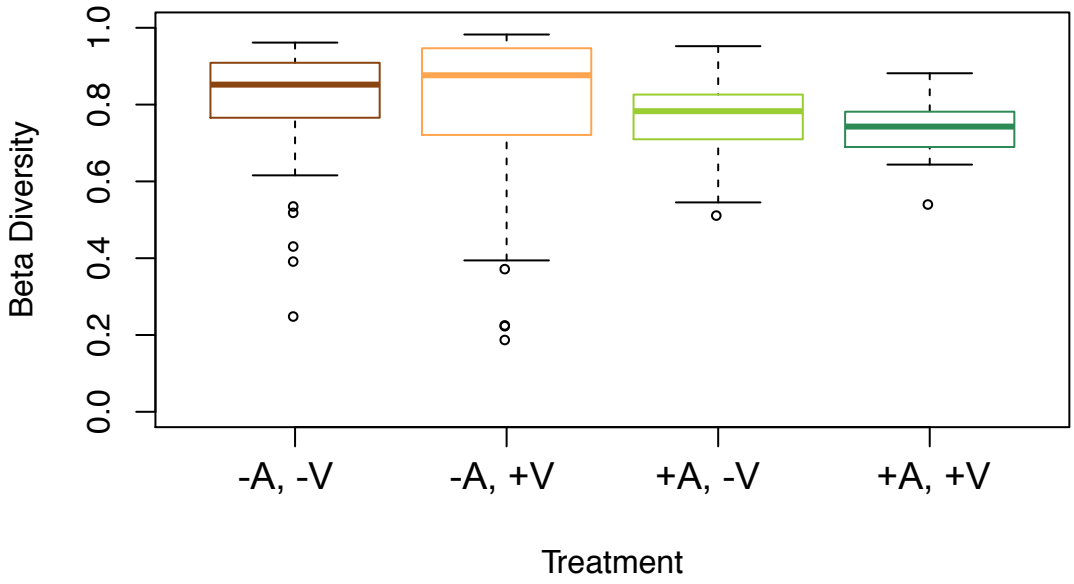


Figure 3.7: Relative abundance of OTUs in the *Vibrio* genus (mean ± SE), known to increase after exposure to stressors as well as contains major pathogenic groups. There was a slight increase in *Vibrios* in the presence of Algae, but it was not significant ($p > 0.05$).

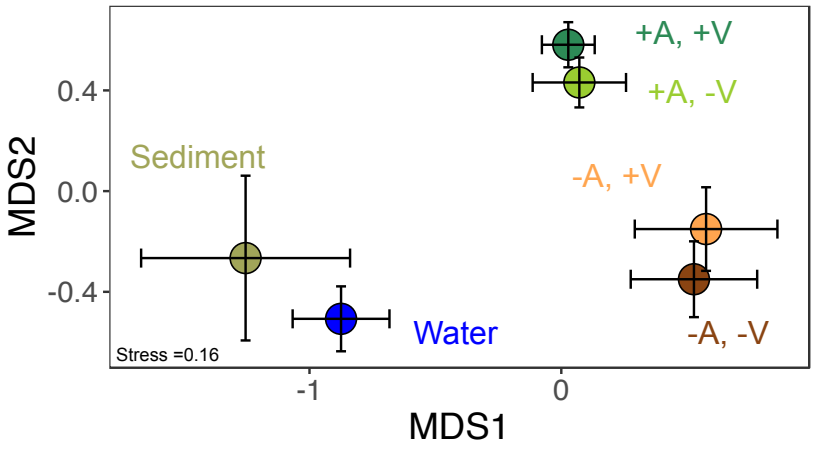
3.Appendix



3A Figure 1: Mean ± SE of alpha diversity differences among sample type (water, sediment and coral). Samples were rarefied to the same depth (11597 sequences/sample). Coral is a combination of all four treatments in the main body of the paper. Corals, sediment and water showed differences in Shannon diversity ($F_{1,49} = 3.32$, $p = 0.04$); Rarefied Richness ($F_{1,49} = 3.44$, $p = 0.04$) and a trend for Chao1($F_{1,49} = 2.8$, $p = 0.07$), according to three separate one way ANOVAs.



3A Figure 2: Boxplot of beta diversity within a treatment, based on Bray-Curtis distance matrices of data summarized by family. Data are based on pairwise distances among samples. Values closer to 1 indicate more variation across samples in a treatment; values closer to 0 indicates less variance within a treatment. The dark line indicates the median value, the top and bottom of the box are the 25th and 75th percentiles, whiskers give 1.5x IQR, and individual points correspond to outliers.



3A Figure 3: nMDS plot with water and sediment. Points are mean (± SE) for communities in each treatment in nMDS space. There are significant differences across sample types (PERMANOVA, p = 0.001, based on 999 permutations).

CHAPTER 4

ALGAE AND VERMETIDS INFLUENCE DIFFERENT ASPECTS OF CORAL PHENOTYPE [3]

[3] To be submitted to *PLoS One*, and authored by A.L. Brown, E.K. Lipp, and C.W. Osenberg.

Abstract

Interaction modifications occur when a third species modifies a pairwise interaction. On coral reefs, algae compete with corals for space and can reduce their growth and percent cover. A third sessile species (a vermetid gastropod) can also have negative effects on coral by reducing coral growth, survival and photophysiology. Because vermetids affect coral through their mucus nets, and because these nets might exacerbate the mechanisms by which algae affect corals, I hypothesized that vermetids intensified the deleterious effects of algae on corals. To test my hypothesis, I conducted manipulative field experiments in which I tested the effects of algae and vermetids on corals, as well as the effects of corals and vermetids on algae. I conducted this study twice, once in 2013 (46 days) and once in 2014 (63 days). I quantified algal biomass and algal density (in 2013), coral growth rates (via calcification and lateral extension in 2013 and 2014), and coral microbial communities (via sequencing 16S rDNA in 2014 only). I found no evidence for interaction modifications. Coral lateral growth declined due to algae, and calcification rate decreased as vermetid mucus net cover increased. Vermetid mucus nets did not affect algal biomass or density, indicating no evidence for density-mediated indirect interactions. I also observed no clear effects of vermetids or algae on coral microbes on experimental corals. My results show vermetids and algae affect different aspects of the coral phenotype but that these effects are not likely mediated via a shift in the microbiome. These results indicate that although algae and vermetids have deleterious effects on corals, these stressors do not combine synergistically.

Introduction

Species rarely interact in isolation. Pairwise interactions, such as competition or predation, can change in the presence of a third species (Wootton 1994). Density-mediated indirect effects arise when a third species alters the density of one of the interacting species, thus changing the overall intensity or outcome of a pairwise interaction. Trophic cascades are a common example of a density-mediated indirect interaction: e.g., sea urchins consume kelp, but in the presence of a third species (sea otters), the density of sea urchins is reduced through consumption by otters, which leads to an increase in kelp (Estes & Palmisano 1974).

The presence of a third species can also lead to changes in the way two species interact. An interaction modification (also known as a higher order interaction, Vandermeer 1969, or a trait-mediated indirect interaction, Werner and Peacor 2003) arises when a third species changes the strength or direction of a pairwise interaction without a change in the density of the focal species. These trait-mediated effects can exceed the magnitude of density-mediated effects (Schmitz et al. 2004).

One way in which interaction modifications occur is when the third species changes the physical environment in which two other species interact. For example, in mesic forest systems, saplings outcompete understory shrub species in sunlit conditions. But in the presence of pine trees (which shade the environment), shrubs outcompete saplings (Pagès et al. 2003). Interaction modifications also arise through changes in the phenotype of the interacting species (i.e., trait-mediated indirect interactions). For example, the presence of predator cues lead to morphological changes in competing wood and leopard frogs, and weaken the competitive effects of wood frogs (Relyea 2000). However, morphological traits are not the only aspect of the phenotype that can

be affected by interaction modifiers, their host-associated microbial communities can change as well.

From humans to sponges, all species exist with microbial symbionts, forming a "holobiont" (the host and its microbes: Mindell 1992, Zilber Rosenberg and Rosenberg 2008, Rosenberg and Rosenberg 2018). Microbes can respond to changes in the abiotic environment (e.g., microbes on the surface of crustose coralline algae change in response to temperature: Webster et al. 2011) and to biotic interactions (e.g., human-human contact leads to microbial sharing, and convergence of microbiomes when people share common spaces: Lax et al. 2014). Combinations of stressors (e.g., nutrients and predators: Shaver et al. 2017; or ocean acidification and increased temperature: Webster et al. 2017) can also lead to changes in host-associated microbial communities. However, no studies have viewed microbes as an aspect of the host phenotype that can change in response to other organisms and thus potentially mediate the strength of species interactions. Here, I examined interaction modifications, as potentially mediated through changes in microbial communities, on a coral reef.

Coral reefs are the most diverse ecosystems on earth (Bellwood et al. 2004). Currently, they are experiencing a multitude of stressors (Hughes et al. 2007, reviewed in Harborne et al. 2017) that increase damage to corals, and allow other species to colonize newly exposed habitat next to living coral. Colonization of these openings on coral reefs by other organisms lead to direct interactions between corals and these benthic-space occupiers, like macroalgae (i.e., macroscopic algae). Macroalgae negatively impact corals through shading (Box & Mumby 2007), abrasion (River & Edmunds 2001), overgrowth (Jompa & McCook 2002), and allelopathy (Rasher 2010). Additionally, the effects of algae on coral can be mediated through the microbial community (Smith et al. 2006). Algae leach dissolved organic carbon, which promotes microbial

growth, and can lead to shifts in the alpha diversity and beta-diversity and composition of microbial communities that inhabit coral surfaces (Nelson et al. 2013, Vega Thurber et al. 2012, Zaneveld et al. 2016). These changes in microbes can lead to hypoxia (Haas et al. 2013) or disease (Nugues et al. 2004), and can cause coral death (Smith et al. 2006). However, the mechanisms underlying the effects of algae on corals can change depending on the physical environment.

One aspect of the physical environment that modifies coral-algal interactions is water flow (Brown & Carpenter 2014). High flow reduces hypoxia, which likely limits the deleterious effects of algae on corals (Wangpraseurt et al. 2012, Brown & Carpenter 2013, Jorrisen et al. 2016). Additionally, water flow can lead to changes in microbial communities, and buffer the effects of other stressors (e.g., temperature, Lee et al. 2017). Thus, a species that can modify the water flow environment can likely modify coral-algal interactions. One such species is the vermetid gastropod, *Ceraesignum maximum* (*C. maximum*), a ubiquitous member of shallow reefs on coral reefs in the South Pacific and Red Sea (Hadfield et al. 1976).

Ceraesignum maximum is a sessile gastropod that is frequently observed embedded in the coral matrix. To feed, the gastropod casts out a mucus net, with which it collects plankton from the water column. After 30-40 minutes, the snail retracts the net, consuming the net and its contents. At high densities (>8 per m^2), these nets consistently cover coral surfaces (pers.obs). *C. maximum* decrease the growth, survival and photophysiology of corals (Shima et al. 2010, 2013, 2015), presumably because of effects mediated through the mucus net. Mucus nets reduce water flow at the surface of corals (Brown and Osenberg 2018), which has the potential to alter microbial communities associated with the corals. Indeed, the likely mechanisms underlying vermetid-coral interactions are similar to the mechanisms underlying coral-algal interactions.

Thus, it is possible that vermetids may intensify effects of algae on corals by enhancing the mechanism underlying the effects of algae on corals. In other words, it is likely that vermetids modify the strength of the interaction between coral and algae.

I tested the hypotheses that vermetids enhance the deleterious effects of algae on corals using manipulative field experiments, repeated in two consecutive years (2013 and 2014). In 2013, I also examined the effects of vermetids and corals on algae to determine if there were any density-mediated effects of vermetids on coral-algal interactions. In 2014, I further explored if possible effects were mediated, in part, through changes in the coral's microbial community.

Methods

Experiments were conducted in the back reef environment on the northshore of Mo'orea, French Polynesia (S17° 28.466' W149° 47.313'), where vermetid gastropods reach high abundances (up to 30 snails m^{-2}, Shima et al. 2010, but see Brown et al. 2016), and their nets often overly regions in which live coral and algae abut. On the backreef of Mo'orea, the most common coral-algal interaction arises between massive *Porites* and algal turf (Brown and Carpenter 2015). Algal turf is a functional group made up of a collection of filaments that belong to a multiphyletic functional group. Massive *Porites* is a species complex of ambiguous taxonomy (Forsman et al. 2009, 2015) but often assigned to *Porites lobata*, *Porites lutea* or *Porites austrialiensis*. Field experiments ran for 46 days (May - July) in 2013 and for 63 days (May - August) in 2014.

Field Manipulation

Cores were removed from a reef using a pneumatic drill with a hole saw attachment (diameter = 5 cm). The hole saw was positioned so that half of the core consisted of live massive *Porites* and

half of the core was algal turf. In 2013, I collected 90 cores, and, in 2014, I collected 52 cores. Cores were brought to the Richard B. Gump Marine Station, where I sawed off excess calcium carbonate with a Dremel (©) to create cores of equal height (~1.5cm). In 2013, I created three types of cores: controls ("+Algae"; with both algae and coral intact); "-Algae" (in which I scraped algae off half of the core using tweezers and/or razor blades but left the coral intact) and "-Coral" (in which I removed coral tissue using a waterpik, but left the algae intact). In 2014, I created only two types of cores: "+Algae" (with both algae and coral intact) and "-Algae" (in which I removed algae from half of the core). Each core was then affixed to its own labeled plastic mesh base using marine epoxy (©Splash Zone), photographed, buoyant weighed (Davies 1989). In 2013, I also estimated the ash free dry mass of algae (on all cores with algae) by scraping off two, 1x1 cm^2 areas from each core, combining that algae in tinfoil weigh boats, drying them at 37^oC, measuring dry mass, and then combusting the sample in a muffle furnace at 500^oC and reweighing the sample. Cores were then taken to the field and either placed directly into the experiment (see below; in 2013) or acclimated to field conditions for 1 week before going into their treatments (in 2014).

Thirty (in 2013) or 26 (in 2014) reefs with similar densities of vermetids were found within an area of approximately 2000 m^2. Reefs were originally formed by massive *Porites* colonies, but at the time of the experiment were mostly dead coral (due to the effects of *Acanthaster plancii* and a cyclone, Adam et al. 2011). Each reef had ~7 *C. maximum* on the top where cages were eventually placed. Half of the reefs were assigned to one of two treatments: "+ Vermetids" and "- Vermetids". I removed snails using small chisels and wire hooks from the reefs in the –Vermetid treatment, vermetids were not manipulated on the +Vermetid reefs. One cage (diameter = 25-55cm, height = 35cm, diameters based on size of the flat-area on top of reefs

where cage was placed) made of plastic mesh (2cm) was affixed to bommies using U-nails. One of each of the three (2013) or two (2014) types of cores was placed in each cage. Each week, cages were scrubbed, and algae were plucked off of coral-only treatments using tweezers. Additionally, the proportion of the core that was covered in vermetid mucus was visually estimated once each week (see Appendix 4A.Figure 2). At the end of the experiments, cores were retrieved. Cores were re-photographed and reweighed using the buoyant mass technique (Davies 1989). In 2014, for cores with algae, I scraped off all of the algae and determined their ash-free dry mass. Experiments ran from May-July 2013 (46 days) and from May-August (63 days).

Changes in coral

Mass

Coral buoyant mass was converted to skeletal accretion using equations from (Davies 1989). Change in skeletal mass was calculated as:

$$\textbf{Growth rate}: G = (M_f - M_i)/S_i)/t \quad (1)$$

where M = skeletal mass at either the final (f) or initial (i) sampling date, S_i = initial surface area, of living coral and t is the duration of the experiment (either 46 or 63 days). Initial surface area was determined by tracing the outline of the coral from the photographs using ImageJ (Schneider et al. 2012).

Lateral Extension

I also used the photographs taken at the start and end of the experiment to quantify the amount of lateral extension in the zone of the algal-coral interaction. To determine lateral extension towards or away from the coral-algal interface, a line was drawn through the coral side of the core (Appendix 4A.Fig 1, "Reference Line") approximately parallel to the border

separating the coral and algae, and two additional lines were drawn perpendicular to the reference line. The lines were matched in the final and initial photos using landmarks on the mesh grid to which the core was affixed. The area of live coral (A) demarcated by these three lines and the leading edge of the coral was measured using ImageJ (Schneider et al. 2012). This area was divided by the length of the reference line between the two other lines (L) to yield the average distance of the coral edge from the reference line. I then subtracted the initial distance from the final distance and divided this difference by the duration of the experiment to estimate the daily rate of lateral extension:

Lateral Extension Rate: $E = (A_f/L - A_i/L)/t$ (2)

Changes in Algae

Algal biomass and density

Area of algae was estimated from photographs in 2014 by tracing the area of the core covered by algae using ImageJ (Schneider et al. 2012). Algae growing on the Z-spar epoxy was excluded from these estimates. To calculate change in algal density, AFDM was divided by the area sampled (i.e., by A_F at the end of the experiment and by 2 cm^2 at the start, Eq. 3). I also quantified the change in total algal biomass by subtracting the initial estimated AFDM of algae (scaled up to the area of the algae that covered the core based on the 2 cm^2 sample) from the algal density scaled up to the total area at the end of the experiment (Eq. 4).

Change in algal density (g/cm^2): $D = AFDM_F/A_F - AFDM_I/2$ (3)

Change in algal biomass (g): $B = (AFDM_F/cm^2)*A_F - (AFDM_I/cm^2)*A_I$ (4)

For the AFDM samples, several samples were lost so (n =13) for –Coral, -Vermetid and 10 for +Coral, -Vermetid, and (n = 10) for –Coral, +Vermetid, and (n = 11) +Coral, + Vermetid.

Microbial communities

In 2014, I sampled microbial communities on corals (n = 43), algal turf (n = 24), vermetid nets (n = 13), and sediment (n = 2) the day before the end of the experiment to determine how interactions with algae and vermetids influenced coral microbes. I collected vermetid mucus nets by swirling the nets onto sterile cotton swabs. To sample coral microbes, I gently agitated the surface of the coral (to waft away sediments and vermetid mucus), and then collected the coral mucus using a 10-ml syringe. To sample the algal turf, I used tweezers to remove algal turf filaments and collected them into a whirlpak ©. In between algal turf collections I waved the tweezers in the water column to reduce any cross-contamination. I collected sediment samples by scooping sediment into whirlpaks ©. Samples were brought back to the lab on ice. Samples of coral mucus had settled to the end of the syringe and this mucus was inserted into 1.5ml microcentrifuge tubes, spun down (at 10,000 RPM, for five minutes) and any excess water was removed. Algal samples were placed in 1.5ml tubes (at a volume of ~200ul, loosely packed). I placed 0.5µl of sediment in 1.5ml centrifuge tubes. Samples were frozen in a -80°C freezer and transported frozen to UGA, where they were transferred to a -20°C freezer.

Samples were extracted using Qiagen PowerSoil kits and sent to IMR CGEB (http://cgeb-imr.ca/) for sequencing, where the V4 region of the 16s rDNA gene was amplified, using primers 515F/806R. Sequencing was performed on an Illumina MiSeq platform.

Bioinformatics were completed using the QIIME (v 1.9, Caporaso et al. 2010) pipeline. Only forward reads were used for analysis. Reads were de-multiplexed using the default

parameters in QIIME and checked for chimeras (incorrectly merged sequences) using usearch61 (Edgar 2010, Edgar et al. 2011). After chimera removal, operational taxonomic units (OTUs) were assembled based on 97% sequence similarity (uclust, Edgar 2010, Caporaso et al. 2010), Taxonomy was assigned using open reference frame OTU-picking and with Greengenes, as the reference database (McDonald et al. 2012, Werner et al. 2012). The OTU biom and phylogenetic tree files were then transferred to R for further filtering and analysis using the phyloseq (McMurdie and Holmes 2013) and vegan packages (Oksanen et al. 2017). In phyloseq, mitochondrial and chloroplast sequences were removed and only sequences from the kingdom Bacteria were analyzed.

Data Analysis

I conducted two basic types of analyses. First, I analyzed the data using the planned design, examining the main effects of vermetids (presence vs. absence) and algae (presence vs. absence) and their interaction. For testing coral effects on algae, I looked also at coral presence and absence. However, due to high variation in vermetid mucus net cover in the cores exposed to vermetids, I conducted a secondary analysis in which I focused on the treatments with vermetids and used mucus net cover as a continuous covariate (see Appendix 4A.Figure 2 for data on mucus cover). All analyses included reef as a random effect (random intercept) because three (in 2013) and two (in 2014) types of cores were placed on the same experimental reef. Vermetid presence (or net cover), algal presence (or coral presence) and year (when applicable) were modeled as fixed effects. Data were analyzed using the nlme package (Pinheiro et al. 2018) in R (R Core Team 2016, v 3.3.2).

For microbial data, I analyzed three different measures of alpha diversity (Rarefied richness, Chao1 richness, and Shannon diversity) using the phyloseq package in R (McMurdie

and Holmes 2013). For richness, Chao1 and Shannon diversity, I rarefied samples to an even depth. Statistical analyses were completed as above, for both the classical analysis, and the analysis that used mucus net cover as a covariate. To analyze effects of algae and vermetids on composition of the coral microbiome I converted total sequence count per sample to relative abundances and conducted a PERMANOVA on the Bray-Curtis dissimilarity matrix using the crossed design (Algae x Vermetid) with 999 permutations in the vegan package (Oksanen et al. 2017). Additionally, I used nMDS to visualize differences across the groups (Oksanen et al. 2017) in two dimensions.

Results

Effects of vermetids and algae on coral

Algae reduced lateral extension by 20%, but this effect was not significant ($F_{1,72}$ = 2.99, p = 0.09), and I did not observe a significant effect of vermetids, year or any interaction between vermetids and algae (Appendix 4B.Table 1a, 4B.Figure 1a). The change in buoyant mass was affected by year (1.9x higher buoyant mass in 2014, Appendix 4B.Table 1a, 4B.Figure 2b), but I observed no effects of algae, vermetids or an interaction between the two on corals. Thus, theses analyses provide no evidence of an interaction modification as originally hypothesized. However, I observed high variability in mucus net cover, which limited the power of these analyses. Therefore, I analyzed the data from the +Vermetid treatments using Net Cover as a covariate. In doing so, I observed potential non-linearity in the data, but due to limitations in the spread of the data at low net cover, I could not estimate the curve. I decided to focus on comparisons with mucus net cover (only + Vermetid treatments), algae and year. Thus, I

analyzed the vermetid present (fixed effects: net cover, algal presence/absence and time, random effect: reef), as described in the methods.

Change in Coral Mass

In the presence of vermetids, change in coral mass decreased with increasing mucus net cover (Fig 4.1, Net Cover $F_{1,18} = 8.61$, $p = 0.009$). Additionally, the effect of algae depended on year (interaction between algae and year: $F_{1,18} = 4.68$, $p = 0.04$), which was likely driven by higher coral growth in 2014 compared to the lower growth in 2013. I also observed a main effect of year ($F_{1,22} = 29.74$, $p < 0.001$), which I cautiously interpret due to the presence of an interaction between algae and year, but it is likely driven by higher growth over all in 2014 compared to 2013. There were no significant interactions between algae and net cover ($F_{1,18} = 0.006$, $p = 0.94$), nor was there a three-way interaction ($F_{1,18} = 0.004$, $p = 0.95$).

Lateral Extension

Corals tended to expand in the absence of algae, but recede in the presence of algae (Fig 4.2, $F_{1,20} = 4.95$, $p = 0.038$). Higher vermetid mucus net cover was associated with decreased lateral extension of corals, although this effect was not significant (Fig 4.2, $F_{1,20} = 3.84$, $p = 0.060$). There also was an indication of an interaction between year and algal treatment, although this effect also was not significant (Fig 4.2, $F_{1,20} = 3.47$, $p = 0.070$). There was no significant interaction between algae and net cover ($F_{1,20} = 0.42$, $p = 0.52$), nor was there a three way interaction ($F_{1,20} = 1.49$, $p = 0.24$).

Effects of coral and vermetids on algae

I observed no main or interactive effects of corals or vermetids on algal density (Appendix 4B.Table 1b) or on total algal biomass (Appendix 4B.Table 1b). Due to the high variation in net cover, I analyzed the data from the +Vermetid reefs using net cover as a covariate.

Algal Density and Biomass

I observed an average change in algal density of 0.003±0.009 g cm^2 (mean ± sd), but no significant effects of coral treatments or vermetid mucus nets (Fig 4.3a Coral: $F_{1,6} = 0.25$, $p = 0.64$; Net Cover = $F_{1,6} = 2.11$, $p = 0.20$; Coral x Net Cover $F_{1,6} = 2.32$, $p = 0.18$). Algal biomass was reduced by 34% in the presence of corals (Fig 4.3b: $F_{1,7} = 5.45$, $p = 0.05$), but there was no significant effect of vermetid net cover or an interaction between coral treatment and mucus net cover (Net Cover: $F_{1,7} = 0.29$, $p = 0.61$; Net Cover x Coral: $F_{1,7} = 1.24$, $p = 0.30$).

Microbial effects

Before quality filtering, the number of sequences per sample ranged from 2,850 – 36,360, after filtering there were 2,019-30,968 sequences. Microbial communities associated with corals, algae, vermetids and sediment were readily distinguished (Appendix 4C. Figure 1). In the coral samples, I found 18,998 different OTUs from 25 phyla. Of the 25 phyla in the samples, only 12 comprised more than 0.1% of a sample. The most abundant phyla were Proteobacteria and Planctomycetes, which collectively comprised 30-40% across samples (Fig 4.4, Appendix 4C. Figure 2 for family composition). Cyanobacteria and Firmicutes, and Bacteroides collectively made up about 20% of the samples. In general, the diversity of coral-associated microbes increased in the presence of algae, although only the algal effect on Chao 1

richness was significant ($F_{1,27} = 4.59$, $p = 0.04$; see also 4C. Table 1 for Rarefied Observed Richness and Shannon Diversity).

As before, because of the variable net cover, I also compared alpha diversity metrics for the treatments with vermetids using mucus net cover as a covariate. Alpha diversity measures did not vary due to net cover (Fig. 4.5: Rarefied richness: $F_{1,6} = 0.34$, $p = 0.58$; Chao1 richness: $F_{1,6} = 3.05$, $p = 0.13$, Shannon diversity: $F_{1,6} = 0.08$, $p = 0.78$). Microbial diversity increased in algal presence, but these differences were not significant (Rarefied richness: $F_{1,6} = 1.12$, $p = 0.33$; Chao1 richness: $F_{1,6} = 5.01$, $p = 0.066$; Shannon diversity = $F_{1,6} = 0.67$, $p = 0.44$). I observed no interactions between algae and vermetid net cover (Rarefied richness: $F_{1,6} = 0.37$, $p = 0.56$; Chao1 richness: $F_{1,6} = 0.07$, $p = 0.79$;; Shannon diversity: $F_{1,6} = 0.44$, $p = 0.53$).

I did not observe any clear differences in composition among treatments (PERMANOVA: Algae: P= 0.167, Vermetid: P= 0.612, Algae*Vermetid: P = 0.965). This is evident by the considerable overlap among treatment groups on the nMDS plot (Fig 4.6).

Discussion

I expected vermetids to exacerbate the effects of algae on coral growth and their microbial community. I failed to find strong evidence in support of this hypothesis and, therefore, conclude that vermetids do not appreciably modify coral-algal interactions or microbial communities associated with coral.

I did, however, find effects of algae and mucus net cover on different aspects of coral growth phenotypic traits. Although, previous studies suggest that algal turf can outcompete corals (McCook 2001, Jompa & McCook 2003, Quan-Young & Espinoza-Avalos 2006). I only observed significant, negative effects of algae on lateral extension but not buoyant mass,

indicating that algae likely have a greater effect on new coral tissue production than on calcification. Thus, these results suggest corals are more vulnerable to algal competition at their growing edges.

Vermetid net cover primarily affects calcification (i.e., from tissue already present). My results expand on the findings of Shima (et al. 2010), and bolster the hypothesis that vermetid effects on coral phenotypic traits are mediated through their mucus nets. However, I suggest at low mucus net cover, vermetids do not lead to deleterious effects on coral, and can have minimal impact on coral phenotypic traits (i.e., calcification rate). Thus, the deleterious effects of vermetids on coral growth and the implications that has for coral morphology (i.e., flattening) observed in other studies (Colgan 1985, Shima et al. 2010, 2013, 2015), can likely only happen if vermetid nets coverage is consistently 75-100%.

Interestingly, I also observed different effects by year. Although cores were from the same collection site, placed on reefs in the same area, and experiments were conducted at the same time of year (the austral winter) corals accrued skeletal mass faster in 2014. As calcification increased across all treatments, the greater calcification rate in 2014 was likely driven by climatic or environmental variables: e.g., a 1^{o}C annual temperature increase can increase calcification by ~4.5% (Bessat and Buigues 2001).

Effects of vermetids on corals could arise if vermetids changed algal biomass or algal density, thus influencing competitive effects of algae on corals. However, I showed algal density and biomass were not affected by mucus nets (4.Fig 3), indicating that vermetids have little to no effect on algae, and thus cannot influence coral-algal interactions through this indirect mechanism. However, the effects of vermetids on coral-algal interactions are likely underestimated, because of another indirect mechanism by which vermetids can affect coral-

algal competition. Vermetid mucus nets reduce grazing on algae (Tootell & Steele 2014), thus, in the presence of herbivory, there may be density-mediated indirect effects of vermetids on coral-algal interactions. As a result, I suggest that the effect of algae on coral lateral extension, would likely be exacerbated in the presence of herbivory and vermetids.

I hypothesized the mechanism underlying the effects of vermetids on coral-algal interactions was the alteration in the physical and chemical microenvironment surrounding coral-algal interactions (Brown and Osenberg 2018). Furthermore, I expected that the presence of vermetids and algae would shift the microbial communities on coral surfaces and increase alpha diversity. I also expected that the combined effects of vermetids and algae would be greater than the additive effect of either group alone. I did not observe this pattern. Algae increased microbial diversity; however, vermetids did not and there was no evidence of a synergism between the two factors. Although I did not observe an effect of algae, although other studies suggest that algae increase alpha diversity (Barott et al. 2011, Vega Thurber et al. 2012, Lee et al. 2017, Pratte et al. 2017). One explanation for the lack of effect of algae (and vermetids) is that only deep sequencing (i.e., more reads per sample) reveals differences because that captures more rare groups (i.e., Barott et al. 2011 used a minimum of 9,000 reads per sample). However, differences among groups (with and without algae) have also been observed for samples <1000 reads/sample (Pratte et al. 2017, 500 reads/sample).

In contrast to the experimental results reported here, I previously conducted an observational study (Ch 3), that demonstrated differences in both alpha diversity and composition due to the presence of algae, and limited combined effects of both algae and vermetids on coral surface microbial communities. In the observational study, I collected samples from corals that had likely been in contact with algae and vermetids for a long time. On

the other hand the experimental study imposed treatments for 63 days. These disparity in results and approach suggest two hypotheses: 1) microbial communities may respond more slowly than expected, such that chronic, prolonged exposure may be needed for microbial communities to diverge in composition; or 2) a history of algal (and possibly vermetid) contact leads to irreversible changes in microbial communities on corals, so that although the addition of algae might have demonstrable effects on microbes, the removal of algae might not produce appreciable effects on coral microbial communities. Both of these hypotheses require a better understanding of the dynamics of microbial communities over time. There are few longitudinal studies currently on coral microbial communities, consequently the lasting effects of macro-organism interactions on coral microbial communities are not well established. One possible explanation is that after contact with algae, microbial communities are altered, likely due to the proliferation of new members, which makes returning to a previous microbial community state difficult. This phenomenon is similar to species invasions to an ecosystem and the resulting changes in both the biotic and abiotic environment: once the invader establishes and proliferates, it is hard to remove from an ecosystem (and not just because of the eradication method; Myers et al. 2000, Strayer et al. 2006). In microbial systems, such as the human gut, the introduction of pathogens (i.e., *Clostridium difficile*), can lead to sweeping microbial and physiological changes, which are difficult to reverse (Louzopone et al. 2012). Alpha diversity in the samples without vermetids and algae was 40% greater in the experimental study relative to the observational study (~700 unique OTUs, compared to ~500 unique OTUs, Fig 3.3), even though the rarefaction depth in the experimental study was only 1/5 of that used in the observational study (2091 vs. >11,000 sequences/sample). Therefore, it is possible that the history of algal contact led to changes in coral microbial communities that were not reversed after algal removal.

However, stressor removal can lead to changes in microbial communities (Ch 5), although microbial community composition reflects previous interaction environments (Ch 5).

These results emphasize the need for longitudinal studies to understand how or if stressors affect coral microbial communities, and how microbial communities influence the rest of the coral holobiont. Instead, it is likely the mechanism underlying vermetid effects are due to physical changes created by vermetid mucus nets, which include reducing water flow (but not altering light; Brown and Osenberg 2018), potentially reducing food availability for corals (Allen-Jacobson 2018) or allelochemical effects (Klöppel et al. 2013).

Although indirect interactions are common in nature, few studies examine interaction modifications that are not based on trophic interactions (Relyea & Yurewicz 2002, Pagès et al. 2003). The primary purpose of my study was to provide one of the first studies of interaction modifications in a coral reef ecosystem. I did not observe interaction modifications as I anticipated, however I did observe that vermetids and algae had effects on different aspects of the coral phenotype. Algae negatively affected lateral extension and vermetids negatively affected calcification as mucus net cover increases. Thus, if vermetids are in high enough densities to consistently cover nearby coral-algal interactions, in the long-term, they can lead to flatter corals that are shrinking around the margins due to algal contact.

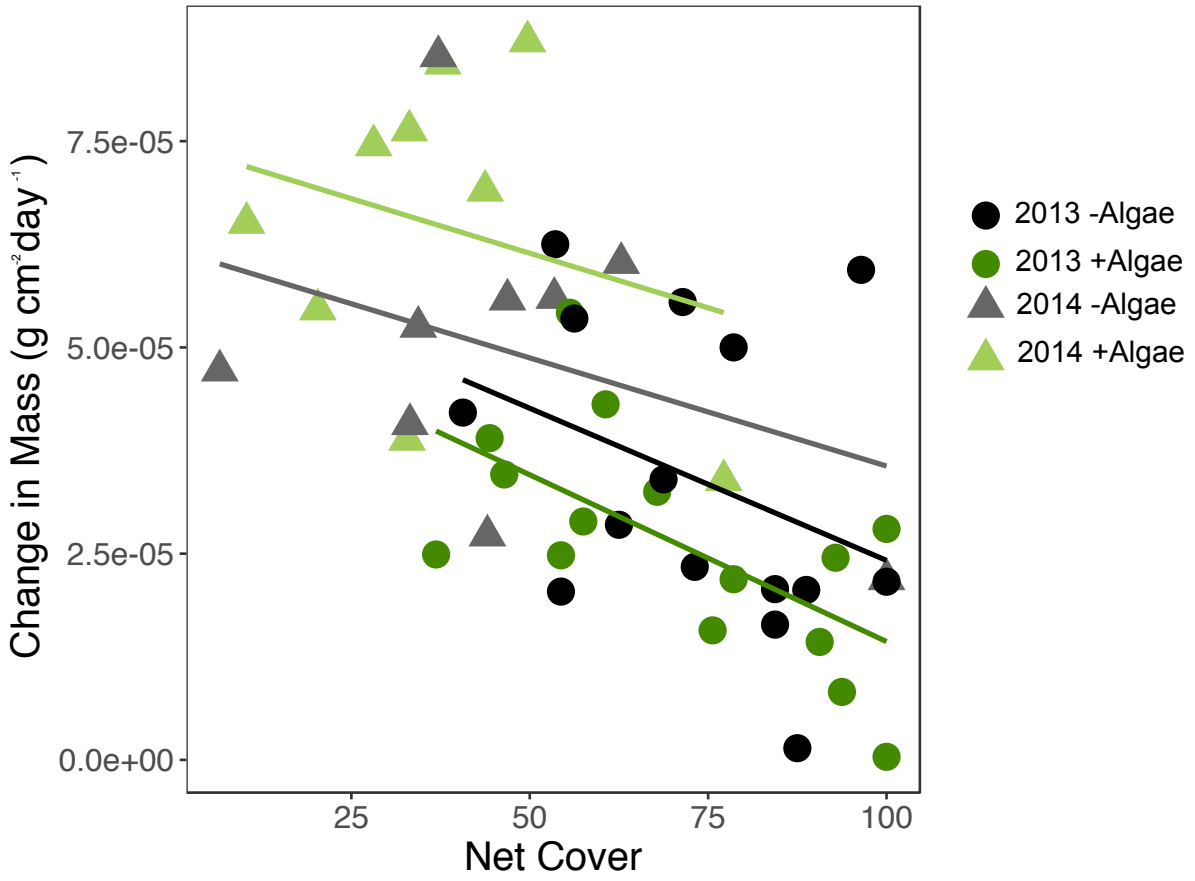


Figure 4.1: Change in mass per initial surface area per day as a function of vermetid net cover, year, and algal treatment. Black circles give results for the –Algae treatment from 2013, dark green circles give results for the + Algae treatment from 2013, and gray and light green triangles give results for the –Algae and + Algae treatments respectively from 2014. Net Cover is based on the average net cover across the entire experiment. I observed a significant effect of net cover, where increasing net cover led to decreases in coral mass. I also observed an interaction between year and algal presence.

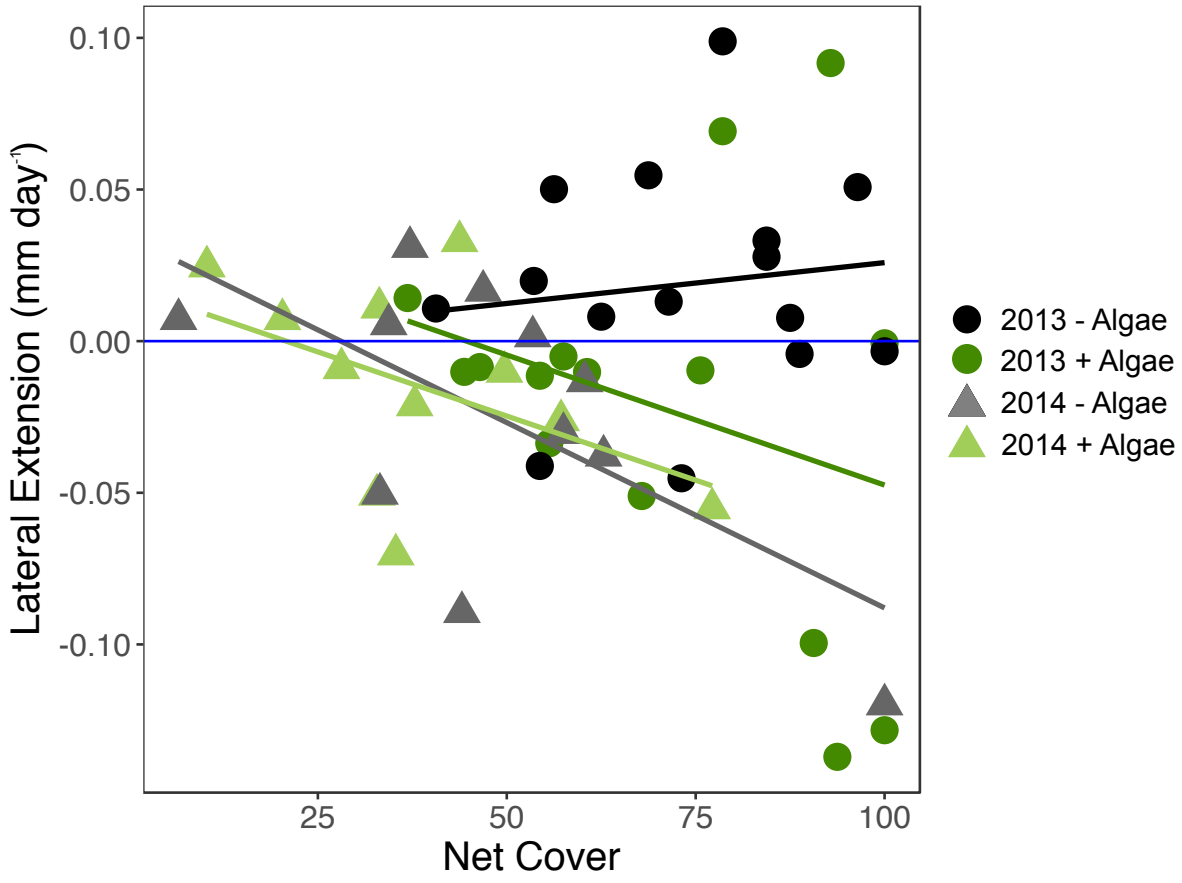


Figure 4.2. Lateral extension (mm day^{-1}) as a function of vermetid net cover, year and algal treatment. Points above the blue line (at 0) indicate growth, whereas points below the blue line indicate recession away from the interface. Black circles give results for the –Algae treatment from 2013, dark green circles give results for the + Algae treatment from 2013, and gray and light green triangles give results for the –Algae and + Algae treatments respectively from 2014. I observed a significant effect of algae. See the text for more statistical information.

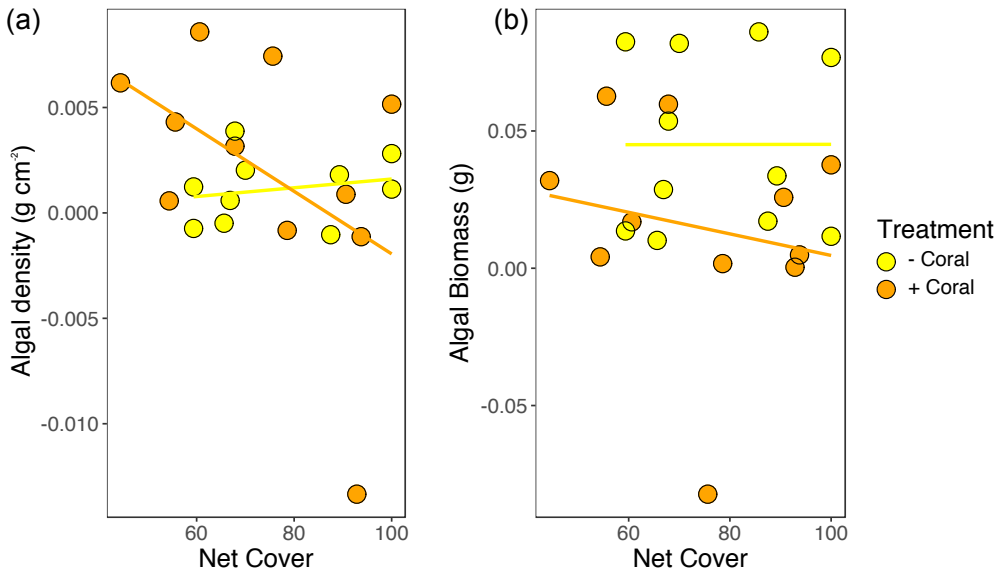


Figure 4.3. (a) Change in algal density (Ash-Free Dry Mass g/cm^2) and (b) Change in algal biomass (g). Colors represent the presence (orange) and absence (yellow) of coral. In the presence of vermetids, I did not observe effects of mucus net cover or coral presence on AFDM (Surface: $F_{1,6} = 0.24$, $p = 0.63$; Net Cover: $F_{1,6} = 2.1$, $p = 0.20$; Surface x Net Cover: $F_{1,6} = 2.33$, $p = 0.18$). I observed a negative effect of coral presence on biomass (Coral: $F_{1,7} = 5.45$, $p = 0.05$), but no effect of net cover or an interaction with coral presence on algal biomass (Vermetid: $F_{1,7} = 0.29$, $p = 0.61$; Vermetid x Coral: $F_{1,7} = 1.24$, $p = 0.30$).

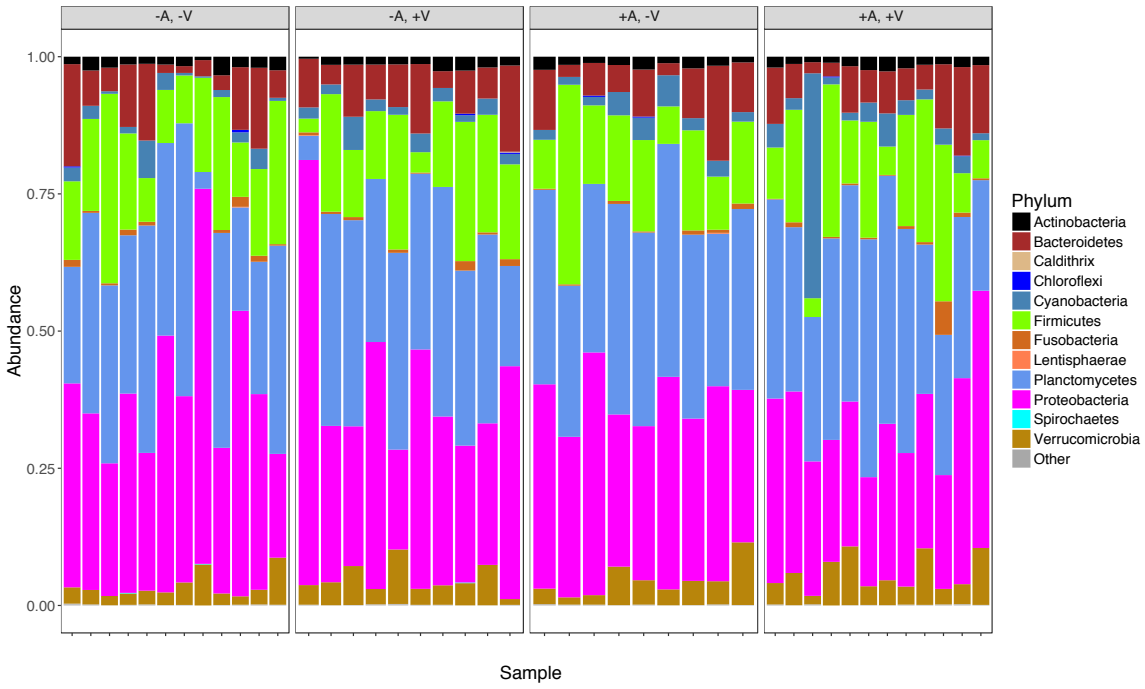


Figure 4.4. The relative abundance of all samples of phyla that represent > 0.1% of a sample. Colors indicate different phyla. The "Other" category represents the groups that represent < 0.1% of samples and includes 13 phyla.

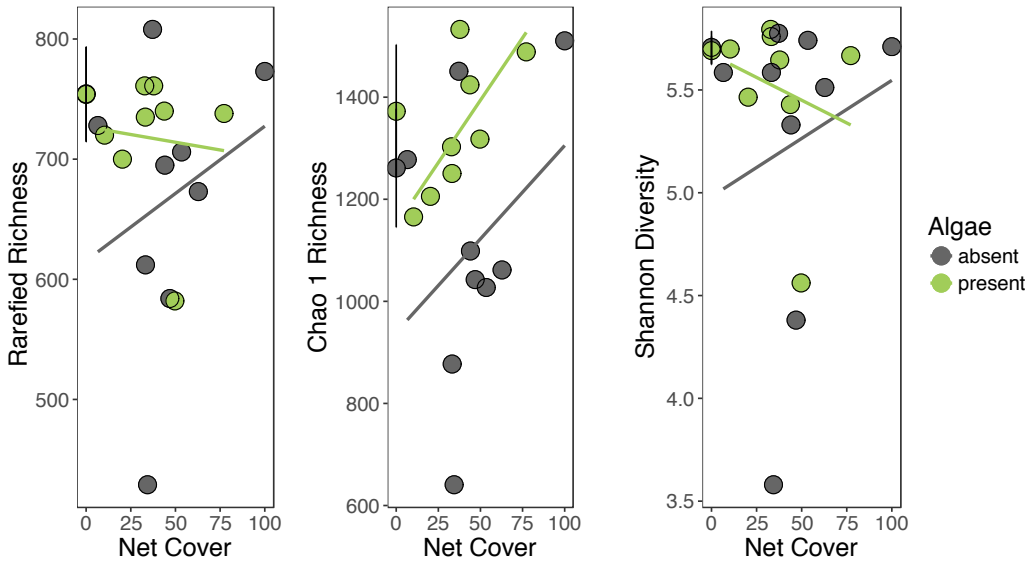


Figure 4.5. Rarefied richness, Chao1 richness, Shannon diversity of the microbial community inhabiting coral surface mucus in the presence (green) and absence (gray) of algae as a function of increasing mucus net cover in the 2014 experiment. The vermetid absent treatments are represented by mean ± SE. I observed a significant effect of algae on Chao1 richness but no effects of vermetids or algae on Shannon diversity or Rarefied richness.

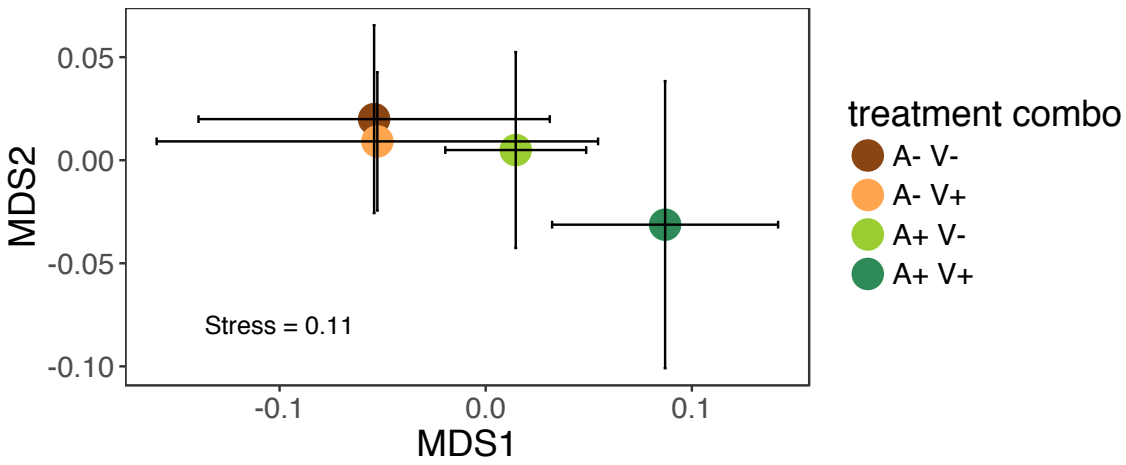
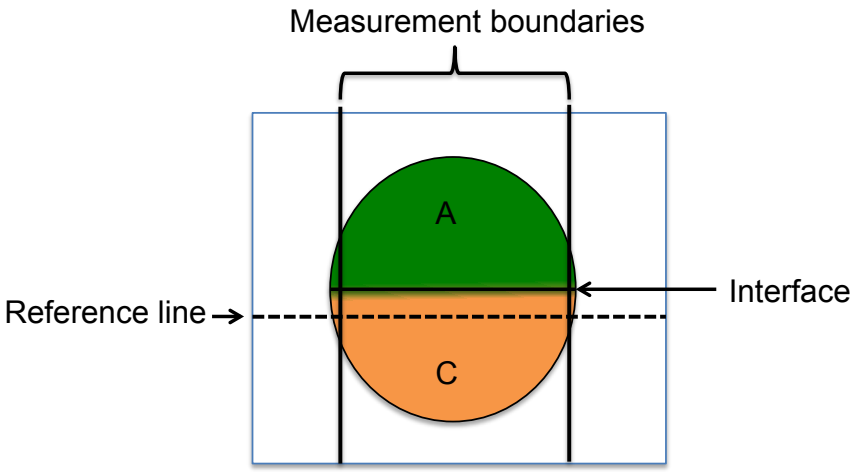


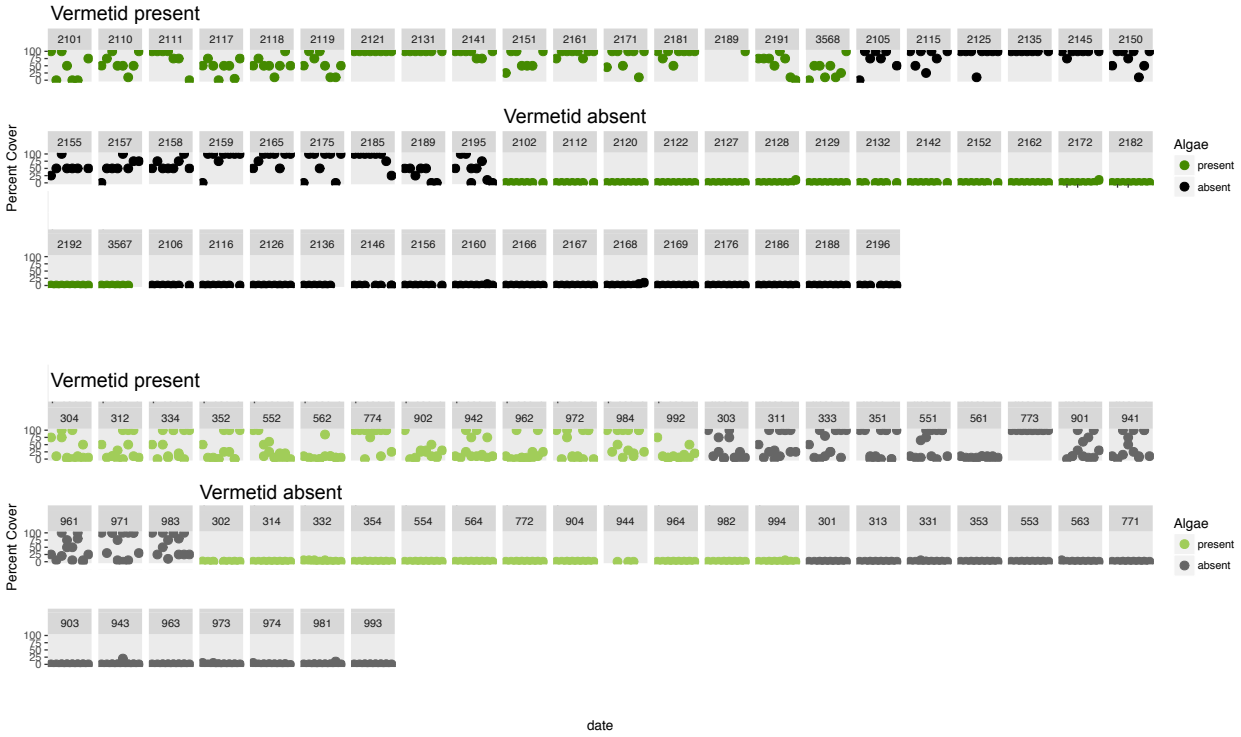
Figure 4.6. nMDS plot based on Bray-Curtis dissimilarity on the relative abundance of sequences in each Phylum. Points and error bars give mean ± SE for the four treatment groups. There were no significant effects of Algae or Vermetids.

4.**Appendices**

Appendix 4A



4A. Figure 1. Schematic of how linear extension was measured. The circle is the core, with algae represented by green (A) and coral represented by orange (C). The "Reference line" indicates the line drawn and where measurements were made between the coral and the interface. The interface indicates the part of the core where the algae and coral are touching. The area between the reference line, interface, and measurements boundaries were measured in the photographs taken at the start and end of the experiments. See Equation 2 for information about converting the areal measurement to a linear measurement.



4A. Figure 2. Mucus net cover over time on cores with and without algae and with and without vermetids. Each facet represents a core and gives the percent mucus net cover each week during the experiment. Colors indicate algal presence and absence. Plots include net coverage from 2013 (algal presence dark green, algal absence is black) and 2014 (algal presence is light green and algal absence is gray). For analyses, weekly net cover values were averaged.

Appendix 4B. Classical statistical design results

4B.Table 1

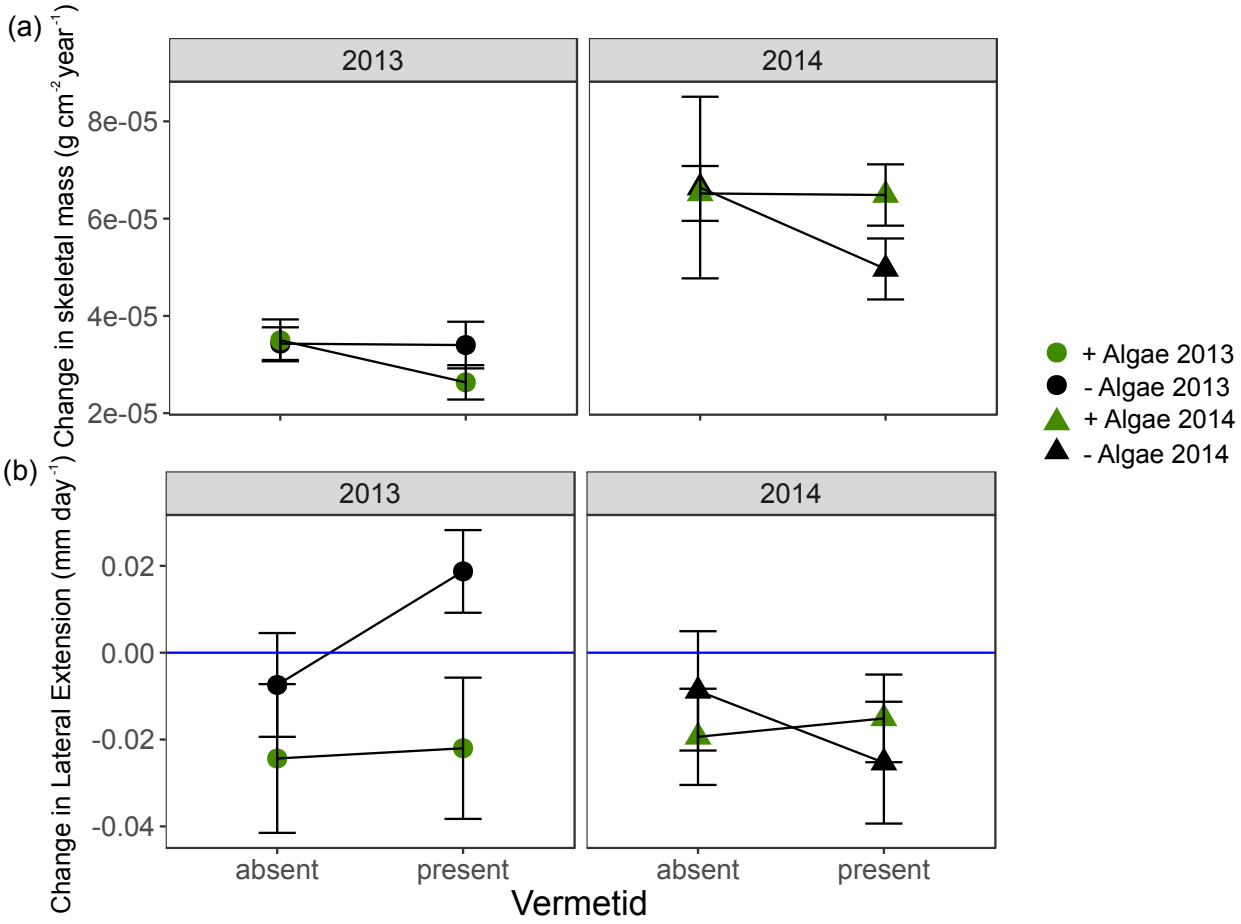
F-table evaluating effects of vermetids, and algae on coral growth as measured by the change in mass and lateral extension. Significant effects ($p<0.05$) are given in **bold**; results with $0.10<p<0.05$ are indicated in *italics*. I observed no significant effects involving vermetids or algae.

	Mass Change				**Lateral Extension**			
	numDF	denDF	F-value	p-value	numDF	denDF	F-value	p-value
Year	1	24	31.47	**<.0001**	1	24	0.73	0.40
Vermetids	1	68	1.38	0.24	1	72	0.34	0.56
Algae	1	68	0.01	0.94	*1*	*72*	*2.99*	*0.09*
year x Vermetids	1	68	0.15	0.70	1	72	1.09	0.30
year x Algae	1	68	0.84	0.36	1	72	2.14	0.15
Vermetids x Algae	1	68	0.02	0.89	1	72	0.07	0.80
year x Vermetids x Algae	1	68	1.37	0.25	1	72	1.30	0.26

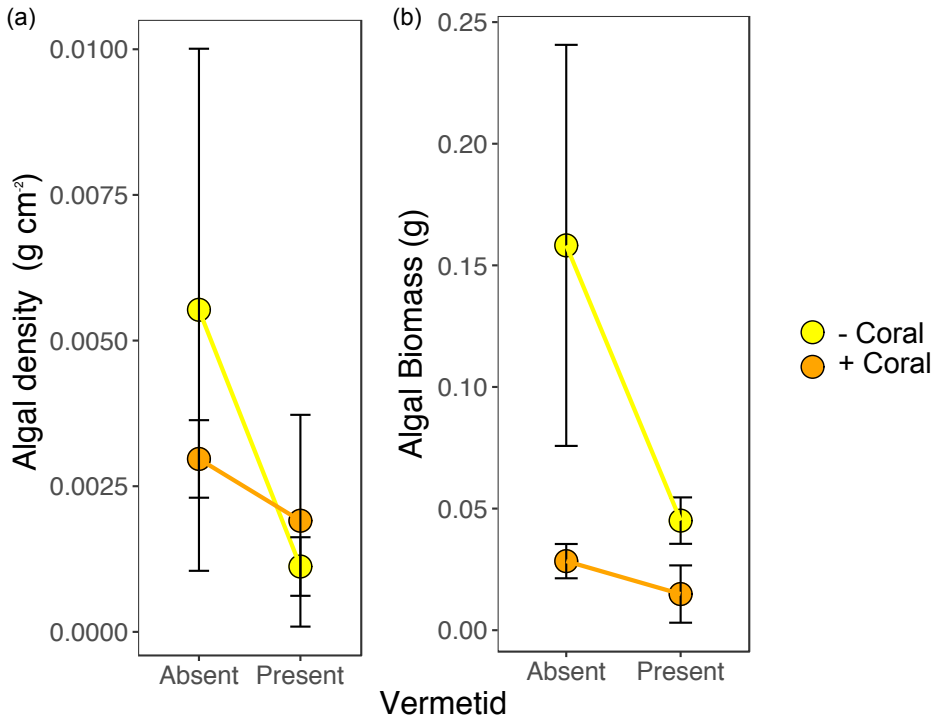
4B.Table 2

F-table evaluating effects of corals and vermetids on algal density and algal biomass. Significant effects ($p<0.05$) are given in **bold**; results with $0.10<p<0.05$ are indicated in *italics*. I observed no significant effects involving vermetids or coral

	Algal density				**Algal Biomass**			
	numDF	denDF	F-value	p-value	numDF	denDF	F-value	p-value
Vermetids	1	30	1.58	0.22	1	30	1.88	0.18
Coral	1	30	0.45	0.51	*1*	*30*	*3.17*	*0.09*
Coral x Vermetid	1	30	1.79	0.19	1	30	1.09	0.30



4B.Figure 1. Results of the original crossed design (vermetid x algae) for 2013 (left panels) and 2014 (right panels). (a) Change in skeletal mass and (b) change in lateral extension, where points above the blue line indicate growth and points below the blue line indicate recession away from the interface. Data are mean ± se, and colors indicate the presence (green) and absence (black) of algae. There is a significant effect of year on calcification, but no other significant effects (see 4B Table 1)

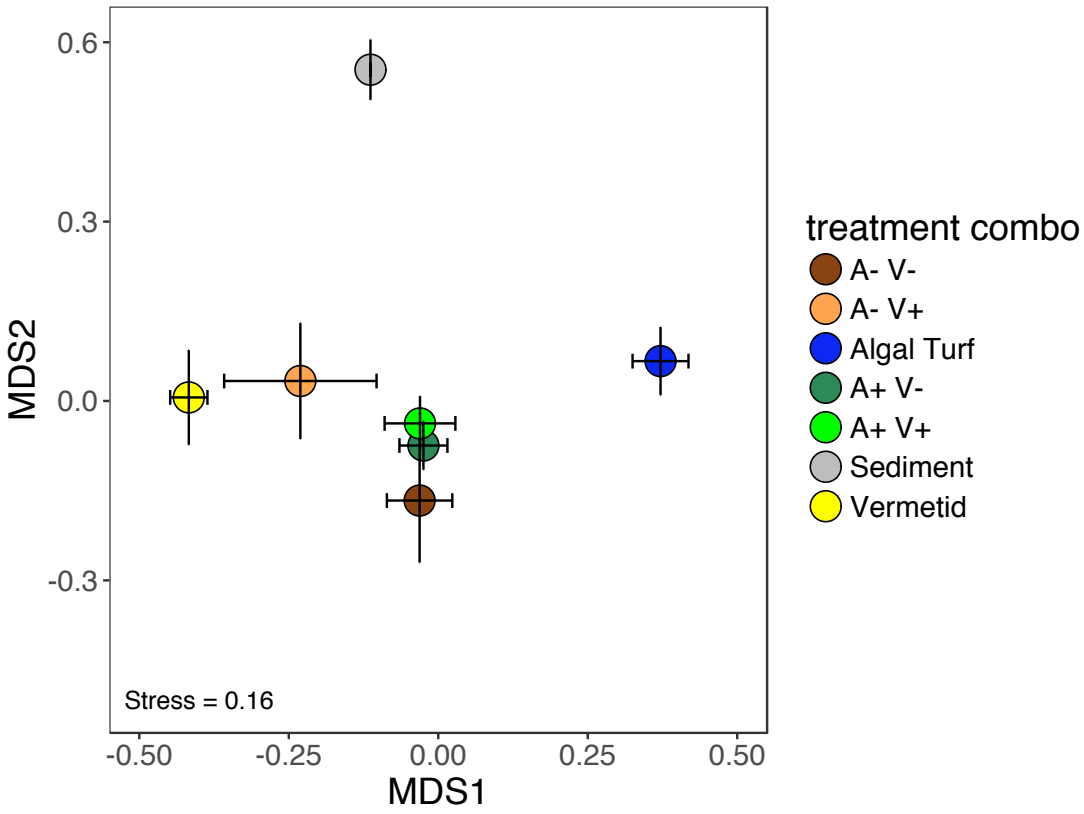


4B.Figure 2. Results of the traditional vermetid x coral treatments on (a) algal density and (b) algal biomass. Data are mean ± se, and colors indicate the absence of coral (yellow) and presence of coral (orange). I observed no significant effects of vermetids or corals on algal density or algal biomass.

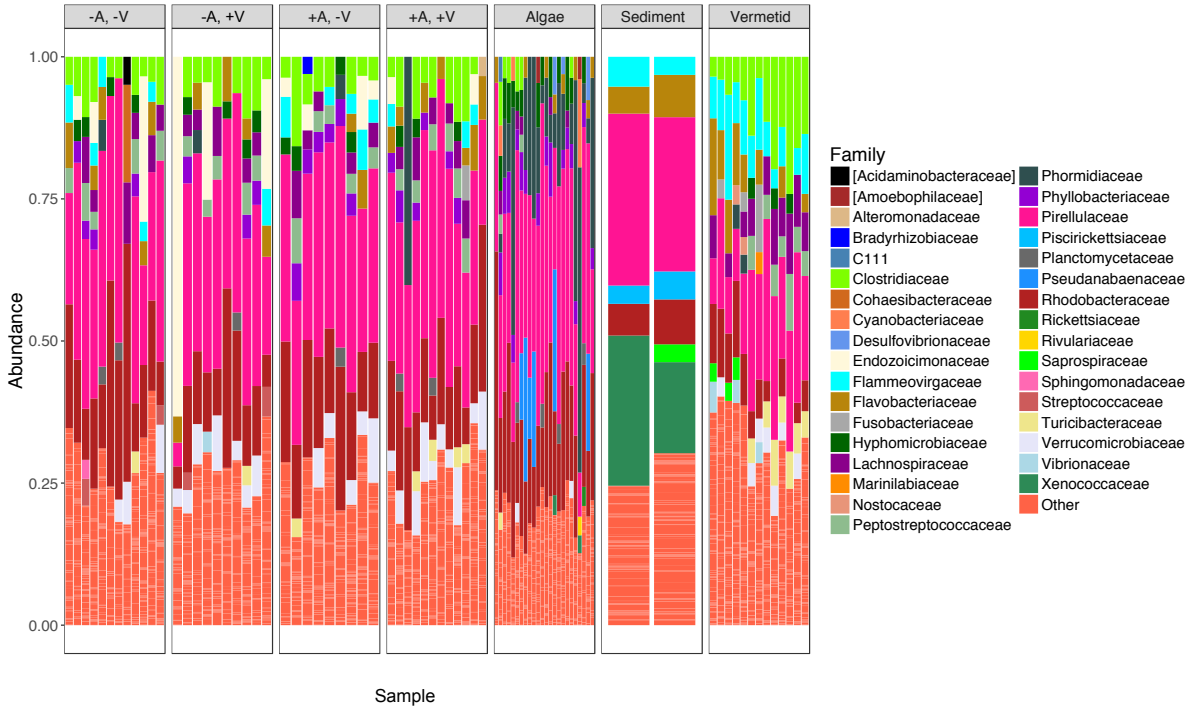
Appendix 4C: Additional microbial diversity

4C.Table 1: Results of diversity analyses using the crossed statistical design. Bolded values are significant.

		numerator DF	denominator DF	F-value	p-value
Chao1	Algae	1	27	4.59	**0.04**
	Vermetid	1	27	0.48	0.49
	Algae x Vermetid	1	27	0.82	0.37
Shannon	Algae	1	27	1.77	0.19
	Vermetid	1	27	1.85	0.18
	Algae x Vermetid	1	27	0.37	0.55
Observed	Algae	1	27	1.64	0.21
	Vermetid	1	27	1.05	0.31
	Algae x Vermetid	1	27	0.48	0.49



4C.Figure 1. nMDS plot based on Bray-Curtis dissimilarity on the relative abundance of sequences in each family. Both the experimental samples, algal, mucus net and sediment samples are shown. Dark brown points indicate –Algae, -Vermetid treatments; light orange treatments indicate -Algae, +Vermetids; neon green indicates +Algae, -Vermetids; and dark green indicates +Algae, +Vermetids. Gray, yellow and blue indicate sediment, vermetid and algal samples. According to a PERMANOVA analysis, there are significant differences among the treatment combinations and sample types (Sample type: $p = 0.001$). Stress in MDS plots are a measure of goodness of fit, values between 0.1 and 0.2 are considered "adequate fit".



4C.Figure 2. Relative abundance of OTUs summarized at the family level for treatment corals, algal samples, sediment samples and vermetid mucus net samples. The “Other” category represents families that are <3% in abundance in samples, and include 245 low abundant families.

CHAPTER 5

EXTENDED PHENOTYPES ON CORAL REEFS: CRYPTIC PHENOTYPES AND SPECIES INTERACTIONS[4]

[4] To be submitted to *Ecology Letters*, and authored by AL Brown, EK Lipp, JP Wares, JS Shima, and CW Osenberg

Abstract

If phenotypic plasticity is adaptive, when environments differ due to the presence or absence of a stressor, prior exposure should weaken the effects of the stressor. Here, we consider plasticity of the extended phenotype including the response of microbes that are associated with a focal organism. We tested our hypothesis using corals that varied in their prior exposure to vermetid gastropods, an organism known to reduce the growth and survival of corals. We conducted several manipulative experiments to test the effects of vermetids on corals that differed in their past exposure to vermetids. First, we examined linear extension on reefs that differed in vermetid exposure. We found lower linear extension on reefs where vermetids were present or experimentally removed compared to reefs where vermetids were naturally absent. These results suggest evidence of a lasting effect of vermetid presence. Following this experiment, we conducted two reciprocal transplant studies. We measured a suite of phenotypic traits (coral growth, tissue thickness, *Symbiodinium* densities, microbial diversity and composition) of experimental corals. For the reciprocal transplant studies, we observed negative effects of vermetids, for both previous exposure contexts, but faster calcification rates of corals previously exposed to vermetids. Corals without previous exposure to vermetids had thicker tissues and higher *Symbiodinium* densities, compared to coral previously exposed to vermetids. Additionally, microbial alpha diversity of corals transplanted to reefs with vermetids increased relative to those transplanted to reefs without vermetids. Both prior and current exposure to vermetids led to changes in microbial composition. The second experiment, which spanned a massive die off of vermetids across French Polynesia, showed depressed growth of corals previously exposed to vermetids. We suggest the lowered growth may be due to the spill-over of the putative pathogen that led to the die-off of vermetids. Reefs with and without vermetids were

almost completely associated with two different mitotypes, highlighting there are likely genetic differences that contribute to the observed phenotypes. Vermetids were only found on the fast-growing phenotype that were characterized by thin-tissues, and that likely had a history of disturbance. Thus, we suggest vermetid absence and presence is part of the extended phenotype of these corals. Coral genotype establishes different trajectories, with thin-tissue types being more prone to disturbance and subsequent colonization by vermetids. The other mitotype has thicker tissue and is more resistant to disturbance and thus colonization by vermetids.

Introduction

Abiotic factors like temperature and rainfall, as well as biotic factors like predation and competition, can lead to changes in phenotypic traits, through plasticity or local adaptation (i.e., genotypic differentiation). Local adaptation arises if gene flow is low and natural selection differs between two local populations. As a results the phenotypes in each population has higher fitness than would the phenotype from the other populations (Kawecki and Ebert 2004). Adaptive phenotypic plasticity, alternatively, arises when a single genotype leads to different phenotypes when developing in two different environments, yielding a higher fitness in the two habitats that could be accomplished by a fixed phenotype (Schlichting and Pigliucci 1998, Ghalambor et al. 2007). For example, Trinidadian guppies show local adaptation to high predator environments: populations from high predator areas grow to larger sizes than those from low predator populations when placed in a common environment. The guppies also show adaptive plasticity: predator environment dictates their location in the water column, no matter which population they originated from (Torres-Dowdall et al. 2012). Phenotypic plasticity in morphological traits, induced by predators, has been shown in wide range of taxa, including *Daphinia* (Dodson 1974), amphibians (Relyea 2004), rocky intertidal snails and barnacles

(Trussell 2000, Raimondi et al. 2000), as well as fish (Brönmark and Petterson 1994, Reznick and Travis 1996). Competitors can also induce phenotypic shifts in species traits. For example, competition with leopard frogs causes the tadpoles of leopard frogs to reduce their growth rates and increase their mouth length and width, (Relyea 2000).

Although phenotypes are often quantified using morphological, physiological, and behavioral traits of the focal organism, phenotypes can extend beyond the traditional concept of an individual (*sensu* Dawkins 1976). A classic example of an extended phenotype is a beaver dam. Although not physically attached to, or a fully integrated part of a beaver, the construction of a dam affects the individual's fitness (i.e., in terms of survival). Recently, the extended phenotype has expanded to include host-associated microbial symbionts. Most individuals consist of both host and microbial symbionts, which together constitute a holobiont (Margulis 1990). Additionally, Zilber-Rosenberg and Rosenberg (2008) proposed that the holobiont is a unit of selection, an idea that has been embraced conceptually by others (reviewed in Roughgarden 2017). Thus, the host-associated microbial communities can be considered a phenotypic trait, subject to change in different biotic and abiotic conditions, and likely to differentially influence the performance of the host under different environmental conditions.

Implicit in the concept of adaptive phenotypic plasticity or local adaptation is the idea that a phenotype performs best in an environment that it has been exposed to previously. For example, if a snail grows a thicker shell in the presence of crayfish predators, it will be less vulnerable to predation by crayfish (*sensu* Auld and Relyea 2011). Microbial communities can also aid in adapting a host to a particular environment. For example, plant-associated soil microbes that were previously exposed to drought conditions increased the ability of plants to

withstand the deleterious effects of drought (Lau et al. 2014). Here, we examine a similar phenomenon on coral reefs.

Scleractinian corals are calcifying holobionts composed of host tissues, as well as bacterial, archaeal and eukaryotic symbionts. *Symbiodinium* are microalgal cells that live within the host tissues and transfer photosynthesis-derived products to the coral host, thus acting as a major food-acquisition source for the holobiont. The bacterial and archeael (hereafter I will refer to these groups as microbial) communities are highly diverse, and are found in coral tissues and in the coral surface mucus layer (SML). Although the functions of these microbial communities are not well understood, they are hypothesized to play roles in immunity (i.e., protection from pathogens; Reshef et al. 2006, Bourne and Webster 2013, Krediet et al. 2013) and nutrient cycling (Bourne and Webster 2013).

Corals exhibit extreme phenotypic plasticity. Water flow, light and temperature are associated with morphologically plastic responses of corals (Bruno and Edmunds 1997, Muko et al. 2000, Smith et al. 2007, Padilla-Gamiño et al. 2012). For some groups, morphological changes are drastic, in which some species can shift from mounding to plating form with depth (Muko et al. 2000). Others show differences in branch diameter and corallite structure with water flow (Bruno and Edmunds 1997). Calcification rate is also plastic and subject to environmental conditions; it decreases with turbidity (Padilla-Gamiño et al. 2012) and increases with temperature (Lough and Barnes 2000).

Coral symbionts are responsive to changes in the environment, often giving rise to responses that are beneficial to the coral. For example, different clades of *Symbiodinium* confer differential sensitivity to thermal stress. Thus, exposure to increased temperature can cause corals to expel maladaptive symbionts and acquire adaptive symbionts: i.e., the adaptive

bleaching hypothesis (Buddemeier et al. 1993, Baker et al. 2004). Changes in the microbial communities can similarly be beneficial (and sometimes harmful). The coral surface mucus layer is the first line of defense of corals against incoming pathogens, and microbes in this carbohydrate-rich layer are hypothesized to prevent pathogen invasion (probiotic coral hypothesis, Reshef et al. 2006, Krediet et al. 2013, Peixoto et al. 2017). However, competitive interactions can favor more pathogenic or heterotrophic groups (Barott et al. 2012), and can lead to disease (Nugues et al. 2004).

Another organism known to induce phenotypic changes in corals are vermetid gastropods. Vermetids are sessile gastropods that cast mucus nets to collect food particles from the water column. The largest vermetid is *Ceraesignum maximum*, which are frequently found embedded in the coral matrix in the Indo-Pacific and Red Sea. These vermetids reduce coral calcification (Shima et al. 2010), flatten coral colonies (Colgan 1985, Zvuloni et al. 2008, Shima et al. 2015) and decrease photosynthetic yield of symbiotic *Symbiondinium* (Shima et al. 2015). The putative mechanism underlying these deleterious effects involves the gastropod's mucus nets. The nets impede water flow and influence chemical conditions at the surface of the corals, and likely lead to the build-up of noxious chemicals (Brown and Osenberg 2018). Additionally, the mucus nets contain bioactive compounds (Klöppel et al. 2013), which may have anti-microbial effects. Thus, we hypothesize that vermetid nets might facilitate the build-up of excess cellular metabolites near the surfaces of corals, which could affect an increase in the growth of pathogenic microbes, inducing maladaptive effects.

There is tremendous spatial variation in the densities of vermetids. At the extreme, some coral colonies have vermetids whereas others, even those of the same species are devoid of vermetids (Shima et al. 2010). This marked variation in historical exposure to vermetids allowed

us to ask if prior exposure to vermetids affected a coral's subsequent response to vermetids, and to assess the relative roles of phenotypic plasticity or genetic differentiation in producing phenotypic variation among corals with vs. without vermetids. We therefore conducted three field experiments. First, we removed vermetids from bommies (large coral colonies) and found the linear extension (e.g., outward coral growth) of these bommies failed to increase; growth remained low relative to bommies that historically lacked vermetids. These results contradicted past experimental work, leading us to hypothesize that past work may have demonstrated strong effects because they used "naïve" corals – those that had lacked prior exposure to vermetids. We tested this hypothesis by conducting a reciprocal transplant experiment, in which we quantified changes in coral calcification, tissue thickness, *Symbiodinium* density, and microbial communities, and determined the role of past exposure to vermetids (i.e., reflected by the presence or absence of vermetids from the site at which the coral originated) versus current exposure to vermetids (i.e., reflected by the presence or absence of vermetids from the transplant location). We expected corals with a history of vermetid exposure to show decreased effects of vermetids compared to corals originating from sites without vermetids (i.e., we expected to find a significant interaction between origin environment and transplant environment). If transplant environment had a greater effect on phenotypic traits relative to origin environment, we would conclude that phenotypic plasticity played a major role. In contrast, if origin reef type was found to have a greater effect on phenotypes, we would conclude either that differences were the result of genetic differentiation, plasticity required longer exposures to develop, or plasticity that required exposure during specific developmental windows. To address potential genetic differentiation among corals that occurred with or without vermetids, we also sequenced the coral's mtCOI region and compared sequence divergence among corals.

Methods

The system

All field studies were conducted in the shallow back reef of Moorea, French Polynesia (S17° 28.511' W149° 48.857') within an area 0.04 km^2 area. The backreef is dominated by massive *Porites* corals, a mounding coral species complex with poorly resolved taxonomy (Forsman et al. 2009, 2015), which create mounding structures called "bommies". "Bommies" are once monospecific coral colonies in which the death of coral tissue allows for colonization by other benthic species. Massive *Porites* corals in Mo'orea are, from a taxonomic perspective, visually indistinguishable and appear to include members that have been previously identified as *Porites lobata*, *Porites lutea* and *Porites australiensis*. On the back reef there are massive *Porites* bommies that are devoid of vermetid gastropods (i.e., without previous vermetid exposure) and bommies that have vermetids present (i.e., with previous vermetid exposure). Both types of bommies can be found within several meters of each other.

Linear extension of entire bommies

In 2009, we identified 10 massive *Porites* bommies that had vermetids and 5 bommies that lacked vermetids. Bommies had similar diameters (113.4± 40.6 cm, mean ± SD). Live coral coverage was higher on bommies that lacked vermetids (98.8% ± 1.3, mean ± SD) compared to bommies with vermetids (72.1% ± 12.1 mean ± SD). We experimentally removed vermetids from half of the reefs with vermetids, resulting in three treatments (each with n= 5): vermetids present, vermetids removed, and vermetids naturally absent. In the absence of any historical effects, and based on past studies (e.g., Shima et al. 2010), we expected bommies without vermetids to grow faster than those with vermetids present, and we expected the removal of vermetids to lead to increased growth rates to a degree that was comparable to the growth rate of

bommies that naturally lacked vermetids. On 6 October 2009, we hammered 5 nails into each bommie, distributed in an arc over the top of a bommie, and measured the length of the nail protruding above the surface of the bommie. After nine months (17 July 2010), we returned to the reefs and re-measured the length of each nail still protruding above the coral surface. The average difference in the lengths of the nails provided an estimate of the linear extension of each bommie.

A reciprocal transplant: transplant experiment 1

The first reciprocal transplant experiment took place from 23 February to 9 June, 2015, using 10 bommies with, and 10 without vermetids that were of similar dimensions and depth: height = 61.95 ± 17.5 cm and diameter = 75.9±19.7 cm, vertical distance from the water surface = 2.0 ± 0.3 m ; Mean ± SD. Bommies that lacked vermetids were characterized by high live coral cover (93±4.3%), whereas bommies with vermetids had only 73.6 ±11.3% live coral cover and had 17.9±7 vermetids on them. The remaining cover on reefs was primarily algal turf and crustose coralline algae.

From each reef, we removed four cores using a pneumatic drill with a diamond-tipped hole saw attachment (diameter = 3.81 cm diameter, McMaster Carr). Each core was returned to the lab and excess skeleton was removed using a bandsaw (© Gryphon C40) so that each core was approximately 1.5 cm in height. Each core was then attached to a mesh base using Zspar (© Splashzone). Each core was photographed, buoyantly weighed (Davies 1989), and placed onto a new reef. The four cores from each origin bommie were placed onto four different bommies (but not their origin reef): two bommies with and two bommies without vermetids. Thus, each reef donated, and also received, four transplants, two from each type of reef.

At the conclusion of the experiment (9 June 2015), we retrieved the cores from each reef and brought them back to the lab, where they were photographed and buoyantly weighed. We recovered 68 of 80 cores; those that were lost were likely consumed by corallivores.

After weighing, samples were then cut in half using a bandsaw (© Gryphon C40) and the surface area of each half was measured. One half of each core was then fixed in formalin for tissue thickness analyses. We removed coral tissue from the other half using an airbrush. The tissue slurry was captured in a plastic bag and then poured into a clean 50 ml tube. Volumes ranged from (12-50 ml). The slurry was then homogenized to break up tissue with a meat grinder. One and a half milliliter aliquots were removed for *Symbiodinium* counts and host genetic analyses. Samples taken for *Symbiodinium* counts were preserved in 10% formalin. Samples taken for host genetic analysis were preserved in DMSO. All host samples were stored at room temperature until analysis.

Reciprocal transplant during the vermetid die-off: transplant experiment 2

The second reciprocal transplant experiment took place from 12 July to 21 September 2015 at the same site as the first transplant experiment, but using different bommies. Bommies were (mean±SD) 62.2±17.9 m high, 80.7±20.3 m in diameter, and 1.9±0.5 m from the surface. Bommies that lacked vermetids were (mean±SD) 95±9.9% live coral, whereas bommies with vermetids were 66 ± 12.9% live coral.

The design of this experiment differed slightly from the first. We used five bommies with vermetids and five bommies without vermetids. Ten cores were collected from each bommie and brought back to the lab as described above, however due to mortality of cores from two vermetid absent reefs, we had cores from only three bommies that lacked vermetids. Thus, each bommie then received eight cores: one from each of the three bommies without vermetids

and one from each of the five bommies with vermetids. Cores were re-collected 21 September 2015, re-weighed, cut in half, and processed for tissue thickness and DNA measurements as described above.

By 24 July 2015 all of the *C. maximum* across Mo'orea, French Polynesia experienced a massive die-off (Brown et al. 2016). Thus, the cores in the second experiment experienced the effects of vermetids for only one week. As a result, we expected the effects of the transplant treatment (with vs. without vermetids) to be much reduced compared to the first reciprocal transplant experiment.

Measurements

Skeletal Mass

Buoyant mass was converted to skeletal mass, using equations from Davies (1989). Because cores were of similar size, calcification rate was measured as the difference in skeletal mass divided by time (but not adjusted by initial surface area).

Tissue thickness

After removing tissues from formalin, we dissolved the skeleton in 10% HCl. Tissues were dried to a constant mass. Tissue mass divided by surface area was used to obtain an estimate of tissue thickness (i.e., mass per area).

Symbiodinium Counts

Counts were completed using a hemocytometer, with 10 subsamples per sample. The average cell count was divided by the volume of the hemocytometer chamber and multiplied by total homogenate volume to obtain the total count. The total cell count was then divided by surface area of the half core to determine cell density (no. per area) and by tissue thickness to obtain cell concentration (no. per volume).

Host genetic analyses

DNA samples of corals from both experiments (n=13 vermetid absent, n=15 vermetid present) were brought back to UGA. Samples were centrifuged and the excess buffer was decanted off. DNA extraction was completed using the protocol from Concepcion (et al. 2006), followed by amplification of the mtCOI region. PCRs were run at 95°C for 3 minutes, followed by 34 cycles of 95°C for 30s, 45°C for 30s, 72°C for 1:00, and a final elongation step at 72°C for 5 minutes, and held at 12°C). After successful amplification, Exosap (Exonuclease I and Alkaline Phosphate) was added to clean up the sample, samples were sent to Georgia Genomic Facility for sequencing, sequences were assembled and aligned in Geneious ©, and a tree was constructed using the Tamura-Nei distance model and the Neighbor-joining tree method without an outgroup. Trees were bootstrapped 100 times.

Microbial sampling

Before corals were cored from reefs, microbial samples were collected from each bommie using 12-ml syringes. After gentle agitation of the coral surface, mucus was sucked into the syringe. At the end of the first transplant experiment, but before cores were retrieved, microbial samples were collected from the surface of each core and each bommie. Sediment samples were also collected at the start and end of the first transplant experiment. After samples were collected, they were kept on ice and transported back to the lab.

In the lab, the mucus settled to the base of the syringe, was inserted into a 1.5-ml centrifuge tube, centrifuged at 10,000 RPM for 5 minutes, and excess water was removed. Samples were immediately frozen.

Samples were transported back to the University of Georgia where they were extracted using the protocol from Boström et al. (2010), but with an added initial bead-beating step (0.03g

beads added, and vortexed at max speed for 10 mins). Due to low DNA yields, samples were re-extracted using Qiagen © Powersoil kits. To remove PCR inhibitors, we added equal volumes of magnetic beads in PEG solution (Rudi et al. 1997). Extracted DNA was sent to IMR-CGEB for sequencing on an Illumina platform. The V4/V5 region was sequenced using the 515F and 806R primers.

Bioinformatics were completed on the forward reads using dada2, including chimera removal (Callahan et al. 2016). Taxonomy was assigned using the Greengenes database (DeSantis et al. 2006). Following the dada2 pipeline, we imported data into the R packages phyloseq (McMurdie and Holmes 2013) and vegan for further analysis (Oksanen et al. 2017).

Statistical Analyses

Linear extension was analyzed using ANOVA. Change in skeletal mass, tissue thickness, and *Symbiodinium* densities were analyzed using mixed linear models in the nlme package in R (R Core Team 2015, Bates et al. 2017). Origin reef type and transplant (recipient) reef type (vermetid absent, vermetid present) were treated as fixed effects and origin bommie number and recipient bommie number nested in origin reef were treated as random effects. We also reported the conditional and marginal R^2 for each model, to quantify the variance explained by the fixed effects (i.e., marginal R^2) and the variance explained by both the fixed and random effects (i.e., conditional R^2, piecewiseSEM package in R, Lefcheck 2015).

We compared species richness, Chao1 richness and Shannon diversity. To compare OTU richness (the number of unique sequences per sample), microbial data was rarefied to the lowest number of reads using phyloseq (McMurdie and Holmes 2013). Rarefied OTU richness, Chao1 richness and Shannon diversity were analyzed by treating origin and transplant reef type (i.e.,

with vs. without vermetids) as fixed effects and origin bommie number and transplant bommie number nested in origin as random effects.

All (unrarefied) data were converted to relative abundance per sample and composition was compared at the family level based on Bray-Curtis dissimilarity matrices using a PERMANOVA with 999 permutations. We visualized the data using an nMDS plot in 2 dimensions based on Bray-Curtis dissimilarity matrices. We additionally examined families that explained significant variation across the treatments based on the PERMANOVA, (i.e., those families that had linear model coefficients > 0.005). PERMANOVA and nMDS were conducted using the vegan package (Oksanen et al. 2017). After determining the families that contributed to significant effects among communities, we compared the relative abundances of each family separately using the same mixed effects models described above (fixed effects: origin reef type, transplant reef type, origin x transplant; random effects: origin bommie number, transplant bommie number; nlme package, Bates et al. 2017).

Host genetic data were compared using Hudson's Snn on DNAsp (v 5.10.1) to determine if there was significant genetic differentiation among the corals that had vermetids and did not have vermetids (Hudson 2000). When near 1, Snn indicates high differentiation among the populations.

Results

Linear extension of entire bommies

Bommies with naturally lacked vermetids had linear extension rates that were double those observed for bommies that historically had vermetids whether the vermetids were present or had been removed (Figure 5.1, $F_{2,12} = 27.39$, $p < 0.001$). Thus, vermetid removal did not increase linear extension, a result that appears to contradict past studies (e.g., Shima et al. 2010),

and which suggests either a long-lasting effect of prior exposure to vermetids or variation among corals that host versus do not host vermetids.

Reciprocal transplant experiment 1

Calcification

Origin reef type and transplant reef type each significantly affected a core's change in skeletal mass (origin: $F_{1, 18} = 7.26$, $p = 0.01$; recipient: $F_{1,45} = 30.58$, $p < 0.001$), although the interaction was not significant ($F_{1,45} = 0.224$, $p = 0.638$). We observed variation due to the random effects (marginal $R^2 = 0.25$, conditional $R^2 = 0.94$). Corals accrued ~1.5x more mass when transplanted to bommies without vermetids compared to bommies with vermetids; but they also grew ~1.5x more if they came from reefs that had vermetids (Figure 5.2a). Thus, corals on their home reef type grew at similar rates. This is an example of counter-gradient selection. These results refute our hypothesis that prior vermetid exposure makes corals less sensitive to vermetids.

Tissue thickness

Coral tissues were 1.8x thicker when cores came from bommies without vermetids compared to cores that came from bommies with vermetids (Figure 5.2b, $F_{1,18} = 4.46$, $p = 0.049$). Tissue thickness was not affected by recipient reef type (Figure 5.2b, $F_{1,40} = 0.003$ $p = 0.953$) suggesting that vermetids did not have a short-term effect on tissue thickness. There was no evidence for recipient x origin interaction (Figure 5.2b, $F_{1,40} = 0.017$, $p = 0.896$). There was variation due to the random effects (marginal $R^2 = 0.07$, conditional $R^2 = 0.99$).

Symbiodinium density

Cores that originated on bommies without vermetids had *Symbiodinium* densities that were ~2x greater than corals that originated from bommies with vermetids (Figure 5.2c, Origin:

$F_{1,48} = 90.167$, $p < 0.001$). Cores that were transplanted to bommies without vermetids had, on average, higher densities of *Symbiodinium*, although this difference was not significant (Recipient: $F_{1,46} = 3.97$, $p = 0.052$). Further, there was a suggestion that cores that came from bommies without vermetids were more sensitive to vermetids, although this interaction also was not significant (Recipient x Origin: $F_{1,46} = 2.858$, $p = 0.098$). We again saw variation due to the random effects (R^2 marginal = 0.59, R^2 conditional = 0.98). The differences in *Symbiodinuium* density largely mirrored the variation in tissue thickness. However, even when *Symbiodinium* counts were adjusted to number per gram of coral tissue (and log transformed to normalize data), there was an effect of origin (origin: $F_{1,18} = 12.53$, $p = 0.002$), but no effect of recipient reef ($F_{1,39} = 0.44$, $p = 0.51$) or an interaction ($F_{1,39} = 0.00$, $p = 0.98$).

Microbial diversity

Before filtering out chimeric, mitochondria and chloroplast sequences, we observed 5,644 – 41,650 sequences/sample, with a mean of 21,900. After filtering, there was an average of 5,017 sequences/sample (range: 200 – 35,310). Sequences from treatment corals ranged from 257 – 12,550, which yielded a total of 1876 OTUs. Samples were rarefied to 257 sequences/sample to assess OTU richness.

Microbial alpha diversity increased when cores were transplanted to bommies with vermetids, relative to diversity on bommies without vermetids (Figure 5.3; Chao1: $F_{1,37} = 8.14$, $p = 0.007$; Shannon: $F_{1,37} = 9.45$, $p=0.004$; Observed: $F_{1,37} =9.72$, $p = 0.004$). There were no effects of origin reef type (Chao1: $F_{1,18} =0.99$, $p =0.33$; Shannon: $F_{1,18} = 048$, $p = 0.50$; Observed: $F_{1,18} = 1.11$, $p = 0.31$) nor any interaction between origin and recipient reef type (Chao1: $F_{1,37} = 0.16$, $p = 0.69$, Shannon: $F_{1,37} = 0.001$, $p = 0.98$; Observed: $F_{1,37} =0.20$, $p = 0.66$). We observed variation due to the random effects (Chao1: R^2 marginal = 0.14, R^2 conditional =

0.96; Shannon: R^2 marginal = 0.14, R^2 conditional = 0.92; Observed: R^2 marginal = 0.16, R^2 conditional = 0.93).

Compositional differences and Beta diversity

The composition of microbes depended on origin and recipient reef type (Fig 5.4) but not their interaction (PERMANOVA Origin: $F_{1,55} = 6.03$, p = 0.001; Recipient: $F_{1,55} = 2.36$, p= 0.004; Origin x Recipient: $F_{1,55} = 0.76$, p = 0.72). Interestingly, samples taken from the bommies at the start and end of experiment exhibited different communities that change over time (Appendix 5A Fig. 2,3). Sediment and vermetid mucus net samples differed from coral microbial samples (PERMANOVA: $P < 0.001$).

Several bacterial families contributed to the compositional differences between cores that came from bommies with vs. without vermetids (Fig 5.6). Endozoicimonaceae (Fig 5.6a), Pirellulaceae (Fig 5.6b), Phyllobacteriaceae (Fig 5.6c), Lachnospiraceae (Fig 5.6d), Clostridiaceae (Fig 5.6n) had greater relative abundances on cores originally from bommies with vermetids, whereas Rhodobacteraceae (Fig 5.6e) and Synechococcaceae (Fig 5.6f) were more abundant on corals originally from bommies without vermetids. Several families also changed relative abundance in response to being moved to a different reef type: Flavobacteriaceae (Fig 5.6g), Verrucomicrobiaceae (Fig 5.6o), and Fusobacteriaceae (Fig 5.6h) were more abundant after transplant to bommies with vermetids, whereas Synchechococcaeae (Fig 5.6e), Halomonadaceae (Fig 5.6j) and Streptococcaea (Fig 5.6k) were more abundant when placed on bommies without vermetids. Planctomycetaceae and Cryomorphaceae (Fig. 5.6q,r) showed a significant interaction between origin and recipient reefs, in which corals originally from vermetid present reefs showed more drastic changes to transplantation compared to corals originally from vermetid absent reefs.

Reciprocal transplant during the vermetid die-off: transplant experiment 2

The results from the second transplant experiment showed that cores transplanted to bommies without vermetids grew faster than did cores transplanted to bommies with vermetids (Fig. 5.7a, $F_{1,70} = 4.81$, $p = 0.03$), independent of the source of the cores ($F_{1,70} = 2.19$, $p = 0.14$). However, corals only grew 1.08x faster without vermetids, compared to 1.5x faster in the first experiment (Fig. 5.2a), likely because *C. maximum* died after the first week of the second experiment. We also observed variation due to the random effects (R^2 marginal = 0.29, R^2 conditional = 0.94).

Origin reef type had an effect on coral growth ($F_{1,6} = 5.66$, $p = 0.05$), although this pattern was in the opposite direction as observed in the first transplant experiment. Interestingly, the growth of cores from the reefs without vermetids was similar during the two experiments (~0.02 g d^{-1}). However, the cores originally from bommies with vermetid grew more slowly (by ~40%) in the second experiment, which occurred during the vermetid die-off.

Tissue thickness in the second experiment mirrored the patterns seen in the first transplant experiment: cores originally from bommies without vermetids were significantly thicker than those from bommies with vermetids (5.Fig 7, $F_{1,6} = 17.85$, $p = 0.006$), and there was no evidence of an effect of recipient reef type ($F_{1,70} = 0.04$, $p = 0.84$) nor an interaction ($F_{1,70} =$ 0.36, p 0.56). Tissues were approximately twice as thick during the second transplant experiment compared to the first (compare 5.Figs. 2b and 7b). As in other analyses, there was considerable variation due to the random effects (R^2 marginal = 0.21, R^2 conditional = 0.93)

Host Genetics

We observed significant underlying genetic differentiation between the samples collected from bommies with versus without vermetids (Figure 5.8, Hudson's Snn = 0.93, $p < 0.001$). All but one bommie with vermetids grouped separately from bommies without vermetids. Three distinct sites along the 700bp segment (~0.4% difference) differentiated the two groups of bommies, which was almost entirely associated with the presence and absence of vermetids. For corals, 0.4% sequence divergence represents considerable variation, as sequence divergence among families is often 5% (Shearer and Coffroth 2008).

Discussion

Phenotypic plasticity

Adaptive plasticity can lead to a decreased response to a stressor after secondary exposure. However, phenotypic plasticity is not always adaptive, and changes in phenotypes may not always incur a benefit to an organism. Additionally, phenotypic traits may change in response to the environment (phenotypic plasticity), or differences in phenotypes may be due to genetic differences among populations, early canalization of traits, or time lags in the plasticity of a trait. Based on the results from the linear extension study (*linear extension across bommies*), we expected to observe lasting effects of vermetids on coral traits. However, the results from our reciprocal transplant experiments (*transplant experiments 1 and 2*) show no evidence that prior exposure of corals to vermetids led to reduced effects of vermetids. Thus, we reject our working hypothesis that corals exhibited adaptive phenotypic plasticity with respect to vermetids.

Although we do not have evidence of *adaptive* plasticity, we do see evidence of phenotypic plasticity: cores from both reef types experienced reduced growth when placed on bommies with

vermetids relative to bommies without vermetids (Fig 5.2). Additionally, microbial diversity increased and community composition shifted on cores that were transplanted to bommies with vermetids (compared to those on bommies without vermetids: Fig. 5.5). These changes were likely maladaptive.

Transplantation to bommies with vermetids led to increased microbial richness, and shifts in microbial partners, in particular increases in groups that include anaerobic or facultative anaerobes (i.e., Fusobacteriaceae and Flavobacteriaceae, Staley and Whitman 2010). Because vermetid mucus nets reduce flow and modify the chemical conditions near coral surfaces (Brown and Osenberg 2018), it is possible that higher relative abundances of anaerobes are due to corals experiencing prolonged periods of low oxygen. Increases in these groups may reflect why corals that were transplanted to bommies with vermetids present decreased calcification, e.g., if exposure to prolonged periods of low oxygen leads to decreased coral growth (Fig.5.2). However, microbial community composition and coral calcification rate were primarily determined by highly reflected their origin bommie type.

Cryptic Phenotypes

Many of the differences in the traits we observed did not change when we transplanted cores to a new environment. Cores that had not been previously exposed to vermetids had higher rates of linear extension (*linear extension of bommies*), lower calcification from February – June (*transplant experiment 1*), but higher calcification from July – September (*transplant experiment 2*), thicker tissues, higher densities of *Symbiodinium*, and different microbial communities, relative to cores from bommies with previous exposure to vermetids. We suggest the differences in traits (linear extension, calcification rate, tissue thickness, *Symbiondinium* density and microbial communities) are indicative of a tolerant phenotype (bommies with vermetids) and a

resistant phenotype (bommies that lack vermetids), which are likely tied to the underlying genetic differences across these reefs.

Corals originally exposed and not exposed to vermetids had similar growth rates when placed on their home type reef. However, their growth rates diverged after transplant to a new environment, which revealed difference among these phenotypes: corals previously exposed to vermetids had higher calcification rates than corals previously naïve to vermetids. This is an example of counter-gradient selection (opposing effects of genetics and environment, Levins 1969, Conover and Schultz 1995). These growth rate differences in the first transplant experiment provide evidence for phenotypic differences between the tolerant and resistant phenotypes: the faster growing phenotype can withstand vermetid presence, and are able to maintain a faster growth rate than the vermetid-absent phenotype.

The vermetid-resistant phenotype consistently had thicker tissues than the vermetid-tolerant phenotype, which may modulate the response of these different phenotypes to disturbances. Tissue thickness is indicative of coral stress and resource allocation: thicker tissues indicate high energy investment into energy storage, whereas low tissue thickness is indicative of stressful conditions and/or low energy reserves (Barnes and Lough 1999, Fitt et al. 2001, Anthony et al. 2002, Jacobson et al. 2016, Putnam et al. 2017). Corals from bommies without vermetids (i.e., resistant phenotype) had thicker tissues and higher symbionts densities, which can yield more resources (i.e., energy derived from *Symbiodinium* or energy reserves, Barnes and Lough 1999, Loya et al. 2001) and allow for faster recovery from disturbances events that lead to open space on reefs. Vermetids cannot colonize live coral tissue (Phillips et al. 2014), thus free space on bommies is critical for vermetids to settle successfully. Indeed, the mechanism that may allow vermetids to colonize to reefs is slower recovery over bare areas (i.e., if reefs have thinner

tissues, and less dense *Symbiodinium,* like the vermetid present, tolerant reef type). Additionally, the high percent cover of coral on bommies without vermetids may be why we observed higher linear extension compared to bommies with vermetids or with a history of vermetids (*linear extension of bommies*). At the reef scale, linear extension was likely higher on the vermetid absent bommies because of more contiguous tissue compared to reefs that where live tissue was interrupted by other species that negatively affect coral growth (i.e., vermetid present reefs; Vega Thurber et al. 2012, Shima et al. 2013). Additionally, these two types of reefs may show a trade-off between density and linear extension: the tolerant phenotypes may have denser skeleton (i.e., spaces in the skeleton are filled with calcium carbonate), but lower linear extension, whereas the resistant phenotype may have less dense skeletons, and faster linear extension (Smith et al. 2007). However, corals from vermetid present reefs would need to be more than 4x as dense to account for the inverse relationship between calcification and linear extension, thus differences in density are likely combined with other factors (e.g., live coral cover and nearby polyps contributing to growth and calcification, Hamman 2017).

Some coral microbial traits also vary between these proposed tolerant and resistant phenotypes. Several families had higher relative abundances on one type of reef compared to the other, and did not change with transplantation to the other environment. For example, Endozoicimonaceae had a higher relative abundance on corals previously exposed to vermetids. This is a family of putative coral symbionts, which are hypothesized to act as a probiotic (Glasl et al. 2016). The likelihood of foreign microbial invasion is higher on vermetid present reefs. Because vermetid nets collect particles, including bacteria, from the water column, this likely increases contact between corals and potential pathogens. Thus, this probiotic symbiont family may be selected for in coral mucus in the presence of vermetids to reduce pathogen invasion.

Therefore, the tolerant phenotype (vermetid present reefs) may be more pathogen-resistant than the vermetid absent phenotype.

Some of the phenotypic differences (i.e., growth rate, tissue thickness, *Symbiodinium* density and microbial communities) associated with origin reef type are likely due to the underlying genetic differences among the corals as indicated by the presence of two distinct mitotypes that correlate with the presence versus absence of vermetids. Differences in mtCOI are typically low in corals (Shearer and Coffroth 2008, Wares 2014), suggesting that the observed differences in mtCOI between the two groups of corals may be evolutionarily significant. Yet, massive *Porites* species complex is notoriously difficult to differentiate visually, as they show considerable morphological plasticity, despite genetic diversity (Smith et al. 2007, Forsman et al. 2009, 2015, Boulay et al. 2013). Some massive *Porites* species are able to hybridize (Forsman et al. 2017), which further blurs genetic distinctions between putative species. Additionally, (Schweinsberg et al. 2016) found considerable intracolony genetic variation (although based on nuclear markers) in massive *Porites* corals in the same location where our study was conducted (Mo'orea, French Polynesia). Indeed, the one reef that had vermetids, but a shared mitotype with bommies that lacked vermetids, may indicate hybridization across these coral types (Forsman et al. 2017) or even show intracolony genetic variability (Schweinsberg et al. 2016). The mismatched reef had vermetids despite a mitotype indicative of reefs without vermetids. By comparing its response, we could possibly infer the relative role of environment vs. genetics in producing the observed patterns. This coral had calcification rates and *Symbiodinium* densities similar to the corals with the same mitotype; however, tissue thicknesses were more similar to corals with a history of contact with vermetids (Appendix 5A.Fig 1). This suggests a more complex interpretation, such as hybridization.

In the second transplant experiment, although corals were only exposed to vermetids for one week, we still observed an effect of transplant (albeit a reduced) on calcification (Fig. 5.7), thus vermetids can have lasting effects on coral growth. We also observed drastically lower calcification of corals from reefs previously exposed to vermetids (and no difference in calcification of corals not exposed to vermetids, transplanted back to their home-type reefs). Although the causes of the die-off are unknown, our working hypothesis is that the die-off was due to a pathogen. The pathogen may have spilled over and affected the vermetid present bommies, leading to an overall reduction in growth rates of corals previously exposed to vermetids. Thus, the presence of vermetids can expose bommies to different selective pressures (i.e., pathogens), which may influence the trajectory of bommies on a reef.

Whether or not these phenotypes are due to local adaptation to vermetids or are the result of another selective pressure (i.e., if the phenotypes we observe is an example of exaptation, Gould and Vrba 1982), it is likely these coral types respond similarly to other stressors currently facing coral reefs. Massive *Porites* spp are frequently considered environmentally tolerant corals (Lough and Barnes 1999, Putnam et al. 2012), but this designation may depend on whether the tolerant (fast-growing, disturbance-prone, vermetid present) phenotype or resistant (slow-growing, disturbance and vermetid absent) phenotype is present. Because of the thinner tissues and reduced resource provisioning (i.e., due to sparser densities of *Symbiodinium*), these colonies may be more prone to disturbance, which could lead to overall lower colony growth even if these colonies have the potential for higher calcification rates. Resistant coral colonies, alternatively, may be able to better prevent colonization of other benthic space occupiers (like vermetids), but maybe more vulnerable to potential pathogens. Therefore, if back reef environments are

dominated by the tolerant (potential for faster growth, but thin-tissue, disturbance-prone) phenotype, they may be more likely to shift towards lower coral cover.

Extended phenotypes of Porites *corals*

Several traits differed due to the underlying genetic structure of corals (i.e., calcification, *Symbiodinium* densities and tissue thickness), which we expect to lead to differences in colonization by vermetids to these reefs. We expect that corals with thinner tissues, and lower densities of *Symbiondium* is indicative of lower energetic reserved for tissue growth. Because of this lowered resource potential, these corals likely recover more slowly after a disturbance. The increased space availability provides the opportunity for vermetid colonization. In other systems, underlying genetic variation leads to differences across communities. This concept has been extensively studied in cottonwood trees (reviewed in Whitham et al. 2006). For example, underlying genetic variation in cottonwoods trees can lead to different arthropod communities (Bangert et al. 2006) and differences in aquatic insect emergence (Compson et al. 2016). Additionally, differences in resistance to gypsy moths by pinyon trees lead to differences in community trajectories (reviewed in Whitham et al. 2006). As gypsy moths are part of the extended phenotype of pinyon trees, I suggest vermetids are part of the extended phenotype of massive *Porites*. The presence of vermetids on reefs can lead to decreased herbivory (Tootell and Steele 2014), differences in competition with other benthic species (Jacobson 2018, Ch 4), and deceleration of succession on reefs (Brown, unpublished), and because they only settle to reefs with higher calcification rates, that likely feedback to affect coral colony morphology, leading to flatter and wider corals.

Cryptic variation in phenotypic traits is likely present across different reefs, as may be present in communities besides coral reefs. Here, genetic variation underlies the interactions

between corals and vermetids, likely by determining the rate at which corals recover from disturbances, which allows for the opportunity for vermetid colonization. After colonization, however, vermetids may affect interactions with the coral and other organisms in the reef community, thus can act as a part of the reef's extended phenotype. We highlight that the extended phenotype (including microbes and other interactors like vermetids) is critical to understanding variation in the trajectory of communities.

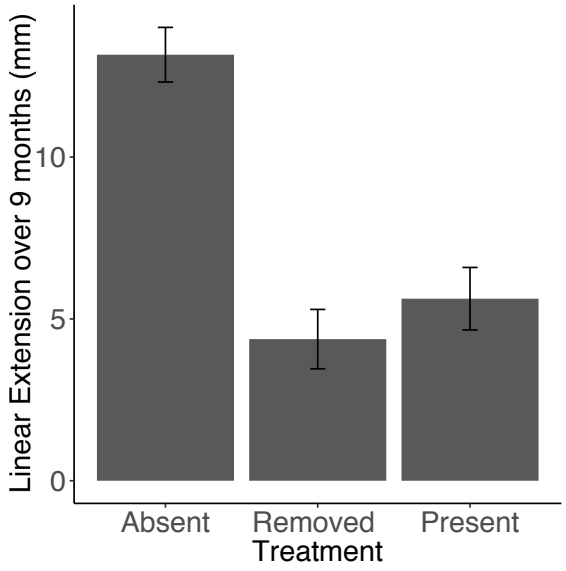


Figure 5.1: Linear extension (mm) of bommies with vermetids naturally absent, with vermetids experimentally removed and with vermetids present. We observed significantly greater linear extension of the bommies without vermetids compared to those that previously had vermetids, even if they were experimentally removed (treatment effect: $F_{2,12}=27.39$, $p < 0.001$). Data provided by Jeffrey S. Shima and Craig W. Osenberg.

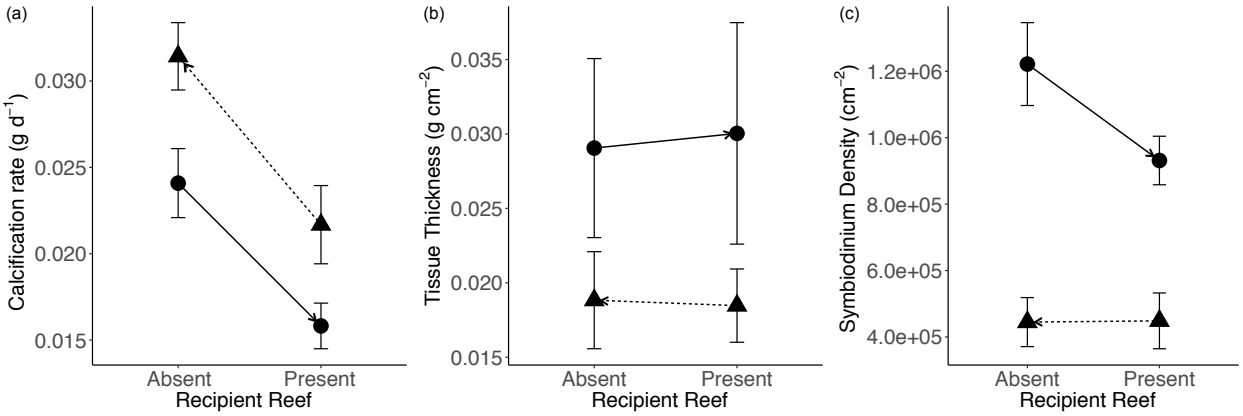


Figure 5.2: Responses of corals from vermetid absent (circles) and vermetid present (triangles) reefs after transplanting to either vermetid present or vermetid absent reefs in the first transplant experiment. Arrows point in the direction of the transplant. Skeletal growth (a) differed based on the origin and transplant reef (Origin: $p = 0.01$, Transplant: $p < 0.0001$). Tissue thickness (b) differed based on origin reef (Origin: $p = 0.049$, Transplant: $p = 0.953$, Origin x Transplant: 0.896). *Symbiodinium* densities showed a significant effect of origin reef ($p < 0.001$), and although not significant, there were trends suggesting an effect of transplant reef type ($p = 0.052$), and an interaction between transplant and origin reef type (Origin x Transplant: $p = 0.098$).

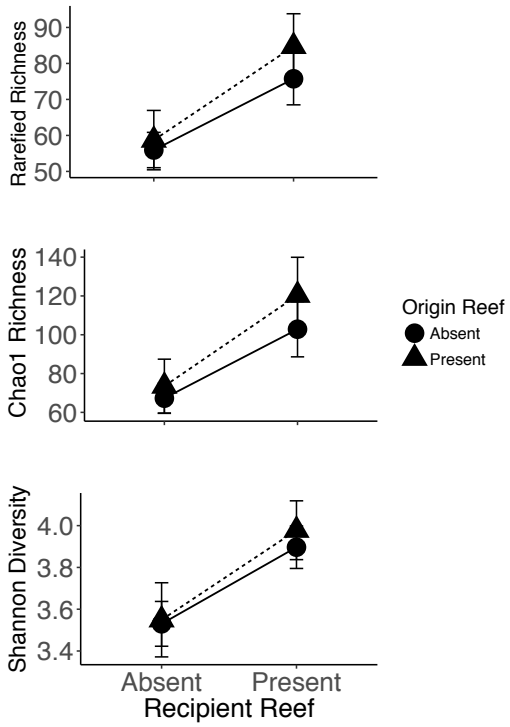


Figure 5.3: Mean± SE of diversity metrics (Rarefied richness, Chao1 richness and Shannon diversity) from the first transplant experiment. For each metric, we observed a significantly higher richness or Shannon diversity after transplanting corals to vermetid reefs for corals from both vermetid and absent reefs (Rarefied: $F_{1,37} = 9,72$, $p = 0.004$; Chao1: $F_{1,37} = 8.14$, $p = 0.007$; Shannon: $F_{1,37} = 9.45$, $p=0.004$).

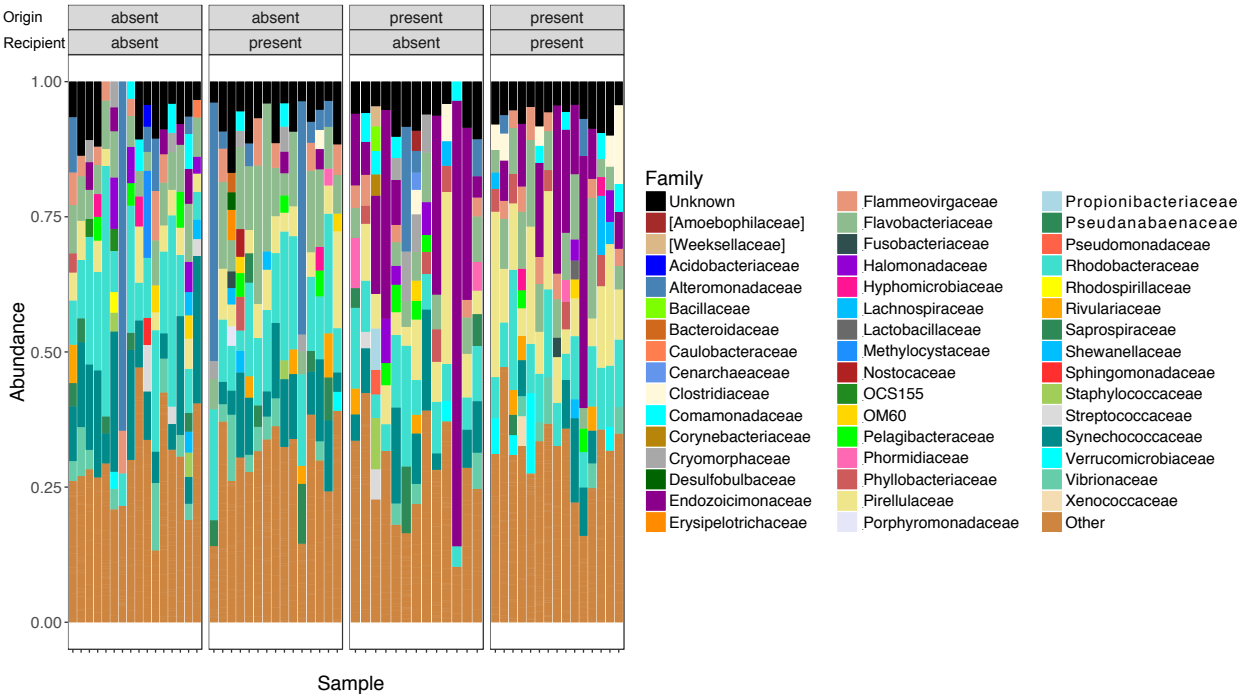


Figure 5.4. Relative abundance of OTUs by Family. Each bar represents a separate sample, and each facet refers to a different treatment (separated by vermetid presence and absence on origin and recipient reefs). Families that represent <3% , of the sample were combined into the "Other" category. The Unknown category represents groups that were not assigned taxonomy.

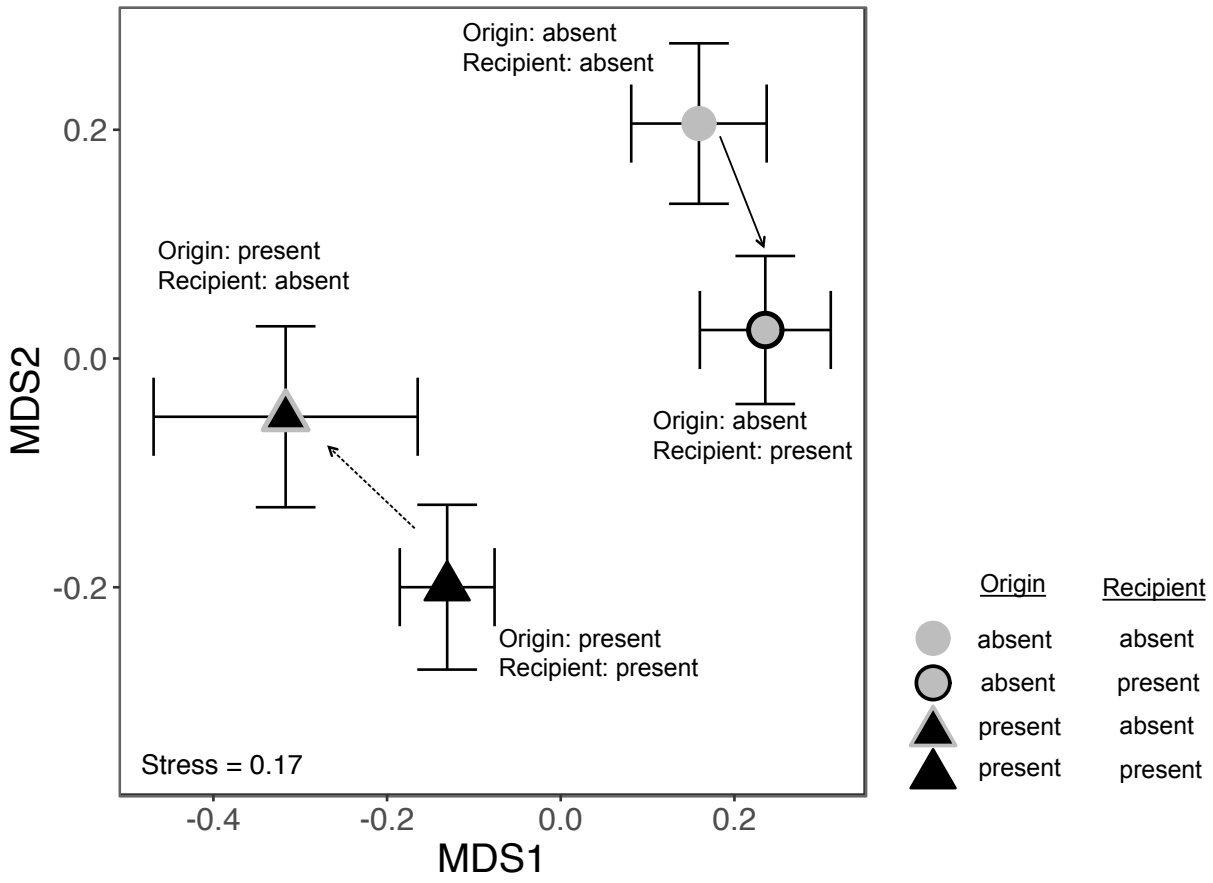


Figure 5.5: Non-metric multidimensional scaling plot, where each dot represents the Mean ± SE across the microbial communities sampled. Data are based on Bray-Curtis dissimilarity matrices. Stress values between 0.1 and 0.2 are deemed "acceptable" (Legendre and Legendre 1998), thus this indicates an acceptable fit. Origin reef types are indicated by circles (absent reefs) and triangles (present reefs). Outline color indicate transplant reef; black indicate transplants to a vermetid present reef, and gray outline indicate corals transplanted to vermetid absent reefs. Arrows indicate direction of transplant to reefs that are different from the origin type. We observed significant differences based on origin reef type ($p = 0.001$) and recipient reef type ($p = 0.001$).

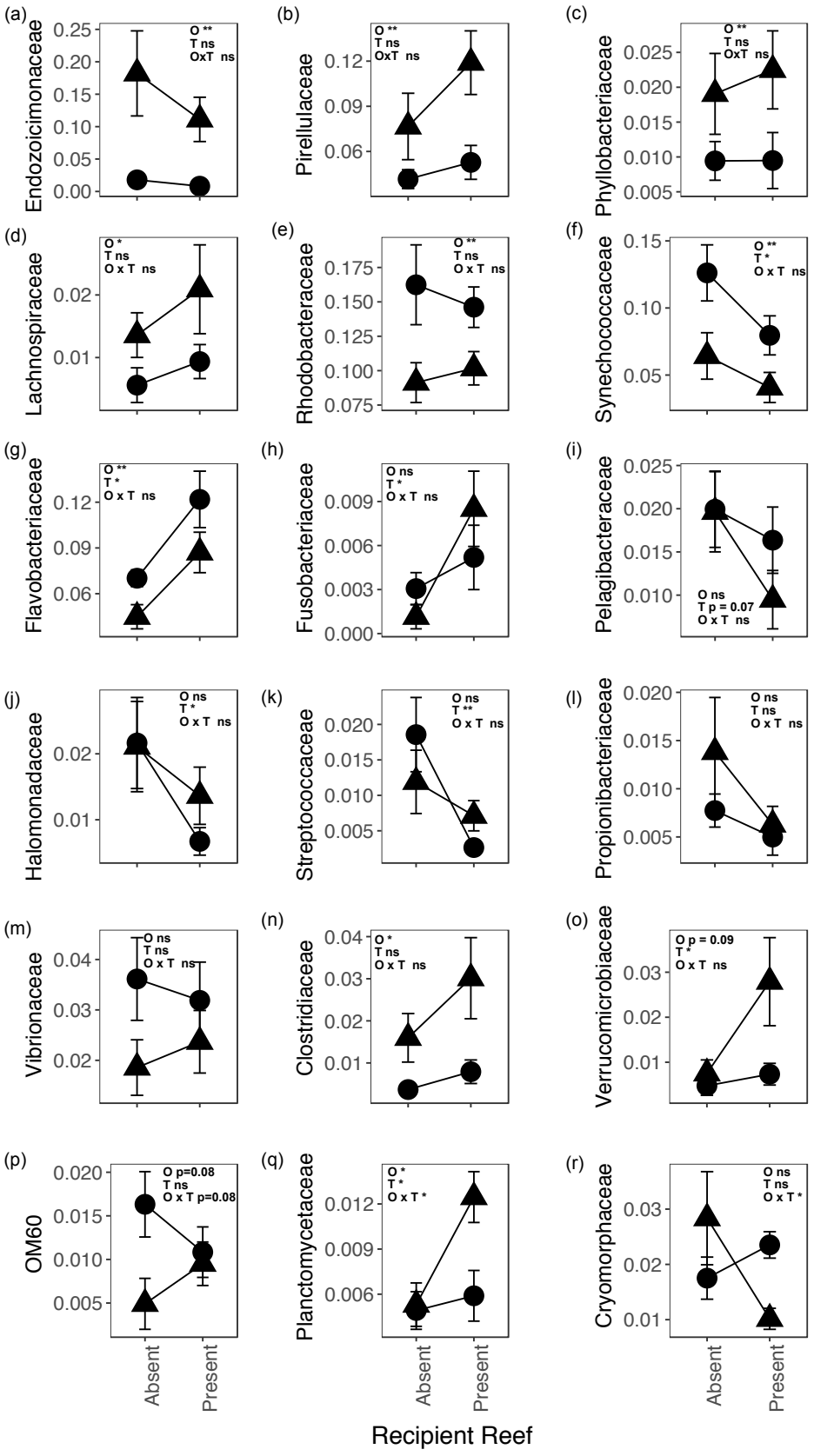

(a)
Endozoicimonaceae
O **
T ns
OxT ns
(b)
Pirellulaceae
O **
T ns
OxT ns
(c)
Phyllobacteriaceae
O **
T ns
OxT ns
(d)
Lachnospiraceae
O *
T ns
O x T ns
(e)
Rhodobacteraceae
O **
T ns
O x T ns
(f)
Synechococcaceae
O **
T *
O x T ns
(g)
Flavobacteriaceae
O **
T *
O x T ns
(h)
Fusobacteriaceae
O ns
T *
O x T ns
(i)
Pelagibacteraceae
O ns
T p = 0.07
O x T ns
(j)
Halomonadaceae
O ns
T *
O x T ns
(k)
Streptococcaceae
O ns
T **
O x T ns
(l)
Propionibacteriaceae
O ns
T ns
O x T ns
(m)
Vibrionaceae
O ns
T ns
O x T ns
(n)
Clostridiaceae
O *
T ns
O x T ns
(o)
Verrucomicrobiaceae
O p = 0.09
T *
O x T ns
(p)
OM60
O p=0.08
T ns
O x T p=0.08
(q)
Planctomycetaceae
O *
T *
O x T *
(r)
Cryomorphaceae
O ns
T ns
O x T *
Absent
Present
Recipient Reef

Figure 5.6a-r. Mean ± SE relative abundance of families that differ depending origin reef, transplant reef or a significant interaction between them. Families were chosen based on coefficients of the PERMANOVA (coefficient >0.005). Circles indicate samples came from vermetid absent reefs originally, and triangles indicate samples came from vermetid present reefs originally. Results of statistical tests are within each plot, O = Origin treatment, T = Transplant treatment, O x T = Origin x Transplant. Significant codes: ns: $p>0.1$; *: $p<0.05$; **: $p=<0.01$, if $0.05>p<0.1$, the p value was provided.

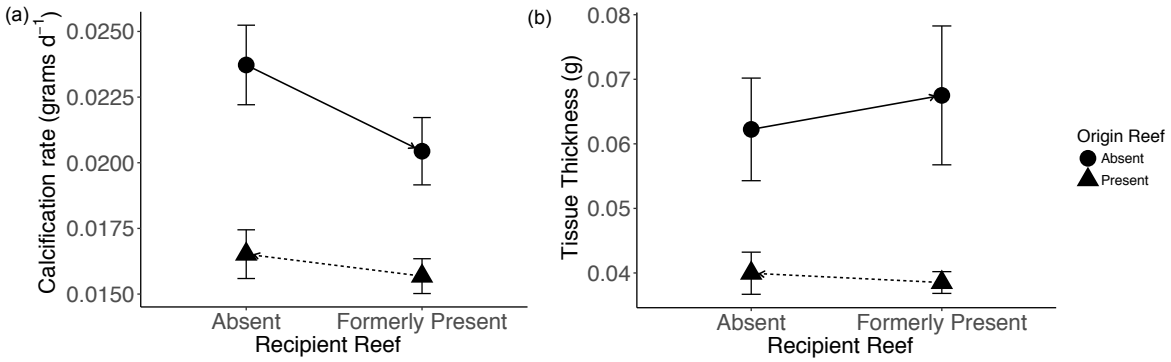


Figure 5.7. Results for the second reciprocal transplant study that spanned the vermetid die-off. Data are mean ± SE for (a) calcification rate, (b) tissue thickness. Circles represent samples from originally vermetid absent reefs, and triangles represent corals from originally vermetid present reefs. Calcification was significantly affected by recipient reef (transplant: $F_{1,70} = 4.8$, $p = 0.03$), and origin reef ($F_{1,6} = 5.67$, $p = 0.05$); there was no significant interaction (O x T: $F_{1,70} = 2.19$, $p = 0.14$). Tissue thickness was affected by origin reef ($F_{1,6} = 17.85$, $p = 0.006$), but there was no effect of recipient reef or an interaction between the two (T: $F_{1,70} = 0.03$, p 0.84, O x T: $F_{1,70} = 0.36$, $p = 0.56$). Refer to Figure 5.2 to compare with results from the first experiment.

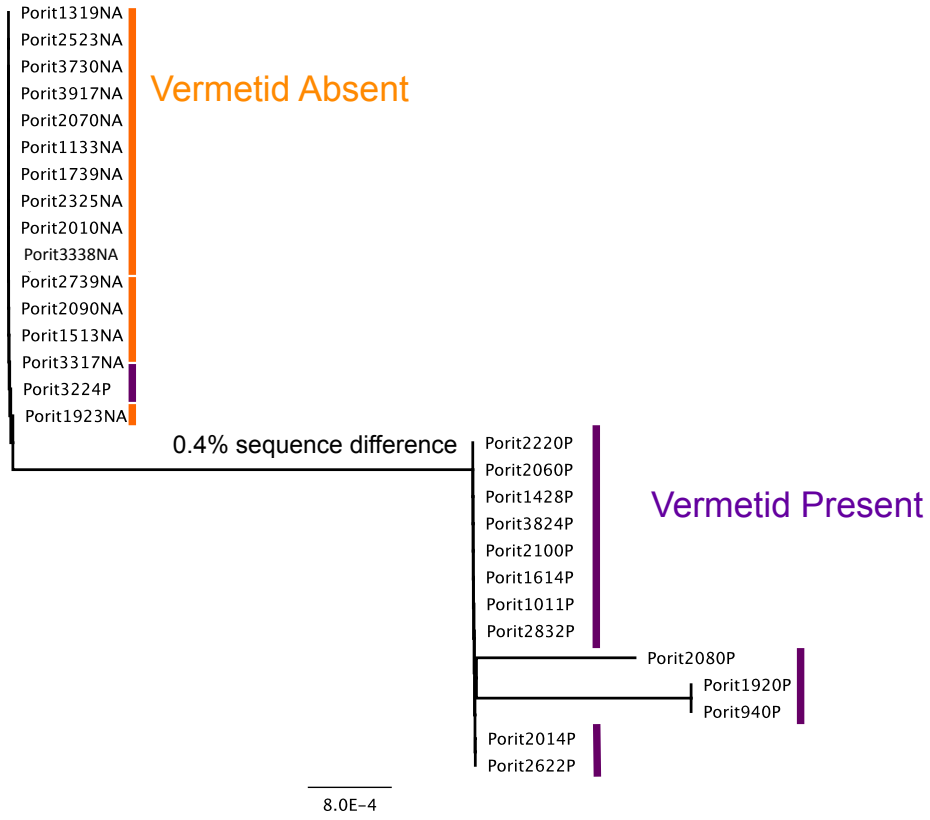
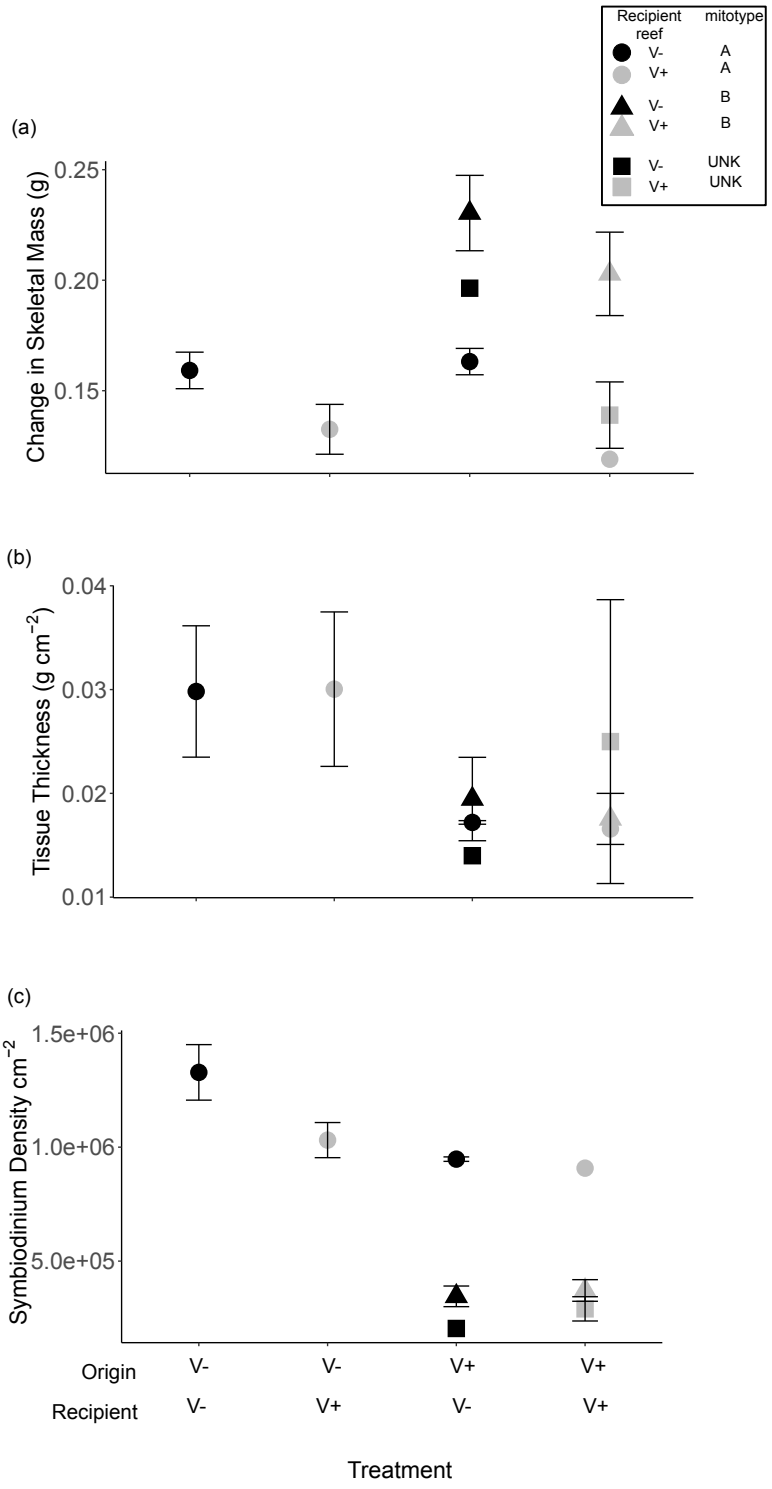


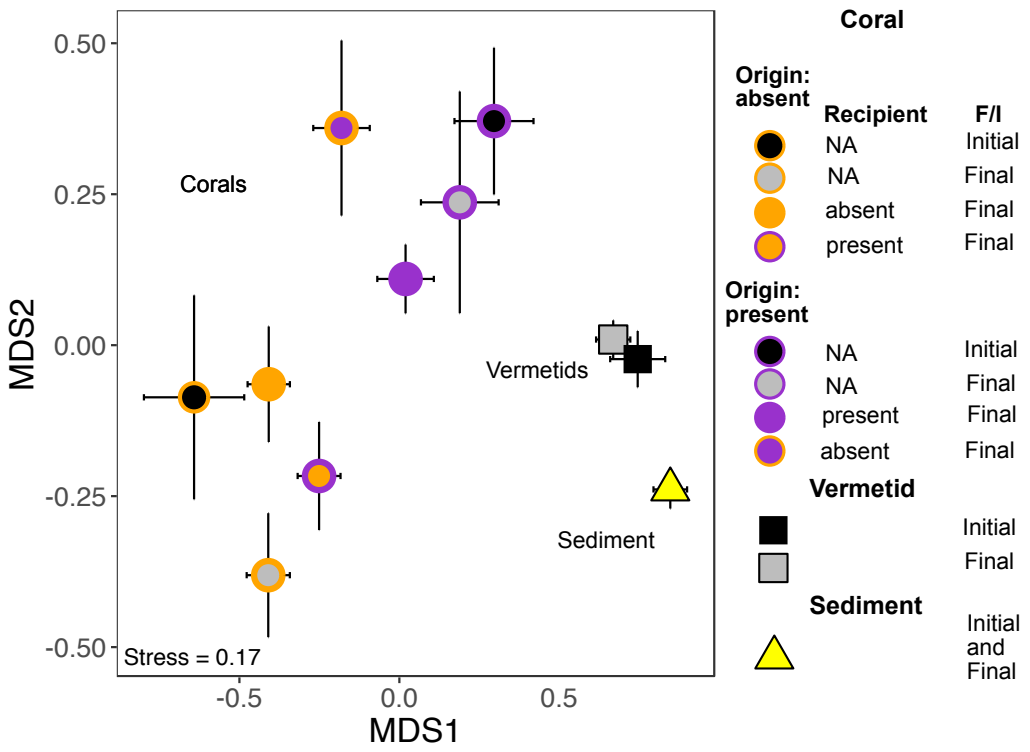
Figure 5.8. Tree based on variation in single nucleotide polymorphisms (SNPs) of MtCO1 gene (mitochondrial cytochrome c oxidase). Groups are significantly different from each other (Hudson's Snn = 0.877, $p < 0.001$). Colors indicate the presence and absence of vermetids: orange indicates bommies (reefs) that lacked vermetids; purple indicates bommies with vermetids.

5.Appendix



5A Figure 1. First transplant experiment: isolating by clade. Mean ± SE of (top panel): change in skeletal mass; (middle panel): tissue mass; (bottom panel): *Symbiodinium* density. Shapes

indicate the underlying host genetics, where circles indicate group A, triangles indicate group B and square is unknown. Colors indicate the transplant environment; black is transplant to a vermetid absent reef and gray is transplant to a vermetid present reef. For all reefs except one, the mitotype indicates the presence or absence of vermetids. The one odd reef (gray and black circle on the right side of the graphs) has the same genetic signature as the vermetid absent reefs (A), but had vermetids present. The unknown mitotype (square) was also from a vermetid present reef.



5.A Figure 2. Non-multidimensional scaling plot, where each dot represents the mean ± SE across the microbial communities sampled from the first transplant experiment. Shapes indicate sample type (corals are circles, triangles are sediment samples, and squares are mucus nets). Colors indicate treatment. Initial indicates microbial samples taken at the beginning of the experiment; final indicates samples taken at the end of the experiment. In the legend "NA" under the recipient reef category indicates microbial communities from the bommies where samples came from and were placed. Data are based on Bray-Curtis dissimilarity matrices in two dimensions. We observed significant differences based on sample type according to PERMANOVA results ($p = 0.0001$).

SUMMARY

Interaction modifications can include induced changes in physio-chemical conditions and phenotypes of individuals. Furthermore, microbes play an important role, both in creating physio-chemical conditions and in determining responses to those conditions, particularly when they are associated with a host. Ecologists have begun to appreciate the wide-reaching associations between organisms and their microbial associations, such as humans and our gut microbiota (Li et al. 2008), or plants and roots microbiota (van Der Heijden 2003). For host-associated microbes, microbial communities can be considered part of an organism's extended phenotype (Dawkins 1976). Thus, in my dissertation, I included microbial assemblages in the concept of phenotypes (microbial communities as traits) that can change in response to environmental stimuli. I focused on the increasingly best-studied holobiont (hosts + microbes): corals.

For my first three data chapters (Chapter 2-4), I focused on interaction modification of coral-algal interactions by vermetids. Macroalgae (hereafter, called algae) decrease coral growth and survival (Jompa and McCook 2003a, 2003b, Quan-Young and Espinoza-Avalos 2006). A hypothesized mechanism underlying the deleterious effects of algae on corals involves the release of dissolved organic carbon (DOC) from the algae (Smith et al. 2006, Nelson et al. 2013). DOC stimulates microbial growth and can lead to shifts in microbial communities and/or hypoxia near coral surfaces (Smith et al. 2006, Haas et al. 2011, 2013, Nelson et al. 2013). These effects can be deleterious to the coral, resulting in decreased growth and survival, and

positively affecting algal cover (Smith et al. 2006). However, the effects of algae on corals are context dependent, and can change depending on the physical environment (*e.g.*, water flow) and the presence of other species (Wangpraseurt et al. 2012, Brown and Carpenter 2013). One such species might be the vermetid gastropod, *Ceraesignum maximum*. *C. maximum* produce a mucus net used for feeding (Kappner et al. 2000). When a mucus net covers a coral that is in close proximity to algae, the net may exacerbate the effects of algae on corals by 1) decreasing water flow; 2) synergistically decreasing coral growth; and/or 3) leading to changes in the coral's microbial communities, favoring species with increased virulence and/or enhancing stress by changing the concentration of dissolved materials (e.g., oxygen or waste products). I tested these hypotheses in my dissertation.

In Chapter 2, I examined how vermetids changed the physio-chemical conditions above corals and corals interacting with algae. Using a series of field surveys and lab flume studies, I found that the mucus net changes the physical and chemical conditions around corals by reducing water flow and lowering oxygen concentrations (Chapter 2). I concluded a likely mechanism underlying the effects of vermetids involves the creation of low flow conditions around corals, especially in the presence of algae because low flow can facilitate the retention of harmful solutes. Additionally, my results suggest that mucus net cover alters microbial metabolism, through changes in microbial respiration or photosynthesis, which led to lower than expected oxygen concentrations in the light. Thus, vermetids create conditions that can modify coral-algal interactions through their effects on microbes.

In Chapter 3, I surveyed microbial communities on corals that were in contact with algae, vermetids or both algae and vermetids to determine if vermetids and algae have synergistic effects on coral microbial communities. Algal presence led to increased alpha diversity, driven

by increases in abundances of pathogenic groups and increased microbial families that can withstand low oxygen conditions. These results suggest algae can reduce the spatial variance across coral microbial communities, likely because algal presence leads to stressful conditions that specific families of microbes tolerate. These results provide evidence that algae may create conditions that promote the hypothesized mechanism of microbially-mediated competition. In contrast, vermetids had no effects on the identity of microbes in the absence of algae, and there was no evidence that vermetids exacerbated the shifts in microbes caused by algae. Thus, although vermetids create conditions that allow for microbially-mediated mechanisms, vermetids did not appreciably change the identity of microbial communities, although they may change microbial metabolic expression (e.g., increased respiration, Chapter 2).

In Chapter 4, I conducted a field experiment where I manipulated the presence and absence of vermetids and algae to determine the trait-mediated effects of vermetids on coral-algal interactions. I focused on three coral traits: calcification, lateral extension, and microbial communities. I conducted two experiments and found different, highly variable results. Increasing vermetid net cover decreased coral calcification, but net cover only weakly reduced lateral extension in one year (this effect was not significant). Algae did not affect calcification, but reduced lateral extension, only in the presence of vermetid mucus nets. These effects however were not mediated by changes to coral microbiomes, as I observed no clear pattern in microbial communities. However, communities generally showed higher alpha diversity in this field experiment compared to the field survey reported in Chapter 3, indicating the corals in the field experiment likely experience higher stress than the field samples, or all experimental corals experienced a lingering algal effect. I concluded that there was not strong evidence for trait-mediated indirect effects on coral-algal interactions, nor was there strong evidence for

deleterious direct effects that had previously been demonstrated for both vermetids and algae on coral growth, lateral extension and microbial communities.

Because of the surprising lack of vermetid effects of different aspects of the coral phenotype, where other studies have found strong vermetid effects, I hypothesized that corals with a history of contact with vermetids allowed for some degree of adaptive phenotypic plasticity to vermetids (Chapter 5). I therefore predicted that the response of coral to vermetids would depend upon their prior history of contact with vermetids: corals that had a history of interacting with vermetids would be less sensitive to vermetids than corals that had not previously interacted with vermetids. I did not find compelling evidence of adaptive phenotypic plasticity to vermetids: corals always showed reduced growth in the presence of vermetids, independent of their historical exposure to vermetids. There was a weak trend suggesting that *Symbiodinium* densities decreased only when corals from reefs without vermetids were subsequently exposed to vermetids. In contrast, there were strong effects of the historical state of bommies on the responses of corals: i.e., coral growth rate, tissue thickness and *Symbiodinium* density, under a common environment, differed depending on whether the coral came from a reef with vermetids (faster growth, thinner tissue, denser *Symbiodinium*) or a reef without vermetids (slower growth, thicker tissue, sparser *Symbiodinium*). Vermetids also increased bacterial richness, however the composition of the community differed depending on both historical reef type and transplant reef type. Interestingly, I also found genetic differences between coral on reefs with vs. without vermetids, indicating there is likely an interplay between coral genetics, the coral phenotype and the coral microbiome that lead to variation in recovery of reefs from disturbances and the likelihood of being colonized by vermetids. I suggest that the two coral mitotypes represent suites of traits that likely lead to differences in their vulnerability to vermetid colonization.

Corals with thicker tissues and higher *Symbiodinium* densities grow slowly but are able to recover quickly from tissue removal or other disturbance events. As a result, vermetids, which cannot settle to live coral, have little chance to colonize these reefs because they have little open space. In contrast, other corals have higher inherent growth rates, but thinner tissue (and lower *Symbiodinium* densities); thus, they are more likely to be disturbed and are probably slower to recover from disturbances. As a result, they have more open space and are more easily colonized by vermetids. Thus, the spatial pattern of vermetid-coral occupancy is likely driven more by coral genetics than it is by differential responses of these corals to vermetids.

The results collectively demonstrate variation in the effects of vermetids and algae on corals, within and among the traits I measured. Algae tend to have stronger localized effects on corals: they mostly influence the growing edge of the coral (i.e., microbial communities and lateral extension), but vermetids have stronger effects on coral calcification, indicating these two groups act on different coral traits, and these aspects of coral phenotype are de-coupled on the time scale of short-term experiments (Chapter 3, Chapter 4). Additionally, the high variability for each trait I measured, especially in Chapter 4, may be due to cryptic genotypic variation among massive *Porites* corals as observed in Chapter 5.

For coral reef ecology, my dissertation suggests reefs may be more resilient to biological stressors than currently considered (Harborne et al. 2017). Because these two common biological stressors can have variable (i.e., no or additive) effects, likely due to the presence of cryptic coral phenotypes, it is possible that corals on disturbed reefs have phenotypes that can persist in the face of benthic community stressors. Additionally, as the "disturbance vulnerable" phenotype contained higher relative abundances of the symbiotic microbial family, Endozoicimonaceae,

these corals may be more pathogen resistant, which could promote resilience of corals even after disturbances.

My dissertation bridges ecology, microbiology and evolutionary ecology, through studies of the potential trait-mediated effects of vermetids on corals and on coral-algal interactions, in which these traits include the coral's microbiome as well as physio-chemical environment surrounding the interacting organisms. The incorporation of trait-mediated effects to include the extended phenotype, especially the microbiome, is an important extension of the growing literature on indirect effects. Furthermore, an active area of study is the relationship between microbial changes and whole-organism measurements, which my dissertation begins to explore. I suggest that many species likely interact by changing the microbial assemblages with which the other species interacts.

REFERENCES

Adam, T. C., R.J. Schmitt, S.J. Holbrook, A.J. Brooks, P.J. Edmunds, R.C. Carpenter, G. Bernardi. 2011. Herbivory, connectivity, and ecosystem resilience: response of a coral reef to a large-scale perturbation. *PloS one* 6(8): e23717.

Allen-Jacobson, L.M. 2018. Life in a colony: growth, morphology, and metabolic scaling. Dissertation. University of Florida

Anderson, M.J., D.C. Walsh DC. 2013. PERMANOVA, ANOSIM, and the Mantel test in the face of heterogeneous dispersions: what null hypothesis are you testing? Ecological Monographs (4):557-74.

Anthony, K. R. N., S. R. Connolly, and B. L. Willis. 2002. Comparative analysis of energy allocation to tissue and skeletal growth in corals. Limnology and Oceanography 47:1417–1429.

Anthony, K.R., D. Kline, G. Diaz-Pulido, S. Dove, O. Hoegh-Guldberg. 2008. Ocean acidification causes bleaching and productivity loss in coral reef builders. Proc Natl Acad Sci USA 105:17442–17446

Apprill, A., H. Q. Marlow, M. Q. Martindale, and M. S. Rappe. 2012. Specificity of Associations between Bacteria and the Coral Pocillopora meandrina during Early Development. Applied and Environmental Microbiology 78:7467–7475.

Armstrong E., L. Yan, K.G. Boyd, P.C. Wright, J.G. Burgess. 2001. The symbiotic role of marine microbes on living surfaces. Hydrobiologia 461:37–40

Augustin, R., S. Fraune, T.C.G. Bosch. 2009. How Hydra senses and destroys microbes. Seminars in Immunology:1–5

Baker, A. C., C. J. Starger, T. R. McClanahan, and P. W. Glynn. 2004. Coral reefs: Corals' adaptive response to climate change. Nature 430: 741–741.

Bangert, R.K., Allan, G.J., Turek, R.J., Wimp, G.M., Meneses, N., Martinsen, G.D., Keim, P. and Whitham, T.G., 2006. From genes to geography: a genetic similarity rule for arthropod community structure at multiple geographic scales. *Molecular Ecology*, *15*(13): 4215-4228.

Bates, D., M. Maechler, B. Bolker, S. Walker. 2015. Fitting Linear Mixed-Effects Models Using lme4. Journal of Statistical Software, 67(1): 1-48. doi:10.18637/jss.v067.i01.

Barnes, D. J., and J. M. Lough. 1992. Systematic variations in the depth of skeleton occupied by coral tissue in massive colonies of Porites from the Great barrier reef. Journal of Experimental Marine Biology and Ecology 159:113–128.

Barnes, D.J. and Lough, J.M., 1999. Porites growth characteristics in a changed environment: Misima Island, Papua New Guinea. Coral Reefs, 18(3): 213-218.

Barott ,K., J. Smith, E. Dinsdale, M. Hatay, S. Sandin. 2009. Hyperspectral and Physiological Analyses of Coral-Algal Interactions. PLoS ONE 4:e8043

Barott, K.L., F. Rohwer. 2012. Unseen players shape benthic competition on coral reefs. Trends in Microbiology:1–8

Barott. K.L., B. Rodriguez-Mueller, M. Youle, K.L. Marhaver, M.J.A. Vermeij, J.E. Smith, F.L. Rohwer (2012a) Microbial to reef scale interactions between the reef-building coral

Montastraea annularis and benthic algae. Proceedings of the Royal Society B: Biological Sciences 279:1655–1664
Barott, K. L., B. Rodriguez-Mueller, M. Youle, K. L. Marhaver, M. J. A. Vermeij, J. E. Smith, and F. L. Rohwer. 2012. Bacterial Associates of Two Caribbean Coral Species Reveal Species-Specific Distribution and Geographic Variability. Proceedings of the Royal Society B: Biological Sciences 279:1655–1664.
Barott, K. L., B. Rodriguez-Mueller, M. Youle, K. L. Marhaver, M. J. A. Vermeij, J. E. Smith, and F. L. Rohwer. 2012. Microbial to reef scale interactions between the reef-building coral Montastraea annularis and benthic algae. Proceedings of the Royal Society B: Biological Sciences 279:1655–1664.
Bell, W. 1983. Bacterial utilization of algal extracellular products. 3. The specificity of algal-bacterial interaction. Limnology and Oceanography 28:1131–1143
Bellwood, D.R., T. Hughes, C. Folke, M. Nystrom. 2004. Confronting the coral reef crisis. *Nature* 429:827–833.
Bertness, M.D., G.H. Leonard, J.M. Levine, P.R. Schmidt, A.O. Ingraham. 1999. Testing the relative contribution of positive and negative interactions in rocky intertidal communities. Ecology, 80(8): 2711-2726.
Bessat, F., and D. Buigues. 2001. Two centuries of variation in coral growth in a massive Porites colony from Moorea (French Polynesia): a response of ocean-atmosphere variability from south central Pacific. Palaeogeography, Palaeoclimatology, Palaeoecology 175:381–392.
Boone, M. D., R. D. Semlitsch, E. E. Little, and M. C. Doyle. 2007. Multiple stressors in amphibian communities: effects of chemical contamination, bullfrogs, and fish. Ecological Applications 17:291–301.
Bordenstein, S.R., K.R. Theis. 2015. Host Biology in Light of the Microbiome: Ten Principles of Holobionts and Hologenomes (MK Waldor, Ed.). PLoS Biology 13:e1002226–23
Boström, K. H., K. Simu, A. Hagstrom, and L. Riemann. 2004. Optimization of DNA extraction for quantitative marine bacterioplankton community analysis. Limnology and Oceanography: Methods 2:365–373.
Boulay, J. N., M. E. Hellberg, J. Cortes, and I. B. Baums. 2013. Unrecognized coral species diversity masks differences in functional ecology. Proceedings of the Royal Society of London. Series B. Biological Sciences 281:20131580–20131580.
Bourne, D. G., and N. S. Webster. 2013. Coral Reef Bacterial Communities. Pages 163–187 *in* The prokaryotes: prokaryotic communities and ecophysiology. Springer Berlin Heidelberg, Berlin, Heidelberg.
Box, S., and P. Mumby. 2007. Effect of macroalgal competition on growth and survival of juvenile Caribbean corals. Marine Ecology Progress Series 342:139–149.
Brönmark, C. and Pettersson, L.B., 1994. Chemical cues from piscivores induce a change in morphology in crucian carp. *Oikos* 70(3): 396-402.
Brown, A. L., and R. C. Carpenter. 2013. Water-flow mediated oxygen dynamics within massive Porites-algal turf interactions. Marine Ecology Progress Series 490:1–10.
Brown, A. L., and R. C. Carpenter. 2015. Water flow influences the mechanisms and outcomes of interactions between massive Porites and coral reef algae. Marine Biology 162:459–468.
Brown, A.L., Frazer, T.K., Shima, J.S. and Osenberg, C.W., 2016. Mass mortality of the vermetid gastropod Ceraesignum maximum. *Coral Reefs 35*(3): 1027-1032.
Brown, A.L. and Osenberg, C.W., 2018. Vermetid gastropods modify physical and chemical conditions above coral–algal interactions. *Oecologia* 186(4): 1091-1099.

Brown, B.E. 1997. Coral bleaching: causes and consequences. Coral Reefs 16:129–138
Bruno, J. F., and P. J. Edmunds. 1997. Clonal variation for phenotypic plasticity in the coral madracis mirabilis. Ecology 78:2177–2190.
Buck, J. C., E. A. Scheessele, R. A. Relyea, and A. R. Blaustein. 2011. The effects of multiple stressors on wetland communities: pesticides, pathogens and competing amphibians. Freshwater Biology 57:61–73.
Buddemeier, R. W., D. F. Bioscience, 1993. 1993. Coral bleaching as an adaptive mechanism. Bioscience 43:320–326.
Callahan, B.J., P.J. McMurdie, M.J. Rosen, A.W. Han, A.J.A. Johnson and S.P. Holmes. 2016. "DADA2: High-resolution sample inference from Illumina amplicon data." *Nature Methods* 13: 581-583. doi: 10.1038/nmeth.3869.
Callaway, R. M. 1997. Positive interactions in plant communities and the individualistic-continuum concept. Oecologia 112(2): 143-149
Caporaso, J. G., C. L. Lauber, W. A. Walters, D. Berg-Lyons, C. A. Lozupone, P. J. Turnbaugh, N. Fierer, and R. Knight. 2011. Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. Proceedings of the National Academy of Sciences of the United States of America 108:4516–4522.
Caporaso, J. G., J. Kuczynski, J. Stombaugh, K. Bittinger, F. D. Bushman, E. K. Costello, N. Fierer, A. G. Peña, J. K. Goodrich, J. I. Gordon, G. A. Huttley, S. T. Kelley, D. Knights, J. E. Koenig, R. E. Ley, C. A. Lozupone, D. McDonald, B. D. Muegge, M. Pirrung, J. Reeder, J. R. Sevinsky, P. J. Turnbaugh, W. A. Walters, J. Widmann, T. Yatsunenko, J. Zaneveld, and R. Knight. 2010. QIIME allows analysis of high-throughput community sequencing data. Nature Methods 7:335–336.
Carpenter, R.C. 1985. Relationships between primary production and ii radiance in coral reef algal communities. Limnology and Oceanography 30(4): 784-793
Carpenter, R., and S. Williams. 1993. Effects of algal turf canopy height and microscale substratum topography on profiles of flow speed in a coral forereef environment. Limnology and Oceanography 38:687–694.
Carpenter, R., S.L. Williams. 2007. Mass transfer limitation of photosynthesis of coral reef algal turfs. Marine Biology, 151(2): 435-450.
Carrier, T.J., A.M. Reitzel.2018. Convergent shifts in host-associated microbial communities across environmentally elicited phenotypes. Nature Communications:1–9
Chalker, B.E. 1981. Simulating light saturation curves for photosynthesis and calcification by reef-building corals. Marine Biology 63: 135-141
Compson, Z.G., Hungate, B.A., Whitham, T.G., Meneses, N., Busby, P.E., Wojtowicz, T., Ford, A.C., Adams, K.J. and Marks, J.C., 2016. Plant genotype influences aquatic‐terrestrial ecosystem linkages through timing and composition of insect emergence. *Ecosphere*, *7*(5) e01331
Concepcion, G. T., Medina, M., & Toonen, R. J. 2006. Noncoding mitochondrial loci for corals. Molecular Ecology Resources *6*(4): 1208-1211.
Crain, C. M., K. Kroeker, and B. S. Halpern. 2008. Interactive and cumulative effects of multiple human stressors in marine systems. Ecology Letters 11:1304–1315.
Darling, E. S., and I. M. Côté. 2008. Quantifying the evidence for ecological synergies. Ecology Letters 11:1278–1286.
Davy, S. K., D. Allemand, and V. M. Weis. 2012. Cell Biology of Cnidarian-Dinoflagellate Symbiosis. Microbiology and Molecular Biology Reviews 76:229–261.

Dawkins, R., 1976. *The extended phenotype: The long reach of the gene*. Oxford University Press.
Dennison, W.C., D.J. Barnes. 1988. Effect of water motion on coral photosynthesis and calcification. Journal of Experimental Marine Biology and Ecology, 115(1): 67-77.
Denny, M.W. 1988. Biology and the Mechanics of the Wave-Swept Environment. Princeton University Press. 329 pp.
DeSantis, T.Z., Hugenholtz, P., Larsen, N., Rojas, M., Brodie, E.L., Keller, K., Huber, T., Dalevi, D., Hu, P. and Andersen, G.L., 2006. Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. *Applied and environmental microbiology 72*(7): 5069-5072.
Dodson, S. I. 1974. Adaptive change in plankton morphology in response to size-selective predation: A new hypothesis of cyclomorphosis. Limnology and Oceanography 19:721–729.
Edgar, R. C. 2010. Search and clustering orders of magnitude faster than BLAST. Bioinformatics 26:2460–2461.
Estes, J. A., and J. F. Palmisano. 1974. Sea otters: their role in structuring nearshore communities. Science 185:1058–1060.
Ezenwa, V.O., N.M. Gerardo, D.W. Inouye, M. Medina, J.B. Xavier.2012. Animal Behavior and the Microbiome. Science 338:198–199
Finelli, C., B. Helmuth, N. Pentcheff, D.Wethey. 2007. Intracolony variability in photosynthesis by corals is affected by water flow: role of oxygen flux. Marine Ecology Progress Series 349:103–110
Fitt, W., B. Brown, M. Warner, and R. Dunne. 2001. Coral bleaching: interpretation of thermal tolerance limits and thermal thresholds in tropical corals. Coral Reefs 20:51–65.
Forsman, Z. H., D. J. Barshis, C. L. Hunter, and R. J. Toonen. 2009. Shape-shifting corals: Molecular markers show morphology is evolutionarily plastic in Porites. BMC Evolutionary Biology 9:45–9.
Forsman, Z. H., I. S. S. Knapp, K. Tisthammer, D. A. R. Eaton, M. Belcaid, and R. J. Toonen. 2017. Coral hybridization or phenotypic variation? Genomic data reveal gene flow between Porites lobata and P. Compressa. Molecular Phylogenetics and Evolution 111:132–148.
Forsman, Z., G. M. Wellington, G. E. Fox, and R. J. Toonen. 2015. Clues to unraveling the coral species problem: distinguishing species from geographic variation in Poritesacross the Pacific with molecular markers and microskeletal traits. PeerJ 3:e751–21.
Garren, M., F. Azam .2011. Corals shed bacteria as a potential mechanism of resilience to organic matter enrichment. ISME J 6:1159–1165
Ghalambor, C., J. McKay, S. Carroll, and D. Reznick. 2007. Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. Ecology 21:394–407.
Gilbert, S. F., J. Sapp, and A. I. Tauber. 2012. A Symbiotic View of Life: We Have Never Been Individuals. The Quarterly Review of Biology 87:325–341.
Glasl, B., G. J. Herndl, and P. R. Frade. 2016. The microbiome of coral surface mucus has a key role in mediating holobiont health and survival upon disturbance 10:2280–2292.
Goh, C-H., D.F. Veliz Vallejos, A.B. Nicotra, U. Mathesius. 2013. The Impact of Beneficial Plant-Associated Microbes on Plant Phenotypic Plasticity. Journal of Chemical Ecology 39:826–839
Gould, S. J., and E. S. Vrba. 1982. Exaptation—a Missing Term in the Science of Form. Paleobiology 8:4–15.

Gowan, J.C., J.S. Tootell, R.C. Carpenter. 2014. The effects of water flow and sedimentation on interactions between massive *Porites* and algal turf. Coral Reefs 33(3): 651-663
Graves S., H.S. Piepho, L. Selzer , S. Dorai-Raj. 2015. multcompView: Visualizations of Paired Comparisons. R package version 0.1-7. https://CRAN.R-project.org/package=multcompView
Haas, A. F., A. K. Gregg, J. E. Smith, M. L. Abieri, M. Hatay, and F. Rohwer. 2013a. Visualization of oxygen distribution patterns caused by coral and algae. PeerJ 1:e106.
Haas, A. F., C. E. Nelson, F. Rohwer, L. Wegley Kelly, S. D. Quistad, C. A. Carlson, J. J. Leichter, M. Hatay, and J. E. Smith. 2013b. Influence of coral and algal exudates on microbially mediated reef metabolism. PeerJ 1:e108.
Haas, A. F., C. E. Nelson, L. Wegley Kelly, C. A. Carlson, F. Rohwer, J. J. Leichter, A. Wyatt, and J. E. Smith. 2011. Effects of Coral Reef Benthic Primary Producers on Dissolved Organic Carbon and Microbial Activity. PLoS ONE 6:e27973.
Hadfield, M.G., E.A. Kay, M.U. Gillette, M.C. Lloyd. 1972. The Vermetidae (Mollusca: Gastropoda) of the Hawaiian Islands. Marine Biology 12:81–98
Hamman, E.A. 2017. Multi-scale spatial patterning of corals and their symbionts. Dissertation, University of Georgia
Harborne, A. R., A. Rogers, Y.-M. Bozec, and P. J. Mumby. 2017. Multiple Stressors and the Functioning of Coral Reefs. Annual Review of Marine Science 9:445–468.
Hartnett, D.C., B. Hetrick, G. Wilson, D.J. Gibson. 1993. Mycorrhizal influence on intra-and interspecific neighbour interactions among co-occurring prairie grasses. J Ecology:787–795
Hauri C., K.E. Fabricius, B. Schaffelke, C. Humphrey. 2010. Chemical and physical environmental conditions underneath mat- and canopy-forming macroalgae, and their effects on understory corals. PLOS ONE 5:e12685
Howells, E.J., V.H. Beltran, N.W. Larsen, L.K. Bay, B.L. Willis, M.J.H. van Oppen. 2011. Coral thermal tolerance shaped by local adaptation of photosymbionts. Nature Climate Change 2:116–120
Hughes, T., M. Rodrigues, D. Bellwood, D. Ceccarelli, O. Hoegh-Guldberg, L. McCook, N. Moltschaniwskyj, M. Pratchett, R. Steneck, B.Willis. 2007. Phase shifts, herbivory, and the resilience of coral reefs to climate change. Current Biology 17:360–365
Hurst, G.D.D. 2017. Extended genomes: symbiosis and evolution. Interface Focus 7:20170001–11
Jacobson, L. M., P. J. Edmunds, E. B. Muller, and R. M. Nisbet. 2016. The implications of reduced metabolic rate in resource-limited corals. Journal of Experimental Biology 219:870–877.
Jompa, J., and L. McCook. 2002a. Effects of competition and herbivory on interactions between a hard coral and a brown alga. Journal of Experimental Marine Biology and Ecology 271:25–39.
Jompa, J., and L. McCook. 2003b. Contrasting effects of turf algae on corals: massive Porites spp. are unaffected by mixed-species turfs, but killed by the red alga Anotrichium tenue. Marine Ecology Progress Series 258:79–86.
Jorgensen B., N. Revsbech. 1985. Diffusive boundary layers and the oxygen uptake of sediments and detritus. Limnology and Oceanography 30:111–122
Jorissen H., C. Skinner, R. Osinga, D. De Beer, M.M. Nugues. 2016. Evidence for water-mediated mechanisms in coral–algal interactions. Proc R Soc B 283: 20161137
Kappner, I., S. M. Al-Moghrabi, and C. Richter. 2000. Mucus-net feeding by the vermetid

gastropod Dendropoma maxima in coral reefs. Marine ecology progress series. 204:309–313.
Kawecki, T. J., and D. Ebert. 2004. Conceptual issues in local adaptation. Ecology Letters 7:1225–1241.
Kemp, K. M., J. R. Westrich, M. S. Alabady, M. L. Edwards, and E. K. Lipp. 2018. Abundance and Multilocus Sequence Analysis of VibrioBacteria Associated with Diseased Elkhorn Coral (Acropora palmata) of the Florida Keys. Applied and Environmental Microbiology 84:e01035.
Kline, D., N. Kuntz, M. Breitbart, N. Knowlton, and F. Rohwer. 2006. Role of elevated organic carbon levels and microbial activity in coral mortality. Marine Ecology Progress Series 314:119–125.
Klöppel, A., F. Brümmer, D. Schwabe, and G. Morlock. 2013. Detection of Bioactive Compounds in the Mucus Nets of Dendropoma maxima, Sowerby 1825 (Prosobranch Gastropod Vermetidae, Mollusca). Journal of Marine Biology 2013:1–9.
Krediet, C. J., K. B. Ritchie, V. J. Paul, and M. Teplitski. 2013. Coral-associated micro-organisms and their roles in promoting coral health and thwarting diseases. Proceedings of the Royal Society of London. Series B. Biological Sciences 280:2012–2328.
Kuffner, I., L. Walters, M. Becerro, V. Paul, R. Ritson-Williams, and K. Beach. 2006. Inhibition of coral recruitment by macroalgae and cyanobacteria. Marine Ecology Progress Series 323:107–117.
Kühl M., Y. Cohen, T. Dalsgaard, B. Jørgensen, N. Revsbech N. 1995. Microenvironment and photosynthesis of zooxanthellae in scleractinian corals studied with microsensors for 0_2, pH and light. Marine Ecology Progress Series 117:159–172
Kuznetsova A., P.B. Brockhoff, R.H.B. Christensen. 2016. lmerTest: Tests in Linear Mixed Effects Models. R package version 2.0-33. https://CRAN.R-project.org/package=lmerTest
Lee, S. T. M., S. K. Davy, S.-L. Tang, and P. S. Kench. 2017. Water flow buffers shifts in bacterial community structure in heat-stressed Acropora muricata. Scientific Reports:1–13.
Lefcheck, J.S. (2015) piecewiseSEM: Piecewise structural equation modeling in Rfor ecology, evolution, and systematics. Methods in Ecology and Evolution. 7(5): 573-579. DOI: 10.1111/2041-210X.12512
Legendre, P. and Legendre, L.F., 2012. *Numerical ecology*. Elsevier.
Lenihan, H. 1999. Physical-biological coupling on oyster reefs: how habitat structure influences individual performance. Ecological Monographs 69:251–275.
Lenth, R.V. 2016. Least-Squares Means: The R Package lsmeans. Journal of Statistical Software, 69(1), 1-33. doi:10.18637/jss.v069.i01
Levins, R. 1969. Thermal Acclimation and Heat Resistance in Drosophila Species. The American Naturalist 103:483–499.
Li M., B. Wang, M. Zhang, M. Rantalainen, S. Wang, H. Zhou, Y. Zhang, J. Shen, X. Pang, M. Zhang, H.Wei, Y. Chen, H. Lu, J. Zuo, M. Su,Y. Qiu, W. Jia, C. Xiao, L.M. Smith, S. Yang, E. Holmes, H. Tang, G. Zhao, J.K. Nicholson, L. Li, L. Zhao. 2008. Symbiotic gut microbes modulate human metabolic phenotypes. Proceedings of the National Academy of Sciences 105:2117–2122
Lough, J., and D. Barnes. 2000. Environmental controls on growth of the massive coral Porites. Journal of Experimental Marine Biology and Ecology 245:225–243.
Loya, Y., Sakai, K., Yamazato, K., Nakano, Y., Sambali, H. and van Woesik, R., 2001. Coral bleaching: the winners and the losers. *Ecology letters*, *4*(2): 122-131.

Martin, M.O., 2002. Predatory prokaryotes: an emerging research opportunity. *Journal of molecular microbiology and Biotechnology*, 4(5): 467-478.
Marubini F., H. Barnett, C. Langdon, M.J. Atkinson. 2001. Dependence of calcification on light and carbonate ion concentration for the hermatypic coral Porites compressa. Marine Ecology Progress Series 220: 153-162
Menge, B. A., and J. P. Sutherland. 1976. Species diversity gradients: synthesis of the roles of predation, competition, and temporal heterogeneity. American Naturalist:351–369.
Metlen, K.L., E.T. Aschehoug, R.M. Callaway. 2013. Competitive outcomes between two exotic invaders are modified by direct and indirect effects of a native conifer. Oikos 122(4): 632-640.
Metlen, K.L., R.M. Callaway. 2015. Native North American pine attenuates the competitive effects of a European invader on native grasses. Biological invasions 17(4): 1227-1237.
McCook, L., 2001. Competition between corals and algal turfs along a gradient of terrestrial influence in the nearshore central Great Barrier Reef. Coral Reefs, 19(4): 419-425.
McDevitt-Irwin, J. M., J. K. Baum, M. Garren, and R. L. Vega Thurber. 2017. Responses of Coral-Associated Bacterial Communities to Local and Global Stressors. Frontiers in Marine Science 4:289–16.
Mera, H., and D. G. Bourne. 2017. Disentangling causation: complex roles of coral-associated microorganisms in disease. Environmental Microbiology 20:431–449.
Meyer, J. L., V. J. Paul, and M. Teplitski. 2014. Community Shifts in the Surface Microbiomes of the Coral Porites astreoides with Unusual Lesions. PLoS ONE 9:e100316.
Mindell, D. P. 1992. Phylogenetic consequences of symbioses: Eukarya and Eubacteria are not monophyletic taxa. BioSystems 27:53–62.
Moberg, F., C. Folke. 1999. Ecological goods and services of coral reef ecosystems. Ecological Economics 29:215–233
Moran, N.A., D.B. Sloan. 2015. The Hologenome Concept: Helpful or Hollow? PLoS Biology 13:e1002311–10
Morrow, K.M., Bourne, D.G., Humphrey, C., Botté, E.S., Laffy, P., Zaneveld, J., Uthicke, S., Fabricius, K.E. and Webster, N.S., 2015. Natural volcanic CO 2 seeps reveal future trajectories for host–microbial associations in corals and sponges. The ISME journal, 9(4): 894-908
Morton, J.E. 1950 Feeding mechanism in the Vermetidae (order Mesogastropoda). Nature 165:923–924
Muko, S., K. Kawasaki, K. SAKAI, F. Takasu, and N. Shigesada. 2000. Morphological plasticity in the coral Porites sillimaniani and its adaptive significance. Bulletin of Marine Science 661:225–239.
Myers, J. H., D. Simberloff, A. M. Kuris, and J. R. Carey. 2000. Eradication revisited: dealing with exotic species. Trends in Ecology & Evolution 15:316–320.
Nasrolahi, A., S.B. Stratil, K.J. Jacob, M. Wahl. 2012. A protective coat of microorganisms on macroalgae: inhibitory effects of bacterial biofilms and epibiotic microbial assemblages on barnacle attachment. FEMS Microbiology Ecology 81:583–595
Neave, M. J., A. Apprill, C. Ferrier-Pagès, and C. R. Voolstra. 2016. Diversity and function of prevalent symbiotic marine bacteria in the genus Endozoicomonas. Applied Microbiology and Biotechnology:1–10.
Nelson, C.E., Alldredge, A.L., McCliment, E.A., Amaral-Zettler, L.A. and Carlson, C.A., 2011. Depleted dissolved organic carbon and distinct bacterial communities in the water column of

a rapid-flushing coral reef ecosystem. *The ISME Journal*, 5(8), p.1374-1387
Nelson, C. E., S. J. Goldberg, L. W. Kelly, A. F. Haas, J. E. Smith, F. Rohwer, and C. A. Carlson. 2013. Coral and macroalgal exudates vary in neutral sugar composition and differentially enrich reef bacterioplankton lineages ISMEJ 1–18.
Nugues, M. M., G. W. Smith, R. J. Hooidonk, M. I. Seabra, and R. P. Bak. 2004. Algal contact as a trigger for coral disease. Ecology Letters 7:919–923.
Odum, H.T., E.P. Odum. 1955. Trophic structure and productivity of a windward coral reef community on Eniwetok Atoll. Ecological Monographs 291–320
Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P. McGlinn, D., Minchin, P.R., O'Hara, R.B., Simpson G.L., Solymos, P., Stevens, M.H.M., Szoecs,E., Wagner, H. (2017). vegan: Community Ecology Package. R package version 2.4-5. https://CRAN.R-project.org/package=vegan
Olsen I. (2014) The Family *Fusobacteriaceae*. In: Rosenberg E., DeLong E.F., Lory S., Stackebrandt E., Thompson F. (eds) The Prokaryotes. Springer, Berlin, Heidelberg
Padilla-Gamiño, J. L., K. M. Hanson, M. Stat, and R. D. Gates. 2012. Phenotypic plasticity of the coral Porites rus: Acclimatization responses to a turbid environment. Journal of Experimental Marine Biology and Ecology 434-435:71–80.
Pagès, J.-P., G. Pache, D. Joud, N. Magnan, and R. Michalet. 2003. Direct and indirect effects of shade on four forest tree seedlings in the French Alps. Ecology 84:2741–2750.
Pandolfi, J.M., Jackson, J.B., Baron, N., Bradbury, R.H., Guzman, H.M., Hughes, T.P., Kappel, C.V., Micheli, F., Ogden, J.C., Possingham, H.P. and Sala, E., 2005. Are US coral reefs on the slippery slope to slime? Science 307:1725–1726
Parkinson, J.E., I.B. Baums. 2014. The extended phenotypes of marine symbioses: ecological and evolutionary consequences of intraspecific genetic diversity in coral–algal associations. Frontiers in Microbiology 5:3518
Peacor, S.D., E.E. Werner. 2001. The contribution of trait-mediated indirect effects to the net effects of a predator. Proceedings of the National Academy of Sciences 98(7): 3904-3908.
Peixoto, R. S., P. M. Rosado, D. C. de A. Leite, A. S. Rosado, and D. G. Bourne. 2017. Beneficial Microorganisms for Corals (BMC): Proposed Mechanisms for Coral Health and Resilience. Frontiers in Microbiology 8:100–16.
Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D. R Core Team (2017). _nlme: Linear and Nonlinear Mixed Effects Models_. R package version 3.1-131, <URL:https://CRAN.R-project.org/package=nlme>.
Phillips, N. E., J. S. Shima, and C. W. Osenberg. 2014. Live coral cover may provide resilience to damage from the vermetid gastropod Dendropoma maximum by preventing larval settlement. Coral Reefs 33:1137–1144.
Pratte, Z. A., G. O. Longo, A. S. Burns, M. E. Hay, and F. J. Stewart. 2017. Contact with turf algae alters the coral microbiome: contact versus systemic impacts. Coral Reefs 37:1–13.
Putnam, H. M., M. Stat, X. Pochon, and R. D. Gates. 2012. Endosymbiotic flexibility associates with environmental sensitivity in scleractinian corals. Proceedings of the Royal Society of London. Series B. Biological Sciences 279:4352–4361.
Putnam, H. M., K. L. Barott, T. D. Ainsworth, and R. D. Gates. 2017. The Vulnerability and Resilience of Reef-Building Corals. Current Biology 27:R528–R540.
Quan-Young, L., and J. Espinoza-Avalos. 2006. Reduction of zooxanthellae density, chlorophyll *a* concentration, and tissue thickness of the coral *Montastraea faveolata* (Scleractinia) when competing with mixed turf algae. Limnology and Oceanography 51:1159–1166.

R Core Team. 2015. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/
Raimondi, P.T., Forde, S.E., Delph, L.F. and Lively, C.M., 2000. Processes structuring communities: evidence for trait-mediated indirect effects through induced polymorphisms. *Oikos*, *91*(2): 353-361.
Rasher, D., and M. Hay. 2010. Chemically rich seaweeds poison corals when not controlled by herbivores. Proceedings of the National Academy of Sciences 107:9683–9688.
Rasher, D. 2010. Seaweed allelopathy degrades the resilience and function of coral reefs. Communicative & Integrative Biology 3:564–566.
Relyea, R. A. 2004. Fine-tuned phenotypes: tadpole plasticity under 16 combinations of predators and competitors. Ecology 85:172–179.
Relyea, R., and K. Yurewicz. 2002. Predicting community outcomes from pairwise interactions: integrating density- and trait-mediated effects. Oecologia 131:569–579.
Relyea, R. A. 2000. Trait-mediated indirect effects in larval anurans: reversing competition with the threat of predation. Ecology 81:2278–2289.
Reshef, L., O. Koren, Y. Loya, I. Zilber-Rosenberg, and E. Rosenberg. 2006. The Coral Probiotic Hypothesis. Environmental Microbiology 8:2068–2073.
Reznick, D., and J. Travis. 1996. The empirical study of adaptation in natural populations.
River, G., and P. Edmunds. 2001. Mechanisms of interaction between macroalgae and scleractinians on a coral reef in Jamaica. Journal of Experimental Marine Biology and Ecology 261:159–172.
Rodriguez, R.J., Henson, J., Van Volkenburgh, E., Hoy, M., Wright, L., Beckwith, F., Kim, Y.O. and Redman, R.S., 2008. Stress tolerance in plants via habitat-adapted symbiosis. *The ISME journal*, *2*(4):404 - 16
Rohwer, F., M. Breitbart, J. Jara, F. Azam, and N. Knowlton. 2001. Diversity of bacteria associated with the Caribbean coral Montastraea franksi. Coral Reefs 20:85–91.
Rosenberg, E. and Zilber-Rosenberg, I., 2018. The hologenome concept of evolution after 10 years. *Microbiome*, *6*(1): 78.
Roughgarden, J. 2017. Holobionts as Units of Selection and a Model of Their Population Dynamics and Evolution. Biological Theory 0:0–0.
Rubinoff, D., and B. S. Holland. 2005. Between Two Extremes: Mitochondrial DNA is neither the Panacea nor the Nemesis of Phylogenetic and Taxonomic Inference. Systematic Biology 54:952–961.
Rudi, K., M. Kroken, O. J. Dahlberg, A. Deggerdal, K. S. Jakobsen, and F. Larsen. 1997. Rapid, universal method to isolate PCR-ready DNA using magnetic beads. BioTechniques 22:506–511.
Rypien, K.L., J.R. Ward, F. Azam. 2010. Antagonistic interactions among coral-associated bacteria. Environmental Microbiology 12:28–39
Safriel, U.N. 1975. The role of vermetid gastropods in the formation of Mediterranean and Atlantic reefs. Oecologia 20:85-101
Shearer, T.L. and M.A. Coffroth. 2008. Barcoding corals: limited by interspecific divergence, not intraspecfici variation. Molecular Ecology Resources 8: 247-255
Schlichting, C. D. and M. Pigliucci. 1998. *Phenotypic evolution: a reaction norm perspective*. Sinauer Associates Incorporated.
Schmitz, O. J., V. Krivan, and O. Ovadia. 2004. Trophic cascades: the primacy of trait-mediated indirect interactions. Ecology Letters 7:153–163.

Schneider, C.A., W.S. Rasband, and K.W. Eliceiri, 2012. NIH Image to ImageJ: 25 years of image analysis. Nature methods *9*(7): 671.
Schweinsberg, M., R. Tollrian, and K. P. Lampert. 2016. Genetic variation in the massive coral *Porites lobata*. Marine Biology 163:1–12.
Sekirov, I., S. L. Russell, L. C. M. Antunes, and B. B. Finlay. 2010. Gut Microbiota in Health and Disease. Physiological Reviews 90:859–904.
Shaver, E.C., A.A. Shantz, R. McMinds, D.E. Burkepile, R.L. Vega Thurber, B.R. Silliman. 2017. Effects of predation and nutrient enrichment on the success and microbiome of a foundational coral. Ecology 98(3):830-9
Shima, J. S., D. McNaughtan, and A. T. Strong. 2015. Vermetid gastropods mediatewithin‐colony variation in coral growth to reduce rugosity. Marine Biology 162:1523–1530.
Shima, J. S., N. E. Phillips, and C. W. Osenberg. 2013. Consistent deleterious effects of vermetid gastropods on coral performance. Journal of Experimental Marine Biology and Ecology 439:1–6.
Shima, J., C. Osenberg, and A. Stier. 2010. The vermetid gastropod Dendropoma maximum reduces coral growth and survival. Biology Letters 6:815.
Smith, J. E., M. Shaw, R. A. Edwards, D. Obura, O. Pantos, E. Sala, S. A. Sandin, S. Smriga, M. Hatay, and F. L. Rohwer. 2006. Indirect effects of algae on coral: algae‐mediated, microbe‐induced coral mortality. Ecology Letters 9:835–845.
Smith, L.W., Barshis, D. and Birkeland, C., 2007. Phenotypic plasticity for skeletal growth, density and calcification of Porites lobata in response to habitat type. *Coral Reefs*, *26*(3): 559-567
Stat, M., X. Pochon, R. Cowie, and R. D. Gates. 2009. Specificity in communities of Symbiodinium in corals from Johnston Atoll. Marine ecology progress series. Oldendorf 386:83–96.
Steneck, R., and M. Dethier. 1994. A functional group approach to the structure of algal-dominated communities. Oikos 69:476–498.
Stier, A. C., C. S. McKeon, C. W. Osenberg, and J. S. Shima. 2010. Guard crabs alleviate deleterious effects of vermetid snails on a branching coral. Coral Reefs 29:1019–1022.
Stocking J.B., J.P. Rippe, and M.A. Reidenbach. 2016. Structure and dynamics of turbulent boundary layer flow over healthy and algae-covered corals. Coral Reefs 35(3): 1047-1059. 10.1007/s00338-016-1446-8
Staley, JT, Whitman WB (2010) Family I. Fusobacteriaceae fam. nov. *The Bacteroidetes, Spirochaetes, Tenericutes (Mollicutes), Acidobacteria, Fibrobacteres, Fusobacteria, Dictyoglomi, Gemmatimonadetes, Lentisphaerae, Verrucomicrobia, Chlamydiae, and Planctomycetes*, p.748.
Strayer, D.L., Eviner, V.T., Jeschke, J.M. and Pace, M.L., 2006. Understanding the long-term effects of species invasions. *Trends in ecology & evolution*, *21*(11): 645-651.
Sweet, M. J., A. Croquer, and J. C. Bythell. 2010. Bacterial assemblages differ between compartments within the coral holobiont. Coral Reefs 30:39–52.
Sweet, M. J., A. Croquer, and J. C. Bythell. 2011. Dynamics of bacterial community development in the reef coral Acropora muricata following experimental antibiotic treatment - Springer. Coral Reefs.
Sweet, M. J., J. C. Bythell, and M. M. Nugues. 2013. Algae as Reservoirs for Coral Pathogens. PLoS ONE 8:e69717.
Tilman, D., F. Isbell, and J. M. Cowles. 2014. Biodiversity and Ecosystem Functioning. Annual

Review of Ecology, Evolution, and Systematics 45:471–493.
Todd, P. 2008. Morphological plasticity in scleractinian corals. Biological Reviews 83:315–337
Tootell, J. S., and M. A. Steele. 2014. Vermetid gastropods reduce foraging by herbivorous fishes on algae on coral reefs. Coral Reefs 33:1145–1151.
Torres-Dowdall, J., C. A. Handelsman, D. N. Reznick, and C. K. Ghalambor. 2012. Local adaptation and the evolution of phenotypic plasticity in trinidadian guppies (poecilia reticulata). Evolution 66:3432–3443.
Trussell, G. C. 2000. Phenotypic clines, plasticity, and morphological trade‐offs in an intertidal snail. Evolution 54:151–166.
van der Heijden M.G.A., R.D. Bardgett, N.M. van Straalen. 2008. The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. Ecology Letters 11:296–310
Vandermeer, J.H. 1969. The competitive structure of communities: an experimental approach with protozoa. Ecology 362–371
Vega Thurber, R., D. E. Burkepile, A. M. S. Correa, A. R. Thurber, A. A. Shantz, R. Welsh, C. Pritchard, and S. Rosales. 2012. Macroalgae Decrease Growth and Alter Microbial Community Structure of the Reef-Building Coral, Porites astreoides. PLoS ONE 7:e44246. Vega Thurber, R., D. Willner-Hall, B. Rodriguez-Mueller, C. Desnues, R. A. Edwards, F. Angly, E. Dinsdale, L. Kelly, and F. Rohwer. 2009. Metagenomic analysis of stressed coral holobionts. Environmental Microbiology 11:2148–2163.
Vermeij, M. 2006. Early life-history dynamics of Caribbean coral species on artificial substratum: the importance of competition, growth and variation in life-history strategy. Coral Reefs 25:59–71.
Vermeij, M., J. Smith, C. Smith, R. Vega Thurber, and S. Sandin. 2009. Survival and settlement success of coral planulae: independent and synergistic effects of macroalgae and microbes. Oecologia 159:325–336.
Vezzulli, L., M. Previati, C. Pruzzo, A. Marchese, D. G. Bourne, C. Cerrano, the VibrioSea Consortium. 2010. Vibrio infections triggering mass mortality events in a warming Mediterranean Sea. Environmental Microbiology 12:2007–2019.
Wagner, M. R., D. S. Lundberg, D. Coleman-Derr, S. G. Tringe, J. L. Dangl, and T. Mitchell-Olds. 2014. Natural soil microbes alter flowering phenology and the intensity of selection on flowering time in a wild Arabidopsis relative. Ecology Letters 17:717–726.
Wahl, M., F. Goecke, A. Labes, S. Dobretsov, and F. Weinberger. 2012. The Second Skin: Ecological Role of Epibiotic Biofilms on Marine Organisms. Frontiers in Microbiology 3:1–21.
Wares, J.P. 2014. Mitochondrial cytochrome b sequence data are not an improvement for species identification in scleractinian corals. PeerJ 2:e564
Wangpraseurt, D., M. Weber, H. Røy, L. Polerecky, D. De Beer, Suharsono, and M. M. Nugues. 2012. In Situ Oxygen Dynamics in Coral-Algal Interactions. PLoS ONE 7:e31192.
Webster, N. S., A. P. Negri, E. S. Botté, P. W. Laffy, F. Flores, S. Noonan, C. Schmidt, and S. Uthicke. 2016. Host-associated coral reef microbes respond to the cumulative pressures of ocean warming and ocean acidification. Scientific Reports:1–9.
Wegley, L., Edwards, R., Rodriguez-Brito, B., Liu, H. and Rohwer, F., 2007. Metagenomic analysis of the microbial community associated with the coral Porites astreoides. Environmental microbiology, 9(11): 2707-2719.
Werner E., S.D. Peacor. 2003. A review of trait-mediated indirect interactions in ecological

communities. Ecology 84:1083–1100
Whitham, T. G., J. K. Bailey, J. A. Schweitzer, S. M. Shuster, R. K. Bangert, C. J. LeRoy, E. V. Lonsdorf, G. J. Allan, S. P. DiFazio, B. M. Potts, D. G. Fischer, C. A. Gehring, R. L. Lindroth, J. C. Marks, S. C. Hart, G. M. Wimp, and S. C. Wooley. 2006. A framework for community and ecosystem genetics: from genes to ecosystems. Nature Reviews Genetics 7:510–523.
Wild, C., M. Huettel, A. Klueter, S. Kremb, M. Rasheed, B. Jørgensen. 2004. Coral mucus functions as an energy carrier and particle trap in the reef ecosystem. Nature 428:66–70
Wild, C., W. Niggl, M. Naumann, A. Haas. 2010. Organic matter release by Red Sea coral reef organisms—potential effects on microbial activity and in situ O2 availability. Mar Ecol Prog Ser 411:61–71
Wimp, G. M., G. D. Martinsen, K. D. Floate, R. K. Bangert, and T. G. Whitham. 2005. Plant genetic determinants of arthropod community structure and diversity. Evolution 59:61–69.
Wootton, J. T. 1994. The nature and consequences of indirect effects in ecological communities. Annual Review of Ecology and Systematics 25:443–466.
Zaneveld, J. R., A. A. Shantz, C. E. Pritchard, R. McMinds, J. E. R. O. M. P. Payet, R. Welsh, A. M. S. Correa, N. P. Lemoine, S. Rosales, C. Fuchs, J. A. Maynard, D. E. Burkepile, and R. V. Thurber. 2016. Overfishing and nutrient pollution interact with temperature to disrupt coral reefs down to microbial scales. Nature Communications 7:1–12.
Zaneveld, J. R., R. McMinds, and R. V. Thurber. 2017. Stress and stability: applying the Anna Karenina principle to animal microbiomes. Nature Publishing Group 2:1–8.
Zilber-Rosenberg I, Rosenberg E (2008) Role of microorganisms in the evolution of animals and plants: the hologenome theory of evolution. *FEMS Microbiology Reviews* 32:723–735.
Zill, J. A., M. A. Gil, and C. W. Osenberg. 2017. When environmental factors become stressors: interactive effects of vermetid gastropods and sedimentation on corals. Biology Letters 13:20160957.
Zvuloni, A., R. Armoza-Zvuloni, and Y. Loya. 2008. Structural deformation of branching corals associated with the vermetid gastropod Dendropoma maxima. Marine Ecology Progress Series 363:103–108.