Harnessing Coral Resilience through Nursery-to-Reef Restoration Processes

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Final Report

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Management Summary

The aim of this study was to identify coral traits indicative of resistance and/or resilience to environmental conditions through the evaluation of coral microbiomes and metabolomics during collection, propagation, and grow-out within an in-water coral nursery. Survival analysis found genotypic specific differences in survival over the course of this project. In addition, corals originally collected from West Turtle in the middle Florida Keys experienced the lowest survival. Smaller colonies of *C. natans* had greater survival than larger corals, however most of the large corals that experienced mortality were collected from West Turtle.

Microbiome analysis indicated that similar microbial communities were present in wild *C. natans* and *D. labyrinthiformis* regardless of collection location and significant changes in these communities were not detected after two months in the nursery. Instead, the microbiome appeared to be genotype specific and the microbial community varied over time. Metabolomic analysis found distinct differences between corals before and after two months in the nursery. Coral metabolomes from Pickles Patch in the upper Florida Keys differed from corals collected further south on the reef tract, but after two months in the nursery a significant shift in the metabolomes of West Turtle corals was observed relative to corals from other locations. These results suggest a potential link between coral survival and metabolomic traits. Investigation into specific features of the metabolome that shifted is ongoing to determine their possible influence on survival, resistance, and/or resilience to environmental stressors.

Given the potential application of microbiome and metabolome analysis in nursery-to-reef restoration processes and the general paucity of quantitative health metrics available for the majority of the threatened stony coral species propagated for restoration, we recommend consideration for these tools in the development of region-wide strategies for the restoration of FCR.

Executive Summary

The summer of 2023 highlighted the need to scale up the production and diversity of boulder corals kept in coral nurseries through the identification and propagation of resistant and/or resilient coral species and genotypes to mitigate losses from future environmental disturbances. To this end, *Colpophyllia natans* and Diploria labyrinthiformis were collected from nearshore reefs in the upper, middle, and lower Florida Keys. Each coral was then sampled for microbiome and metabolome analysis, fragmented, and placed in FWC's in-water coral nursery. Sampling was repeated after two months in the nursery to assess changes in the microbiome and metabolome due to translocation. While in the nursery, corals were monitored routinely to assess survival and note overall health. Survival analyses revealed genotype specific differences with the lowest survival observed in both species collected from West Turtle in the middle Florida Keys. Survival of *C. natans* was influenced by the size of coral collected, with larger corals exhibiting higher mortality. This was reflected in both linear regression analysis and survival curves. However, five of the six largest *C. natans* corals were collected from West Turtle, precluding the interpretation of this relationship due to interaction effects and unknown confounding factors. Coral size did not have a significant effect on survival trends of *D. labyrinthiformis*.

Microbiome analysis identified similar microbial communities in *C. natans* and *D. labyrinthiformis* that did not vary greatly over time or across collection locations, and less than 2% of the variation in microbial communities was explained by coral species or timepoint. Microbial community differences were influenced primarily by coral specimen, and 23% of the community variation was explained by the interaction between genotype and sampling timepoint. Changes in specific microbial families due to translocation were detected, with generally decreasing ammonia-oxidizing archaea and increasing anaerobes observed in nursery corals.

Similar trends resulted from metabolome analysis of *C. natans* and *D. labyrinthiformis* and indicated a shift in the metabolome over time and after translocation, with less variability between the metabolomes of corals collected across the upper, middle and lower Florida Keys relative to propagated corals after two months in the nursery. This was also reflected in Shannon diversity indices where statistically significant differences in metabolomes between both species and timepoint were observed. Metabolome diversity was greater in *D. labyrinthiformis* than *C. natans*, and increased diversity was found in nursery corals than the wild collected colonies.

Analyses are ongoing to identify specific biological pathways perturbed by the translocation of these species to an in-water nursery. Preliminary findings suggest a distinct change in carnitine after translocation. PCAs showed significant location-based differences in metabolomes for both species. Corals collected from Pickles Patch had unique metabolome communities relative to West Turtle and Looe, but after two months in the nursery changes in the metabolome of West Turtle corals differed from corals originating from the other sites. This shift in the metabolome of West Turtle corals combined with the higher mortality observed in corals from this location suggest the potential role of metabolites in coral survival and warrant further investigation to determine their utility as predictors of health for selective coral propagation.

This study highlighted some of the challenges in identifying resilient corals for use in restoration while applying novel tools to investigate how coral propagation processes influence microbial and metabolite communities of two threatened stony coral species. These data may help us to better differentiate healthy coral genotypes from those with higher probability of mortality. However, further analysis into the role of specific microbes and metabolites in survival is needed, in addition to baseline data for other stony coral species used by practitioners, to evaluate the utility of these tools in improving restoration success.

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1. BACKGROUND

In the summer of 2023, Disturbance Response Monitoring surveys revealed that greater than 70% of the reefs across the Florida Keys experienced severe bleaching (>50% bleached) with bleaching prevalence varying between species (Stein and Ruzicka 2023). Many reef-building species (*Colpophyllia natans, Montastraea cavernosa, Orbicella* spp.) experienced higher levels of bleaching but rates of mortality varied (Stein *et al.* 2024). *C. natans* reported >55% bleaching prevalence but <10% decrease in abundance (Stein *et al.* 2024). *M. cavernosa* experienced >60% bleaching but 1% mortality (Stein *et al.* 2024). Of the reef building corals, *Orbicella* spp. experienced the highest rate of bleaching (>80%) and mortality (>15%) (Stein *et al.* 2024). These relatively low mortality rates suggested a level of resilience in coral individuals as has been shown in other coral populations (McCarthy *et al.,* 2024).

Florida Fish and Wildlife Conservation Commission's (FWC) Coral Nursery experienced significant mortality following the summer 2023 bleaching event and responded by focusing its coral husbandry efforts on identifying and propagating presumably resistant and/or resilient boulder coral species and genotypes to mitigate losses from future environmental disturbances. This project focused on *C. natans* and *D. labyrinthiformis,* two reef-building species that are of particular importance due to their susceptibility to stony coral tissue loss disease (SCTLD) and subsequent losses on coral reefs (Alvarez-Filip *et al.*, 2022; Toth *et al.*, 2023). Corals were collected from reefs across the upper, middle, and lower Florida Keys with the aim of collecting and propagating corals shown to be resilient in the face of disease and warming

ocean temperatures.

As technology progresses, new methods are being used to characterize the response of corals to a variety of stressors. Many of these have been used to examine disease, specifically characterizing how corals respond to SCTLD. The response of the coral holobiont to disease has been examined using a variety of methods including genotyping, proteomics, transcriptomics, metabolomics, and microbiome analysis with differences found between apparently healthy and diseased corals (Deutsch *et al.*, 2021; Brown *et al.*, 2022; Traylor-Knowles *et al.*, 2022; Schul *et al.*, 2023). These methods can be used to develop disease biomarkers and for diagnostic purposes as they have for human medicine. These tools are also being used to characterize corals and discover the mechanisms underlying how they may respond to other stressors (Rubin *et al.*, 2021). Due to the novelty of these methods in their application to corals, specifically proteomics, transcriptomics, and metabolomics, there is a fundamental need for these data from a diversity of species to establish baselines for "healthy" corals (Traylor-Knowles *et al.*, 2022).

The microbiome associated with a coral has been shown to change based on coral species with some shifting with different environmental conditions while others remain the same (Voolstra and Ziegler, 2020; Strudwick *et al.*, 2022). Of particular importance for this study, some species of corals have been shown to shift their microbiome when being moved from the source location to a coral nursery and shifting yet again when outplanted (Strudwick *et al.*, 2022, 2023). This is important when targeting corals for restoration as the microbiome has been shown to play an important role in pathogen defense, metabolic cycling, and thermal tolerance (Voolstra and Ziegler, 2020). Additionally, the metabolome has been linked to factors including the microbiome, genotype, and current and past environmental conditions (Traylor-Knowles *et al.*, 2022). It is this ever-shifting nature of coral characteristics in combination with restoration efforts increasing worldwide that makes it vitally important to understand how the translocation of a source coral to an in-water nursery and then to outplant sites affects the coral holobiont and the resilience and resistance that comes with it.

Results from this study will aid restoration practitioners by examining temporal changes in microbial and metabolomic characteristics of healthy corals relative to coral genotype, source, and nursery environmental parameters. Understanding the microbiomes and metabolomes of *C. natans* and *D. labyrinthiformis* and how they shift based on a variety of factors is a necessary step for refining methods for restoration success, particularly given the challenges Florida's Coral Reef faces with the chronic persistence of SCTLD and extreme temperatures. This study has the capacity to establish a baseline of a "healthy" coral which is crucial to understanding how these characteristics may change in a diseased or otherwise unhealthy coral.

2. METHODS

2.1. In-water collection and grow-out

Corals collected for this experiment came from corals of opportunity (COOs) found unsecured at three different inshore patch reefs encompassing the upper, middle and lower Florida Keys. Five individuals each of *Colpophyllia natans* and *Diploria labyrinthiformis* were collected. Corals were collected and measured (length x width x height) to the nearest cm,

percent living tissue was recorded, and coral were cleaned before transport. This cleaning process involved removing dead portions of skeleton and any non-coral organisms (sponges, octocorals, etc.) using a hammer and chisel. Corals were transported in damp bubble wrap to Keys Marine Laboratory (KML) where they were immediately placed in seawater tanks.

Within 24 hours of collection, samples were taken for microbiome and metabolome analysis. Corals were handled with gloved hands throughout the sampling process at KML. Three locations on each coral >5 cm apart were chosen for the tissue sampling. Using a Gryphon diamond bandsaw, three 2 cm^2 tissue samples were collected from each genotype for metabolome analysis and three 1 cm^2 samples from each genotype were collected for microbiome analysis. The 2 cm^2 and 1 cm^2 tissue samples were each taken from the same location on the coral to obtain matched microbiome and metabolome samples. Sampling locations were predominately along the edges of the corals due to constraints with the height of the bandsaw. As much skeleton as possible was removed from each sample without bottoming out the polyps. The bandsaw was cleaned thoroughly with ethanol between each genotype to reduce cross-contamination. After sampling, tissue pieces were stored in individual sterile containers (Whirl-Pak® sampling bags) in FWC's -80 °C freezer before shipment.

After initial samples were collected, all corals were fragmented and mounted on cement pucks. These pucks were transported to FWC's in-water coral nursery where they were routinely monitored to track genotypic differences in growth and survival. After 2 months in the coral nursery, 3 pucks from each genotype were collected. Only pucks that were visually healthy (i.e. no active tissue loss or discoloration) were selected for this sampling. Each genotype was transported in water in individual bags to FWC's South Florida Regional Laboratory. Each puck was divided in two using a Gryphon diamond bandsaw to provide a sample for microbiome analysis (~1 cm^2) and a sample for metabolome analysis (~2 cm^2). Each sample was placed in labeled Whirl-Pak® sampling bags and stored in FWC's -80 °C freezer. The bandsaw was cleaned with ethanol between each genotype to reduce cross-contamination and gloves were worn throughout sampling and switched between genotypes.

All microbiome samples were shipped on dry ice to the University of Florida (UF) for processing and analysis. All metabolome samples were shipped on dry ice to the Smithsonian Marine Station (SMS) for processing and the resulting extracts were sent to Georgia Institute of Technology (GT).

After propagation, coral fragments were translocated to FWC's Coral Nursery by FWC vessels and staff. Coral genotype groups were installed onto coral trees with multiple genotypes per tree. Corals were routinely monitored for a variety of conditions including overall growth and survival, disease, predation, and bleaching. Environmental parameters within the nursery were monitored throughout the duration of this project. Survival of each genotype was analyzed in R using Kaplan-Meier Survival Curves and analyzed using a Cox proportional hazards regression model.

2.2. Microbiome analysis

At the University of Florida, the microbiomes of *C. natans* and *D. labyrinthiformis* were characterized by sequencing the V4 region of the 16S rRNA gene on an Illumina MiSeq with paired 150-bp reads using the standardized Earth Microbiome Project protocol (Caporaso *et al.*, 2016) for 16S rRNA gene libraries, a well-established method used by the Meyer lab (Meyer *et al.*, 2016a, Meyer *et al.*, 2016b, Meyer *et al.*, 2019). All PCR was performed with no-template controls (negative controls). Only samples with un-contaminated negative controls were sequenced. In addition, extraction blanks were sequenced with unique barcodes during the preparation of 16S rRNA gene libraries. The efficiency of each sequencing run was assessed by the proportion of reads identified as having the submitted barcodes. The quality of sequencing reads was assessed with fastqc. All raw sequence data will be submitted to the National Center for Biotechnology Information and made available for public access.

Community structure was analyzed for changes between the start of the experiment and after two months within the in-water coral nursery. Microbial community structure was also analyzed in relation to coral genotype and changes in environmental conditions during the study period. Data analysis was performed in R with packages such as DADA2, phyloseq, vegan, and ANCOM-BC, using established methods. All bioinformatic scripts and code associated with this microbiome analysis will be made publicly available through the Meyer lab's github page (https://github.com/meyermicrobiolab).

2.3. Metabolome analysis

At the Smithsonian Marine Station, samples were removed from the whirl-paks using ETOH sterilized forceps. Samples were placed onto a clean piece of foil, and sample size and depth (skeleton and flesh) recorded to the tenth of a cm. Samples were transferred to a prewashed (MeOH 3x), pre-weighed and labeled 20 ml scintillation vial, and dry weight was recorded to 0.1 mg. Samples were processed in batches alongside solvent controls (usually 11 samples and one control per batch). Processing involved a series of extractions in 2:2:1 EtOAc:MeOH:H20. All samples were weighed, and 5 (±0.3) mg. portions of each extract were weighed into solvent resistant Eppendorf tubes before shipment to Georgia Institute of Technology (GT).

The metabolomes of *C. natans* and *D. labyrinthiformis* were characterized using ultra high-performance liquid chromatography (LC) coupled with high resolution mass spectrometry (MS) as described in Deustch *et al.* (2021) following the guidelines published by the metabolomics standards institute (Sumner *et al.*, 2007). A total of 180 coral extracts of *C. natans* and *D. labyrinthiformis* were received by GT for metabolomic analysis. Extracts were resuspended in 100% methanol with the internal standard sulfadimethoxine. LC/MS data were then acquired on a Thermo Fisher Exploris 240 utilizing an AquireX workflow, allowing for identification and subsequent MS2 fragmentation of features which in a traditional workflow would not have been selected for fragmentation. GT uses molecular networking for data reduction that is performed by displaying identical fragmentation spectra as a single node and by clustering similar spectra as connected nodes in the form of networks (Wang *et al.*, 2016). Similarities in MS2 fragmentation spectra relate to similarities in chemical structures. Such MS2-based similarity approaches also allow automatic identification of known compounds by comparing experimental MS2 spectra with spectra available in mass spectral repositories such

as the National Institute of Standards and Technology (NIST), in-house libraries hosted by Global Natural Products Social (GNPS). In addition to this approach, GT employed various emerging de novo identification of metabolites by combining MS2LDA based analysis with network analysis (Deutsch *et al.*, 2022). MS2LDA analysis identifies the presence of shared chemical substructures in the entire metabolomics data, thus enabling researchers to reliably predict structure families present in the untargeted metabolomics data. Such an analysis allows researchers to decipher if a certain sub-structural scaffold is enriched in a sample type (e.g., enrichment of phenolic compounds, indoles and terpenes). In addition, we utilized an in-silico method, SIRIUS with CSI:FingerID, to annotate unknown compounds and to predict chemical classes of detected compounds (known and unknowns). SIRIUS accurately predicts the chemical formula of the measured precursor ion and of each fragment detected in the MS/MS spectra. It then generates a fragmentation tree of reactions leading to each fragment from the precursor ion. For the predicted chemical formula, the platform then matches molecular fingerprints generated from fragmentation trees of candidate compounds present in structural databases such as PubChem, and Dictionary of Natural Products. Thus, it is an in-silico method that allows annotations of compounds by the matching of common substructures. Data were then processed utilizing Compound Discoverer 3.4. A visual representation of the metabolomic workflow employed can be found in Figure 1 below.

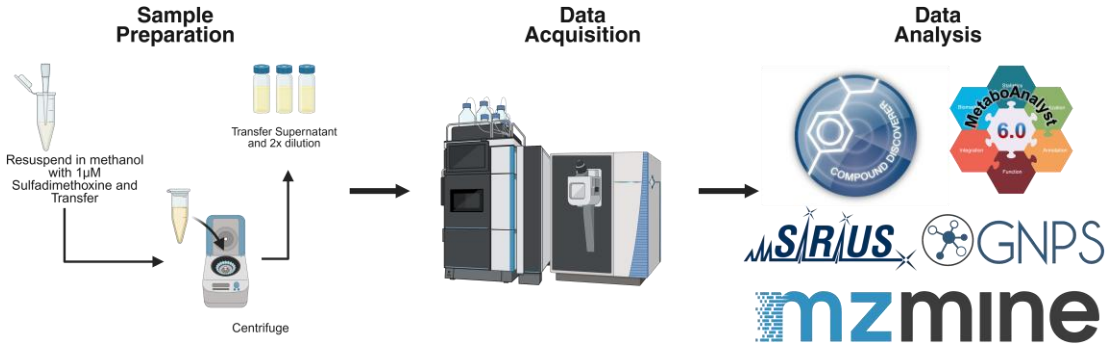

Figure 1. *The workflow illustrates the steps for metabolomic profiling, beginning with sample preparation where coral tissue is resuspended in 100% methanol with the internal standard sulfadimethoxine, centrifuged, and the supernatant is transferred and diluted. In the data acquisition phase, prepared samples are analyzed using liquid chromatography-mass spectrometry (LC-MS). The final step is data analysis, which includes compound identification and statistical processing using platforms such as Compound Discoverer, SIRIUS, GNPS, MZmine, and MetaboAnalyst 6.0.*

Significant features were identified through ANOVA analysis followed with Fisher's Least Significant Difference post-hoc test within Metaboanalyst. Features that could not be annotated were subjected to SIRIUS to reveal potential class information. Several principal component analyses (PCAs) were generated in order to visualize the metabolome shifts in both CNAT and DLAB samples collected in triplicate in the wild and collected after 2 months of being placed into nursery conditions. These

All raw LC-MS data will be submitted to the MassIVE respository https://massive.ucsd.edu/ProteoSAFe/static/massive.jsp. In addition, all bioinformatic scripts

and code associated with metabolome analysis will be made publicly available through the Garg Lab's github page (https://github.com/Garg-Lab).

3. RESULTS

3.1. In-water collection and grow-out

A total of thirty unique corals were collected for this study, 15 each of *C. natans* and *D. labyrinthiformis* divided across three different inshore patch reefs encompassing the upper, middle and lower Florida Keys. Collection sites were a patch reef inshore of Pickles Reef (Pickles Patch 25.00843, -80.45875) in the upper Keys, West Turtle Shoal (West Turtle 24.70176, -80.96360) in the middle Keys, and a patch reef inshore of Looe Key Reef (Looe Inshore 24.578660, -81.438670) in the Lower keys (Figure 2).

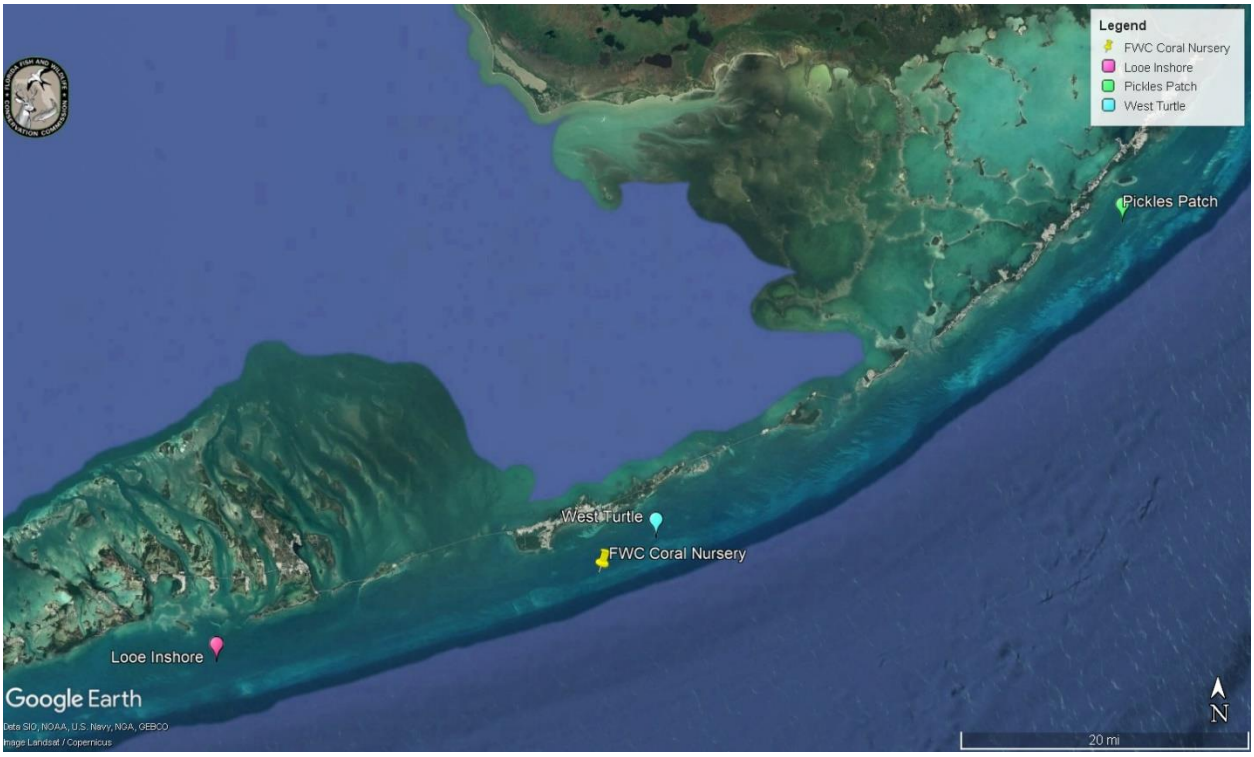

Figure 2*.Google Earth map of the Florida Keys showing coral collection sites in the Upper (Pickles patch shown in green), middle (West Turtle shown in blue) and Lower (Looe Inshore shown in pink) Florida Keys. The FWC in-water Coral Nursery is indicated in yellow. The map highlights the surrounding shallow reef areas, seagrass beds, and landmasses of the Florida Keys. A north arrow and scale bar (20 miles) are included for orientation. Color-coded markers correspond to the legend in the top-right corner for site identification.*

Corals were collected from West Turtle on July 30, 2024 and August 16, 2024. The *C. natans* from West Turtle ranged in size from 5,400 to 25,181 cm^3 living area while the *D. labyrinthiformis* ranged from 1,529 to 7862 cm^3 living area. After propagation, corals collected

from West Turtle were placed in the nursery on August 15, 2024 and September 6, 2024, and three pucks of each genotype were collected October 15, 2024 and November 12, 2024.

All corals from Looe Inshore were collected on September 16, 2024. The Looe Inshore *C. natans* ranged from 2,803 to 11,250 cm^3 while the *D. labyrinthiformis* ranged from 210 to 2,250 cm^3. Corals originally collected from Looe were placed in the nursery on October 15, 2024 and 2-month samples collected December 19, 2024.

Coral collections at Pickles Patch occurred on September 20, 2024, for *D. labyrinthiformis*, and between September 20–23, 2024, for *C. natans*. Coral collected from Pickles Patch were on average smaller than those found at the other two sites. *C. natans* ranged from 320 to 1,296 cm^3 whereas *D. labyrinthiformis* ranged in size from 400 to 1,764 cm^3. Corals collected from the Pickles Patch collection were placed in the nursery on October 15, 2024 and 2-month samples were collected December 19, 2024.

Monthly coral monitoring resulted in significant differences observed in survival between individual genotypes (Figure 3 A-B). This was especially evident in the *C. natans* where survival, as of 287 in the nursery, ranged from 12% in the poorest performing genotype CN021 to 100% in the five top-performing genotypes. In the poorest performing genotypes, initial instances of mortality appeared associated with overgrowth from algae and later mortality appeared associated with bivalves and other encrusting organisms. (Figure 4). Although all coral structures experience biofouling, which is cleaned as part of routine maintenance, these genotypes experienced biofouling on the coral fragments themselves. This is unusual as corals have mechanisms to prevent competition by other sessile organisms. Survival analysis showed a difference based on collection location with West Turtle having significantly lower survival (p value < 0.001) (Figure 5). Furthermore, survival curves showed a difference in survival of *C. natans* according to size (Figure 6A) with 10,000 cm^3 affecting the division in survival. Linear regression analysis indicated that ~50% of the variation in survival can be explained by the initial size (Figure 6B). Overall larger corals had lower survival over the monitoring period.

D. labyrinthiformis also showed genotypic-specific differences in survival which ranged from 38% in one genotype to 100% in three genotypes. Mortality by tissue loss primarily occurred within the first 2 months of holding corals within the nursery (Figure 7). Survival curves (Figure 8) indicated that, similar to *C. natans*, *D. labyrinthiformis* at West Turtle had significantly lower survival (p value < 0.001). The relationship between size and survival in *D. labyrinthiformis* is less clear, as linear regression indicating that ~25% of the variation in survival was explained by the initial size with smaller corals faring better, while survival curves indicated that larger corals had greater survival (Figure 9 A-B).

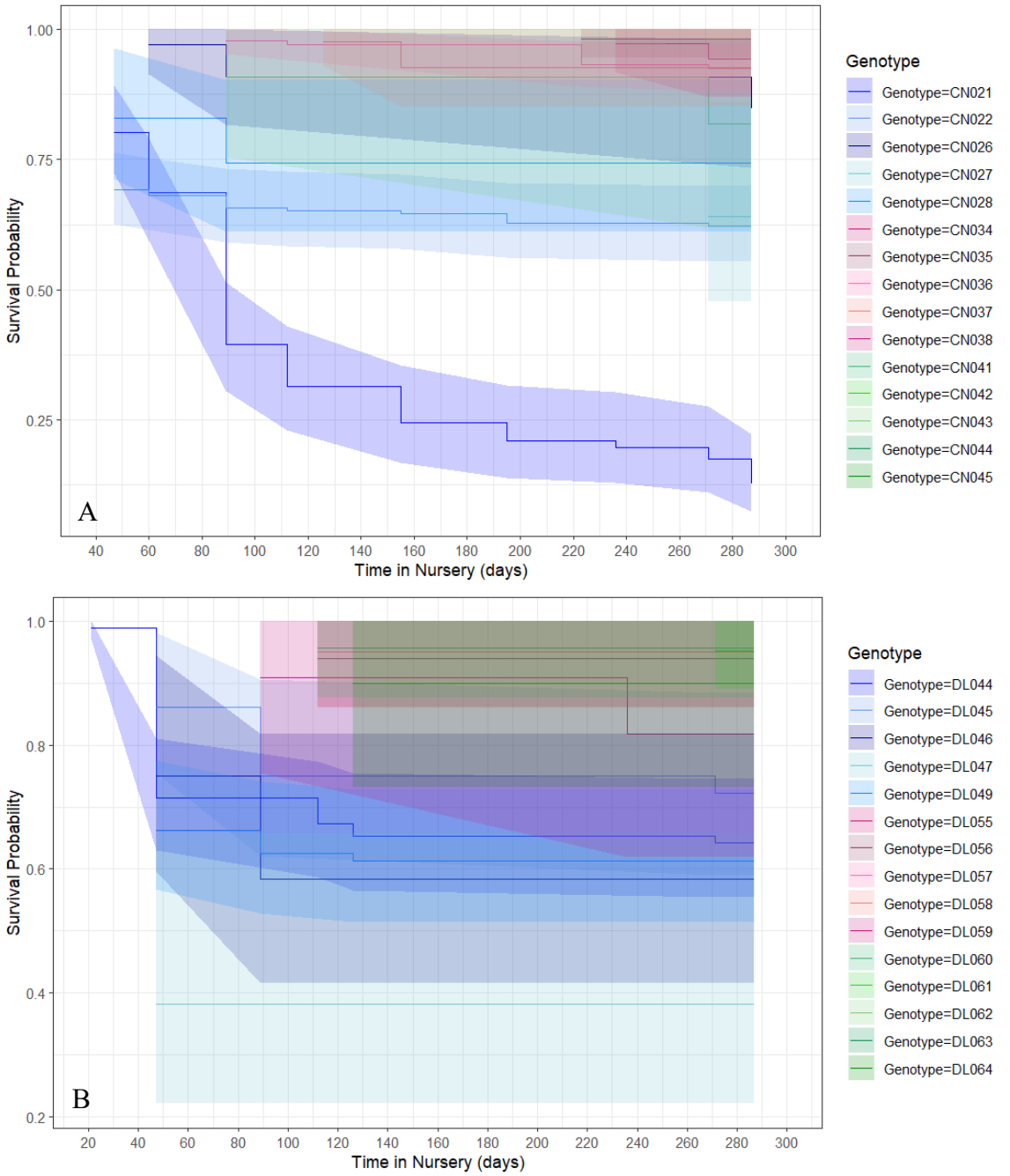

Figure 3. *Kaplan-Meier survival curves by genotype for C. natans (A) and D. labyrinthiformis (B). Line plot displaying the estimated survival probabilities of individual coral genotypes over time (days) in a nursery setting. Each colored line represents a genotype, with shaded ribbons indicating 95% confidence intervals. The y-axis shows survival probability from 0 to 1, and the x-axis shows time in the nursery up to 287 days. The legends on the right of each graph list genotypes, each color-coded to match the respective survival curve.*

Figure 4. *Image of genotype CN021 in FWC's in-water coral nursery being overgrown by a variety of encrusting organisms. Picture was taken 3/26/25.*

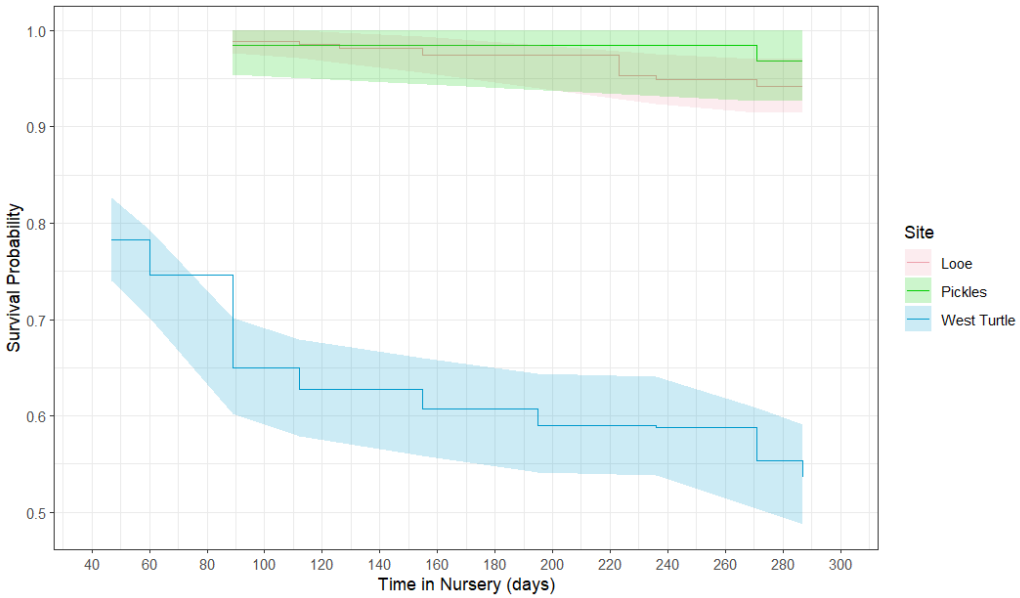

Figure 5. *Kaplan-Meier survival curves by location for C. natans. Line plot displaying the estimated survival probabilities of corals by location over time (days) in a nursery setting. Each colored line represents a location, with shaded ribbons indicating 95% confidence intervals. The y-axis shows survival probability from 0 to 1, and the x-axis shows time in the nursery up to 287 days. The legends on the right of each graph list locations, each color-coded to match the respective survival curve.*

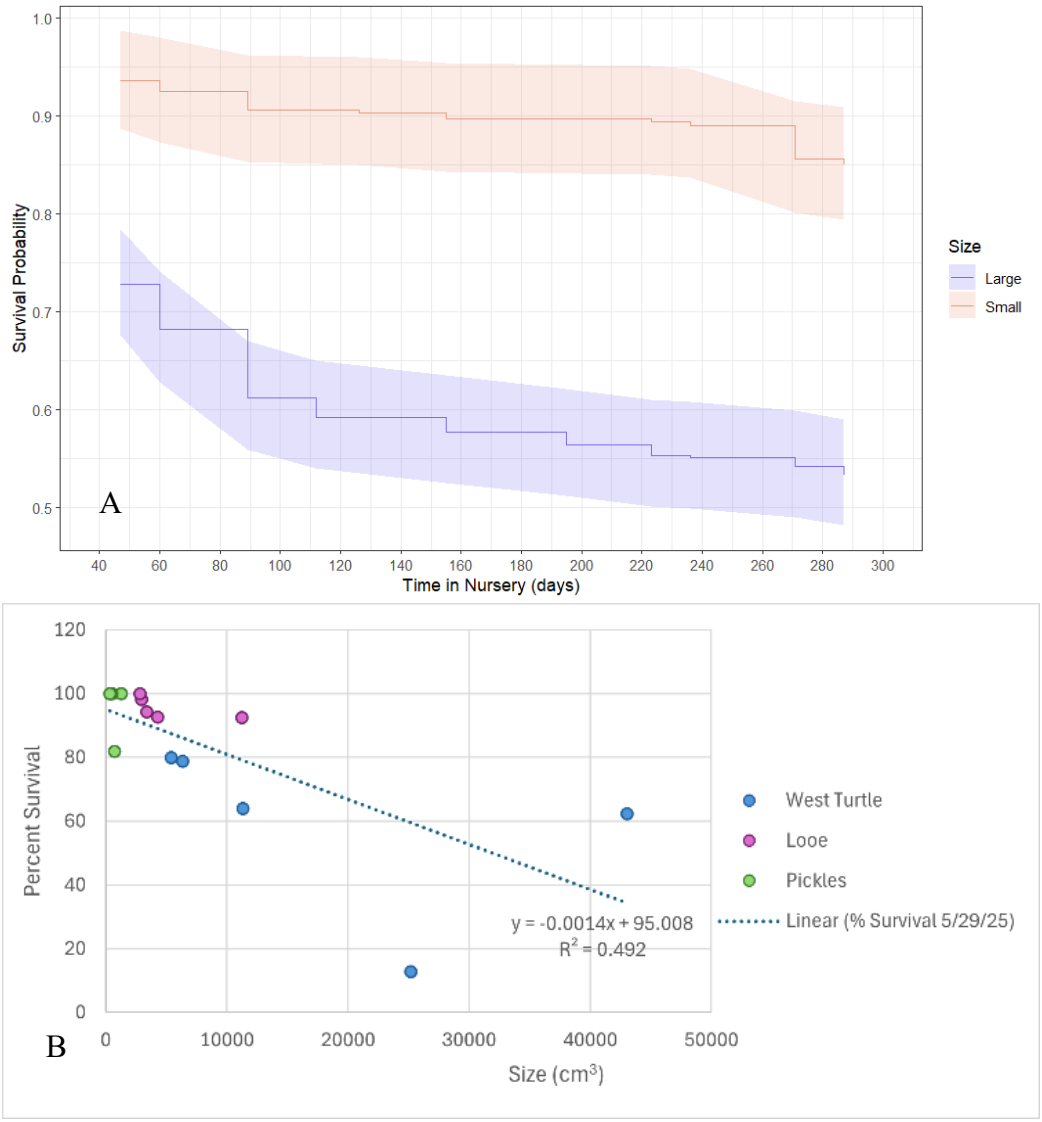

Figure 6. *Survival of C. natans by size shown by both Kaplan-Meier survival curves (A) and a linear regression (B). Line plot (A) displays the estimated survival probabilities of corals by size over time (days) in a nursery setting. Each colored line represents a location, with shaded ribbons indicating 95% confidence intervals. The y-axis shows survival probability from 0 to 1, and the x-axis shows time in the nursery up to 287 days. The legends on the right of each graph list sizes, each color-coded to match the respective survival curve. Scatter plot (B) displays unique coral genotypes with size on the x-axis and survival time on the y-axis. Each point is color coded for collection location. A linear regression line is overlaid on the plot, showing a negative relationship between size and survival time. The regression equation is y = -0.0014x + 95.008, with an R^2 value of 0.492, indicating that approximately 49.2% of the variation in survival time can be explained by coral size.*

Figure 7*. Image of D. labyrinthiformis in coral nursery displaying tissue loss. Picture taken 10/1/24.*

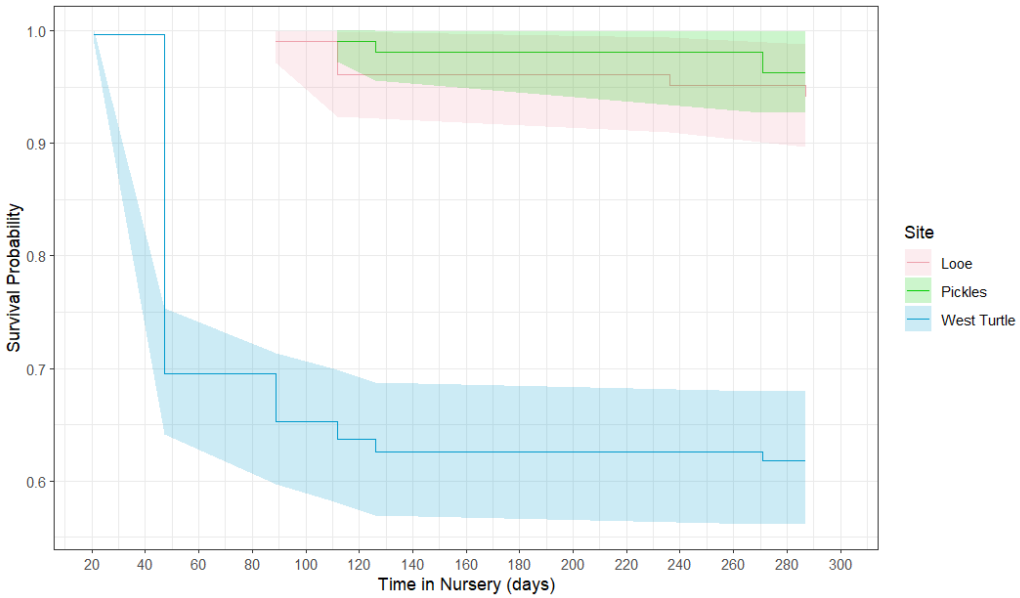

Figure 8*. Kaplan-Meier survival curves by location for D. labyrinthiformis. Line plot displaying the estimated survival probabilities of corals by location over time (days) in a nursery setting. Each colored line represents a location, with shaded ribbons indicating 95% confidence intervals. The y-axis shows survival probability from 0 to 1, and the x-axis shows time in the nursery up to 287 days. The legends on the right of each graph list locations, each color-coded to match the respective survival curve.*

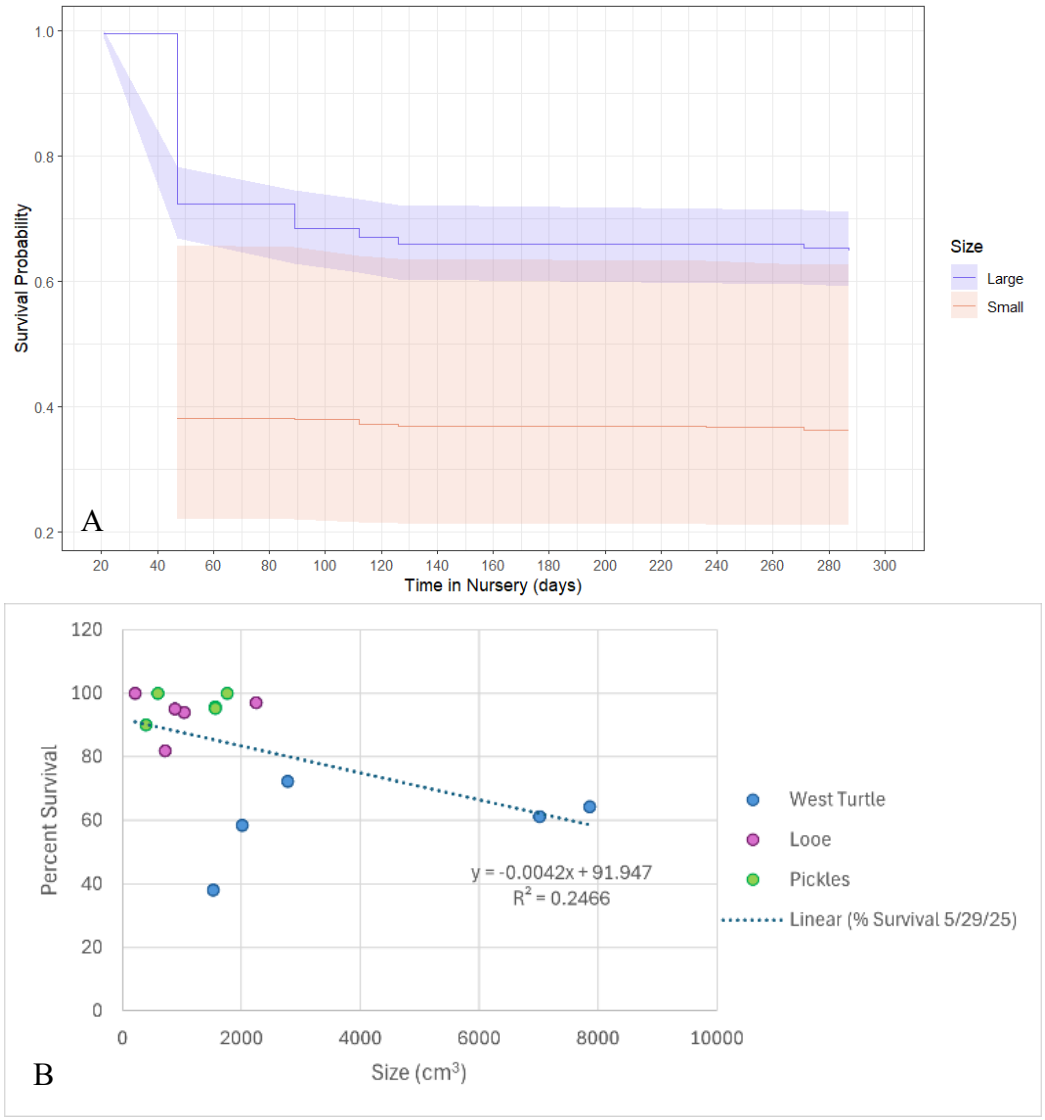

Figure 9. *Survival of D. labyrinthiformis by size shown by both Kaplan-Meier survival curves (A) and a linear regression (B). Line plot (A) displays the estimated survival probabilities of corals by size over time (days) in a nursery setting. Each colored line represents a size bin, with shaded ribbons indicating 95% confidence intervals. The y-axis shows survival probability from 0 to 1, and the x-axis shows time in the nursery up to 287 days. The legends on the right of each graph list sizes, each color-coded to match the respective survival curve. Scatter plot (B) displays unique coral genotypes with size on the x-axis and survival time on the y-axis. Each point is color coded for collection location. A linear regression line is overlaid on the plot, showing a negative relationship between size and survival time. The regression equation is y = -0.0042x + 91.947, with an R² value of 0.2466, indicating that approximately 24.66% of the variation in survival time can be explained by coral size.*

***Table 1**. This table displays data collected on the study corals, including collection date, geographic coordinates (latitude and longitude), collection site name, FWC coral genotype label, species (CNAT – C. natans, DLAB – D. labyrinthiformis), and physical measurements (length, width, height in centimeters). It also includes the initial percentage of living tissue, calculated size in cubic centimeters, and percent alive as of May 29, 2025.*

Collection Date	Lat	Long	Site	Genotype	Species	Length (cm)	Width (cm)	Height (cm)	Initial % Living	Size (cm^3)	% Alive 5/29/25
7/30/2024	24.70176	-80.9636	West Turtle	CN021	CNAT	61	43	24	40	25180.80	12.79
7/30/2024	24.70176	-80.9636	West Turtle	CN022	CNAT	57	51	37	40	43023.60	62.29
8/16/2024	24.70176	-80.9636	West Turtle	CN026	CNAT	31	16	15	85	6324.00	78.79
8/16/2024	24.70176	-80.9636	West Turtle	CN027	CNAT	36	35	12	75	11340.00	64.00
8/16/2024	24.70176	-80.9636	West Turtle	CN028	CNAT	30	25	8	90	5400.00	80.00
9/16/2024	24.57866	-81.4387	Looe Inshore	CN034	CNAT	25	15	10	90	3375.00	94.29
9/16/2024	24.57866	-81.4387	Looe Inshore	CN035	CNAT	34	18	8	60	2937.60	98.11
9/16/2024	24.57866	-81.4387	Looe Inshore	CN036	CNAT	50	25	10	90	11250.00	92.48
9/16/2024	24.57866	-81.4387	Looe Inshore	CN037	CNAT	38	15	15	50	4275.00	92.68
9/16/2024	24.57866	-81.4387	Looe Inshore	CN038	CNAT	49	13	11	40	2802.80	100
9/20/2024	25.00984	-80.4575	Pickles Patch	CN041	CNAT	18	16	5	90	1296.00	100
9/20/2024	25.00984	-80.4575	Pickles Patch	CN042	CNAT	11	11	4	100	484.00	100
9/20/2024	25.00984	-80.4575	Pickles Patch	CN043	CNAT	15	13	4	95	741.00	81.82
9/23/2024	25.00385	-80.4556	Pickles Patch	CN044	CNAT	9	10	4	100	360.00	100
9/23/2024	25.00385	-80.4556	Pickles Patch	CN045	CNAT	10	8	4	100	320.00	100
7/30/2024	24.70176	-80.9636	West Turtle	DL044	DLAB	28	24	13	90	7862.40	64.29
7/30/2024	24.70176	-80.9636	West Turtle	DL045	DLAB	21	17	13	60	2784.60	72.22
7/30/2024	24.70176	-80.9636	West Turtle	DL046	DLAB	14	18	8	100	2016.00	58.33
7/30/2024	24.70176	-80.9636	West Turtle	DL047	DLAB	15	13	8	98	1528.80	38.10
7/30/2024	24.70176	-80.9636	West Turtle	DL049	DLAB	30	26	18	50	7020.00	61.25
9/16/2024	24.57866	-81.4387	Looe Inshore	DL055	DLAB	20	15	15	50	2250.00	97.97
9/16/2024	24.57866	-81.4387	Looe Inshore	DL056	DLAB	13	10	8	100	1040.00	93.94
9/16/2024	24.57866	-81.4387	Looe Inshore	DL057	DLAB	7	6	5	100	210.00	100
9/16/2024	24.57866	-81.4387	Looe Inshore	DL058	DLAB	11	10	8	100	880.00	95.00

9/16/2024	24.57866	-81.4387	Looe Inshore	DL059	DLAB	9	10	8	100	720.00	81.82
9/20/2024	25.00843	-80.4588	Pickles Patch	DL060	DLAB	20	13	6	100	1560.00	95.65
9/20/2024	25.00843	-80.4588	Pickles Patch	DL061	DLAB	15	13	8	100	1560.00	95.24
9/20/2024	25.00843	-80.4588	Pickles Patch	DL062	DLAB	10	12	5	100	600.00	100
9/20/2024	25.00843	-80.4588	Pickles Patch	DL063	DLAB	18	14	7	100	1764.00	100
9/20/2024	25.00843	-80.4588	Pickles Patch	DL064	DLAB	10	10	4	100	400.00	90.00

3.2. Microbiome analysis

A total of 180 coral tissue samples were received for microbiome analysis, with 3 replicate samples per original coral colony at each time point. We successfully extracted DNA and sequenced 16S rRNA gene libraries for all 180 samples. After sequence quality-filtering, there was an average of 4,497 reads per sample. Of these libraries, 18 samples had fewer than 500 reads and were removed from further analysis. An additional 10 samples were removed that only contained 16S rRNA sequences from mitochondria and chloroplasts (i.e. contained no bacterial reads). All 28 low-quality libraries were from the initial collection timepoint, leaving only 69% of the initial samples for analysis. Because of the replication in samples per coral colony, only one colony (CN042) did not have microbiome composition data from the initial timepoint. All other colonies had one or more successful microbiome libraries. In contrast, all 90 samples from the 2-month collection time point resulted in high-quality libraries. Pending additional funding, we would repeat the amplification and sequencing of the low-quality amplicon libraries. The QC metrics from the sequencing run show that while the run passed all instrumental QC standards, only 76% of the reads were high-quality compared to other runs from our lab that typically have 85 to 95% high-quality reads. Since all samples were sequenced on the same run and the DNA extractions appeared normal, the low-quality initial samples may have had less microbial biomass.

3.2.1. Beta diversity of microbial communities

When examining the 152 successful 16S rRNA libraries, microbial community composition varied by coral species (PERMANOVA R^2 = 0.01626, p = 0.05), timepoint (PERMANOVA R^2 = 0.01444, p = 0.05), and the interaction of species and time (PERMANOVA R^2 = 0.01568, p = 0.05), but the variation explained by these factors was very low (< 2% of the variation was explained by these factors). Overall, microbial composition was similar between the coral species and timepoints, as indicated in the high overlap among samples in the principal components analysis (Figure 10). If we considered only the samples from the 2-month timepoint which had the full complement of samples, giving an even sampling scheme, variation in the microbial composition was not explained by site, controlling for coral species, by colony, or by coral species. This further supports that the overall microbial composition did not vary strongly by the factors examined here. However, changes were observed in specific microbial taxa between the coral species and timepoints, as described below.

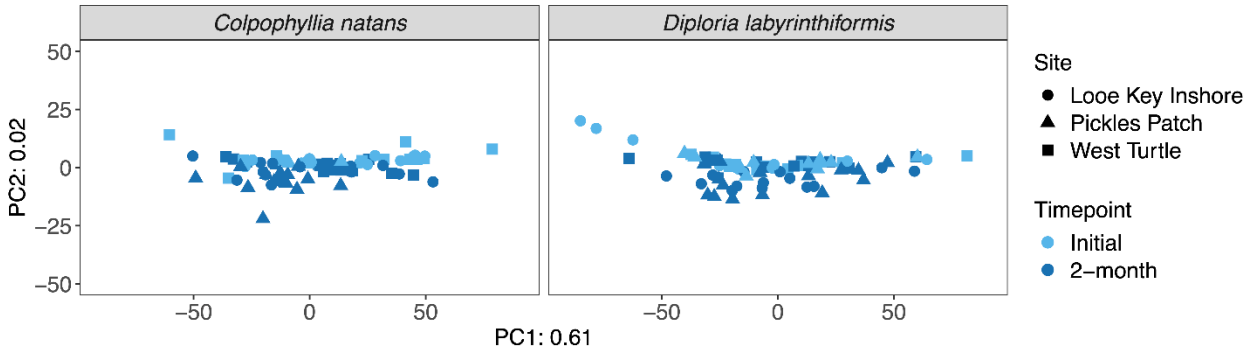

***Figure 10**. Principal components analysis of center-log-ratio transformed bacterial read counts. The ordination is faceted by coral species for visual clarity, with point shapes indicating the collection site and color indicating the collection timepoint for each sample. Principal component 1 explains 61% of the variation in microbial communities, while principal component 2 explains only 2%.*

3.2.2. *Common microbial taxa*

Analysis of the 152 successful 16S rRNA libraries showed that taxa common in both coral species and at both timepoints include bacterial strains classified only to the Gammaproteobacterial order Ga0077536 and bacterial strains in the families Amoebophilaceae, Vibrionaceae, Terasakiellaceae, and Paracoccaceae (Figure10). While the unclassified strain of Ga0077536 is difficult to place ecologically, the Meyer lab has detected unclassified Gammaproteobacteria in the microbiomes of several coral species. In addition, the remaining predominant taxa have been regularly associated with corals. The family Amoebophilaceae and the genus *Candidatus*. Amoebophilus are likely endosymbionts (living inside the coral tissue rather than on the surface) (Apprill *et al.*, 2016) and are common in Caribbean corals (Huggett and Apprill, 2019). Members of the Vibrionaceae are core members of the cnidarian microbiome (McCauley *et al.*, 2023). The recently named family Terasakiellaceae of the order Rhodospiralles (Hördt *et al.*, 2020) has previously been associated with stony coral tissue loss disease (SCTLD) (Rosales *et al.*, 2020), but the Meyer lab has also detected this group in seven species of apparently healthy bouldering corals, including *D. labyrinthiformis* sampled in Belize and the Cayman Islands before the arrival of SCTLD (Schul, et al, *in prep*). The family Paracoccaceae of the order Rhodobacterales was primarily represented here by a strain that was an exact sequence match to the type-strain of *Ruegeria profundi*, which was isolated from the Red Sea. *Ruegeria* has been commonly detected in coral microbiomes from both healthy and diseased individuals. Lastly, the family Blastocatellaceae of the phylum Acidobacteria was abundant primarily in *C. natans*. To our knowledge, this bacterial family has not been previously reported in corals.

3.2.3. *Microbial taxa that changed over time*

Differential abundance analysis showed that the families Amoebophilaceae and Nitrosopumilaceae were more abundant at the initial timepoint in both coral species. As described above, bacteria in the family Amoebophilaceae are likely endosymbiotic in coral tissue. Nitrosopumilaceae are aerobic ammonia-oxidizing archaea that are common globally in sediments but are not commonly detected in corals. We detected three amplicon sequence variants of Nitrosopumilaceae whose closest BLAST match was from sediment in a *Zostera* seagrass meadow. These Nitrosopumilaceae sequences were found only in the initial timepoint samples of both coral species, suggesting that the nursery conditions did not favor its continued growth. Lastly, Blastocatellaceae were more abundant at the initial timepoint in *C. natans*, while Cyanobacteriaceae were more abundant initially in *D. labyrinthiformis,* particularly in colonies DL045 and DL046 (Figure 10).

At the two-month timepoint, five families were more abundant in both coral species: the common Vibrionaceae and Terasakiellaceae families and the less common Hyphomonadaceae, Desulfovibrionaceae, and an unclassified family of Campylobacterales (Figure 11). Two closely related strains of Hyphomonadaceae strains were detected, both classified as the genus *Hyphomonas*, which have been detected in biofilms associated with artificial reef structures

(Sajid *et al.*, 2024), suggesting that *Hyphomonas* could be colonizing the coral fragments from the adjacent nursery structure. Members of Desulfovibrionaceae and Campylobacterales are most likely anaerobic bacteria and were often detected in the same colonies with higher relative abundances of Phormidiaceae. Only one strain of Phormidiaceae was detected and its closest BLAST match was 99.6% similar to the reference strain of *Dapis pnigousa* (Engene *et al.*, 2018). *D. pnigousa* are filamentous red cyanobacteria capable of forming algal blooms and may be part of the algal biofilms that encroached coral propagates in the nursery which potentially support low oxygen niches that favor the growth of Desulfovibrionaceae and Campylobacterales.

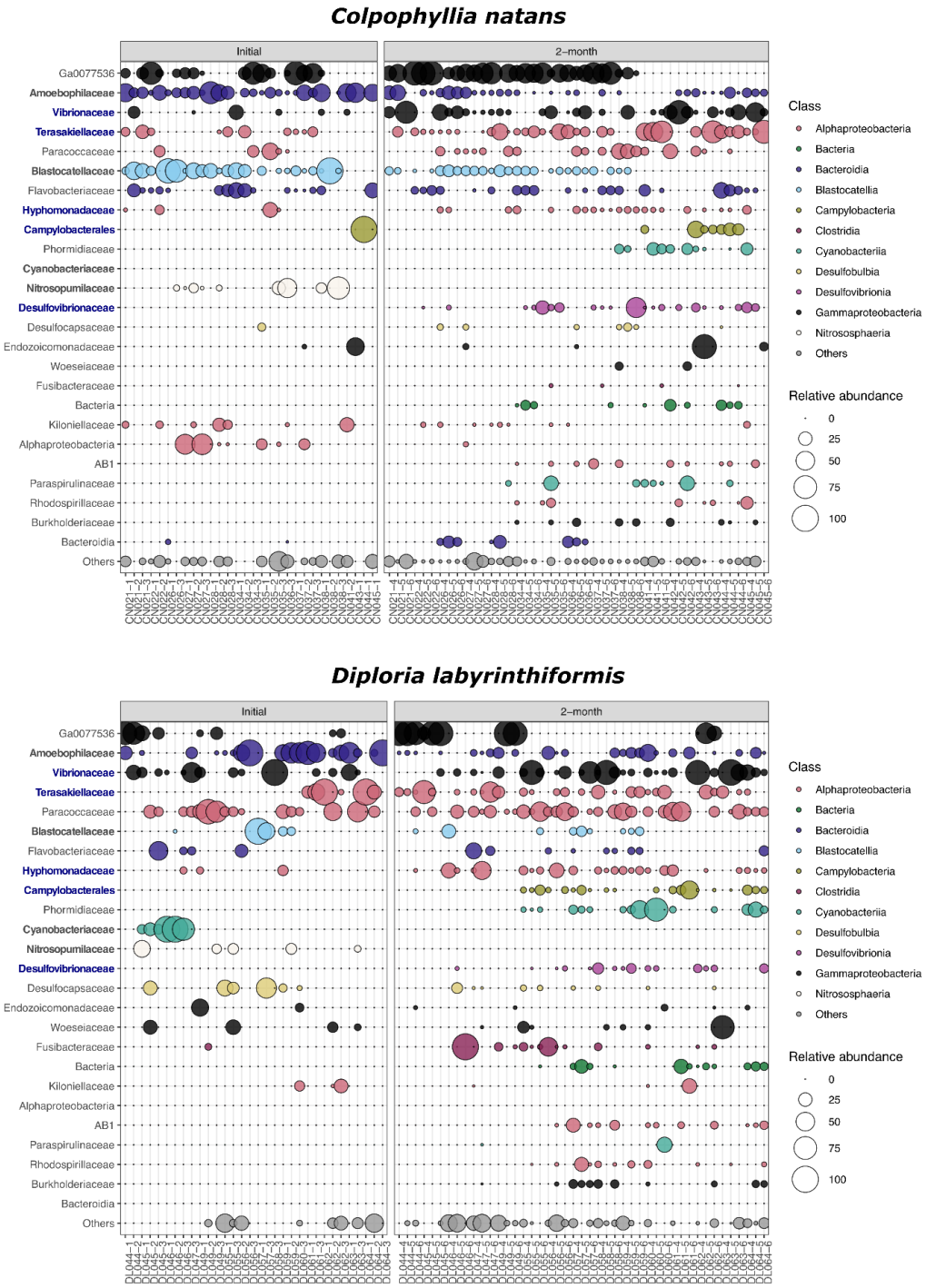

Figure 11*. Relative abundances of the top 25 bacterial and archaeal families detected in Colpophyllia natans (top panel) and Diploria labyrinthiformis (bottom panel) microbiomes at the initial collection timepoint and after two months in the land-based coral nursery. The relative abundances of all remaining families (n = 21) are shown as "Others". Families are colored by taxonomic classes. Names of families in black bold were more abundant in the initial timepoint and names in blue bold were more abundant at the 2-month timepoint.*

3.3. Metabolome analysis

180 coral extracts of *Colpophyllia natans* and *Diploria labyrinthiformis* were received for metabolomic analysis. Triplicates were taken from each individual coral twice, once when collected in the wild then again after 2 months within nursery. LC/MS data was acquired for each extract and then processed utilizing Compound Discoverer 3.4. Post-processing filtering was also employed within Compound Discoverer 3.4 bringing the number of features from 16487 to 4613. Significant features were identified through ANOVA analysis followed by Fisher's Least Significant Difference post-hoc test within Metaboanalyst. Features that could not be annotated were subjected to SIRIUS to reveal potential class information. One class of molecules was initially identified, carnitines. A heatmap of features annotated as carnitines was generated (Figure 12), carnitines were detected in higher intensity in corals in nursery as opposed to the wild. Further investigation into this trend will be conducted.

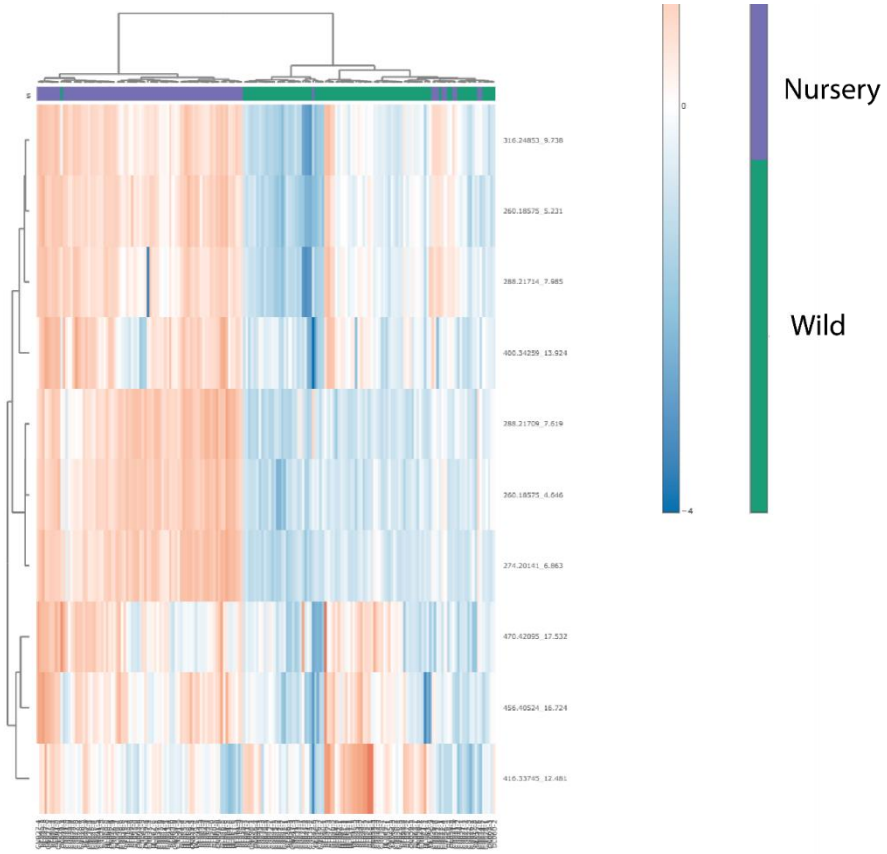

Figure 12. *Heatmap of Differentially Abundant Carnitines in Coral Samples from Nursery and Wild Populations. This heatmap illustrates the relative abundance of carnitine compounds (rows) across individual coral samples (columns), clustered by sample source. Hierarchical clustering along the top dendrogram groups samples based on similarity in carnitine profiles. Color gradients from blue (low abundance) to red (high abundance) represent standardized z-scores of carnitine levels. The colored annotation bar above the heatmap distinguishes between nursery (purple) and wild (green) coral samples. Carnitines on the right are labeled with their mass-to-charge ratio and retention time.*

Several principal component analyses (PCAs) were generated to visualize the metabolome shifts in both *C. natans* and *D. labyrinthiformis* samples collected in triplicate in the wild and collected after 2 months of being placed into nursery conditions. Several perspectives were leveraged, an analysis comparing inter-reef variation, an individual species approach, and finally a holistic analysis including both species to identify the general effect that placing a coral within an in-water nursery has on the metabolome. Upon initial collection, PCAs show that *C. natans and D. labyrinthiformis* at Looe Inshore reef and West Turtle reef cluster tightly together while Pickles Patch reef is differentially clustered (Figures 13, 15). In *C. natans* this trend is further validated thorough hierarchical clustering analysis (HCA) which reveals a separate cluster for Pickles Patch reef (Figure 14). Several analyses demonstrate a trend of metabolomes becoming more variable after two months within nursery while wild corals' metabolomes appear more similar (Figures 17-25). After their 2-month period within the FWC nursery samples do not cluster by original location in the same way as initial samples, instead it appears that West Turtle is clustering out from the rest of the collection locations (Figures 22,25).

Finally, Shannon diversity index analysis was performed to measure the alpha diversity within the samples (Figures 26-27). ANOVA confirmed the statistical significance in both species ($F = 71.54$, $p = 87e\text{-}15$), and wild and nursery ($F = 87.24$, $p = 2e\text{-}16$). Thus, the breadth of features is higher within nursery corals than wild as well as *D. labyrinthiformis* than *C. natans*.

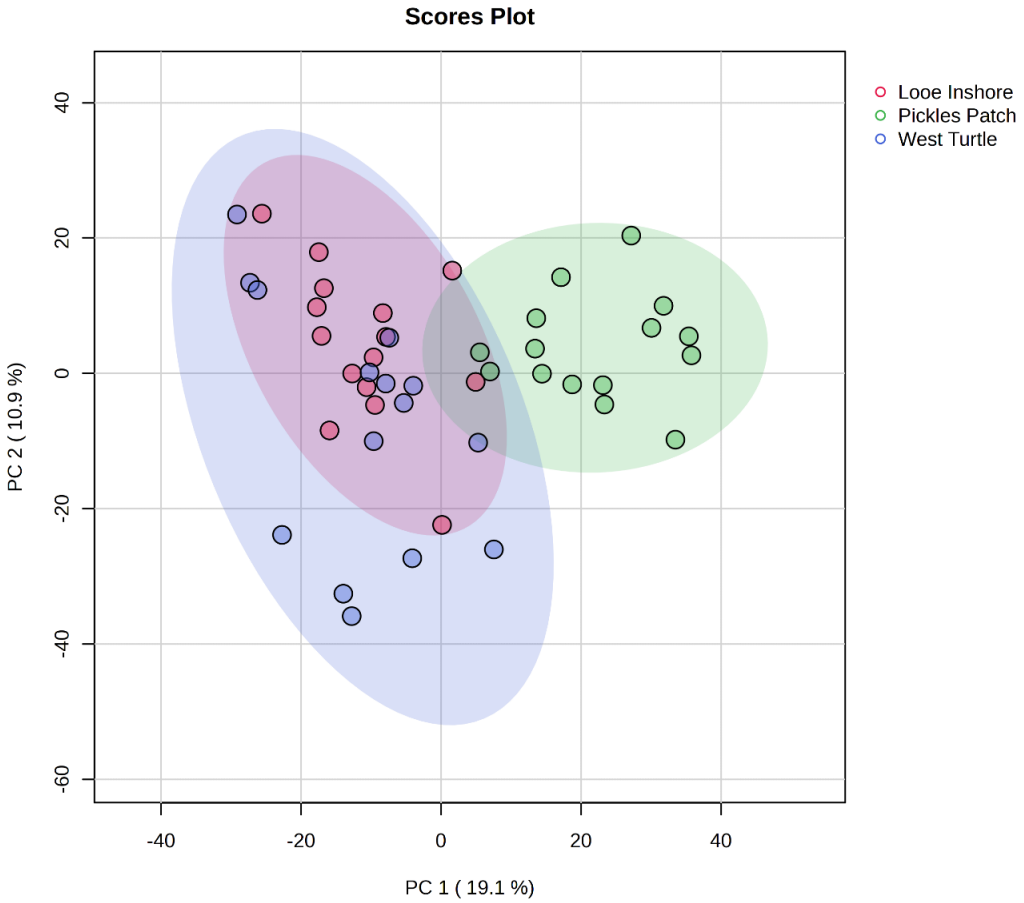

Figure 13. *Principal Component Analysis (PCA) Scores Plot Showing Metabolome Differences within C. natans by Source. The PCA scores plot illustrates the variation in metabolome composition across three locations: Looe Inshore (pink), Pickles Patch (green), and West Turtle (blue). Each point represents an individual sample, and ellipses encompass 95% confidence intervals for each group. The first principal component (PC1) explains 19.1% of the variance, and the second (PC2) explains 10.9%.*

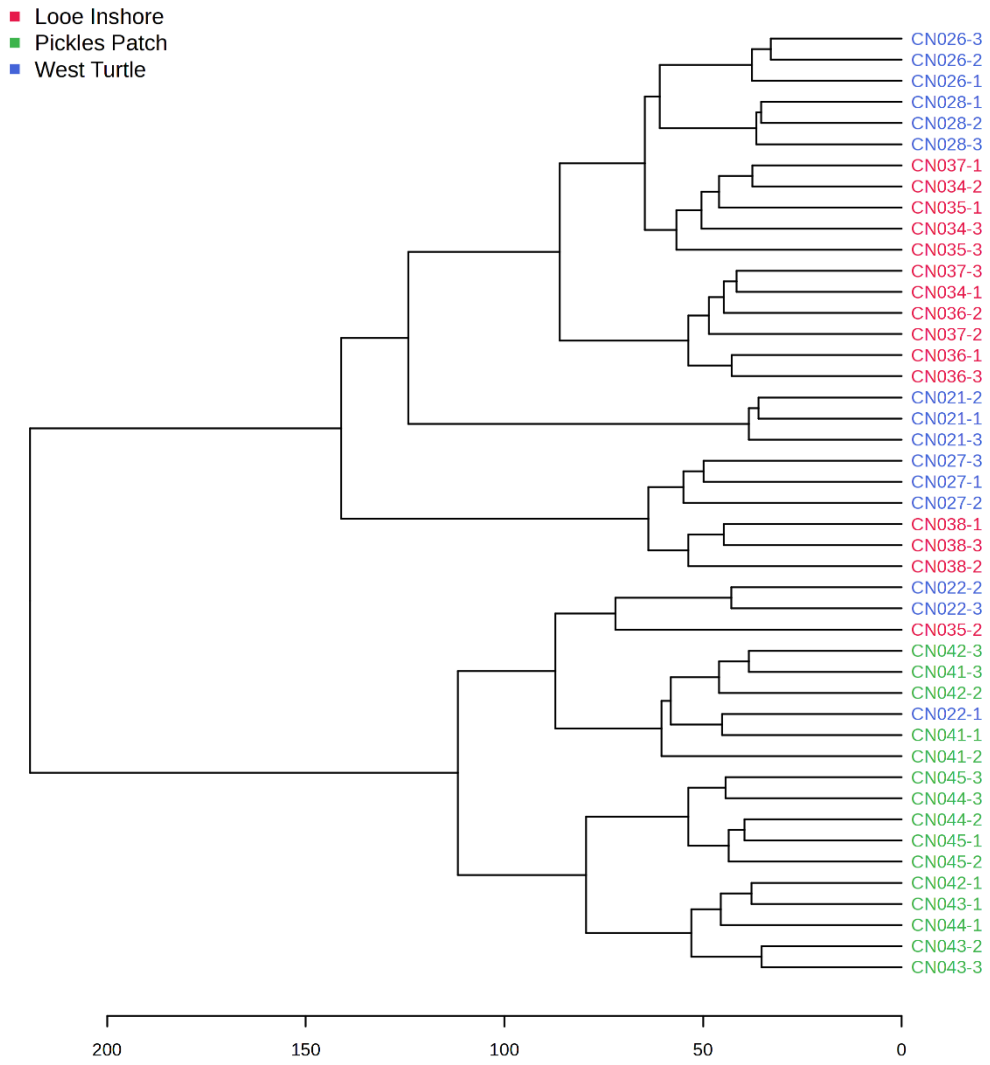

Figure 14. *Dendrogram showing hierarchical clustering showing metabolome differences within C. natans by source. Each sample is represented with genotype listed and the variation in metabolome composition across three locations are color coded: Looe Inshore (red), Pickles Patch (green), and West Turtle (blue).*

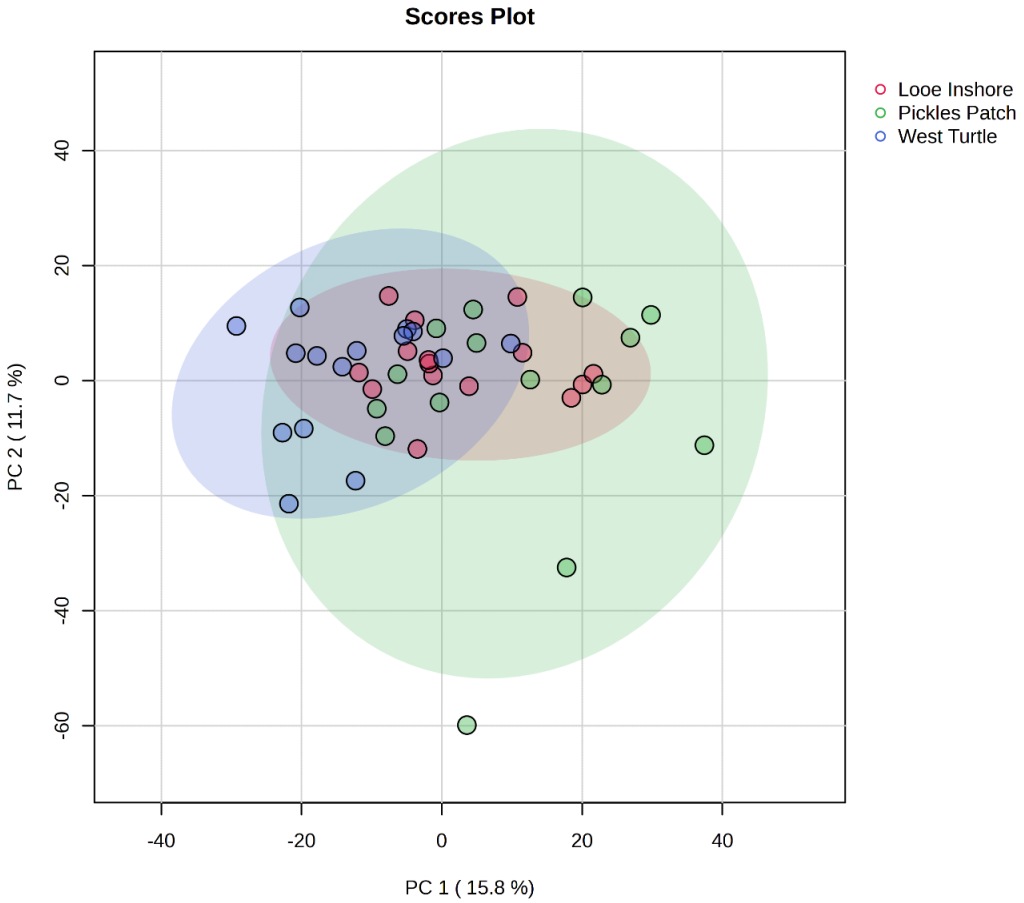

Figure 15. *Principal Component Analysis (PCA) Scores Plot Showing Metabolome Differences within D. labyrinthiformis by Source. The PCA scores plot illustrates the variation in metabolome composition across three locations: Looe Inshore (pink), Pickles Patch (green), and West Turtle (blue). Each point represents an individual sample, and ellipses encompass 95% confidence intervals for each group. The first principal component (PC1) explains 15.8% of the variance, and the second (PC2) explains 11.7%.*

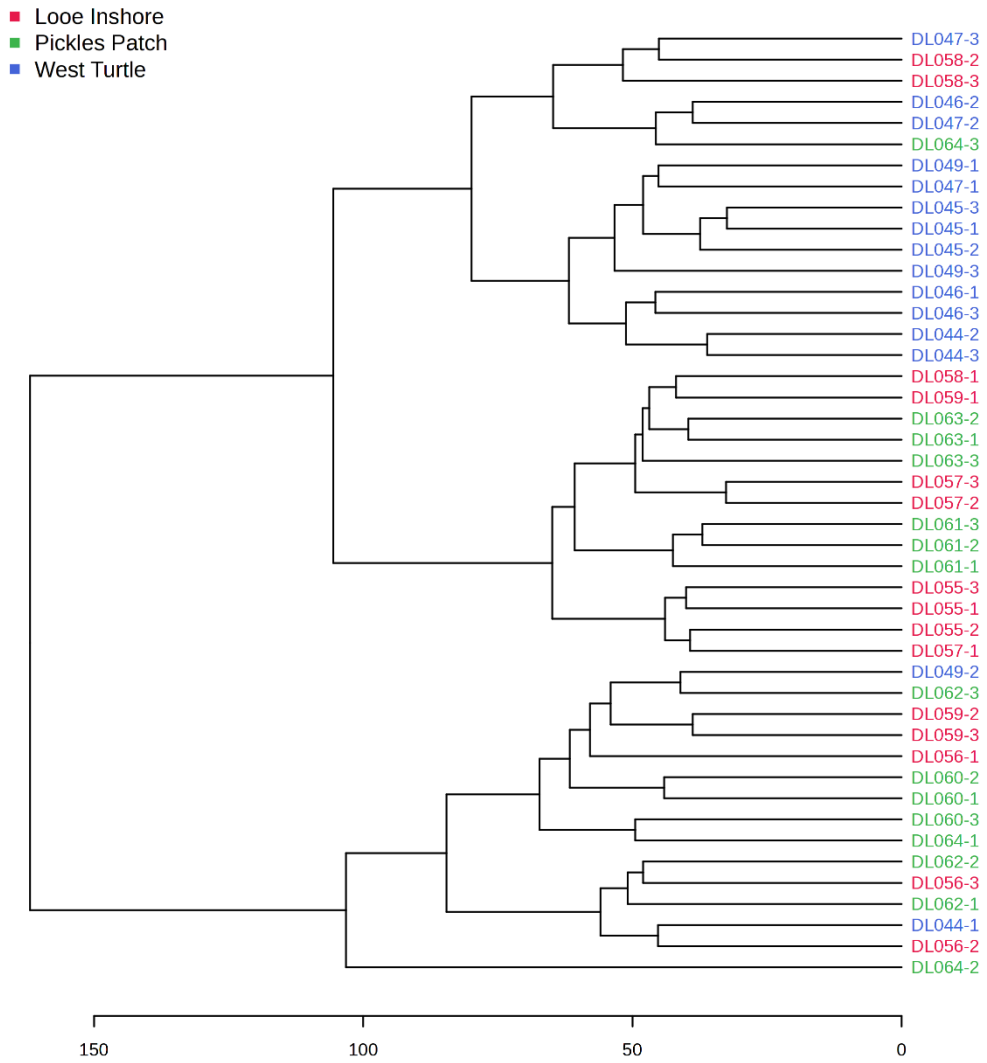

Figure 16. *Dendrogram showing hierarchical clustering showing metabolome differences within D. labyrinthiformis by source. Each sample is represented with genotype listed and the variation in metabolome composition across three locations are color coded: Looe Inshore (red), Pickles Patch (green), and West Turtle (blue).*

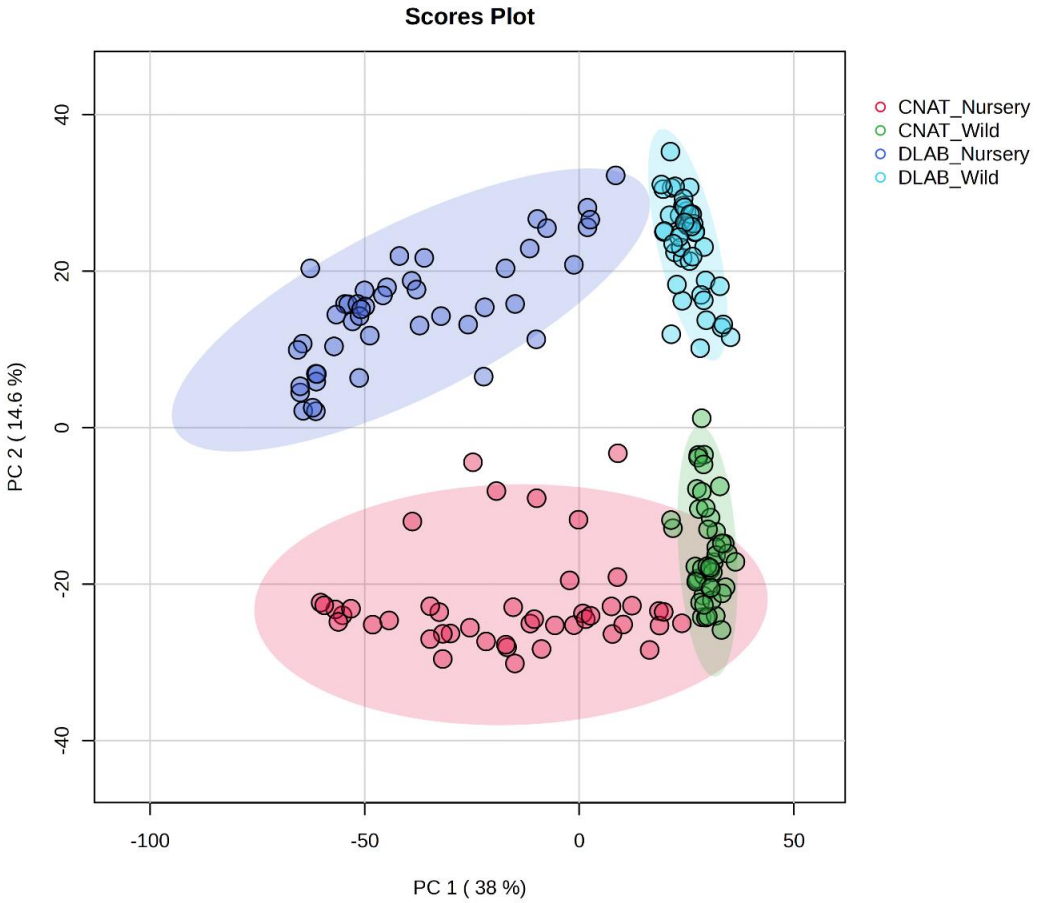

Figure 17. *Principal Component Analysis (PCA) Scores Plot Showing Metabolome Differences by Coral Species and Source. The PCA scores plot illustrates the variation in metabolome composition across four coral groups: CNAT_Nursery (pink), CNAT_Wild (green), DLAB_Nursery (blue), and DLAB_Wild (light blue). Each point represents an individual sample, and ellipses encompass 95% confidence intervals for each group. The first principal component (PC1) explains 38% of the variance, and the second (PC2) explains 14.6%.*

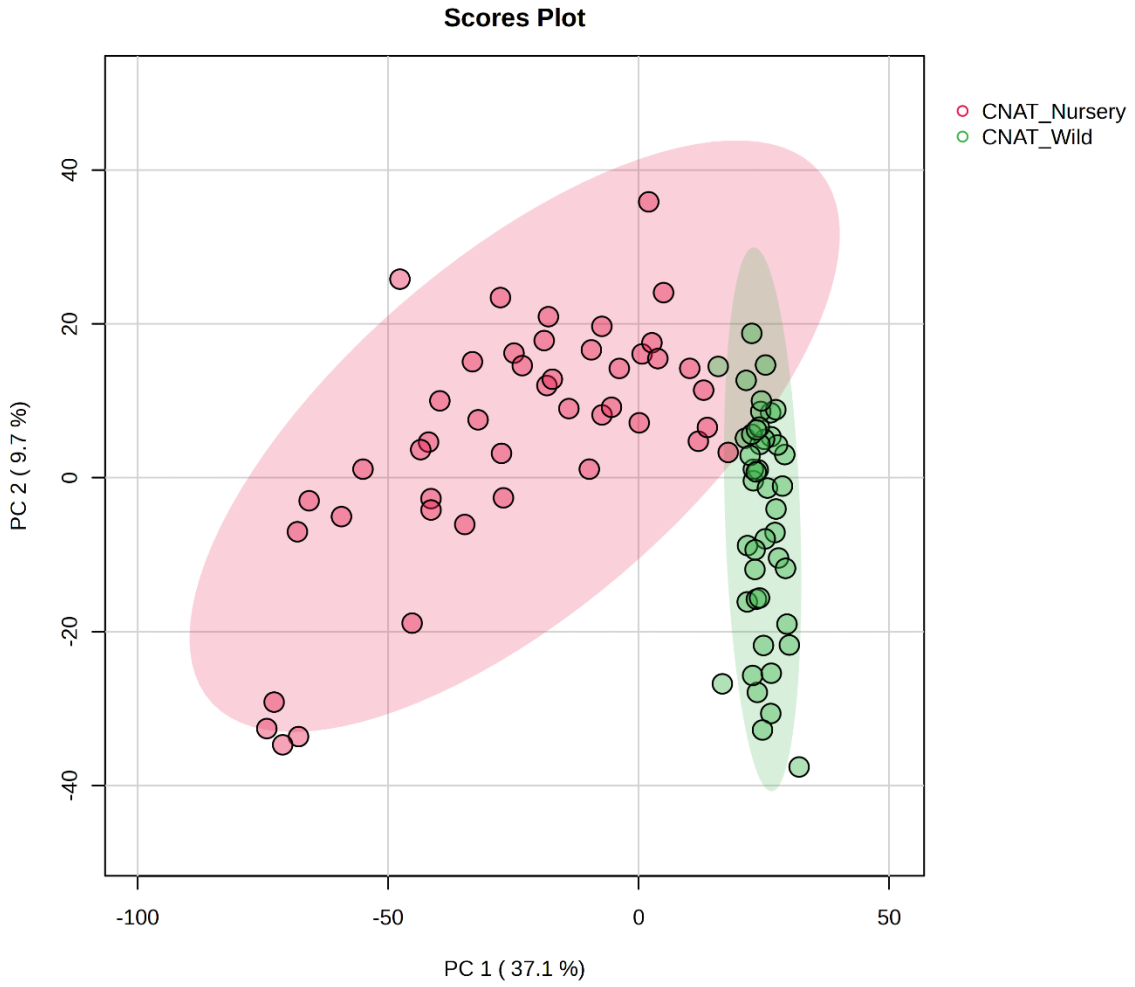

Figure 18. *Principal Component Analysis (PCA) Scores Plot Showing Metabolome Differences within C. natans by location (Initial Wild vs 2-month Nursery). The PCA scores plot illustrates the variation in metabolome composition across two coral groups: CNAT_Nursery (pink), CNAT_Wild (green). Each point represents an individual sample, and ellipses encompass 95% confidence intervals for each group. The first principal component (PC1) explains 37.1% of the variance, and the second (PC2) explains 9.7%.*

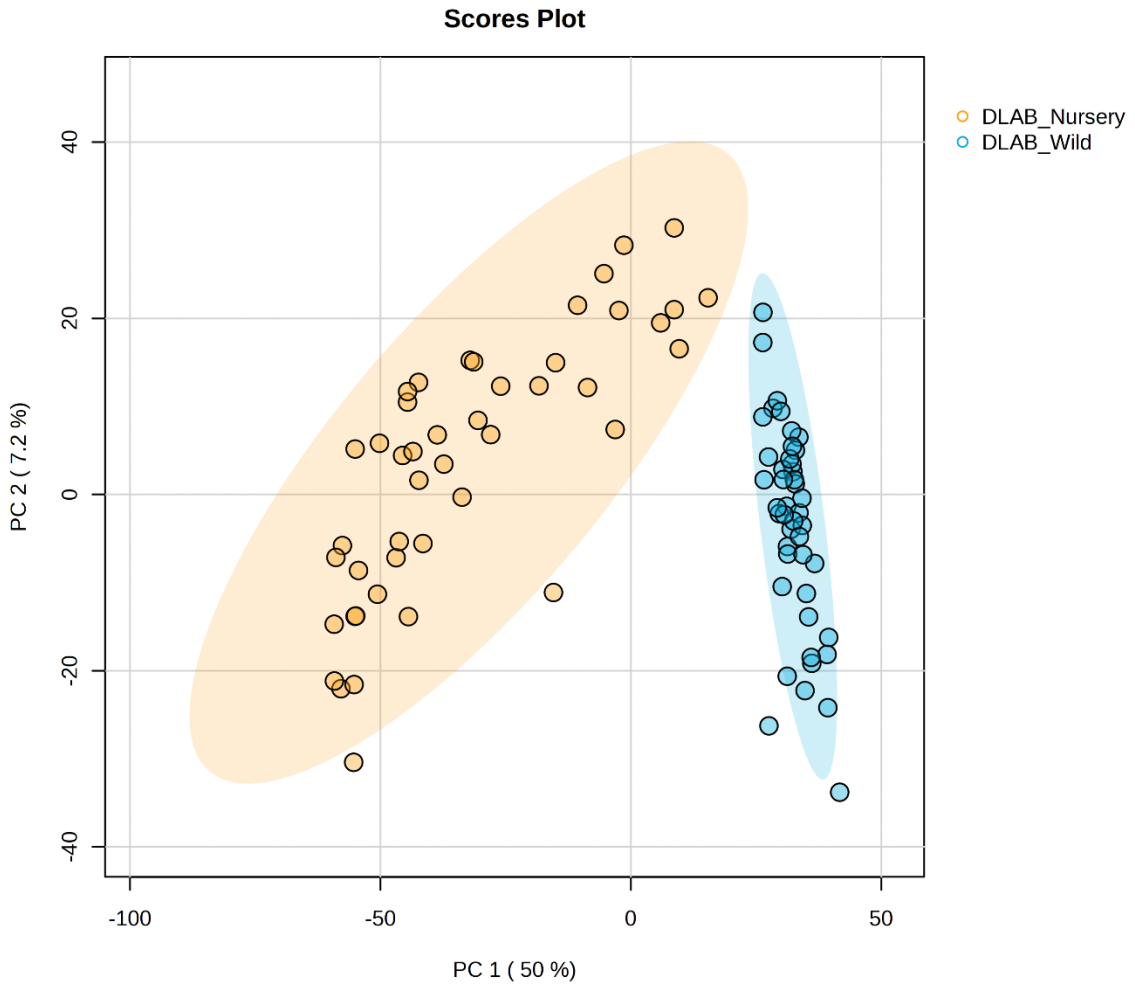

Figure 19. *Principal Component Analysis (PCA) Scores Plot Showing Metabolome Differences within D. labyrinthiformis by location (Initial Wild vs 2-month Nursery). The PCA scores plot illustrates the variation in metabolome composition across two coral groups: DLAB Nursery (yellow), and DLAB Wild (light blue). Each point represents an individual sample, and ellipses encompass 95% confidence intervals for each group. The first principal component (PC1) explains 50% of the variance, and the second (PC2) explains 7.2%.*

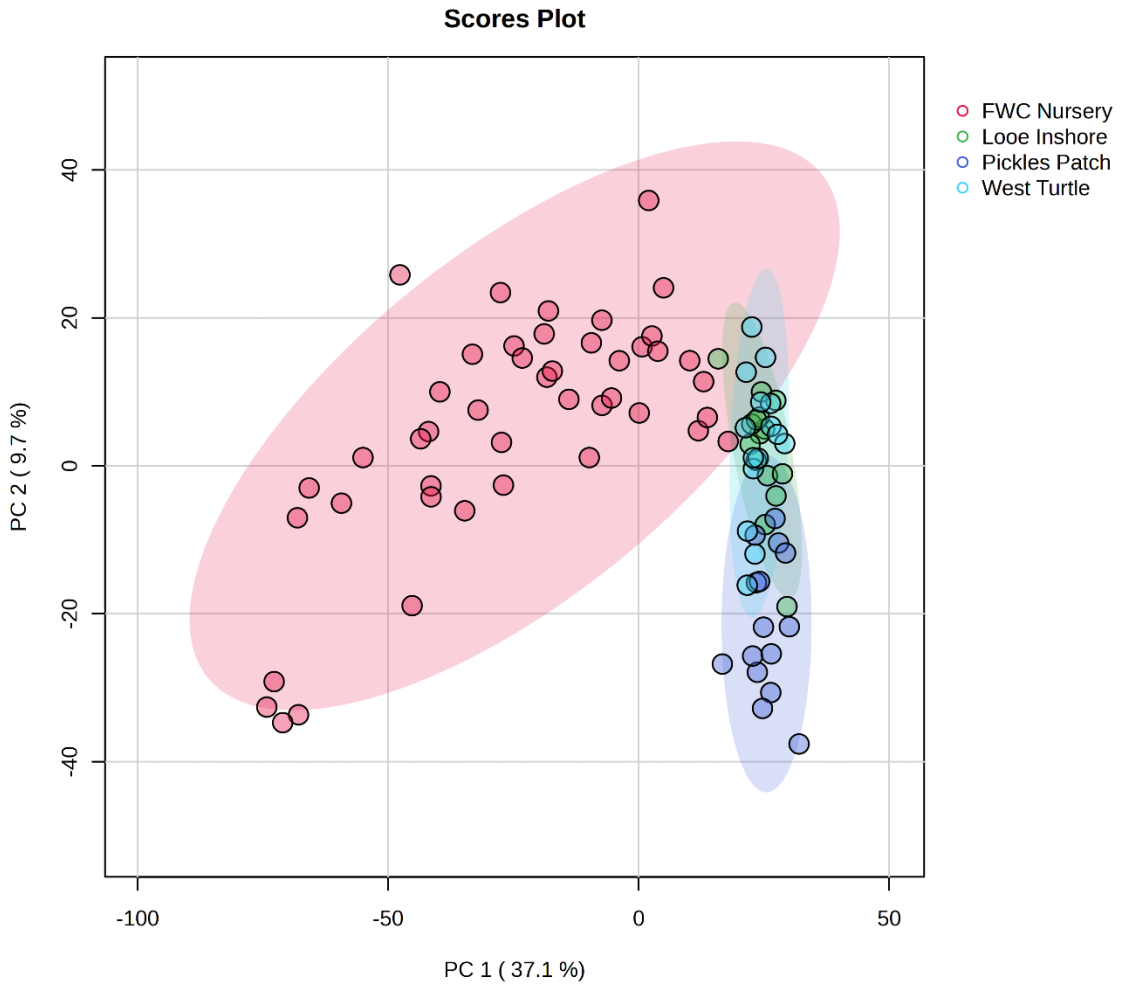

Figure 20. *Principal Component Analysis (PCA) Scores Plot Showing Metabolome Differences within C. natans by Original Source versus all nursery combined. The PCA scores plot illustrates the variation in metabolome composition across four locations: FWC Nursery (pink), Looe Inshore (green), Pickles Patch (Blue) and West Turtle (Light blue). Each point represents an individual sample, and ellipses encompass 95% confidence intervals for each group. The first principal component (PC1) explains 37.1% of the variance, and the second (PC2) explains 9.7%.*

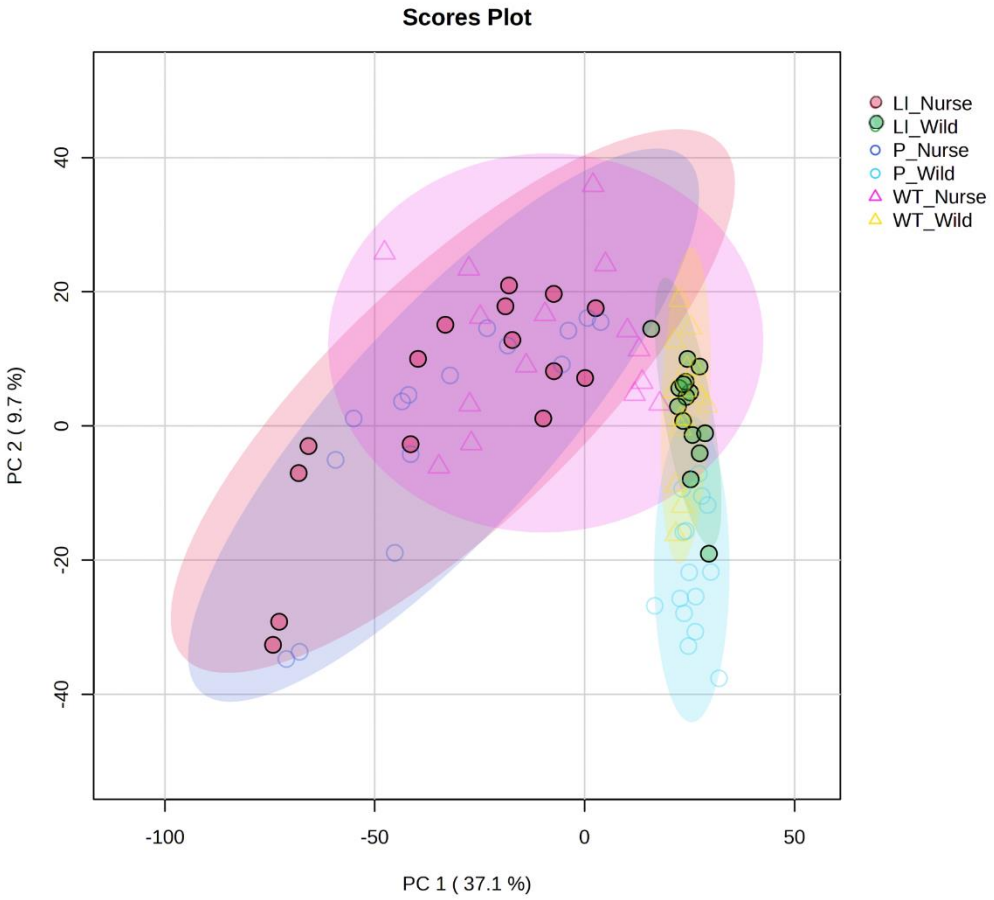

Figure 21. *Principal Component Analysis (PCA) Scores Plot Showing Metabolome Differences within C. natans by Original Collection location both at the original sampling and after 2 months in the Nursery. The PCA scores plot illustrates the variation in metabolome composition across three locations and two time points for six unique ellipses: Looe Inshore Nursery (solid pink circles), Looe Inshore Wild (solid green), Pickles Patch Nursery (Open Dark Blue circles), Pickles Patch Wild (Open Light Blue circles), West Turtle Nursery (Open Pink Triangles) and West Turtle Wild (Open Yellow Triangles). Each point represents an individual sample, and ellipses encompass 95% confidence intervals for each group. The first principal component (PC1) explains 37.1% of the variance, and the second (PC2) explains 9.7%.*

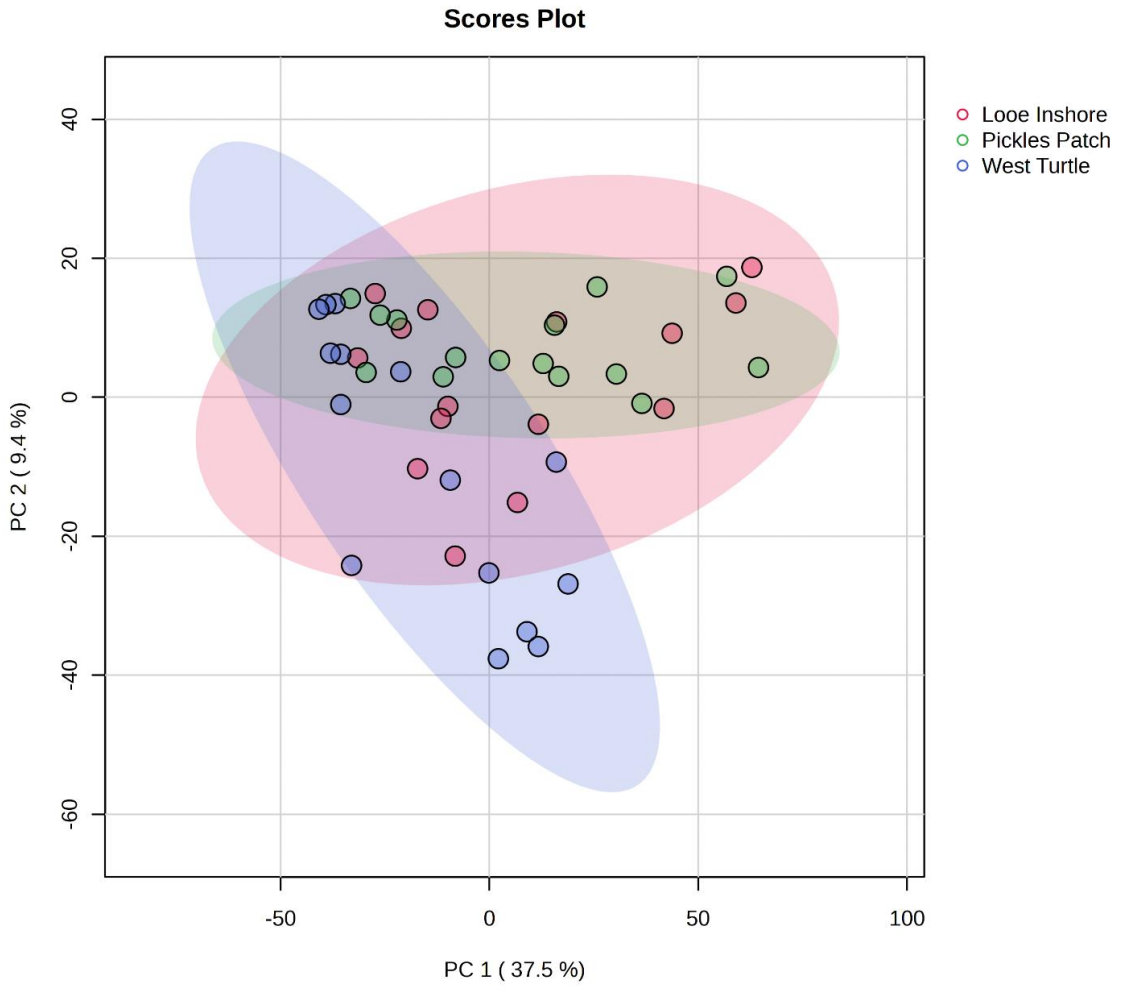

Figure 22. *Principal Component Analysis (PCA) Scores Plot Showing Metabolome Differences within C. natans by Original Collection location after 2 months in the Nursery. The PCA scores plot illustrates the variation in metabolome composition across three locations: Looe Inshore (pink), Pickles Patch (green), and West Turtle (blue). Each point represents an individual sample, and ellipses encompass 95% confidence intervals for each group. The first principal component (PC1) explains 37.5% of the variance, and the second (PC2) explains 9.4%.*

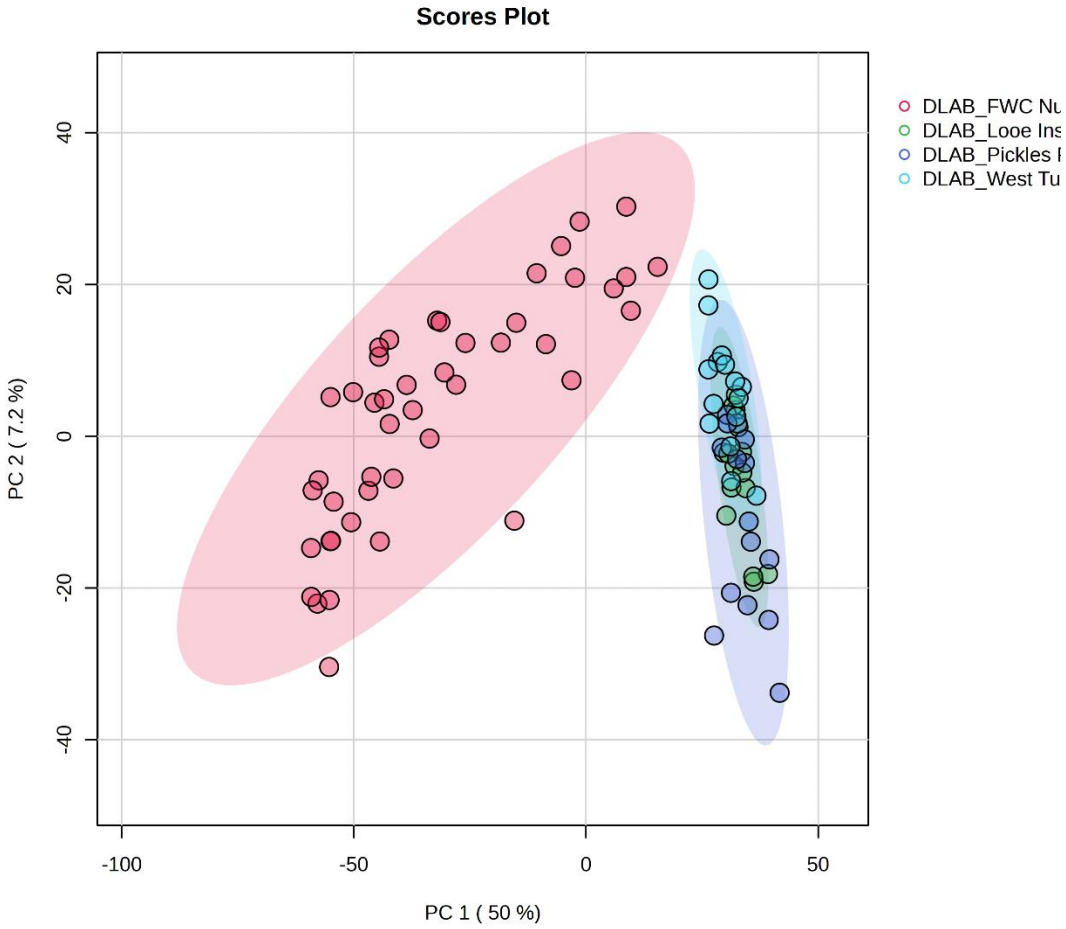

Figure 23. *Principal Component Analysis (PCA) Scores Plot Showing Metabolome Differences within D. labyrinthiformis by Original Source versus all Nursery combined. The PCA scores plot illustrates the variation in metabolome composition across four locations: FWC Nursery (pink), Looe Inshore (green), Pickles Patch (Blue) and West Turtle (Light blue). Each point represents an individual sample, and ellipses encompass 95% confidence intervals for each group. The first principal component (PC1) explains 50% of the variance, and the second (PC2) explains 7.2%.*

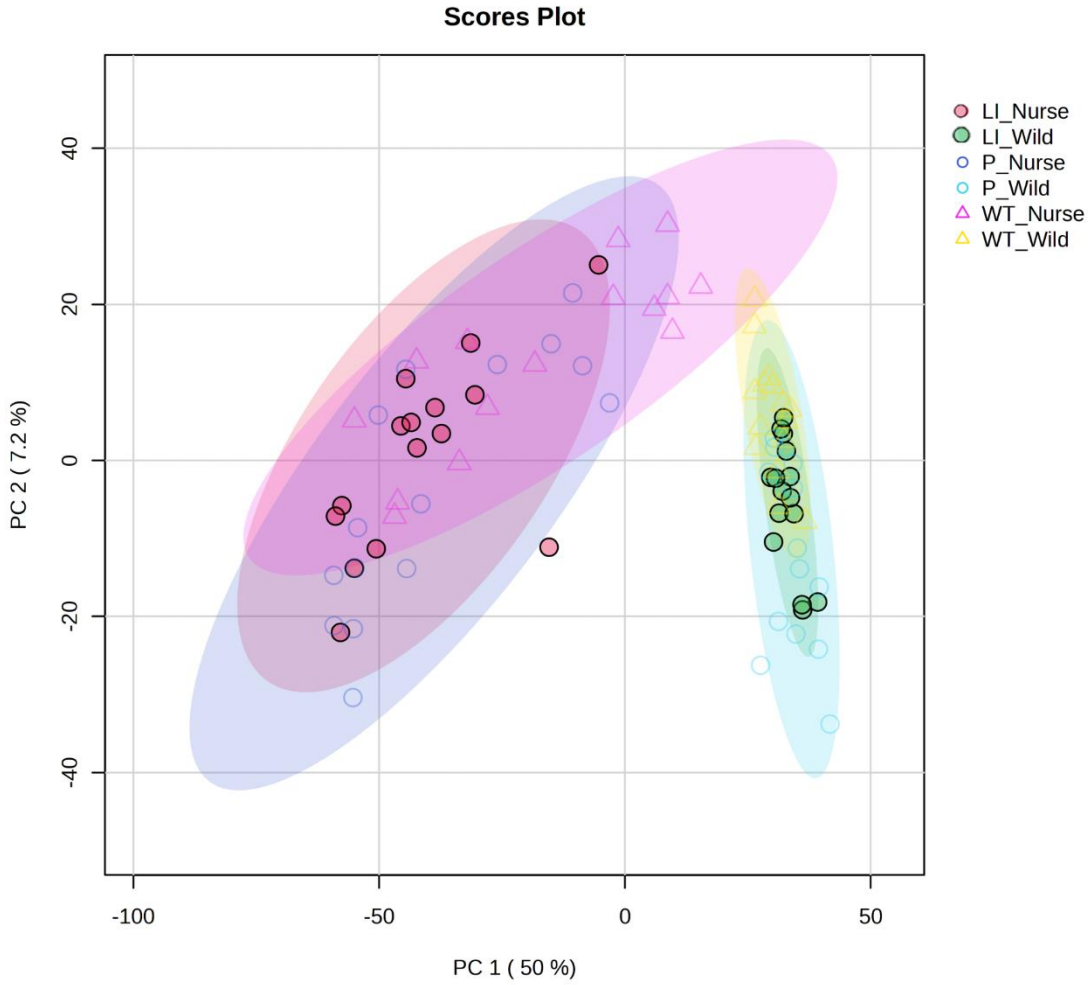

Figure 24. *Principal Component Analysis (PCA) Scores Plot Showing Metabolome Differences within D. labyrinthiformis by Original Collection location both at the original sampling and after 2 months in the Nursery. The PCA scores plot illustrates the variation in metabolome composition across three locations and two time points for six unique ellipses: Looe Inshore Nursery (solid pink circles), Looe Inshore Wild (solid green), Pickles Patch Nursery (Open Dark Blue circles), Pickles Patch Wild (Open Light Blue circles), West Turtle Nursery (Open Pink Triangles) and West Turtle Wild (Open Yellow Triangles). Each point represents an individual sample, and ellipses encompass 95% confidence intervals for each group. The first principal component (PC1) explains 50% of the variance, and the second (PC2) explains 7.2%.*

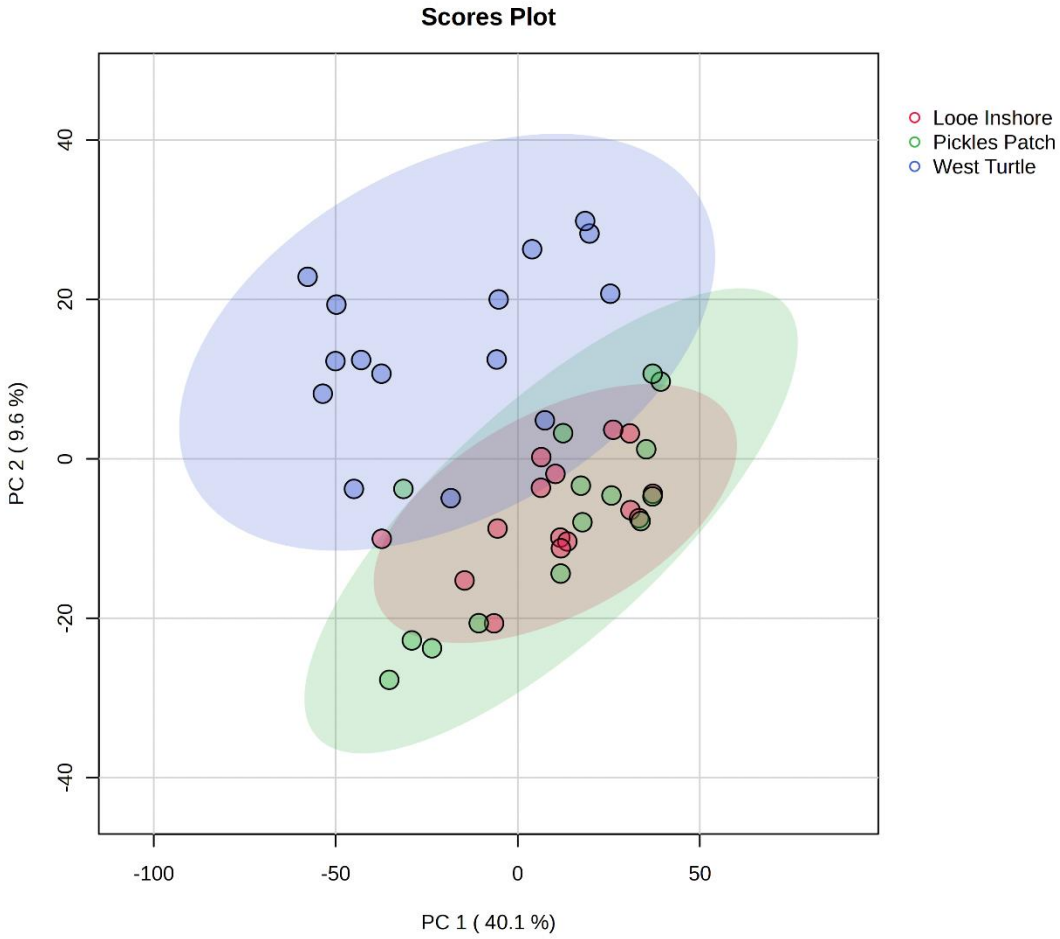

Figure 25. *Principal Component Analysis (PCA) Scores Plot Showing Metabolome Differences within D. labyrinthiformis by Original Collection location after 2 months in the Nursery. The PCA scores plot illustrates the variation in metabolome composition across three locations: Looe Inshore (pink), Pickles Patch (green), and West Turtle (blue). Each point represents an individual sample, and ellipses encompass 95% confidence intervals for each group. The first principal component (PC1) explains 40.1% of the variance, and the second (PC2) explains 9.6%.*

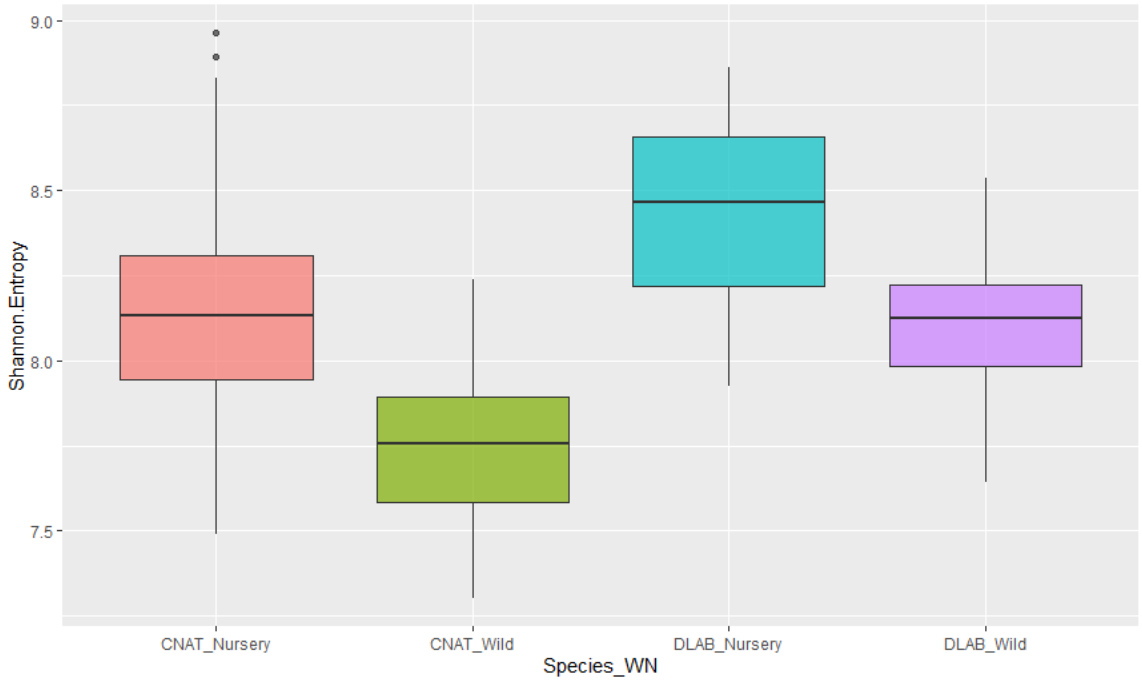

Figure 26. *Boxplots of Shannon entropy by species and nursery state. This boxplot displays the distribution of Shannon entropy values—a measure of alpha diversity—across coral species at first collection and after two months in the nursery; CNAT Nursery (pink), CNAT Wild (green), DLAB Nursery (blue), DLAB Wild (purple). Each box represents the interquartile range (IQR) with the horizontal line indicating the median. Whiskers extend to 1.5 times the IQR, and outliers are shown as individual points. Shannon entropy values indicate that differences between species and wild versus nursery are statistically significant.*

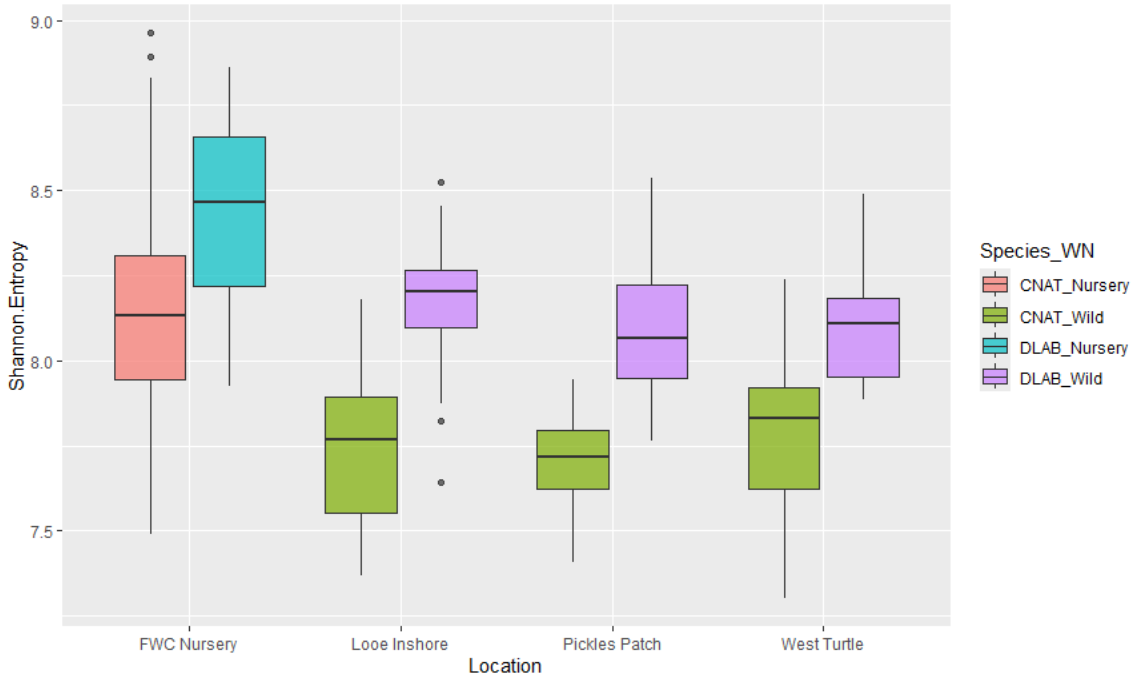

Figure 27. *Boxplots of Shannon entropy by species, sampling location (Nursery vs Wild), and collection location. This boxplot displays the distribution of Shannon entropy values, a measure of alpha diversity, by coral species and sampling location. The sampling location is displayed along the x-axis and color coded by species. All nursery samples are combined into two boxes, CNAT Nursery (pink) and DLAB Nursery (blue), while all original CNAT Wild (green) and DLAB Wild (purple) are separated out by location (Looe Inshore, Pickles Patch, and West Turtle. Each box represents the interquartile range (IQR) with the horizontal line indicating the median. Whiskers extend to 1.5 times the IQR, and outliers are shown as individual points. Higher Shannon entropy values for D. labyrinthiformis samples indicate greater diversity compared to C. natans.*

4. DISCUSSION AND MANAGEMENT RECOMMENDATIONS

4.1. Discussion

The project aimed to understand how the translocation of presumed resilient corals from a reef to an in-water nursery affects the coral microbiome and metabolomics and the resilience and resistance that comes with those characteristics. Additionally, we aimed to identify microbiome and metabolomic characteristics that may correlate with higher survival, growth, and resilience.

Based on routine monitoring we were able to determine that there were distinct genotypic differences in survival within the coral nursery. We also saw some differences based on collection location. Specifically, corals of both species originally collected from West Turtle in Marathon had the lowest percent survival. This finding was unexpected due to this site being geographically closest to the coral nursery and therefore presumed to be the most similar in terms of environmental conditions. In addition to the poorest performing genotypes having been collected from West Turtle, the *C. natans* with the highest mortality were also the largest

coral collected. These larger corals can also be assumed to be older as compared to the smaller corals which could contribute to the differences seen. Older corals may be less inclined to grow which may have contributed to higher rates of biofouling on the coral fragments and lower survival. The exact nature of this relationship is unknown and warrants further study. In addition, because five of the six largest *C. natans* were collected from West Turtle it is not possible to differentiate the effects of collection location and the size of the coral when collected.

Analysis of the microbiome of these corals both at initial collection and after 2-months in the nursery yielded some trends but did not directly link to survival. Overall, we detected similar microbial communities in the propagates of two brain corals, *C. natans* and *D. labyrinthiformis.* Microbial composition was only slightly (<2%) influenced by the coral species and timepoint. Instead, variation in microbial composition was explained by the interaction of colony and time. In addition, microbial composition did not vary based on the original collection site, suggesting that the microbial community may be curated more by the coral host than by prevailing environmental conditions. These analyses indicate that specific microbial taxa changed over time in a colony-dependent manner. Overall, the microbiome of *C. natans* and *D. labyrinthiformis* was relatively stable over the two-month time period between samplings which may indicate that any resilience that the microbiome conveys to changing conditions, such as bleaching, may persist.

Metabolome analysis showed more distinct trends compared to those shown in microbiome analysis. Analysis of initial samples showed that both *C. natans* and *D. labyrinthiformis* collected from Pickles Patch were distinctly different from those collected from West Turtle and Looe Inshore. Reasons for this may include the geology of the Florida Keys and the age of the corals collected. Pickles Patch is in the Upper Keys which means that this reef is less influenced by inputs from the Gulf side as compared to West Turtle and Looe Inshore, both of which are close to channels between the Gulf side and Ocean side waters. In addition, the corals of opportunity that we could collect at Pickles Patch were small compared to the other two sites, especially West Turtle. Again, this may indicate that these corals are of a different age which may influence metabolomics.

After two months in the coral nursery, there were significant changes in metabolomes. Overall, there was a much larger variance after being in the coral nursery and a higher Shannon diversity index. Importantly, as it relates to the initial goal of determining the relationship between survival and metabolomics, colonies originally collected from West Turtle clustered out from those collected from the other two sites. Further analysis into the specific components that led to this clustering may help us better understand the metabolome helps a genotype to survive in an in-water coral nursery.

The next step will be to see how different coral genotypes respond to outplanting both in regard to growth and survival and how the microbiome and metabolome may or may not shift. In addition, we will continue to monitor these corals in the nursery as they experience their first summer since collection.

4.2. Management Recommendations

- Survival trends during propagation and grow-out were influenced by genotype and collection location. Therefore, collection of wild corals of opportunity from a broad area along the reef tract is recommended to enhance genetic diversity of biomass produced for restoration, which allows for the selection of genotypes demonstrating higher survival probability.
- Coral microbial communities were more variable between genotypes than collection locations, further indicating that the incorporation of greater genetic diversity during propagation may help to increase the persistence of resilient microbiomes within nursery stocks for restoration.
- Coral metabolomes can be used to identify metabolites linked to resilience and survival. Further investigation into the role of specific metabolites in growth and survival is needed to identify potential health indicators for upscaling the propagation of resilient corals for restoration.
- Given the potential application of microbiome and metabolome analysis in nursery-to-reef restoration processes and the paucity of quantitative health metrics available for the majority of the threatened stony coral species propagated for restoration, we recommend consideration for these tools in the development of region-wide strategies and priorities for the restoration of FCR. Initially applied to help identify coral disease, these tools can similarly be used to select healthy and resilient coral stock for improved restoration success.

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