GROWTH RATES OF BRANCHING *LITHOPHYLLUM* CORALLINE ALGAE ACROSS REEF HABITATS IN GUAM

BY

REILLY KLEVEN

A thesis submitted in partial fulfillment of the

requirements for the degree of

MASTER OF SCIENCE

IN

BIOLOGY

SUPERVISORY COMMITTEE

Dr. Tom Schils, Chair

Dr. Matthew S. Mills

Dr. Brett Taylor

Dr. Guillermo Diaz-Pulido

UNIVERSITY OF GUAM

2024

AN ABSTRACT OF THE THESIS of Reilly Kleven for the Master of Science in Biology presented, August 2, 2024

Title: Growth Rates of Branching *Lithophyllum* Coralline Algae Across Reef Habitats in Guam

Approved: Tom Schils

Tom Schils, PhD, Chair, Thesis Committee

Acknowledgements

I would like to sincerely thank my advisor, Dr. Tom Schils for all his input and support throughout my master's degree. I have learned so much from him and I am very thankful for his guidance throughout this journey. I would also like to thank my committee members, Dr. Matthew Mills, Dr. Brett Taylor, and Dr. Guillermo Diaz-Pulido for their support and counsel. I would like to thank my mom, and the rest of my family and friends, who have been my unwavering support system the past three years. Lastly, this work would have not been possible without my source of funding, NSF EPSCoR.

This material is based upon work supported by the University of Guam, the National Science Foundation under Grant Number OIA-1946352. Any opinions, findings, and conclusions or recommendations expressed herein are those of the author(s) and do not necessarily reflect the views of the National Science Foundation. The views expressed herein are those of the author(s) and do not necessarily reflect the views of NSF or any of its sub agencies.

List of Figures

Figure 7. Comparison of growth rates between location and season based on surface area measurements.

Figure 8. Comparison of growth rates between location and season based on volume measurements.

Table of Contents

Abstract

Coralline algae are dominant benthic organisms on tropical reefs and play a crucial role in the carbon and carbonate cycles of these ecosystems. They contribute equally, or greater to reef carbonate production than scleractinian corals, emphasizing their role in the maintenance of a net positive reef accretion state as climate change continues to reduce coral cover. This study developed a reliable method for long-term, non-destructive documentation of growth *in situ* for a tropical branching *Lithophyllum* species originating from different reef environments in Guam, a typical forereef environment (Pago Bay) and one with seasonally increased nutrients and sediment (Togcha Bay). The relationship between growth and seasonality was also explored. Growth was significantly higher during the dry than wet season, with an average dry season growth of 6.21 ± 3.18 mm^3/day and 3.12 ± 1.34 mm^3/day for the two reef sites, Pago and Togcha Bay. Growth was also significantly higher during the wet season at Pago than Togcha Bay, with an average of 1.15 ± .261 mm^3/day and .462 ± .149 mm^3/day, respectively. This study reveals accurate values for *Lithophyllum* growth across different seasons which is crucial for attempting paleo-environmental reconstructions and understanding the present and future contribution of these organisms to reef accretion. It also emphasizes the complex role that environmental variables play in coralline algal growth.

Introduction

Crustose coralline algae (CCA) are dominant benthic reef organisms with a global distribution, from polar to tropical waters (Johansen 1981; Martin & Gattuso, 2009; Schäfer *et* al., 2011; Martin *et al.*, 2013). CCA also have a very broad vertical distribution, ranging from the supralittoral to mesophotic zones (Schubert *et al.*, 2023). CCA provide a variety of ecosystem

services for reef environments (Littler *et al.*, 1985; Martin *et al.*, 2013; Weiss & Martindale, 2017). They build and stabilize reef frameworks by depositing calcium carbonate as extracellular skeletons of Mg-calcite prisms in the cell walls (Lewis *et al*., 2017; Smith *et al*., 2012). These skeletons contribute to the resilience and recovery potential of reefs, providing protection from disturbances such as storm events and bioerosion (Doropoulos *et al*., 2012). Corallines act as important reef cementers, decreasing the likelihood of structural collapse by reinforcing caves and cavities (Weiss & Martindale, 2017). Additionally, they produce chemicals and contain associated bacterial communities that promote the settlement of invertebrate larvae such as coral (Morse *et al*., 1980). Coralline algae also play a crucial role in the carbon and carbonate cycle of coastal reef ecosystems (Martin *et al.*, 2013). However, their contribution to net calcium carbonate production on coral reefs remains unquantified, warranting additional empirical studies to evaluate their potential as carbon sinks (van der Heijden & Kamenos, 2015; Lewis & Diaz-Pulido, 2017). Obtaining these data becomes increasingly more important as the effects of climate change continue to reduce coral cover (Cornwall *et al*., 2023). Following shifts in environmental conditions and disturbance events, coral-dominated reefs may transition to an alternative stable state comprised of CCA-dominated communities. This phenomenon has been observed below the upper subtidal on the forereefs of Guam (Schils 2023). After an island-wide bleaching event in 2017, ghost skeletons of *Acropora abrotanoides* were quickly colonized by *Lithophyllum* species. Yet, it is unknown whether this transition is merely a short-term event. Coralline algae contribute equally or greater to reef carbonate production than scleractinian corals, emphasizing their role in the maintenance of a net positive reef accretion state (Cornwall *et al*., 2023). However, the long-term impacts of such phase shifts on the carbonate budget of coral reefs remains unknown (Schils 2023).

Calcification and growth rates of coralline algae are impacted by a variety of factors, such as species type and environmental conditions (Villas Bôas *et al*., 2005; Darrenougue *et al*., 2013). Growth is primarily influenced by water temperature, carbonate saturation state, desiccation, light, and intensity of grazing (Figueiredo *et al.*, 2000; Villas Bôas *et al*., 2005; Kuffner *et* al., 2008; Pulecio-Plaza *et al*., 2023). A study in the Gulf of California demonstrated much faster growth rates, calculated by apical tip extension, for *Lithophyllum margaritae* in summer months (5.02 ± 1.16 mm/year) than winter months (0.83 ± 0.16 mm/year), revealing a strong influence of light regime and temperature on algal growth (Steller *et al*., 2007). Growth can also be regulated by hydrodynamic energy. Corallines existing at leeward sites exhibit fast, vertical growth forming protuberant and branched shapes, while those existing at windward sites experience slower growth with flat, compact shapes (Villas Bôas *et al*., 2005). Epiphytes can also slow coralline algal growth by inhibiting light and nutrient absorption. However, wave action and grazing by herbivores help control epiphytes. The productivity of corallines is also affected by sedimentation (Schäfer *et al*., 2011). *Lithophyllum* species growing off the Pacific coast of Panama in areas with large amounts of sediment derived from rivers experience a tenfold decrease in carbonate production. Additionally, *Lithothamnion* beds in Brazil demonstrated a 70% reduction in net photosynthetic production rates due to sediment cover (Riul *et al*., 2008). Ultimately, there are many local ecological drivers influencing growth and calcification rates (Pulecio-Plaza *et al*., 2023). The growth and calcification of reef-builders *Porolithon antillarum* and *Lithophyllum* cf. *kaiseri*, found in the Caribbean Sea, have been observed to increase during periods of coastal upwelling. Corallines are also vulnerable to the effects of ocean acidification and global warming, resulting in decreased growth and calcification rates (Lewis & Diaz-Pulido, 2017).

Amongst marine calcifying organisms, coralline algae are believed to be some of the most sensitive to the effects of ocean acidification (Martin & Gattuso, 2009; Lewis & Diaz-Pulido, 2017). They are absent in naturally acidified seawater where other calcifying species can exist (Martin & Gattuso, 2009). For many, this is partially due to their skeletal composition of magnesium calcite, which has a lower saturation state than aragonite (Jokiel *et al*., 2008; Kuffner *et al*., 2008; Martin *et al*., 2013). As pH levels decrease, it becomes increasingly more difficult for coralline algae to deposit calcium carbonate in their cell walls (Jokiel *et al*., 2008). Because of their sensitivity to acidification, they serve as sentinels for assessing the impacts of climate change (Lewis & Diaz-Pulido, 2017). Methodologies used in the past to evaluate the impacts of acidification on corallines possess limitations such as, the use of closed systems, minimal treatment replication, and short-term experimental designs (Jokiel *et al*., 2008; Martin & Gattuso, 2009). Some recently published studies do not all corroborate this idea of corallines being particularly sensitive to the effects of ocean acidification and warming (Cornwall *et al*., 2020; Cornwall *et al*., 2019; Pinna *et al*., 2022). A meta-analysis of 14 studies found that the mean effect of elevated temperature on coralline algal accretion becomes significant only at 5.23°C above the temperatures that each author deemed as the "control" (Cornwall *et al*., 2019). This was found to be consistent for studies in cool and warm temperate areas, as well as on tropical coral reefs. Additionally, CCA species with shorter generation times, high phenotypic plasticity, or broad thermal tolerance may be more adaptable to ocean temperature increases. An *in situ* study conducted on *Lithophyllum stictiforme* found an increase in growth of algal thickness when transplanted from a cold to warmer site, outperforming the local algae (Pinna *et al*., 2022). Conceptacles of *L. stictiforme* were also produced in the warmer conditions, suggesting this species is more resistant than previously believed. The study emphasizes the importance of *in*

situ experiments; laboratory studies may be misleading as real natural variability cannot be replicated inside a tank. Moving forward, accurate methods to assess the impact of ocean acidification and warming on coralline algae growth and calcification are essential to understand and predict the effects of climate change on coral reef systems (Lewis & Diaz-Pulido, 2017).

In general, baseline data on the growth and calcification of coralline algae are deficient. The number of studies on coralline algae lags that of other calcifying marine organisms, such as corals (Agegian, 1981; Lewis & Diaz-Pulido, 2017). Many experimental techniques to quantify the growth and calcification of CCA have been employed, each with their own restrictions (Lewis & Diaz-Pulido, 2017). For example, chemical marker staining (Lewis & Diaz-Pulido, 2017), growth band counting (Darrenougue *et al*., 2013), Mg/Ca ratio cycles (Halfer *et al*., 2000), buoyant weight (Johnson *et al*., 2014), and alkalinity anomaly measurements (Chisholm & Gattuso, 1991) have all been used to estimate calcification, dissolution, and growth rate with varying levels of accuracy and success. For example, one study using both the buoyant weight and alkalinity anomaly methods to document growth found discrepancies in their results, where the buoyant weight method showed a two-fold greater rate of calcification than the alkalinity anomaly method for the same coralline alga (Steller *et al*., 2007). These studies also used destructive measurement techniques, making it impossible to monitor growth over multiple time intervals over a longer time frame. 3D scanning is a promising technique to monitor the growth rate of coralline algae due to its minimally invasive nature. This technology is comparable to X-ray computed tomography (X-ray CT) or photogrammetry (Reichert *et al*., 2016). However, CT scanners are a costly investment and photogrammetry has limitations in accuracy. Image-based 3D scanning records a succession of 2D images and uses mathematical models to detect 3D point clouds. The point clouds are fused to create polygonal 3D meshes of scanned objects. Although

such devices have never been used to study algae, they have been utilized to quantify the surface area and volume of scleractinian corals. Such studies have found 3D scanning to be highly precise and reliably reproducible (Reichert *et al*., 2016). This technology is comparable to X-ray computed tomography (X-ray CT) or micro-computed tomography (μ-CT). Although not extensively used with coralline algae, CT scanning has been widely utilized for observing annual coral banding and was used for the first time on corallines by Bressan *et al*. (2007). More recently this equipment has allowed for the visualization of both external and internal features, such as branching pattern, shape of conceptacles, buried epiphytes, and sand pockets (Torrano-Silva *et al*. 2015). Peña *et al*. (2021) employed micro-computed tomography to analyze crust thickness in response to differences in CO_2 levels. Other studies have used the technology to visualize and measure growth bands as a function of seasonality (Chan *et al.*, 2017, Lewis *et al*., 2017b). Micro-CT is a fast, nondestructive, and high-resolution technique that does not endanger the survival of live specimens (Torrano-Silva *et al*., 2015).

Most studies on the growth and calcification of coralline algae have been conducted in polar or temperate regions, demonstrating a dire need to expand such studies to the tropics (Schäfer *et al*., 2011). Knowledge on *Lithophyllum* growth in tropical waters is lacking and is based off only a few studies. Obtaining accurate values for coralline growth and calcification rates across different seasons is crucial for attempting paleo-environmental reconstructions and understanding the present and future contribution of these organisms to reef accretion (Darrenougue *et al*., 2013).

Statement of Purpose

The goal of this study was to quantify and compare the growth rate of a fruticose *Lithophyllum* species inhabiting two unique reef environments in Guam, and to test the efficacy of a novel experimental design. This study aimed to develop a reliable method for long-term, non-destructive documentation of growth *in situ*. Growth rates were compared between a *Lithophyllum* species originating from two different reef environments: a reef channel with seasonally increased nutrient and sediment loads in comparison to typical forereef habitats in Guam. Although the same species, this *Lithophyllum* algae displays varying growth morphologies at the two differing sites. This study strived to answer whether source location influences the growth dynamics of the two populations. Growth rates were observed for samples that have been transplanted from their place of origin to the contrasting reef environment and in a laboratory setting. These data were generated for further use to assess *Lithophyllum'*s contribution to reef growth in present and future environmental conditions.

Materials & Methods

Study Organism

A common and widespread species of *Lithophyllum* was selected as the study organism, as *Lithophyllum* species are among the most abundant calcifiers on Guam's reefs and thrive well after episodes of mass coral mortality. *Lithophyllum* species 8, as identified in Mills *et al*. (2022), was utilized in the study. The branching morphology of *Lithophyllum* sp. 8 also makes the algae a suitable specimen for 3D scanning. To confirm the samples collected were *Lithophyllum* sp. 8, DNA sequencing was performed. The DNA from each sample was extracted, amplified, and sequenced. A new, single-edged razor blade was used to retrieve tissue from each plant. The

tissue was then placed in a 1.5 mL Eppendorf tube for DNA extraction. The extraction was performed using QIAGEN DNeasy Blood and Tissues kits following the manufacturer's bench protocol (Qiagen Inc., Valencia, CA). Chloroplast photosystem II thylakoid membrane protein D1, *psb*A (about 950 base pairs), was amplified by polymerase chain reaction (PCR). The *psb*A marker was selected due to the high success rate of amplification. The primers used to amplify this gene were *psb*AF and *psb*AR2 (Yoon *et al*., 2002). Protocols for *psb*A amplification were created by Mills & Schils (2021). Additionally, mitochondrial cytochrome c oxidase subunit 1 DNA barcode region, COI-5P (about 664 base pairs), was amplified by PCR. The forward primer used to amplify this DNA barcode region was TS_COI_F01_10 (Mills & Schils, 2021) and reverse primer was GWSRx (Saunders & McDevit, 2012). Protocols COI-5P for amplification were created by Mills & Schils (2021). Species were identified using phylogenetic analysis based on the maximum likelihood method.

Sample Collection

Samples of *Lithophyllum* sp. 8 were collected from Pago Bay and Togcha Bay (Figure 3). Using SCUBA, seven individuals or plants were photographed and collected using a hammer and chisel from each study site. Samples were collected from their habitats in Togcha and Pago Bay, at approximately 2 and 6 meters respectively. Samples were placed in seawater-filled plastic bags for transportation from the collection site to the University of Guam Marine Laboratory. There, these source plants were maintained in flow-through tanks supplied with seawater from Pago Bay. The plants were gently cleaned of epiphytes and sediment with a toothbrush and given a couple days to acclimate to the tank environment. Seven plants were collected from each site to

account for mortality that may occur during transportation or the acclimation period, and five plants from each were used in the study.

Experimental Design

The growth rate of individual clippings, or branches, from each of the ten sample plants were tracked over the course of fourteen months. One-to-three-centimeter branches with one or two growth axes were selected for the study. Individual branches were clipped from the ten source plants using tweezers, sanded at the base with a Dremel rotary tool, and placed in a dish inside a flow-through seawater tank. Each branch was secured into an appropriately sized drill hole at the top of a nylon thumb screw using super glue (Figure 4). Nylon screws were selected for the study to avoid corrosion, which would affect the growth of the fragments. To prevent excessive stress and branch mortality, the glue was given three minutes to dry, and the newly attached sample was screwed into a base plate and placed back in a flow-through seawater tank. The base plates were 27 cm by 13.5 cm and created with a table saw from eight-foot composite decking boards. Ten threaded 0.635 cm holes were drilled vertically into the base plates, allowing for the attachment of the 0.635 cm thread nylon screws. A total of 15 composite base plates were used in the study, each holding ten individual branches, one from every source plant. Each plate was assigned a number and labeled on the bottom with a permanent marker. Branches from the same source plants were placed in the same location on each base plate. Each screw was given a labeled washer secured with a nut to indicate which source plant the fragment originated from. This degree of replication was chosen to account for branch mortality, breakage, or loss throughout the study. Onset MX2202 HOBO Pendant MX temperature and light loggers were

attached with zip ties to small holes that were drilled into the bottom end of each base plate (Onset, Bourne, MA).

Study Sites

Sample plates were installed at both the collection sites, Pago and Togcha Bay (Figure 3), at depths where the source plants were collected. Pago Bay represents a typical forereef habitat for Guam. This site had an average temperature of 30.1°C and range of 29.1-31.8°C during the wet season (August-November 2022) (Figure 1). During the dry season (December 2022-March 2023), Pago's temperature ranged from 27.1-31.3°C and had an average of 28.9°C. Conversely, Togcha Bay consists of a channel with increased nutrient and sediment levels. During the wet season, we recorded a median temperature of 30.2°C and a range of 27.8-36.2°C. During the dry season, the median temperature was 28.9°C and ranged from 26.2-35.3°C (Figure 1). Light values (lux) were only recorded during the wet season due to logger malfunctioning. Lux was converted into Photosynthetically Active Radiation (PAR) (µmol photons $m^{-2} \cdot s^{-1}$) based on water depth. During the sunniest hours of the day, 9AM-3PM, Pago and Togcha's average PAR levels were 78.3 and 70 µmol photons $m^{-2} \cdot s^{-1}$, respectively. (Figure 2). Along with these environmental differences, *Lithophyllum* growing at these two sites have distinct morphologies (Figure 3). At Pago Bay, plants were observed to have shorter, more fused branches than those at Togcha Bay.

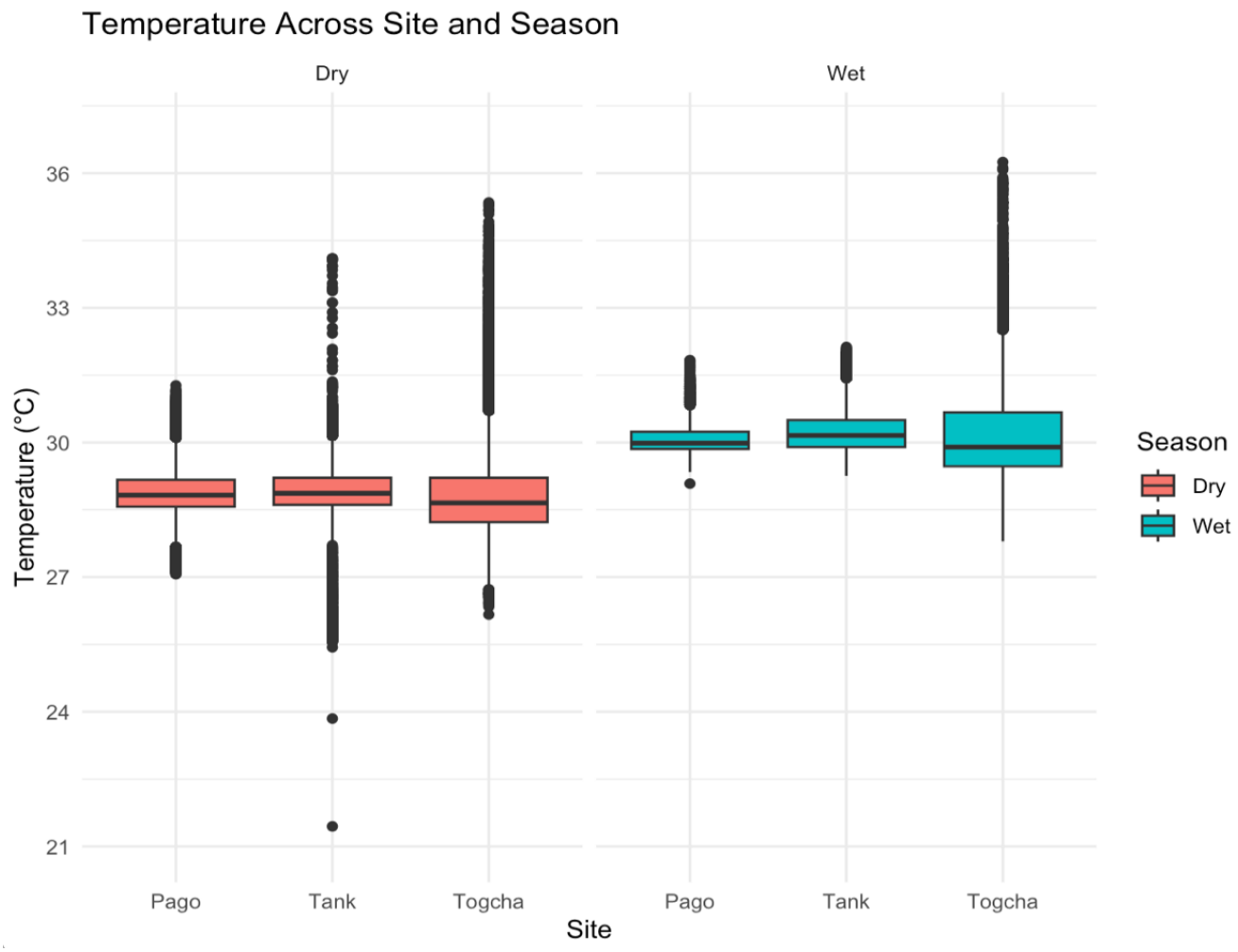

Figure 1. Temperature values (°C) for each study site: Pago Bay, UOG Marine Lab tank, and Togcha Bay during wet and dry season.

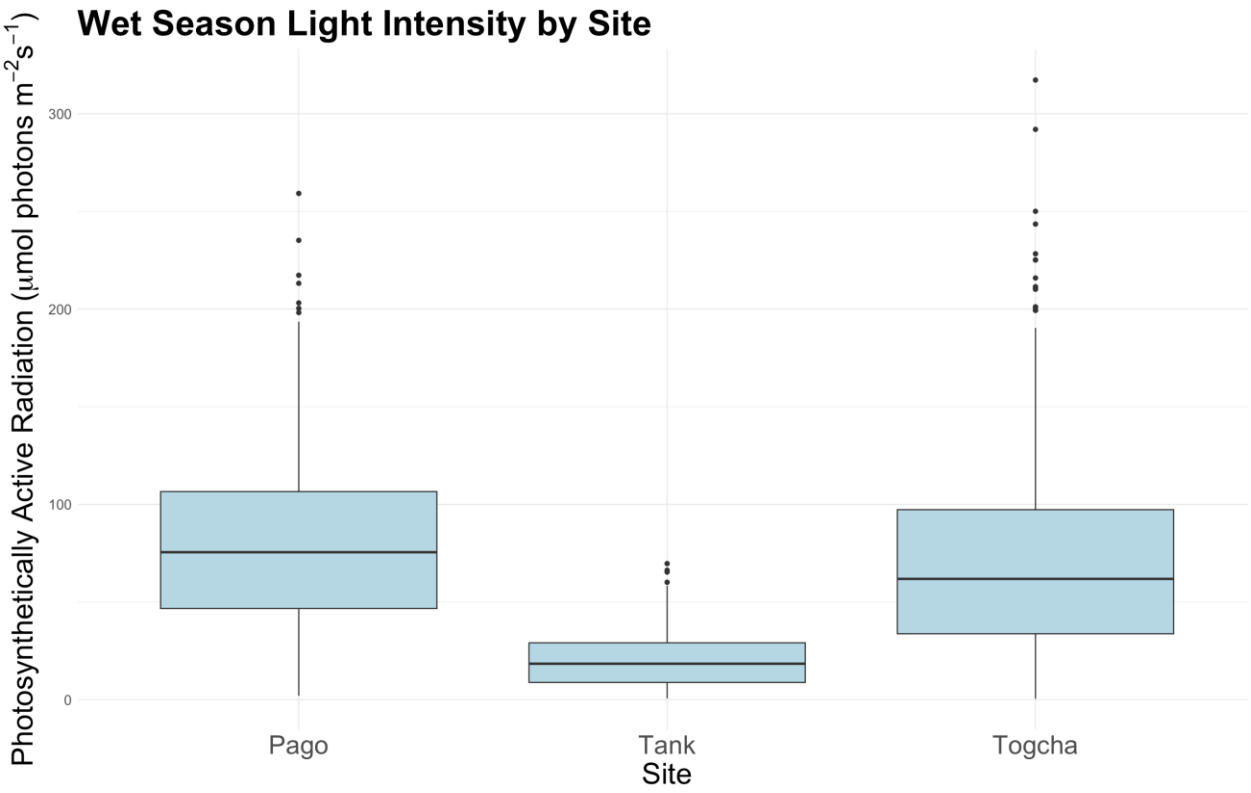

Figure 2. Photosynthetically active radiation (PAR) (µmol photons $m^{-2} \cdot s^{-1}$) values during the wet season (August-November 2022) for all sites, Pago Bay, Togcha Bay, and UOG Marine Lab tanks.

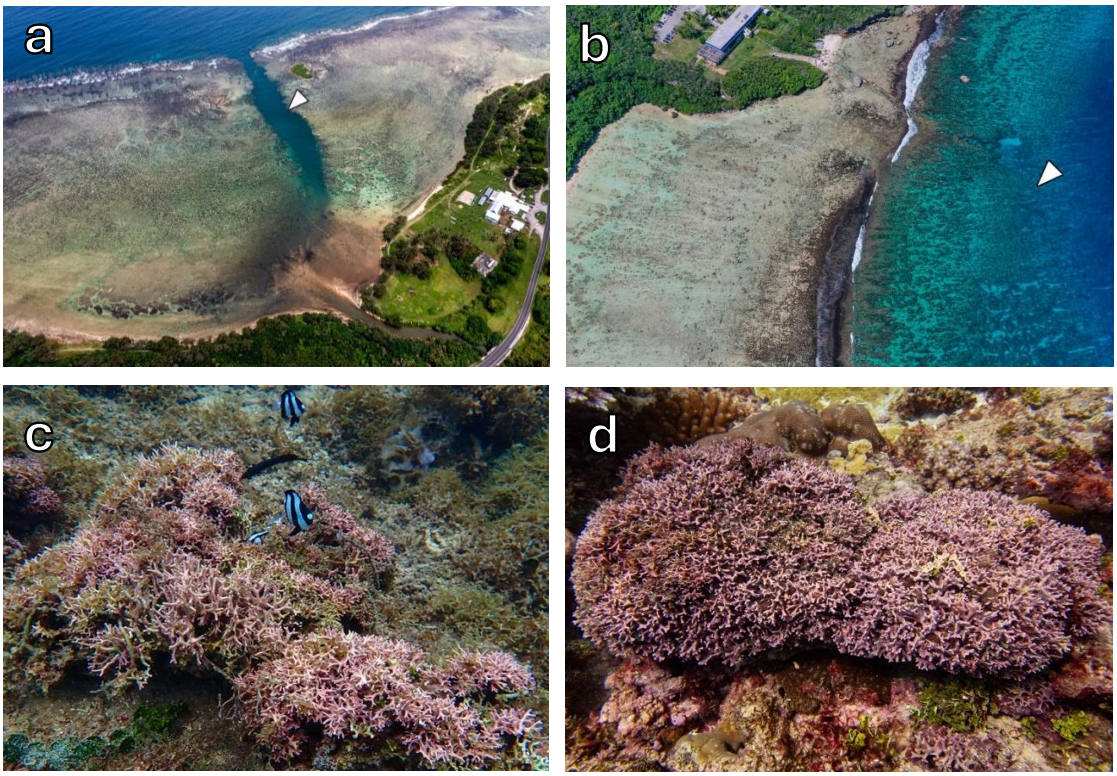

Figure 3. Study sites, Togcha and Pago Bay, and *Lithophyllum* plants existing at each. (**a**) Togcha Bay channel, the white arrowhead points to where samples were installed. (**b**) Pago Bay, the white arrowhead points to where samples were installed. (**c**) *Lithophyllum* plant growing in Togcha Bay, displaying a morphology with less fused branches and longer growth axes. (**d**) *Lithophyllum* plant growing in Pago Bay, displaying a morphology with tightly fused branches and shorter growth axes.

Five of the plates with samples were installed onto flat, rocky substrate in Pago Bay at the same depth and area as the samples were collected (Figure 4). This was repeated with five of the plates at Togcha Bay (Figure 4). To transport and deploy the plates, they were attached to the inside walls of a rectangular plastic crate while submerged in a flow-through tank at the UOG Marine Laboratory. Two zip ties were threaded through the holes in the center of the plates, wrapped around both sides, and attached to the holes lining the crate. To deploy five of the plates

in Pago Bay, the crate was quickly walked from the UOG Marine Laboratory to the entrance of the water and submerged immediately in the shallow waters. Using SCUBA, the crate was transported to the study site and attached to the threaded stock. To deploy five of the plates in Togcha Bay, the crate was placed in a cooler filled with seawater and driven to the site. The cooler was secured with rope in the back of a truck. Extra buckets of seawater were taken to replenish the cooler as needed. SCUBA was used to swim the crate and samples to the study site where the plates were fixed.

The remaining five base plates and samples remained in the flow-through tanks for the duration of the study. Two layers of mesh shade cloths attached with zip ties to a PVC-pipe frame were placed over the tank. This amount of shading was necessary as we observed branch mortality in preliminary experiments when light exposure was higher. The average temperature in the tank for the wet season was 28.9°C and ranged from 29.3-32.1°C (Figure 1). The average temperature during the dry season was 30.2°C and ranged from 21.4-34.1°C (Figure 1). Between the hours of 9AM-3PM, the average PAR was 20 µmol photons $m^{-2} \cdot s^{-1}$ (Figure 2). Tank samples were gently cleaned with a toothbrush once or twice a week, when necessary, to remove epiphytes and sediment. The tank was drained and cleaned once a week to remove sediment and other algal growth.

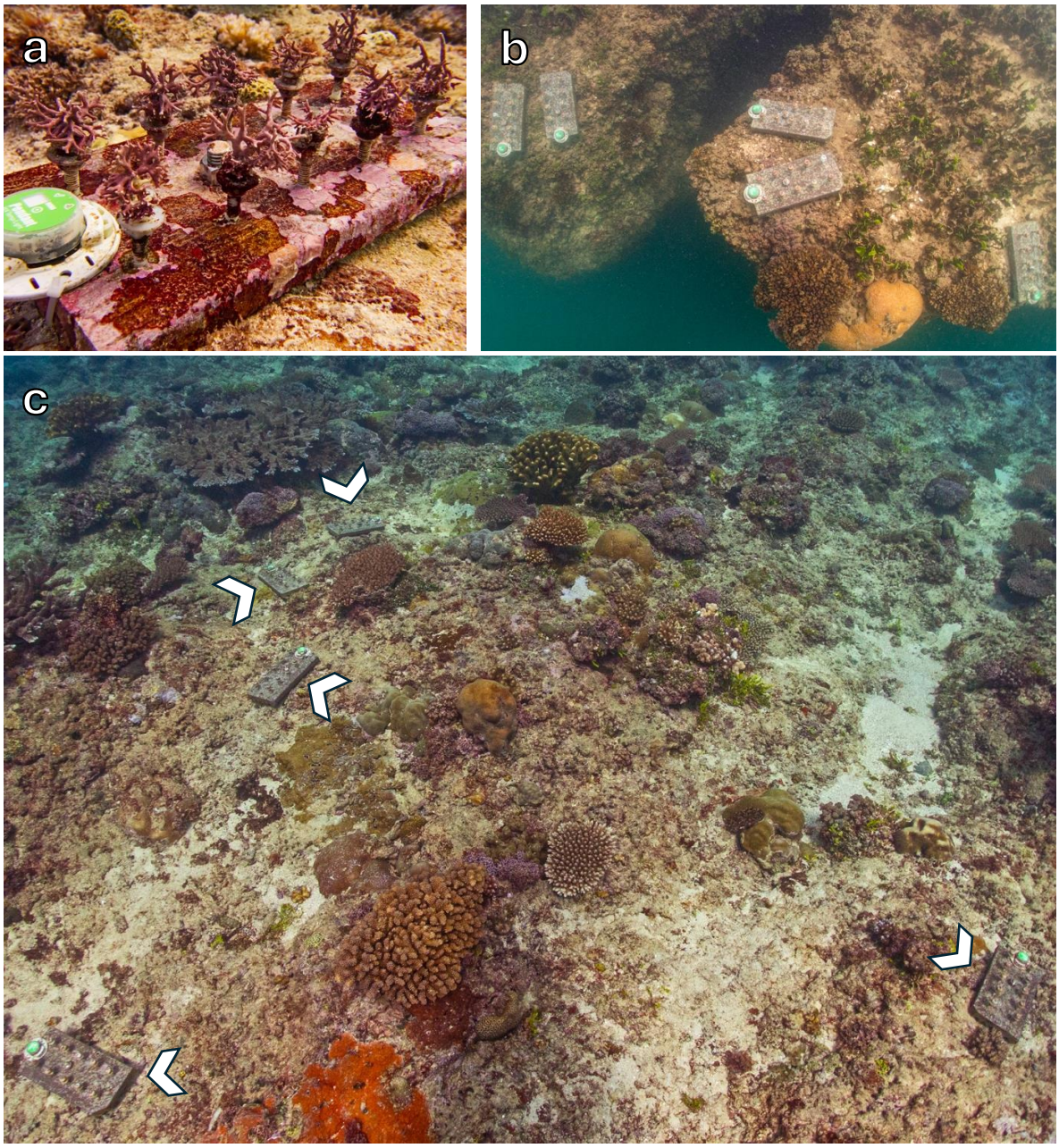

Figure 4. *Lithophyllum* samples mounted on base plates and installed on the reef. (**a**) Close-up image of one of the base plates installed on the reef at Pago Bay. Samples are mounted on top of nylon thumb screws which are secured to a composite decking board. (**b**) Five base plates with samples installed on the reef at Togcha Bay at 2 meters. (**c**) Five base plates with samples installed on the reef at Pago Bay at 6 meters, pointed out with white arrowheads.

Obtaining Growth Measurements

Once a month, or every few months depending on the season, the composite plates were retrieved from Togcha and Pago Bay with SCUBA using zip ties and a crate, just as during the initial installation phase. If ocean conditions were not safe for SCUBA diving, retrieval was postponed until they improved. During the dry season, ocean conditions prevented retrieval of the samples for two or three months at a time. In May 2023, Typhoon Mawar hit the island of Guam, preventing retrieval of the samples until October 2023. Our samples did not appear to be affected by the typhoon; branch loss and breakage was consistent with what was observed throughout the rest of the experiment. At Pago Bay, the plates were attached to the crate with zip ties underwater, quickly walked from the entrance of the water, placed back in the flow-through tank system, and cleaned with a toothbrush. At Togcha, the crate and plates were placed in a seawater filled cooler in the same manner as the installation and driven back to the Marine Laboratory, where they were immediately placed in the tank system and cleaned. Heavy crustose coralline algal growth was removed from the temperature and light logger sensors using a razor blade. Logger data was downloaded at this time. Scans of the fragments mounted on nylon screws was taken using an Artec Micro 3D scanner to generate surface area and volume measurements. This device has 3D accuracy of up to 10 μm and 3D resolution of up to 29 μm. One screw was removed from the base plate at a time and transported from the tank in a cup of seawater to be scanned. The scanner settings were kept consistent with a scanning path of “small complex” at high resolution. Immediately after the 3.5-minute scan was completed, the screw was placed in a cup filled with seawater and returned to its position on the plate in the tank. This was necessary to prevent stress and mortality of the fragments. The cup was replenished with new seawater and the scanning process was repeated for each sample on the base plates.

Following the retrieval day for each site, the plates were re-installed onto the threaded stock residing on the reef. The tank samples were scanned in conjunction with those from both reef sites. Fragments that broke, but were still healthy, were kept in the experiment but data prior to the breakage was omitted. Two healthy fragments from each of the source plants were attached to screws and maintained in the flow-through tank as backups for those that experienced mortality or were lost. At the time of replacement, the 3D scan of the new healthy branch was taken as the new "time zero" measurement. When replacements occurred, a new backup fragment was made for that source plant to ensure adequate backups were available for the duration of the study.

After seven months of growth, the samples grown on the reef at Pago and Togcha Bay increased to a size and complexity that no longer allowed for scanning with the Artec Micro 3D scanner. Moving forward, samples were scanned using a Bruker Skyscan 1273 micro-computed tomography (microCT) scanner. Because the largest standing stock of *Lithophyllum* on Guam exists at Pago Bay and preliminary results pointed to this site having the most optimal conditions for its growth, the experiment was continued with only the samples growing there. A nut was glued to the bottom of a 14 cm by 8 cm empty jar and the sample was screwed into the nut, sitting in an upright position for scanning. Prior to scanning, the machine's settings were calibrated in the software SkyScan 1273. The lowest resolution, 768x486, was used for all the scans to decrease scan time and ensure the samples stayed alive. When performing trial runs with the Skyscan 1273, it was determined that there was minimal difference between the scan quality of low (768x486) and high (3072x1944) resolution scans. Both resolutions captured the entire sample, thus we determined the low-resolution scan was suitable for obtaining surface area and volume measurements. Scans taken with high resolution were too lengthy to keep the samples

alive, which was an important facet of the study. The 1 mm Al + .2mm Cu filter was used, as we found it to produce the best quality images for the *Lithophyllum* samples. A "large" focal spot and pixel size of 205.9 µm were used. Scanning options "continuous rotation" and "119 seconds" were selected and the rest of the settings were kept as default. This scanning time allowed for about two minutes to set up the sample inside the scanner and did not exceed the four-minute mark, which was about the maximum time the samples could withstand being outside of the water. Samples were removed one by one from seawater and placed directly into the scanner. Once the scan was complete, the sample was placed back into seawater, and the process was repeated with all samples.

Processing 3D Scans from the Artec Scanner

All scans were processed using Artec Studio Edition 17 (Artec 3D, Senningerberg, Luxembourg). First, the scan was duplicated so one unedited version existed as a backup. Then, the screw was removed from the scan using the eraser ("rectangular selection") feature listed under "editor." This function allowed for a horizontal cut at the base of the sample, removing the head of the screw and down. Under "tools," "global registration" and "sharp fusion" were applied to the scan. The settings under "sharp fusion" were altered to turn off the hole filling feature and turn on the erroneous frame exclusion feature. The "small object filter" (polygon count 5000) was applied. To fill any areas that the scan may have missed, the "fix holes" function was used. First, under "edges," the base edge of the sample was smoothed at a strength of 1.0 and the base hole was filled with the "flat" function. Other existing holes on the sample were filled with the "smooth" function. To repair hard to connect holes, the "bridges" feature

was used under the “fix holes” tab. Once all the holes were fixed, the model was measured for surface area and volume under the “measures” tab.

Processing Images from the Micro-CT Scanner

The TIF files generated by the Skyscan 1273 were opened in NRecon (v. 2.0, Bruker Corporation, Billerica, MA) to reconstruct the images. After the dataset was loaded, the “start” tab was selected in the reconstruction box. The “position” was changed by dragging the horizontal green line to a dense portion of the scan, indicating which slice to use for previewing. Then, the “preview” button, allowing for the alteration of various parameters, was clicked to reconstruct the chosen slice. This displayed a histogram under the “output” tab. This function determined the dynamic data range when transforming real numbers into integers. The histogram was changed into a logarithmic scale by double clicking the left mouse button on the plot. The image range was changed by dragging the green vertical line to the position where the black plotted line hits “0” on the x-axis. Then the “settings” tab was selected, allowing the profile window on the toolbar to be clicked. The profile showed two curves, profiles along the central horizontal line and average over the full vertical line. The more similar the two curves, the better the scan, meaning the sample had zero to minimal movement. If the two curves were not aligned, a new scan was performed and the sample was further secured. To check for beam-hardening from linear transformation in the software, a portion with the same density was selected on the sample. A line was drawn through the chosen spot starting and ending from the air surrounding the sample. The desired shape of the plot generated by this function was rectangular. If a “cupping” (“U” shape) phenomenon was seen for the middle values on the plot, the beam-hardening percentage (%) was adjusted under the “settings” tab. Misalignment, shown by

artifacts such as stars or crosses on the scan, was corrected by selecting the “fine tuning” function under the “start” tab. The circle for “post-alignment (0.0)” was selected, number of trials was set to 5, and parameter steps was set to 0.5. “Start” was selected to view a series of five preview reconstructions. The image with the highest clarity and least artifacts was selected. Under the “output” tab, the file format was changed to BMP, region of interest (ROI) was set to the size of the container used to scan each sample, and the yellow vertical lines on the sides of the software screen were adjusted to capture the entire sample. The finalized reconstructions were viewed in CTVox (v. 3.3.0 r1412, Bruker Corporation, Billerica, MA).

The screw was removed from the scan using the software Amira with the segmentation editor tool (v. 5.3.3, Thermo Fisher Scientific, Waltham, MA). These scans were then converted into a 3D image in the same software. The BMP files were opened and pixel size, found on the text document that is created in association with the reconstruction files, were copied into the three voxel size sections in the popup box. Then the “isosurface” function was selected in the box in the top left corner of the software. The frequency was adjusted to capture the entire sample and eliminate artifacts. Finally, the reconstruction was exported as an STL file to allow the 3D models to be opened in Artec Studio.

Growth Rate Comparisons

Growth rates were compared through volume (mm^3) and surface area (mm^2) measurements obtained from the 3D scans. These measurements were divided equally across the number of days within each time interval. The proportional percent of increase per day of surface area and volume was calculated for all samples with at least three consecutive time points during the wet season and the time point during the dry season. Samples that did not meet this

requirement were not included in the analyses. All analyses were performed in the statistical software environment R (v. 4.4.0, RStudio Team, Boston, MA).

Due to mortality and breakage, some source populations did not have the same number of replicates as at the start of the experiment. To account for this, the proportional percent of increase for each source plant replicate was averaged for each transplantation site, source location, and season. PERMANOVAs were then performed to explore the effects of location, source population, and season on growth rates. A pairwise Wilcoxon rank sum test was used as a post hoc test with p-values adjusted using the Bonferroni correction.

To decipher how much total growth was experienced across the samples growing in Pago Bay, descriptive statistics were performed to compare the size of samples that survived the entirety of the experiment.

Lastly, growth rates were compared to what was reported by Lewis & Diaz-Pulido (2017) for *Lithophyllum pygmaeum* as it is one of the only studies that has recorded growth rates of tropical branching *Lithophyllum* plants. The study reports growth as a function of monthly linear extension, or vertical growth in μm. This metric was obtained from the data set by measuring the 3D scans in Artec Studio. During the wet season, branches were measured with no additional growth axes. However, due to increased morphological complexity during the dry season, this approach could not be repeated. Instead, branches that had grown into a forked shape were selected, randomly choosing one side of the fork to measure. Branchlets with multiple growth axes were avoided to best capture linear growth for that particular branch. For both studies, one to six branchlets were measured for all samples. Branchlet growth was averaged for each sample and divided by the length of the growth time interval to achieve daily increase values. Growth

data for the wet and dry season were combined and the two time intervals in Lewis & Diaz-Pulido (2017) and explored their relationship with a PERMANOVA and a pairwise Wilcoxon rank sum test as a post hoc test.

Results

Successful DNA extraction, amplification, and maximum likelihood analyses confirmed that all source plants used in the study were the same species.

A PERMANOVA demonstrated source population was not a significant factor of growth rate (surface area: F= .96, df= 1, p= .344, volume: F= .25, df= 2, p= .626) (Figures 5 & 6). The average growth rates for plants sourced from Pago and Togcha Bay and transplanted to Pago Bay were 7.67 ± 6.25 mm^2/day and 6.50 ± 5.60 mm^2/day (based on surface area) and 3.72 ± 3.14 mm^3/day and 3.41 ± 3.69 mm^3/day (based on volume), respectively (Figures 5 & 6). The average growth rates for plants sourced from Pago and Togcha Bay and transplanted to Togcha Bay were 4.38 ± 4.18 mm^2/day and 3.74 ± 3.71 mm^2/day (based on surface area) and 1.87 ± 1.76 mm^3/day and 1.59 ± 1.60 mm^3/day (based on volume), respectively (Figures 5 & 6). This allowed us to pool the growth data from both source populations within each study site for the rest of the analyses.

Location and season were significant factors for growth rates (Location: surface area: F= 40.27, df= 2, p= <0.01, volume: F= 28.11, df= 2, p= <0.01; Season: surface area: F= 69.23, df= 1, p= <0.01, volume: F= 46.95, df= 1, p= <0.01) (Figures 7 & 8). A pairwise Wilcoxon rank-sum test displayed a significant difference in growth rates between Pago and Togcha Bay for the wet (surface area: p= <0.01, volume: p= <0.01) but not the dry (surface area: p=.80, volume: p=.15) season. Growth rates were significantly higher for samples at Pago than Togcha Bay during the

wet season. A significant difference in growth rates was observed between the samples growing in the tank and both Pago and Togcha Bay for the wet and dry season (Pago and tank: Wet: surface area: $p = <0.01$, volume: $p = <0.01$, Dry: surface area: $p = <0.01$, volume: $p = <0.01$; Togcha and tank: Wet: surface area: $p = <0.01$, volume: $p = <0.01$, Dry: surface area: $p = <0.01$, volume: $p = <0.01$) (Figures 7 & 8). Surface area and volume growth rates, respectively, during the wet season at Pago Bay were $2.62 \pm .65$ mm^2/day and $1.15 \pm .261$ mm^3/day, at Togcha Bay were $1.09 \pm .30$ mm^2/day and $.462 \pm .149$ mm^3/day, and in the tank were $.25 \pm .08$ mm^2/day and $.11 \pm .04$ mm^3/day. Surface area and volume growth rates during the dry season at Pago Bay were 11.9 ± 4.87 mm^2/day and 6.21 ± 3.18 mm^3/day, at Togcha Bay were 7.33 ± 3.17 mm^2/day and 3.12 ± 1.34 mm^3/day, and in the tank were $.25 \pm .09$ mm^2/day and $.11 \pm .05$ mm^3/day.

The test also showed a difference between the growth rates across the wet and dry season for Togcha and Pago Bay (Togcha Bay: surface area: $p = <0.01$, volume: $p = <0.01$; Pago Bay: surface area: $p = <0.01$, volume: $p = <0.01$), but not for the those in the tank (surface area: $p = 1$, volume: $p = 1$) (Figures 7 & 8). Growth rates were significantly lower during the wet season than dry season for samples at Pago Bay and Togcha Bay.

Surface area and volume values generated by the 3D scanner at the start of the experiment were compared to those generated by the micro-CT scanner at the end. 17 samples survived for the entire duration of the study without breakage. Surface area and volume measurements at the start had an average of 109.75 mm^2 and 37.75 mm^3, a range of 49.94-289.29 mm^2 and 16.48-119.43 mm^3, respectively. At the end of the experiment, surface area and volume measurements had an average of 9475.355 mm^2 and 5353.419 mm^3, with a range of 4918.14-22683.52 mm^2 and 2378.3-12807.7 mm^3, respectively. Based on surface area measurements samples increased anywhere from 20-166 times in size over the 14 months.

A PERMANOVA and pairwise Wilcoxon rank sum test revealed that there was a significant difference between the linear growth rates observed by Lewis & Diaz-Pulido (2017) and what we observed at Pago (p= <0.01) and Togcha Bay (p= <0.01) (Figure 9). Both Pago and Togcha Bay displayed significantly higher linear growth rates than those reported by Lewis & Diaz-Pulido (2017). The average linear extension for Pago Bay, Togcha Bay, and Lewis & Diaz-Pulido's 2017 study were 65.6 ± 14.8 µm/day, 52.9 ± 12.4 µm/day, and 20.2 ± 5.85 µm/day, respectively.

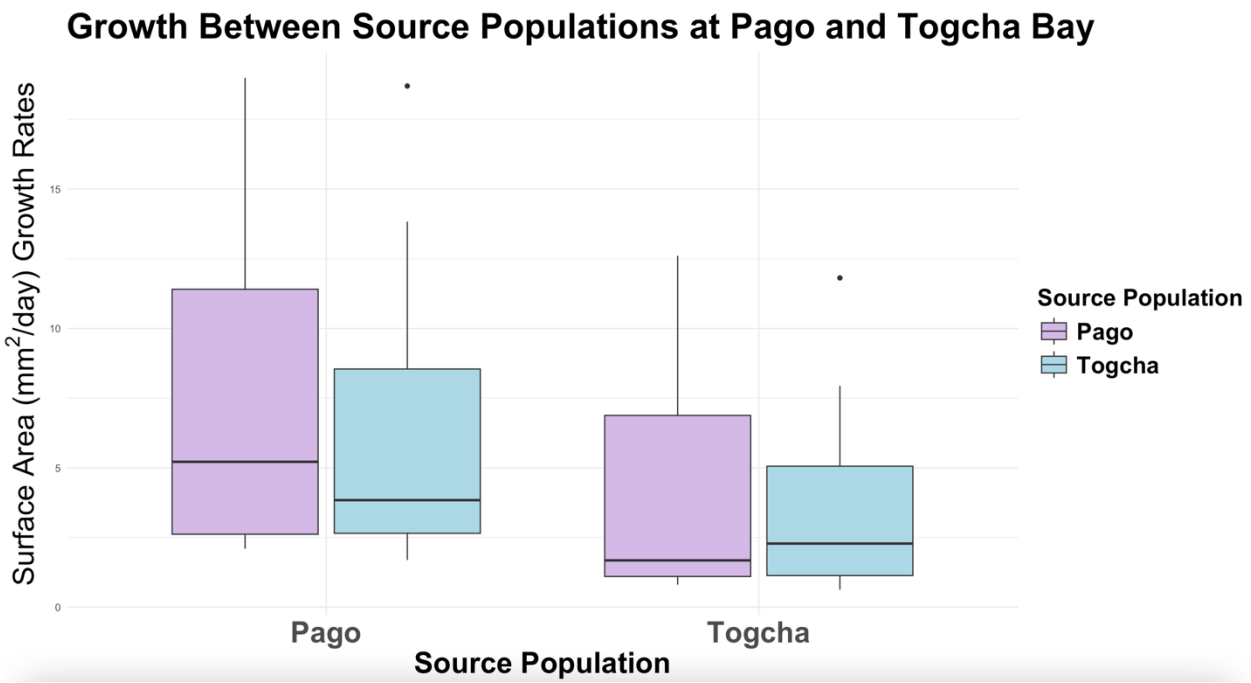

Figure 5. Growth rates based on surface area for source populations Pago and Togcha Bay at both study sites.

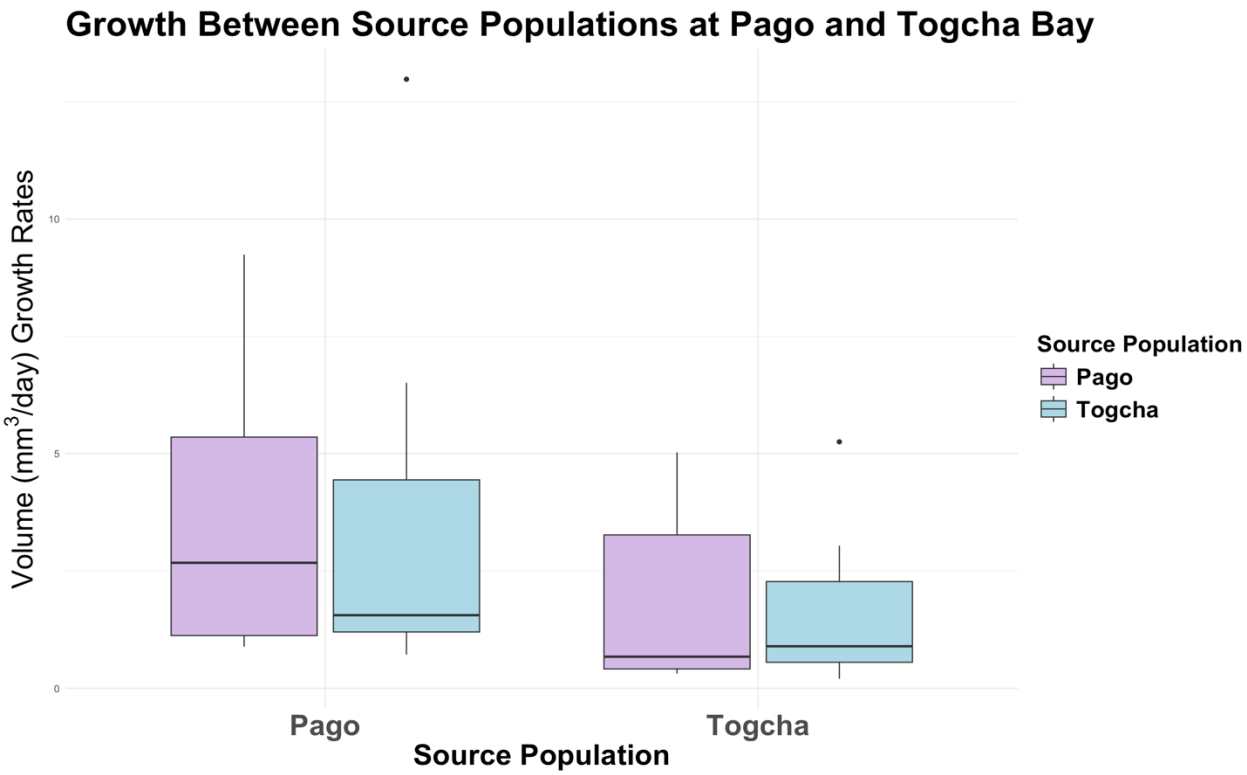

Figure 6. Growth rates based on volume for source populations Pago and Togcha Bay at both study sites.

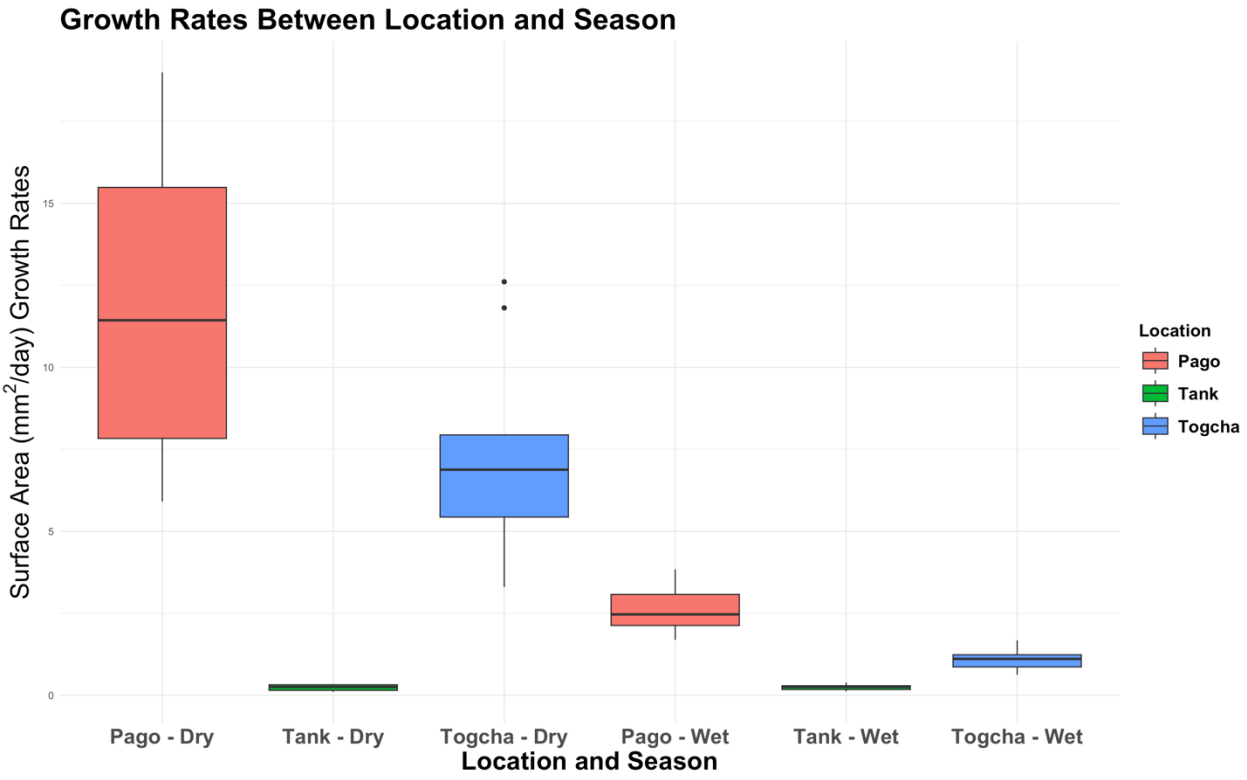

Figure 7. Comparison of growth rates between location and season based on surface area measurements.

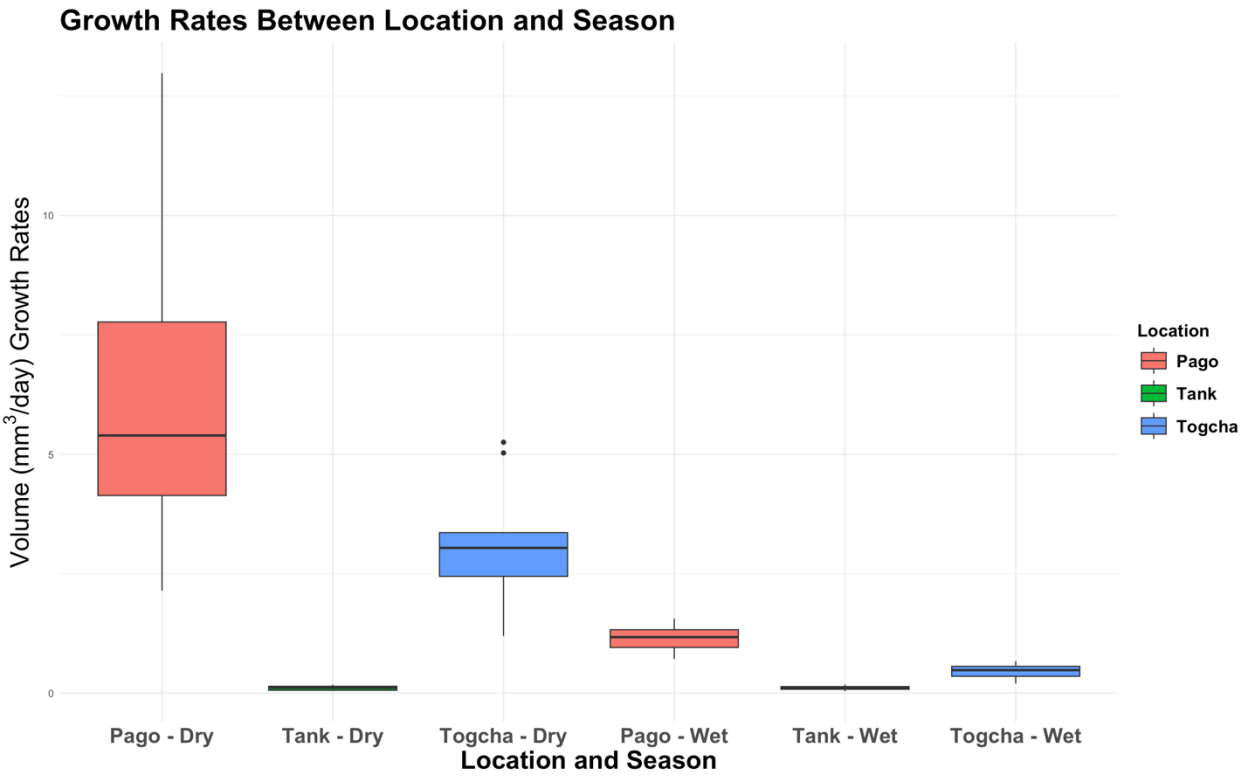

Figure 8. Comparison of growth rates between location and season based on volume measurements.

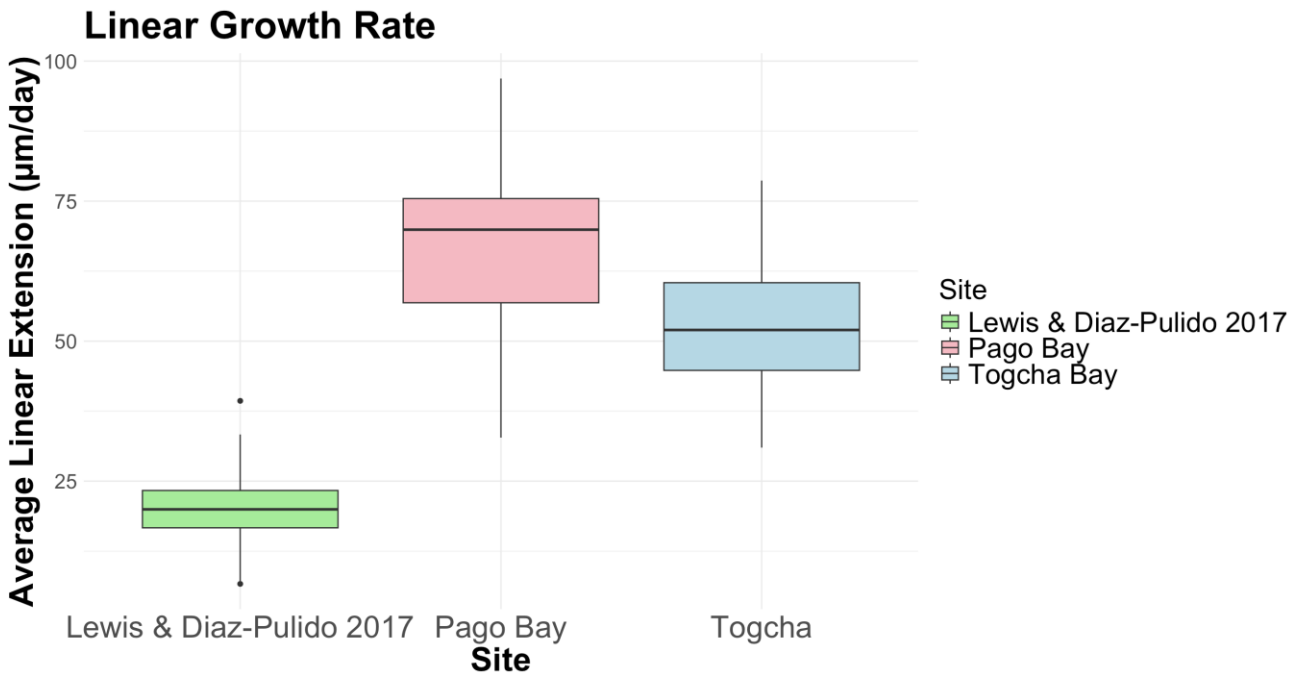

Figure 9. Average linear extension (µm/day) for *Lithophyllum* samples in Lewis & Diaz-Pulido 2017, Pago Bay, and Togcha Bay.

Discussion

One of the main goals of this study was to develop a reliable method for long-term, non-destructive monitoring of *Lithophyllum* growth *in situ*. This goal was reached by understanding the physiological constraints involved in handling the samples and the use of novel measurement techniques with high accuracy and precision. This is the first study of its kind for the Micronesia region and one of the longest growth studies on coralline algae. It is one of just a few studies providing baseline data on the growth of the genus *Lithophyllum* and delves into various growth-determining environmental factors. After developing a scientifically robust experimental design, we were able to explore the growth patterns of one *Lithophyllum* species inhabiting two contrasting reef habitats in Guam. The results confirm that there is a large difference in growth between samples from the forereef systems compared to reef channels, the former growing at a

much faster rate. This can likely be explained by the dissimilarity in environmental conditions between these habitats. One reason may be due to the increased irradiance at Pago Bay, as calcification rates of coralline algae are directly related to photosynthetic rates (McCoy & Kamenos, 2015; Lewis *et al*., 2017). Temperature is also a key component in coralline growth and calcification rates. Field studies on *Lithophyllum margaritae* from the Gulf of California displayed a large difference in growth rates in winter (0.83 ± 0.06 mm yr^{-1}) than summer (5.02 ± 1.16 mm yr^{-1}), where temperatures dramatically ranged from 18°C to 30.5°C across the two seasons (Steller *et al*., 2007). Significantly higher temperatures were recorded at Togcha than Pago Bay during the wet season, reaching a maximum of 36.25°C at the former. Although the growth and calcification of coralline algae typically increases with temperature, values above their thermal tolerance may have adverse effects on growth and lead to mortality (Martin & Gattuso, 2009). A study on the tropical coralline algae *Porolithon gardineri* observed a 50% decrease in growth rate and mortality events under elevated temperatures of 2.5-4.5°C (Agegian, 1985). No significant tissue bleaching was observed in our samples; however, it may be possible that the extreme temperatures experienced at Togcha Bay led to elevated stress levels, inhibiting growth rates. However, no significant difference existed between the growth of samples sourced from Pago versus Togcha Bay and grown in Togcha Bay, suggesting those originating from Pago Bay were able to quickly adapt to the higher temperatures. This suggests the importance of this species as ocean warming progresses, as it appears to have a large thermal tolerance range.

Another significant difference between the reefs in Pago and Togcha Bay is increased freshwater, nutrient, and sediment output from Togcha River. Substantial sediment cover and epiphytic algal growth were observed within the channel at Togcha Bay and may have negatively impacted *Lithophyllum* samples' growth rates. Large amounts of sediment are known to inhibit

light penetration and partly suppress carbonate production, as seen with rhodoliths growing along the Pacific coast of Panama (Schäfer *et al*., 2011). Sedimentation can act as a vector for pollution, such as organic pollutants, sewage, and heavy metals (Comfort *et al*., 2019). Noncalcareous macroalgae may also benefit from the elevated nutrients levels within the channel, leading to competition for light and space; another possible explanation for the significantly slower growth rates observed at Togcha Bay. Increased nutrients, such as phosphate, have been observed to negatively impact the growth and calcification of *Lithophyllum kotschyanum* at sewage-affected reefs in Zanzibar (Björk *et al*., 1995). This emphasizes the vulnerability of this genus and other coralline algal groups situated on reefs in proximity to freshwater river outputs, watersheds, and wastewater treatment facilities; all of which are present in Guam.

The stability of water conditions is also variable between Pago and Togcha Bay. Currents and flow at Pago Bay are much more stable than at Togcha Bay, and the mixing of the water column is greatly reduced within the reef channel. Hydrodynamic conditions control the rate at which dissolved gases and inorganic compounds move throughout the water column (Finelli *et al*., 2006). Increased flow may enhance photosynthesis by facilitating the removal of oxygen, thereby promoting growth (Comfort *et al*., 2019). Salinity, temperature, and organic matter also fluctuates at Togcha Bay, which can cause heightened stress for the algae growing there. The differences we observed between samples grown at Togcha and Pago Bay reinforce the notion that not one, but probably many environmental conditions simultaneously influence growth rates.

The source location of all the samples did not have an influence on their growth rates once they were transplanted to a new site. For instance, fragments sourced from Togcha Bay did not show significant differences from those sourced from Pago Bay when both were grown at the

Pago Bay site. Likewise, samples sourced from Pago Bay and Togcha Bay showed similar performance when grown in the tank environment. Additionally, algal fragments sourced from Pago Bay and transplanted to Togcha Bay exhibited significantly slower growth rates compared to those returned to their native environment. This demonstrates that reef environment plays a more significant role than the origin of source plants or their initial morphology.

Another question addressed in this study was whether growth studies can be accurately mimicked in an *ex situ* environment, such as the UOG Marine Lab's flow-through tanks. Our results revealed a significant difference between the growth rates of samples in the tank and both *in situ* environments. A key difference to note between the tank and reef environments is the level of irradiance. The light values in the tank were significantly lower than those at Pago and Togcha Bay. However, we found it necessary to control light exposure in the tank with two layers of mesh shade cloth to prevent bleaching of our samples. Another obvious difference between the field and lab environments in our study, as with many other studies, was the degree of water motion (Agegian, 1981). We supplied as high of water flow as possible with our tank system, however, it was probably much lower than both *in situ* environments. The volume of water in the tank that the samples were grown in may have also played a role in buffering many physiochemical processes. These factors likely played a role in the much lower growth rates observed in the tank environment. These findings highlight the challenges of replicating reef environments in a lab setting, given uncontrollable constraints. It also highlights the potential misconceptions that can arise from relying exclusively on *ex situ* experiments. If we had based our conclusions solely on the samples grown in a tank environment, we would have significantly underestimated the actual growth rates occurring on Guam's reefs. This is an important

consideration for past growth studies that solely relied on *ex situ* experiments, as algal growth rates might not reflect those in their natural setting.

Conducting a long-term study was essential for investigating the role of seasonality in growth, as reported for other regions (Short *et* al., 2015; Lewis *et* al., 2017; Pulecio-Plaza *et al*., 2023). A significant difference in growth rate was detected between Guam's wet and dry season for samples at Pago and Togcha Bay, but not for those grown in the tank. At both Pago and Togcha Bay, growth rates were significantly higher during the dry than wet season. These differences were anticipated due to the distinct environmental conditions of each season. The wet season in Guam is marked by heavy rainfall, resulting in increased river runoff, brackish water, and decreased salinity. The study site at Pago Bay is far removed from freshwater input at the mouth of the Lonfit River; whereas the reef channel at Togcha Bay is greatly affected by freshwater from the river. Elevated levels of turbidity are likely to hinder photosynthetic rates during the wet season (Comfort *et al*., 2019). Higher water temperatures during the wet season may also slow growth. Understanding the role that various environmental and seasonal variables play with respect to the growth of coralline algae is essential to begin to predict their responses to environmental change.

Most of the literature available on coralline algae growth uses linear branch extension as the metric of growth. However, during preliminary experiments, we observed that most energy for growth was directed towards developing additional growth axes, rather than elongating the existing branches. Additionally, protuberances of the same alga can have different growth rates (Caragnano *et al.,* 2016). Because of this, methods capturing changes in the entirety of the algal sample over time may be a lot more informative than linear extension values. When comparing our results to those of Lewis & Diaz-Pulido's (2017), we found linear growth rates to be much

larger at both of our study sites than what they reported. This may be explained by differences in local environmental factors, their study's small sample size, or short duration. Additionally, chemical staining has the potential to have toxic effects on algae and their growth (Lewis & Diaz-Pulido 2017). Other regions in the tropics have also reported much smaller growth rates for the genus *Lithophyllum*, and coralline algae in general than what was observed in our study. A study taking place in the Red Sea using Alizarin staining documented a daily mean marginal elongation rate of about 24 µm/day for *Lithophyllum kotschyanum*, comparable to those reported by Lewis and Diaz-Pulido (2017) (Caragnano *et al*., 2016). Another recently published paper found the average vertical growth of a cryptic non-branching *Lithophyllum* species in French Polynesia to be <1 to 3 µm/day by Alizarin staining. The variability in growth within our study, as well as in comparison to others, shows that corallines are very sensitive to environmental conditions and their growth patterns vary largely in different localities, microhabitats, seasonally, and interannually. However, coralline growth and calcification remains largely understudied. In order to accurately represent coralline species in conservation plans and carbon budget modeling, a lot of work needs to be done to explore growth patterns at a species level. As coral bleaching events become more frequent and severe, the significance of coralline algae increases. These algae are likely to play a crucial role in preserving tropical reef carbonate structures as environmental change continues (Cornwall *et al*., 2020).

References

Adey, W.H., Vassar, J.M. (1975). Colonization, succession and growth rates of tropical crustose coralline algae (Rhodophyta, Cryptonemiales). Phycologia, 14: 55-69.

Agegian, C.R. (1981). Growth of the branched coralline alga, *Porolithon gardineri* (Foslie) in the Hawaiian Archipelago. Proceedings of the Fourth International Coral Reef Symposium, 2: 419-423.

Agegian, C.R. (1985). The biogeochemical ecology of *Porolithon gardineri* (Foslie). PhD thesis, University of Hawaii, Honolulu, 178 pp.

Björk, M., Mohammed S.M., Björklund, A., Semesi, A. (1995). Coralline algae, important coral-reef builders threatened by pollution. Ambio, 24: 502-505.

Caragnano, A., Basso, D., Rodondi, G. (2016). Growth rates and ecology of coralline rhodoliths from the Ras Ghamila back reef lagoon, Red Sea. Marine Ecology, 37: 713-726.

Chisholm, J.R.M., Gattuso, J.P. (1991). Validation of the alkalinity anomaly technique for investigation calcification and photosynthesis in coral reef communities. Limnology and Oceanography, 36: 1232–1239.

Comfort, C.M., Walker, G.O., McManus, M.A., Fujimura, A.G., Ostrander, C.E., Donaldson, T.J. (2019). Physical dynamics of the reef flat, channel, and fore reef areas of a fringing reef embayment: An oceanographic study of Pago Bay, Guam. Regional Studies in Marine Science, 31: 100740.

Cornwall, C.E., Carlot, J., Branson, O. Courtney, T.A., Harvey, B.P., Perry, C.T., Andersson, A.J., Diaz-Pulido, G., Johnson, M.D., Kennedy, E., Krieger, E.C., Mallela, J., McCoy, S.J., Nugues, M.M., Quinter, E., Ross, C.L., Ryan, E., Saderne, V., Comeau, S. (2023). Crustose coralline algae can contribute more than corals to coral reef carbonate production. Communication Earth & Environment, 4: 1-12.

Cornwall, C.E., Comeau, S., DeCarlo, T.M., Larcombe, E., Moore, B., Giltrow, K., Puerzer, F., D'Alexis, Q., McCulloch, M.T. (2020). A coralline alga gains tolerance to ocean acidification over multiple generations of exposure. Nature Climate Change, 10: 143-146.

Cornwall, C.E., Diaz-Pulido, G., Comeua, S. (2019). Impacts of ocean warming on coralline algal calcification: meta-analysis, knowledge gaps, and key recommendations for future research. Frontiers in Marine Science, 186: 1-10.

Darrenougue, N., De Deckker, P., Payri, C., Eggins, S., Fallon, S. (2013). Growth and chronology of the rhodolith-forming, coralline red alga *Sporolithon durum*. Marine Ecology Progress, 474: 105-119.

Doropoulos, C., Ward, S., Diaz-Pulido, G., Hoegh-Guldberg, O., Mumby, P. J. (2012). Ocean acidification reduces coral recruitment by disrupting intimate larval-algal settlement interactions. Ecology Letters, 15: 338– 346.

Figueiredo M.A.O., Kain J.M., Norton, T.A. (2000). Responses of crustose corallines to epiphyte and canopy cover. Journal of Phycology, 36: 17-24.

Finelli, C.M., Helmuth, B.S.T., Pentcheff, N.D., Wethey, D.S. (2006). Water flow influences oxygen transport and photosynthetic efficiency in corals. Coral Reefs, 25: 47-57.

Halfar, J., Zack, T., Kronz, A., Zachos, J.C. (2000). Growth and high-resolution paleoenvironmental signals of rhodoliths (coralline red algae): a new biogenic archive. Journal of Geophysical Research: Oceans, 105: 2107–22116.

Johansen, H.W. (1981). Coralline algae, a first synthesis. 1-239. CRC Press, Florida.

Johnson, M.D., Moriarty, V.W., Carpenter, R.C. (2014). Acclimatization of the crustose coralline alga *Porolithon onkodes* to variable pCO(2). Public Library of Science One, 9: e87678.

Jokiel, P.L., Rogers, K.S., Kuffner, I.B., Andersson, A.J., Cox, E.F., Mackenzie, F.T. (2008). Ocean Acidification and calcifying reef organisms: a mesocosm investigation. Coral Reefs, 27: 473-483.

Kuffner, I.B., Andersson, A.J., Jokiel, P.L., Rogers, K.S., Mackenzie, F.T. (2008). Decreased abundance of crustose coralline algae due to ocean acidification. Nature Geoscience, 1: 114-117.

Lewis, B., Diaz-Pulido, G. (2017). Suitability of three fluorochrome markers for obtaining *in situ* growth rates of coralline algae. Journal of Experimental Marine Biology and Ecology, 490: 64-73.

Lewis B., Kennedy E.V., Diaz-Pulido, G. (2017). Seasonal growth and calcification of a reef-building crustose coralline alga on the Great Barrier Reef. Marine Ecology Progress Series, 568: 73-86.

Littler, M.M., Littler, D.S., Blair, S.M., Norris, J.N. (1985). Deepest known plant life discovered on an uncharted seamount. Science, 227: 57–59.

Martin, S., Gattuso, J.P. (2009). Response of Mediterranean coralline algae to ocean acidification and elevated temperature. Global Change Biology, 15: 2089-2100.

Martin, S., Charnoz A., Gattuso, J.P. (2013). Photosynthesis, respiration, and calcification in the Mediterranean crustose coralline alga Lithophyllum cabiochae (Corallinales, Rhodophyta). European Journal of Phycology, 48: 163-172.

Martin, S., Cohu, S., Vignot, C., Zimmerman, G., Jean-Pierre, G. (2013). One-year experiment on the physiological response of the Mediterranean crustose coralline alga *Lithophyllum cabiochae.* Ecology and Evolution, 3: 676-693.

McCoy, S.J. (2013). Morphology of the crustose coralline alga Pseudolithophyllum muricatum (Corallinales, Rhodophyta) responds to 30 years of ocean acidification in the Northeast Pacific. Journal of Phycology, 49: 830-837.

Mills, M.S., Deinhart, M.E., Heagy, M.N., Schils, T. (2022). Small tropical islands as hotspots of crustose calcifying red algal diversity and endemism. Frontiers in Marine Science, 9: 898308.

Mills, M.S., Schils, T. (2021). The habitat-modifying red alga *Ramicrusta* on Pacific reefs: A new generic record for the Tropical Northwestern Pacific and the description of four new species from Guam. Public Library of Science, 16: e0259336.

Peña, V., Harvey, B.P., Agostini, S., Porzio, L., Milazzo, M., Horta, P., Le Gall, L., Hall-Spencer, J.M. (2021). Major loss of coralline algal diversity in response to ocean acidification. Global Change Biology, 27: 4785-4798.

Pinna, F., Caragnano, A., Piazzi, L., Ragazzola, F., Stipcich, P., Rindi, F., Ceccherelli, G. (2022). The Mediterranean bioconstructor *Lithophyllum stictiforme* shows adaptability to future warming. Frontiers in Marine Science, 9: 930750.

Pulecio-Plaza, L., Diaz-Pulido, G., García-Urueña, R. (2023). Seasonal upwelling conditions promote growth and calcification in reef-building coralline algae. Journal of Phycology, 59: 908-925.

Reichert, J., Schellenberg, J., Schubert, P., Wilke, T. (2016). 3D scanning as a highly precise, reproducible, and minimally invasive method for surface area and volume measurements of scleractinian corals. Limnology and Oceanography: Methods, 14: 518-526.

Riul, P., Targino, C.H., Da No´brega Farias, J., Tenuis Visscher, P., Antuneshorta, P. (2008). Decrease in *Lithothamnion* sp. (Rhodophyta) primary production due to the deposition of a thin sediment layer: Journal of the Marine Biological Association of the United Kingdom, 88: 17–19.

Saunders, G.W., McDevit, D.C. (2012). Methods for DNA barcoding photosynthetic protists emphasizing the macroalgae and diatoms. Methods in Molecular Biology, 858: 207-222.

Schäfer, P., Fortunato, H., Bader, B., Liebetrau, V., Bauch, T., Reijmer, J.J.G. (2011). Growth rates and carbonate production by coralline red algae in upwelling and non-upwelling settings along the pacific coast of Panama. Palaios, 26: 420-432.

Schils, T. (2023). Branching *Lithophyllum* coralline algae: dominant reef builders on herbivory-depressed tropical reefs after high coral mortality. Diversity, 15: 1-5.

Schubert, N., Schoenrock, K.M., Aguirre, J., Kamenos, N.A., Silva, J., Horta, P.A., Hofmann, L.C. (2020). Editorial: Coralline Algae: Globally Distributed Ecosystem Engineers. Frontiers in Marine Science, 7: 352.

Short, J., Foster, T., Falter, J., Kendrick, G.A., Mcculloch, M.T. (2015). Crustose coralline algal growth, calcification and mortality following a marine heatwave in Western Australia. Continental Shelf Research, 106: 38-44.

Smith A.M., Sutherland J.E., Kregting L., Farr T.J., Winter D.J. (2012). Phylomineralogy of the coralline red algae: correlation of skeletal mineralogy with molecular phylogeny. Phytochemistry, 81: 97-108.

Steller, D.L., Hernandez-Ayon, J.M., Riosmena-Rodriguez, R., Cabello-Pasini, A. (2007). Effect of temperature on photosynthesis, growth and calcification rates of the free-living coralline alga *Lithophyllum margaritae*. Ciencias Marianas, 33: 441–456.

Torrano-Silva, B.N., Ferreira, S.G., Oliveira, M.C. (2015). Unveiling privacy: Advances in microtomography of coralline algae. Micron, 72: 34-38.

van der Heijden, L.H., Kamenos, N.A. (2015). Reviews and syntheses: calculating the global contribution of coralline algae to total carbon burial. Biogeosciences, 12: 6429-6441.

Villas Bôas, A.B., Figueiredo, M.A. de O. (2004). Are anti-fouling mechanisms in coralline alga species-specific? Brazilian Journal of Oceanography, 52: 11-18.

Villas Bôas A.B., Figueiredo M.A. de O., Villaça, R.C. (2005). Colonization and growth of crustose coralline algae (Corallinales, Rhodophyta) on the Rocas Atoll. Brazilian Journal of Oceanography, 53: 147-156.

Weiss, A. & Martindale, R.C. (2017). Crustose coralline algae increased framework and diversity on ancient coral reefs. Public Library of Science, 12: e0181637.

Yoon, H.S., Hackett, J.D., Bhattacharya, D. (2002). A single origin of the peridinin- and fucoxanthin-containing plastids in dinoflagellates through tertiary endosymbiosis. Proceedings of the National Academy of Sciences of the United States of America, 9: 11724–11729.