The Role of Zooplankton on Coral Physiology and Ecology

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Dedication

To my parents, Janis and Bill Dwyer, for their constant support and encouragement.
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Abstract of Dissertation

Coral health is threatened by increasing water temperatures causing more frequent and higher intensity bleaching events. This dissertation investigates the role of zooplankton on coral health in Bocas del Toro, Panama. While previous studies have shown certain coral species are able to survive bleaching events through heterotrophic plasticity, little focus has been placed on the role of zooplankton themselves in the natural setting on coral reefs. The following dissertation describes spatial and temporal changes in zooplankton communities accessible to corals, and whether an increased abundance of the natural zooplankton community will provide faster recovery to *Porites furcata* after a heat-stress event.

The highest zooplankton abundances and diversity of zooplankton taxa occurred on the offshore site in fall 2016. No differences in abundance were seen between sites during winter 2017. The enhanced feeding experiment showed that symbiont metrics, including symbiont density, chlorophyll concentrations, and photosynthetic efficiency were not significantly different across feeding treatments of heat-stressed corals. However, the ambient temperature enhanced fed treatment showed an increase in symbiont metrics compared to other treatments, except for photosynthesis rates, which decreased and were lower than all other treatments. This could be explained by *P. furcata’s* ability to decouple its heterotrophic and autotrophic capabilities and capitalize on the enhanced zooplankton availability. No significant differences were found between treatments on the coral host physiology at the end of the five-week recovery. Therefore, the natural conditions of the nearshore coral reef, provide the necessary resources for corals to recover from heat-stress in five weeks.
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Introduction

Coral reefs worldwide are experiencing devastating stress events due to environmental changes, including increased disease outbreaks, ocean acidification and water temperatures (Hoegh-Guldberg et al., 2007; Bruno et al., 2007; Maynard et al., 2014). All of these environmental changes threaten coral health from rapid tissue loss due to disease, limited access to calcium carbonate for growth, or limiting their energy intake from the breakdown of their symbiotic algae during bleaching events (Ainsworth et al., 2007; Andersson & Gledhill, 2013; Hoegh-Guldberg 1999). While all of these environmental changes present serious risks to coral health, corals are likely most threatened by increasing water temperatures (Hughes et al., 2003; Hoegh-Guldberg et al., 2017). Increased water temperatures cause a breakdown in the symbiotic relationship between the coral animal and their photosynthetic endosymbiotic dinoflagellates (*Symbiodinium*) that live inside of them. When those symbionts leave, the coral loses its vibrant colors, hence the term coral bleaching, and the benefits the symbionts provided with photosynthesis. However, depending on the coral animal itself, and the specific environmental conditions during and following the bleaching event, some corals can survive and reinstate their relationship with their symbionts after the bleaching event has passed. A variety of recovery mechanisms exist, including symbiotic plasticity (Silverstein et al., 2015), stored energy reserves (Rodrigues & Grottoli, 2007; Anthony et al., 2009), or heterotrophic plasticity (Grottoli et al., 2006; Houlbrèque & Ferrier-Pagès, 2009; Levas et al., 2013). The latter refers to corals shifting their behavior from actively feeding on plankton and particles in the water column to supplement the energy and benefits they receive from their photosynthetic symbionts, to actively feeding to meet all of their metabolic needs. This dissertation further explores the role of zooplankton on coral reefs to better understand their ability to be a resource for corals recovering from bleaching.
Overview of heterotrophy in corals

Healthy scleractinian corals rely on their symbiotic algae for energy, but additional nutrients and energy come from heterotrophy ranging from dissolved organic carbon (DOC) to nano-plankton (Houlbereque et al., 2004), to macrozooplankton (Sebens et al., 1996). Early studies determined coral feeding occurs either by tentacle capture, using mucus filaments to entangle and capture prey, or a combination of the two (Lewis & Price, 1975). Originally, it was thought corals with high surface area to volume ratios (i.e., branching or plating morphologies) relied more on photosynthesis and corals with low surface area to volume ratios (i.e., mounding morphologies) and large polyps relied more on heterotrophy (Porter, 1976). However, due to the trade-offs between polyp size and surface area, when taking prey size into account, branching and plating morphologies have also displayed heterotrophic strategies (Sebens, 1979). Corals with smaller polyps can work together to capture larger plankton. At increased flow speeds, branching or plating morphologies with larger surface areas can excel in capturing plankton because of their increased surface area and their ability to create turbulent eddies around their more complex morphologies. Therefore, it is important to continue investigating how feeding behaviors impact coral health across species with various morphologies, across individuals with different thermal-stress histories, and across various reefs with different environmental parameters.

Benefits of Heterotrophy

Heterotrophy is an important component for Cladocora caespitosa, as feeding rates increased in dark environments with limited light availability. However, in high light intensities, heterotrophy was also important as tissue growth only occurred in high light with food availability (Hoogenboom et al., 2010). Heterotrophy also allows for increased light and dark calcifications rates, as well as increased protein concentrations for Stylophora pistillata (Houlbrèque et al.,
How much a coral depends on heterotrophic feeding to maintain its energy budget depends on its depth, light availability, and its feeding behaviors.

**Heterotrophy Under Stress**

While heterotrophy is important for maintaining basic physiological needs, such as growth, photosynthesis, and protein development, heterotrophic plasticity is also a recovery mechanism when corals are experiencing stressful environmental conditions. Anthony et al. (2000) showed heterotrophic plasticity in a symbiotic coral for the first time when *Goniastrea retiformis* was exposed to two months of shading and suspended particulate matter (SPM). Normally, SPM restricts light availability, however *G. retiformis* was able to double its rate of particle feeding with a high SPM treatment to fully compensate for its decreased photosynthetic capabilities. Regardless of the light and SPM conditions, this coral was able to increase skeletal and tissue growth. Meanwhile, *Porites cylindrica* were exposed to the same conditions and did not increase its feeding rates. Instead, its tissue mass and lipid content decreased when in shaded and high-SPM conditions. Increased coral heterotrophy also can lead to continued growth in corals suffering from ocean acidification conditions (Edmunds, 2011). However, these conditions seem to limit the feeding ability at least for *S. pistillata*, and continued acidifying conditions may prevent corals from being able to use heterotrophic plasticity as a recovery mechanism (Houlbrèque et al., 2015).

Increased heterotrophy can also be used as a recovery mechanism from heat stress events, but is variable across coral species (Gottoli et al., 2006). *Montipora capitata* lost color and decreased photosynthetic rates and chlorophyll concentrations during bleaching and after six weeks of recovery under ambient conditions on the reef. Despite the lack of symbiont recovery, after six
weeks of natural recovery on the reef, the coral host showed full recovery in its total energy reserves and biomass. *Porites compressa* showed visible bleaching and decreased chlorophyll concentrations and photosynthetic rates, but after six weeks of recovery began to display a pale brown appearance and some increases in chlorophyll concentrations and photosynthetic rates. However, their total energy reserves and total biomass continued decreasing during recovery. To better understand the role of heterotrophic plasticity, a feeding experiment was conducted on the reef which revealed no difference in feeding rates for *P. compressa* between bleaching and non-bleaching corals. The feeding rate of bleached *M. capitata*, however, increased five-fold relative to that of non-bleached corals, which means their entire metabolic demand could be covered by heterotrophy (Grottoli et al., 2006).

While heterotrophic plasticity appears to be a promising solution for corals, increased feeding rates have been documented up to 11 months after a heat-stress event, indicating that either this response is an acclimatization to allow the coral to be prepared for future bleaching events or it takes more than 11 months for corals to fully recover from a bleaching event (Hughes & Grottoli, 2013). This study further found that the heterotrophic carbon obtained through this plastic response during the early stages of recovery is not assimilated, but rather is used immediately to meet metabolic needs or is lost through mucus. Since the non-bleached corals do not increase their heterotrophic carbon, it is likely that the bleached corals remain in a recovery phase with their elevated feeding behavior. However, further studies are needed to investigate how corals use heterotrophic plasticity after exposure to heat stress events.
*Acropora intermedia* that were fed prior to heat stress events started them with the highest protein concentrations, dropped approximately 70% during bleaching, and then increased throughout the recovery phase. Conversely, unfed corals regardless of heat stress had lower protein concentrations going into bleaching, continued to decline during bleaching and then stabilized during recovery. Only fed heated corals increased protein levels during the recovery phase. These levels were approximately three times higher than those of heat-stressed unfed corals (Connolly et al., 2012). This experiment showed different responses between January and July, where in July there was no difference in skeletal growth between starved and fed treatments, indicating there may be another source of carbon in the seawater in July. Or it could have been due to coral seasonal changes for biomass characteristics affecting growth potential. Additionally, it could be due to seasonal variation in zooplankton community dynamics. Therefore, it is important to continue exploring the role of heterotrophic plasticity in other coral species and in other coral regions, to try to better understand what is driving the ability for some corals to use heterotrophic plasticity, and whether this is a long-term sustainable recovery mechanism from bleaching.

Even fewer studies that conducted heterotrophic plasticity feeding experiments incorporated natural zooplankton community dynamics. Both lab and field studies indicate that corals rely on a diverse diet. Coral ingestion rates in lab experiments were proportional to prey concentrations for particles less than 100 µm. Within this size class, nanoflagellates contributed 84 - 94 % of the total carbon and 52 - 85 % of the total nitrogen ingested for zooxanthellate corals, *Stylophora pistillata* and *Galaxea fascicularis* (Houlbrèque et al., 2004). In Le Prevoyante, Mayotte Island, particles < 10 um accounted for 74 % of the chlorophyll-a concentration and 47 % of the total
living carbon (Houlbrèque et al., 2006). Chlorophyll-a concentrations, as well as picoplankton groups, were depleted by 30 - 45 % over a patch reef compared to an adjacent sandy-bottom, but nanoflagellate concentrations were unchanged between these two areas (Houlbrèque et al., 2006). This suggests selective grazing of picoplankton by the benthic community. Corals prey on macrozooplankton as gut content analysis revealed copepods, amphipods, polychaetes, salps, eggs, and various taxa’s larvae in their digestive track (Porter, 1974). A more recent study evaluated the effect of zooplankton abundance over a lunar cycle and its effect on two corals species’ feeding rates (Palardy et al., 2006). This study found coral feeding rates are proportional to ambient zooplankton concentrations, and the proportional contribution of zooplankton taxa to coral diet varied throughout the lunar cycle. This likely is because critical taxa like crab zoea, isopods and amphipod abundances varied significantly at different phases in the lunar cycle. This variation results in corals relying on detritus and bacteria when zooplankton are not readily available. However, this study calculated feeding rates based on only one hour of feeding after corals were starved during the day prior to feeding. While this is an appropriate method to ensure zooplankton consumed can be identified before they are digested, it limits the ability to understand how corals are utilizing the zooplankton community outside of that single hour of feeding.

### Zooplankton on Coral Reefs

Building a better understanding of zooplankton community dynamics on coral reefs is an important component of assessing the future of coral reefs with increasing heat-stress events because they will also impact zooplankton. Many zooplankton do not live or stay planktonic for more than one year, allowing for the coupling of climate and population dynamics seen in long term data sets (Hays et al., 2005). Zooplankton’s physiological processes, such as growth rate
and generation time, are highly sensitive to temperature (Mauchline, 1998). Warming of 0.5 °C resulted in a 70% decline in zooplankton abundance (Roemmich & McGowan, 1995). This large decline may also be an indirect effect of temperature increase, which limits upwelling and restricts nutrient availability in the surface layer. However, without consistent monitoring of zooplankton communities, it will be difficult to know the impact of heat stress events on their abundance and community composition, let alone how these changes may impact corals’ ability to rely on them to make up the energy lost from their symbionts during and immediately following heat-stress events. Connolly et al. (2012) exemplified how differences in availability of heterotrophic energy among reefs are an important factor in the variability found between different corals’ ability to rapidly recover from heat-stress events. Therefore, corals with enhanced feeding opportunities are better prepared to overcome stress events because they can use the increased resources from zooplankton to both strengthen their symbionts and maintain or strengthen their protein and lipid reserves.

**Dissertation Objectives**

This dissertation aims to better understand the role of zooplankton on coral health. First, in Chapter 1, zooplankton community dynamics are explored on an offshore and nearshore reef in Bocas del Toro, Panama. Abundance and diversity are determined and compared across sites, season, and lunar phase to better understand the spatial and temporal variation in zooplankton availability to corals. Other environmental parameters, including temperature, nutrients, and dissolution rates, were monitored to help explain both zooplankton dynamics and overall coral health.
Based on the results from Chapter 1, Chapters 2 and 3 aim to better determine if increased zooplankton abundance alone, is an important factor in allowing a quicker recovery from bleaching for *Porites furcata*, on a nearshore reef in Bocas del Toro, Panama. Bleached and non-bleached corals were each placed in an ambient and an enhanced feeding treatment on the nearshore reef, separated only by a large coral head. Symbiont quality and concentrations (Chapter 2) and coral host physiology (Chapter 3) were measured before heat-stress, immediately following heat-stress, and after five weeks of recovery in their respective feeding treatments. This timeframe was based on a previous study indicating it takes up to two weeks to observe a difference between fed and starved corals, and up to five weeks for significant changes in tissue biomass of proteins, chlorophyll, and symbiont densities (Houlbrèque et al., 2003). The aim of these chapters was to assess if a better understanding of zooplankton community dynamics can lead to better estimations of coral recovery from heat-stress events.
Literature Cited


Spatial and Temporal Differences in Zooplankton Communities between an Offshore and a Nearshore reef in Bocas del Toro, Panama

Abstract

Zooplankton are an important component of coral diets to supplement nutrients not provided by photosynthesis and can be an important recovery mechanism from heat-stress induced bleaching in some corals. However, zooplankton community dynamics are not well known on coral reefs, making it difficult to assess their ability to protect corals through repeated future heat-stress events. This study compares zooplankton community dynamics between an offshore coral reef and a nearshore coral reef in Bocas del Toro, Panama during fall 2016. Daytime monitoring occurred at each site during each of the eight lunar phases to determine differences in zooplankton abundance, diversity, and overall community composition available to corals. The following winter (2017), sampling was conducted weekly at each site to compare seasonal changes within and across the sites. Nighttime zooplankton community dynamics were compared at the nearshore site only. The offshore site had significantly higher zooplankton abundance during fall 2016, but no differences in abundances were seen between sites in winter 2017. The offshore site had a higher relative abundance of zooplankton taxa other than copepods compared to the nearshore site. These other zooplankton taxa may be easier to capture and provide more nutritional value than copepods. Our results revealed large spatial and temporal variation in zooplankton availability to corals, indicating the importance of better understanding zooplankton spatial distributions and community dynamics over coral reefs to assess their ability to consistently be a recovery mechanism for coral bleaching with continued and increasing heat-stress events.
Introduction

Zooplankton are an important food source for many planktivorous species living on coral reefs (Glynn, 1973; Alldredge and King, 2009). However, relatively little is known about zooplankton community dynamics on coral reefs (Heidelberg et al, 2004). While zooplankton spatial patchiness makes it difficult to quantify zooplankton communities in all aquatic systems, determining zooplankton community dynamics is even more difficult on coral reefs due to the complex and delicate topography, increased predation impacts, the demersal and oceanic zooplankton populations occurring simultaneously on the reef, and other changing environmental factors (Yahel et al., 2005). Coral reefs present an increased risk of predation for zooplankton compared to open ocean environments, with many sessile planktivores covering the benthic environment on coral reefs (Hamner et al., 2007). However, these sessile planktivores, such as corals, can provide refuge for some zooplankton. Demersal zooplankton may remain hidden in the benthos during the day and migrate higher in the water column to feed at night (King and Alldrevege, 1977; Ohlhurt, 1982). Oceanic zooplankton, move on and off the reef with currents, providing a high turnover of zooplankton on reefs that are more exposed to the open ocean and that have higher flow rates (Alldredge and King, 2009).

Nakajima et al. (2017) compiled the results of studies that investigated coral reef zooplankton communities over the past 45 years. Variation in methodology, the sampling time of day and sampling season made it difficult to make accurate comparisons over space and time. These zooplankton communities are likely shifting with the same environmental changes affecting the corals (Richardson et al., 2008), but without robust zooplankton data available for coral reefs, it is difficult to understand the magnitude of how they are affected. For example, Carrillo-Baltodano & Morales-Ramirez (2016), recently described the zooplankton community
on a coral reef in Limon, Costa Rica, but the only dataset they could directly compare to was from 1984. In this system zooplankton abundance was nearly 20 times higher than that described in 1984. Without any other references within this 30-year time period, it is difficult to determine the cause for the observed increases. Additionally, methodologies have changed over the past 30 years, making it difficult to replicate the study effectively, and it is unclear if better methods for collection and identification resulted in a higher abundance being discovered. Additionally, because coral reef environments can vary drastically across sites due to different environmental conditions such as flow (Alldredge and King et al., 2009), distance from land (D’Croz et al., 2005), and fish abundance (Hammner et al., 2007; Leray et al., 2019), it is difficult to broadly apply knowledge about zooplankton dynamics over space and time. Therefore, it is essential for more studies to investigate zooplankton community dynamics to start gathering baseline information as our coral reef environments continue to change.

Zooplankton communities are patchy as migratory plankton are transported on and off the reef with currents, and demersal zooplankton remain within the substrate during the day and rise to the top of the water column at night to feed. To develop improved zooplankton monitoring, it is essential to account for as many environmental factors that could impact zooplankton communities as possible to help predict spatial and temporal variability. Nighttime sampling on coral reefs has been studied more intensely than daytime zooplankton communities on coral reefs, because previously it was thought that demersal zooplankton living on the reef and rising to the surface to feed at night were the primary source of zooplankton for corals and coral reef fishes (Alldredge and King, 1977). Other studies have focused on nighttime sampling due to the effects of the lunar cycle and the changing illumination of the moon (Gliwicz 1986; Alldredge and King, 1980). However, many corals and other dominant reef species feed on zooplankton
during the day, making it important to evaluate daytime zooplankton communities on coral reefs as well. Hamner et al. (2007) examined mesozooplankton and fish egg abundances along with daytime zooplankton sampling in Palau, finding high variability and, thus, limited predictability within lunar phases. However, they did see highly significant spawning patterns associated with lunar phases for surgeonfish and parrotfish, indicating the importance of recognizing the role of spawning patterns on daytime zooplankton communities and food availability to coral reef planktivores across a lunar cycle.

Healthy corals gain energy from the products of their symbiotic algae and from actively feeding on plankton in the water column around them. Different corals rely on these two energy capture mechanisms in various proportions, but both are important for corals to meet their daily metabolic requirements (Sorokin, 1973; Houlbroque & Ferrier-Pagès, 2009). The benefits of increased heterotrophy can be allocated in various ways. For *Stylophora pistillata* increased feeding on zooplankton resulted in continued photosynthetic function or replacement of degraded photosynthetic complexes (Hoogenboom et al., 2012), an increase in metals involved in photosynthesis and anti-oxidant protection (Ferrier-Pagès et al., 2018), and increased calcification rate and tissue growth (Ferrier-Pagès et al., 2003). Some corals have used heterotrophic plasticity in a variety of stressful environments, including high turbidity (Anthony & Fabricius, 2000) and bleaching (Grotolli et al., 2006).

Unfortunately, due to the poor understanding of zooplankton community dynamics on coral reefs, especially in recent years, it is difficult to accurately predict the role zooplankton can have in helping corals recover from bleaching events. It is likely increased water temperatures will impact zooplankton communities as well (Richardson et al., 2008). This study aims to address the current lack of knowledge of daytime spatial and temporal zooplankton community
dynamics on coral reefs. Specifically, the lunar cycle is associated with this study’s daytime sampling methodology to attempt to better understand if it affects variation in zooplankton communities. Sampling each lunar phase provided a regular sampling schedule in addition to exploring whether daytime zooplankton variation can be associated with previous nights’ moonlight cues, triggering spawning, distribution, or behavioral changes for specific taxa.

Methods

Study Site and Sampling Design

This study was conducted on two coral reefs in Bocas del Toro, Panama at the Smithsonian Tropical Research Institute. One nearshore reef adjacent to mangroves and one offshore reef were selected as study sites. These reefs do not follow the typical reef zonation patterns seen throughout the Caribbean. Our nearshore and offshore classification are comparing the proximity of each of these separate reefs to the nearest landmass. The nearshore site was located just off of the main Bocas del Toro island inside the semi-enclosed lagoon of Almirante Bay. The offshore site was outside of the Almirante Bay about 25 km away from the nearshore site, and was approximately 1 km away from the nearest island. Due its location outside of Almirante Bay, the offshore reef was more exposed to currents coming from the open ocean (Fig 1.1). Using SCUBA, three-20 m permanent transects were created on each reef at 7 m depth. Zooplankton were collected by divers towing a plankton net (0.5 m diameter, 40 µm mesh) directly over corals along each transect to ensure sampling of the zooplankton community available to the corals (Alldredge and King, 2009).
In fall 2016, sampling was conducted midday during one day of each of the eight lunar phases, starting with the waning crescent moon phase on September 27 and ending in the last quarter phase on October 22 (Fig 1.2). This resulted in the collection of 48 samples. Three of these fall 2016 samples were not preserved well enough for accurate sample processing. Missing samples included one from each site during the waxing crescent phase and one during the waning crescent phase at the nearshore site. Environmental measurements taken on the reefs during fall 2016 include temperature, nitrate concentrations, and dissolution rates. All environmental measurements were collected along the permanent transects at 7 m. Temperature was measured by placing a HOBO temperature logger on one of the permanent transects (7 m depth) at each site for the duration of sampling, recording every 15 minutes. Dissolution rates were used to compare water motion regimes between the benthic environments at the offshore and nearshore site (Jokiel and Morrissey, 1993). Three dissolution balls were weighed prior to each deployment at each site (7 m depth). Each dissolution ball was secured at the start of each permanent transect, and left for 48-72 hours between the waxing crescent and the first quarter, waxing gibbous and full moon, and waning gibbous and last quarter phases. After collection off the reef, the dissolution balls were dried for 24 hours at 60 °C and weighed. Dissolution rates were calculated by the difference in beginning and end weights and standardized by the amount of time deployed. Nutrient sampling was conducted by collecting a single water sample at each site in a 50 mL falcon tube during the waning and waxing gibbous phases and kept in a cooler on the boat until they were stored in a -20 °C freezer. A VCl₃ reduction was used for the spectrophotometric determination of nitrate on a GENESYS 30 visible spectrophotometer (Thermo Scientific) measured at 540 nm (modified protocol from Garcia-Robledo et al., 2014).
In winter 2017, zooplankton samples were collected on the nearshore site once a week for five weeks starting during the waning crescent phase on Feb 22 and end in the waning crescent phase on March 22 (Fig 1.2). Due to logistical constraints and weather-related issues, the offshore site was only sampled during the day once a week for three weeks, starting with the waxing gibbous on March 8 and ending with the waning crescent on March 22. Nighttime zooplankton tows were conducted a few hours after sunset using the same methodology as the daytime zooplankton samples, but due to safety reasons, they were only done at the nearshore site. Temperature was measured the same as described above at the nearshore site. Temperature data for the offshore site was provided by a colleague, Sara Williams of Northeastern University, from a nearby reef. Water samples were taken during the waning crescent, waning gibbous, waxing crescent and waxing gibbous and nitrate measurements were processed as cited above. Ammonium (NH$_4^+$) was measured colorimetrically on a GENESYS 30 visible spectrophotometer (Thermo Scientific) following protocols from Solórzano (1969).

**Zooplankton Sampling Processing**

After each dive, zooplankton samples were immediately filtered with a 40 µm mesh into 50 ml falcon tubes and kept in a cooler until they could be stored in a -20° C freezer. They were later viewed under an AZ100 Nikon Macroscope at 40x magnification. Subsamples were counted until 200 individuals were tallied and each subsample was always counted in its entirety. Total abundance was calculated for individuals per cubic meter by taking the number of individuals counted divided by the product of the proportion of the sample counted and the total volume of water towed by the zooplankton net. Diversity parameters are reported in taxa richness (number
of unique taxa identified in each sample), the Inverse Simpson Index, and overall community composition to better account for the relative abundance of the various taxa identified.

**Analysis**

Abundance, taxa richness and the Inverse Simpson Index were analyzed in R Studio 3.5.2. All data were tested for normality and homoscedasticity. Data that did not meet either assumption were either log or square root transformed. Three-way ANOVAs were used to compare sampling season, site, and lunar phase as fixed effects. However, because I only included lunar phases that were sampled at both sites and seasons, subsetting the tested data, I then followed up with two-way ANOVAs to compare site and lunar phase within each sampling season, and one-way ANOVAs were used to determine the effect of lunar phase and site individually within each sampling season to assess all available data. To further explore differences in abundance across lunar cycle phases and abundance incorporating all samples taken, a regression was run using a generalized additive model with the mgcv package (Wood, 2006). The best-fitting regression model was the log of the abundance being drawn from indicator variables for site and season added together with the predicted outcome of a smooth function. The smooth function is cubic shrinkage spline and was predicted by the number of days sampling occurred from the full moon, with the full moon considered as day zero. The spline function complexity was also modified by the number of knots across its domain. The Akaike information criterion (AIC) score was used to select the optimal number of knots (13). The abundance data was over-dispersed, so the family of the response variable was modeled with a Tweedie distribution.
To compare community composition, total count data were converted to relative abundance to determine differences between communities as a whole over both space and time using the vegan package in R Studio. The Adonis function was used to perform a permutational multivariate analysis of variance using distance matrices on the data to determine significant differences in overall zooplankton community composition. The same breakdown of ANOVAs was repeated for this analysis. A one-way ANOVA was used to compare nitrate concentration across sites for each sampling season individually. One-way ANOVAs were used to compare dissolution rates across sites for the entire lunar cycle with three sampling points combined, and also to compare each of the three sampling points taken within each site. For multi-factorial ANOVAs with significant interactions, a pairwise posthoc test was run using the lsmeans package and for one-way ANOVAs, posthoc Tukey HSD tests were used for all significant results. When this resulted in unequal variances, a log transformation was performed. Statistical tests were made at the 5 % significance level.

Results

Environmental Parameters

Temperature

Throughout the fall 2016 lunar cycle sampling, offshore temperatures ranged from 30.1 °C to 30.9 °C and nearshore temperatures ranged from 30.0 °C to 31.1 °C. In winter 2017, during the three weeks where both sites were sampled from March 8 – March 22, the temperature ranged from 27.67 °C to 28.9 °C at the offshore site and 27.57 °C to 29.15 °C at the nearshore site. During the two weeks of only nearshore sampling from Feb 22 – March 7, the temperature ranged from 27.08 °C to 30.15 °C.
**Nutrients**

During fall 2016, offshore individually sampled nitrate values ranged from 1.31 µM during the waxing crescent, 4.56 µM during the waxing gibbous, and 3.94 µM during the waning gibbous. Nearshore values were lower than the offshore during the waxing gibbous with 2.35 µM, but similar to the offshore during the waning gibbous with 3.92 µM (Fig 1.3). During winter 2017, there were no significant differences found overall for nitrate or ammonium levels between the offshore and nearshore sites. Nitrate levels start at 0.93 µM at the offshore site during the waxing crescent, then increased to 2.82 during the waxing gibbous, and then dropped to 0.69 µM during the waning crescent. Nearshore nitrate levels started during the first waning crescent sampling at 1.2 µM, then increased to 2.3 µM during the waxing crescent and 2.1 µM in Waxing Gibbous, then decreased below detection during waning gibbous and then increased back to 2.3 µM during the final waning crescent sampling. Offshore ammonium levels ranged from 4.5 µM during the waxing crescent, increased to 14.68 µM during the waxing gibbous, then returned to 4.5 µM during the waning gibbous. At the nearshore site, ammonium levels were 7.2 µM during the first waning crescent, 9.4 µM during the waxing crescent, increased to 15.6 µM during the waxing gibbous and then dropped to 5.8 at the waning gibbous.

**Flow (Dissolution Rates)**

Dissolution rates were significantly higher on the offshore reef, both overall and at every individual sampling point (p < 0.001). The average offshore dissolution rate was 0.674 grams/hour compared to the nearshore dissolution rate of 0.410 grams/hour. Within the offshore site alone, the three sampling points all had dissolution rates significantly different from each other (p < 0.006). During the first sampling point during the waxing crescent phase, the
dissolution rate was 0.930 g/h, then dropped to 0.615 g/h from the waxing gibbous to the full moon, and then down to 0.476 g/h during the waning gibbous into the last quarter. The nearshore had no significant change in dissolution rates from 0.457 g/h during the waxing crescent to 0.428 g/h from the waxing gibbous into the full moon. However, both were significantly higher than the dissolution rate of 0.346 g/h from the waning gibbous into the last quarter (p < 0.004).

Zooplankton Community Seasonal Comparison

There was a significant interaction among year, site, and lunar phase for taxa richness (p = 0.001) and Simpson’s Inverse Diversity Index (p = 0.001). Taxa richness was significantly higher during the 2017 offshore waxing gibbous phase with seven taxa found compared to the 2016 nearshore waning gibbous, 2017 nearshore waxing gibbous, and the offshore 2017 waning gibbous, each with three to four taxa found (Fig 1.4a; p < 0.03). Simpson’s Inverse was 1.64 during 2017 offshore waning gibbous, which was significantly lower than all of the 2016 offshore communities and the 2017 offshore waxing gibbous and the 2017 nearshore waning gibbous (Fig 1.4b; p < 0.04). The 2017 offshore waning crescent Inverse Simpson Index was 1.74 and was significantly lower than the 2016 offshore waning gibbous and 2017 offshore waning gibbous (Fig 1.4b; p = 0.002).

There was a significant interaction between year and site for zooplankton abundance, with the offshore fall 2016 zooplankton community being significantly larger than the offshore community in winter 2017 and the nearshore communities during both sampling seasons, by almost four times (Fig 1.4c; p < 0.001). Fall 2016 zooplankton abundance was significantly
higher than the nearshore zooplankton abundance and significantly higher than both sites in winter 2017 (Fig 1.4d; p < 0.001).

There was a significant interaction for community composition between year and site (p = 0.007). Significant differences were found between phases of the moon both across and between the offshore and nearshore reef communities in fall 2016 (p < 0.002) and winter 2017 (p < 0.04). There was more variation found between communities across 2017 sampling than in 2016 (Fig 1.5). Additionally, in 2017 there are more distinct differences between nearshore and offshore zooplankton communities than in 2016.

**Zooplankton Community Full Lunar Cycle Comparison (Oct 2016)**

During fall 2016, zooplankton daytime abundance was three times higher on the offshore reef than the nearshore reef (p < 0.001). Zooplankton abundances were significantly higher during the full moon at both sites than at all other lunar phases. The nearshore community during the full moon was significantly higher than all other phases by two and a half to ten times higher (Fig 1.6a; p < 0.03) with no other significant differences seen between any other phases. At the offshore site alone, zooplankton abundances during the full moon phase were significantly higher than all other phases, ranging from two to twelve times higher (Fig 1.6b; p < 0.001). During the waxing gibbous phase abundance was significantly higher than during the waxing crescent, new moon, first quarter and waning crescent phases, ranging from three to six times higher (Fig 1.6b; p < 0.04).
There was a significant interaction between site and lunar phase for the Inverse Simpson Diversity Index (Fig 1.6c; p < 0.001). The Inverse Simpson’s Index was 1.26 during the nearshore first quarter and was significantly lower than all other phases at both sites, except for the nearshore waxing crescent (p < 0.003). The nearshore waxing crescent community had an Inverse Simpson Index of 1.44, which was significantly lower than all phases at both sites, except for the nearshore full moon and offshore first quarter (p < 0.05).

During fall 2016 sampling, there was a significant interaction between site and phase of the moon on zooplankton community composition (p = 0.01). At both sites, copepods and copepod nauplii are the dominant taxa throughout the entire lunar phase (Fig 1.5). However, at the offshore site, there is a higher relative abundance of barnacle nauplii during the waxing crescent and first quarter lunar phases, as well as an increase of chaetognaths during the waxing crescent, full moon, and waning gibbous phases. Further, more larvaceans were observed during the waning gibbous and last quarter phases at the offshore site (Fig 1.5; Table 1). At the nearshore site, only abundances of larvaceans appear to be of higher relative abundance, in addition to copepods and copepod nauplii, during the waxing gibbous and full moon phases (Fig 1.5; Table 2). At both sites, copepod nauplii decreased from the waxing crescent to the first quarter lunar phase, while adult copepods are increasing during this time. At the nearshore site, a more drastic transition occurred between these two groups as the adult copepods reach close to 100 % relative abundance, whereas they are less than 75 % of the relative abundance at the offshore site. During the waxing gibbous both groups return to making up close to 50 % of the relative copepod community. During the full moon, the offshore site had a slight increase in adult copepods and a slight decrease in copepod nauplii, whereas the nearshore site had an increase of almost 10 %
relative abundance in copepod nauplii and the same decrease in the adult copepod community. The communities return to near 50% relative abundance for the waning gibbous and last quarter phases at both sites.

The offshore zooplankton community richness was significantly higher with an average of just over five individual taxa (range: 4-8) compared to nearshore richness with an average of four individual taxa (range: 2-7) (p = 0.001). Offshore zooplankton richness was significantly higher during the full moon compared to the last quarter phase, with nearly twice the number of different taxa present (n = 7.3; p = 0.02). No significant differences were seen between lunar phases at the nearshore site.

2017 Winter Zooplankton Community Comparison

There were no significant differences in daytime zooplankton abundances across or within sites in winter 2017 (p = 0.814; p = 0.915) across the lunar phases or overall (p = 0.341; p = 0.295). A significant interaction was found for species richness between site and lunar phase during the daytime winter 2017 sampling. The offshore community during the waxing gibbous phase had a significantly higher richness, with on average seven individual taxa (range: 5-8), compared to the approximately four individual taxa (range: 3-5) found at the nearshore waxing gibbous phase and waning gibbous phase at both sites (Fig 1.7a; p < 0.05). The offshore communities alone also had significantly higher taxa richness during the waxing gibbous phase than the waning gibbous phase (Fig 1.7a; p < 0.02). There were no differences in the nearshore communities between lunar phases.
A significant interaction was found between site and phase for Simpson’s Inverse Index in winter 2017 (Fig 1.7b; p = 0.006). The offshore waning gibbous and waning crescent communities had an Inverse Simpson's Index below 1.75, which was significantly lower than the offshore waxing gibbous communities (Fig 1.7b; p < 0.02). No significant differences in diversity are seen between lunar phases across sites or within the nearshore site alone.

During winter 2017 sampling, there was a significant interaction observed for community composition between site and phase (Fig 1.5; p = 0.006). However, zooplankton community composition at the offshore site did not differ among lunar phases. In contrast, significant differences were found between lunar phases for zooplankton communities at the nearshore site (Fig 1.5; p = 0.002). The community was dominated by copepods, both adult and naupliar stages. At the offshore site, only chaetognaths had a slightly higher relative abundance during the waxing gibbous phase (Fig 1.5; Table 3). At the nearshore site, no other taxa made a large contribution to the zooplankton community (Fig 1.5; Table 4). However, distinctly different patterns were observed between the adult and nauplii copepods between sites. Throughout all five sampling points from waxing crescent to waxing crescent at the nearshore site, adult copepods slowly increased from about 25 % to just over 50 % relative abundance and copepod nauplii slowly decreased from about 75 % to 25 % relative abundance. During the three sampling points at the offshore site, adult copepods decreased from under 50 % relative abundance during the waxing gibbous phase to just below 25 % relative abundance during the waning gibbous and slightly increased again at the waning crescent phase. Copepod nauplii increased from 50 % relative abundance during the waxing gibbous phase to just under 75 % relative abundance during the waning gibbous phase and then slightly decreased during the waning crescent.
Nighttime zooplankton community abundance was more than 50% lower than daytime abundances (Fig 1.8a; p < 0.002). There were no differences in abundances among lunar phases when including nighttime sampling.

Taxa richness was 1.5 times higher for nighttime communities compared to daytime communities (p < 0.001), but there were no differences among lunar phases. There was a significant interaction between site and lunar phase for the Inverse Simpson Diversity Index (Fig 1.8b; p = 0.006). The nighttime waning gibbous Inverse Simpson Index was significantly higher than the daytime first waning crescent and waxing crescent lunar phases and the nighttime waning crescent phase (Fig 1.8b; p < 0.05). The nighttime waxing gibbous and second waning crescent phases were both significantly higher than the first daytime waning crescent and nighttime waning crescent (Fig 1.8b; p < 0.04). Finally, the nighttime waxing crescent was significantly higher than the nighttime waning crescent (Fig 1.8b; p < 0.04).

When comparing the daytime and nighttime zooplankton communities measured during the winter 2017 sampling, there were significant differences between day and nighttime zooplankton communities (p = 0.004), and between lunar phases (p = 0.001). The nighttime samples had the largest variation observed between zooplankton communities in this analysis (Fig 1.8c; Table 5). Additionally, cumaceans and polychaetes were only found in nighttime samples, and no daytime samples regardless of site or season. During the waxing gibbous phase, around 10% of the relative abundance was from chaetognaths. Larvaceans made up about 5% of the relative abundance from all phases, except the first waning crescent. Decapod zoea made up about 5% of the relative abundance during the first waning crescent and crab zoea abundance alone was just
under 5% during this phase. Isopods made up about 5% of the relative abundance during the final waning crescent phase. Ostracods made about 5% of the relative abundance during the waxing gibbous and final waning crescent phases. Adult and nauplius copepods had similar, but slightly different patterns during the daytime and nighttime samples. Adult copepods increased in relative abundance throughout the lunar phase sampling. During the daytime sampling, they started at the first waning crescent phase making up about 25% of the zooplankton community and increased to almost 60% by the final waning crescent sampling. During the nighttime sampling adult zooplankton started making up about 40% of the zooplankton community during the first waning crescent and waxing crescent phases, then increased to about 60% in the waxing gibbous and waning gibbous phases, and then increased to almost 80% in the final waning crescent phase. Nauplius copepods decreased from about 75% of the population down to 40% by the final waning crescent phase in the daytime communities. During the nighttime communities, the nauplius copepod population started at about 55% during the first waning crescent and waxing crescent phases, then decreased to 30-35% relative abundance during the waxing and waning gibbous phases, and then dropped to 10% in the final waning crescent phase.

Discussion

Across all temporal and spatial changes in zooplankton availability, the most striking was the significant increase in zooplankton abundance found in the offshore reef in fall 2016. However, since both sites had similar temperature ranges within sampling seasons, temperature is not likely to be a driving factor in different zooplankton abundances or communities between sites or across time. Temperature may be more important to consider when looking at time scales
longer than seasonal differences, as this may impact zooplankton physiology, survival, and fitness over time (Richardson et al., 2008). Temperature may also play an important role on shorter time scales, in terms of their nutritional availability to coral reef planktivores. Garzke et al. (2016) found that adult copepods decreased in abundance, while their content of saturated fatty acids increased with increased water temperatures.

The temporal and seasonal variation of nutrient concentrations found in this study reached nitrate and ammonium concentrations not typically seen on oligotrophic coral reefs. However, the reefs in Bocas del Toro, especially the nearshore reefs, are known to have higher nutrient enrichment due to run-off from land previously used as banana plantations, in addition to increasing development occurring on the island itself, and lower freshwater or oceanic input in its enclosed position within Almirante Bay (D’Croz et al., 2005; Guzman et al., 2005; Cramer, 2013).

Additionally, over the past decade, hypoxia has been documented on Bocas reefs, and higher nutrient concentrations could be responsible for these events (Altieri et al., 2017).

A correlation between higher nitrate concentrations and higher zooplankton abundances was previously reported on Bocas del Toro coral reefs (D’Croz et al., 2005). Interestingly, in the current study, the significantly higher zooplankton abundance during the offshore fall 2016 full moon did not co-occur with the highest nitrate concentrations. Increased nutrients may result in higher phytoplankton concentrations and, thus, increased food availability for zooplankton. However, increased food resources do not instantaneously result in increased zooplankton reproduction and growth, so collected nutrient results do not directly explain the significant increase in zooplankton observed in our study during the full moon. Rather, our results of unusually high nitrate and ammonium concentrations could suggest the large increase in zooplankton during the full moon, was the result of a previous increased nutrient conditions prior
to our sampling. This highlights the importance of continued sampling of environmental parameters to better understand and predict zooplankton availability to corals.

The most distinguishable difference between the offshore and nearshore environments is the significantly higher dissolution rates found on the offshore reef. These results are expected as the nearshore site is located in a protected semi-enclosed lagoon, compared to the offshore site which is less protected and directly connected to the open ocean. Flow generally occurs from the southeast to the northwest in this region (D’Croz et al., 2005), but detailed studies of currents in and out of Almirante Bay are unknown, but an understanding of the currents could determine the source of the zooplankton introduced to each reef. Zooplankton dynamics can be controlled by specific hydrodynamics, resulting in the flushing or retention of zooplankton on reefs (Carleton et al., 2001). A greater understanding of the local currents within these reefs could provide more insight into the zooplankton patterns seen in the present study. It is known that inside Almirante Bay, water conditions are driven more by the introduction of oceanic water than from runoff and freshwater inputs, based on historically similar salinity and nutrient measurement (D’Croz et al., 2005). Despite not knowing the specific water movements in and out of Almirante Bay, increased flow in the offshore site means increased zooplankton were likely transported on and off the reef, explaining not only differences in abundance observed between sites during one of the sampling seasons, but also the increased diversity seen at the offshore site, which is consistent with previous findings (Alldredge and King et al., 2009).

Site Differences

The increased abundance, diversity, and more unique zooplankton taxa community composition seen at the offshore site is likely due to the increased exposure to oceanic waters moving on and
off the reef compared to the more protected nearshore site (Guzman et al., 2005). The similarity between sites seen in winter 2017 may potentially be due to the lower rainfall in the winter, compared to the fall rainy season, resulting in less runoff leading to higher nutrient levels (D’Croz et al., 2005). The higher zooplankton diversity seen in the offshore site is consistent with previous studies (Valentin and Monteiro-Ribas, 1993; Darnis et al., 2008). This consistent diversity of the offshore zooplankton community is important when considering how the zooplankton community acts as a resource for corals recovering from heat-stress. Not all zooplankton are physically captured by corals (Sebens et al., 1996; Palardy et al., 2008). Some corals may increase their feeding behavior in comparison to others, and some may be better equipped for capturing zooplankton compared to others (Porter 1976, Sebens et al., 1996; Palardy et al., 2005). Previous studies have reported that the most common taxa captured by corals, determined by gut content dissections, are crab zoea, amphipods, and polychaetes, which all have poor swimming abilities and fit within the ideal size range for capture by coral tentacles of 200 – 500 µm (Nakajima et al., 2008). Leray et al., (2019) experimented with new molecular eDNA techniques to determine coral diets from their tissue and found that *Pocillopora eydouxi* consumed copepods, shrimp larvae, and polychaetes. While copepods are the dominant taxa found throughout our samples, they can have a relatively fast avoidance response and are often able to detect a moving coral tentacle and use avoidance behavior to prevent capture (Heidelberg et al., 2004).

Secondly, even considering only zooplankton taxa that are reliably captured by corals, there is variation in their nutritional quality. According to Glynn (1973), larvaceans have the highest C: N ratios (10.5), followed by barnacle nauplii and zoea (4.71), copepods have the third-highest (4.33) and chaetognatha and polychaetes have the lowest at just over 3.5. These ratios are
important for understanding how corals are benefiting from consuming the zooplankton around them. Consumption of zooplankton by corals is important for both the coral host metabolism and the coral symbionts (Johannes, et al., 1970; Glynn, 1973). Therefore, it is important to consider zooplankton community composition and their individual nutritional qualities in addition to zooplankton abundance when evaluating zooplankton communities as a food source for whole communities or ecosystems.

**Day and Night Differences**

Nighttime zooplankton abundances were unexpectedly significantly lower than daytime abundances, despite taxa richness and the Inverse Simpson Index often being significantly higher than daytime communities throughout sampling points. However, our average abundance of 200 individuals/m$^3$, is the minimum of the range found from previous studies on coral reefs using net tows (Houlbrèque, et al., 2004). Additionally, Ohlhorst (1982) found many copepods (adult and juvenile), barnacle nauplii, and larvaceans are of greater or equal abundance during the day than at night when sampling with emergence traps. While other studies consistently show an increase in zooplankton abundance during nighttime sampling, this is usually due to the introduction of demersal zooplankton in addition to oceanic zooplankton (Houlbrèque et al., 2004). However, since sampling occurred directly above the corals only a few hours after sunset, it is possible we sampled in the depleted bottom layer (Yahel et al., 2005). We assume that the lower nighttime zooplankton abundances are due to the fact that (1) demersal zooplankton had probably already risen off of the reef and moved higher into the water column, and not yet returned to the safety of the substrate, and (2) due to heavy predation from corals and planktivorous fish. If we had sampled higher in the water column or at the surface, or for a longer duration directly over the corals we may have seen the expected increase in nighttime zooplankton abundance by capturing
more of the demersal zooplankton community. Alternatively, our sampling methodology of using a net towed by SCUBA divers instead of using pumps may have contributed to the lower nighttime zooplankton abundance. Our net tow consisted of swimming 20 m across a reef, which took about five minutes, rather than a pump in a single location sampling continuously for one hour. It is also possible zooplankton were able to better avoid capture by the net compared to the pumps.

Zooplankton taxa community composition varied more between nighttime samples than daytime samples. This is likely due to the physical effect of changes in moonlight, which can change behavior of specific zooplankton taxa, resulting in copepods and zoea dominating zooplankton communities due to their response to light cues, and for other taxa like ostracods and decapod larvae to be more common with less moonlight exposure (Alledge & King, 2009). Leray et al. 2019, also showed similar patterns of an increase in crab zoea abundance during the night, and high abundance of copepods regardless of time of day. Their study found a large component of the zooplankton community to be eggs and gastropods, which were not included in our data analysis. Previous studies have shown that demersal zooplankton taxa are found in nighttime net tows along with oceanic zooplankton taxa, but their abundance is much lower than when captured by emergence traps (Alldredge and King, 1977). The sampling timing and methodological differences may explain our unexpected lower zooplankton abundance since we did not find a depleted layer during the day as the previous studies observed. On the other hand, our zooplankton community composition was similar to other studies (Yahel et al., 2005; Holzman et al., 2005).
**Lunar Phase Differences**

Our zooplankton taxa community composition results did not reveal any patterns explaining the variation in zooplankton abundance seen across lunar phases both within and across sites. It is known that variation in zooplankton communities can be driven by the distribution and behavior of, and interaction between, individual taxa (Carleton et al., 2001). However, the present study does not evaluate diversity within copepod genus and species. As copepods were the majority of the zooplankton community, it is possible there was an increase of certain copepod species, responsible for the variation in total abundance seen across lunar phases due to behavior or spawning. For example, common copepod genera found on coral reefs, including *Acartia*, *Oithona*, and *Centropages*, may be aggregated in dense swarms, which when collected in a net tow can result in densities higher than the surrounding average plankton density by an order of 2-3 magnitudes (Omori & Hamner 1982; Carleton & Hamner, 2007). These swarms allow for protection against predation and result in increased reproduction opportunity, and allow for populations to grow on the reef (Hamner & Carleton, 1979). Light appears to be an important cue for the formation of the swarms, and at night the swarms break up, which allows for less feeding competition (Buskey et al., 1996). Additionally, many copepods may unintentionally form temporary swarms from the impact of physical changes in the water resulting in copepods switching their swimming speeds or directions. Physical environmental changes, such temperature or salinity, may impact mean copepod density (Harder, 1968; Pineda, 1991).

Additionally, while there is limited knowledge of zooplankton taxa behavior, even less is known about individual zooplankton behavior, and associate variation, which also could be a large component of zooplankton patchiness, indicating the importance of further research (Hamner, 1988).
Unfortunately, a second full lunar cycle sampling was not possible in winter 2017, restricting our ability to better determine which patterns in fall 2016 might be directly related to lunar phase impacts. Based on the sampling completed, it is likely the increased flow and potentially previously increased nutrient levels at the offshore site, could explain these patterns. These results are an important contribution to beginning to better understand and predict zooplankton availability to corals, but continued sampling is needed to determine how closely these environmental effects are correlated with lunar phase to determine if lunar phase could be a reliable predictor of zooplankton availability to corals.

**Seasonal Differences**

Our results indicate that during fall 2016, the offshore zooplankton community was higher in abundance and diversity (Fig 4). The community composition was similar to other studies with copepods dominating the community and barnacle nauplii and larvaceans being two of the next most common taxa (Heidelberg et al., 2004). Again, a better understanding of the specific copepod taxa community dynamics may provide insight as the copepod swarming described above has been reported to have seasonal patterns between different copepod taxa (Hamner & Carleton, 1979). The increased abundance and diversity of the offshore zooplankton community in fall 2016, resulted in those corals being provided with better resources in comparison to the nearshore reef and the winter 2017 reef systems. In Bocas del Toro, October often has water temperatures 2 °C above ambient (~28 °C), indicating a routinely stressful time for corals (Neal et al., 2014). Therefore, this greater abundance of diverse zooplankton taxa may be an important resource, allowing these corals to be less susceptible and show greater recovery from bleaching events.
Larval zooplankton are present in zooplankton samples throughout the year, but are more common in the latter half of the year when reproduction is more concentrated (Glynn, 1973). The latter half of the year is the rainy season (D’Croz et al., 2005), which has been shown, along with increased wind velocities, to impact plankton populations (Glynn, 1973). Additionally, the zooplankton’s nutritional value can vary greatly across seasons, which is usually attributed to changes in the age distribution of individuals and overall species composition of the zooplankton community throughout the year (Platt et al., 1968). In that study, the overall abundance of zooplankton showed a 100-fold variation throughout sampling, and a 14-fold variation was found in the dry weight of the standing stock. The seasonal changes in total zooplankton calories per unit volume of water were lower than any other available index of zooplankton standing stock by about a seven to eight-fold variation. This means that despite the remarkable seasonal progression of how energy is presented to higher trophic levels, the actual amount available to the community may stay relatively constant.

While both sites experience almost annual stress events, the offshore site shows fewer corals susceptible to bleaching and overall shows greater recovery from bleaching that does occur (personal observation). These results of higher and more diverse zooplankton communities accessible to corals throughout a month in which they are typically experiencing heat-stress, emphasizes the importance of better understanding zooplankton availability to corals during heat stress events, as it may be a contributing factor in assessing a coral’s ability to recover. Previous studies looking at zooplankton composition have also had mixed results with seasonal differences in zooplankton communities (Houlbrèque et al., 2004), indicating the need for increased sampling to better understand seasonal patterns and what is or is not driving change.
Chlorophyll-a concentrations were not measured during this study, and satellite derived sea surface chlorophyll-a is not readily available at a fine enough scale to compare across sites and across seasons for this study. However, incorporating localized chlorophyll-a measurements would be critical for future studies as Fox et al. (2018) showed a connection between primary production patterns and the nutrition of coral communities. Increased chlorophyll-a concentration resulted in the carbon isotope signatures of coral hosts more closely aligning with coral reef zooplankton carbon isotopes. This indicates that corals will increase heterotrophy as a function of food availability when considering a regional or global scale. Comparing these results at a local level will provide more insight into specific coral host behavior and the ability to capitalize on increased food availability.

**Conclusion/Further Directions**

The design of this study was to collect zooplankton immediately available to corals by swimming with a plankton net directly above the reef. However, due to the varied topography of corals along the transects, we were not swimming at a constant depth throughout each transect. Allderege & King (2009) showed that zooplankton abundance had a greater variation between a few centimeters of depth than between day and night or between seasons. Their results led them to conclude that the combination of the height of the corals and their specific proximity to zooplankton may be an important indicator for the ability of corals to grow faster or better recover from bleaching. Therefore, it is important to acknowledge the difficulties of determining the true food availability to all corals on coral reefs.

This study focused exclusively on mesozooplankton communities, but it is important to also consider micro, nano or picoplankton (Houlbrèque, 2003) and phytoplankton concentrations
(Pinto-Coeho et al., 2005). Understanding the changes in coral reef zooplankton community structure, biodiversity, and overall abundances over the coming years is extremely important in understanding coral reef health, and how rapidly it is changing (Chiba et al., 2018). To do this effectively, it is critical that a uniform and accessible zooplankton sampling protocol is produced that can be used to effectively compare across time and across reefs as best as possible. While the various sampling methodologies have their pros and cons, it is important that these discrepancies do not limit our ability to effectively track zooplankton abundance across time and space. Therefore, continued efforts must be made for more routine sampling to occur to document and understand these changes in the future (Chiba et al., 2018).

An improved understanding of zooplankton community dynamics is important to aquatic systems beyond coral reefs. Many larval and adult species rely on zooplankton regularly at specific time intervals, and if these are changing based on environmental cues or factors, this can cause regime shifts. For example, Möllmann et al. (2008) described a shift in dominance between two copepods, resulting in a decline of the cod population and an increase in the sprat population. Zooplankton also play an important role in the rate of change seen in physical parameter shifts in the ocean through their role in the biological pump. Much of the CO$_2$ fixed by phytoplankton is eaten by zooplankton and sinks to the seabed when the zooplankton die, allowing this carbon to be kept in sediment and out of the carbon cycle (Richardson, 2008).

Our main takeaway from this study is that major spatial and temporal differences were observed in zooplankton community dynamics across an offshore and nearshore coral reef in Bocas del Toro, Panama. The most abundant and diverse zooplankton communities were found during a period of heat-stress, on a reef showing less susceptibility and greater recovery from bleaching
events. Better understanding of how this zooplankton community specifically is being utilized as part of corals’ diet, could provide important insight for coral reef management, to better understand the patterns of zooplankton on coral reefs, and if this resource will continue to allow heterotrophic plasticity to protect reefs from heat-stress.
Literature Cited


Ferrier-Pagès C., Sauzeat, L., Balter. 2018. Coral Bleaching is linked to the capacity of the animal host to supply essential metals to the symbionts. Global Change Biology, 24(7), 3145-3157.


Fig 1.1. Map of Study Sites in Bocas del Toro, Panama. Blue indicates the nearshore site inside Almirante Bay, a semi-enclosed lagoon. Orange indicates the offshore site, just outside of Almirante Bay and more exposed to the open ocean.
Figure 1.2 Zooplankton Sampling Occurrences Across Season, Site, and Lunar Phase. Sampling occurrences for each site are noted above with lunar phases sampled in 2016 above the images and those sampled in 2017 below the images.
Fig 1.3. Nitrate Concentrations. Individual nitrate measurements (µM) taken throughout both sampling seasons. Nearshore sites are open symbols and offshore sites are filled. Circles represent sampling in fall 2016 and triangles represent sampling in winter 2017. The offshore and nearshore nitrate values were identical in fall 2016 and are overlapping for the Waning Gibbous phase.
Figure 1.4. Seasonal Comparison between Zooplankton Communities. a) Zooplankton taxa richness (number of individual taxa), b) Inverse Simpson Index. In panels (a) and (b), fall 2016 sampling are the lighter colors, and winter 2017 are darker. c) Average zooplankton abundance for each site in each sampling season (individuals/m$^3$). d) Raw zooplankton abundance fitted with spline-based on the number of days sampling took place from the full moon. Nearshore sites are represented in blue and offshore in orange. Error bars represent standard error and letters denote levels of significance within each panel.
Fig 1.5. Zooplankton Taxa Relative Abundance. Panels are the left represent the relative abundance of zooplankton in fall 2016 and the right for winter 2017. The top row is the offshore site and the bottom row is the nearshore site. The five most common taxa have their individual relative abundances displayed. All other taxa up make up less than 5% of the community and are grouped together in the ‘other’ category. See Table 1 for a breakdown of the full community description.
Fig 1.6. Fall 2016 Zooplankton Community Dynamics. a) Nearshore zooplankton abundance (individuals/m$^3$). b) Offshore zooplankton abundance (individuals/m$^3$), and c) Inverse Simpson Index. In all panels, the nearshore site is represented in blue and the offshore in orange. Error bars represent standard error and letters denote significant differences within each panel.
Fig 1.7. Winter 2017 Zooplankton Community Dynamics. a) Species Richness (number of individual taxa), and b) Inverse Simpson Interaction. Nearshore sites are represented in blue and offshore sites in orange. Error bars represent standard error and letters denote significant differences within each panel.
Figure 1.8. Daytime and Nighttime Zooplankton Community Differences. a) Zooplankton abundance (individuals/m$^3$), b) Inverse Simpson Diversity Index, c) zooplankton taxa relative abundance, the most abundant nine taxa are plotted, and the rest are combined into other. See Table 2 for further breakdown of other species.
Table 1.1 2016 Offshore Zooplankton Taxa Abundance

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Table 2. 2016 Nearshore Zooplankton Taxa Abundance

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Table 3. 2017 Offshore Zooplankton Taxa Abundance

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Effect of Enhanced Feeding on Symbiont Quality in *Porites furcata* after Heat-Stress Events

Abstract
Coral reefs are threatened by the increasing prevalence of heat-stress events, resulting in coral bleaching and a loss of energy resources. Although some corals can compensate for this energy loss through increased feeding, only limited knowledge exists on the role food availability plays in this recovery mechanism. This study explores the effect of enhanced feeding availability on natural zooplankton communities on the symbiont quality in *Porites furcata*. Corals were collected and separated into heat-stress or control temperature treatments in the lab and then returned to the reef for five weeks of recovery in one of the following four treatments: Ambient Fed – Ambient Temperature, Ambient Fed – Heated Temperature, Enhanced Fed – Ambient Temperature, and Enhanced Fed – Heated Temperature. Measurements of symbiont densities, chlorophyll concentrations, maximum quantum yield, photosynthesis rates, and photosynthetic efficiency were taken before and after recovery. Heat-stressed corals recovered to control values in all parameters during the five weeks of recovery regardless of feeding treatment. Non-stressed (control) corals with enhanced feeding showed increases in quantum yield, chlorophyll concentration, and symbiont densities compared to all other treatments, while heat-stressed corals in the ambient fed treatment showed a significant increase in photosynthesis rates and photosynthetic efficiency. Enhanced zooplankton allowed non-stressed corals to build up their symbiont densities and symbiont qualities, and heat-stressed corals to regain their symbiont qualities. Therefore, understanding zooplankton abundance on coral reefs will enhance our ability to predict how reefs will recover from heat-stress events.
Introduction

Healthy symbiotic corals gain energy and nutrients through two mechanisms: photosynthesis and heterotrophy (Goreau et al., 1971). Single-celled dinoflagellate symbionts live inside the coral colony's inner gastrodermal layer and translocate products from photosynthesis to the coral animal (Oakley & Davy, 2018). All coral symbionts are from the family Symbiodiniaceae but consist of many different species and are associated with different coral hosts, geographic location, and ecological breadth (LaJeunesse et al., 2018). Heterotrophic feeding occurs for most corals predominantly at night, when photosynthesis is not occurring and when zooplankton vertically migrate higher in the water column are thus, are more available as a food resource to the reef (Yahel et al., 2005; Heidelberg, et al., 2010). The ratio of photosynthetic to heterotrophic energy gain differs between coral species and is dependent on environmental conditions (Houlbrèque et al., 2009).

Gustafsson et al. (2013) provides a basic framework explaining the importance of gaining nutrients from both mechanisms for corals in order to remain healthy from internal exchange and recycling of essential nutrients. Symbionts are able to translocate up to 95% of their photosynthetic production and selectively leak amino acids, sugars, complex carbohydrates, and small peptides to their coral host (Hoegh-Guldberg et al., 1999). Heterotrophic feeding provides the coral with nitrogen, phosphorous, and other important nutrients that are not consistently available through photosynthesis (Houlbrèque and Ferrier-Pagès, 2009). Such heterotrophic carbon and nitrogen can be allocated directly to coral tissues and subcellular structures for host lipid stores (Krueger et al., 2018).
Numerous studies using a variety of coral species have shown that the health of heterotrophically starved corals significantly declines, both in terms of symbiont density and function, compared to fed corals (Hoogenboom et al., 2012; Titlyanov et al., 2001; Houlbrèque and Ferrier-Pagès, 2009). Chlorophyll concentrations and photosynthesis rates are higher in fed than starved corals but can take over one month for this difference to be relevant (Houlbrèque et al., 2003). After tracking host assimilation of photosynthates in *Stylophora pistillata*, no differences were found between fed or starved corals for this metric; however, symbionts themselves had a 10% increase in assimilation in fed corals, and carbon from photosynthesis was directed towards host lipid bodies (Krueger et al., 2018).

When corals are exposed to heat-stress through increasingly warming ocean temperatures, this disrupts the relationship between corals and their symbionts, known as coral bleaching, and results in depletion of the energy and nutrients translocated to the coral from photosynthesis (Oakley & Davy, 2018). This has resulted in approximately a 50-75% decline in live coral cover in nearly all regions of the world over the past 30-40 years (Bruno et al., 2019). For over a decade, studies have been investigating the role of heterotrophic plasticity as a recovery mechanism from coral bleaching (Grottoli et al., 2006; Palardy et al., 2005; Levas et al., 2015). Additional studies indicate heterotrophy can play an important role in susceptibility to bleaching before and during heat stress events (Hoogenboom et al., 2012). Ferrier-Pagès et al. (2010) supported this by showing that despite different coral species showing increased or decreased feeding rates in response to thermal stress, fed corals of all species consistently prevented damage to the symbiont’s photosystems, whereas starved corals showed a decrease in electron transport and photosynthetic rates in addition to decreased chlorophyll concentrations and
photoinhibition in photosystem II. Recent studies suggest important metals such as manganese, magnesium, and iron that are used in photosynthesis and for anti-oxidant production are only accumulated through heterotrophic feeding (Ferrier-Pagès et al., 2018). These metals have been found in tissues of corals exposed to thermal stress that did not bleach and were found to be drastically decreased in corals that did show bleaching. Additionally, the corals which were heated and fed did not show bleaching nor any decrease in their photosynthetic performance. These resources from feeding have been found to mitigate bleaching impacts by providing nutrients to maintain photosynthetic functioning or to replace damaged photosynthetic complexes (Gustafsson et al., 2013).

Although zooplankton are important to coral resistance and recovery from heat events, little attention has been directed towards understanding this in the context of natural zooplankton communities on coral reefs. Knowledge of zooplankton community dynamics on specific reefs could be an important indicator of why some reefs are more severely impacted by heat stress events than others in close proximity with both experiencing the same thermal histories. Previous studies have shown enhanced feeding opportunities do not result in increased feeding rates or increased benefit for coral symbionts (Palardy et al., 2008; Ferrier-Pagès et al., 2003). However, in order to determine accurate feeding rates, corals must be starved prior to feeding exposure and allowed to feed for a short time (about an hour) to ensure an accurate calculation of zooplankton ingested over time. Although these methods are important for accurately determining feeding effort and ability in response to thermal stress, they do not portray an accurate account of coral use of natural zooplankton as a resource over the duration of a recovery period.
Our study was designed to address whether enhanced natural zooplankton accessibility throughout a five-week recovery period on a reef, increased the ability of *Porites furcata* symbionts to recover from heat stress compared to *P. furcata* symbionts exposed only to natural ambient zooplankton communities. The metrics of interest were maximum quantum yield, chlorophyll concentrations, symbiont densities, and photosynthetic rates.

**Methods**

*Site Description*

This study was conducted at site Punta Caracol inside Almirante Bay near the Smithsonian Tropical Research Institute (STRI) field station in Panama. *P. furcata* was used in this study because previous work at this site confirmed it was an abundant coral species and previously displayed heterotrophic plasticity in response to stress events (Seeman, 2013).

*Experimental Description*

On 4 January 2017, 18 fragments were collected from four *P. furcata* colonies. They were immediately transported to 50-gallon water tables with water flowing indirectly from the lagoon outside STRI, located approximately 3 km from the collection site, allowing for similar water quality conditions with ambient ocean temperature ~ 29 °C at both locations. All coral fragments were acclimated under these conditions for three weeks. All corals were split between two 10-gallon tanks, one kept at the ambient temperature (~29 °C) and one exposed to heat-stress. One tank was used for each temperature treatment to ensure all corals within a treatment underwent the same temperature exposure, which cannot be accurately controlled for across multiple tanks due to frequent power outages and unpredictable changes in water flow. Heat stress was
introduced by increasing water temperature to 30 °C and then 0.5 °C each day up to 32 °C, the maximum water temperature recorded in the area over the past decade, and kept at 32 °C for two weeks until the first visible signs of bleaching were noticed, at which time the water temperature was brought down to 31 °C. It took another ten days at 31 °C until the majority of fragments displayed visible signs of bleaching, and all corals were returned to ambient temperatures for three days to take post-stress measurements.

From each colony, 12 coral fragments were used for the duration of the experiment. The remaining six coral fragments from each colony were sacrificed to estimate symbiont densities and chlorophyll concentrations for post-stress measurements for both heat-stressed and ambient corals (Fig 2.1). The post-heat-stress measurements were used to confirm whether heat-stressed corals were impacted by the stress when compared to pre-stress measurements and at the beginning of recovery measurements. These measurements were used to calculate the percent change within treatment over the duration of the recovery period.

After post heat-stress measurements, all 48 corals were then returned to the reef they were collected from for a five-week recovery period in either an enhanced or ambient feeding treatment, resulting in the creation of four experimental treatments: Ambient Fed – Ambient Temperature (AFA), Ambient Fed – Heated Temperature (AFH), Enhanced Fed – Ambient Temperature (EFA), and Enhanced Fed – Heated Temperature (EFH) (Fig 2.1). The enhanced feeding treatment was created with an underwater light system programmed using an Arduino microcontroller and powered by Duracell D-cell batteries inside a waterproof case. The light source consisted of five white LEDs that gave off approximately 5 µmol photons/(m² sec) of
light, ensuring it would attract plankton but would not allow for net primary production. The ambient feeding treatment was separated from the enhanced feeding treatment by a large mounding coral to ensure the only difference in environmental conditions was plankton availability.

Corals were monitored weekly both during the day and night. To quantitatively compare the difference in zooplankton abundance between the enhanced and ambient feeding treatments, 60 ml syringes were used to collect plankton directly above the corals in triplicate. At the end of the recovery period, all corals were collected from the reef and brought back to the lab for final measurements.

Measurements

*Seawater Conditions*

Seawater samples to determine carbonate chemistry were collected in 250 ml borosilicate glass bottles sealed with a greased stopper and were immediately poisoned with 250 µl saturated HgCl solution. Additional seawater samples were collected in 50 ml Falcon (Fisher Scientific) tubes to determine salinity, pH, and nutrient measurements. Samples were collected from the field site during the initial coral fragment collection and weekly during both the laboratory heat-stress period and *in situ* throughout the recovery period on the reef. Seawater carbonate chemistry conditions were determined with high precision measurements (calibrated with Certified Reference Materials) of total alkalinity (TA) and dissolved inorganic carbon (DIC) via closed-cell potentiometric titration and coulometry, respectively, using a VINDTA 3C (Marianda) system in the Justin Ries Laboratory at Northeastern University. The program CO2SYS (Lewis
et al., 1998) was used to calculate seawater $p$CO$_2$, pH, [CO$_3^{2-}$], [HCO$_3^-$], aqueous CO$_2$, and calcite saturation state from measured DIC, TA, temperature, and salinity. Ammonium (NH$_4^+$) was measured colorimetrically on a GENESYS 30 visible spectrophotometer (Thermo Scientific) following protocols from Solórzano (1969). Water temperature was recorded throughout the experiment with each treatment using Onset HOBO data loggers recording every 15 minutes.

**Maximum Quantum Yield ($F_v/F_m$)**

A Diving PAM (Pulse Amplitude Modulation) fluorometer (Walz) was used to take baseline, post-stress, mid-recovery, and final recovery measurements for maximum quantum yield ($F_v/F_m$) of Photosystem II (PSII). $F_v$ is the difference between minimum ($F_o$) and maximum ($F_m$) fluorescence, which was calculated by measurements made by the PAM fluorometer on dark-adapted photosystems (Finelli et al., 2006). This is the only parameter monitored at the mid-recovery time point as it could be taken on the reef without disturbing the corals during recovery. All fragments were dark-adapted for at least one hour prior to measurement to ensure their photosystems were completely shut down to determine an accurate measurement of photosynthetic efficiency. Each coral had five measurements taken on different areas to obtain an average maximum quantum yield ($F_v/F_m$) for each coral.

**Surface Area Calculations**

Surfaces areas were calculated using the novel methodology of 3D modeling that accounts for changes in surface area throughout the duration of the experiment. I designed the protocol used for this experiment which consisted of creating 3D models by taking a video of each fragment from multiple angles starting at the base and rising to the top of the coral. These videos were
split into 250 still images and uploaded into either PhotoCap (Autodesk) or Photoscan (Agisoft). The base of the corals was a known distance that was used to calculate a scale bar for each individual model. The rendered models were then cropped so only live tissue remained in the model and the above software was used to calculate surface area.

*Symbiont Density, Chlorophyll a, & Chlorophyll c2 Calculations*

All corals, including the nine preserved for pre- and post-stress measurements, were put in a -20 °C freezer immediately after final measurements were taken at the end of each stage of the experiment. After corals had been frozen for at least 24 hours, tissue was removed with an airbrush with filtered seawater, homogenized, and stored in 50 ml Falcon (Fisher Scientific) tubes at -20 °C until ready to be processed. Frozen samples were thawed in a fridge and the following process was conducted in the dark. The samples were centrifuged at 4100 RPM for 20 minutes, the supernatant discarded, and the symbionts resuspended in 1 ml of filtered seawater. This solution was vortexed and a 50 µl subsample was taken and kept in the fridge to measure symbiont densities later that same day. The remaining solution was centrifuged again at 13,000 RPM for 13 minutes, the supernatant removed, and 0.5 ml of acetone was added. A metal spatula was used to crush the pellet up in acetone and then was rinsed with another 0.5 ml acetone into the sample to ensure no chlorophyll was lost. Samples were left in the fridge for extraction for 24 hours and then centrifuged for 10 min at 13,000 RPM. Absorbance values were then taken on a Nanodrop microvolume spectrophotometer (Thermo Scientific) and final chlorophyll concentrations were determined according to Jeffrey and Humphrey (1975). These concentrations were normalized by surface area of live tissue. Symbiont densities were determined by pipetting 10 µl into a hemocytometer and counting at least 300 cells. This was
repeated three times for each coral and an average was taken to determine a final symbiont density normalized by the surface area of live tissue.

**Gross Photosynthesis Rates and Photosynthetic Efficiency**

Ten clear plastic 500 ml chambers were filled with seawater filtered on 0.45 mm GF/F filters. An optical fluorescence oxygen sensor (PyroScience Firesting) was placed inside nine of these chambers. All sensors were calibrated with 100% oxygen saturation at sea level pressure. The PyroScience Firesting fiberoptic cables were attached to continuously read the oxygen levels inside the chambers. Eight of these chambers were used to determine photosynthesis and respiration rates on the corals and one served as a control. The final chamber had a temperature sensor used to automatically compensate oxygen fluctuation based on any minimal temperature changes. All chambers were placed on magnetic stir plates to ensure continuous mixing. Change in oxygen concentration was measured for one hour while corals were exposed to aquarium LED lights to determine net photosynthesis. Corals were then kept in complete darkness for one hour to ensure no photosynthesis occurred, and a change in oxygen concentration was measured for another hour in complete darkness to determine respiration rates. This process was repeated twice a day for three consecutive days to obtain measurements for all 48 coral fragments. Rates were normalized to surface area. Gross photosynthesis was calculated by adding the absolute values of net photosynthesis and respiration. Photosynthetic efficiency was calculated by dividing gross photosynthesis by the absolute value of respiration.
Statistical Analyses

The effect of food availability on both heat-stressed and non-heat stressed symbionts in the corals was analyzed by comparing the differences in parameters measured across treatments at the end of the recovery period, as well as the percent change between each parameter measured at the beginning and end of the recovery. For chlorophyll and symbiont density which required the destruction of samples, heat-stressed and non-heat-stressed population averages were calculated during the post-stress time point. These population averages were used as the beginning recovery measurement to compare all experimental corals to the termination of the experiment to calculate the percent change from beginning to end of recovery.

Statistical analysis and resulting figures were developed using R Studio Version 1.1.463. Data were checked for normality by viewing a histogram of the data distribution and by calculating a skewness value from the R package ‘moments’. If data did not fit a normal distribution, square root or Box-Cox transformations were applied to the data. If this resulted in a normal distribution and equal variances, results were analyzed using a two-way linear mixed-effects model with fixed effects of temperature and feeding and random effects of colony and predation. (Neither colony nor predation had a significant effect on the analysis). If the data could not be transformed to a normal distribution, a generalized linear mixed-effects model was applied with the same effects. Statistical tests were made at the 5 % significance level.

Various levels of predation did occur on some corals throughout the recovery period on the reef. Both predation and colony were included as a random effect in all statistical models but had no significant effect on the model, and therefore did not interfere with the results of this experiment.
Three frags were lost entirely to predation so were not included in any of the analyses. Additionally, two individuals with surface areas less than 2 cm² were removed for chlorophyll a and c2 analysis. For symbiont densities and photosynthesis rates and quantum efficiency analyses, only one individual less than 1 cm² was removed from the analysis.

**Results**

Water chemistry results showed $\rho$CO₂ values both on the reef and in the tanks were consistently below 500 ppm. Ammonium levels were variable both on the reef and in the tanks. During collection, the reef concentration was 7 µM. Ammonium levels at the beginning of heat stress showed elevated levels of 50 µM for the heated tank and 22 µM for the ambient tank, while measurements taken two weeks later showed both heated and ambient tanks had ammonium levels of 14 µM. During the first four weeks of recovery on the reef ammonium levels were 7 µM, 9 µM, 15 µM, and 6 µM, respectively.

A large amount of zooplankton were visible by divers directly above the corals in the enhanced feeding treatment was observed during every weekly night dive during the five weeks of recovery, and zooplankton samples showed the enhanced feeding treatment ranged from a 3-fold to 20-fold increase in zooplankton abundance compared to the ambient feeding treatment, throughout the five-week recovery period. Despite the difference in overall abundance, there was still high diversity in both feeding treatments including copepods, nauplii, cladocerans, larvaceans, chaetognaths, and isopods.
Both feeding treatments experienced predation effects but GoPro video cameras in underwater housings deployed overnight, as well as observations during the weekly night dives, revealed only one occurrence of a parrotfish present at the enhanced feeding treatment. During daytime dives during the five-week recovery period, damselfish were commonly found near both enhanced and ambient feeding treatments. At the end of the experiment, three corals were no longer present, three corals were mostly eaten, three corals were partially eaten, and eight had just tips that were eaten. These predation events occurred across all four colonies and all four treatments.

*Maximum Quantum Yield ($F_v/F_m$)*

After two weeks of recovery on the reef, non-stressed corals with enhanced feeding had statistically significantly higher $F_v/F_m$ values compared to stressed corals in both feeding treatments (Fig 2.2; $p < 0.04$). The interaction between temperature and feeding was significant ($p = 0.03$), while no significant differences were found between temperature or feeding treatments themselves. After both five weeks of recovery, no significant differences were seen in $F_v/F_m$ values across treatments or the interaction between treatments. Although there was a slight decrease seen across all treatments in $F_v/F_m$ values over the last two weeks of recovery, there was no significant difference found between $F_v/F_m$ values between mid- and end-recovery. Corals exposed to heat stress showed a significant increase in the percent change of $F_v/F_m$ values from the beginning to mid-recovery and beginning to end of recovery (Fig 2.2; $p = 0.02$; $p = 0.04$).
Chlorophyll and Symbiont Densities

Heat-stressed corals had 24% lower chlorophyll a and 23% lower chlorophyll c2 concentrations than non-heat stressed corals at the beginning of recovery. After five weeks of recovery on the reef, chlorophyll a and chlorophyll c2 values were significantly higher in the enhanced-feeding treatments regardless of the effect of temperature (Fig 3; p = 0.013; p = 0.03). For both chlorophyll a and c2, corals in the enhanced-feeding treatment that were not exposed to heat stress, had significantly higher chlorophyll concentrations than corals exposed to heat stress regardless of feeding treatment (p = 0.003; p < 0.006). Enhanced-feeding corals regardless of temperature had a significant increase in the percent change of chlorophyll a and chlorophyll c2 concentration from the beginning to end of recovery (p < 0.029; p < 0.03). At the beginning of the recovery period, heat-stressed corals showed a 61% decrease in symbiont density compared to non-stressed corals. At the end of five weeks of recovery, corals exposed to enhanced feeding had significantly higher symbiont density than corals exposed to ambient feeding regardless of heat stress (p = 0.038). From the beginning to the end of the recovery period, corals exposed to heat stress showed a significant increase in their percent change in symbiont density compared to non-stressed corals regardless of feeding treatment (Fig 2.3; p < 0.004). Additionally, all heat-stressed corals showed a significant increase in percent change of symbiont densities compared to non-stressed ambient fed corals (p < 0.02).

Photosynthetic Rates & Photosynthetic Efficiency

Heat-stressed corals with ambient feeding had significantly higher photosynthetic rates compared to non-stressed corals exposed to enhanced feeding (Figure 4; p = 0.03), but no difference between individual treatments was seen in photosynthetic efficiency. Heat-stressed corals had
significantly increased percent change in their photosynthetic rates and efficiencies from the beginning to the end of the recovery period compared to non-heat stressed corals (Fig 2.4; p < 0.001).

**Discussion**

This study demonstrates that heat-stressed corals regardless of feeding treatment showed significant increases in the percent change of symbiont density, maximum quantum yield, photosynthesis rates, and photosynthetic efficiency from beginning to the end of recovery. Both chlorophyll and c2 concentrations showed a significant increase in percent change from beginning to end of recovery in enhanced fed corals compared to ambient fed corals regardless of exposure to heat stress. We also found that regardless of feeding treatment, all corals exposed to heat stress showed full recovery in their symbiont functioning after five weeks of recovery on the reef. Despite the increased symbiont quality in enhanced fed corals at the end of recovery, these corals showed significantly lower photosynthetic rates compared to ambient fed corals, regardless of heat stress.

*Enhanced Feeding without Heat Stress*

Non-stressed corals in the enhanced feeding treatment had significantly higher chlorophyll a and c2 concentrations than heat-stressed corals regardless of feeding, and also had the highest symbiont densities of all treatments. Although statistically, the enhanced feeding treatment regardless of temperature showed a significant increase in symbiont density, it is also due to the increase in the non-stressed enhanced fed treatment alone. Few studies have looked at the effect of increased natural zooplankton abundance on corals compared to ambient concentrations.
Ferrier-Pagès et al. (2003), found no significant difference in symbiont qualities of corals fed for three hours equivalent concentrations of natural plankton either two or six times a week. In contrast, many other studies have shown that fed corals have significantly higher symbiont densities and chlorophyll concentrations compared to starved corals (Muscatine et al., 1989; Ferrier-Pagès et al., 2003; Houlbrèque et al., 2003; Houlbrèque et al., 2009; Titlyanov et al., 2001) indicating enhanced zooplankton availability to non-stressed corals allows for either the coral host or symbionts to allocate more resources to chlorophyll production for *P. furcata*.

Additionally, feeding has been shown to be a critical factor for corals needing to increase symbiont density under low light conditions (Titlyanov et al., 2001). Translocation of photosynthates from the symbiont to the coral host is most efficient in well-fed corals under ambient conditions (Tremblay et al., 2014). Therefore, it is unlikely the increased symbiont densities are due to the symbionts no longer providing to the coral host, despite its heterotrophic behavior. Recent technology has traced heterotrophic carbon and nitrogen from traced brine-shrimp to better understand how heterotrophic resources are allocated to the coral host and symbiont in ambient and heat-stress conditions, and better explain the role of feeding in increase chlorophyll and symbiont density as seen in the present study (Krueger et al., 2018).

Regularly fed corals use the heterotrophic input to provide coral symbionts with N and P, in addition to increasing symbiont densities and host tissue thickness. Due to the short time period (~6 hours) for heterotrophic C and N to be assimilated into the symbionts, it appears that the symbionts are able to directly assimilate N from coral heterotrophy rather than recycled from host metabolism. This direct acquisition of nutrients from heterotrophy by the symbionts is
mainly from respiratory CO$_2$ and organic and inorganic N from food breakdown. Increased metabolic CO$_2$ may indicate the coral host is allocating heterotrophic food for catabolic activity. Under elevated temperatures would improve energy production for the coral host while also likely enhancing symbionts by allowing for increased nutrient fixation and internal recycling, but further analysis is being conducted to determine if this is happening. However, symbionts in regularly fed corals, do not rely only on heterotrophic input, as these regularly fed corals also show a significant increase in autotrophic C assimilation regardless of exposure to heat stress, and this assimilated carbon is primarily used as a benefit for the symbionts themselves, resulting in individual cell and population growth. However, coral feeding did not mitigate the autotrophic acquisition of C or N during heat-stress. Hughes et al., 2010 demonstrates the two principle ways that heterotrophic C is acquired by the coral host and symbionts; (1) symbionts receive DIC from heterotrophic carbon respired by coral host implying recycling of heterotrophic carbon in the coral holobiont or (2) direct transport from coral host to symbionts as DOC. Skeletons from corals that have been bleached displayed are not really isotopically enriched, it appears heterotrophic carbon is primarily translocated from the coral host to the symbionts as DOC. This pathway of organic carbon to the symbionts during a heat-stress event allows the symbionts to better maintain and recover from the stress event. In summary, regardless of if the symbionts are improved from heterotrophic feeding via DIC from coral host respiration or directly accumulating the organic C or N, feeding is an important resource for both stressed and non-stressed corals to build up their symbiont quality.

Maximum quantum yield was also significantly higher for non-stressed enhanced fed corals after two weeks of recovery, further solidifying the impact enhanced feeding can have on symbiont
quality in healthy corals. After five weeks of recovery there were no significant differences found between treatments due to a slight decline in maximum quantum yield across all treatments, but no significant differences were found in maximum quantum yield between two weeks and five weeks of recovery and the slight observed declines still resulted in healthy Fv/Fm ratios across all treatments. Non-stressed enhanced fed corals also showed significantly lower photosynthetic rates compared to heat-stressed ambient fed corals. Non-stressed enhanced fed corals appeared to decrease photosynthesis during the recovery period, whereas ambient fed heat-stressed corals increased photosynthetic rates above the non-stressed ambient fed control corals. These results indicate that increased natural zooplankton abundance on coral reefs impacts the ability of coral symbionts to increase chlorophyll concentration and maximum quantum yield, while simultaneously decreasing photosynthetic rates.

Identifiable Patterns

It is known that P. furcata in Bocas del Toro, Panama, has been exposed to repeated heat stress events over the past decade (Neal et al., 2017). Previous studies showed corals lose their heterotrophic plasticity to further protect themselves from future heat-stress events after repeat heat-stress events (Grottoli et al., 2014; Levas et al., 2015; Schoepf et al., 2013). This study presents hopeful results that if natural food availability is present in a high enough concentration during the non-stressful environmental interval, corals can take advantage of this resource after exposure to repeated heat-stress events, to better protect themselves from future stress events. Our study is the first to show enhanced feeding in situ results in corals resulting in significantly lower photosynthetic rates and efficiencies compared to corals exposed to ambient feeding, despite strong symbiont quality. Previous studies looked at the effect of fed versus starved corals.
and found fed corals increased photosynthetic rates under a variety of light, temperature, and feeding conditions (Houlbrèque et al., 2009; Hoogenboom et al., 2012; Lyndby et al., 2019). Our results showing a significant increase in photosynthetic rates of heat-stressed corals exposed to ambient feeding compared to non-stressed corals with enhanced feeding are supported by prior studies which found that *P. furcata* in Bocas del Toro, Panama, have the ability to regulate their symbiotic interactions and alternate from a strong exchange between the host and symbiont and to one suppressing autotrophic activity (Seeman et al., 2012; Leinfelder et al., 2012). Our study goes a step further, showing that when *P. furcata* are presented with enhanced feeding opportunities, these additional nutrient and carbon inputs allow for a significant decrease in photosynthetic rates, resulting in a decrease of photosynthetic efficiency. We propose this decoupling is a result of exposure to frequent bleaching events over the past decade, and that corals maximize the benefits of heterotrophic plasticity by keeping the majority of the carbon and nutrients gained through heterotrophy for the coral host’s basic needs, rather than providing it to their symbionts. This may better protect them from future heat-stress events since increased photosynthetic rates do not always increase the transfer of photosynthetic products to the coral host, as symbionts may keep them for their own requirements (Swanson and Hoegh-Guldberg, 1998).

Even with lower photosynthetic rates, corals with enhanced feeding had significantly higher chlorophyll concentrations and symbiont densities than corals under ambient feeding, regardless of exposure to temperature. This indicates that the coral host may allocate its resources to keep its symbionts at an optimal functioning level but it appears to limit the amount of carbon transferred to be used for photosynthesis, as evidenced by non-stressed corals with enhanced
feeding more than doubled their chlorophyll-a concentration. We surmise this allows corals to be better prepared to be able to withstand future heat-stress events.

Our findings that enhanced zooplankton abundance is a stronger resource for coral resistance rather than resiliency to heat stress are supported by previous studies that suggest that corals that are fed have healthier symbionts because food supply can help prevent damage to symbionts by supplying nitrogen or other nutrients (Borell and Bischof, 2008; Ferrier-Pagès et al., 2010; Hoogenboom et al., 2012). Our results now suggest that if planktonic food is at a high enough concentration, corals may decrease their photosynthetic rates as a result of the increased energy and nutrients available to them from the environment. Both of these findings support the takeaway from previous studies that coral host metabolic status strongly influences symbiont responses to physical stresses (Hoogenboom et al., 2012).

**Improvements/Future Directions**

These results are important in understanding the effect of feeding on coral health, but continued studies are needed as different corals may respond differently to temperature stress and food availability, especially depending on other environmental parameters and thermal stress histories (Gustafsson et al., 2013). High variability was found within colonies and within treatments for both chlorophyll concentration and symbiont densities, as has been found in previous studies with other coral species (Bessel-Browne, 2013). Increasing replication to better account for this variability will be valuable when further exploring these relationships on other species in other reef environments.
Feeding rates were initially incorporated into our experimental design but were removed due to logistical constraints. This information would provide a greater understanding on the impacts of variation in feeding rates throughout the duration of recovery for corals exposed to heat stress with and without enhanced feeding, as previous studies have found no difference between ambient and enhanced feeding treatments over just a few days into recovery (Palardy et al., 2008). Another important insight that manipulation of feeding rates could provide is whether or not it is the act of increased feeding enabled by enhanced zooplankton abundance that initiates the resilience response, or if feeding rates stay constant, but an increased abundance of zooplankton with either a higher nutritional quality, or an increase in zooplankton that are more easily captured by corals, are providing corals with nutrients that are not available under conditions when zooplankton abundance is lower. Testing this will also be critical for implementing monitoring strategies that incorporate zooplankton community dynamics to better understand the resources available to corals for both resistance and recovery from heat-stress events.

Conclusions

Our study supports previous findings that zooplankton abundance is an important factor in coral health and suggests that for certain corals, enhanced zooplankton availability is important in advance of heat-stress events to produce corals with strong symbiont qualities. Corals exposed to enhanced feeding regardless of heat-stress showed decreased photosynthetic rates, despite increased symbiont densities and chlorophyll concentrations. Our findings show that benefits from enhanced zooplankton availability are used by both P. furcata individuals that are immediately recovering from heat-stress and individuals that are not. However, based on their
recovery status, these individuals may allocate the heterotrophic benefits differently between increasing symbiont quality and meeting the basic needs of the coral host.


Ferrier-Pagès, C., Sauzeat, L., Valter, V. 2018. Coral bleaching is linked to the capacity of the animal host to supply essential metals to the symbionts. Global Change Biology, 24(7), 3145-3157.


Figure 2.1. Experimental design
Figure 2.2. Maximum quantum yield (Fv/Fm) after (a) two weeks of recovery, (b) five weeks of recovery, (c) percent change after two weeks recovery, and (d) percent change after five weeks of recovery. Error bars represent standard error.
Figure 2.3. Chlorophyll concentrations and symbiont densities
(a-c) Chlorophyll and symbiont concentrations after five weeks of recovery (d-f) Percent Change in chlorophyll and symbiont concentrations from beginning to end of recovery. Error bars represent standard error.
Figure 2.4. Gross photosynthetic rates and efficiencies
End values of gross photosynthesis (a) and photosynthetic efficiency (b). Percent change in gross photosynthesis (c) and photosynthetic efficiency (d) from beginning to end of recovery. Error bars represent standard error.
Coral host physiology under the effect of enhanced feeding and heat-stress

Abstract

Coral reefs are threatened by the increasing prevalence of heat-stress events, resulting in coral bleaching and a loss of energy resources for the host. Although some corals are able to compensate for this energy loss through increased feeding behavior, there is limited knowledge on how food availability affects this recovery mechanism. This study explores the effect of enhanced feeding availability of natural zooplankton communities on recovery in Porites furcata after heat stress in waters off of Panama. Corals were collected and divided into heat-stress or control treatments that were conducted in the laboratory. After corals showed visible signs of bleaching, they were placed back on the reef for five weeks of recovery in one of the following four treatments: Ambient Fed – Ambient Temp, Ambient Fed – Heat Stressed, Enhanced Fed – Ambient Temp, and Enhanced Fed – Heat Stressed. Non-stressed corals showed increased growth rates compared to heat-stressed corals regardless of feeding treatment during recovery. Therefore, enhanced zooplankton availability does not allow heat-stress corals to compensate for the lower growth rate that occurred during heat-stress. Heat-stressed corals showed no significant difference between non-stressed corals for both protein concentration and respiration rates regardless of feeding treatment. This indicates that the ambient zooplankton availability allows for full recovery for basic metabolic needs after five weeks of recovery from heat stress. Future studies could investigate if faster recovery for heat-stress corals occurred after two or three weeks of enhanced feeding availability during recovery on the reef.
Introduction

Coral reefs are subjected to multiple stressors, the largest threat being increasing warming of our oceans (Bruno et al., 2007; Hoegh-Guldberg et al., 2007, 2017; Hughes et al., 2018). This warming disrupts the relationship between corals and their algal symbionts, resulting in decreased energy and resources from photosynthesis, a phenomenon known as coral bleaching (Hoegh-Guldberg et al., 1999). Depending on the frequency, severity, and duration of heat-stress events, some corals have the ability to recover from these heat-stress events through a multitude of recovery mechanisms (Baker et al., 2008), ranging from increased heat shock proteins (Sharp et al., 1997), switching of symbiont types (Buddemeier & Fautin, 1993; Baker et al., 2004), or drawing on energy reserves (Rodrigues and Grottoli, 2007). Another important recovery mechanism, first shown by Grottoli et al. (2006), is that certain coral species have the ability to use heterotrophy to recover from coral bleaching. The entirety of their metabolic demands may be met by this external resource, zooplankton.

Numerous studies have examined the mechanism behind the role of heterotrophy in coral recovery from bleaching to better understand to what extent heterotrophy can protect coral reefs as heat-stress events become more prevalent from global warming. Feeding provides additional nitrogen that increases the metabolic capacity of the symbionts, allowing photosynthesis to continue under heat stress, prior to complete bleaching (Borell et al., 2008). Additionally, fed corals have increased concentrations of metals in their tissues important for symbiont function (Ferrier-Pagès et al., 2018). Increased heterotrophy has also been found to specifically benefit the coral host by providing nutrients to restore or maintain energy reserves (Grottoli et al., 2006).

Despite all of the research focused on heterotrophic plasticity in corals as a recovery mechanism from bleaching, less work has been done to better understand zooplankton community dynamics
on coral reefs. It is known that zooplankton abundance varies greatly over reefs on both daily 
(Heidelber et al., 2010) and monthly time scales, usually in coordination with the lunar cycle 
(Palardy et al., 2006). Additionally, zooplankton populations are also threatened by warming 
ocean temperatures, and their populations may be decreasing over time as the oceans acidify 
(Smith et al., 2016). Without a clear understanding of present-day zooplankton community 
dynamics, it is extremely difficult to predict the future role of heterotrophic plasticity in corals as 
the oceans warm (Richardson, 2008).

Coral reefs in Bocas del Toro, Panama have experienced multiple heat-stress events over the past 
decade (Neal et al., 2017), and many species are not recovering, although surviving colonies 
appear increasingly stress-tolerant (personal observations). Offshore corals often have higher 
zooplankton abundance and decreased susceptibility from stress events compared to nearshore 
corals (personal observation). Although numerous studies have shown the resistance and 
resilience that fed versus starved corals have to thermal stress (Houlbrèque 2009; Borell and 
Birschof, 2008; Ferrier-Pagès et al., 2003), this study investigates a more applied question: does 
enhanced feeding on natural zooplankton post heat stress improve recovery for Porites furcata? 
This study predicts heat-stressed corals in the enhanced feeding treatment will have increased 
protein concentrations, respiration rates, and growth rates, similar to those of the control corals 
after five weeks of recovery, while heat-stressed corals in an ambient feeding treatment will have 
a significantly lower value for these metrics.
Methods

Site Description

The field site was located in Punta Caracol inside Almirante Bay near the Smithsonian Tropical Research Institute (STRI) field station in Bocas del Toro, Panama. *Porites furcata* was used because previous work at this site confirmed it was an abundant coral and displayed heterotrophic plasticity in response to stress events (Seeman, 2013).

Experimental Design

On 4 January 2017, 21 fragments were collected from four *P. furcata* colonies. They were immediately transported to 50-gallon water tables with water flowing indirectly from the lagoon outside STRI, approximately 3 km from the collection site, allowing for similar temperature conditions (~29 °C). From each colony, 12 coral fragments were used for the duration of the experiment. The remaining nine coral fragments from each colony were sacrificed to estimate protein concentrations for baseline measurements and post-stress measurements, three in ambient and three in heat-stressed conditions. All coral fragments were acclimated under these conditions for three weeks while pre-stress measurements were taken (Fig. 2).

All 48 corals were split between two 10-gallon tanks, one kept at the ambient temperature (~29 °C) and one exposed to heat stress. One tank was used for each temperature treatment to ensure all corals within a treatment underwent the same temperature exposure, which cannot be accurately controlled for across multiple tanks due to frequent power outages and unpredictable changes in water flow.
Heat stress was introduced by increasing water temperature to 30 °C and then 0.5 °C each day up to 32 °C, the maximum water temperature recorded in the area over the past decade. The tanks were then kept at 32 °C for two weeks until the first visible signs of bleaching were noticed, and then the water temperature was reduced to 31 °C. It took another ten days at 31 °C until the majority of fragments displayed visible signs of bleaching, and all corals were returned to ambient temperature for three days to take post-stress measurements.

After post-stress measurements, all 48 coral fragments were then returned to the reef they were collected from for a five-week recovery period. Half of each of the corals from the heat-stressed and non-heat-stressed treatments were placed in an ambient feeding treatment and the other half were placed in an enhanced feeding treatment, created with an underwater light powered using an Arduino microcontroller and Duracell D batteries inside a waterproof housing. The light consisted of 5 LEDs, which gave off approximately 5 μmol photons/(m² sec) of light, a level low enough to prevent significant photosynthesis, but still attract plankton. The ambient feeding treatment was separated from the enhanced feeding treatment by a large mounding coral to ensure the only difference in environmental conditions was plankton availability.

Corals were monitored weekly both during the day and night. To quantitatively compare the difference in zooplankton abundance between the enhanced and ambient feeding treatments, 60 ml syringes were used to collect plankton directly above the corals, in triplicate. At the end of the recovery period, all corals were collected from the reef and brought back to the lab for final measurements.

Measurements

**Seawater Conditions**
Seawater samples to determine carbonate chemistry were collected in 250 ml borosilicate glass bottles sealed with a greased stopper and were immediately poisoned with 250 µl saturated HgCl solution. Additional seawater samples were collected in 50 ml Falcon (Fisher Scientific) tubes to determine salinity, pH, and nutrient measurements. Samples were collected from the field site during the initial coral fragment collection and weekly during both the laboratory heat stress period and in situ throughout the recovery period on the reef. Seawater carbonate chemistry conditions were determined with high precision measurements (calibrated with Certified Reference Materials) of total alkalinity (TA) and dissolved inorganic carbon (DIC) via closed-cell potentiometric titration and coulometry, respectively, using a VINDTA 3C (Marianda) system in the Justin Ries Laboratory at Northeastern University. The program CO2SYS (Lewis et al., 1998) was used to calculate seawater pCO₂ pH, [CO₃²⁻], [HCO₃⁻], aqueous CO₂, and calcite saturation state from measured DIC, TA, temperature, and salinity. Ammonium (NH₄⁺) was measured colorimetrically on a GENESYS 30 visible spectrophotometer (Thermo Scientific) following protocols from Solórzano (1969). Water temperature was recorded throughout the experiment with each treatment using Onset HOBO data loggers recording every 15 minutes.

Surface Area Calculations

Surface area was used to normalize symbiont density, chlorophyll content, protein concentrations, and photosynthesis/respiration rates. Surfaces areas were calculated using a novel methodology of 3D modeling that accounts for changes in surface area throughout the duration of the experiment. I designed the protocol used to create 3D models by taking a video of each fragment from multiple angles starting at the base and rising to the top of the coral. These measurements were done underwater to prevent the least amount of stress to the frag during this process. The fragment was placed on a small turntable inside of a tank and an underwater camera
was used to take the videos. Each video was separated into 250 still images. These images were sorted visually to remove any frames that were out of place or blurry. The remaining images were then uploaded into the software programs Remake PhotoCap (Autodesk) and Photoscan (Agisoft) to construct the 3D models. The base of the corals was a known distance that was used to calculate a scale bar for each individual model. The rendered models were then cropped so only live tissue remained in the model and the above software was used to calculate surface area.

*Growth Rates & Protein Concentrations*

Calcification rates were measured in all experimental frags by using the buoyant weight technique (Jokiel et al., 1978). Measurements were first taken after two days of acclimation in the lab and again after five weeks of recovery on the reef. A subset of these fragments also had dry weights taken to construct a linear regression between buoyant weight and dry weight to confirm a high correlation between the two ($R^2 = 0.999$). The linear regression was used to calculate dry weights for the remaining corals and the change in dry weight from beginning to end of the experiment was then normalized by surface area. Linear extension growth rates were estimated by dividing the normalized calcification rate by the average density of the coral skeletons (2.6 g/cm$^2$). The average density was calculated using the Archimedes principle. After each fragment had been in the -20 °C freezer for at least 24 hours, the tissue was airbrushed into Falcon (Fisher Scientific) tubes with filtered seawater to be preserved for protein concentration analysis using a BCA assay with measurement using a Nanodrop microvolume spectrophotometer (Thermo Scientific) (Brown, et al., 1989). Results were then normalized by fragment surface area.
**Respiration Rates**

Eight clear plastic 500 ml chambers were filled with seawater filtered on 0.45 mm GF/F filters. An optical fluorescence oxygen sensor (PyroScience Firesting) was placed inside seven chambers. All sensors were calibrated with 100% oxygen saturation at sea level pressure. The PyroScience Firesting fiberoptic cables were attached to continuously read the oxygen levels inside the chambers. Six of these chambers were used to determine photosynthesis and respiration rates on the corals and one was a control to account for any oxygen change occurring in the filtered seawater. The final chamber had a temperature sensor used to automatically compensate oxygen fluctuation based on any minimal temperature changes. Magnetic stir plates were used to ensure continuous mixing. Change in oxygen was measured for one hour while corals were exposed to aquarium LED lights to determine net photosynthesis (gross photosynthesis – respiration). Corals were then kept in complete darkness for one hour to ensure no photosynthesis was occurring, and change in oxygen was measured for another hour in complete darkness to determine respiration rates. This process was repeated twice a day for three consecutive days to obtain measurements for all 48 coral fragments. Rates were calculated by the change in oxygen over time and normalized by fragment surface area.

**Statistical Analyses**

The effect of food availability on both heat-stressed and non-heat stressed corals were analyzed by comparing the differences in parameters measured across treatments at each of the three-time points of the experiment: Baseline, Post-Stress, and Recovery. For protein analysis, which requires destruction of samples methodology, a baseline, post-stress heat-stressed, and post-
stress non-heat-stressed population average were calculated from the sacrificed fragments at each time point.

Statistical analysis and resulting figures were developed using R Studio Version 1.1.463 and JMP 14 Pro. Data were checked for normality by viewing a histogram of the data distribution and calculating a skewness value from R package 'moments'. If data did not fit a normal distribution, a square-root or Box-Cox transformation was applied. If this resulted in a normal distribution, results were analyzed using a 2-way linear mixed-effects model with the fixed effect of temperature, feeding as an interaction, and colony and injury as random effects. Statistical tests were made at the 5% significance level.

After recovery, three fragments were missing and two had experienced predation resulting in a surface area less than 2 cm$^2$ and were removed from protein and respiration analyses. An additional 14 coral individuals experienced varying levels of predation. For protein and respiration analyses predation was included as a random effect and determined to not be a significant factor in the experiment results. These 14 frags were removed from the calcification rate and linear extension rate analyses. For the calcification rate and linear extension rate analysis, a one-way ANOVA was run for the effect of colony and resulted in no significant effect so colony was not included in further analysis. All anovas were run as type III to account for the unequal sample sizes due to the removal of individuals due to predation.

JMP 14 Pro was used to conduct a principal component analysis to determine the main components of variation in the symbiont parameters measured in chapter 2. This analysis was selected to better understand which symbiont parameters impact growth, without leading to confounding results due to co-variation among multiple symbiont parameters. One-way
ANOVA.s were run to test the effect of each principal component on calcification and linear extension rates. Two-way ANOVAs were run to test the effect of the interaction between each principal component and treatment as well as the interaction between both principal components on calcification and linear extension rates.

Results

Water chemistry results showed pCO$_2$ values both on the reef and in the tanks were all below 500 µatm. Ammonium levels were variable both on the reef and in the tanks. During collection, the reef concentration was 7 µM. Ammonium levels at the beginning of the three-week heat stress period showed elevated levels of 50 µM for the heated tank and 22 µM for the ambient tank, but measurements taken two weeks later showed both heated and ambient tanks had ammonium levels of 14 µM. During recovery on the reef, sequential weekly ammonium levels were 7 µM, 9 µM, 15 µM, and 6 µM. During baseline acclimation, corals were exposed to temperatures ranging from 23 - 32 °C (Fig. 1). During the heat-stress period, ambient water temperature varied between 26 - 31 °C and the heat-stressed water temperature varied 29 - 34 °C. During recovery on the reef, corals in both feeding treatments experienced water temperatures ranging from 27 - 30 °C.

A swarm of zooplankton directly above the corals in the enhanced feeding treatment was observed during every weekly night dive during the five weeks of recovery. The syringe showed the enhanced feeding treatment ranged from a 3-fold to a 20-fold increase in zooplankton abundance compared to the ambient feeding treatment throughout the five-week recovery period. Despite the difference in overall abundance, there was still high diversity in both feeding treatments including copepods, nauplii, cladocerans, larvaceans, chaetognaths, and isopods.
Both treatments experienced predation effects. Prior to deployment of the experiment, this threat was considered, but caging was decided against considering the implications it could play in feeding behavior and food availability, as well as other effects such as shading due to algal growth. The light was deployed overnight with GoPro underwater cameras positioned to monitor fish presence in the light, but no fish were found during these trials, supporting no need for caging. During daytime dives during the five-week recovery period, damselfish were commonly found near both treatments and on one night dive, a parrotfish was seen around the enhanced feeding treatment. At the end of the experiment, three frags were no longer present, three frags were mostly eaten, three frags were partially eaten, and eight had just tips that were eaten. These predation events were spread across all four colonies and all four treatments. These observations, along with our statistical analysis, confirm predation was not increased by the light of the enhanced feeding treatment. However, predation is a common additional stressor that corals can face during recovery from thermal stress.

**Protein Concentration, and Respiration**

No significant differences in protein concentrations (Fig. 2) or respiration rates (Fig. 3) were found across treatments during baseline and recovery measurements. A significant increase in protein concentration was found in ambient corals compared to heat-stressed corals during post-stress measurements (p = 0.006). Post-stress respiration rates for heat-stressed corals were significantly lower than ambient corals (p < 0.001).

**Growth**

Despite the decreased sample size among treatments due to predation, colony still had no effect on calcification rate (p = 0.3812). Heat-stressed corals had significantly lower growth (40%)
regardless of feeding treatment (Fig. 5; p < 0.02). No noticeable differences in growth rates were seen between the two heat-stressed treatments, and both showed significantly lower growth rates compared to only non-stressed enhanced feeding treatment (p < 0.002). The same patterns were seen for the linear extension growth rate across treatments. The principal components analysis revealed two main components explaining 72% of the variance in the symbiont parameters (Fig 6; Table 1). Principal Component 1 (PC1) consisted of both chlorophyll and chlorophyll c concentrations and symbiont density and consisted of almost 52% of the variance. Symbiont density shows a strong negative relationship only for enhanced fed heat-stressed corals ($R^2 = 0.642$). No correlations were seen between either chlorophyll concentration and calcification rate ($R^2 < 0.25$). These three symbiont parameters are closely aligned, as they all can be a metric of symbiont productions. Enhanced-fed non-stressed corals show a trend of higher positive values on the PC1 axis compared to other treatments (Fig 6). While there is not a clear separation along the PC1 axis for temperature, non-heat-stressed corals tend to have a higher position on the PC1 axis than heat-stressed corals. Principal Component 2 (PC2) explains 20% of the variance and is a linear combination of gross photosynthesis and maximum quantum yield measurements. These parameters are aligned with how the symbiont is functioning. For enhanced fed corals, there is a positive relationship between calcification and both gross photosynthesis and maximum quantum yield. Gross photosynthesis explains 50% and 58% of variation in calcification rate for both enhanced feeding treatments. The maximum quantum yield explains 50% of variation in calcification for the enhanced-fed ambient temperature treatment. Again, enhanced-fed non-heat-stressed corals are overall more associated with higher numbers along the PC2 axis than corals from other treatments. However, we do see more variation among treatments along the PC2 axis than the PC1 axis.
Independently neither principal component had a significant effect on calcification rate independently (PC1 p = 0.178; PC2 = 0.057). However, each principal component did have a significant interaction with treatment. The enhanced fed heat-stressed corals were significantly different from the enhanced fed ambient corals (p=0.022) and the ambient-fed non-heat stressed corals (p = 0.029). The enhanced fed ambient corals were also significantly different from the ambient-fed heat-stressed corals (p = 0.045). The significant interaction between treatment and PC2 resulted in a significant difference found between the calcification rates of the enhanced-fed ambient temperature treatments and both heat-stress treatments regardless of feeding (p = 0.007; p = 0.01). Additionally, when both principal components are included in the model without treatment, PC2 alone shows a significant effect on calcification rate (p = 0.042).

Discussion

Environmental Conditions

The spike in ammonium concentration observed during the heat-stress period is likely due to a crack in the aquaria water filtration system that resulted in the shutdown of the filtered seawater. After shutdown, only raw seawater came through the system. To attempt to mitigate these effects, tanks were cleaned every other day to remove the increased algal concentration coming through the seawater, but the initial influx did seem to have an impact, especially with the higher temperatures. This increase in ammonium may have helped the corals increase their thermal tolerance and be less susceptible to the heat-stress, resulting in the prolonged heat-stress period that was needed to show signs of bleaching, as ammonium is known to stimulate photosynthesis (Morris et al., 2019).
The change in temperature variability throughout the time points of the experiment is likely a result of the corals being located in different volumes of water (Fig. 1). The water table used for coral acclimatization had a larger surface area, allowing for a greater change in temperature during day and night, creating a different type of environment than when the corals were split into smaller tanks during the heat-stress period. Variability then decreased again when corals were kept on the reef for recovery. Due to the differences in these systems, comparisons between treatments will only be made within and not across time points.

**Protein Concentration & Respiration Rates**

Our study revealed that all corals began the experiment with similar protein concentrations and baseline respiration rates. Following exposure to heat-stress, the heat-stressed corals had significantly lower protein concentrations and respiration rates compared to non-stressed corals, as expected since decreased respiration can be a mechanism for corals to conserve energy during times of stress (Rodrigues and Grottoli, 2007). However, heat-stressed corals in both feeding treatments showed no significant differences in protein concentrations or respiration rates compared to non-stressed corals after five weeks of recovery on the reef. These results are similar to previous studies that have found protein concentrations do not show variation between heat-stressed and non-stressed corals within five weeks of recovery from heat-stress (Grottoli et al., 2014; Levas et al., 2018). Previous studies also found no differences between non-stressed and repeat bleached corals after five to six weeks of recovery (Schoepf 2013, Levas et. al, 2013).

Although the enhanced feeding treatment did not show a significant effect in our study, it is promising that after only five weeks of recovery, all corals showed full recovery in the ambient environment.
The heat-stressed ambient fed corals did show the highest respiration rates compared to all other treatments. Increased respiration can be explained by an increase in metabolic energy being used to repair mechanisms damaged during heat-stress (Levas et al., 2013). This excess heterotrophic carbon may be rapidly catabolized to meet metabolic demand or lost through mucus expression rather than assimilated into coral tissue or translocated to symbionts, as previous studies found increased feeding rates did not result in a difference in the expression of heterotrophic carbon between stress and non-stressed corals (Hughes & Grottoli, 2013). Therefore, it appears that the ambient fed heat-stressed corals may still be at the end of their recovery from heat stress, and if we had ended the experiment earlier, we may have seen a significant effect of enhanced feeding leading to quicker recovery at least for metabolism. We also see the enhanced fed non-heat-stressed corals appears to have decreased their respiration more than the ambient fed non-heat-stressed corals, to a similar level of the heat-stressed ambient fed corals. Decreased respiration is likely allowing the coral host to better conserve its energy reserves, which was also found in Montipora capitata, known to display heterotrophic plasticity after heat-stress (Rodrigues & Grottoli, 2007).

**Growth Rates**

Heat-stressed corals decreased their growth by 40% compared to non-stressed corals, regardless of feeding treatment over the course of the experiment. Although other studies have shown no difference in growth between stressed and control corals after repeated heat stress events (Levas et al., 2013; Grottoli et al 2014), our study kept corals in heat stress past conditions seen historically on the reef until they were visibly impacted. Therefore, our results show that when temperatures broke this threshold, growth was not a parameter that was able to recover within
five weeks, even with exposure to enhanced feeding. This can be explained by the coral allocating its resources to other metrics for protection and rehabilitation from heat stress, as has been seen in other *Porites* species (Levas et al., 2013). Additionally, it has been found that non-stressed corals assimilate photoautotrophically acquired carbon for short-term metabolic needs and calcification, and carbon from heterotrophy is directed to the coral tissue or symbionts (Hughes et al., 2010). The benefits of stressed corals using heterotrophic plasticity as a recovery mechanism are typically used to meet basic metabolic needs and symbiont quality over growth (Rodrigues and Grottoli, 2006).

Therefore, it is important to determine how effects on food supply and temperature on symbiont quantity and quality can subsequently affect coral growth. Symbiont production (symbiont density and chlorophyll concentration), function (gross photosynthesis and maximum quantum yield), and the treatments predict significant variation in calcification rate. Again, enhanced-fed ambient corals show significantly higher calcification and linear extension rates than both of the heat-stressed corals regardless of feeding, with increasing symbiont production and function. An added level of significance between treatments was found when incorporating PC1 representing symbiont quality. Heat-stressed enhanced-fed corals showed an opposite pattern of lower calcification rates with higher symbiont densities but were still only significantly different from the ambient-fed ambient temperature treatment. This is likely due to a few of the enhanced-fed heat-stress corals having higher symbiont densities than the ambient-fed ambient corals. When both principal components are included to test their individual and interactive effects on calcification and linear extension rate, only PC2 representing symbiont function has a significant effect. While it is expected that increased photosynthesis results in lower calcification rates (Goreau 1959, Hughest et al., 2010), these results show that like nutrient enrichment, enhanced
feeding availability can stimulate both colony growth and photosynthesis (Ferrier-Pagès et al., 2003). This is promising, that even though after five weeks, enhanced feeding did not increase growth rates, perhaps after a longer exposure, corals will be able to successfully increase their growth rates. Additionally, it is important to note, calcification rate was only measured from the beginning to end of the experiment, and there is no direct comparison of the growth rate for the recovery portion of the experiment alone.

Conclusions and Roadmap for Future Work

Enhanced feeding availability has not shown to have a significant change in feeding rates for stressed or non-stressed corals (Ferrier-Pagès et al., 2003; Palardy et al., 2008). However, to calculate feeding rates, corals must be starved prior to exposure to feeding, and are only allowed to feed for a specific period of time, usually from one to three hours, to determine an accurate rate. Ferrier-Pagès et al., 2003 did determine that increased zooplankton availability led to the increased total number of zooplankton captured by corals and that despite uncontrolled variation in zooplankton concentrations fed to their experimental corals, feeding never reached saturation. However, this study focused statistically on the number of feeding occurrences, rather than the overall differences in feeding availability over a continuous time period.

The present study was designed to determine the effect of long-term enhanced feeding opportunities rather than the immediate effect of enhanced feeding rates. However, all corals regardless of feeding treatment showed a full recovery in protein concentrations and respiration rates within five weeks. A shorter-term experiment might reveal that enhanced feeding allowed corals to recover more quickly. With the number of heat-stress events predicted to increase in the coming years, it is important to gain a better understanding of how zooplankton on coral reefs
may allow corals that show heterotrophic plasticity capabilities to recover from heat stress events. Additionally, a prolonged experiment could better assess if enhanced feeding results in recovery of growth rates more quickly than ambient feeding during recovery. A final component to address would be the use of a non-destructive methodology to calculate feeding rates to better understand how much effort corals put into extra feeding when extra food is available.


**Literature Cited**


Ferrier-Pagès, C., Sauzeat, L., Balter. 2018. Coral Bleaching is linked to the capacity of the animal host to supply essential metals to the symbionts. Global Change Biology, 24(7), 3145-3157.


Figure 3.1 Water temperatures throughout the experiment. The water temperature was monitored every 15 minutes with an Onset HOBO logger. Light blue indicates baseline temperatures, dark blue indicates ambient temperature during stress-period, red indicates temperatures heat-stress temperatures, and green and dark blue represent the enhanced fed and ambient fed water temperatures on the reef during recovery, respectively.
Figure 3.2. Protein concentration (mg cm$^2$). Protein concentration was normalized to surface area. From left to right, concentrations represent Baseline, Post-Stress Ambient, Post-Stress Heat, Recovery Ambient Fed – Ambient Temperature, Recovery Ambient Fed – Heat-Stressed, Recovery Enhanced Fed – Ambient Temperature, and Recovery Enhanced Fed – Heat Stressed. Error bars represent standard error.
Figure 3.3. Respiration Rates (µmol oxygen cm\(^{-2}\) hour\(^{-1}\)) a) Baseline Respiration Rates, b) Post-Stress Respiration Rates, c) Recovery Respiration Rates. In all panels from left to right, treatments are displayed as Ambient Fed – Ambient Temperature, Ambient Fed – Heat-stressed, Enhanced Fed – Ambient Temperature, and Enhanced Fed – Heat-Stressed. Respiration rates were normalized to surface area. Error bars represent standard error.
Figure 3.4. Net calcification rates (mg cm\(^{-2}\) day\(^{-1}\)). Calcification rates were calculated from the beginning to the end of the experiment. From left to right, values indicate Ambient Fed – Ambient Temperature, Ambient Fed – Heat-stressed, Enhanced Fed – Ambient Temperature, and Enhanced Fed – Heat-Stressed. Calcification rates are normalized to surface area. Error bars represent standard error.
Figure 3.5. Component scores from Principal Components Analysis of symbiont data. Points indicate individual colony scores and color denotes temperature and feeding treatment combinations. Principal component one (PC1) indicates dependence of symbiont density and chlorophyll concentrations and principal component 2 (PC2) indicates dependence of gross photosynthesis and maximum quantum yield measurements.
Table 1. Principal Components Analysis variable loadings and correlations

<table>
<thead>
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<th>Component</th>
<th>Symbiont Parameter</th>
<th>Loading</th>
<th>Correlation</th>
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<tbody>
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<td>PC2: Symbiont Function</td>
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<tr>
<td></td>
<td>Maximum quantum yield</td>
<td>0.87001</td>
<td>0.81147</td>
</tr>
</tbody>
</table>
Conclusion

Research Summary

The large zooplankton spatial and temporal variability found on coral reefs makes it difficult to understand their dynamics, and without increased continued and consistent monitoring, it will be difficult to know how this critical food source is changing with warming ocean temperatures. In Bocas del Toro, Panama, the offshore site had a higher abundance of hard coral cover and diversity, and was less susceptible and had faster recovery from bleaching events compared to the nearshore reef (personal observation). The offshore reef also had higher nitrate and higher flow, and this likely lead to our observed increased zooplankton abundance on these reefs. Our results indicate that during fall 2016 sampling, the offshore environment was a more ideal environment for corals recovering from heat stress. Knowing that some corals do rely on heterotrophic plasticity to recover from bleaching events, it is pertinent that zooplankton community dynamics are incorporated in the predictions of coral recovery after heat-stress events.

The most intriguing result of the present study is in the full recovery of symbiont parameters in heat-stressed corals regardless of feeding, but a decrease in photosynthesis rates for non-stressed enhanced fed corals that had significantly higher symbiont density and chlorophyll concentrations. This suggests that *Porites furcata*, is able to decouple its heterotrophic intake from its symbiotic partner, as previously discussed in Seeman et al., (2013). However, the present study is the first time this is shown by comparing photosynthetic rates. A possible explanation for this behavior is that Bocas del Toro reefs have been subjected to multiple annual heat-stress temperatures for the past 14 years, and corals may be conditioned to utilize the
enhanced feeding availability to prepare for future heat-stress events. Heat-stressed corals' basic physiology showed full recovery in both feeding treatments, but growth rate was still significantly lower in heat-stress corals regardless of feeding treatment. This means the ambient zooplankton conditions are able to help corals meet their metabolic needs, but not maintain growth rates.

**Future Directions**

My dissertation provides an improved understanding of the role of zooplankton on coral reefs but, more research is needed for a fully comprehensive understanding of zooplankton dynamics on coral reefs, specifically in relation to coral health. Increased comparisons of differences in zooplankton communities across reefs with varying hard coral cover and bleaching susceptibility will provide important further insight for monitoring purposes to determine if zooplankton communities can be used to predict the severity of bleaching events on certain reefs. Increased sampling on the same reef over time will allow for a better understanding of temporal changes over both seasonal and lunar cycle patterns. Increased sampling will also allow insight for changes in zooplankton communities over time due to environmental conditions affecting zooplankton physiology, reproduction, and survivorship. In addition to sampling for abundance and diversity metrics, it also critical to measure the nutritional value of the zooplankton the reefs, as this could be changing over time due to environmental changes as well and may also have cascading impacts on coral health.

Additional experiments looking at the specific role of natural zooplankton availability to corals, both in terms of abundance and community composition, will also provide a greater
understanding of the long-term option for corals to use heterotrophic plasticity to recover from bleaching. Running a similar experiment to this one, but with a shorter duration may provide insight into how increased zooplankton abundance enhances coral recovery from bleaching. Incorporating in the experimental design, a better way to measure how symbiont variability impacts growth will also be important. Additionally, lab-based experiments comparing the bleaching recovery time for corals fed offshore and nearshore zooplankton communities would provide more insight into what proportion of the zooplankton community is comprised of zooplankton important to the coral’s diet. Since there were distinctive community composition differences, zooplankton abundance alone may not be a clear indicator of how the zooplankton community contributes to bleaching recovery. If one community has a higher abundance of copepods with strong avoidance behaviors or less nutritional taxa, this will not be beneficial for the corals.
Literature Cited