doi: 10.25923/9nrg-fd06

Evaluating the Use of Live Rock in Closed-System Aquaria with Mesophotic Corals

Coral Propagation Technique Development Project

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August 2026

DWH MDBC Technical Memorandum 004

DWH Mesophotic & Deep Benthic Communities Restoration

Suggested Citation

Aquilina-Beck, A., Rojano, M., Burdine, W., Salgado, E., Greig, T., and Etnoyer, P. (2026). Evaluating the Use of Live Rock in Closed-System Aquaria with Mesophotic Corals. DWH MDBC Technical Memorandum 004. https://doi.org/10.25923/9nrg-fd06

Acknowledgments

The authors would like to thank all of those who helped with the collection of the mesophotic live rock material on the MDBC expedition aboard the MV *Tyson B* from June 1–9, 2024 (cruise ID: TB2401). The live rock was collected by a technical diving team led by Jake Emmert of Moody Gardens and Chris Gardner of NOAA. Thanks to Jonathan Quigley (USGS) for providing the SOP on keeping and transporting live rock; and to Mark McCauley (USGS) and Julia Johnstone (CSS/NOAA) for help designing this study. Special thanks to all of the reviewers of this document. Funding for this work was provided by the Mesophotic and Deep Benthic Communities Coral Propagation Technique Development project, which was selected by the Open Ocean Trustee Implementation Group to restore natural resources injured by the 2010 *Deepwater Horizon* oil spill.

For more information on MDBC Restoration, please visit:

https://www.gulfspillrestoration.noaa.gov/restoration-areas/open-ocean

https://coastalscience.noaa.gov/science-areas/restoration/gulf-mdbc-restoration/

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Deepwater Horizon **Mesophotic and Deep Benthic Communities Restoration**

This report is part of the NOAA Mesophotic and Deep Benthic Communities (MDBC) Series of publications that share the results of work conducted by the Deepwater Horizon MDBC restoration projects.

The 2010 *Deepwater Horizon* oil spill was an unprecedented event. Approximately 3.2 million barrels of oil were released into the deep ocean over nearly three months. The plume of oil moved throughout the water column, formed surface slicks that cumulatively covered an area the size of Virginia, and washed oil onto at least 1,300 miles of shoreline habitats. More than 770 square miles (2,000 square kilometers) of deep benthic habitat were injured by the oil spill, including areas surrounding the *Deepwater Horizon* wellhead and parts of the mesophotic reef complex located at the edge of the Mississippi–Alabama continental shelf.

Under the Oil Pollution Act, state and federal natural resource trustees conducted a Natural Resource Damage Assessment (NRDA). The Trustees assessed damages, quantifying the unprecedented injuries to natural resources and lost services. They also developed a programmatic restoration plan to restore injured resources and compensate the public for lost services.

In April 2016, a settlement was finalized that included up to $8.8 billion in funding for the Deepwater Horizon Trustees to restore the natural resource injuries caused by the oil spill as described in their programmatic restoration plan, Final Programmatic Damage Assessment and Restoration Plan and Final Programmatic Environmental Impact Statement. The Deepwater Horizon Open Ocean Trustee Implementation Group is responsible for restoring natural resources and their services within the Open Ocean Restoration Area that were injured by the oil spill. The Open Ocean Trustees include NOAA, U.S. Department of the Interior, U.S. Environmental Protection Agency, and U.S. Department of Agriculture.

Since 2019, the Open Ocean Trustee Implementation Group has committed more than $150 million to implement four restoration projects to address the injury to MDBC. The MDBC projects are: Mapping, Ground-Truthing, and Predictive Habitat Modeling; Habitat Assessment and Evaluation; Coral Propagation Technique Development; and Active Management and Protection. NOAA and the Department of the Interior are implementing the projects, in cooperation with a range of partners, over eight years.

Together, the projects take a phased approach to meet the challenges involved in restoring deep-sea habitats. Challenges to restoration include a limited scientific understanding of these communities, limited experience with restoration at the depths at which these communities occur, and remote locations that limit accessibility.

More information about *Deepwater Horizon* restoration and the MDBC restoration projects is available at: https://www.gulfspillrestoration.noaa.gov.

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Acronyms

5mC	5-methylcytosine
ANOVA	analysis of variance
ASV	amplicon sequence variant
BC	before corals
CCA	crustose coralline algae
CPT	Coral Propagation Technique Development Team
CTD	conductivity, temperature, and depth
Control/C1	bare-rock tank
DESeq	differential expression analysis
DWH	*Deepwater Horizon*
ELISA	enzyme-linked immunosorbent assay
g5mC	global 5-methylcytosine
HML	Hollings Marine Laboratory
MDBC	Mesophotic and Deep Benthic Communities
MS-AL/E3	mesophotic Mississippi-Alabama Shelf rock/experimental tank 3
OD	optical density
PBS	phosphate-buffered saline
PCoA	principal coordinate analysis
PCR	polymerase chain reaction
PERMANOVA	permutational multivariate analysis of variance
Rd	received
ROV	remotely operated vehicle
rRNA	ribosomal ribonucleic acid
WFL/E2	shallow West Florida Shelf rock/experimental tank 2
TLE	total linear extension
T=0	initial time point
T=6mo	six months
T=3mo	three months

Executive Summary

The use of "live rock," defined as hard substrate consisting of microbial communities capable of breaking down harmful waste by-products, is valued in the aquarium trade and among aquarists. These bacterial communities can improve the health of aquarium inhabitants and the water in which they live. Careful scientific evaluations of the risks and benefits of adding live rocks to experimental aquarium systems are few. This study was conducted to determine the effect of live rock on the health of three octocoral species kept in aquaria: *Muricea pendula*, *Swiftia exserta*, and *Thesea nivea*. The corals were used to assess whether benefits gained by the presence of live rock are also dependent upon the source of the rock. This study documented water quality, coral health and growth, and microbial communities of corals and rocks over six months in experimental aquaria. The results of this investigation will aid husbandry practices for these coral species and others, which are among those injured by the *Deepwater Horizon* disaster and targeted for restoration.

This study utilized two different sources of live rock in order to compare their effects on coral health and growth. One type was commercially available from an aquaculture facility off the shallow waters of the West Florida (WFL) Shelf near Tampa Bay, Florida, USA (tbsaltwater.com); while the other live rock was manually collected from the mesophotic zone by divers near coral collection sites on the Mississippi-Alabama (MS-AL) Shelf south of Pensacola Bay, Florida. Utilizing both commercial shallow-water live rock along with rock from the mesophotic zone allows for utility and functionality comparisons.

Coral health was measured using a variety of traditional visual metrics alongside more novel quantitative cellular metrics such as microbiome 16S ribosomal ribonucleic acid (rRNA) gene amplicon and enzyme-linked immunosorbent assays. Visually, coral health peaked at three months in the presence of live rocks. Most corals in all three species either increased or maintained stable polyp number, linear growth, or tissue thickness at three months. However, positive visual appearances were lost by most corals at six months. *Swiftia exserta* colonies exhibited less overall decline in all treatment tanks. *Thesea nivea*'s visual metrics showed positive changes in tanks with live rocks. *Muricea pendula* corals had the most decline overall across all treatments. However, positive gains in polyp number, extension, and thickness were observed in the mesophotic rock treatment at three months. Declines observed at six months in all coral replicates across treatments may have been attributed to water quality changes during the last two months of the experiment. Multiple metrics used for evaluating corals in the presence of live rock show that, despite coral species-specific declines, the presence of live rock benefits mesophotic corals.

Evaluating 16S rRNA gene amplicon data gave valuable insight into bacterial changes in different species of corals based on tank conditioning with and without live rock. Comparing coral species through time showed there was a considerable change in bacterial community makeup among the corals with the exception of *Swiftia exserta*, which was consistent regardless of time and treatment. Analysis of the coral tissue top 20 bacterial communities showed *Thesea nivea* and *Muricea pendula* contained greater amounts of bacteria involved in overall health within live rock treatments. Both types of sourced live rock contain bacteria that break down organic matter and

combat algae growth. Initially, shallow WFL Shelf live rock and mesophotic MS-AL Shelf live rock contained similarly high levels of alpha diversity and abundances between 4 and 5. Mesophotic MS-AL Shelf and shallow WFL Shelf live rock maintained populations of bacteria that are commonly associated with crustose coralline algae and, further, are putatively involved in the breakdown of organic matter.

In conclusion, variability in coral microbiomes resulted from exposure to different live rock treatments, reflected by the bacterial communities present in coral replicates and species. Both shallow WFL Shelf live rock and mesophotic MS-AL Shelf live rock offered benefits to corals by reducing organic buildup and controlling algae loads. This study bolsters evidence that life support systems for mesophotic corals can benefit from the provision of live rock as a stabilizer to maintain balance in the environmental conditions of husbandry facilities. The availability and ease of obtaining aquacultured live rock from commercial vendors should be considered given its wide availability, the substantial coordinated effort required to obtain mesophotic live rock from the field, as well as the potential of such collections to impact natural habitats.

1. Introduction

The term “live rock” refers to any piece of hard substrate, typically dead coral skeleton, that has been submerged long enough to acquire a microscopic community of encrusting algae and invertebrates capable of breaking down harmful waste by-products of living organisms (Delbeek and Sprung, 1994; Simões et al., 2017). Live rock has been used in the hobbyist aquarium trade for many years, where it has been noted to improve water quality and help maintain healthier saltwater environments for animals that are difficult to keep in captivity (Simões et al., 2017). Beginning in the mid-1980s, most of the live rock for the hobbyist aquarium industry was harvested off the coast of Florida, up to 300 tons per year, until a closure on wild harvesting was instituted in 1997 (Falls et al., 2003; Simões et al., 2017). After similar closures in Hawaii, live rock is now primarily cultured sustainably by the use of limestone substrate in waters off the Florida coast. Within the scientific community, the benefits of using live rock in aquariums have been relayed. Early documentation in the scientific literature of the use of live rock in shallow coral aquariums was reviewed in Carlson (1987). Since then, studies have examined live rock’s effectiveness specifically as an aid to filtration acting as a biofilter, removing nitrogen compounds from coral aquaria (Yuen et al., 2009). However, evaluating mesophotic live rock with mesophotic coral communities has not been addressed.

In this study, the effectiveness of two types of live rock for improving the health of three mesophotic octocoral species maintained in laboratory aquarium conditions was assessed. Visual and cellular metrics were compared to evaluate health and growth in corals held for six months in closed-system aquaria with one of three types of rock: (a) unconditioned bare limestone, (b) commercially available live rock sourced from shallow West Florida (WFL) Shelf waters, or (c) live rock sourced from mesophotic sites similar to and near where corals were collected. Standard coral health assessment metrics were chosen to be comparable to other studies, such as polyp count, total linear extension (TLE), tissue color, and tissue thickness (Lange and Etnoyer, 2024; Lasker et al., 2003; DeLeo et al., 2016), while also testing new metrics that could reveal more subtle indicators of coral condition (lipoprotein content and global DNA methylation) prior to decline. Mesophotic corals grow at very slow rates in aquaria (0.2 mm–1.5 cm/year) depending on the species of coral and location (Lange and Etnoyer, 2024; Kahng et al., 2014; Weinstein et al., 2016). These results not only provide an assessment of the impact of live rock on coral health but also support the comparison between live rock sources. Documenting the difference between mesophotic live rock, which requires additional effort, cost, and difficulty to obtain, with readily available commercial live rock for purchase has direct implications for mission planning and future husbandry practices. The results of this experiment will therefore inform both field- and lab-based Mesophotic and Deep Benthic Communities (MDBC) project activities, as well as potentially add new tools for assessing fine-scale changes in coral health.

2. Methods

2.1 Rock Collection

Mesophotic live rock was collected by divers (permit: SRP # F/SER24:KO) aboard the contracted vessel MV *Tyson B* during the June 1–9, 2024, MDBC expedition TB2401 in the Gulf of America (formerly, the Gulf of Mexico [Exec. Order No. 14172, 2025]). Ten individual mesophotic live rock pieces of various sizes were obtained from 60-m depth, near the edge of the Mississippi-Alabama (MS-AL) Shelf south of Pensacola, Florida (site ID DSC, 29.913798, −87.205983). Rocks were collected at depth into a crate, which was then lifted to the surface. Aboard the vessel, they were put into an insulated cooler with constantly aerated site water collected from depth via Niskin bottles deployed on a conductivity, temperature, and depth (CTD) rosette. Rocks were then transferred within the aerated cooler by vehicle to a saltwater quarantine tank at the Hollings Marine Laboratory (HML) in Charleston, South Carolina. Once aboard the vessel, rocks were scraped with a sterile spatula to collect microbiome samples for 16S ribosomal ribonucleic acid (rRNA) analysis, which were preserved in RNAlater (Thermo Fisher Scientific) and stored at 4°C until being transferred to the laboratory's −80°C freezer until processing. Mesophotic live rock was placed in a saltwater quarantine tank for acclimation at HML.

Commercial live rock was purchased from Tampa Bay Saltwater (tbsaltwater.com) "base rock" in June 2024. These rocks were farmed at a depth of 7 m on the West Florida Shelf for a period of anywhere from 1–25 years. The rocks were packaged and transported overnight by freight airline. Once received at HML, rocks were scraped as described above and placed into a separate quarantine saltwater tank. Bare rock was also purchased from an aquarium supplier (bulkreefsupply.com) in June 2024. Bare rocks were washed in deionized water, scraped to collect microbiome samples for 16S rRNA analysis, and placed into a separate saltwater tank for quarantine.

All natural and commercial rocks underwent a five-week quarantine at HML, during which any invertebrate associates were removed, and water quality was continuously monitored. No associates were identified in the bare rock. The mesophotic live rock associates were not taxonomically identified but included: one squat lobster, two sea urchins, and one anemone. Only rocks whose associates could be removed were used in this study.

Scrapes of all rocks for 16S rRNA gene amplicon analysis were conducted at the time of receipt, (received, "Rd"), two weeks after rocks were put into their respective treatment tanks before the addition of corals ("BC") T=14 days, three months ("3mo"), and six months ("6mo") following the start of the study. Rocks were individually measured with a tape measure for length, width, and height and weighed. Surface area was obtained by recording the volume of saltwater displaced by each rock (Cooper and Testa, 2001). Rocks were then divided into each respective tank such that there were similar amounts of total rock weight and total rock surface area in each tank. Total rock weight and total rock surface area in each tank were first tested for normality and homogeneity of variance to verify statistical test assumptions were met. Using a one-way analysis of variance (ANOVA) examining rock weights, no significant differences between tanks ($p = 0.8$) were observed. A non-parametric Kruskal–Wallis test for displacement revealed no significant differences between

tanks ($p > 0.1$). Total bare rock weight: 4,724 g ± 217 g, surface area: 2,680 cm^3 ± 115 cm^3; shallow West Florida Shelf rock weight: 4,815 g ± 485 g, surface area: 2,235 cm^3 ± 168 cm^3; mesophotic MS-AL Shelf rock weight: 5,316 g ± 457 g, surface area: 2,185 cm^3 ± 162 cm^3.

2.2 Coral Collection and Experimental Setup

Coral colonies of the species *Swiftia exserta* (Ellis and Solander, 1786), *Thesea nivea* (Deichmann, 1936), and *Muricea pendula* (Verrill, 1864) were collected during cruises aboard the RV *Point Sur*, utilizing the remotely operated vehicle (ROV) *Mohawk* (owned by the National Marine Sanctuary Foundation and operated by the University of North Carolina at Wilmington's Undersea Vehicle Program) in June 2022 and October 2023. All corals were collected following standard operating procedures developed by the MDBC Coral Propagation Technique Development (CPT) project teams. Briefly, the ROV was lowered to the designated collection site using a high resolution video feed and a robotic five-function manipulator arm (ECA Hytec, ARM Micro 5E, France). Corals were identified, less than 10% of the colony was cut, and the coral fragment was placed into a sample container filled with saltwater from depth, integrated into an extendable and retractable skid attached to the ROV and brought on board. Once on board the vessel, the corals were tagged and floated in a 3.7-liter container holding water collected from depth by means of Niskin bottles deployed on a CTD rosette. Water changes were performed every 12 hr with freshly collected water from depth. Live samples were transported back to HML in the same containers with freshly changed water held in an insulated cooler at 18°C–22°C. All samples arrived at HML within 24 hr of docking.

Upon the initiation of the study, five colonies of each species were cut into three fragments of at least 15 cm in length, when possible. Due to limitations with *M. pendula* starting material, some of these fragments were smaller (5 cm). Each of these fragments was fixed onto an E-Marco-400 Aquascaping Mortar (FL, USA) base using SeaTak (PA, USA) aquarium glue and kept in a community saltwater tank for one week before being installed in an experimental tank. One fragment was chosen at random from each colony and put into one of the three treatment tanks containing: the control bare rock (C1), shallow WFL Shelf live rock (E2/WFL), and mesophotic MS-AL Shelf rock (E3/MS-AL) for a total of fifteen coral individuals per tank represented by five fragments of each species. An additional 2.5-cm fragment of each original colony was flash frozen as a "T=initial" sample to be used in triglyceride lipoprotein and global DNA methylation analyses. An additional 2.5-cm piece of each colony was collected and preserved in RNAlater as a "T=0" sample for 16*S* rRNA gene amplicon analysis. All collected samples were stored at −80°C until processed.

Corals were maintained in the experimental tanks for six months, beginning July 29, 2024, and ending on January 31, 2025. Corals were fed a slurry consisting of a quarter teaspoon (1.23 mL) each of Reef-Roids (Polyplab), Pro-Coral Zooton (Tropic Marin), and frozen rotifers twice daily Monday–Friday and once on Saturday/Sunday. All tanks were kept at a 50% blue light with a 9 Light:15 Dark cycle with 30 min of 25% intensity before and afterwards to simulate dawn and dusk, respectively.

2.3 Collection Methods

2.3.1 Tank Water Quality Collection

The three 50-gallon (189 L) aquarium systems were conditioned prior to introduction of rocks or corals; all tanks were cycled with filtered saltwater for four weeks to ensure removal of any harmful chemicals and adjust for water quality and filtration prior to any addition of corals or rocks. Filtered seawater was obtained from Charleston Harbor estuary (N 32°45''11.52"; W 79° 53'58.31"), filtered (5 µm), UV-sterilized, activated carbon filtered (5 µm), and adjusted when necessary to obtain optimal salinity of 36 ppm. Rocks were added to the experimental tanks so as to have similar weight and surface area in each treatment. Water quality and nutrient metrics (Table 1; Figure 1) were obtained weekly using a YSI ProQuatro Multiparameter Meter and LaMotte WaterLink Spin Touch FX photometer (Forestry Suppliers, USA). Ten-percent water changes occurred weekly throughout the experiment.

Table 1. Water quality metrics in treatment tanks with rocks prior to the introduction of corals. Rocks were maintained in the treatment tanks for two weeks before corals were added. These water quality values were obtained one week prior to the addition of corals.

Metric	Bare Rock/ Control Tank	Shallow West FL Shelf Live Rock/ E2 tank	Mesophotic MS-AL Shelf Live Rock/ E3 Tank
Temperature (°C)	21.4	21	21.6
Salinity (ppm)	36.4	36.4	36.4
pH	8.17	8.18	8.14
Alkalinity (ppt)	8.4	8.7	8.3
Nitrates (mg/L)	1.6	1.8	2.1
Nitrites (mg/L)	0.001	0.005	0.001
Ammonia (µmol/L)	0	0	0
Calcium (ppm)	430	440	460
Magnesium (mmol/L)	1,480	1,520	1,520
Phosphate (ppm)	0	0	0

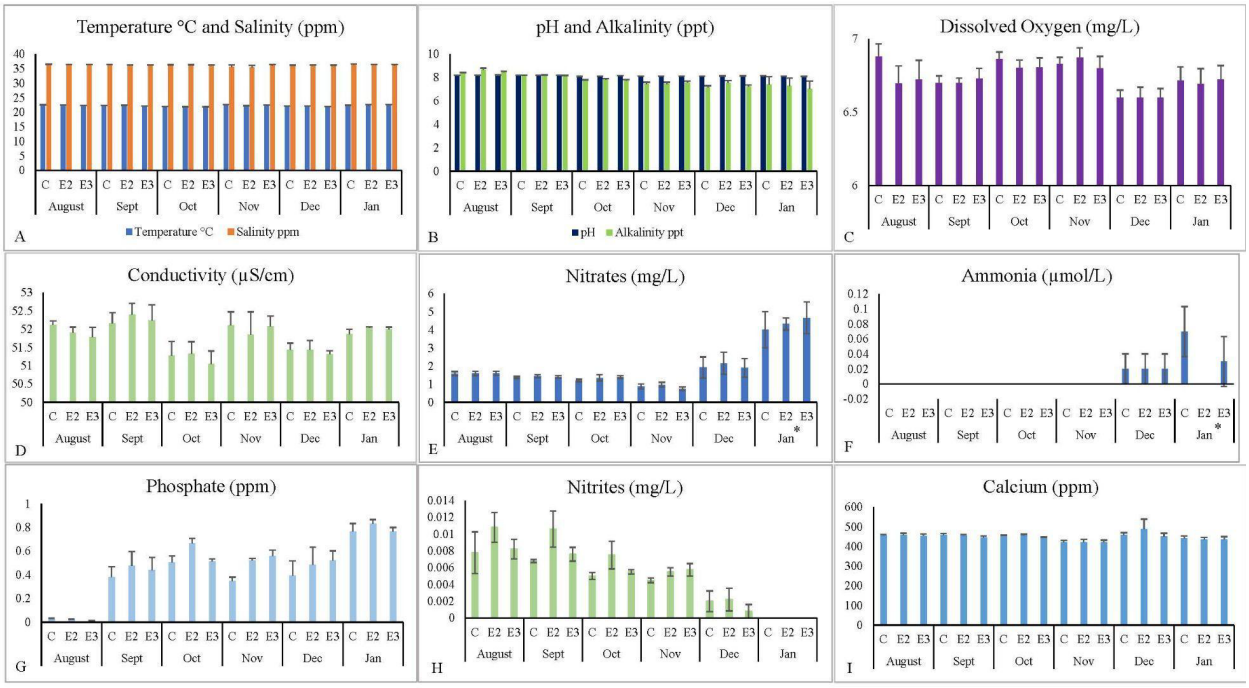


Figure 1. Water quality metrics for each treatment tank across six months. Water quality was measured weekly for six months in each tank (A–I). Averages and standard errors were calculated for all. C = bare rock tank, E2 = shallow WFL Shelf rock tank, E3 = mesophotic MS-AL Shelf rock tank. Units of measure are displayed next to each water quality metric. Numerical values equal to zero do not have bars present. Significant differences observed among nitrates and ammonia during month six of the experiment (*), $p < 0.001$.

2.3.2 Photography and ImageJ Analysis

Each coral was photographed on a PVC base fitted with a centimeter scale bar, using a Canon EOS RP DSLR camera. Photographs of each fragment were collected at the start of the study, three months, and six months. Visual metrics for TLE and tissue thickness were calculated by ImageJ software (National Institutes of Health, USA). Polyp numbers were estimated by visually counting polyps on five representative branches per fragment. Counts were averaged and multiplied by the branch lengths to estimate polyps per branch, which were then summed to obtain an estimated total polyp count per fragment.

2.3.3 16S rRNA Gene Amplicon Collection

Each rock type was photographed at zero days and six months. Rock surfaces were scraped at zero days, three months, and six months with a sterile spatula to collect surface-associated microbial communities. Samples were added to 5-mL Eppendorf tubes with sufficient RNAlater to fully submerge the collected material. Tubes were stored upright in a −80°C freezer until processed for DNA extraction using the Qiagen DNeasy PowerBiofilm Kit. The following modifications were made to the manufacturer's protocol: Particulate material was removed from the 5-mL tube with a sterile spatula and transferred to a pre-weighed, sterile 2-mL Eppendorf tube. Excess RNAlater was removed, and tubes were reweighed to normalize sample mass across all samples. Samples were rinsed with sterile 1X phosphate-buffered saline (PBS) solution followed by centrifugation at

13,000× g at room temperature for 1 min to remove residual buffer. A kit blank control was prepared using sterile 1X PBS solution and processed identically to rock samples.

All 45 individual coral fragments were photographed at zero days, three months, and six months. Coral tissue samples were collected at six months using sterile techniques. All tools were autoclaved prior to use. Between samples of the same species in the same treatment tank, scissors and forceps were bleached with 10% bleach solution, rinsed with Milli-Q water, and then dried with 70% ethanol to avoid contamination between samples. Separate tool sets were used for each treatment tank and coral species. A 2.5-cm snip was taken from each coral fragment and placed either into a clean tube containing RNAlater (for 16S rRNA amplicon sampling) or flash frozen in liquid nitrogen (for colorimetric assays). When insufficient tissue remained for all downstream analyses, samples for 16S rRNA amplicon sequencing were prioritized over lipoprotein and DNA methylation analyses.

2.4. Colorimetric Assays

2.4.1. Triglyceride Colorimetric Assay

A Triglyceride Colorimetric Assay Kit (EEA028 Invitrogen, USA) was used to assess total triglyceride content in coral tissue samples. The assay protocol followed the recommended procedure with minor modifications. A preliminary pilot test was conducted to determine the minimal amount of tissue needed in order to elicit a measurable response. The mean tissue mass for all samples analyzed exceeded this threshold (0.112 g ± 0.05 g). All tissues were maintained on dry ice before homogenization, and at 4°C after homogenization thereafter, unless otherwise specified. Tissues were homogenized in 2-mL microcentrifuge tubes using a Retsch Tissue Lyser II (Qiagen, USA) and cold, pre-cleaned 5-mm stainless steel beads. Briefly, beads were washed with RNaseZap (Thermo Fisher Scientific, USA), rinsed three times with Milli-Q water, dried with 70% ethanol, and autoclaved prior to use. Tissues were homogenized at a 9:1 ratio of the volume of anhydrous ethanol (mL) to equal tissue mass (g). Homogenates were centrifuged at 1,500 × g at 4°C, and the supernatant was transferred to a new tube and kept on ice. The assay was conducted following the recommended protocol, including preparation of samples, blanks, and standards. Samples were run in triplicate, and an enzyme reagent was added simultaneously using a multichannel pipette to initiate the reaction. Plates were incubated at 37°C for 10 min, after which optical densities (ODs) were measured at 510 nm using a Biotek Synergy HTX microplate reader (Agilent, USA). OD values were averaged, and calculated triglyceride (TG) amounts were assessed through the following equation where c = concentration of standard, f = dilution factor, m = weight of tissue (in grams), and v = volume of anhydrous ethanol (in milliliters).

$$TG = [(\Delta A_1/\Delta A_2) \times c \times f]/(m/v)$$

Samples lacking sufficient tissue mass for comparison with the original colony were excluded from analysis (Table 2).

Table 2. Metadata of all coral tissue samples included for either triglyceride lipoprotein or DNA methylation assay. (+) = samples included in 16S rRNA gene amplicon processing; a = initial samples (at zero days); z = final samples (at six months); * = samples excluded from Metabarcoding; x = samples processed but not included due to QA/QC limitations. If not designated with a symbol, then samples were not able to be processed due to minimal healthy tissue availability.

Samples	Assays		
	Triglyceride Lipoprotein	**DNA Methylation**	**Metabarcoding**
***M. pendula* 108**			
M. pendula # 108.1			a/z
M. pendula # 108.2			a/z
M. pendula # 108.3			a/z
***M. pendula* 115**	+	+	
M. pendula # 115.1			a/z
M. pendula # 115.2	+	+	a/z
M. pendula # 115.3	+	+	a/z
***M. pendula* 110**		+	
M. pendula # 110.1			a/z
M. pendula # 110.2		+	a/z
M. pendula # 110.3			a/z
***M. pendula* 146**			
M. pendula # 146.1			a/z
M. pendula # 146.2			a/z
M. pendula # 146.3			a/z
***M. pendula* 105**			
M. pendula # 105.1			a/z
M. pendula # 105.2			a/z
M. pendula # 105.3			a/z
***S. exserta* 79a**		x	
S. exserta # 79a.1	+	+	a/z
S. exserta # 79a.2		+	a/z
S. exserta # 79a.3		+	a/z
***S. exserta* 16a**	+	+	
S. exserta # 16a.1	+	+	a/z
S. exserta # 16a.2	+	+	a/z
S. exserta # 16a.3	x	+	a/z
***S. exserta* 20**	+	+	
S. exserta # 20.1	+	+	a/z
S. exserta # 20.2	x	+	a/z
S. exserta # 20.3	+	x	a/z
***S. exserta* 81**	+	+	
S. exserta # 81.1	x		a/z

Samples	Assays		
	Triglyceride Lipoprotein	**DNA Methylation**	**Metabarcoding**
S. exserta # 81.2		+	a/z
S. exserta # 81.3	+	+	a/z
***S. exserta* 15**	+	+	
S. exserta # 15.1	+	+	a/z
S. exserta # 15.2	+	+	a/z*
S. exserta # 15.3	x	+	a/z
***T. nivea* 59**	+	x	
T. nivea # 59.1	x	x	a*/z*
T. nivea # 59.2	+	+	a
T. nivea # 59.3			a*/z*
***T. nivea* 17**	+	+	
T. nivea # 17.1	+	x	a*/z*
T. nivea # 17.2	+	+	a/z
T. nivea # 17.3	+	x	a*/z*
***T. nivea* 005**	+	x	
T. nivea # 005.1		x	a/z
T. nivea # 005.2		x	a/z
T. nivea # 005.3		+	a/z
***T. nivea* 68a**	+	+	
T. nivea # 68a.1	+	x	a/z
T. nivea # 68a.2	+	+	a/z
T. nivea # 68a.3		x	a/z
***T. nivea* 004**	+	+	
T. nivea # 004.1	+	x	a/z
T. nivea # 004.2		+	a/z
T. nivea # 004.3	+	+	a/z
			a= initial sample z= final sample
	x= sample lost in bead beating step	x= samples not included due to quality of tissue OD<90 µg/L	*samples extracted but not run due to space limitations on flowcell and restrictions on ordering

2.4.2 MethylFlash Global DNA Methylation

Global DNA methylation levels were quantified using the MethylFlash Global DNA Methylation (5-mC) ELISA Kit (P-1030; EpigenTek, USA), which measures the percentage of 5-methylcytosine (5mC) present in genomic DNA. Coral tissue samples were snipped at zero days and six months, and extracted using the Qiagen Blood and Tissue Kit (Qiagen, USA). Samples were normalized to 90 ng/µL DNA and 260/280 absorbance ratios averaging 1.82 ± 0.07. Samples were processed as directed in the manufacturer's protocol. The extracted DNA was bound to the strip wells, and detection antibodies were bound to the DNA. After wash steps, a color developer solution was added, and samples were allowed to incubate at room temperature (21°C) before the plate was read. Optical Densities ODs were measured with a Biotek Synergy HTX microplate reader (Agilent, USA). The temperature of the run was recorded at 23°C at 450-nm absorbance. Samples and standards were processed in duplicate to accommodate a single 96-well plate. The resulting calculated standard slope of 0.387, with an accompanying R^2 value of 0.9997, indicated high linearity. Percent global DNA methylation was calculated by the following equation:

5mC % = ((sample OD−negative control OD)/(slope * normalized concentration))*100

Samples that were not collected due to tissue availability or that failed to meet this extraction criteria were excluded from the analysis (Table 2).

2.4.3 Statistical Analysis

Statistical analyses were conducted using JMP12 software (Statistical Discovery v.12). Data were examined for normality and homogeneity of variance to verify statistical assumptions. A one-way ANOVA was applied to all response variables associated with colorimetric assays. A significance threshold of $p \leq 0.05$ was used to identify statistically significant effects. Post hoc comparisons were conducted using Tukey–Kramer tests for pairwise group comparisons and Dunnett's tests for comparisons against control treatments, where applicable.

2.4.4 16S rRNA Gene Amplicon Analysis

Two 2.5-cm tissue snips were collected from each individual coral fragment at the time of fragmentation and again at six months. Tissue samples were stored in cryovials containing RNAlater and kept in the −80°C freezer until processed. DNA was extracted using the DNeasy Power Biofilm kit (Qiagen, USA) following the manufacturer's instructions. Samples were processed following the Illumina 16S rRNA Sequencing Library protocol including amplification polymerase chain reaction (PCR), indexing, normalization, and quality control. Only samples that passed all quality assurance/quality control metrics were carried on through to sequencing (Table 2). Briefly, extracted DNA was used to amplify the V3/V4 hypervariable region with primer pairs: 341 Forward (5' CCTACGGGNGGCWGCAG 3') and 805 Reverse (5' GACTACHVGGGTATCTAATCC 3') (Klindworth et al., 2013). After PCR cleanup, indexing PCR was performed using index set A, followed by a second PCR cleanup. Libraries were validated and quantified using an Agilent Bioanalyzer system with a DNA 1000 Kit (5067-1504) and QuBit 2.0 Fluorometer (Thermo Fisher Scientific, Q32866) with dsDNA broad range assay kit (Thermo Fisher Scientific, Q32853). Libraries

were normalized to 4 nM, pooled, and sequenced to a final concentration of 5 pM with a 25% PhiX control DNA spike-in. Sequencing was performed on an Illumina MiSeq platform using the MiSeq reagent V3 600 cycle kit (Illumina MS1023003) with a paired reading of 300 bp. No-template PCR controls, as well as blank kit samples with only 1X PBS, were used as negative controls. Run metrics were 98.5% clusters passing filter and 6,217 MB estimated yield.

2.4.5 Bioinformatics Analysis

Sequence data were processed using the DADA2 pipeline (version 1.26.0; Callahan et al., 2016) in RStudio v4.2.3 ("Shortstop Beagle"). Raw demultiplexed reads were filtered, trimmed using TruncLen (255, 225), dereplicated, and merged, and amplicon sequence variants (ASVs) were inferred for 96 samples. Taxonomic assignment was performed using the SILVA nr99 v138.1 reference database. Following data filtering and trimming, 86%–89% of reads were retained, and approximately 2% of sequences were identified as chimeras and subsequently removed. Two control kit blank samples, one no-template PCR control, along with ASVs classified as chloroplasts, mitochondria, and eukaryotes were also excluded. A prevalence threshold of 2 was applied, which resulted in 1,267 remaining taxa in 81 samples. Samples were rarified to a depth of 486 sequences prior to ordination and diversity analyses. Principle coordinate analyses (PCoA), and alpha and beta diversity indexes were calculated using the *phyloseq* package (version 1.42.0; McMurdie and Holmes, 2013).

Downstream community analysis applied the following packages: DESeq2 was used to normalize counts and conduct statistical tests; Vegan and pairwiseAdonis packages were used to generate distance matrices and obtain beta diversities. Statistical relevance was generated through permutational multivariate ANOVA (PERMANOVA), group dispersion, and pairwise PERMANOVA statistical metrics. Alpha diversities were obtained through the use of Shannon diversity index, and statistical measurements were analyzed through ANOVA.

3. Results and Discussion

3.1 Water Quality

In the present study, all three tanks maintained a stable pH of approximately 8 regardless of rock treatment, indicating that even the bare rock, which was primarily composed of calcium carbonate, likely acted as a buffering agent contributing to water quality stabilization (Figure 1). Previous studies have reported pH values measuring close to 8 in aquariums with live rock and lower in aquariums without live rock (Yuen et al., 2009). The inclusion of live rock lowers harmful concentrations of ammonia, nitrates, and nitrites (Yuen et al., 2009). The presence of live rock alone, in the absence of mechanical filtration, can maintain acceptable water quality for approximately 5–7 days; however, proper aquarium filtration in addition to live rock is necessary to affect positive water quality over longer periods of time (Yuen et al., 2009; Li et al., 2017). In this study, there were no significant differences in nitrite concentrations over time among treatments (ANOVA $p = 0.8$). However, all tanks showed a spike in ammonia and nitrate concentrations during the final two months of the experiment (months five and six), which was statistically significant at month six (nitrate and ammonia $p < 0.001$). No individualized care of water chemistry, separate from weekly water changes, was administered to these experimental tanks as this would have altered the experiment, rendering some experimental tanks with different care than others and possibly masking any effects of live rock to the system. Conditions that promote algal growth in closed-system aquaria often result from elevated nitrate concentrations, which can occur when filtration efficiency declines or feeding rates are excessive. Degradation of water quality can negatively affect the health and growth of marine invertebrates (Delbeek and Sprung, 1994; Grguric et al., 2005). This measured increase of ammonia is likely a contributing factor to declining overall water quality and may have negatively affected coral health during the final months of the experiment (Figure 1). Specifically, the fleece roller filters (ClariSea, model SK-3000), which were used in this experiment, did not perform as expected. Though these filters were designed to automatically advance to clean fleece, they often did not and required manual daily adjustments. Furthermore, the addition of granular ferric oxide, which helps with neutralizing phosphates in closed aquaria, was intentionally omitted due to uncertainty of live rock efficiency in mitigating phosphates. This, coupled with adverse weather limiting access to the building during the last month of the experiment, compounded care and may have led to rapid declining conditions throughout the experimental tanks.

3.2 Visual Metric Analysis (TLE, Polyp Number, Thickness)

All coral fragments were visually assessed in a binary way to initially appear either healthy (no apparent concerns) or stressed (exhibiting a skinny or pale appearance or tissue loss). Fragments exhibiting compromised initial conditions such as appearing skinny, exhibiting a pale tissue color, or showing skeleton were intentionally retained to evaluate whether live rock treatments could facilitate recovery. A full visual report is noted in Table 3. All *M. pendula* corals initially appeared visually healthy; however, these fragments were smaller relative to the other species fragments (Figure 2). Most corals appeared visually healthy at three months, with some individuals measuring increased length and polyp number (Figure 3). By six months, however, any visual or measured gains in total length or polyp number had been lost, and coral health declined in all treatments (Table 3 and Figure 3). Only a few individual replicates of *S. exserta* and *T. nivea*, along with one replicate of *M. pendula*, continued to thrive through the end of the experiment at six months. Mortality observed among *M. pendula* replicates may reflect multiple contributing factors including a small initial fragment size, species-specific fragility, and decrease in water quality during the last month of the study.

Table 3. Visual coral health scoring. Each individual species of coral was assessed initially, after three months, and at the end of the six months for each treatment tank. Observations were recorded, and a color was designated for each observation. A coral that was initially in good health was given a score of "good" and colored in light green. Corals that were seen as skinny or lacked color (pale) were recorded as such and colored as light orange. Corals at three months that decreased in visual health from the initial assessment were recorded as such and given a color of medium orange. Corals that showed positive growth compared to the initial assessment after three months were recorded as such and given a color of dark green. Corals that decreased in health from the three-month assessment were recorded as such and given a dark orange color. Text was changed to red if the corals underwent dramatic changes compared to the three-month assessments. Corals with no visual signs of change were labeled as "no change" and left white. MP = *Muricea pendula*, SE = *Swiftia exserta*, TN = *Thesea nivea.* Numbers next to abbreviations represent the fragment number identifier of that coral.

Species	Replicate	Initial Visuals			After 3 Months			After 6 Months (Compared to 3 Months)		
		C	E2	E3	C	E2	E3	C	E2	E3
M. pendula	108	small frag but good	small frag but good	good	tissue loss mostly dead	mostly dead/lots of tissue loss	tissue loss	mostly dead	mostly dead	dead
M. pendula	115	small frag but good	good	good	skinny/tissue loss	maybe some growth	maintaining	skinny/tissue loss	good	no change
M. pendula	110	small frag but good	good	good	tissue/skeleton loss	losing polyps distally	maintaining	mostly dead	tissue loss/ skinny	dead
M. pendula	146	small frag but good	good	good	some tissue loss	slight growth	slight tissue loss	mostly dead	mostly dead	dead
M. pendula	105	small frag but good	small frag but good	good	tissue loss mostly dead	tissue loss	tissue loss	dead	tissue loss/skinny	dead
S. exserta	79a	skinny/ pale	skinny	skinny/ pale	color loss	grew in height	maybe one more polyp	skinny	skinny	skinny
S. exserta	16a	good	good	good	color loss	grew in height and more polyps	grew, kept color, more polyps	great	skinny	skinny
S. exserta	20	good	good	pale	color loss/slight growth if any	grew in height	grew more polyps	skinny/pale	skinny/pale	skinny/pale
S. exserta	81	good	good	good	slight growth if any	grew a polyp	Grew, more polyps	skinny	skinny	skinny
S. exserta	15	skinny	skinny	skinny	slight growth if any	right branch growth	paler in color	skinny/pale	skinny	skinny/pale
T. nivea	59	good	good	good	tissue/skeleton loss	slightly shorter	paler in color	dead	skinny	dead
T. nivea	17	pale	good	good	tissue/skeleton loss	no change	thicker	lots of skeleton	no change	skinny/pale
T. nivea	005	skeleton showing	good	good	tissue/skeleton loss	lost height	tissue loss distally	lots of skeleton	tissue/ skeleton loss	no change
T. nivea	68a	detritus on it	detritus on it	good	tissue/skeleton loss	tissue loss	grew vertically and distally	lots of skeleton	skinny	skinny
T. nivea	004	skeleton showing	skeleton showing	skeleton showing	skeleton receded	lost a branch that was dead initially	lost a branch -more tissue loss distally	lots of skeleton	good	good
Color indicator:		**started off visually as not in the best of health**	**started visually in good health**	**NA**	**visually started to decline**	**visual positive growth documented**	**no change**	**compared to 3 months decrease in visual health**	**compared to 3 months increase in visual health**	**no change**

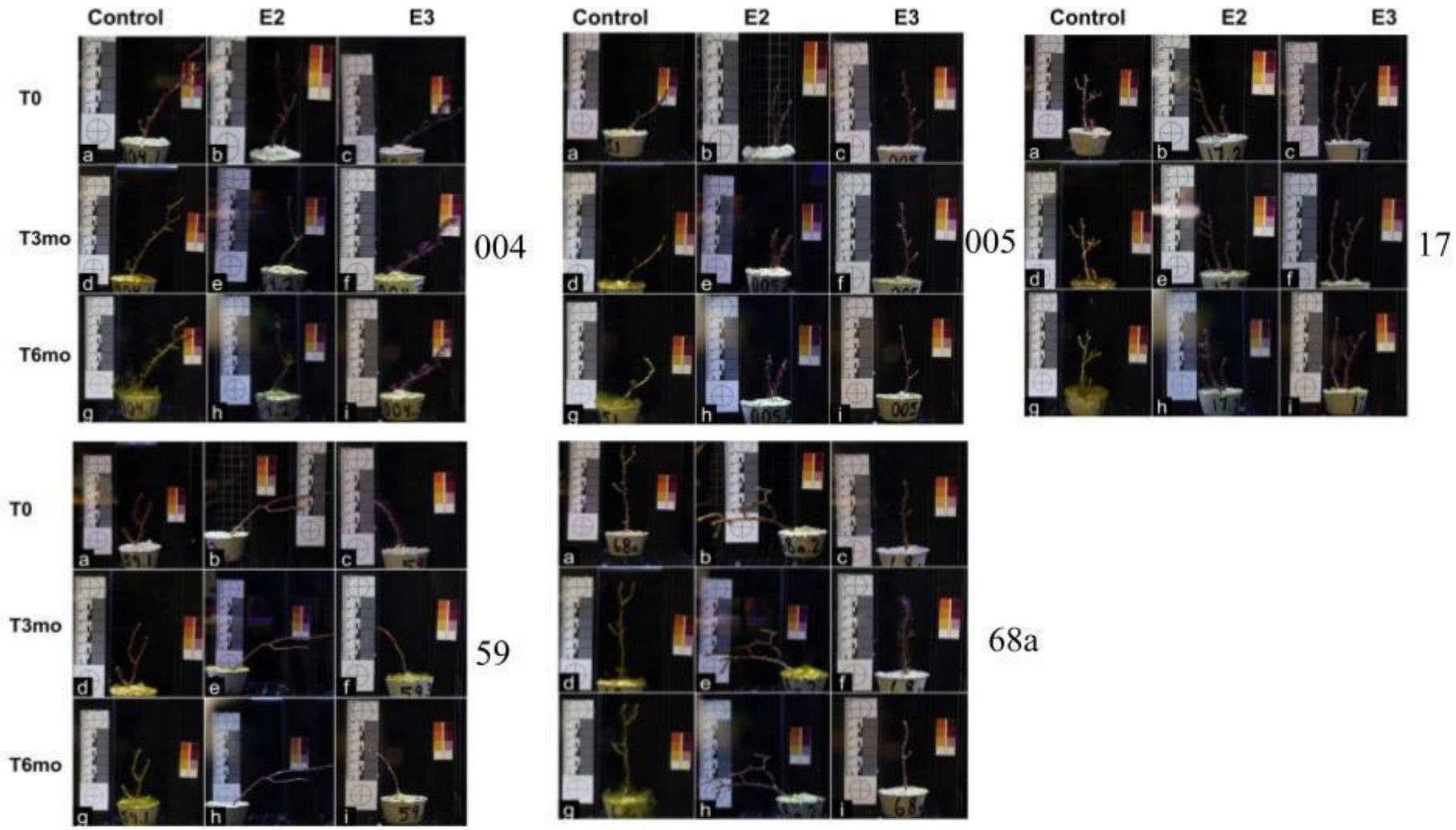


Figure 2a. Composite photographs of *Thesea nivea* per treatment tank throughout time. Individual photographs of coral replicates were taken at zero days (T0), three months (T3mo), and six months (T6mo). Tiles (a), (d), and (g) represent the control/bare rock tank; (b), (e), and (h) represent the shallow WFL Shelf rock tank/E2; and (c), (f), and (j) represent the mesophotic MS-AL Shelf rock tank/E3. The replicate number is listed to the right of the composite photographs. Photo credit: Allisan Aquilina-Beck (CSS/NOAA).

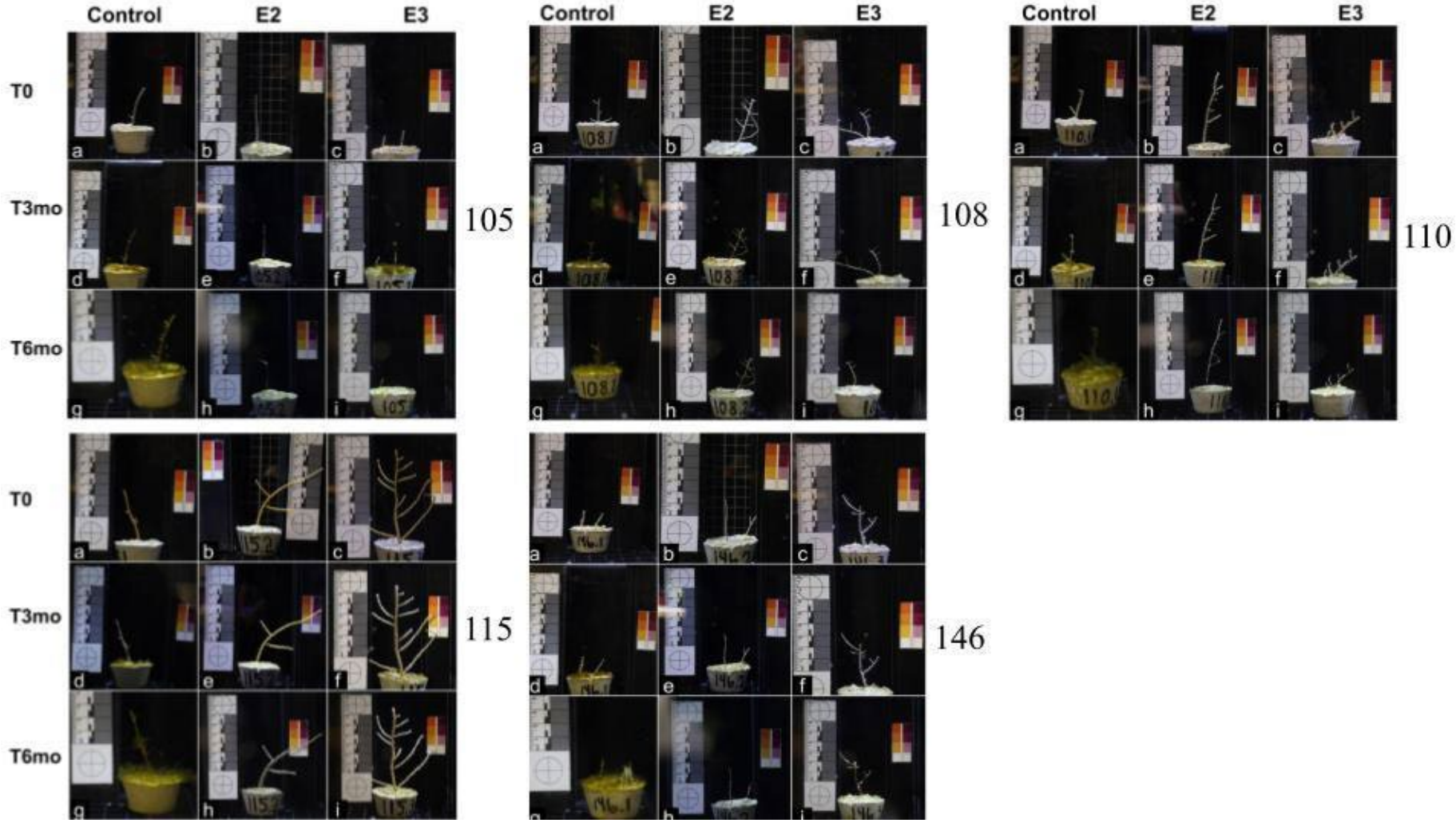


Figure 2b. Composite photographs of *Muricea pendula* per treatment tank throughout time. Individual photographs of coral replicates were taken at zero days (T0), three months (T3mo), and six months (T6mo). Tiles (a), (d), and (g) represent the control/bare rock tank; (b), (e), and (h) represent the shallow WFL Shelf rock tank/E2; and (c), (f), and (j) represent the mesophotic MS-AL Shelf rock tank/E3. The replicate number is listed to the right of the composite photographs. Photo credit: Allisan Aquilina-Beck (CSS/NOAA).

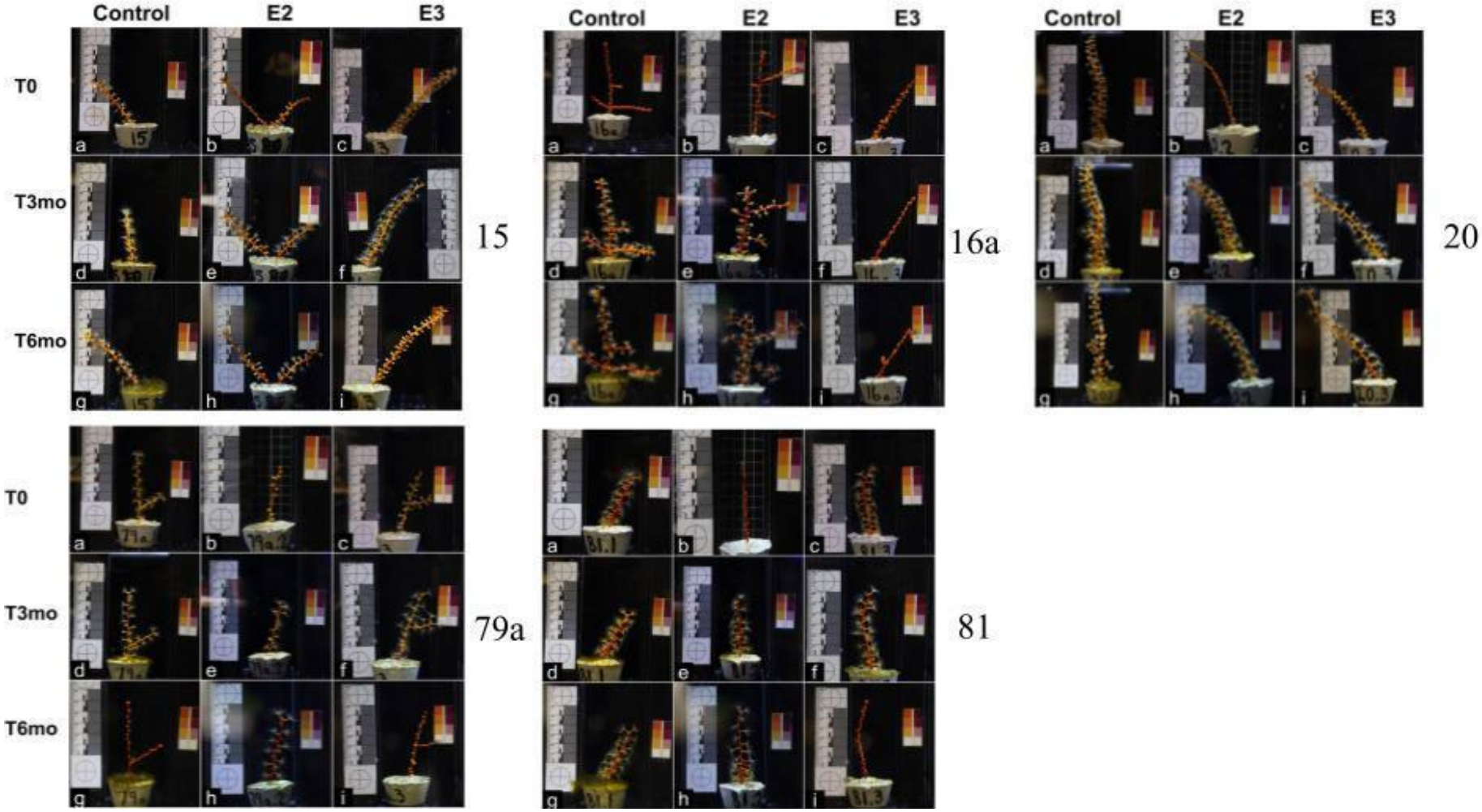


Figure 2c. Composite photographs of *Swiftia exserta* per treatment tank throughout time. Individual photographs of coral replicates were taken at zero days (T0), three months (T3mo), and six months (T6mo). Tiles (a), (d), and (g) represent the control/bare rock tank; (b), (e), and (h) represent the shallow WFL Shelf rock tank/E2; and (c), (f), and (j) represent the mesophotic MS-AL Shelf rock tank/E3. The replicate number is listed to the right of the composite photographs. Photo credit: Allisan Aquilina-Beck (CSS/NOAA).

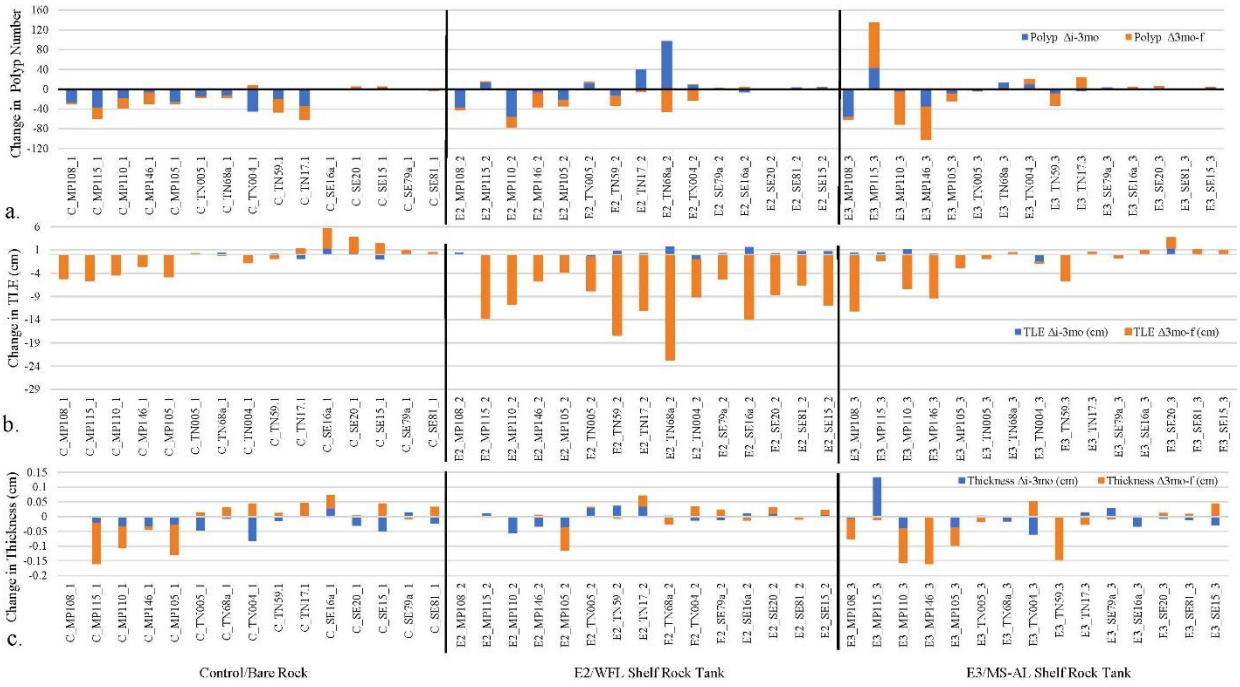


Figure 3. Average change in metrics per coral per treatment, in three-month increments. Individual coral samples for all three treatments plotted change in: polyp number (a), total linear extension (TLE) (b), and thickness (c) from initial measure (i) to three months (blue color), and from three months to six months (final, f) (orange color). The graphs represent metrics collected from the images shown in Figure 2. In all graphs, the first group of samples are representative of the bare rock treatment tank (C), the middle grouping of samples are representative of the shallow WFL Shelf rock tank (E2), and the last grouping of samples reflect measurements from the mesophotic MS-AL Shelf rock tank (E3).

3.3 Colorimetric Assays

3.3.1 Triglyceride Lipoprotein

Lipids are a primary energy source for hard and soft corals alike, with tissue lipid concentrations varying according to reproductive state, respiration, cellular renewal, and symbiont activity (Imbs and Dembitsky, 2023). Healthy cells use these energy reserves for metabolism and body processes. Thus, when corals are sustaining growth, this assay could provide a tangible metric to otherwise subjective visual measures. At six months, the majority of the coral fragments appeared skinny, pale, or had tissue loss across all treatment tanks (Table 3), which made calculating quantifiable triglyceride levels challenging. Overall, triglyceride concentrations were significantly lower ($p \leq 0.05$) at six months relative to initial source colony samples (Figure 4). Triglyceride readings varied among species and within replicates, with some concentrations recorded close to or below zero. Variability of total lipids has been noted among coral species in other papers (Imbs and Dembitsky, 2023). This result likely reflects depletion of energy reserves in order to maintain cellular processes as corals faced increasing demands. This was noted specifically in gorgonian corals that underwent bleaching events causing the lipid content to drop considerably from 19%–33% to 3%–17% (Imbs and Dembitsky, 2023). Though this is mostly seen in the experimental replicates of *T. nivea*, source colony fragments' initial triglyceride amounts were recorded near or below zero in three of the four replicates analyzed (Figure 4), indicating these corals were in a challenged state prior to the start of the experiment.

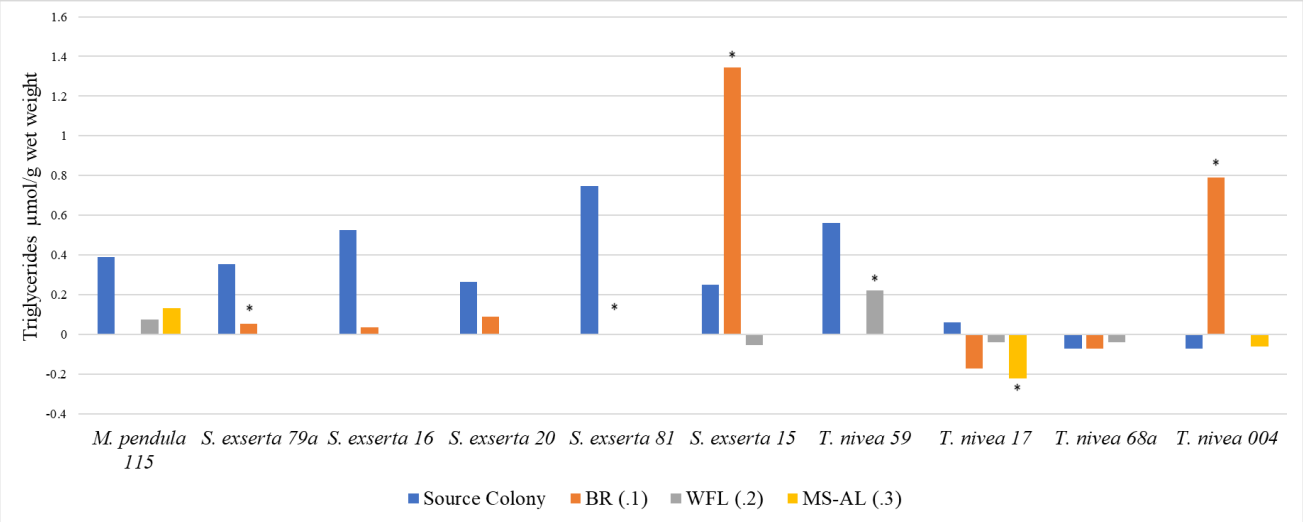


Figure 4. Triglyceride ELISA assay on all three coral species. Small samples of coral were collected from the original source colony and again at six months from individual coral fragments. Samples from each species were run when there was enough tissue. BR = bare rock samples, WFL = West Florida Shelf samples; MS-AL = Mississippi-Alabama Shelf samples. An "x" of the designating color indicates treatment tank samples that had a reading of zero. *M. pendula* n = 3, *S. exserta* n = 13, *T. nivea* n = 12. One-way ANOVA was used to determine statistical significance of differences in triglycerides compared to the source colony of that sample, with (*) representing $p \leq 0.05$. ELISA = enzyme-linked immunosorbent assay.

Two coral samples, *S. exserta* (15.1) and *T. nivea* (004.1), exhibited marked increases in triglyceride lipoprotein concentrations among the bare rock treatment (Figure 4). Notably, these samples also exhibited filamentous algal growth on coral polyps, and despite manual removal efforts, residual algae remained entangled within the coral polyps. As a result, algal biomass was coextracted with coral tissue and likely contributed to elevated triglyceride measurements. Future studies should either exclude samples such as these from the assay or, as in this case, take meticulous notes as to the sample's visual appearance.

The single sample of *M. pendula*, #115, is an example in which both live rock treatments resulted in positive triglyceride measurements, even though the final triglyceride concentrations were lower than the original source colony fragment. However, the visual and morphometric indicators recorded for this sample suggested improved coral condition despite low triglyceride measures. Given the overall decline in coral health toward the end of the study, these results may accurately reflect the physiological stress of corals at the end of the study. Sampling at the three-month time point, particularly when coral tissues were not receding, may have yielded clearer treatment-level differences in lipid concentration.

This study represents the use of a rapid and quantitative measurement of lipoprotein to assess health in mesophotic octocorals of the Gulf of America. This assay provides results within a day and leverages archived frozen tissue samples, making it a promising tool for early detection of sublethal stress in aquarium-held corals.

3.3.2 Global DNA Methylation

Assessing DNA methylation within a cell provides information about gene activity, which, when correlated with growth and disease recovery, can offer valuable information about coral health. DNA methylation is involved in homeostasis between cellular function and the environment, in addition to inducing a measured response to stress in cnidarians (Yu et al., 2024). External environmental factors can influence this system, thus giving insight into negative environmental impacts on an organism and its ability to acclimate and adapt to changes. This complex system involves three actions in order to adjust gene expression: catalyze methyl groups, remove methyl groups, and bind to methyl groups (Moore et al., 2013). Together, these modifications are involved in regulating gene function by altering methylation structures and patterns. This assay measured the ratio of total percentage of methylated DNA compared to overall DNA in a sample. Similar to the triglyceride lipoprotein assay, it delivers data within one day and relies on previously acquired samples for comparison.

DNA methylation in invertebrates occurs in gene bodies throughout the genome. Invertebrates have either constitutively expressed highly methylated gene bodies, termed housekeeping genes, or low methylated gene bodies such as those involved in cell signaling (Dixon et al., 2014; Dimond and Roberts, 2016; Abbott et al., 2024). DNA methylation values have been reported across diverse invertebrate taxa, including sponges, tunicates, and nematodes (Regev et al., 1998). Walling (2020) reported percentage global methylation (global 5-methylcytosine [g5mC]) levels ranging from 0–6.9 g5mC across 76 different species of octocorals. Observed values in the present study are within this reported range (0.1–1.1 g5mC). Lower amounts of g5mC could indicate changes in gene function in response to changing environmental stressors depending upon the species. Establishing methylation baselines for these three octocoral species contributes valuable reference data for future studies.

Gene body global methylation levels equal to or greater than the original source colony fragment could indicate normal steady-state cellular functions; however, most samples exhibited decline in the experimental tanks, which resulted in decreased methylation compared to source colony fragments (Figure 5). This could indicate an activation of low methylated gene bodies responding to changing, potentially stressful conditions at six months. However, confirmation of specific genes undergoing stress responses would need to be determined through more detailed methods such as RNA sequencing or quantitative PCR. Such commonly activated pathways corresponding to low methylation levels are reported to be involved in stress response, immune responses, and cellular processes (Alam et al., 2015; Moore et al., 2013). Thus, changes reported in global methylation levels indicate potential regulatory responses rather than specific stress pathways.

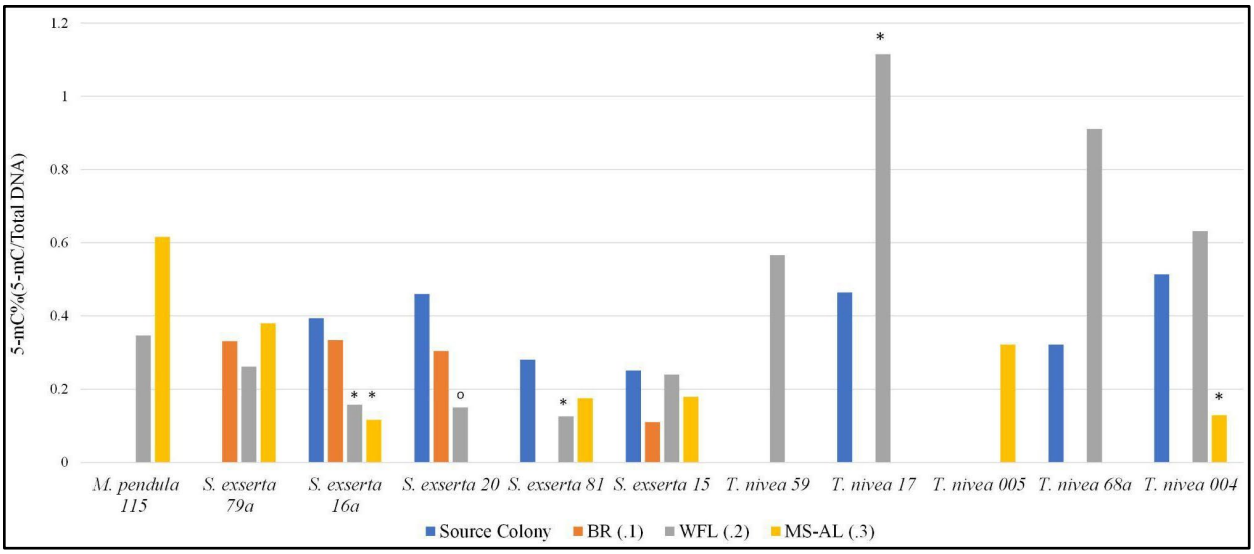


Figure 5. Percent total DNA methylation. Samples were compared to initial (source colony) levels when data were available. Initial methylation levels from source colony samples were variable between and within species. A low sample number resulted from a lack of available tissue or insufficient starting concentration. The graph represents samples from at least one of the three coral species tested in source colonies, bare rock (BR), shallow West Florida Shelf (WFL) rock, and mesophotic Mississippi-Alabama Shelf rock (MS-AL). Greater measures of methylation were observed in *T. nivea* corals kept with shallow WFL Shelf rock. *S. exserta* corals (*) showed a statistically significant response compared to the source colony fragment. One-way ANOVA was used to determine statistical significance, with (*) representing $p < 0.05$ and (o) $p = 0.0535$.

The same *M. pendula* sample, #115, analyzed in the triglyceride assay was also measured for DNA methylation levels. However, since the original source colony sample was not analyzed due to low DNA concentration, no claim can be made regarding the impact of the live rock treatments on recovery or improvement in health according to DNA methylation values for this replicate. However, considering positive triglyceride levels for this sample in live rock treatments, the combination of remaining positive energy reserves, together with relatively high levels of DNA methylation (compared to other species in the live rock treatments), suggests that this replicate was under minimal cellular stress and sustaining stable methylation.

Among *S. exserta*, three of the five replicates among live rock treatments showed significantly reduced methylation relative to source colony samples ($p < 0.05$) despite appearing visually similar across treatments. This discrepancy highlights the utility of DNA methylation as a sensitive indicator of sublethal stress. Combined with near-zero triglyceride reserves, these patterns are consistent with observed visually declining physiological conditions described as skinny or pale.

T. nivea had the three replicates that could be compared to initial source colony levels. Shallow WFL Shelf rock treatment samples in this group were consistently higher than original source colony samples. Of these, #17 and #004 were noted to have increased levels of tissue thickness (Figure 3). The same sample, *T. nivea* #004 in the MS-AL treatment, by contrast, had significantly reduced levels compared to the original source colony ($p < 0.05$). None of the bare rock treatment *T. nivea* samples had tissue to analyze, due to poor performance in this tank. The correlation between high

global methylation readings and increased measured thickness seen in shallow WFL Shelf samples has been related to increased tissue growth and cellular proliferation (Zhang et al., 2017). Though thickening of cellular tissues is commonly linked with human disease, thickening of coral tissue is associated with a response to stress and acclimation (Qin et al., 2020).

Overall, global DNA methylation provided quantitative insight into health status beyond visual assessments. Analyzing these metrics for coral health could provide quick confirmation of a healthy state or mark initial decline. When interpreted alongside triglyceride measurements, these assays collectively indicate whether corals are maintaining stable cellular function or experiencing metabolic stress.

Considering these two colorimetric assay results together, samples that gave positive triglyceride results and higher levels of global methylation support the conclusion that some corals were maintaining good cellular functions and not in a stressful state. Conversely, lower or zero scores for triglyceride lipoprotein, combined with decreased methylation levels in coral tissues, may suggest that the tissues are depleting remaining energy reserves to meet the heightened demands of maintaining stable cellular processes.

3.4 16S rRNA Gene Amplicon Survey Results

Coral tissue and rock scrapings were analyzed for changes in community microbiome structure. Analyzing the entire dataset of corals and rocks together revealed distinct bacterial assemblages associated with individual coral species and treatments, which shifted by the end of the experiment. Specifically, *M. pendula* samples began (0d, “C0” samples) with a microbiome characterized by a large fraction of the phylum Firmicutes (shown in green in Figure 6), while at six months (6mo), Firmicutes was mostly gone or considerably depressed among the bare rock treatment tank (“C6” samples). Within the shallow WFL Shelf rock treatment (“E2”), samples retained this phylum at high levels in two samples (E2-6-MP-146; E2-6-MP-115). It was retained in only one of the five corals in the mesophotic MS-AL Shelf rock treatment sample (E3-6-115). This is the same *M. pendula* replicate #115 previously analyzed in colorimetric assays that showed positive results. Maintaining its initial microbial makeup may have contributed to this replicate’s success in the live rock tanks. Similarly, shifts in starting community microbiome compared to end community microbiome were seen in the other two coral species. *T. nivea* began with large portions of Proteobacteria and was significantly depressed by six months in the bare rock and shallow WFL Shelf rock treatment tanks. The mesophotic MS-AL Shelf rock treatment tank samples for *T. nivea* (“E3”) maintained Proteobacteria when compared to starting communities (“E3-0”). *S. exserta* lost large proportions of Proteobacteria and gained Firmicutes by six months in sample E3-6-SE15.3.

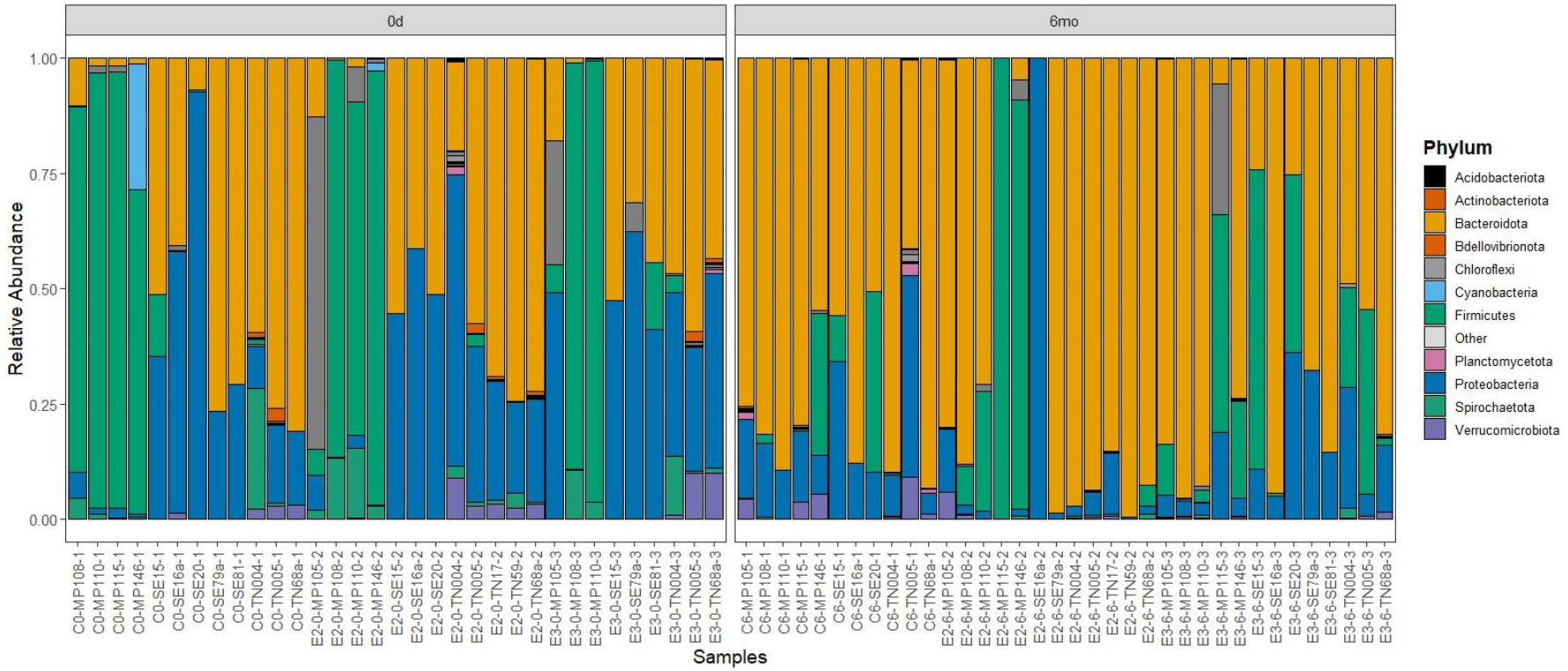


Figure 6. Proportional abundance of top 20 bacteria phyla. A) Proportional abundance of bacteria (phylum) in coral tissues only at zero days (0d) and six months (6mo). Treatment tanks are labeled as C (bare rock tank), E2 (shallow WFL Shelf rock tank), and E3 (mesophotic MS-AL Shelf rock tank). Individual coral samples are labeled on the x-axis, where MP = *M. pendula*, SE = *S. exserta*, and TN = *T. nivea*. Graph displays the changes in bacterial makeup individual samples underwent throughout time.

As seen in the coral tissue, the three different rock treatments also exhibited differences in their initial bacterial makeup upon collection (received, Rd), before corals were added to the tanks (BC), and again at three months to six months, showing differences in phyla over time (Figure 7). To gain more clarity on these shifts over time regarding coral and rock bacterial communities, the following analysis focused first on how different coral bacterial communities differ over time in all three rock treatments, then evaluated changes in live rock bacterial communities over time.

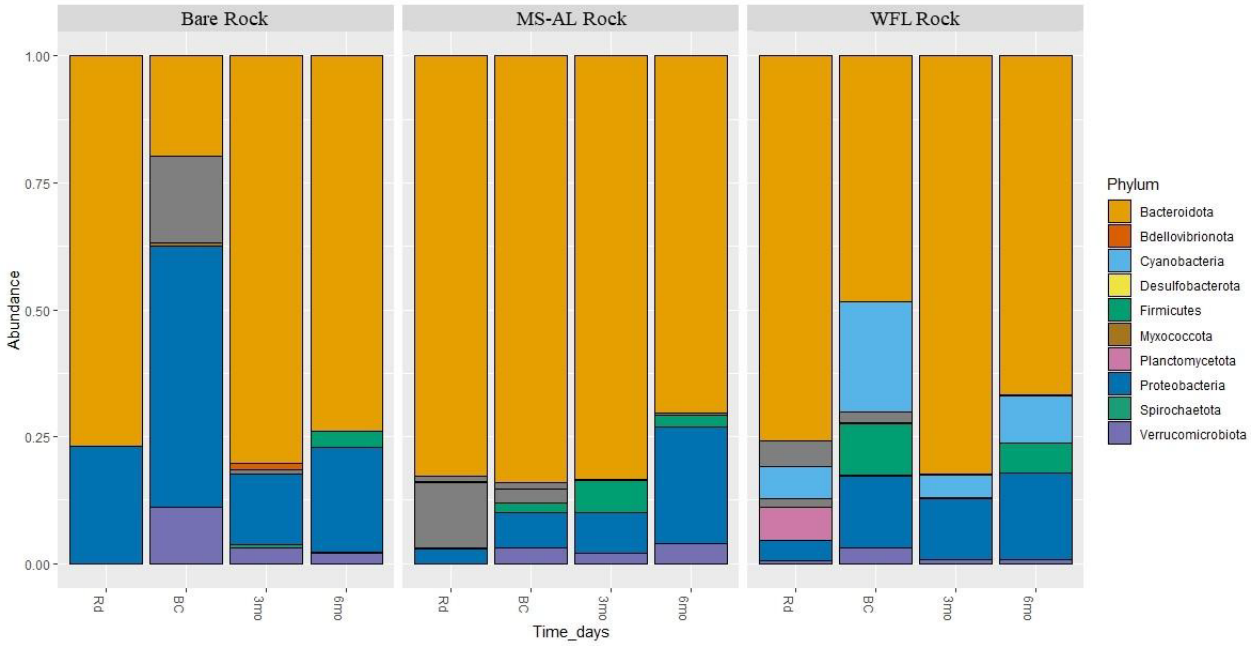


Figure 7. Proportional abundance of top 20 bacteria phylum. The graph reflects the proportional abundances of bacteria on different rock treatments by phylum, with time plotted on the x-axis and each rock type labeled above. The graph displays the top 20 most abundant bacteria at each time point. Changes in microbial types and abundances can be seen as time progresses for each rock type. Rd = received; BC = before corals; 3mos = three months.

When describing coral tissues, the treatment tank descriptors: C/BR, E2, and E3 will be used. Conversely, when describing analysis involving rocks the descriptors: Bare Rock, WFL Rock, and MS-AL Rock will be used.

3.4.1 Coral Tissue Analysis

A PCoA plot of coral tissue revealed clustering of samples primarily by coral species rather than treatment or time (Figure 8). *M. pendula* replicates exhibited greater separation and grouping with both *S. exserta* and all *T. nivea* samples. However, a PERMANOVA confirmed differences between species to be statistically significant ($p = 0.0001$). Evaluating species richness and relative abundance within samples (alpha diversity analysis) using Shannon indices revealed significant differences among species over time ($p = 0.0246$; Figure 9).

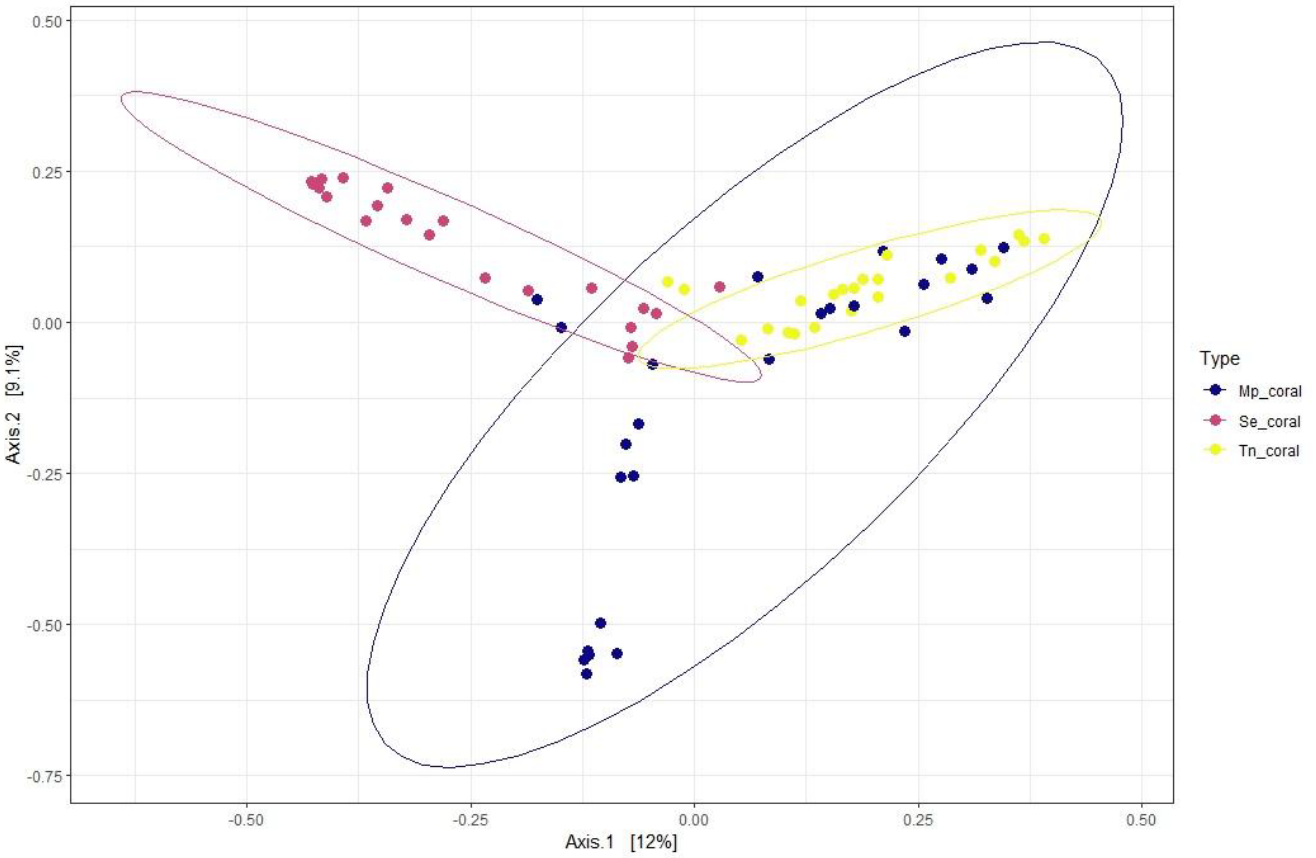


Figure 8. Orientation plot of coral species, illustrating the relationship between all coral species initially and at six months. Each species of coral is graphed, regardless of treatment condition, displaying groupings within each species, with the tightest groupings among *S. exserta* (green dots) and *T. nivea* (blue dots). *M. pendula* had the widest grouping, showing similarities with *T. nivea*. Adonis PERMANOVA significance measured at p = 0.0001.

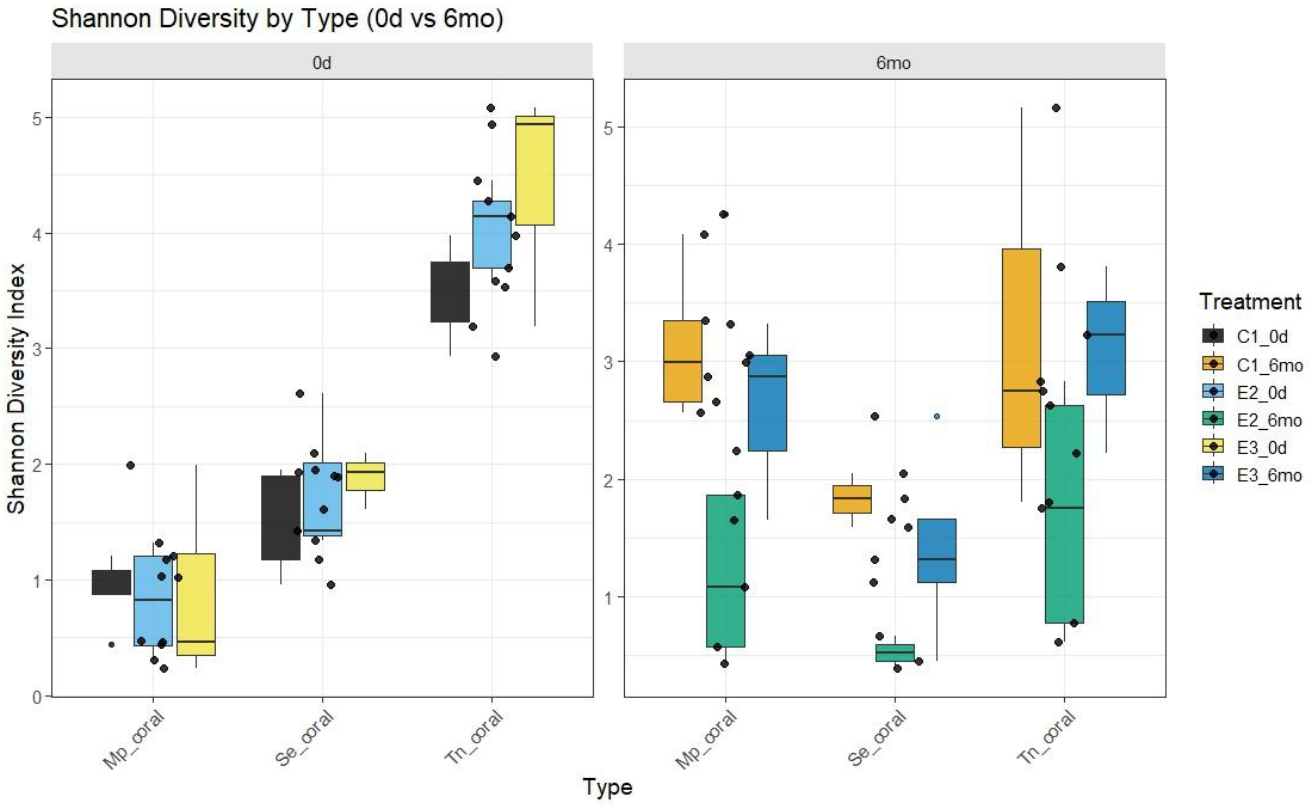


Figure 9. Alpha diversity of microbial communities in coral tissue. Shannon diversity measures in all coral tissues initially (0d) and at six months (6mo) for each treatment tank. Black = bare rock treatment tank (C1) 0d, Light blue = shallow WFL Shelf rock treatment tank (E2) 0d, Yellow = mesophotic MS-AL Shelf rock treatment tank (E3) 0d, Orange= bare rock treatment tank (C1) six months, Green = shallow WFL Shelf rock treatment tank (E3) six months, Dark blue = mesophotic MS-AL Shelf rock treatment tank (E3) six months. Kruskal-Wallis statistical test for Shannon Diversity between each coral species and length of time is significant p = 0.0246. Alpha diversity of coral species within initial treatments of C1_0 (p = 0.02), E2_0 (p = 0.01), E3_0 (p = 0.05) were all significantly different from each other, while the six-month data for corals E3_6mo neared significance (p = 0.055).

In order to clearly identify the changing bacterial taxa and identify differences within the coral species over time, relative abundance plots were generated for the top five bacterial families for each species replicate between initial and final timepoints (Figure 10). This measures the proportion of the top bacterial families compared to the overall bacterial community. A shift in community composition was observed across all treatment in all three species. In order to determine which bacterial families were significantly different, a further detailed analysis was conducted.

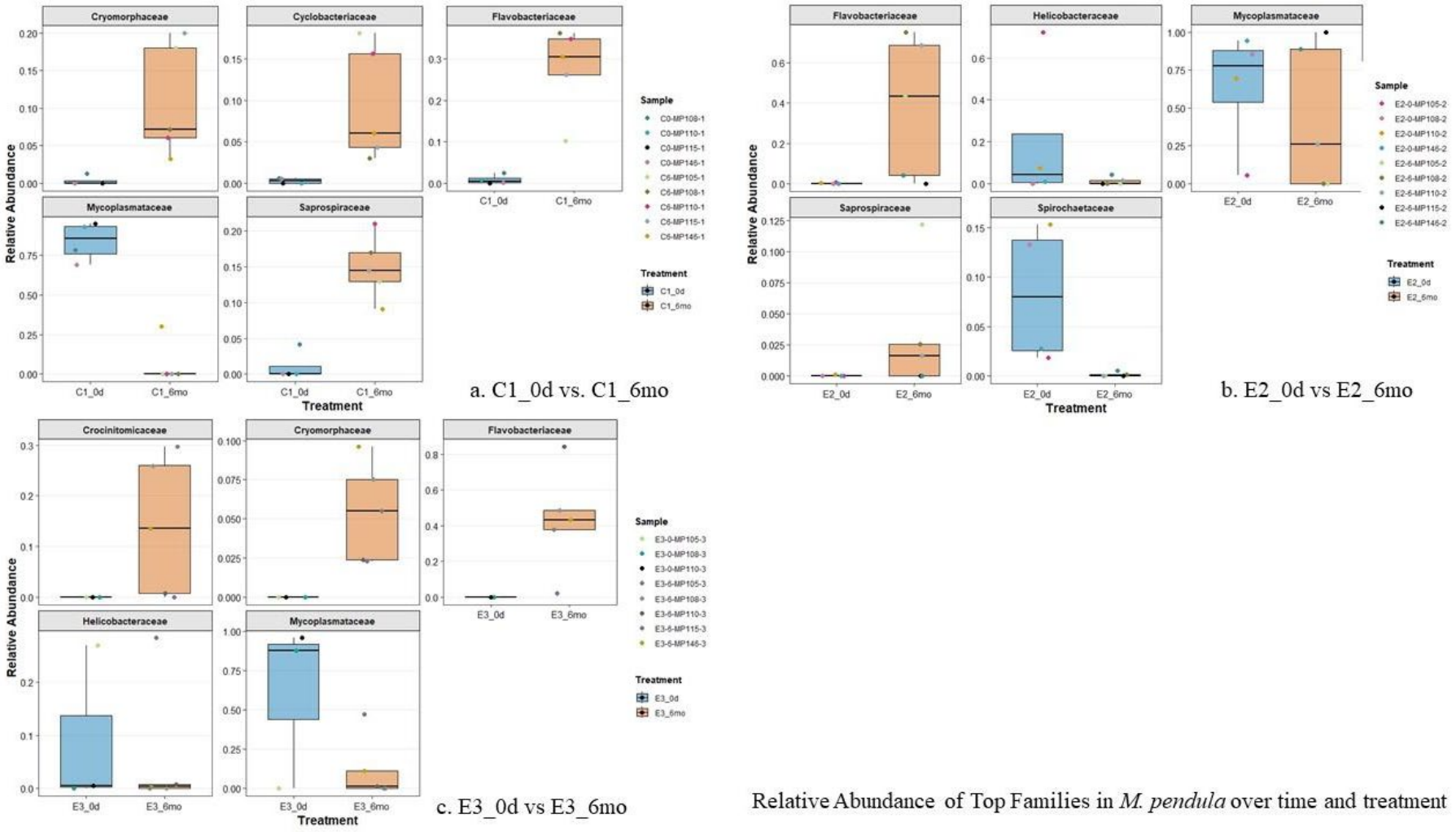


Figure 10.1. Relative abundance of top five families in *Muricea pendula* coral tissues over time and treatment and species. Most abundant bacterial families identified in *M. pendula* per treatment tank over time, initial 0d, and final 6mo in (a) C1 tank, (b) E2 tank, (c) E3 tank.

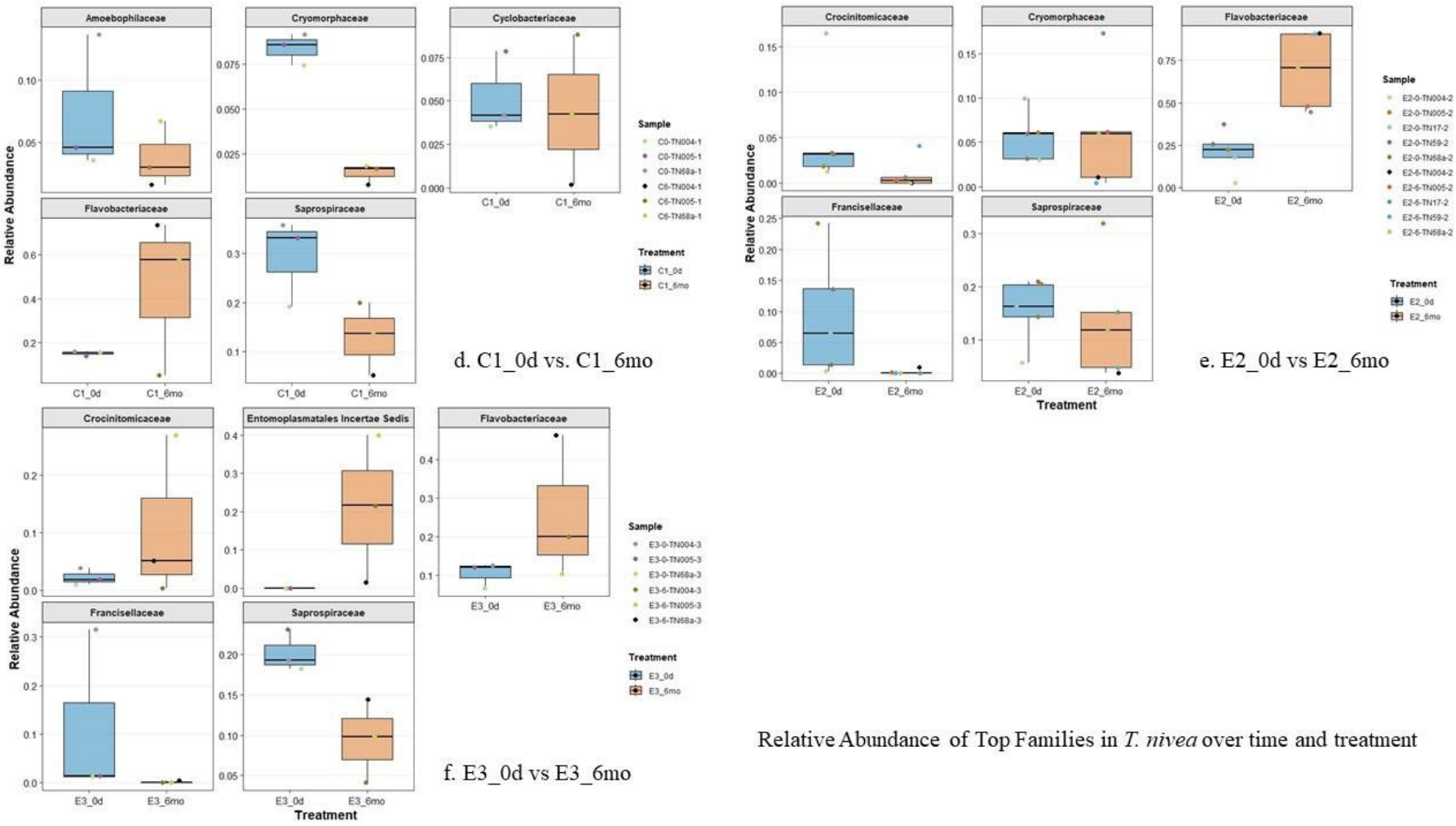


Figure 10.2 . Relative abundance of top five families in *Thesea nivea* coral tissues over time and treatment and species. Most abundant bacterial families identified in *T. nivea* per treatment tank over time, initial 0d, and final 6mo in initial 0d and final 6mo (d) C1 tank, (e) E2 tank, (f) E3 tank.

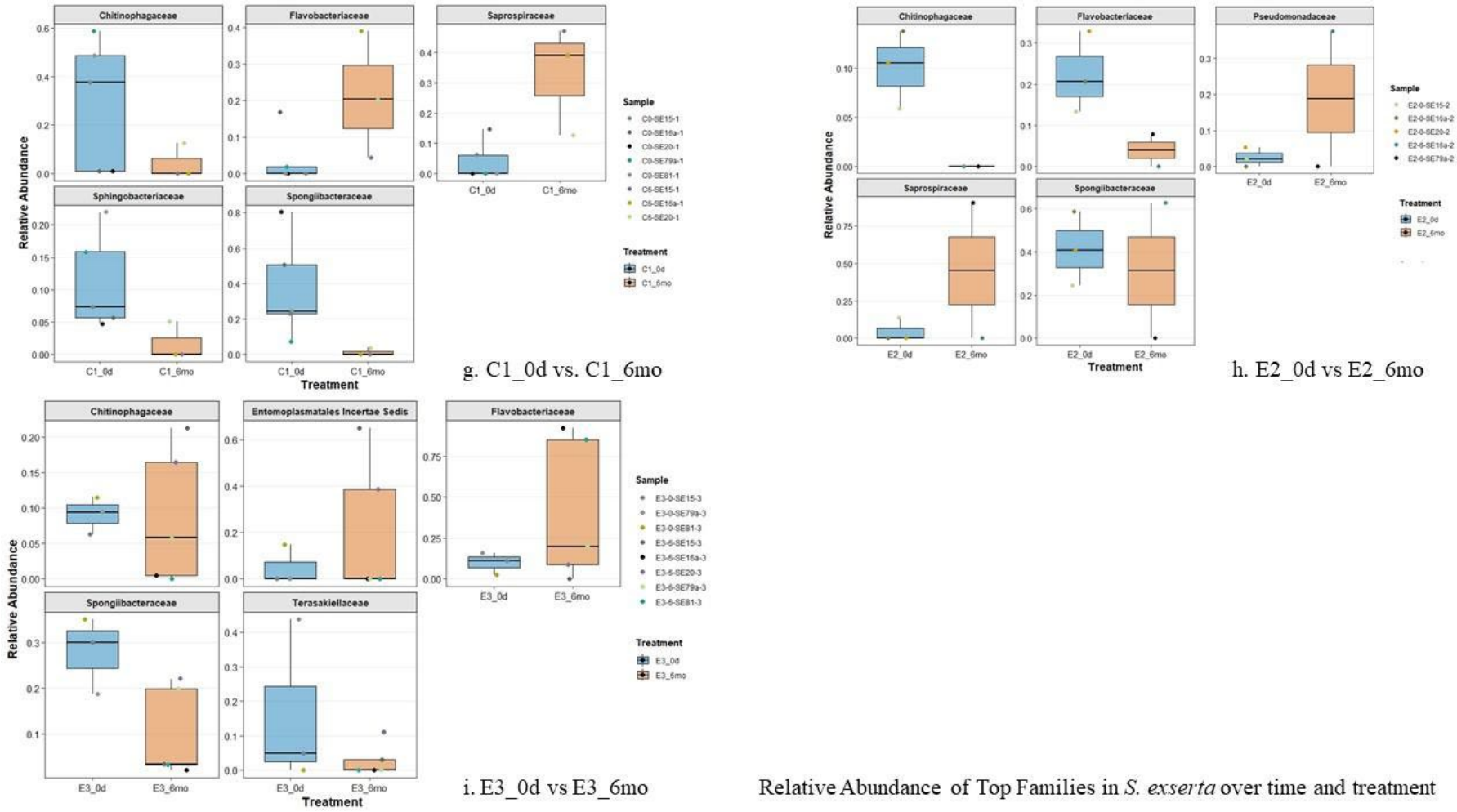


Figure 10.3 Relative abundance of top five families in *Swiftia exserta* coral tissues over time and treatment and species. Most abundant bacterial families identified in *S. exserta* per treatment tank over time, initial 0d, and final 6mo in (g) C1 tank, (h) E2 tank, and (i) E3 tank.

Table 4 details the top 20 most-abundant taxa that were statistically significant ($p < 0.05$) across all treatments within each coral species. In the bare rock treatment group, both pathogenic taxa and taxa associated with fighting infection were identified. This was observed in *M. pendula* corals and points to the challenges this species underwent to survive. In this treatment, only one taxon negatively influencing coral health was associated with *T. nivea*, Crocinitomicaceae (Glasl et al., 2016; Lu et al., 2025), found in coral mucus. The majority of taxa found associated with *S. exserta* in the bare rock treatment are correlated with immune response, breaking down organics, and algicidal activity. These results suggest that *S. exserta* was less affected by pathogenic bacteria.

Table 4. Top 20 log2 fold change coral tissue bacteria enriched at six months. The top significantly abundant coral bacterial families identified ($p < 0.05$) are indicated along with their descriptions and the total number of ASVs for each bacterial taxon per coral species for each experimental treatment. ASV = amplicon sequence variant.

Species	Family	Treatment	Coral ASV Count	Notes
M. pendula	Cryomorphaceae	(C) Bare Rock	2381	Possibly pathogenic (Woyke et al., 2011; Rosales et al., 2019)
	Cyclobacteriaceae	(C) Bare Rock	2187	Degrades polysaccharides, helps with nutrient cycling and breaking down of organic matter; roles in carbohydrate metabolism and antibiotic resistance (Pinnaka and Tanuku, 2014)
	Francisellaceae	(C) Bare Rock	682	Possibly pathogenic (Larson et al., 2016)
	Simkaniaceae	(C) Bare Rock	534	Found in juvenile coral tissues, considered to be an important energy source for host and overall holobiont health (Miller and Bentlage, 2024)
	NS9 marine group	(C) Bare Rock	241	Associated with diseased and lesioned corals (Apprill et al., 2013)
	Saprospiraceae	(C) Bare Rock	2318	Break down complex organic compounds (Lu et al., 2022); some species with algicidal activity (McIlroy and Nielsen, 2014; Furusawa et al., 2003)
	Flavobacteriaceae	(E2) TB	8521	Break down complex organic polymers, such as polysaccharides and proteins (WoRMS, 2025; Gavriilidou et al., 2020)
	Flavobacteriaceae	(E3) Meso	65810	Break down complex organic polymers, such as polysaccharides and proteins (WoRMS, 2025; Gavriilidou et al., 2020)
	Crocinitomicaceae	(E3) Meso	8660	Commonly found in marine environments and coral mucus-associated microbiomes; associated with macroalgae community and declining coral health (Glasl et al., 2016; Lu et al., 2025)
	Cryomorphaceae	(E3) Meso	4705	Possibly pathogenic (Rosales et al., 2019)
	Entomoplasmataceae	(E3) Meso	2685	Both are found in coral tissues only contributing to overall health; may involve evolutionary relationships (van de Water et al., 2018)
	Mycoplasmataceae	(E3) Meso	8700	

Species	Family	Treatment	Coral ASV Count	Notes
T. nivea	Crocinitomicaceae	(C) Bare Rock	361	Commonly found in marine environments and coral mucus-associated microbiomes; associated with macroalgae community and declining coral health (Glasl et al., 2016; Lu et al., 2025)
	Cryomorphaceae	(E2) TB	2434	Possibly pathogenic (Rosales et al., 2019)
	Flavobacteriaceae	(E2) TB	26418	Break down complex organic polymers, such as polysaccharides and proteins (WoRMS, 2025; Gavriilidou et al., 2020)
	Crocinitomicaceae	(E3) Meso	543	Commonly found in marine environments and coral mucus-associated microbiomes; associated with macroalgae community and declining coral health (Glasl et al., 2016; Lu et al., 2025)
	Cryomorphaceae	(E3) Meso	319	Possibly pathogenic (Rosales et al., 2019)
	Cyclobacteriaceae	(E3) Meso	199	Degrades polysaccharides; helps with nutrient cycling and breaking down of organic matter; roles in carbohydrate metabolism and antibiotic resistance (Pinnaka and Tanuku, 2014)
	Flavobacteriaceae	(E3) Meso	1630	Break down complex organic polymers, such as polysaccharides and proteins (WoRMS, 2025; Gavriilidou et al., 2020)
	Saprospiraceae	(E3) Meso	587	Break down complex organic compounds (Lu et al., 2022); some species with algicidal activity (McIlroy and Nielsen, 2014; Furusawa et al., 2003)
	Entomoplasmataceae	(E3) Meso	779	Immune response; recycling of carbon, nitrogen, and sulfur (van de Water et al., 2018; Tully et al., 1993)
	GWF2-29-10	(E3) Meso	40	Function unknown
	Halieaceae	(E3) Meso	61	Associated with macroalgae microbiomes (Pozas-Schacre et al., 2025; Li et al., 2023)
	Spongiibacteraceae	(E3) Meso	173	Associated with nitrogen fixation and sulfur metabolism (Rola et al., 2025)
S. exserta	Entomoplasmataceae	(C) Bare Rock	1106	Immune response, recycling of carbon, nitrogen, and sulfur (van de Water et al., 2018; Tully et al., 1993)
	Saprospiraceae	(C) Bare Rock	662	Break down complex organic compounds (Lu et al., 2022); some species with algicidal activity (McIlroy and Nielsen, 2014; Furusawa et al., 2003)
	Flavobacteriaceae	(C) Bare Rock	732	Break down complex organic polymers, such as polysaccharides and proteins (WoRMS, 2025; Gavriilidou et al., 2020)

Species	Family	Treatment	Coral ASV Count	Notes
S. exserta (continued)	Flavobacteriaceae	(E2) TB	25	Break down complex organic polymers, such as polysaccharides and proteins (WoRMS, 2025; Gavriilidou et al., 2020)
	Saprospiraceae	(E2) TB	284	Break down complex organic compounds (Lu et al., 2022); some species with algicidal activity (McIlroy and Nielsen, 2014; Furusawa et al., 2003)
	Flavobacteriaceae	(E3) Meso	1247	Break down complex organic polymers, such as polysaccharides and proteins (WoRMS, 2025; Gavriilidou et al., 2020)
	Saprospiraceae	(E3) Meso	19	Break down complex organic compounds (Lu et al., 2022); some species with algicidal activity (McIlroy and Nielsen, 2014; Furusawa et al., 2003)
	Chitinophagaceae	(E3) Meso	167	Associated with coral disease and stress (Mannochio-Russo et al., 2023)
	Pseudomonadaceae	(E3) Meso	5	Associated with coral bleaching and/or disease (Li et al., 2014)
	Spongiibacteraceae	(E3) Meso	93	Associated with nitrogen fixation and sulfur metabolism (Rola et al., 2025)

The shallow WFL Shelf rock treatment had fewer changing microbiome taxa associated with each coral host species. Of those identified as significantly present, Flavobacteriaceae were predominant and common to all three coral species. This group is documented to break down complex organics and proteins (WoRMS, 2025; Gavriilidou et al., 2020). Within the mesophotic MS-AL Shelf live rock treatment, a range of bacterial taxa was identified associating with all three coral species. Among *M. pendula* corals, both bacterial taxa related to disease and those that contributed to overall health were identified, as in the bare rock treatment. The seven most abundant microbial taxa found associated with *T. nivea* were positively involved in immune response, health of the corals, and breaking down algal cells. The most abundant microbial taxa found associated with *S. exserta* are characterized with both positive and negative coral health.

In order to evaluate and compare treatment groups as a whole, as well as to identify significant differences measured between all the replicates over time, a pairwise PERMANOVA was conducted. Collectively, no statistical differences were initially shown between the corals housed in each treatment tank at T=0, represented by $p > 0.05$ white squares (Figure 11a). Therefore, the three coral species were equally represented among each treatment type. However, when evaluated at six months, E2 and E3 treatments were significantly different from C1 ($p = 0.018$, $p = 0.003$, respectively). Significant differences were also shown between E2 and E3 corals at six months, with both treatments having values of $p = 0.016$. These data show that the microbiomes associated with corals in each rock treatment became different over time.

Considering the five coral replicates at initial and final time points within treatment groups (Figure 11b), the results become more complicated. Since all species had a dissimilar survival at six months, a large variation existed within the data, which reduced statistical power. This is consistent with data from visually scored health metrics; however, refinement of these comparisons, using amplicon data, indicated the importance of microbiome stability as a marker of coral resilience among species. *M. pendula* replicates showed a significance value of p = 0.0548 between live rock treatments compared to bare rock (p = 0.0548, graphed at 0.055 in Figure 11b). Though this is just outside significance of p = 0.05, the data suggest slightly better performance of this species under live rock treatments than without live rock present. *T. nivea* replicates also show p values = 0.0548, though in this case, the differences were shown between the two live rock treatments (p = 0.0548, graphed at 0.055 in Figure 11b).

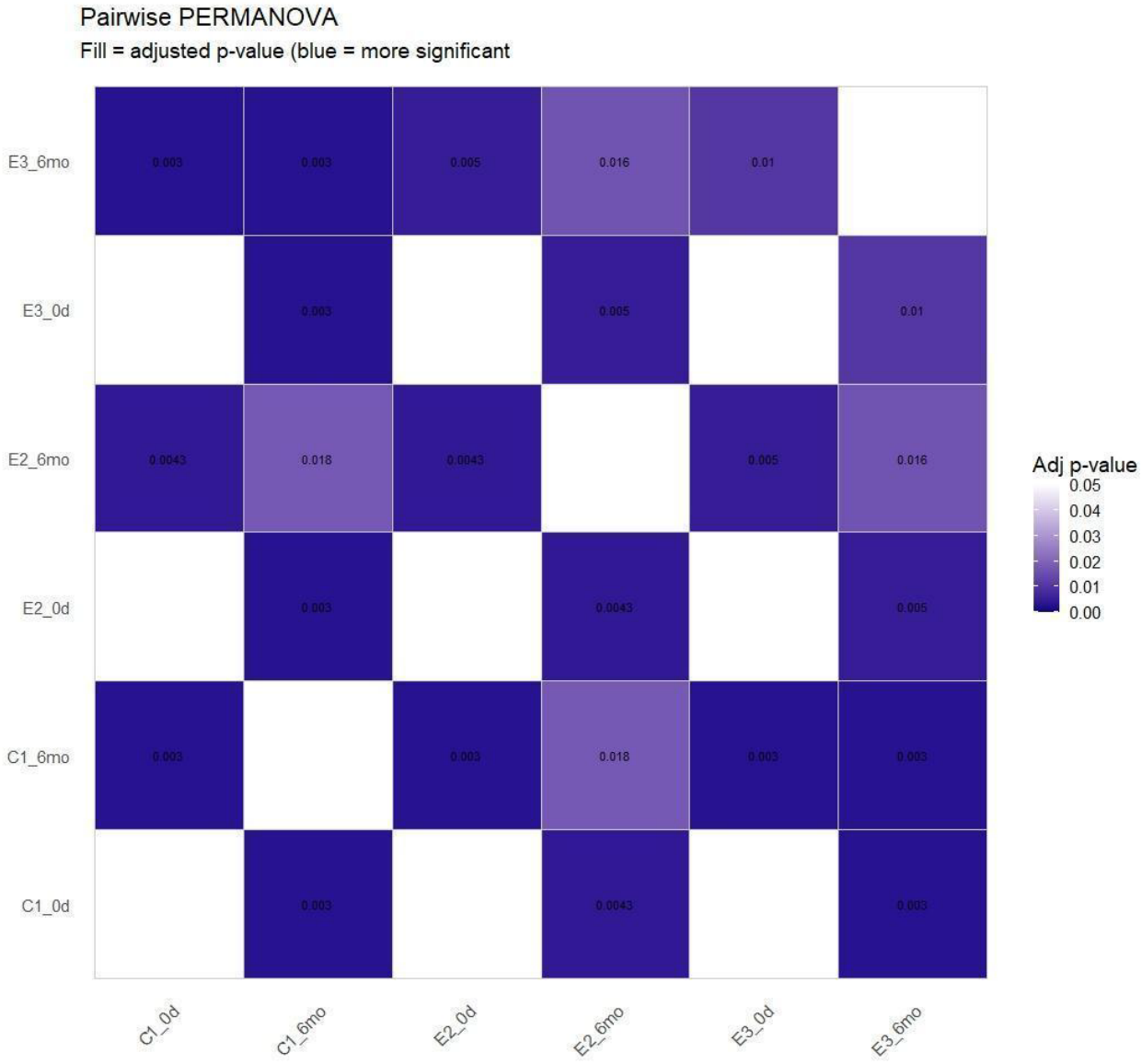


Figure 11a. Pairwise Adonis PERMANOVA heat map. Statistical significance was evaluated for each treatment tank (C1, E2, E3) holding all coral species (Tn = *T. nivea*, Mp = *M. pendula*, Se = *S. exserta*) over time (i = initial, 0d; f = final, 6mo). PERMANOVA annotated for adjusted p values displayed in each square, whereby a darker color represents higher significance (0.00). Nonsignificant cells are white.

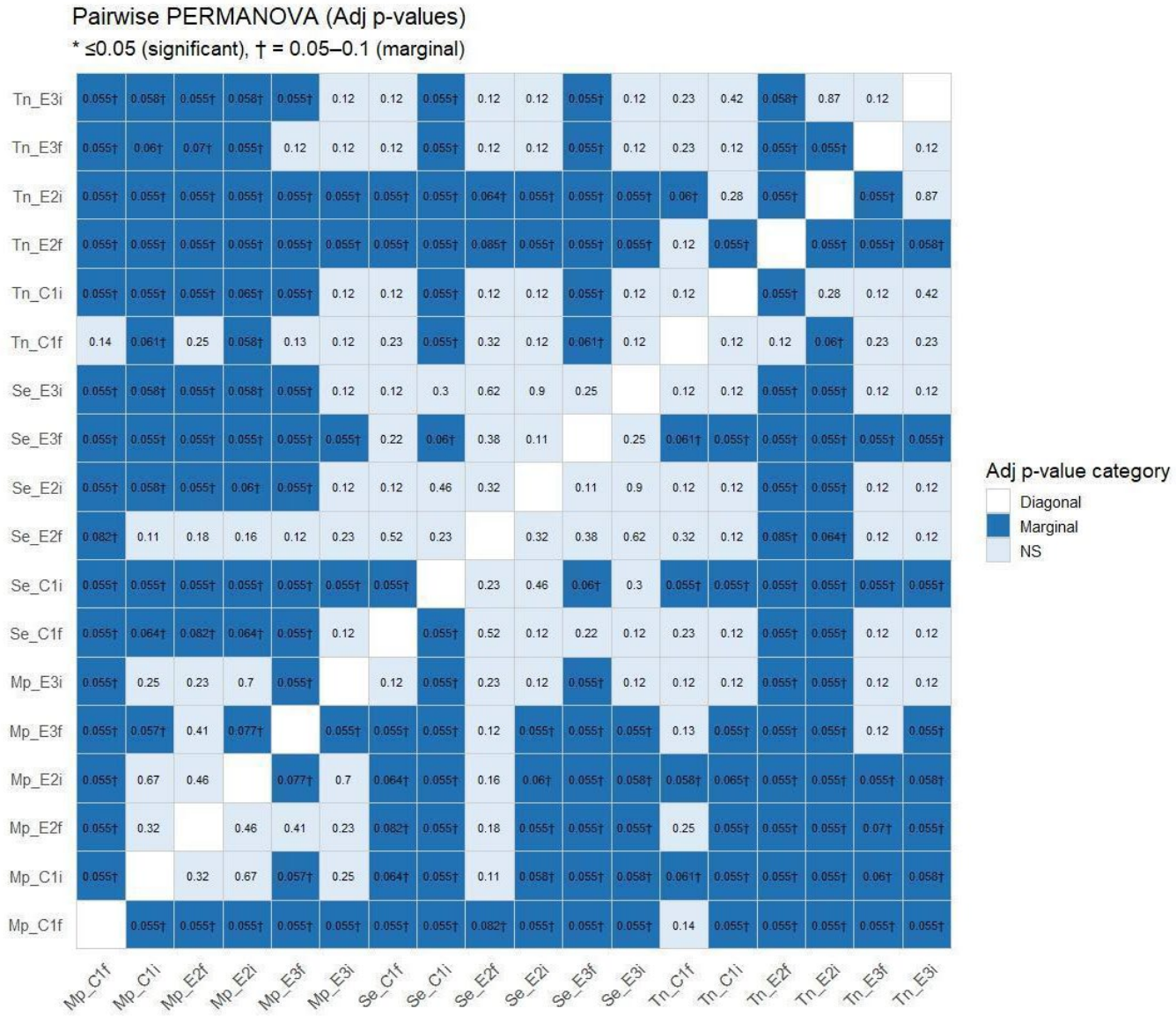
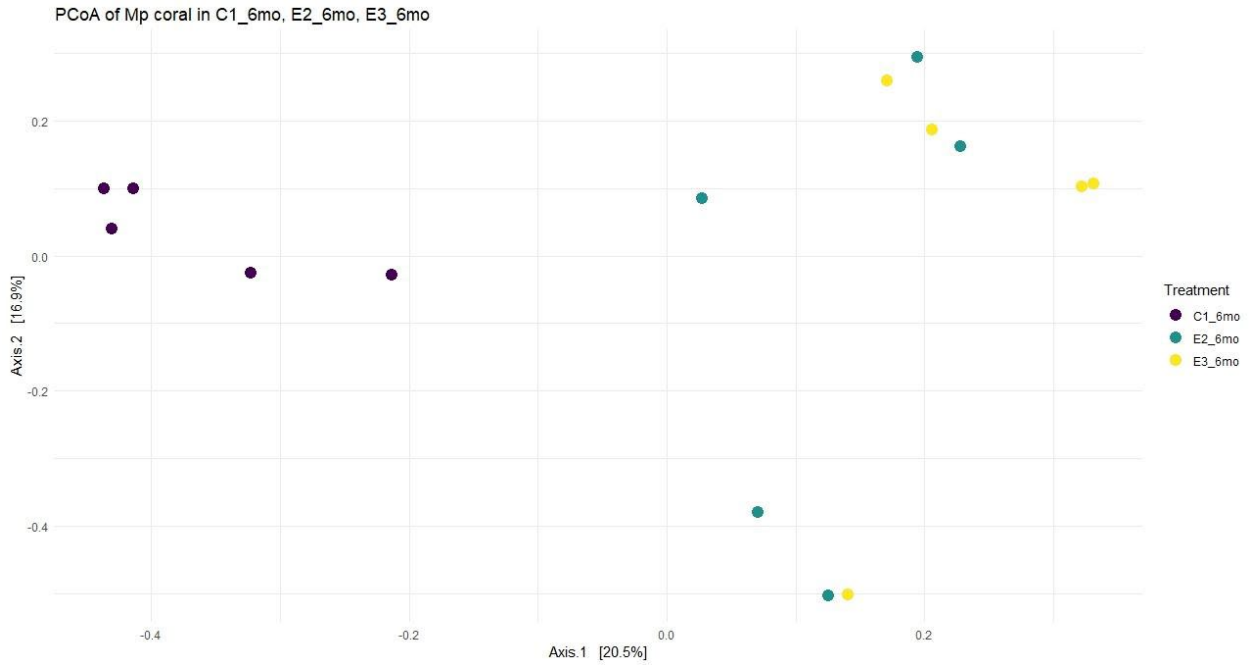
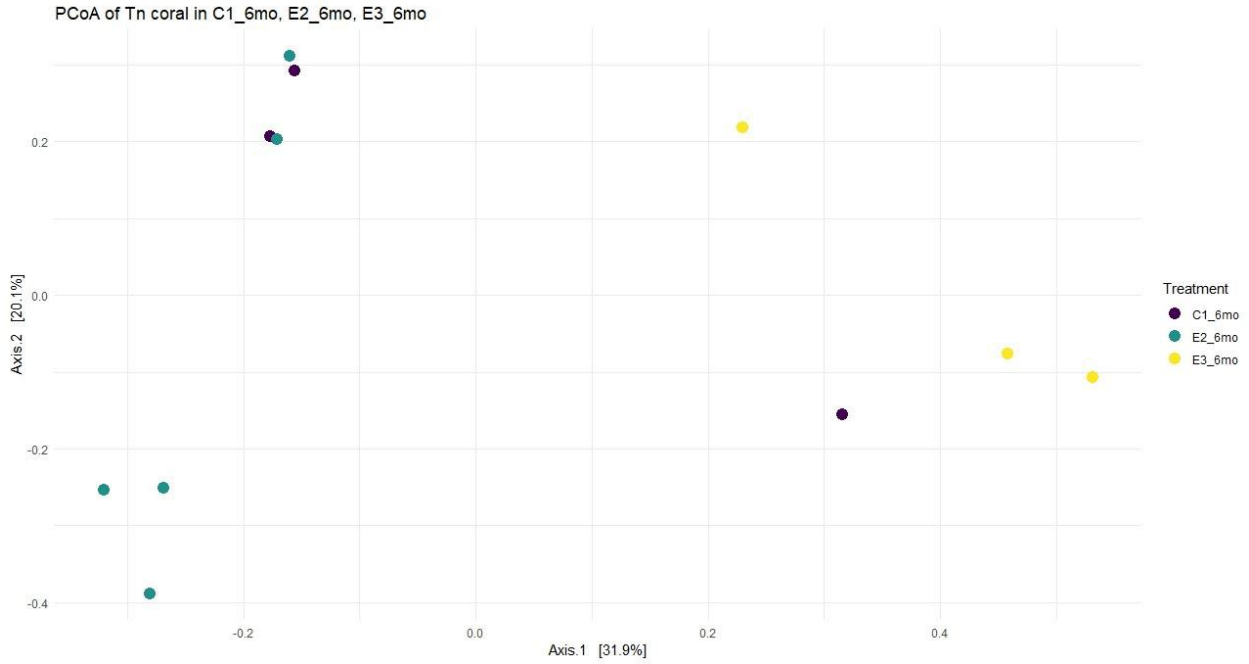


Figure 11b. Pairwise Adonis PERMANOVA heat map with coral species represented within each treatment. Statistical significance was evaluated for all coral replicate species (Tn = *T. nivea*, Mp = *M. pendula*, Se = *S. exserta*) within each treatment tank (C1, E2, E3) over time (i = initial, 0d; f = final, 6mo). PERMANOVA annotated for adjusted p values displayed in each square, whereby a darker color represents higher significance (0.00). Nonsignificant cells are white.

A PCoA using six-month data points of either *M. pendula* or *T. nivea* replicates was evaluated in order to clarify the pairwise PERMANOVA results further (Figure 12). *M. pendula* replicates (Figure 12a) show bare rock tank (C1) grouping together and separately from other treatment replicates. This grouping explains the marginal differences noted in the heat map between bare rock and live rock treatments. The *T. nivea* replicates (Figure 12b) have a single replicate from both the bare rock (C1) and mesophotic MS-AL Shelf rock (E3) treatments that are separate from the others, complicating the data. Among the *T. nivea* replicates, there is a tight grouping of three replicates among the E2. The close grouping between C1 and E2 coral replicates explains the non-significance identified between the two treatments. Contrary to *M. pendula* findings, there was no significance detected between the live rock treatments compared to bare rock in *T. nivea.* This large dispersion among C1 and E3 corals is most likely due to the mix of healthy and sick corals among all treatments.



(a)



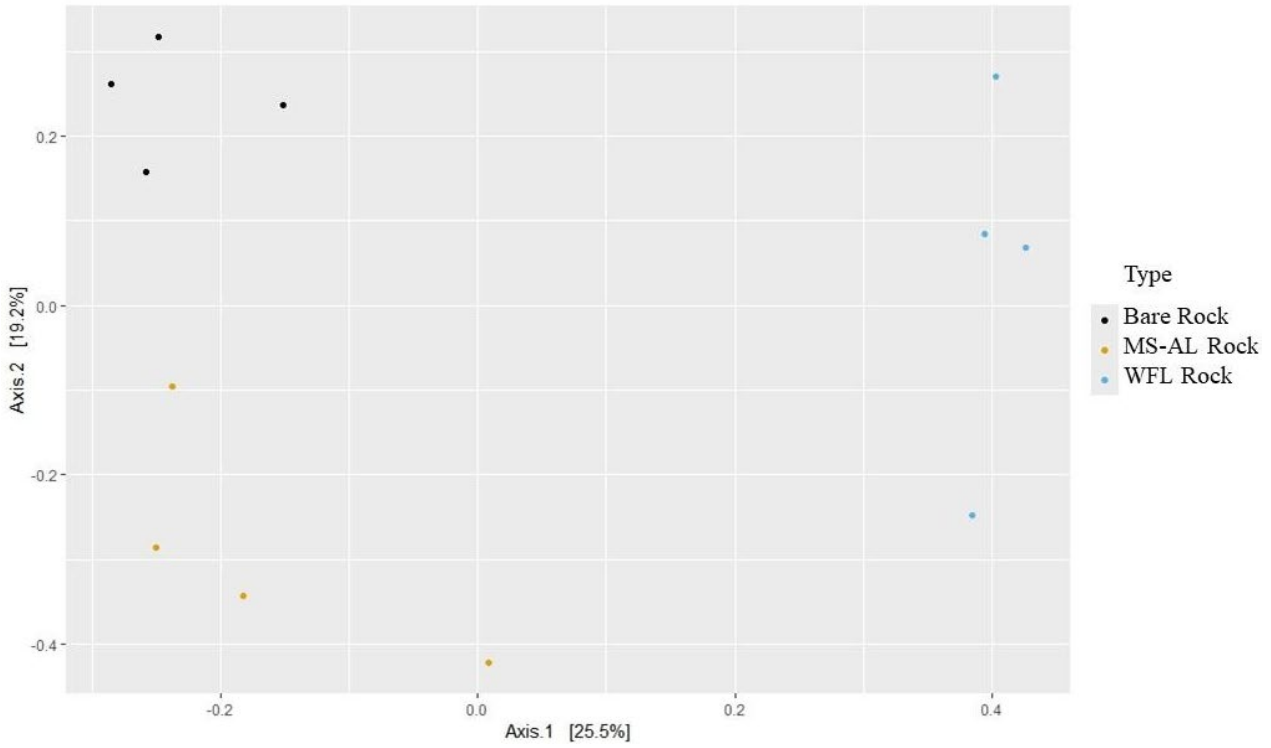
(b)

Figure 12. Principal coordinate analysis (PCoA) of both (a) *M. pendula* and (b) *T. nivea* within all treatments at six months. PCoA of each replicate among the three treatments, C1 (bare rock), E2 (shallow WFL Shelf rock), and E3 (mesophotic MS-AL Shelf rock) at six months.

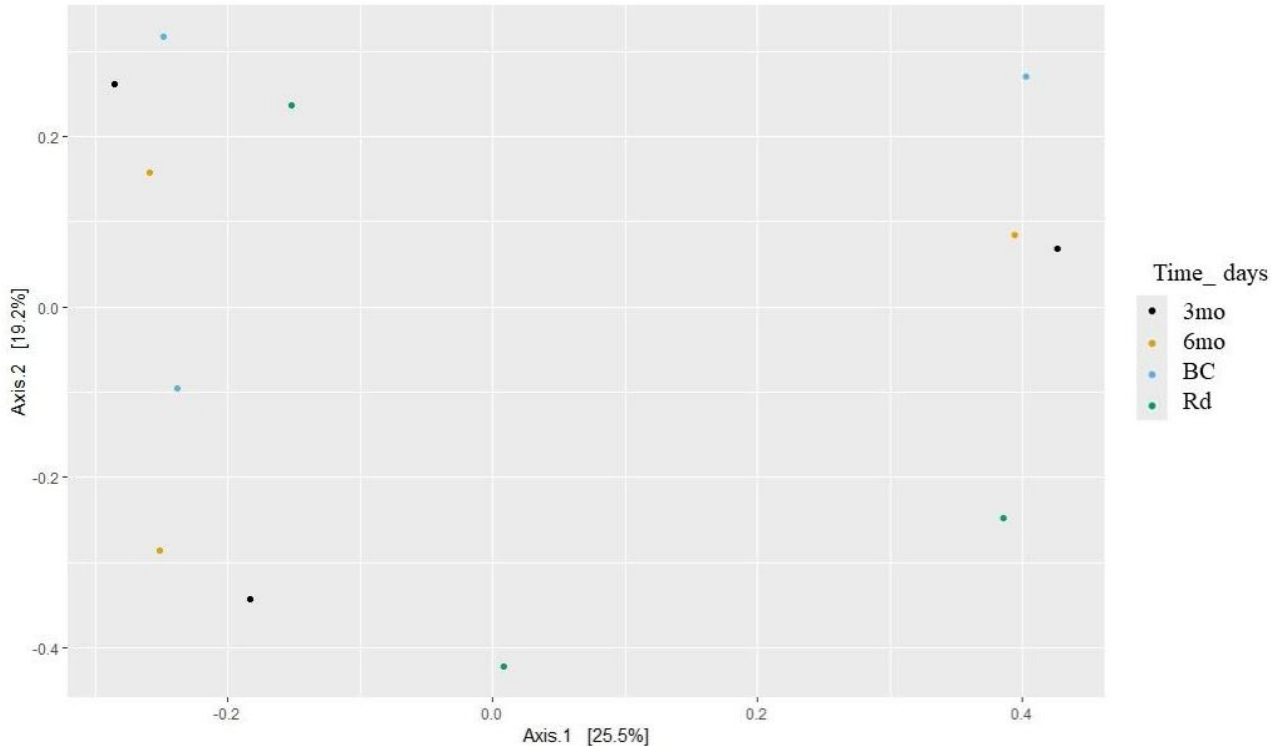
Since these sample results include a mix of healthy and sick corals by six months, results are confounded. All *M. pendula* replicates died in the bare rock treatment, with two replicates remaining in live rock treatments (#110 and #115). Metabarcoding results suggest *M. pendula* may be more susceptible to challenging environmental conditions and might benefit from the presence of live rock in the tanks. Additionally, anecdotal evidence supports the idea that *M. pendula* require lower temperatures and, due to smaller polyp sizes, may also require smaller food particle size. Future controlled experiments are required to confirm these hypotheses.

3.4.2 Rock Analysis

A PCoA of Bray–Curtis DESeq2 normalized data (Figure 13a) revealed each rock type clustered separately from each other, displaying distinct microbial community composition among treatments. Mesophotic MS-AL Shelf rock exhibited greater temporal change than the other two rock types, which clustered closer to one another over time (Figure 13b). The largest shifts in microbial communities occurred between the “Received” and “Before Corals” time points across all rock types, while the three- and six-month time points maintain similar proximity.



(a)



(b)

Figure 13. 16S rRNA V3-V4 gene region PCoA Bray–Curtis DESeq2 normalized plot comparing all rock treatments. Normalized 16S rRNA bacterial data were evaluated through Bray–Curtis dissimilarity metric within DESeq normalized data. Higher values on the x-axis indicate greater dissimilarity between the two time periods. (a) Relationship among the three rock types Adonis PERMANOVA significance measured at p = 0.0003. (b) Normalized DESeq data with time variable shows the three treatments: Bare Rock, MS-AL Rock, and WFL Rock at all time points three months (3mo), six months (6mo), before corals (BC), and received (Rd).

Alpha diversity metrics (Figure 14) illustrate shifts in microbial community richness and abundance over time. Shannon diversity estimates indicated a higher overall richness at time of receipt (Rd) in mesophotic MS-AL Shelf rocks compared to shallow WFL Shelf rocks. By six months, however, this relationship reversed, with the shallow WFL Shelf rock maintaining higher Shannon diversity. The initial differences likely reflect variation in the natural environments where these two rocks were collected (e.g., depth, light/region/associated fauna, as noted in Methods section 2.1) (Ricklefs, 2006). As expected, the bare rock exhibited minimal microbial diversity upon receipt and showed the largest proportional increase in diversity prior to coral addition. Differences and similarities between the two live rock types were plotted comparing family taxa numbers at receipt and six months (Figure 15a,b). The taxa in mesophotic MS-AL Shelf rock observed upon receipt gave insight into the native mesophotic environment. Specifically, the Fusobacteriaceae, which were more abundant in the mesophotic MS-AL Shelf rock, are known to be enriched in deeper, low-oxygen environments and have been reported in oiled sediment on the Gulf of America seafloor following the *Deepwater Horizon* (DWH) spill (Gutierrez et al., 2016).

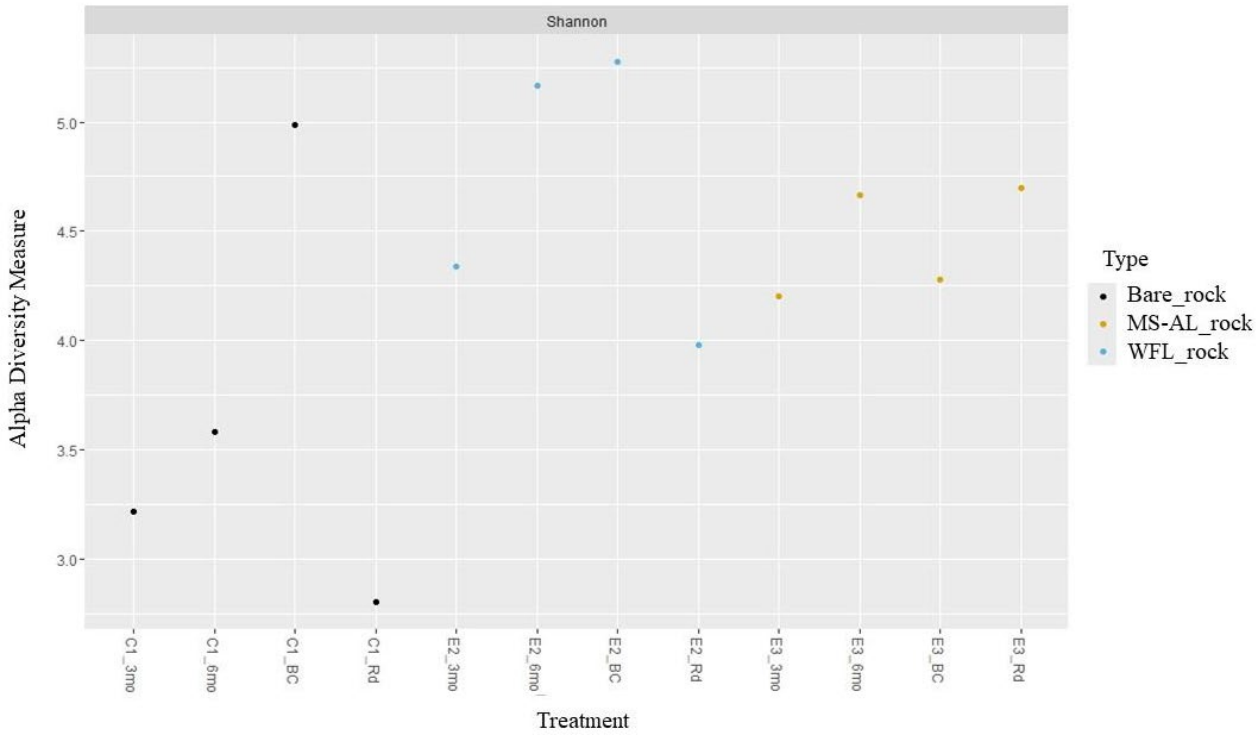
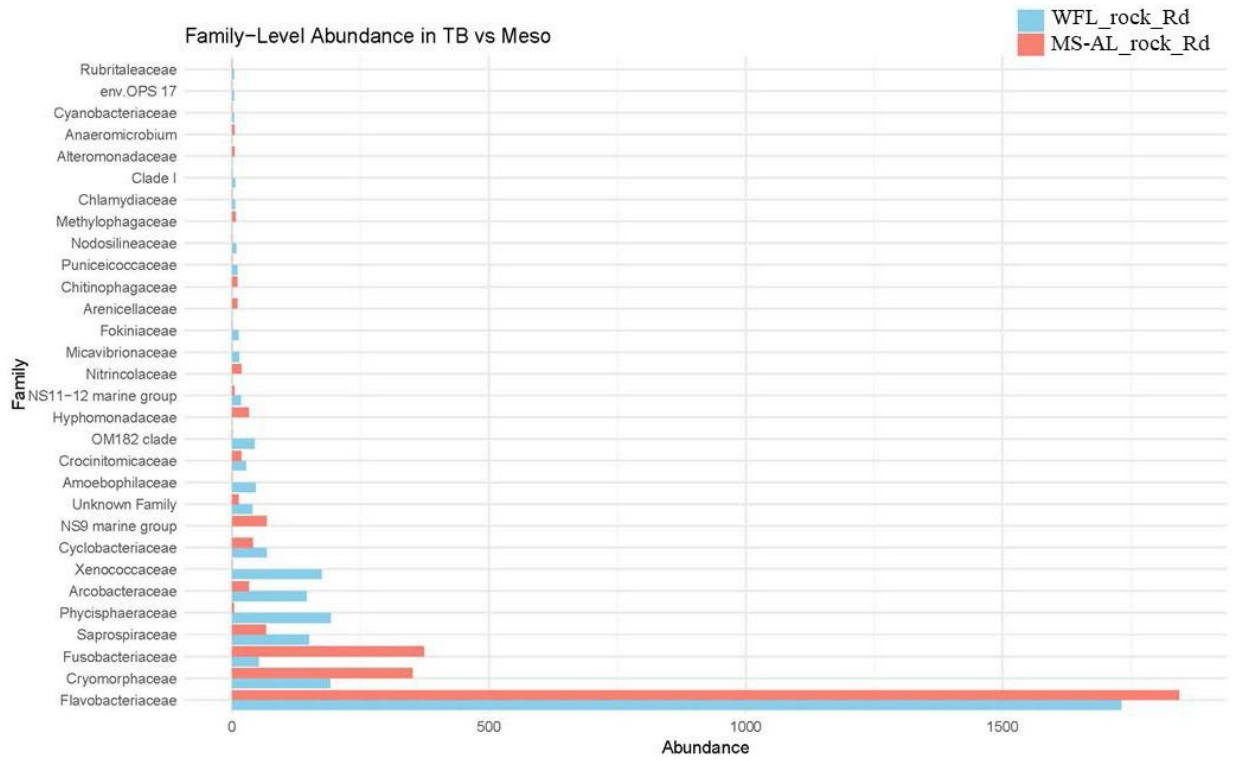
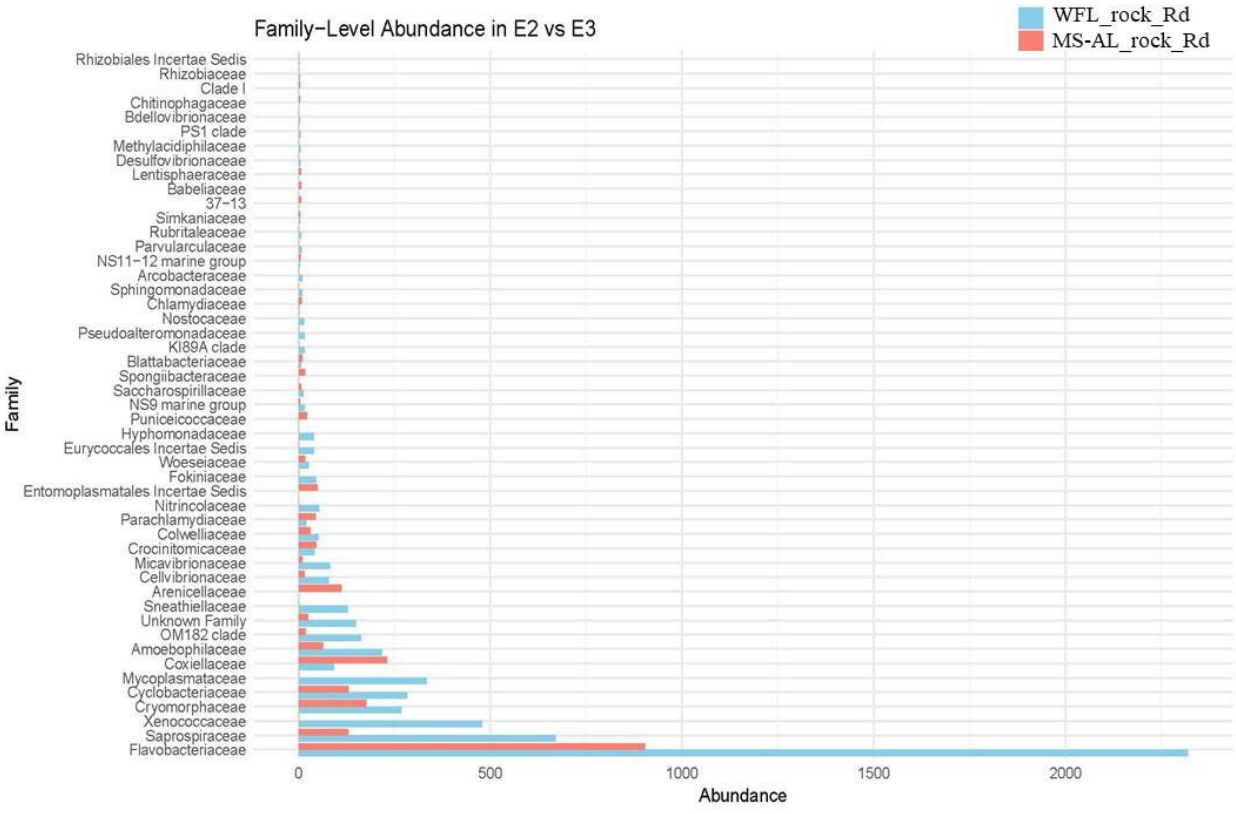


Figure 14. Alpha diversity of rock samples plotting Shannon diversity metrics. MS-AL = mesophotic Mississippi-Alabama Shelf rock, WFL = shallow West Florida Shelf rock; Treatments are labeled by rock type: C1 (bare rock), E2 (shallow WFL Shelf rock), and E3 (mesophotic MS-AL Shelf rock); and time point: three months (3mo), six months (6mo), before corals (BC), and received (Rd).



(a)



(b)

Figure 15. Taxonomic family abundances in shallow West Florida (WFL) Shelf rock compared to mesophotic Mississippi-Alabama Shelf rock (MS-AL) at two different time points for taxa values > 0 in abundance. (a) WFL and MS-AL rock family abundances when rocks were initially received. (b) WFL and MS-AL rock family abundances at the 6-month time point (6mo). WFL rock is represented in blue, and the MS-AL rock is represented in red for both graphs.

Cryomorphaceae, also associated with mesophotic MS-AL Shelf rock, are common among marine environments, have functional nitrogen-fixing cyanobacteria, and are associated with areas of high organic bioloads (Bowman, 2020). In contrast, Xenococcaceae, predominately observed in WFL Shelf rock, are commonly found in shallow-water environments, thrive in low light levels, and tolerate marginal oxygen levels (Sun et al., 2025). Similar conditions were observed within the experimental aquarium environment. Flavobacteriaceae, a family known for breaking down complex organic matter, were present in both live rock types. These compositional differences likely reflect the distinct depth and light environments of origin for the two live rock sources. Previous studies have shown that different crustose coralline algae (CCA) species can have unique associated microbial taxa among different populations. Several taxa identified in Table 5 were also reported by Quinlan et al. (2019) and were associated with high light levels and water flow, conditions not typical of mesophotic habitat, explaining their lower relative abundance in the mesophotic MS-AL Shelf rock. The MS-AL rock may therefore be more sensitive to aquarium conditions, as evidenced by a greater reduction in ASVs in the same bacterial family over time, whereas the WFL live rock may be more resilient to frequent shifts in water quality due to its shallow-water origin.

Table 5. Bacterial taxa and related number of ASVs identified specific to rock treatments only. Bacterial families with an asterisk (*) have been associated with the presence of crustose coralline algae. The other families were identified through relative abundance observations specifically occurring on the live rock in this study. The bolded Saprospiraceae is a family that contains some species of bacteria known to have algicidal activity. ASV = amplicon sequence variant; Rd = received; BC = before corals; 3mo = three months; 6mo = six months.

Bacterial Family	Sampled	Bare Rock/ASVs	West FL Shelf Rock/ASVs	MS-AL Rock/ASVs
*Cytophagales	Rd	3	7	40
	BC	0	5	54
	3mo	0	27	30
	6mo	48	96	28
*Cyanobacteriales	Rd	0	185	0
	BC	0	237	0
	3mo	0	66	0
	6mo	0	447	0
*Flavobacteriaceae	Rd	0	1,731	1,844
	BC	85	303	381
	3mo	5	790	193
	6mo	15	1,137	225
*Sphingobacteriales	Rd	0	20	5
	BC	0	4	4
	3mo	0	3	0
	6mo	0	4	5
Arcobacteraceae	Rd	0	145	32
	BC	0	0	12
	3mo	0	2	0
	6mo	0	9	0
Cryomorphaceae	Rd	0	191	351
	BC	0	0	0
	3mo	1	36	61
	6mo	108	21	26
Fusobacteriaceae	Rd	0	51	374
	BC	0	0	0
	3mo	0	0	0
	6mo	0	0	0
Saprospiraceae	Rd	0	149	66
	BC	0	36	4
	3mo	0	31	8
	6mo	0	434	22

Both live rock types were received with substantial coverage of CCA (Figure 16 b1, c1). While several CCA-specific bacterial taxa were identified (Table 5), four key CCA-associated groups, Cytophagales, Cyanobacteriales, Flavobacteriaceae, and Sphingobacteriales, were particularly prevalent in the WFL Shelf rock. Cyanobacteriales increased in ASV abundance by six months in WFL Shelf rock, a trend also visually supported by increased cyanobacteria at the phylum level (Figure 17). In contrast, mesophotic MS-AL Shelf live rock showed an overall decline in ASV richness by the end of the study. Visual observations of both live rocks show loss of color compared to the initial intake photographs (Figure 16 b/b1, c/c1). Despite this visual decline, amplicon sequence data showed both live rock types retained beneficial bacteria taxa involved in organic matter degradation and nutrient cycling. Consistent with coral tissue results, Saprospiraceae were present in both live rock treatments, potentially contributing to suppressed algal growth. The ASVs identified in this family included Aureispira and (formerly) Lewinella, which are known to degrade algae cells and complex organic matter (McIlroy and Nielsen, 2014). Lewinella was formerly part of Saprospiraceae but split into its own family Lewinellaceae (Hahnke et al., 2016). Saprospiraceae ASVs could not all be classified to the genus level; thus, their specific functional role remains unclear. In contrast to the live rock, Saprospiraceae were absent from the bare rock treatment, where extensive brown and green algae growth was observed on rocks, coral polyps, and coral bases by six months (Figures 3 and 16a).

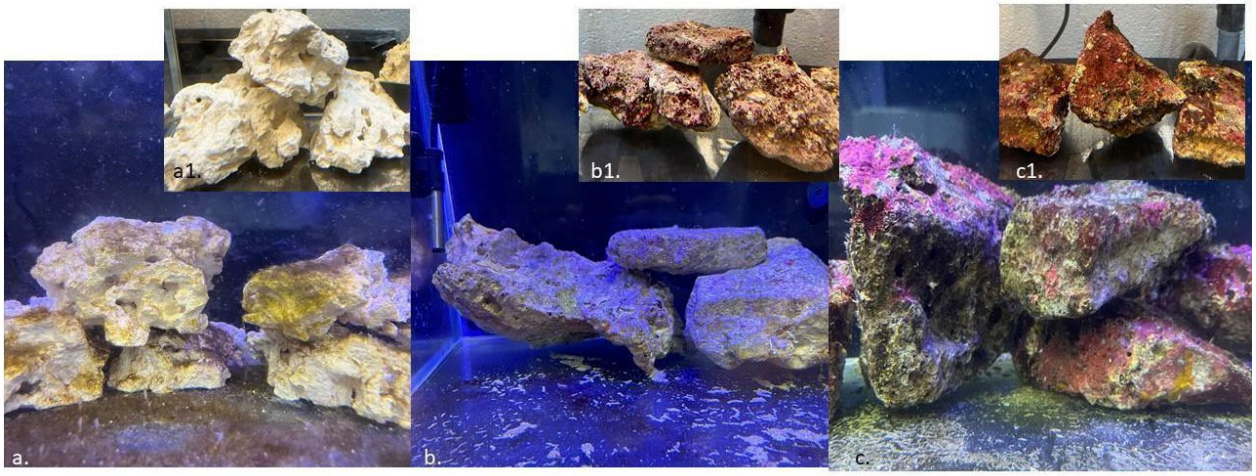


Figure 16. Live rock photographs taken at initial and final time points for all rock types. Bare rock (a1) at initial placement before addition of corals and (a) final picture taken at six months. Shallow West Florida Shelf rock (b1) at initial placement before addition of corals and (b) final picture taken at six months in blue light. Mesophotic Mississippi-Alabama Shelf rock (c1) is shown at initial placement before addition of corals and (c) at six months. Photo credit: Allisan Aquilina-Beck (CSS/NOAA).

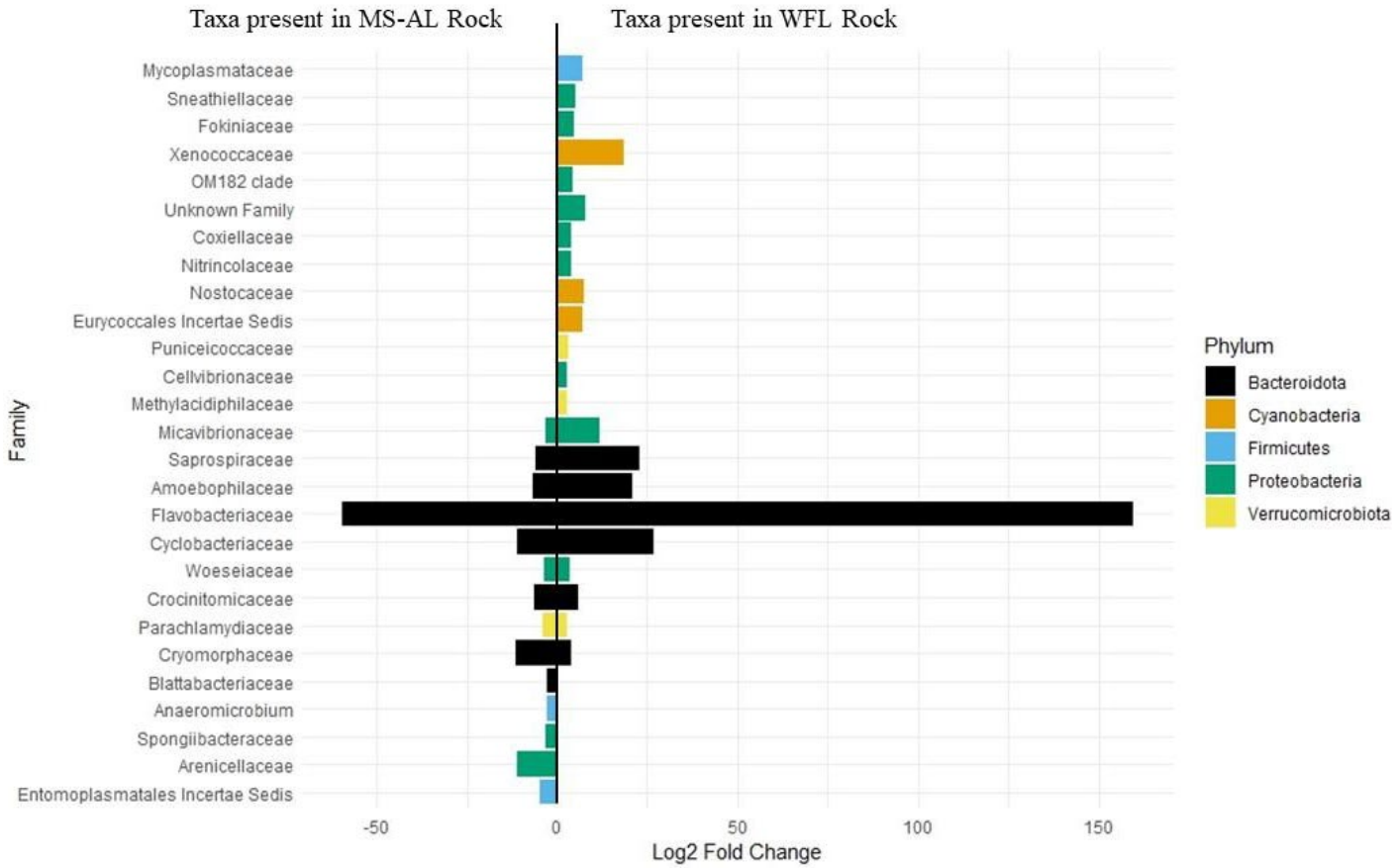


Figure 17. Differentially abundant taxa between shallow west Florida shelf rock compared with mesophotic Mississippi-Alabama Shelf rock across all time points. Graph represents significant taxa filtered where $p < 0.05$, for both mesophotic MS-AL Shelf rock and shallow WFL Shelf rock. Bacterial family is located on the y-axis, and the associated phylum taxonomy is indicated by color. Enhanced taxa in shallow WFL rock are indicated by values greater than 0.

4. Conclusions

The goal of this study was to determine the influence of the addition of different types of live rock on mesophotic coral health within closed-system aquaria. The initial three-month results indicated a positive effect with the inclusion of live rock among corals. This was especially seen in species such as *Muricea pendula* and *Thesea nivea*. Internal CPT project team laboratory protocols have been updated to specify the use of live rock within aquaria housing corals in closed systems. Some partner laboratories in the CPT project team network have already implemented these updated live rock protocols. There is consensus that live rock presence benefits mesophotic corals and that the ideal live rock is from the native mesophotic environment for long-term use. Commercial cultured rock from the shallow WFL Shelf has similarly been shown to provide long-term benefits in aquatic environments of warmer saltwater and bright light and is capable of conferring water quality benefits specifically to husbandry aquaria for CCA and mesophotic coral larvae growth (personal communication, December 3, 2025, USGS Wetland and Aquatic Research Center, Gainesville, Florida laboratory).

The study was compromised by the slow decline in water quality over time, which was not addressed through active intervention so as not to mask any positive effects of live rock inclusion. Future studies will graph conditions in real time in order to identify small changes and terminate the study before harmful decline occurs. Conditions that promote algal growth in closed-system aquaria often result from elevated nitrate concentrations, which can occur when filtration efficiency declines or feeding rates are excessive. Degradation of water quality can negatively affect the health and growth of marine invertebrates (Delbeek and Sprung, 1994; Grguric et al., 2005). Despite elevated nitrate levels across all treatments at six months, this study demonstrates that the presence of live rock provided measurable benefits, including suppression of algal overgrowth and slowed coral decline under stressful conditions.

At three months, corals exhibited visual signs of growth including increased polyp number compared to bare rock treatment. However, by six months, colorimetric assays revealed physiological stress, indicated by altered DNA methylation levels and reduced triglyceride levels. These qualitative metrics, applied here for the first time to mesophotic corals, gave objective measures of coral condition and may serve as early indicators of stress prior to visible decline.

The three species of corals, *M. pendula*, *T. nivea*, and *S. exserta*, displayed different tolerance levels to stress exposure. *M. pendula* exhibited the greatest decline, whereas *S. exserta* showed the highest survival across all treatments. Mesophotic octocorals are known to possess chemical defense mechanisms, which are attributed to highly pigmented tissues (Syamsi et al., 2025). The combination of the intensity of color and polyp number is an adaptation that could aid in metabolite production with changing environmental cues (Syamsi et al., 2025). *S. exserta* and *T. nivea* are both brightly colored (orange and purple, respectively), whereas the *M. pendula* used in this study were white, potentially contributing to their greater susceptibility to stress, particularly in the bare rock treatment. Additionally, *M. pendula* may require more specialized care overall. Morphologically, this species has a smaller polyp size than the other mesophotic species studied here, thus requiring smaller food particle size. Other recent anecdotal observations reveal lower aquarium temperatures between 20.6°C and 21.7°C assist in the survival of this species (personal observations by CPT aquarium staff).

Both *M. pendula* and *T. nivea* exhibited improved survival in live rock treatments compared to bare rock, with mesophotic MS-AL Shelf rock resulting in slightly better metrics than shallow WFL Shelf rock. *S. exserta* had the highest survival in all replicates and remained stable across all three treatments, suggesting greater

physiological resilience and adaptability. Together, these data indicate that live rock confers clear benefits to mesophotic octocorals, particularly for species more susceptible to stress.

Soft corals are known to produce antiviral, anti-inflammatory, cytotoxic, steroidal glycosides, including prostaglandins (Chen et al., 2016; Syamsi et al. 2025). Studying the presence of these properties specifically in species identified as injured due to the DWH oil spill (Etnoyer et al., 2016) could provide valuable insight into species-specific resilience mechanisms and inform restoration strategies.

Rock scrapings collected across all treatments revealed a temporary decline in taxa diversity at three months, followed by a partial increase by six months. Bacterial taxa associated with nutrient cycling and the breakdown of complex organic polysaccharides and proteins were present in both live rock types. The presence of a natural algicide, identified in both live rock treatments, further demonstrates the beneficial nature live rocks have in stabilizing aquarium conditions. The decrease in bacterial abundance in mesophotic MS-AL Shelf rock may reflect sensitivity to deteriorating water quality conditions during the latter stages of the experiment. Preliminary observations from non-experimental holding tanks suggest that microbial communities on live rock continue to shift under captive conditions, particularly affecting CCA-associated taxa (data not shown). Long-term monitoring is therefore essential, and presently ongoing, to understand how different live rock microbiomes change in captivity.

Overall, live rock presence improved coral survival and reduced algal accumulation, particularly during the first three months of the experiment. With refinements to fragment size and water quality control, cohabitation of mesophotic corals with live rock can yield positive outcomes (Table 6). While mesophotic rock is logistically challenging to obtain, the temperature and light conditions present in mesophotic coral husbandry aquaria are similar to natural conditions where the rocks are found. Commercially available live rock represents an alternative for captive coral husbandry, but continued long-term evaluation will be necessary to assess the persistence of beneficial microbial communities under specific mesophotic aquarium conditions.

Table 6. Summary chart of results and related recommendations. BR = bare rock.

Metrics Used	Anticipated Result	Actual Result	Conclusions	Experimental Recommendations
Water Quality	Stability in nitrates, low or 0 ammonia, pH = 8 in live rock tanks.	Spike in nitrates and ammonia, and a drop in dissolved oxygen occurred 5 weeks prior to the end of the experiment.	Corals were responding to and adversely affected by decreasing water quality in every treatment.	Use trusted filter equipment; condition experimental tanks similar to parameters used in holding tanks (e.g., add granular ferric oxide). Future studies should address coral species-specific preferences such as food particle size and temperature.
Visual: Polyp Number, Total Linear Extension, Thickness	Increase in growth, increase polyp count, sustained thickness.	Overall decrease in polyp number in the last three months across BR and West Florida Shelf rock tanks.	Corals were improving at three months in live rock tanks; however, any gains were lost by six months due to decrease in water quality. Minimum algae buildup observed in the live rock experimental tanks.	Start with all healthy corals, ideally freshly collected; fragment at a minimum size of 7.6 cm, incorporate artificial intelligence/machine learning to assess polyp counts and minimize human error.

Metrics Used	Anticipated Result	Actual Result	Conclusions	Experimental Recommendations
Triglyceride Lipoprotein Levels	Increased or similar amounts to initial snips.	All three species had statistically lower levels compared to initial snips.	Due to poor coral health at six months, no one species nor one treatment performed better.	Lipoprotein metric can be a quick, insightful measure of coral health but needs to be examined before severe visual decline in order to obtain changing energy levels.
DNA Methylation Levels	High levels, indicative of normal steady-state functions.	Variability existed in coral replicates; stress response measures were observed in live rock samples of *S. exserta* where decline was less obvious.	The species most affected by declining health, *T. nivea* and *M. pendula,* measured stable levels in live rock treatments.	This metric is useful in quickly assessing coral stress when corals seem visually stable. Assessments should be conducted before decline.
16S rRNA Gene Amplicon Survey Results	(Coral Tissue) Bacteria associated with healthy coral tissue; (Live Rock) Bacteria associated with breaking down organics/associated with increasing water quality (BR); less abundant bacteria associated with aiding in water quality	(Coral Tissue): confirmed and supported other metrics of decline at six months; (Live Rock): continuously changing microbiome throughout time; bacteria aiding in breakdown of organics and algae.	Live rock contains beneficial bacteria to combat algae growth and maintain water quality. Species-specific responses were apparent with *M. pendula*, which measured statistically similar in live rock vs. BR tanks. *T. nivea* suggested a difference in performance between the live rock treatments.	16S rRNA gene amplicon survey results supported the above conclusions and helped to explain complex relationships between species and treatments. It additionally improved understanding as to changing bacteria within coral tissue and rocks. Recommend to include this metric in future studies in order to examine changing bacterial community loads and identify pathogens.

Summary:

16S survey results helped explain relationships across species and treatments, suggesting corals in live rock treatments had statistically significant improvements in performance over bare rock treatments. Both live rock treatments maintained helpful bacteria, with shallow WFL Shelf rock containing more ASVs related to crustose coralline algae (CCA) species despite visual loss of color at six months. However, long-term holding of live rocks in controlled conditions suggests mesophotic MS-AL Shelf rock retains more CCA and helpful bacteria with constant low light and low temperature (data not included in this study).

Adapting lessons learned from this experiment, any further experimentation should include: using tested and reliable filtration units, maintaining stable tank water conditioning, ≥ three replicate tanks per treatment; two treatments such as an inert glass block and mesophotic rock; ≥ eight replicate healthy corals at 7.6-cm initial length; one or two white or lightly colored coral species, running a duration of four months or prior to visual decline; and using the same four metrics (water quality, visual, colorimetric assays, and 16S rRNA gene amplicon surveys) to assess treatment effects on coral health.

Data Accessibility

Summary values and code from this analysis can be downloaded from GitHub:

https://github.com/NOAA-Key-Species-and-Bioinformatics/Evaluating-the-Use-of-Live-Rock-in-Closed-System-Aquaria-with-Mesophotic-Corals

Raw FASTQ files and metadata are available on NCBI:

http://www.ncbi.nlm.nih.gov/bioproject/1477137

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