

**THE GROWTH RESPONSE OF THE HAWAIIAN BLUE OCTOCORAL,
Sarcothelia edmondsoni, TO VARIOUS NITRATE CONCENTRATIONS**

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ABSTRACT

Shifts in coral reef benthic community species composition have been observed in response to changes in water quality, such as conditions of elevated nutrients from urban development and agriculture runoff. Species that display a predictable biological response to environmental conditions (bioindicators) are needed as cost-effective tools for assessing water quality and environmental change. The endemic Hawaiian species of Blue Octocoral, *Sarcothelia edmondsoni*, has been suggested as a potential bioindicator of land-based water quality pollution because it is notably abundant in multiple locations near heavily developed coastlines on Hawai'i Island. Using laboratory experiments, the growth of Blue Octocoral was documented under three treatment levels of NO_3^- concentration; below (0.5x), equal ($\sim 1 \mu\text{mol/L NO}_3^-$), and above (1.5x) the ambient *in situ* concentration in the octocoral habitat in Hilo Bay, Hawai'i Island. Blue Octocoral growth was observed using two metrics: polyp growth and tissue expansion. Enrichment of NO_3^- had a negative effect on Blue Octocoral growth in the experiment. Colonies subjected to the above ambient NO_3^- concentration treatment showed less growth and altered growth patterns compared to colonies subjected to lower NO_3^- concentrations. The Blue Octocoral growth response to NO_3^- concentration is informative of the NO_3^- enrichment influence in nearshore marine ecosystems and is novel information for an endemic species to Hawaii. This study suggests that the Blue Octocoral is not a suitable bioindicator of land based nutrient pollution in nearshore marine ecosystems of Hawai'i.

INTRODUCTION

Human activities, such as fossil fuel combustion and fertilizer use in agriculture and industrial processes, have produced an increase in anthropogenic-sourced nitrogen (Galloway et al. 2008), which is transferred from terrestrial environments to streams and marine ecosystems (Vitousek et al. 1997). Eutrophication, or increased production due to enrichment of inorganic nutrients, has a notable impact on coral reef ecosystems because the organisms are adapted to very low-nutrient conditions. Nutrient enrichment is recognized as a chronic stressor that can alter ecosystem function and reduce species recovery capacity following a damaging or stressful event (Costa et al. 2008). Increased nutrients in coral reef ecosystems has been observed to alter ecosystem function by fueling primary productivity, cause benthic species composition to shift towards algal or zoanthid (soft coral) dominance, and impair Scleractinian coral health and survival (Fabricius et al. 2005, Costa et al. 2008, Parsons et al. 2008, Wiedenmann et al. 2013). The peak nitrogen tolerance determined for coral reef ecosystems has been variable among studies over time, reported at concentrations in the range 1 - 4.51 μM nitrate (Guan et al. 2015, Costa et al. 2008, Kleypas 1997). The tolerance to nutrient enrichment that any coral reef ecosystem processes is dependent on the water quality conditions to which organisms of the ecosystem were adapted.

Dissolved compounds such as nutrients are not visibly detectable and must be evaluated using laboratory analysis, which is a large expense in research that requires repetitive measurements due to high spatial variability and fluctuation over short time frames. Organisms that respond predictably to water quality changes may assist the evaluation of anthropogenic alteration of water quality. Zoanthid species in Brazil, Puerto Rico, Australia, and Maui, Hawai'i, are noted to be dominant in regions subject to human-driven eutrophication, and some are considered to be reliable indicators of water quality pollution (Fabricius et al. 2005, Costa et al. 2008, Hernandez-Delgado et al. 2008, Amato et al. 2016). Seasonal increase in abundance of a

pollution-indicative zoanthid species (*Palythoa*) and benthic algae coverage has been observed in correlation with seasonal nutrient enrichment in Bahia, Brazil (Costa et al. 2008). Indicator species are desirable as cost-effective and efficient means for evaluating water quality (Carignan & Villard 2002) by providing an alternative to laboratory water sample analysis.

Anthropogenic nutrient pollution is an issue increasingly affecting coral reefs in Hawai'i. Water quality monitoring on Hawai'i Island has shown that eutrophication of aquatic ecosystems correlates with population growth and increasing land development (Parsons et al. 2008), as well as amount of agriculture land within a watershed (Dailer et al. 2012, Michaud & Wiegner 2011). Wastewater discharge (Miller-Pierce et al. 2016, Richardson et al. 2015), septic systems, cesspools, agricultural land (Michaud & Wiegner 2011), and near coastal water injection wells (Dailer et al. 2012, Bishop et al. 2015) have been identified as sources of nitrogen pollution. Submarine groundwater discharge (SGD) is the major hydrologic connection between the land and ocean environments for most of Hawai'i Island (Grossman et al. 2010), delivering nutrients, dissolved gasses, and trace minerals to the nearshore marine environment (Lecher & Mackey 2018, Raikow et al. 2008, Knee et al. 2008). In addition to SGD, surface runoff and river discharge also provide a large connection for water transfer from land to sea, primarily on the east (windward) side of Hawai'i Island. Nitrate (NO_3^-) is the primary form of inorganic nitrogen that is delivered to the ocean and the dominant form present in the marine water column (Libes 2009). Monitoring the effects of NO_3^- enrichment is key to understanding anthropogenic impact to marine ecosystems in Hawai'i.

In the nearshore marine environment surrounding Hawai'i Island, the Hawaiian Blue Octocoral, *Sarcothelia edmondsoni* (Family Xenidiidae), has been observed to be notably abundant at several locations subject to anthropogenic altered water quality. The Blue Octocoral is a zooxanthellate soft coral capable of mixotrophic feeding, which allows the holobiont to obtain nutrition from algal symbionts within the coral tissue and from heterotrophic feeding

(McFadden et al. 2014). The Blue Octocoral is endemic to the Hawaiian Islands, and is unique in the way the polyps rise straight from the stolon and form a mat of polyps rather than a branching structure. Surveys by Division of Aquatic Resources on Hawai'i Island report that the "distribution and percent cover of *Sarcothelia edmondsoni* appears to be centered around the developed regions of the West Hawai'i coastline" (Walsh et al. 2013). The species has also been noted as abundant at locations in Hilo Bay on the east side of Hawai'i Island and in Kaloko-Honokōhau National Historical Park in west Hawaii (Marrack et al. 2014, Weigerman et al. 2014, National Park Service unpublished data). Both locations receive a large flux of land-sourced ground water input containing anthropogenic-produced nutrients. This patchy pattern of Blue Octocoral abundance in spatial correlation with developed shorelines and anthropogenic altered water quality has led to the consideration of this species as a possible indicator of nutrient pollution (Knee et al. 2008, Parsons et al. 2008, Walsh et al. 2013, Marrack et al. 2014).

This experiment was developed to investigate Blue Octocoral as a potential nutrient pollution indicator species by evaluating octocoral growth in three treatments of varying concentrations of biologically available nitrogen; specifically nitrate (NO_3^-). The primary objective of this experiment was to determine if NO_3^- concentration, within a range surrounding the observed ambient coastal concentration, has an effect on octocoral growth. This experiment utilized novel approaches for octocoral transplanting, husbandry, and growth evaluation (Appendix I). This study provides an evaluation of the endemic Hawaiian Blue Octocoral, *Sarcothelia edmondsoni*, as a potential biological indicator species for water quality.

METHODS

Study Site

Octocoral collection for this study took place on Keahua Reef, located outside of the Hilo Bay breakwater on the windward side of Hawai'i Island (Figure 1), where there is an abundant

population of Blue Octocoral. The site is exposed to year-round wave action and winter storms and swells. The water quality of the site is influenced by submarine groundwater discharge (SGD), two rivers that feed into Hilo Bay on the inside of the breakwater, and a submarine sewage outfall that is located on the outside of the breakwater. These water pathways transport land-based pollutants, sediments, and nutrients from the surrounding urbanized area into the bay. During 2017-2018, the area had a mean (\pm SD) sea surface temperature of 25.5 ± 1.0 °C, with a maximum monthly average of 27.4 °C during September 2018 and minimum of 24.38 °C during March 2018 (Rayner et al. 2019). Near to the breakwater, the habitat is rock and boulder with rubble patches and a 3-5 m depth range. Moving offshore of the breakwater, the environment shifts to basalt ridges with sand and rubble channels, moderate coral cover, and a gradual increase in bathymetry to 10-15 m depths. The Hawaiian Blue Octocoral is present throughout the habitat near the breakwater and moving offshore, showing greatest abundance in the rock and boulder habitat (pers. obs.). Scleractinian coral cover is lower and zoanthids are visibly more abundant in the boulder habitat as well. Colonies of octocoral range from approximately 5-100 cm² and most frequently occupy the sides of large boulders, exposed sides of small fixed rocks, and dead colonies of Cauliflower Coral (*Pocillopora meandrina*). Colonies of octocoral were observed in aquaria for the experiment to allow control of water quality and creation of treatment conditions.

In Situ Water Quality & Sampling

Triplicate benthic water samples for nutrient testing were collected in sterile, acid washed, polypropylene plastic bottles at three sites, East, Middle, and West, located approximately 50 m apart within the octocoral collection area on 10 dates, approximately monthly, from November 2017 through November 2018 (Figure 1). Water samples were collected 10 cm above the benthic substrate, filtered through a 0.7- μ m filter (GF/F, Whatman) and stored frozen until analysis for nutrient concentrations. Samples were analyzed at the

University of Hawai'i at Hilo Analytical Laboratory on a Lachat Quickchem 8500 Series 2™ using standard methods to determine $\text{NO}_3^- + \text{NO}_2^-$ (Detection Limit [DL] 0.07 $\mu\text{mol/L}$, USEPA 353.4), PO_4^{3-} (DL 0.03 $\mu\text{mol/L}$, USEPA 365.5), and NH_4^+ (DL 0.36 $\mu\text{mol/L}$, USEPA 349) concentrations. The concentration of $\text{NO}_3^- + \text{NO}_2^-$ was assumed to be largely NO_3^- because NO_3^- typically comprises 90% of total inorganic nitrogen in the marine environment (Libes 2009). If nutrient concentrations were undetectable, they were assumed to be half of the detection limit. The average *in situ* concentrations were used to determine nutrient concentrations for the experimental treatments.

Coral Collection & Husbandry

Collection of live rock harboring multiple colonies of Blue Octocoral was performed by freediving under the permission of the *State of Hawaii, Division of Land and Natural Resources Special Activities Permit No. 2019-18*. Live rock was obtained at a minimum 1-m distance from one another in order to capture genetic variation within the sites and ensure a single colony was not fragmented to create multiple experimental colonies. Fragments of colonies of octocoral were separated from the live rock and transplanted onto plates made from debris cobbles of mounding stony coral (*Porites lobata*) skeleton (Appendix I-B). The octocoral tissue was initially attached to the plates with *Super Glue™*. Transplanting colonies onto new substrate was necessary to eliminate nuisance organisms, predators living within the live rock, and to provide a flat surface for accurate growth measurement. All colonies were distributed among 2 large tanks and acclimated to the aquarium conditions for 2-week after transplant and prior to the start of each experimental trial. Water for octocoral husbandry was sourced from a 500-gallon aquarium system with undetectable inorganic nutrient concentrations (Appendix I-C). Nutrients were adjusted using HACH® potassium nitrate standard (KNO_3) and Fisher Scientific® potassium phosphate monobasic (KH_2PO_4) standard solutions to create desired NO_3^- and PO_4^{3-} concentrations. For octocoral acclimation, nutrients were enriched to be equal to the ambient *in*

situ NO₃⁻ and PO₄³⁻ concentrations. During experiment trials, colonies were kept in 2-L aquariums without flow-through and received a 90% water changes every other day to refresh nutrient conditions and maintain a salinity of 34-35 ppt. This frequency of water change was determined after observation of nutrient fluxes in these aquaria and frequency of water changes in similar coral experiments (Serrano et al. 2018). Oxygen supply and minor water movement were provided by *Tetra 77851 Whisper Air Pump* and aeration stones and octocoral were subjected to 10/14-hr light/dark cycle exposure to $90 \pm 5 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ from an LED light. Aquarium temperature was maintained at standard coral reef aquarium conditions, 23.5-24.5 °C, using *Hydor* submersible glass aquarium heaters (Godinot 2011, Rosset et al. 2017).

Experimental Nutrient Treatments

Three experimental treatments were based on the mean (\pm SD) 2017-2018 ambient *in situ* NO₃⁻ concentrations: '*ambient*' ($1.01 \pm 0.65 \mu\text{mol/L NO}_3^-$), *below* (0.5x ambient), and *greater* (1.5x ambient). PO₄³⁻ concentration in all experimental treatments was maintained equal to the *in situ* ambient PO₄³⁻ concentration ($0.084 \pm 0.055 \mu\text{mol/L PO}_4^{3-}$). Three aquariums replicated each NO₃⁻ treatment level (n = 3/treatment). Water samples were collected weekly from each aquarium both immediately following a water change and 24 hours after a water change to analyze PO₄³⁻ and NO₃⁻ + NO₂⁻ concentration. The NO₃⁻ + NO₂⁻ concentrations of experiment aquaria were assumed to represent the NO₃⁻ concentration because KNO₃ (NO₃⁻) was the form of inorganic nitrogen added to create treatment conditions.

Growth Experiment

Two eight-week long trials were conducted to evaluate the growth of octocoral colonies in NO₃⁻ treatments. Aquariums were randomly reassigned a position on the shelf under the light fixture after each water change to avoid laboratory condition blocks. This created a randomized block design, where each aquarium was considered a block and individual colonies within the

aquariums were unique sample units. Colonies were randomly assigned to the treatment aquariums after the acclimation period and before the start of each trial.

The mean starting tissue area of colonies in Trial 1 was $33.26 \pm 13.44 \text{ mm}^2$, and the mean number of polyps was 24 ± 12 . The number of colonies in each aquarium was based on random assignment of colonies to aquariums and colony survival. Colonies that died within the first two weeks of a trial and two colonies that merged together were removed from the experiment (Appendix I-D). In Trial 1, there were three or four colonies in each aquarium for a total of 26 colonies ($n = 26$). Random assignment of colonies to aquariums was unsuccessful in creating equal variation of starting colony size (area or polyp count) among treatments and resulted in more large colonies in the greater than ambient treatment. Starting size correlated with experimental treatments in Trial 1 (Figure 2). This correlation was a confounding aspect of the analysis in Trial 1, however random assignment of colonies to tanks in Trial 2 resulted in equal variation in starting size among tanks. The mean starting size of colonies in Trial 2 was $17.74 \pm 18.16 \text{ mm}^2$ tissue area and 7 ± 6 polyps. Number of colonies in each aquarium was increased to four or five during Trial 2 for a total sample size of 42 colonies ($n = 42$).

Octocoral growth was quantified using two methods: 1) measurement (mm^2) of new tissue area (*New Area*) and 2) the number of new polyps (*New Polyps*) between week one and eight. Photos of each colony with a scale bar for size reference were captured at the start and end of each trial, and photos were analyzed in *Program Image J* (Figure 3, Schneider 2012). Colonies were also analyzed under a dissecting microscope to ensure accurate polyp count.

Statistical Analysis

Nutrient data from the aquarium treatments were analyzed independently for each trial using a one-way-analysis of variance to determine if nutrient conditions were successfully different among treatment groups.

Patterns of octocoral growth among treatments for each experiment were investigated through a separate analysis of each growth parameter, *New Area* (mm²) and *New Polyps* (quantity), for each trial. Linear mixed effect models were conducted in *R* using package '*lme4*' (Bates et al. 2015), to investigate how the dependent variables for growth were influenced by the random effect and various combinations of fixed effects of the experiment. Mixed effect models allowed for analysis of data that may be clustered in order to acknowledge the possibility for intra-cluster correlation (Zurr et al. 2009). Growth data were analyzed through mixed effect models to account for the fact that multiple colonies were nested within a common aquarium environment (*Tank*). It has become common practice to account for the possibility of an experimental block effect (Zurr et al. 2009, Edmunds 2011). The fixed effects included in the analysis for this experiment were *Treatment* and colony size *Start Area* or *Start Count*, corresponding to the dependent growth metric *New Area* or *New Polyps*. Each colony in the experiment was considered an independent sample while aquarium unit (*Tank*) was included as a random effect. Models were statistically compared using Akaike Information Criterion (AIC) values and comparing Δ AIC values. Values for Δ AIC were derived by calculating the difference between the AIC of a given model and the lowest AIC value generated. A null model with the random effect of *Tank* was included in the analysis. The AIC comparison method penalizes models for complexity while rewarding simplicity and fit to data (Akaike 1998). Models that receive a lower AIC score are suggested to be a better fit model for explaining the pattern of the dependent variable and addressing the hypothesis at hand (Akaike 1998). Model selection was performed separately colonies in each trial and for each growth metric, *New Area* and *New Polyp*.

All analyses and data visualizations performed for the study at hand were using statistical software *R Studio* (version 3.5.0, R Studio Team 2016).

RESULTS

In Situ Water Quality

Nutrient concentrations of the benthic reef environment were similar throughout the octocoral collection area on Keahua Reef, with seasonal variation observed (Figure 4). The difference among mean $\text{NO}_3^- + \text{NO}_2^-$ concentrations at the sample sites was less than $0.15 \mu\text{mol/L}$ and less than $0.015 \mu\text{mol/L}$ for PO_4^{3-} concentrations. The range of $\text{NO}_3^- + \text{NO}_2^-$ observed throughout the sites was $0.14 - 2.61 \mu\text{mol/L}$ and the range of PO_4^{3-} was $0.015 - 0.28 \mu\text{mol/L}$. The mean NH_4^+ concentration at all sites was less than the detection limit ($0.36 \mu\text{mol/L}$). The mean salinity throughout the collection area was 34.8 ± 0.5 ppt.

Experiment Water Quality

In both trials of the experiment, three distinct treatments of NO_3^- were successfully maintained among aquaria, and the NO_3^- concentration of the *ambient* treatment was similar to the *in situ* concentration (Figure 5). In both trials, PO_4^{3-} concentrations throughout aquaria were slightly lower than the ambient phosphate concentration at the collection site and similar among all experimental treatments (Figure 5). Substantial depletion of nutrients occurred over the 24 hours following a water change (Figure 5). Among all tanks in both trials, the mean (\pm SD) salinity was similar to *in situ* salinity at 35 ± 0.4 ppt, and the mean (\pm SD) temperature was slightly lower than the *in situ* sea surface temperatures at 23.4 ± 0.7 °C.

Octocoral Growth

The average amount of growth (*New Area* or *New Polyps*) exhibited by colonies in Trial 1 was greater than the average growth of colonies in Trial 2 (Table 1). Models explaining *New Area* or *New Polyps* in both trials showed that starting colony area (*Start Area*) or polyp count (*Start Count*) were significant predictors. The amount of growth exhibited by colonies was not in

linear relation to the *Start Area* or *Polyp Count*. Colonies with greater *Start Area* or *Start Count* exhibited more raw growth (*New Area* or *New Polyps*) than smaller colonies, and less proportional growth (*New Area/Start Area* or *New Polyps/Start Count*) than smaller colonies (Figure 6). Treatment was also a significant factor for predicting both *New Area* or *New Polyps* for octocoral colonies in the experiment.

New Area

Octocoral colonies in the *greater* than ambient NO_3^- treatment exhibited less new area growth (*New Area*) and an abnormal growth pattern in comparison to colonies in the *ambient* and *below* ambient NO_3^- nitrate treatments (Figure 7). *Treatment* and starting colony tissue area (*Start Area*) were not significant factors explaining *New Area* of octocoral colonies in Trial 1, however, both the additive and interactive models including these fixed effects were significant for describing *New Area* of colonies in Trial 2 (Table 2). The additive model that includes the fixed effects of *Treatment* and *Start Area* for the growth of colonies in Trial 2 showed that colonies subjected to the *greater* treatment (estimate = -0.807 ± 0.907) exhibited substantially less growth than colonies in the *ambient* and *below* ambient treatments (estimate = 2.024 ± 1.064 , Table 3, Figure 8). This additive model also showed a positive relationship between *Start Area* and *New Area* growth when grouping all colonies in Trial 2 (estimate = 0.110 ± 0.025 , Figure 8). The interactive model showed that there was an abnormal pattern displayed by colonies in the *greater* treatment: colonies subjected to the *ambient* and *below* treatments levels displayed a strong positive relationship between *New Area* and *Start Area*, whereas, colonies in the *greater* treatment displayed almost no relationship between the variables (Figure 9). These data showed that colonies subjected to the greatest NO_3^- concentration showed deterred new area growth with an abnormal relationship between starting size and amount of growth. These data also show that colonies in the lower NO_3^- treatments exhibited the greatest amount of new area growth and a normal relationship between starting area and new area growth.

New Polyps

Octocoral colonies in the *greater* than ambient NO_3^- treatment exhibited less new polyp growth (*New Polyps*) than colonies in the *ambient* and *below* ambient NO_3^- nitrate treatments (Figure 7). The interactive model including fixed effects NO_3^- treatment (*Treatment*) and starting number of polyps (*Start Count*) was significant for predicting number of new polyp growth (*New Polyps*) on colonies in Trial 1. The interactive and additive models including these fixed effects were significant for describing *New Polyps* on colonies in Trial 2 (Table 2). The additive model describing *New Polyps* on colonies in Trial 2 showed that colonies subjected to the *greater* treatment (estimate = -0.237 ± 0.310) exhibited substantially less growth than colonies in the *ambient* and *below* ambient treatments (estimate = 1.786 ± 0.362 , Table 3, Figure 8). Models which include an interaction between these fixed effects showed colonies in the *ambient* and *below* treatments displayed a positive relationship between *Start Count* and *New Polyps*, whereas, colonies in the *greater* treatment showed no relationship or a negative relationship between these variable (Figure 9). These data showed that compared to colonies subjected to ambient and below ambient NO_3^- , colonies subjected to enriched NO_3^- concentration displayed deterred production of new polyps and an altered relationship between starting polyp count and new polyp production.

DISCUSSION

NO_3^- Effects on Growth

The results of this experiment demonstrated that enrichment of NO_3^- was detrimental to Blue Octocoral growth (Figure 8). Similarly, an observational study conducted in Honokōhau Bay of West Hawai'i found that octocoral cover was negatively correlated with anthropogenic-sourced nitrogen ($\delta^{15}\text{N}$) (Parsons et al. 2008). Colony size was identified as a confounding factor in the first trial of the experiment, and therefore, was reasoned to dampen the observation of a treatment effect. Trial 2 results showed that NO_3^- deterred growth and larger colonies

tended to show more growth (Figure 8). The elevated NO_3^- treatment contained a greater number of large colonies in Trial 1, so it was reasonable to assume that colony size dampened the observation of a clear treatment effect. Few experimental studies have investigated the response of Alcyonacean (soft) corals to NO_3^- or nutrient enrichment; however, several studies on Scleractinian corals documents mixed responses of various species to elevated nutrient concentrations. In some studies, nutrient enrichment appeared to increase or have a negligible effect on coral growth (Atkinson et al. 1995, Bongiorni et al. 2003a, Bongiorni et al. 2003b, Tanaka et al. 2010, Serrano et al. 2018). However, other investigations found that elevated nutrients reduced calcification and increased vulnerability to bleaching and temperature or pH induced stress (Ferrier-Pages et al. 2000, Be'raud et al. 2013, Ezzat et al. 2015).

Blue Octocoral in the experiment showed less growth and altered growth patterns in an elevated nitrogen to phosphorus (N:P) ratio, similar to studies on Scleractinian corals which found that some species were sensitive to the N:P ratio. An excess of N without provision of sufficient P has shown to be more detrimental to coral growth and health in comparison to excess P or enrichment of both nutrients (Wiedenmann et al. 2013, Rosset et al. 2015, Ezzat et al. 2015, Rosset et al. 2017). In this experiment, using Blue Octocoral colonies subjected to N:P ratios that were equal to, greater than, and less than that of the ambient conditions, the greatest colony growth was observed in the reduced N:P ratio conditions. This result suggests that Blue Octocoral colonies outside of the Hilo breakwater may be exposed to less desirable conditions for growth than those which the species is adapted to.

The mean benthic concentration of $\text{NO}_3^- + \text{NO}_2^-$ at the octocoral collection site in Hilo Bay is comparable to the high end of the range of benthic $\text{NO}_3^- + \text{NO}_2^-$ concentrations observed around Hawaii Island (Wiegner et al. 2019a, Wiegner et al. 2019b, Abaya et al. 2018). Mean bottom $\text{NO}_3^- + \text{NO}_2^-$ observed within Honokōhau bay and throughout Kaloko-Honokōhau National Historical Park have been observed at lower concentrations than that of the octocoral collection site (Parsons et al. 2008, Raikow et al. 2017). The results of this investigation and the

Honokōhau Bay study (Parsons et al. 2008) suggest that ambient nearshore Hawaii Island NO_3^- + NO_2^- concentrations, assumed to be mostly NO_3^- in the marine environment (Libes 2009), may be enriched conditions compared to what the species is adapted to. As suggested by altered growth pattern in the enriched NO_3^- treatment of this experiment, these concentrations may be close to the threshold for withstanding NO_3^- enrichment that the Blue Octocoral possesses.

Elevated Nutrient Impacts on Blue Octocoral Habitat

The patchy abundance and special distribution of Blue Octocoral in West Hawai'i lead to its suggestion as a possible indicator of anthropogenic alteration to water quality (Parsons et al. 2008, Walsh et al. 2013). This experiment suggests that elevated NO_3^- alone is not likely to be driving high octocoral cover, however, it is valuable to consider the potential explanations for the patterns of abundance of Blue Octocoral that have been observed around Hawai'i Island.

Studies have reported soft coral species abundance to decrease on a gradient of decreasing water quality, however, soft coral coverage is typically greater than stony coral coverage in areas of poor water quality (Parsons et al. 2008, Baum et al. 2016). The observational study in Honokōhau Bay revealed that Blue Octocoral abundance was positively correlated with stressed (diseased, necrotic or bleaching) stony coral abundance (Parsons et al. 2008). Although Blue Octocoral may not exhibit increased growth in conditions of poor water quality or elevated NO_3^- , the species may be a more effective competitor than other benthic species under these conditions. Studies that observed soft coral dominance in conditions of eutrophication and sedimentation have also concluded this condition could be a result of relief from competition from stony coral species (Baum et al. 2016). Although colonies in the elevated NO_3^- conditions of this experiment exhibited deterred growth, colonies survived in all treatment levels and in the aquaria used, which was not a flow-through system. The ability for colonies to acclimate and survive in the experiment conditions suggests the Blue Octocoral to be a robust and adaptable species. Various soft coral species have presented themselves as superior

competitors. Alcyonacean species were observed to cause localized mortality and outcompete stony coral for substrate, although competitive capabilities were species-specific (Sammarco et al. 1983). Observations have shown that although soft corals are affected by storm events, colonies are likely to only suffer partial damage and exhibit fast regeneration following a damaging event (Dai 1991). For this reason, soft coral presence, and often dominance, may be frequently observed in storm swept areas. It has also been stated that a shift in benthic community structure to octocoral dominance is likely to follow an event of a mass Scleractinian coral mortality, such as following a crown of thorns outbreak (Bradbury & Mundy 1989). The Blue Octocoral may exhibit increased abundance in a region due to opportunistic occupancy in habitat space that is not suitable for stony corals.

The correlation between octocoral cover and developed coastlines warrants discussion on the specific conditions of water quality that may be beneficial to this species (Walsh et al. 2013). If octocoral abundance around Hawai'i Island is a result of water quality or environment conditions, it may be more likely that other biological, physical, or chemical factors are involved, possibly in synergistic effect. The photosynthetic activity in soft corals has been observed to be lower than that of stony corals, which indicates that soft corals may rely on heterotrophic feeding (Fabricias & Klumpp 1995). Evaluation of photosynthetic activity of zooxanthellae soft corals found the primary production to respiration ratio of these corals is lower than stony corals, as is the amount of organic carbon transported from the symbiont to the coral host (Fabricius & Klumpp 1995). Heterotrophic feeding is known to be an important part of soft coral energy supply for certain species (Fabricias & Klumpp 1995), and dissolved particulate organic matter (POM) has been observed as an important food source for soft coral-dominated reefs in Australia (Fabricias & Dommissé 2000). Zooxanthellate octocorals, including those in the family Xeniidae, are known to rely on suspended plankton and detritus particles in addition to the food sourced from endosymbiotic algae, however, they rely entirely on water flow to transport

particulate matter to the polyp mouth for heterotrophic consumption (Fabricias 2009). The octocoral habitat outside of the Hilo breakwater is frequently subject to strong surge and wave action, particularly during winter months, which creates high turbidity conditions of suspended POM and sediment (pers. obs.).

If POM is an important water quality characteristic of Blue Octocoral habitat, it is unlikely to be without limits. Members of the family Xennidae, which includes Blue Octocoral, have been reported to be especially sensitive to high turbidity (Fabricius et al. 2012) and are most frequently observed in moderately clear water (Fabricius et al. 2005). Zooxanthelae octocoral are seen to occupy habitat area between high wave action and damaging intertidal zone and depths between high and low light penetration (Fabricius & Klumpp 1995). Observational studies in Australia found zooxanthelae octocoral species richness to decline along a gradient of increasing turbidity (Fabricius & De'ath 2004) and declining water quality in general (Fabricius et al. 2005). In comparison to stony corals, octocorals have been seen to respond strongly and more species specifically to water quality changes (Fabricius et al. 2005). POM plays a role in the nutrition requirement of some soft coral species and could be a factor effecting Blue Octocoral abundance, however, further investigation is required.

Blue Octocoral as an Indicator Species

Indicator species may be used to identify changes in the structure and function of an ecosystem due to disturbance and can assist in determining management actions (Carignan and Villard 2002). Some identified pollution indicator microbial species are reliable enough to act as a sole measure of water quality (Rajendran et al. 2018). The patchy distribution of abundant populations of Blue Octocoral around Hawai'i Island suggests that the species can tolerate some amount of anthropogenic altered water quality conditions (Walsh et al. 2013). However, results of this experiment and *in situ* observations suggest that the Blue Octocoral is more likely to exhibit a negative growth response to enriched NO_3^- (Parsons et al. 2008). If this

species is capable of displaying increased growth in response to some aspects of water quality, it is still not understood sufficiently to provide predictable models. Octocoral of the genus *Sarcothelia* have been observed to exhibit fast bleaching in an aquarium and mass mortality in the nearshore marine environment as a response to elevated temperature (Parrin et al. 2016, Parrin et al. 2012, McCutcheon in review, National Park Service unpublished data). This sensitivity to temperature also makes the Blue Octocoral an undesirable indicator species because reliable pollution indicator species must show fast and continuous response to environmental changes without bottoming out or leveling off (Carignan and Villard 2002). The mass loss of octocoral within Kaloko-Honokōhau National Historical Park that occurred during the 2015 bleaching event concludes that the Blue Octocoral is likely to exhibit a 'bottoming out' effect during high temperature conditions. The response of Blue Octocoral to aspects of water quality is not well enough understood at this point to consider the species a predictable and useful indicator.

CONCLUSION

This investigation presents novel information about the endemic Hawaiian species of octocoral, *S. edmondsoni*. The purpose of this study was to determine the growth response of the Blue Octocoral in varying concentrations of NO_3^- through a laboratory experiment. This study showed that enriched NO_3^- was a deterrent to Blue Octocoral growth. Other studies have observed that on a large spatial scale, the species appears to be abundant in regions subject to anthropogenic alterations to water quality (Walsh 2013). However, the species has demonstrated a negative relationship with declining water quality, including nitrogen, at a single site (Parsons et al. 2008), as supported in this experiment. More information is needed to understand the influences driving high Blue Octocoral abundance at particular sites around Hawai'i Island. Investigation of water quality parameters that relate to fresh land-based water input, such as particulate matter and other ratios of N:P enrichment. The results and

observations provided in this study can be used to further assess the potential of the Blue Octocoral as an indicator of anthropogenic alteration to water quality.

Tables

Table 1. Descriptive statistical data for octocoral growth among NO₃⁻ treatments during two 8-week trials of growth experiment.

Treatment	n	Growth: Area (mm ²)				Growth: Polyps (count)			
		Mean	SD	Min	Max	Mean	SD	Min	Max
<i>Trial 1</i>									
all	26	25.9	16.4	0	59.1	10.2	6.1	1	24
Below	9	30.4	5.3	8.2	51.3	10.4	2.1	0	23
Ambient	10	23.7	5.2	0.0	51.6	8.8	1.9	0	16
Greater	7	23.4	6.8	5.9	59.1	12.0	2.2	6	24
<i>Trial 2</i>									
all	42	2.4	3.7	0.1	18.2	1.1	1.1	0	5
Below	16	2.9	1.1	0.1	18.2	1.8	0.3	0	5
Ambient	11	3.2	1.4	0.1	16.0	1.6	0.3	0	3
Greater	15	1.3	0.4	0.1	5.0	0.2	0.1	0	1

Number of samples (n), mean, standard deviation (SD), minimum observed value (min), and maximum observed value (max).

Table 2. Performance of mixed effect models in explaining octocoral growth in experiment Trial 1 and 2. Models include combinations of fixed effects, *Nitrate Treatment* and starting colony area (*Start Area*) and starting polyp count (*Start Count*), to describe dependent variables for octocoral growth; quantity of new polyps (*New Polyps*) or area (mm²) of tissue expansion (*New Area*). The random effect of experiment tank (intercept) is included in all models.

Model	Fixed Effects Included	n	df	k	AIC	ΔAIC	X ²	p (chi-sq)
Trial 1: Models for New Area								
	Intercept only	26	3	0	<u>224.11</u>	0.00	-	-
	Nitrate Treatment	26	5	2	227.05	2.94	1.06	0.5870
	Nitrate Treatment + Start Area	26	6	1	226.99	2.88	2.05	0.1513
	Nitrate Treatment x Start Area	26	8	2	230.70	6.59	0.28	0.8679
Trial 1: Models for New Polyps								
	Intercept only	26	3	0	171.39	1.35	-	-
	Nitrate Treatment	26	5	2	174.37	4.33	1.02	0.5999
	Nitrate Treatment + Start Count	26	6	1	176.37	6.33	0.01	0.9793
	Nitrate Treatment x Start Count	26	8	2	<u>170.04</u>	0.00	10.33	0.0057*
Trial 2: Models for New Area								
	Intercept only	42	3	0	233.30	20.12	-	-
	Nitrate Treatment	42	5	2	235.35	22.17	1.95	0.3371
	Nitrate Treatment + Start Area	42	6	1	221.93	8.75	15.41	<0.005*
	Nitrate Treatment x Start Area	42	8	2	<u>213.18</u>	0.00	12.74	0.0017*
Trial 2: Models for New Polyps								
	Intercept only	42	3	0	123.80	16.26	-	-
	Nitrate Treatment	42	5	2	115.31	7.77	12.72	0.0017*
	Nitrate Treatment + Start Count	42	6	1	112.50	4.96	4.58	0.0323*
	Nitrate Treatment x Start Count	42	8	2	<u>107.54</u>	0.00	8.95	0.0114*

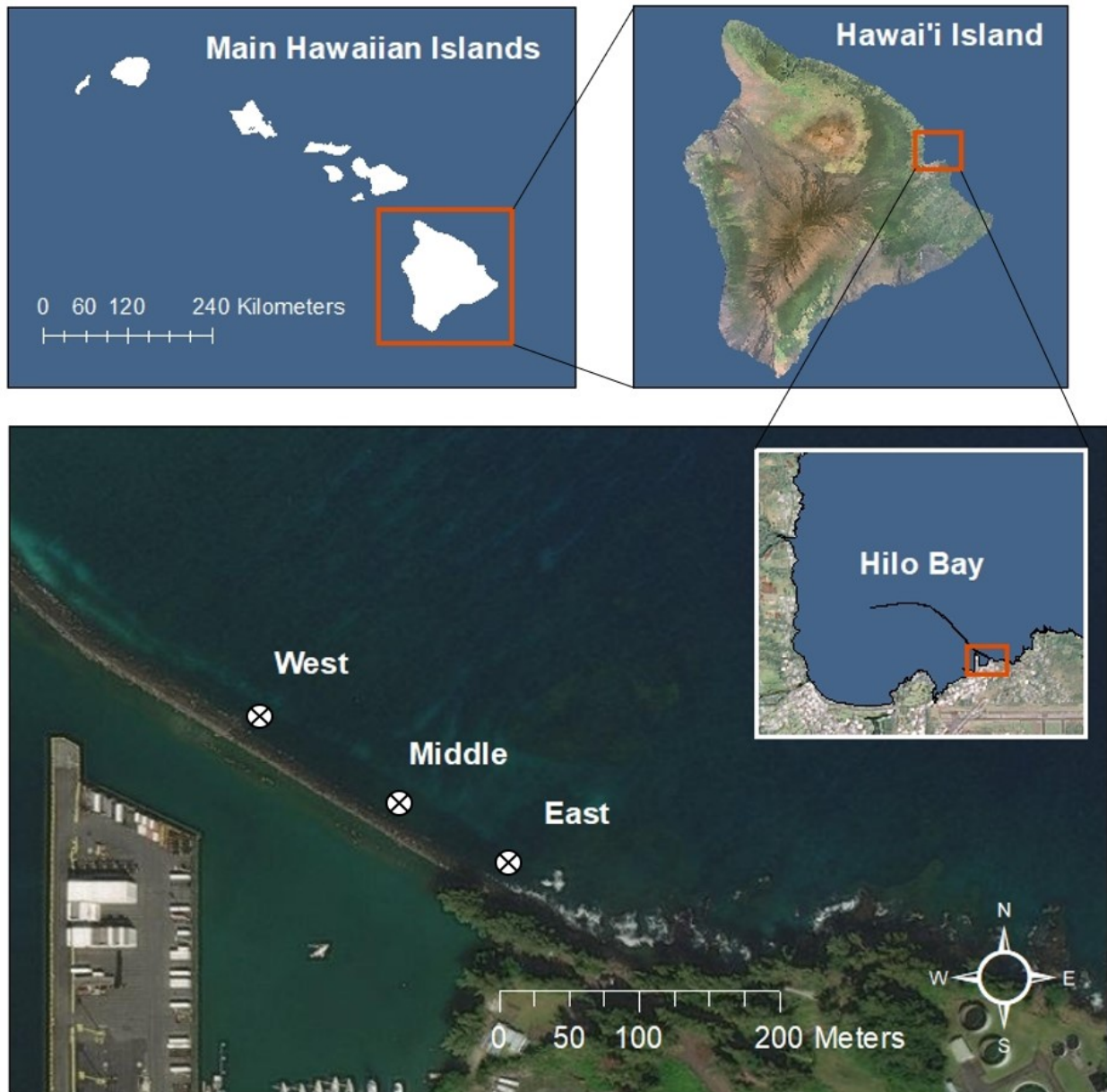
Number of samples (n), degrees of freedom (df), number of predictors (k), value of Akaike Information Criterion (AIC), best AIC score (underlined), difference from lowest AIC score (ΔAIC), Chi-squared test statistic (X²), p-value (p), and significance (*) are provided for each model.

Table 3. Performance of fixed terms among significant models for describing octocoral growth in experiment Trial 1 (A) and Trial 2 (B). Models include interactive (x) or additive (+) combination of fixed effects to predict either quantity of new polyps (*New Polyps*) or area (mm²) of tissue expansion (*New Area*).

Model: Fixed Effects	n	df	Est.	σ	t	p
A) Trial 1						
New Polyps (Nitrate Treatment x Start Count)						
Intercept (Treatment: Greater)	26	25	23.79	6.034	3.943	<0.001*
Treatment - Ambient	26	25	-17.05	7.078	-2.409	0.023*
Treatment - Below	26	25	-27.95	8.164	-3.424	0.002*
Start Count	26	23	-0.325	0.155	-2.099	0.046*
Treatment – Ambient x Start Count	26	25	0.398	0.203	1.958	0.062
Treatment – Below x Start Count	26	22	1.298	0.374	3.458	0.001*
B) Trial 2						
New Polyps (Nitrate Treatment x Start Count)						
Intercept (Treatment: Greater)	42	27	0.145	0.291	0.499	0.621
Treatment - Ambient	42	39	0.920	0.498	1.848	0.072
Treatment - Below	42	20	0.807	0.395	2.039	0.052
Start Count	42	36	0.001	0.023	0.066	0.948
Treatment – Ambient x Start Count	42	33	0.055	0.046	1.195	0.239
Treatment – Below x Start Count	42	41	0.144	0.043	3.361	0.001*
New Polyps (Nitrate Treatment + Start Count)						
Intercept (Treatment: Greater)	42	18	-0.237	0.310	-0.764	0.454
Treatment – Ambient	42	11	1.431	0.378	3.787	0.002*
Treatment – Below	42	9	1.786	0.362	4.931	0.000*
Start Count	42	37	0.042	0.019	2.211	0.033*
New Area (Nitrate Treatment x Start Area)						
Intercept (Treatment: Greater)	42	27	0.824	0.889	0.927	0.359
Treatment - Ambient	42	39	-1.852	1.506	-1.320	0.225
Treatment - Below	42	20	-0.629	1.246	-0.506	0.615
Start Area	42	36	0.026	0.031	0.840	0.405
Treatment – Ambient x Start Area	42	33	0.188	0.057	3.282	0.002*
Treatment – Below x Start Area	42	41	0.152	0.051	3.001	0.004*
New Area (Nitrate Treatment + Start Area)						
Intercept (Treatment: Greater)	42	18	-0.807	0.907	-0.889	0.378
Treatment – Ambient	42	11	1.824	1.169	1.561	0.126
Treatment – Below	42	9	2.024	1.064	1.902	0.064
Start Area	42	37	0.110	0.025	4.316	<0.001*

Number of samples (n), degrees of freedom (df), mixed effect model estimate (est.), standard error (σ), test statistic (t), p-value (p), and significance (*) of individual fixed effects.

Figures



WGS 1984 | <https://coast.noaa.gov/ccapftp/#/>, <https://planning.hawaii.gov/gis/download-gis-data/> | 12/10/2019

Figure 1. Water samples were collected for nutrient analysis at three sites (*West, Middle, and East*) within the octocoral collection area, outside of the breakwater (black line in lower insert) of Hilo Bay on the east side of Hawai'i Island from November 2017 to November 2018. Latitude 19.733 N and Longitude -155.052 W.

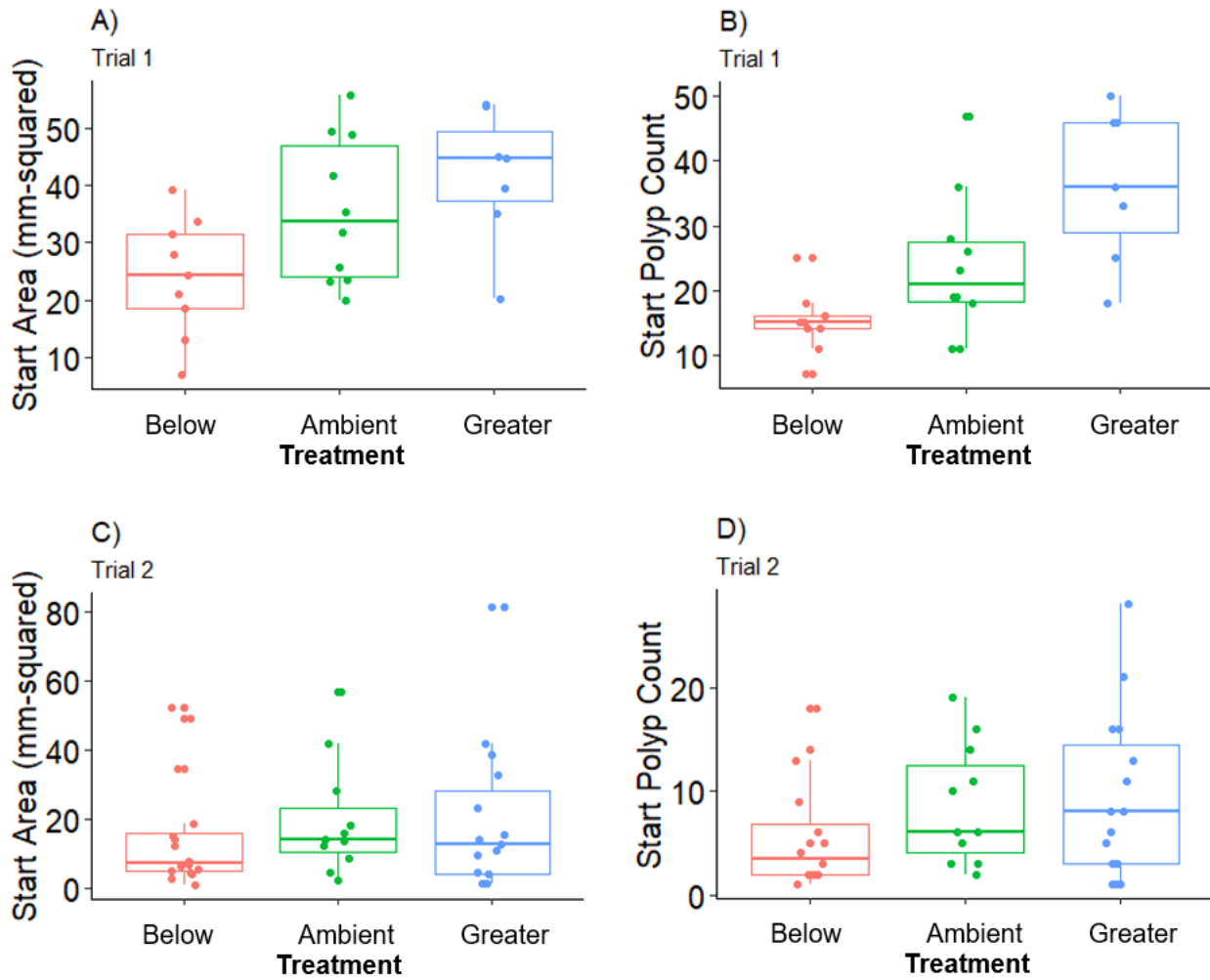


Figure 2. Distribution of starting colony size among three NO_3^- treatments for starting area of colonies in Trial 1 (A), starting polyp count of colonies in Trial 1 (B), starting area of colonies in Trial 2 (C), and starting polyp count of colonies in Trial 2 (D).

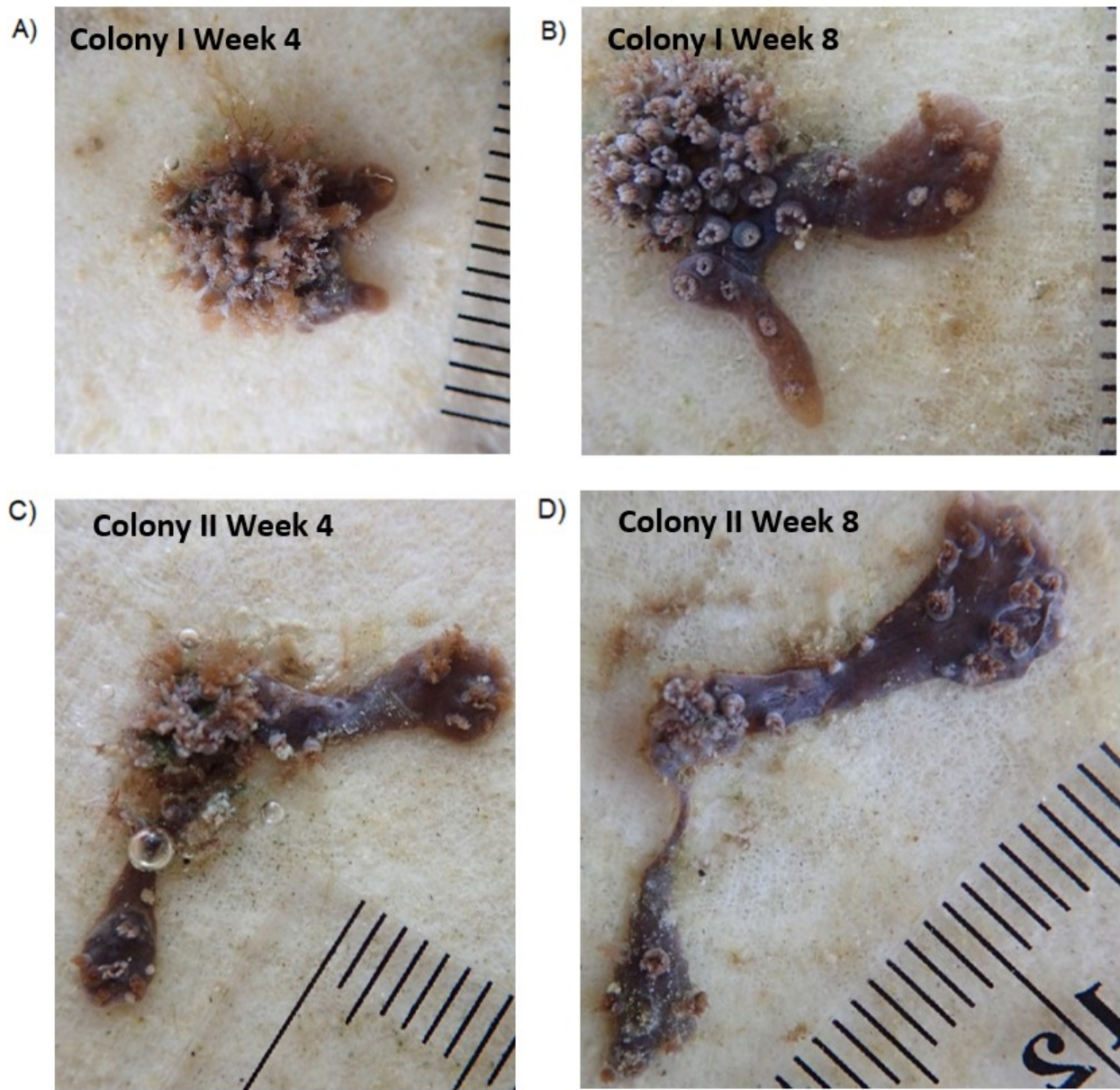


Figure 3. Photographic images for growth measurement of Blue Octocoral colony I during week four (A), and week eight (B) as well as colony II during week four (C), and week eight (D).

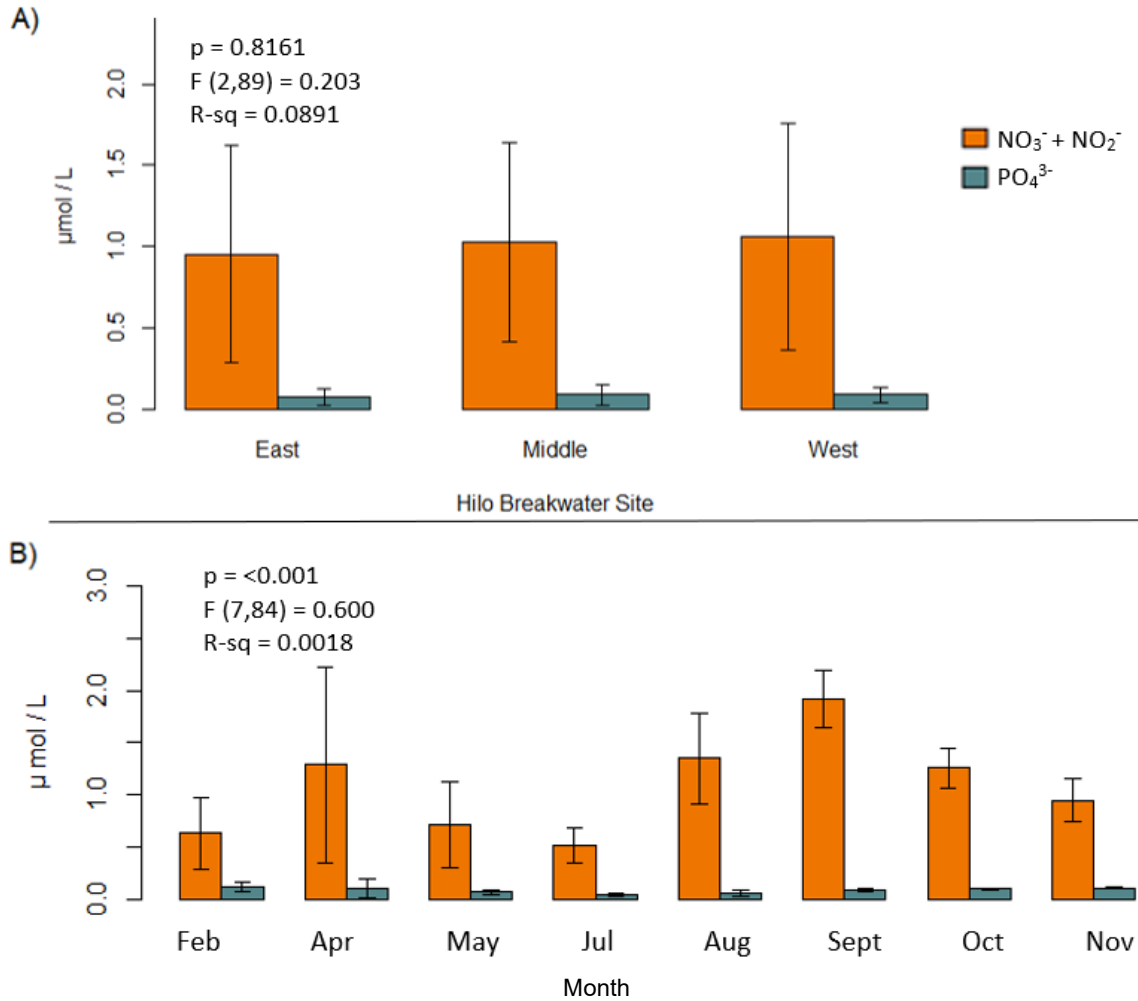


Figure 4. *In situ* nutrient concentrations at Keahua Reef in Hilo Bay, Hawaii Island. Nitrite + nitrate ($\text{NO}_2^- + \text{NO}_3^-$) and phosphate (PO_4^{3-}) concentrations (mean \pm SD) at three sites throughout 13-month sampling period (A), and monthly averages from November 2017 to November 2018 (B). Triplicate water samples ($n=10$) were collected at each site. The month of November includes samples from 2017 and 2018.

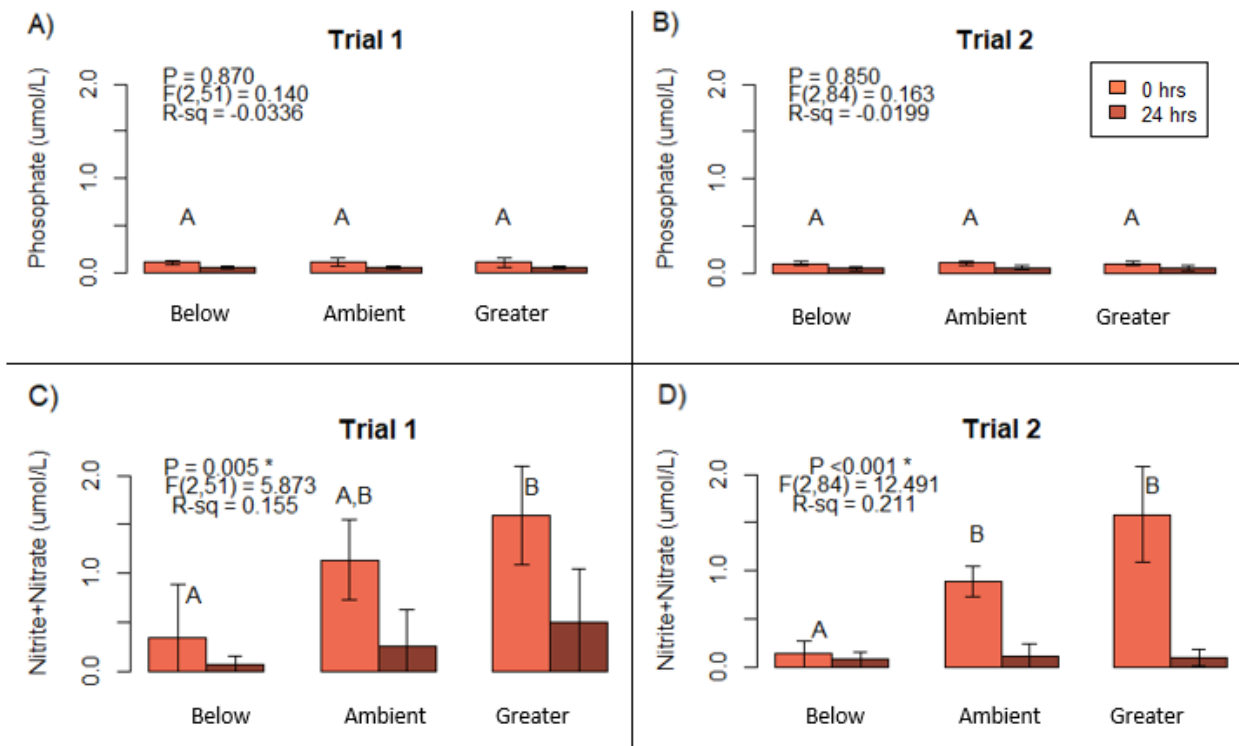


Figure 5. Experiment treatment nutrient concentrations throughout both trials of eight-week octocoral growth experiment. Concentration (mean \pm SD) at the time of water change (0 hrs) and the day following a water change (24 h) for phosphate (PO_4^{3-}) during Trial 1 (A), and Trial 2 (B), and nitrate + nitrite ($\text{NO}_2^- + \text{NO}_3^-$) during Trial 1 (C), and Trial 2 (D). Letter groupings indicate no significant difference in concentration between treatments within each experiment trial as determined by Tukey post hoc test for one-way analysis of variance.

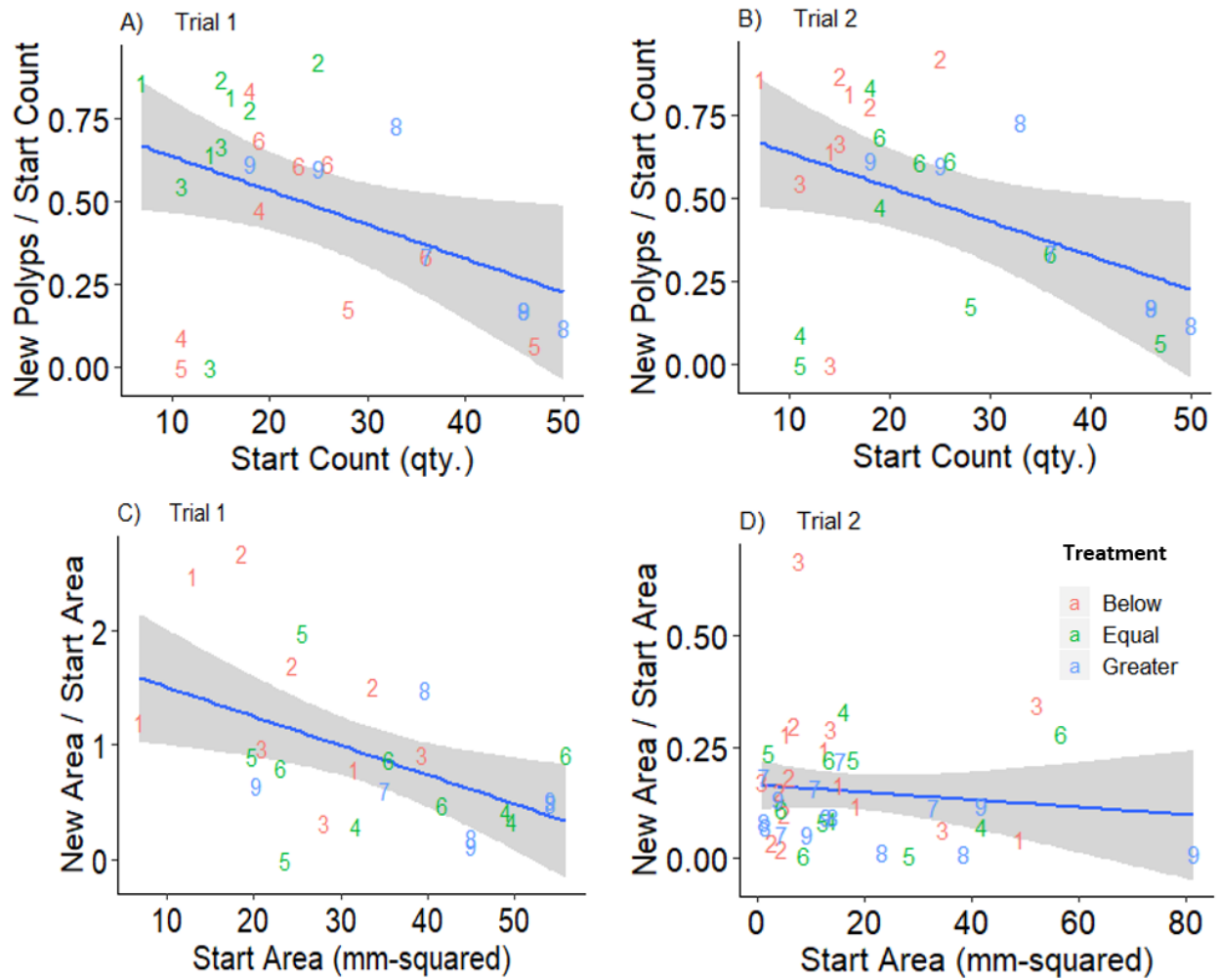


Figure 6. Relationship between proportional growth (*New Area/ Start Area* or *New Polyps/ Start Count*) and starting size of octocoral colonies for the metric of polyp count for Trial 1 (A) and Trial 2 (B), and for the metric of colony tissue area (mm-squared) for Trial 1 (C) and Trial 2 (D). Shading represents the confidence interval of the predicted relationship, coloring indicates treatment level, and numbering indicates aquarium unit.

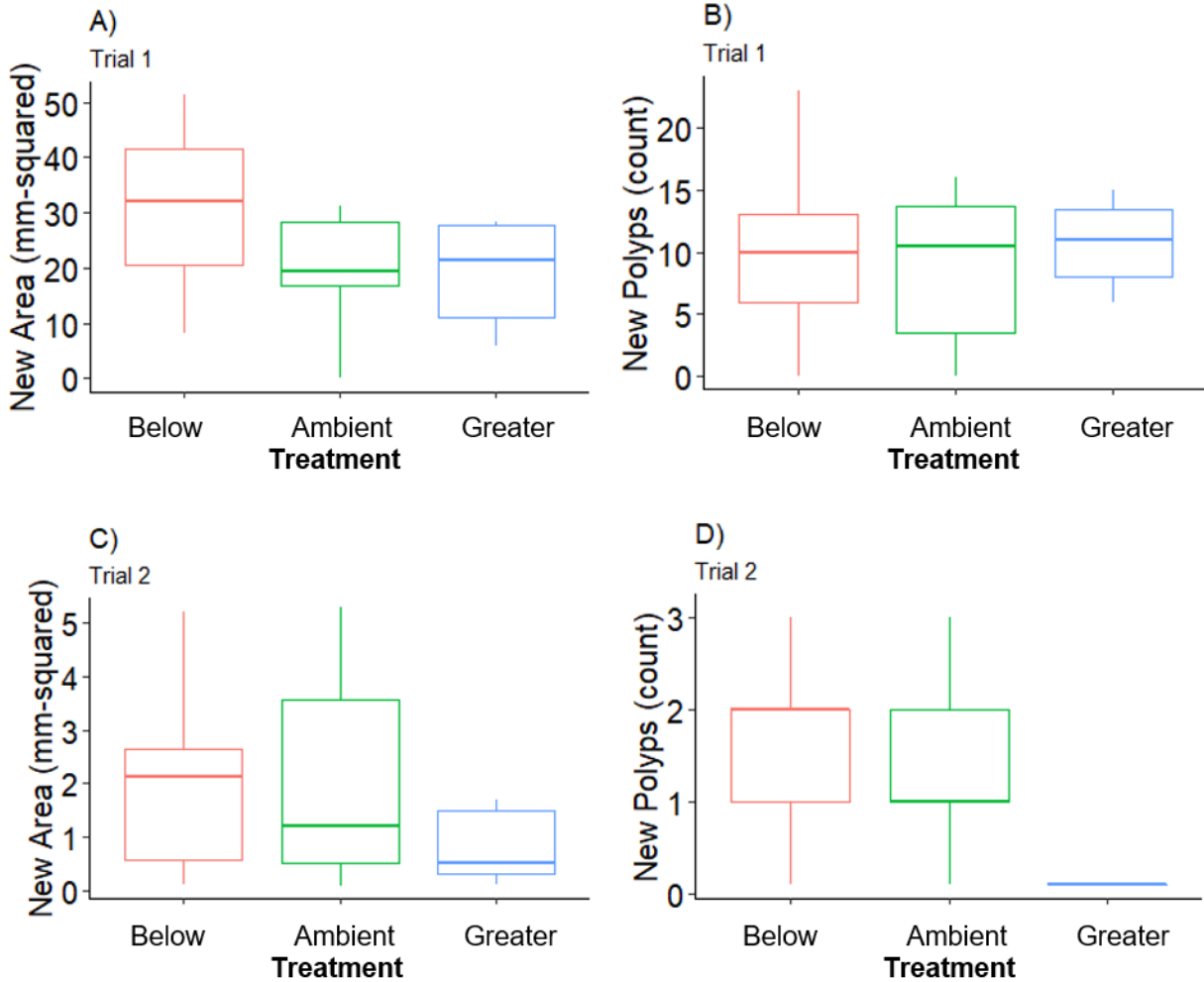


Figure 7. Amount of octocoral growth among three NO₃- treatments at the end of two trials of eight-week growth experiment. End of Trial 1 average area (mm²) of octocoral tissue expansion (*New Area*) (A), and quantity of new polyps (*New Polyps*) (B), as well as end of Trial 2 average area of tissue expansion (*New Area*) (C), and quantity of new polyps (*New Polyps*) (D). Plot whiskers depict the 10th and 90th percentiles and boxes show 25th and 75th percentiles with median value indicated by horizontal line; individual colony measures indicated by points.

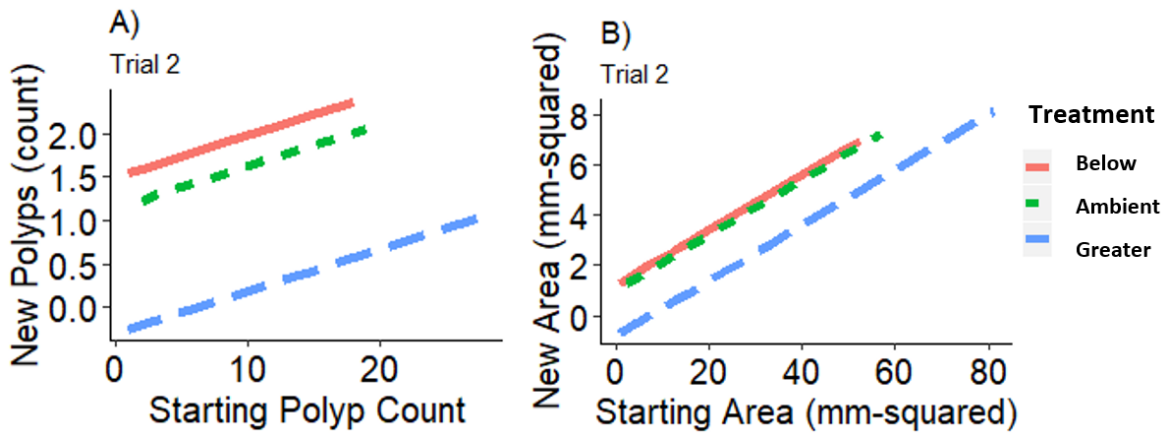


Figure 8. Relationship between octocoral growth and starting size of colonies within Trial 2, with adjusted intercept for colonies in each NO_3^- treatment. Parameters for growth include area (mm^2) of octocoral tissue expansion (*New Area*) (A), and quantity of polyps (*New Polyps*) (B). Treatments of NO_3^- included *below*, *ambient*, and *greater* than ambient NO_3^- concentration during 2017-2018 at the experiment collection site on Keahua Reef outside of the at the Hilo Bay breakwater.

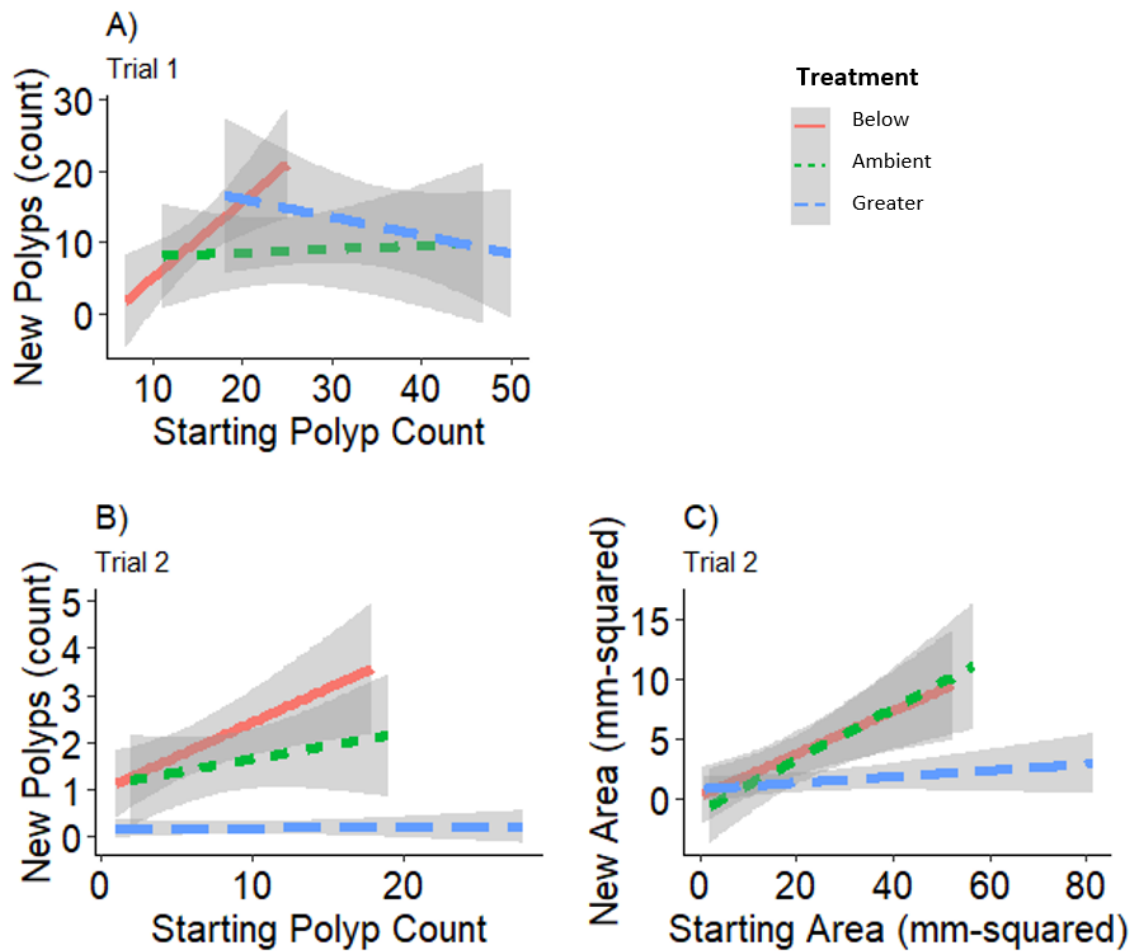


Figure 9. Relationship between growth and starting size of octocoral colonies with adjusted intercept and slope for colonies in each NO_3^- treatment. Parameters for growth include area (mm^2) of octocoral tissue expansion (*New Area*) (A), and quantity of polyps (*New Polyps*) (B). Treatments of NO_3^- included *below*, *ambient*, and *greater* than ambient NO_3^- concentration during 2017-2018 at the experiment collection site on Keahua Reef outside of the at the Hilo Bay breakwater. Shading represents the confidence interval of the predicted relationship.

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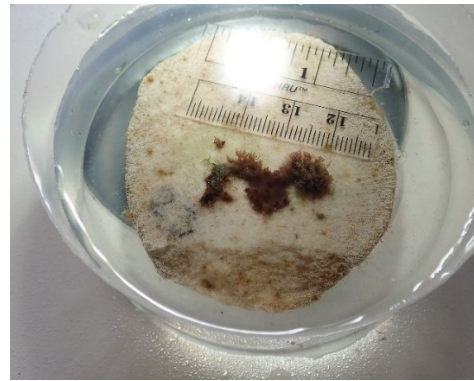
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Appendix I.

This is the first documented experiment of the endemic Hawaiian Blue Octocoral, so this appendix provides a record of additional observations unrelated to the experiment purpose but that may prove useful to future experiments and cultivation of the Blue Octocoral. These observations provide insight on techniques for supporting the Blue Octocoral survival and growth in aquaria.

Notable Success in Methods

- A. *Water Flow*: This experiment has shown that the Hawaiian Blue Octocoral appears to be a hardy species that can withstand the process of transplanting and is capable of survival as well as exhibiting measurable growth in a stagnant aquarium system. Other species of octocoral have exhibited greater growth in aquaria with water movement compared to no water movement (Geteno et al. 2000), however the stagnant systems appear to suffice for the purpose of this growth experiment.
- B. *Transplant Substrate*: The plates made from sliced cobbles of debris Scleractinia coral skeleton were found to be the most desirable surface for transplanting the octocoral onto. Debris cobbles of stony coral were collected from shorelines of east Hawaii Island and sliced using a rock cutter to create roughly 1-cm thick plates at a suitable length and width (<25 cm²) to enable placement of multiple plates into each aquarium. Octocoral colonies exhibited the fastest and strongest attachment to this natural substrate in comparison to other materials they were tried including: glass, frosted glass, scuffed plastic, and cement. This is comparable to an experiment that found octocoral larvae to settle faster and more frequently on natural substrate with an established microbial community (Geteno et al. 2000).

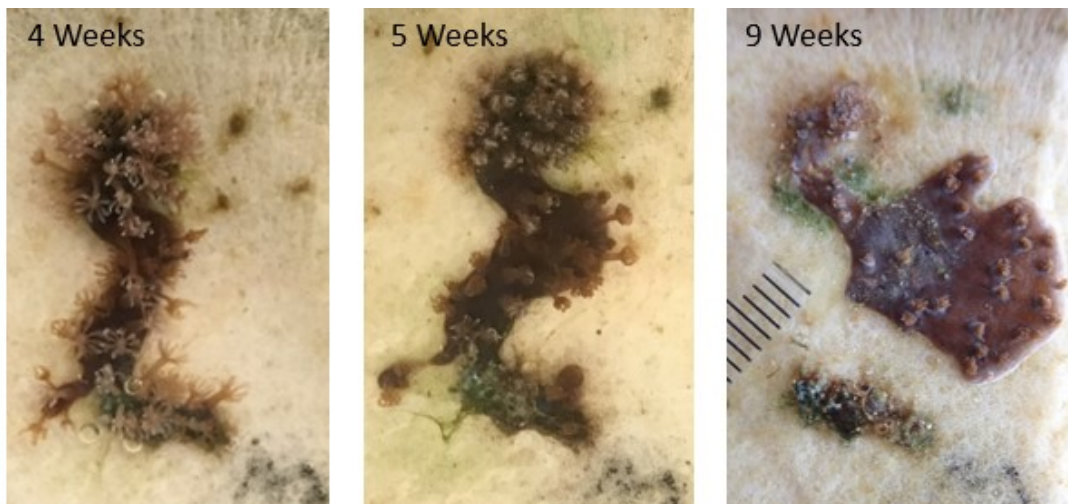


Images Above: Shows several cut plates of debris coral skeleton with octocoral transplanted onto it (Left). Single plate of coral skeleton in a petri dish with part of cm ruler stick laying on top for size reference (Right).

- C. *Water Source*: A source of consistent and low nutrient salt water was required in order to create treatment conditions for this experiment. A 500-gallon aquarium equipped with live rock, macroalgae, a Joubert-Plenum-refugium system, and protein skimmer was found to be the best low nutrient water source for this experiment. About 5-gallons of water was

removed from the 500-gallon system to supply water for each water change on the experiment aquariums. The water removed from the experiment aquariums for each water change was added to the 500-gallon system to allow it to be recycled and subjected to biological cycling of excess nutrients including possible ammonium build up. This strategy was developed after unsuccessful trial of artificial sea water and collection of water from the octocoral collection site. The nutrient concentrations in artificial seawater was not consistent and often times was not low enough to allow for creation of desired concentrations for treatments. Freshly mixed artificial sea water does not contain a microbiome and it's recommended that this biome is allowed to develop before subjecting marine organism to the water. These factors combine made artificial sea water an undesirable water source. Collecting water from the study site was considered, however this was deemed too inefficient for performing water changes every other day and presented the possible obstacle of running in to poor environmental conditions when a collection was necessary.

- D. *Colony Merger*: Two colonies that were growing on a single plate merged together and exhibited a notably greater amount of growth compared to other colonies. This suggests that octocoral may be capable of exhibiting enhanced growth when small fragments are growing in close proximity and are of compatible genotypes to merge. This close placement of many small transplanted fragments has been termed 'microfragmenting' by coral cultivation specialist. Observations of out planted *Scleractinia* coral fragments revealed that microfragmented massive corals produced 10x more tissue than larger fragments (Page et al. 2018).



Images Above: Merged colony growth progression at 4, 5, and 9 weeks after colonies were transplanted onto substrate.

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