Methods to improve the success of SCTLD-susceptible coral species outplanted for restoration: From lab to nursery to reef

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Final Report

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List of Acronyms

Dlab: *Diploria labyrinthiformis*
Pcli: *Pseudodiploria clivosa*
Cnat*:* *Colpophyllia natans*
Mcav: *Montastraea cavernosa*
SCTLD: Stony Coral Tissue Loss Disease

Executive Summary

In this project, we evaluated methods to increase the growth and survivorship of fragments and recruits of four species of SCTDL-susceptible species (*Diploria labyrinthiformis* (Dlab), *Pseudodiploria clivosa* (Pcli), *Colpophyllia natans* (Cnat), *Montastraea cavernosa* (Mcav)) commonly used for restoration in Florida. The growth and survivorship of Dlab and Mcav were enhanced by feeding while ex situ prior to outplanting, but feeding did not influence fish predation rates. The benefits of feeding on growth and survivorship were higher for smaller corals. Coral size had a positive influence on survivorship both ex situ and after outplanting, indicating that keeping corals within nurseries until they reach a threshold size of at least 4 cm in diameter is highly beneficial. Feeding corals ex situ allows for this size threshold to be reached faster. We did not find a direct relationship between feeding and lipid or protein content but a larger sample size or longer feeding periods may be needed to detect such changes. Both Mcav and Dlab showed high survivorship both in situ and ex situ. We found that when growing corals from larvae, the optimum number of recruits to be reared on a single plug is 2-10. This density maximizes both survivorship and the number of surviving genotypes. Through the use of specially designed cement coral bases, we concluded that protection from fish predation is only efficient when fish are prevented from reaching the outplanted Pcli corals through the use of plastic canopies or by embedding the coral fragments 1 cm into the base. The treatments that only provided partial physical protection were ineffective at reducing predation. Lastly, fish predation was not reduced by deploying Dlab in sheltered reef locations or by attaching Cnat right next to unpalatable reef taxa like sponges or zoanthids.

Objectives:

The overarching goal of this research was to streamline and enhance the efficiency of the pipeline that takes colonies of SCTLD-susceptible and other priority species from nursery to reef by testing best practices that may enhance coral success once outplanted and advance our ability to restore populations of SCTLD-susceptible coral species throughout Florida's Coral Reef. The outcomes of this project will be incorporated into an on-going coral disease response effort which seeks to improve understanding about the scale and severity of the coral disease outbreak on Florida's Coral Reef, identify primary and secondary causes, identify management actions to remediate disease impacts, restore affected resources, and ultimately prevent future outbreaks. *Diploria labyrinthiformis*, *Pseudodiploria clivosa*, *Colpophyllia natans*, and *Montastraea cavernosa* (the species used in this project) have been identified by the State of Florida as priority species for propagation and restoration in the "State of Florida Restoration Priorities for Florida's Coral Reef: 2021-2026" report.

Here, we aimed to understand the minimum amount of time and care corals need while being grown ex situ before they are outplanted, as well as understanding the role that colony size and nutritional status play on coral survivorship, growth, and susceptibility to fish predation after outplanting as important steps towards improving the efficiency and cost of the restoration

pipeline. This proposed project was driven by research priorities identified by three working groups (Nursery Propagation, Coral Outplanting Design, Predation) that are part of the SCTLD Restoration Team (RT) (Figure 1).

Goals:

- Evaluate tradeoffs of growing the SCTLD-susceptible species, *D. labyrinthiformis* and *M. cavernosa*, in ex situ vs. in situ nurseries (Task 1, Task 2, Task 4.1, Task 4.2, Task 5).
- Determine whether separating out individual genets (sexual recruits), or leaving aggregated settlers on substrates, is a better strategy for increasing survival, promoting growth and reducing time to outplant (Task 4.2).
- Assess the influence of pre-outplant acclimation on the growth and survivorship of outplanted coral colonies (Task 1, Task 2, Task 5).
- Assess the influence of feeding and environment (lab vs nursery) on lipid tissue content (Task 3).
- Test the influence of substrate orientation on the survival of outplanted coral colonies (Task 6.3).
- Assess how pre-outplant acclimation (including tissue metrics like lipid content) affects predation on outplanted coral colonies (Task 2, Task 3).
- Test the ability for novel physical predation mitigation structures to reduce predation on outplanted coral colonies (Task 6.2).

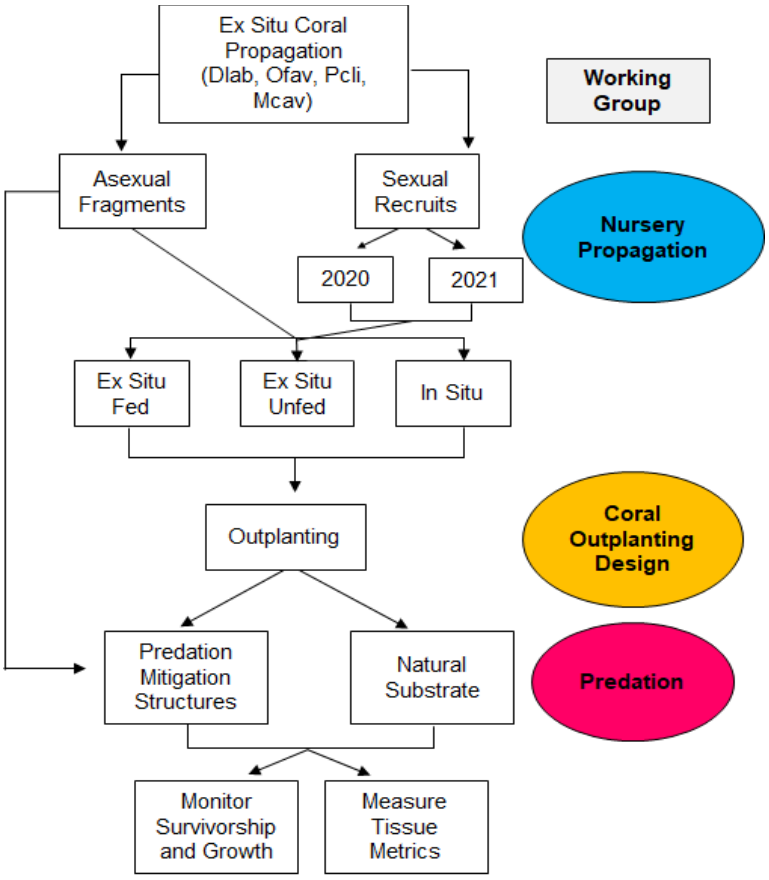

Figure 1. Conceptual diagram of proposed project that integrates top research priorities from the Nursery Propagation, Coral Outplanting Design, and Predation working groups.

Tasks and Research Outcomes

Task 1 (Florida Aquarium): Provide Pre-Conditioned Corals for Outplanting Experiments

The Florida Aquarium (FLAQ) provided 900 colonies of the coral *Diploria labyrinthiformis* (Dlab) grown from lab-spawned larvae for outplanting in this project. Corals were pre-conditioned under three treatments: 1) One third (300) of the corals were kept at FLAQ and fed a diet of premade liquid and powdered invertebrate food for > 3 months, 2) one third (300) were kept at FLAQ but were not supplemented with any food for > 3 months, and 3) one third (300) of the corals were sent to the 3 partners (UM, NSU, MOTE) and deployed (100 each) at in situ nurseries for > 3 months. Half of the recruits were grown from the 2020 spawn and the other half from the 2021 spawn. Corals for the in situ nurseries were delivered to partners in November 2022. Pre-conditioned corals were delivered to partners at NSU (Drs. Figueredo and Gilliam), UM (Dr. Lirman), and Mote (Dr. Muller) in February 2022. FLAQ completed all nursery-transfer paperwork and conducted a veterinary inspection prior to delivery of the corals. Scaled photographs of the 900 Dlab colonies were taken before initiating conditioning treatments, upon deployment to partner in situ nurseries, and after >3 months under treatment conditions. These

photographs were processed by NOAA to quantify live tissue area of Dlab colonies under the three different experimental treatments.

In addition to the 900 Dlab colonies, FLAQ delivered 500 *Colpophyllia natans* (Cnat) colonies spawned in 2020 that were used in Task 6 to evaluate methods to mitigate fish predation by deploying predation deterrent taxa adjacent to corals. These corals, ranging in maximum size between 3-4 cm were delivered to the University of Miami on 4/4 and kept within indoor tanks until outplanting. The corals were split into 2 groups of 250 colonies each and one set was outplanted onto a Miami reef on 4/8 (UM) and another set was outplanted onto a Key Largo Reef (NOAA) on 4/15.

Results

Prior to delivery to the partners for outplanting, corals from the 2020 cohort had 100% survivorship during the ~3 month acclimation period, regardless of acclimation treatment (Figure 2). Corals from the 2021 cohort had lower survivorship. The lowest survivorship for 2021 corals was for the in situ (93%) and Unfed (90%) treatments, whereas the highest was in the Fed treatment (95%).

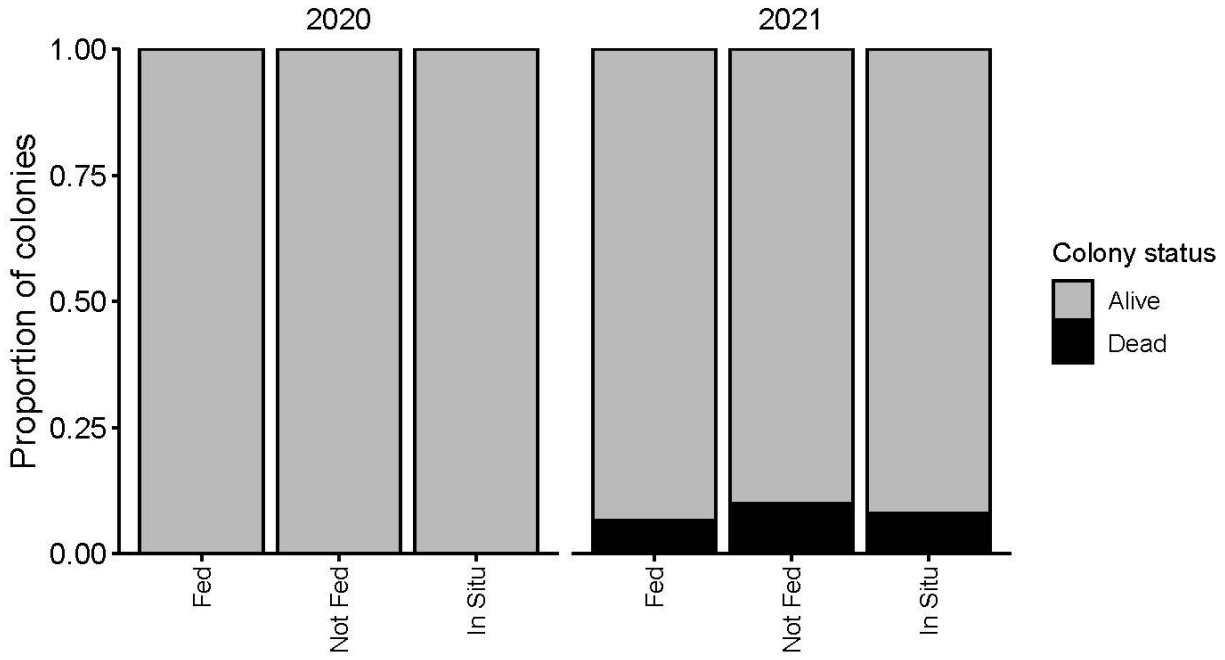

Figure 2. Proportion of colonies alive (gray) and dead (black) for 2020 and 2021 cohorts of *Diploria labyrinthiformis* acclimated under different conditions.

Growth of corals conditioned by FLAQ varied widely between cohorts and among treatments within each cohort (Figure 3 left panel). Coral colonies from the 2020 cohort acclimated at the ex situ facility and fed grew faster than any other acclimation treatment (mean ± SE; 0.04 ± 0.001 cm^2 day^{-1}) and 6x faster than 2020 cohort corals acclimated at in situ coral nurseries (0.006 ± 0.002 cm^2 day^{-1}). Coral colonies from the 2021 cohort that were acclimated at the ex situ facility and not fed had the lowest growth rate (0.002 ± 0.0001 cm^2 day^{-1}), though this did not differ statistically from 2021 Not Fed corals.

Percent change in live area also differed among treatments and cohorts (Figure 3 right panel). Corals from the 2021 cohort had higher increases in live tissue area compared to 2020 cohort corals regardless of what treatment they were acclimated under. However, within the 2021 cohort, corals acclimated at the ex situ facility and fed increased in live tissue area ~2.5x more than corals acclimated under the other two treatments (452 ± 29% vs. 178 ± 12%; 2021 – Not Fed and 176 ± 25%; 2021 – in situ). Corals from the 2020 cohort that were acclimated at in situ coral nurseries had the lowest percent increase in live tissue area among all the cohort x treatment combinations.

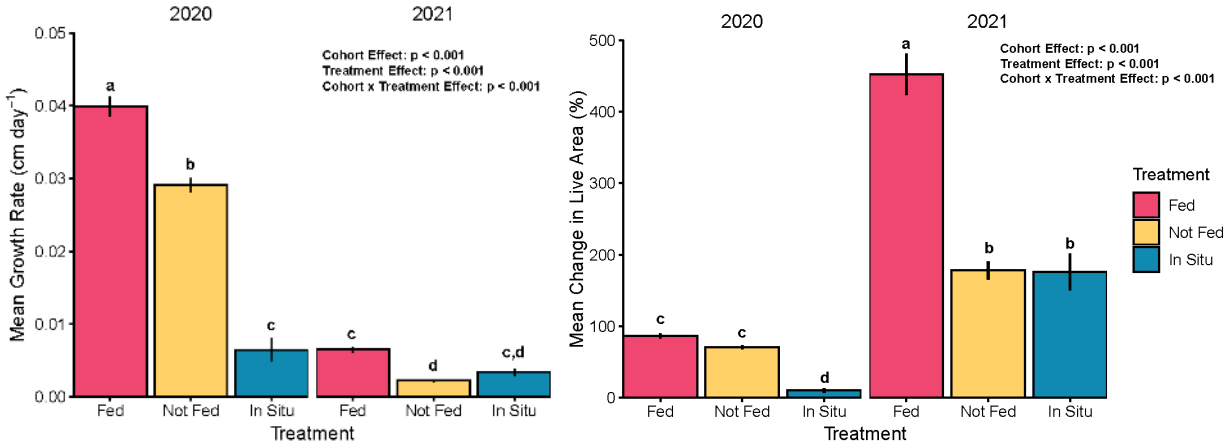

Figure 3. (Left) Mean growth rate (cm^2 day^{-1}) of *Diploria labyrinthiformis* colonies acclimated for 3 months under different conditions at ex situ facilities (Fed and Not Fed) and in situ nurseries (n = 150 per treatment x cohort combination). (Right) Mean percent change in live tissue area of *D. labyrinthiformis* colonies acclimated for 3 months under different conditions at ex situ facilities (Fed and Not Fed) and in situ nurseries (n = 150 per treatment x cohort combination).

Mean growth rates of corals acclimated at in situ coral nurseries differed among cohort x partner combinations (Figure 4 left panel). Corals from the 2020 cohort acclimated at the Mote in situ nursery had the highest growth rates, followed by corals acclimated at the UM in situ nursery. Surprisingly, corals from the 2020 cohort acclimated at the NSU in situ nursery displayed negative growth rates. This was likely due to high predation rates of these corals at the in situ nursery at this location. Patterns in the percent change in live tissue area (Figure 4 right panel**)** also varied among cohort x agency combinations. Similar to the overall acclimation treatment patterns (Figure 3 right panel) corals from the 2021 cohort had the largest increase in mean percent change live tissue area, likely due to their small size at the start of the experiment. Corals acclimated at the NSU in situ nursery displayed either negative (2020 cohort) or no (2021 cohort) mean percent change in live area over the course of the 3-month acclimation period.

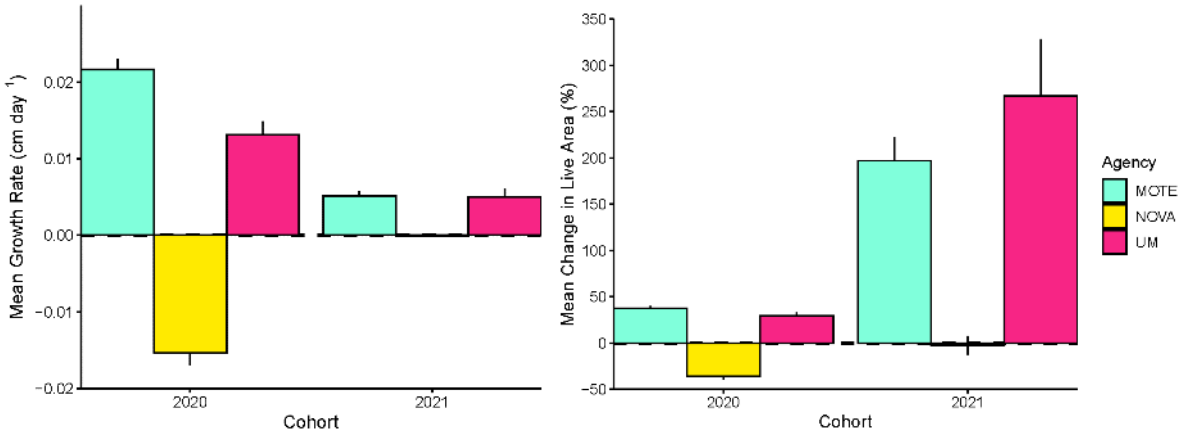

Figure 4. (Left) Mean growth rate (cm^2 day^{-1}) of *Diploria labyrinthiformis* colonies acclimated for 3 months at each of the three in situ coral nurseries operated by project team members Mote Marine Laboratory (Mote; teal), Nova Southeastern University (NOVA; yellow), and the University of Miami (UM; pink). (Right) Mean percent change in live tissue area of *D. labyrinthiformis* colonies acclimated for 3 months at each of the three in situ coral nurseries operated by project team members. n = 50 per agency x cohort combination.

Task 2: (All partners) Outplanting of Conditioned Corals, Dlab

900 *D. labyrinthiformis* (henceforth referred to as "Dlab") corals from 2 cohorts (2020, 2021) were reared under 3 treatments for a period of 3 months prior to outplanting: (1) ex situ + food, (2) ex situ + no food, and (3) in situ nursery. Corals in the (1) ex situ + food and (2) ex situ + no food treatments were grown at the Florida Aquarium. Corals for the in situ nursery treatment were distributed to in situ nurseries run by Nova Southeastern University (Dr. D. Gilliam), the University of Miami (Dr. D. Lirman), and Mote Marine Laboratory (Dr. E. Muller, Dr. H. Koch). After 3 months of conditioning, the colonies grown under each acclimation treatment were outplanted to reefs in Broward, Miami-Dade, and Monroe Counties, onto a single reef within each region. All corals conditioned at FLAQ arrived in excellent condition and were outplanted without any signs of partial mortality in February 2022.

Permits and Site Selection: All permits for outplanting were secured or renewed by partners, with species added to existing permits as needed. One site was selected in each region (Broward, Miami, and Monroe) based on the rationale provided by each partner (see "Sites" file provided as part of Report 1 for a description of the rationale for site selection; Appendix A).

Experimental Design: Corals were outplanted in plots containing six corals each arranged in a 3x2 grid. Each plot contained one coral from each of the cohort (2020 or 2021) x acclimation (ex situ fed, ex situ unfed, or in situ) treatments. Corals within a plot were secured to the reef substrate using cement or epoxy and were placed 15 cm apart and plots were separated by at least 1 m. Plots at Miami and Broward sites were arranged along multiple linear transects as needed to keep all plots within the same reef type. Plots at Monroe County were deployed in a high-rugosity patch reef site on top of dead massive coral colonies. Within plots, corals were deployed in one of 9 predetermined orientations to randomize the position of the different cohorts x treatments corals within plots. Colonies have been tracked individually from the grow out period at FLAQ all the way to outplanting to track individual growth and mortality using photographs. The number of Dlab corals outplanted, excluding the corals sent to FSU for analyses (n = 151), were: NSU = 245

(129 from 2020 and 116 from 2021), UM = 257 (133 from 2020 and 124 from 2021), Mote = 250 (133 from 2020 and 117 from 2021). Total number of corals outplanted in Feb 2022 = 752.

Results

Predation intensity on outplanted corals varied widely among outplant regions and surveys (Figure 5). In the Lower Florida Keys (Mote), the number of corals with bites was highest in week 2, and by Month 3 only corals from the 2021 cohort had been completely removed. In Broward County (NSU), little predation was observed in the first 4 weeks, with <25% of colonies in any cohort x treatment combination being removed or bitten. In Miami-Dade County (UM), predation was extremely high, with 50-75% of corals removed or bitten within the 24 hours of deployment across all cohort x treatment combinations. At the UM site, >75% all cohort x treatment corals were either bitten or completely removed after one week of deployment, except for the 2021 Unfed treatment, which had the lowest predation at this site. The proportion of colonies with new bites decreased at Week 2 and Month 1 and remained low at Month 2 and Month 3. Across all three regions, a higher proportion of 2021 cohort corals were completely removed compared to 2020 cohort corals.

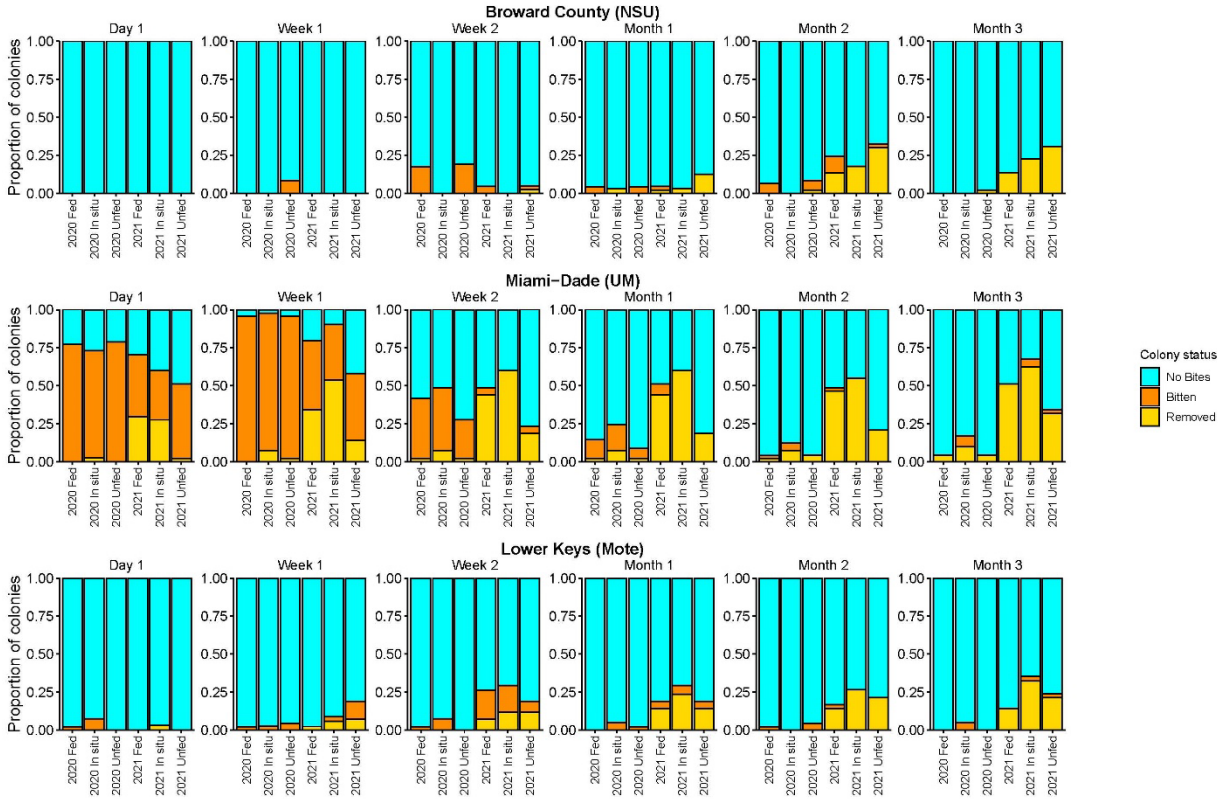

Figure 5. Proportion of outplanted *Diploria labyrinthiformis* colonies recorded as either having no bites (blue), at least 1 new bite from a predator (orange), or completely removed (yellow) at each survey for each outplant site.

Similar to predation intensity, survivorship of outplanted Dlab colonies varied among regions, treatments, and cohorts (Figure 6). Across all three regions, 2021 cohort corals had a higher proportion of colonies recorded as dead compared to the 2020 cohort corals, with the exception of 2020 in situ Dlab in Broward County (NSU), which had the highest mortality out of any cohort x treatment x region combination (80% mortality). In Broward County, 23-52% of 2021 cohort

colonies were dead by Month 3 compared to the UM site where 33-65% of corals from the 2021 cohort were dead after 3 months. Mortality was substantially lower for the 2020 cohort at the UM site, with mortality ranging from 4% (2020 Unfed) to 10% (2020 in situ) after 3 months. At the Lower Keys site (Mote), survivorship was 100% for all 2020 cohort corals outplanted. At this site, the proportion of 2021 cohort colonies recorded as dead by Month 3 ranged from a low of 14% (2021 Fed) to a high of 32% (2021 in situ).

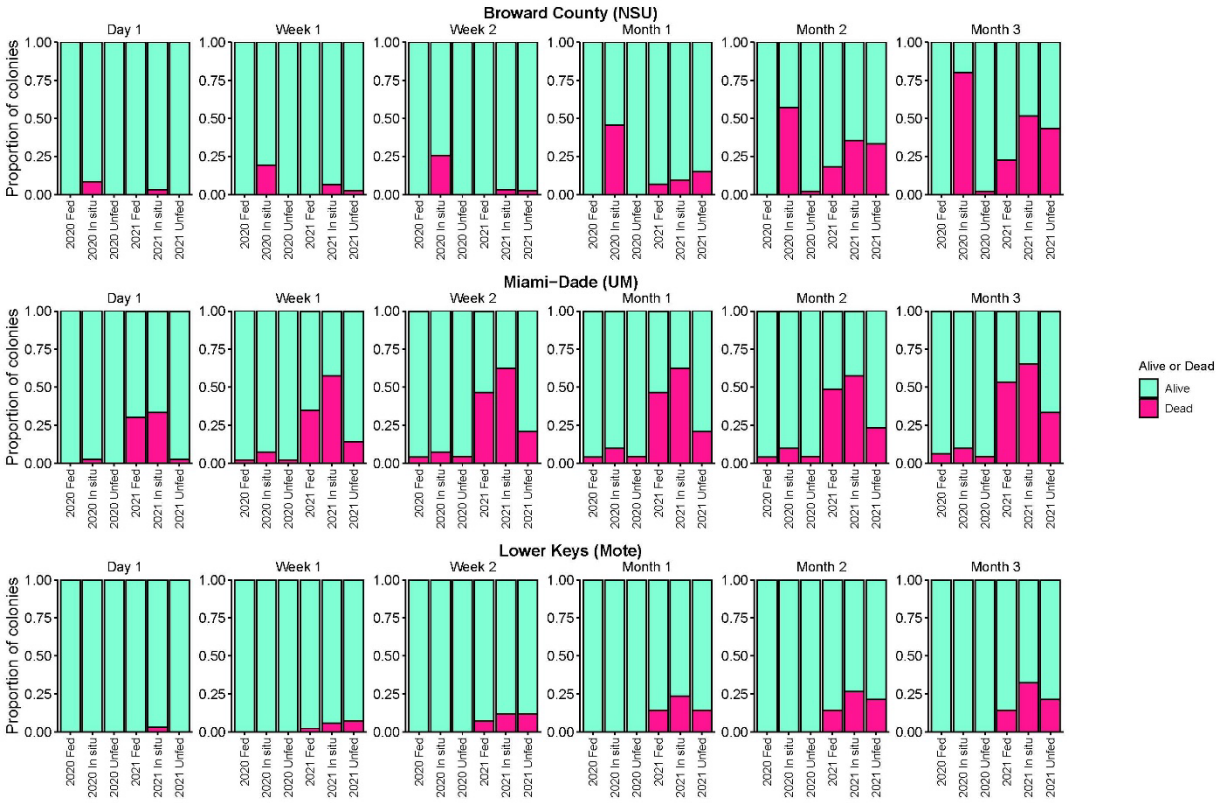

Figure 6. Proportion of outplanted *Diploria labyrinthiformis* colonies recorded as either alive (teal) or dead (pink) during each survey at each outplant site.

Growth rates of outplanted Dlab colonies varied by region, cohort, and treatment over the course of our 3-month experiment. Corals from the 2020 cohort typically had higher growth rates compared to 2021 cohort corals (Figure 7). For 2020 cohort corals outplanted to the Lower Keys (Mote), all treatments displayed positive growth, though Not Fed corals grew at least 2x slower than corals acclimated under Fed or In Situ treatments. Growth rates of 2020 cohort corals outplanted to Broward (NSU) varied widely among acclimation treatments. Fed corals grew fastest, nearly 4x faster than Not Fed corals, whereas In Situ corals had extremely variable growth rates. In Miami (UM), Fed and Not Fed corals from the 2020 cohort displayed negative growth rates due to intense predation, while In Situ corals had variable growth rates. Dlab colonies from the 2021 cohort outplanted to the Lower Keys (Mote) displayed similar patterns in growth rates among acclimation treatments as the 2020 cohort outplanted to this location (Figure 7). In Broward (NSU), 2021 cohort colonies acclimated under Fed and In Situ treatments appeared to have positive mean growth rates over the course of 3 months, whereas Not Fed 2021 cohort corals had negative mean growth rates, suggesting predation or other mortality influenced these corals more than other treatments. In Miami, Fed corals from the 2021 cohort had the highest growth rate on

average, though this was extremely variable. Not Fed corals did not demonstrate measurable growth, whereas in situ corals had negative growth, again likely due to intense predation at this site.

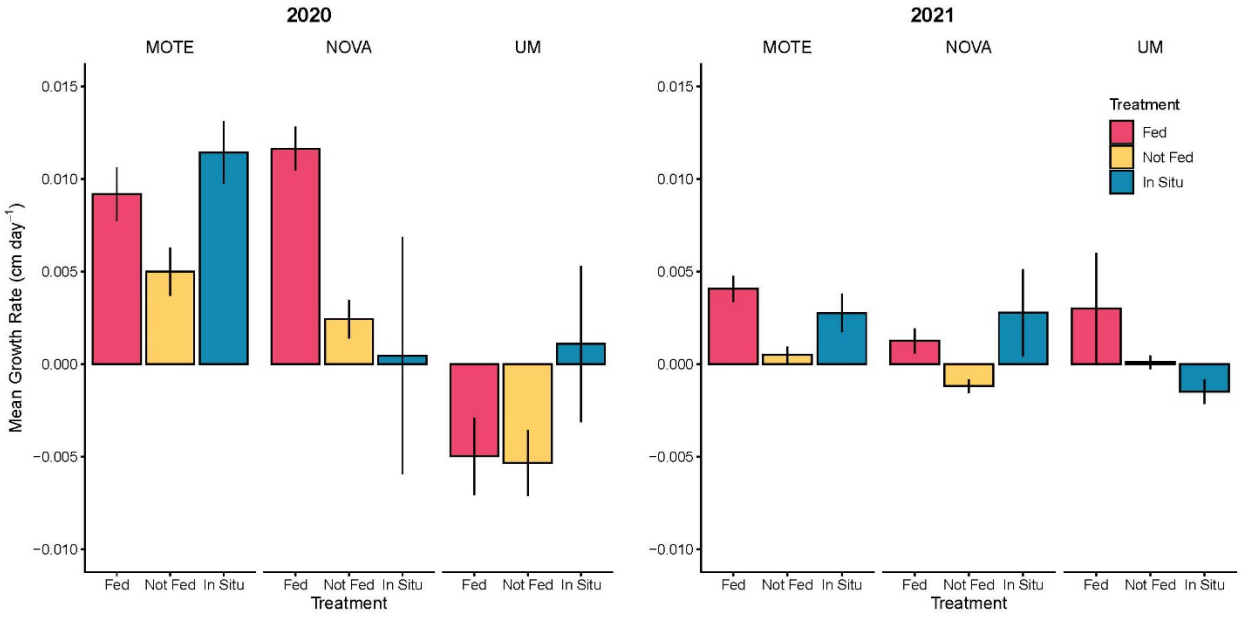

Figure 7. Mean growth rate (±SE , cm^2 day^{-1}) of *Diploria labyrinthiformis* colonies from the 2020 cohort (left) and 2021 cohort (right) after 3 months of being outplanted in each region. Treatments represent the conditions each coral was acclimated under prior to outplanting (Red = Fed; Yellow = Not Fed; Blue = In Situ).

Task 3 (FSU): Measuring Tissue Properties of Conditioned Corals

Previous studies have found that, when outplanted, corals reared at ex situ nurseries are targeted by predators at a higher rate than corals which remained in the ocean. To understand if- and how coral tissue metrics correlate with coral survival and predation intensity, protein and lipid content were quantified for *D. labyrinthiformis* corals prior and after conditioning. *Diploria labirinthiformis* (Dlab) samples (n = 151) were shipped from all project partners to the Shantz lab at Florida State University's Coastal and Marine Lab (CML). Samples were shipped frozen, overnight on dry ice and arrived at the CML still frozen, with the final samples arriving from Mote Marine Lab on February 25th. At the CML, the corals were inventoried and photographed, and immediately stored at -80 C to await preparation and analysis. Because accurate protein and lipid quantification requires processing the colonies in toto, epiphytes, fouling organisms, and external substrate and epoxy were removed before processing. After cleaning and epoxy removal, the colonies were sectioned for the different types of analyses (quantification of Symbiodiniaceae density versus protein and lipid content). All the samples from the 2021 cohort are too small to provide sufficient tissue for analyses of all three physiological metrics. Therefore, 2021 samples were pooled by treatment and rearing location for analysis of average protein and lipid content.

Results

No significant differences in protein (2020 cohort) or lipid content (2020 and 2021 cohorts) ($p > 0.05$) were documented for Dlab corals prior to conditioning. Samples did not provide enough tissue to assess protein content in the 2021 cohort due to small coral sizes. After conditioning (3 months), the total protein content differed significantly among nurseries and treatments ($p < 0.001$). Colonies reared ex situ at FLAQ had the highest protein content, colonies reared in situ at NSU had the lowest total protein content, and UM and MML corals reared in situ contained intermediate protein levels (Tukey's HSD post hoc comparisons; Figure 8 left panel). Lipid content in the 2020 cohort averaged 23.97 ± 10.62% of tissue weight, was highly variable, and did not differ significantly among treatments ($p > 0.61$) (Figure 8). In contrast, lipid content in the 2021 cohort differed significantly among treatments and nurseries ($p = 0.04$). Colonies from the UM and MML in situ nurseries had significantly higher lipid reserves than those grown at the NSU in situ nursery (Figure 8 right panel). Corals conditioned at FLAQ and fed also had larger lipid reserves (19.85 ± 4.16%) than those that were unfed (9.08 ± 1.06%), but this difference was not statistically significant.

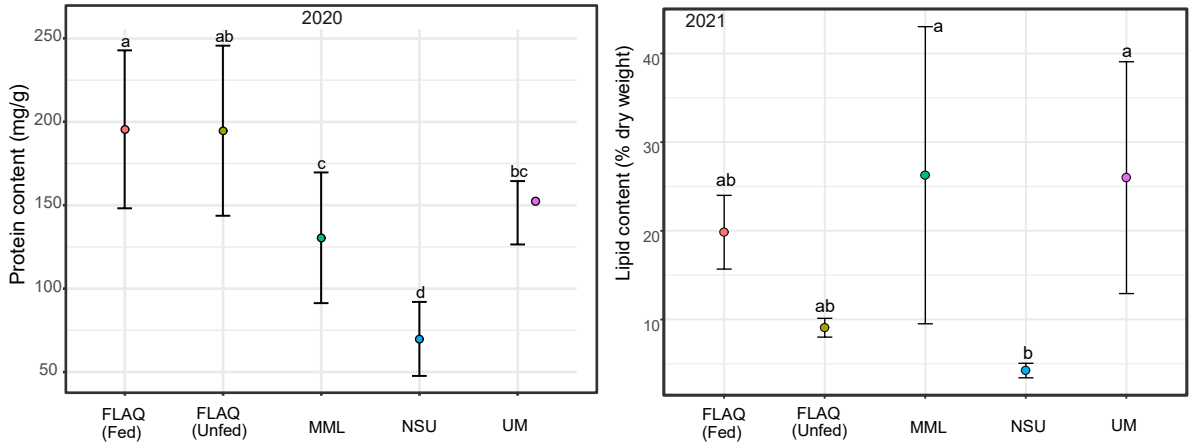

Figure 8: Average (± 1 SD) protein (left panel, 2020 cohort) and lipid (right panel, 2021 cohort) content from corals reared under different conditions. Lowercase letters above treatments signify significant differences detected by Tukey's HSD post hoc analysis. Corals from UM, MML, and NSU were reared within in situ nurseries in each region.

Task 4 (Mote): Growth and survival of Dlab within in situ and ex situ nurseries

Task 4.1: To evaluate tradeoffs of growing *Diploria labyrinthiformis* in ex situ vs. in situ nurseries, 90 coral genets originally spawned and settled in 2020 by FLAQ were fragmented into two pieces and remounted onto new plugs. A representative of each genet remained within Mote's land-based nursery and a representative of each genet was placed within Mote's offshore nursery. Corals were fragmented and deployed to their respective nurseries on 2/19/2022. Measurement photos were taken prior to deployment and at the conclusion of the three month growth period. At the end of the three month study, surface area measurements were taken for all remaining corals using ImageJ analysis. Visual monitoring was also conducted at 1 month intervals to assess survival and general health conditions.

Results

Survival of Dlab in both nurseries was very high (94%) (Table 1). In the field nursery, 148 of 149 corals survived (99.33% survival). In the land-based nursery 132 of 149 corals survived (88.6% survival). At the conclusion of the 3-month study, one coral was lost in the in situ nursery due to falling off of the tree between T2 and T3. Seventeen corals were lost in the ex situ nursery due to an ciliate-related tissue recession event between T2 and T3. As such, these corals were removed from the measurements data.

Table 1. Inventory of the *D. labyrinthiformis* fragments used within the present study at the conclusion of the experiment.

Nursery	2 Replicates	1 Replicate	Single	Total # Corals	# Corals at T3
Ex Situ	DLS1-DLS56	DLS57-DLS86	DLS87-DLS93	149	132
In Situ	DLS1-DLS56	DLS57-DLS86	DLS94-DLS100	149	148

While survivorship was higher in situ, growth showed the opposite patterns as the growth of the Dlab fragments was significantly higher in the ex situ nursery. A one-way ANOVA was conducted to compare the growth between the two locations (ex situ and in situ). Statistically, the growth of the *D. labyrinthiformis* fragments was significantly higher in the ex situ nursery compared with the in situ nursery ($p<0.001$). The average growth of corals housed in the ex situ nursery for 3 months was 1.7925 cm². The average growth of corals housed in the in situ nursery for 3 months was 1.1314 cm² (Figure 9).

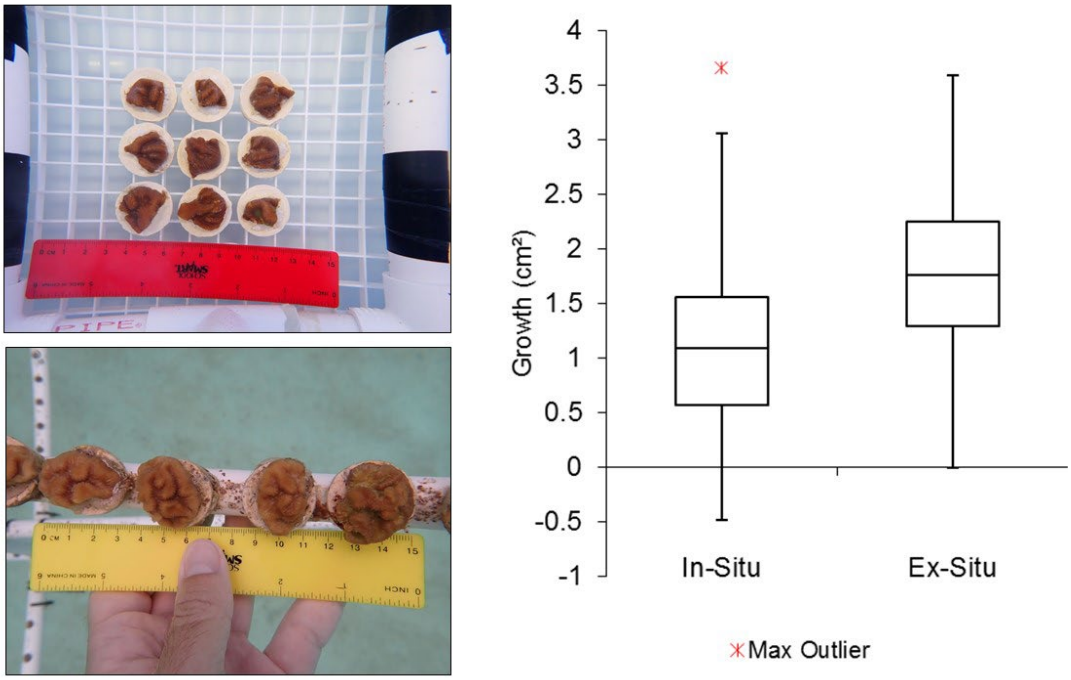

Figure 9. Images of Dlab corals deployed in the land (top left) and field (bottom left) and the land-based nursery. (Right) Average 2-dimensional growth of *D. labyrinthiformis* within the in situ and ex situ nurseries over a three month time period.

A total of 167.45 cm² of tissue grew in the in situ nursery during the 3 months. A total of 236.61cm² of tissue grew in the ex situ nursery during the 3 months. Corals housed in the ex situ nursery were fed twice a week, which could have influenced the significantly higher growth rates within the ex situ nursery compared with the in situ nursery.

There are pros and cons to both in situ and ex situ nurseries. Unknown tissue recession can occur in either type of nursery, but the higher density of corals within a land-based system can lead to health problems spreading faster. *Diploria labyrinthiformis* corals grew significantly faster within the ex situ nursery. Even with a lower survival rate in the land-based nursery and accounting for the tissue that was lost due to mortality, more coral tissue was produced over a 3 month period than in the field-based nursery. It is recommended that *D. labyrinthiformis* corals be grown in both types of environments due to potentials for disease and recession outbreaks in both settings. It would be best to grow them for production for outplanting/research in a land-based nursery while housing a subset of those genotypes within the field-based nursery as a precautionary measure to ensure genotype survival. Further studies can be conducted to compare growth rates of different species in the future to guide best practices of coral production.

Task 4.2: To determine whether separating out individual Dlab genets (sexual recruits) or leaving aggregated settlers on substrates is a better strategy for increasing survival and genetic diversity, promoting growth and reducing time to outplant. Plugs harboring 1, 2-10, or 10+ Dlab sexual recruits were transferred to Mote's offshore nursery. A complementary set of each category were maintained in Mote's land-based nursery for comparison (Figure 10).

It was predicted that having more recruits on a plug (1) reduces the surface area available for fouling (i.e., potentially less interspecific competition), which can negatively affect coral growth and health, (2) may lead to greater intraspecific competition and loss of genotypic diversity if recruits compete, and/or (3) may facilitate rapid colonization of the plug by coral (and/or chimerism) thereby reducing time to outplant. Alternatively, having one or fewer recruits on a plug (1) may take longer for the recruit(s) to cover the surface area of the plug (thereby increasing time to outplant and potential for interspecific competition), and/or (2) result in no/less intraspecific competition and reduced genotypic loss.

Results

Table 2. Results from Task 4.2 experiments

Treatment	Survivorship (Low Density)	Survivorship (Medium Density)	Survivorship (High Density)
Ex situ	20/25 (80%)	25/25 (100%)	20/20 (100%)
In situ	23/25 (92%)	25/25 (100%)	20/20 (100%)

Overall, survivorship was high (80-100%) across treatments. There was no significant difference in survivorship based on nursery type (Cox model, $p = 0.24$), but there was a significant effect of density on survival (Cox model, $p < 0.01$). The Low-density treatments (1 recruit/plug) had lower survivorship, with the ex situ / Low-density treatment having the lowest survival (80%), followed

by the in situ treatment (92%) (Table 1, Figure 11). There were significant effects of nursery type (location) and density (and no significant interaction) on coral growth (change in surface area, cm^2). The in situ treatment had a significantly greater increase in surface area compared to the ex situ treatment. The Medium-density treatments (2-10 recruits/plug) had the greatest increases in surface area, which were not significantly different from the High-density treatments (11+ recruits/plug), but were so from the Low-density treatments. Finally, for both nursery types, the Low-density treatments had the highest percentage of surviving genets, followed by the Medium- and High-density treatments, but the Medium- and High-density treatments still retained a higher final number of genets compared to the Low-density treatments. Except for the in situ Low-density treatment, all other treatments had a significant reduction in the number of surviving genets (paired t-test). However, the High-density treatments had the largest margin of loss. It appears the 'better' strategy would be to rear, in situ, substrates with more than 1 but less than 10 recruits per plug to be able to put out more diversity (than just a single recruit per plug) while still being able to retain most of that diversity and maintain high survivorship.

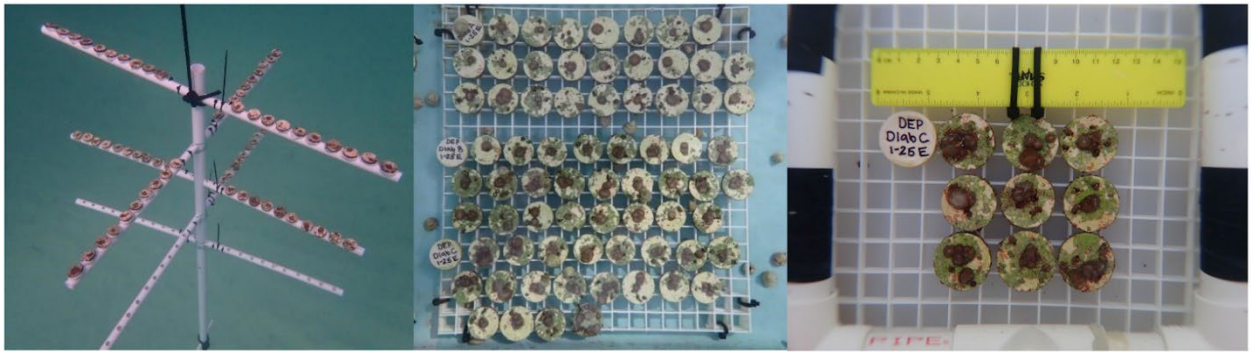

Figure 10. Images of the Dlab corals used for Task 4.2 deployed in Mote's in situ (left) and ex situ (middle) nurseries, along with a representative photograph of how the corals were imaged for growth analysis (right).

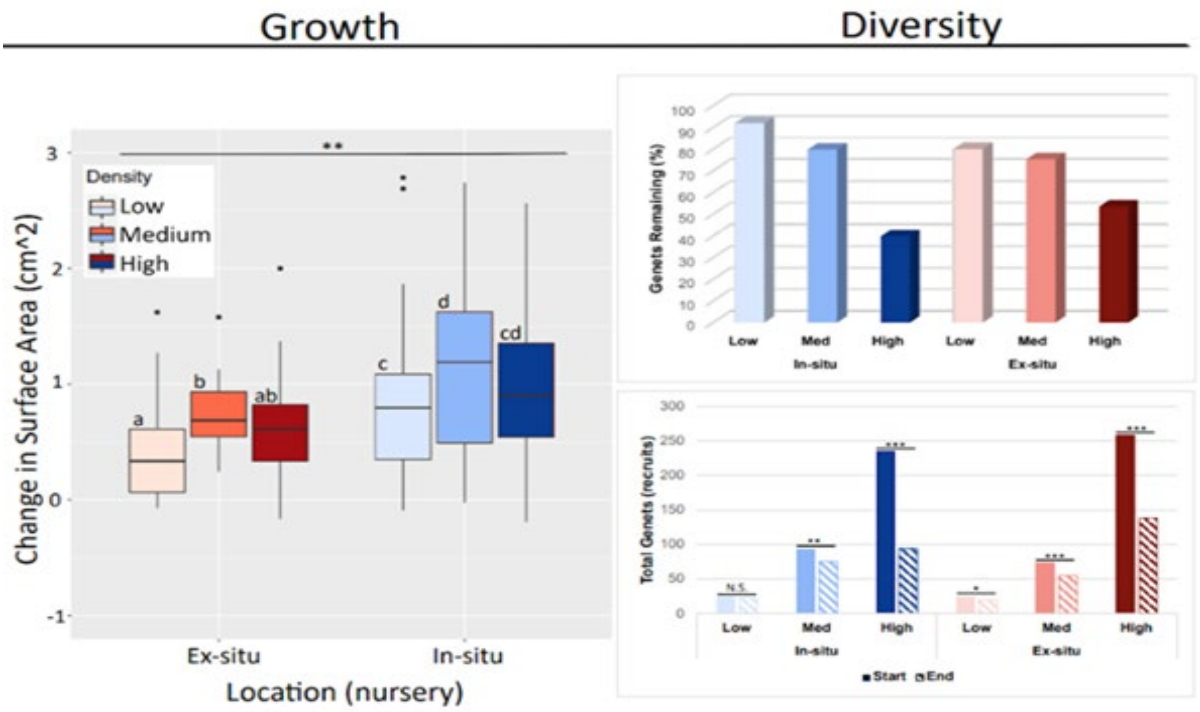

Figure 11. Growth and diversity (genets remaining (top) and total number of genets (bottom) of Dlab based on rearing location (field vs. land-based nursery) and density (number of recruits per plug).

Task 5 (NSU): Outplanting of Conditioned Mcav Corals

To determine if the feeding provided at ex situ nurseries is responsible for the higher predation levels, and ultimately to circumvent it, we tested two alternative pre-outplanting strategies on the coral *Montastraea cavernosa* (Mcav). This task used Mcav sexual recruits and asexually propagated microfragments. One year-old sexual recruits (juveniles) produced from 2020 spawning and Mcav microfragments were reared under the same three acclimation treatments as the main experiment: (1) ex situ + food, (2) ex situ + no food, and (3) in situ nursery for three months prior to outplanting. Ex situ acclimation took place at NSU's land-based facilities, and in situ acclimation was done in the NSU's offshore nursery operated by Dr. Gilliam. Corals were outplanted in February 2022 and monitored after 24-48h, one week, two weeks, one month, two months, and three months (Figure 12).

Results

Nutritional conditioning (lab, fed vs. lab, unfed vs. in-water nursery) of *Montastraea cavernosa* colonies influenced survival over the 3-month monitoring period. By the end of the 3-month monitoring period, there was a survival rate of 81.7% (Figure 13). The highest occurrence of predation occurred between the 2-week and 1-month monitoring periods (95%). Recruits accounted for 96.3% of colony mortality. The highest mortality occurred in unfed recruits (52%). *M. cavernosa* fragments that were conditioned in the nursery had the highest survival rate (87%), while corals in the unfed treatment had the lowest survival rate (74%). Recruits experienced higher predation than the microfragments, accounting for 72% of all predation recorded. Fed and unfed

ex situ treatments experienced similar predation rates (37% and 39% for juveniles, 17% and 10% for fragments, respectively).

Figure 12. Images of the Mcav corals deployed within grids (left). Images of the outplants at time of deployment (top right) and after 3 months (bottom right).

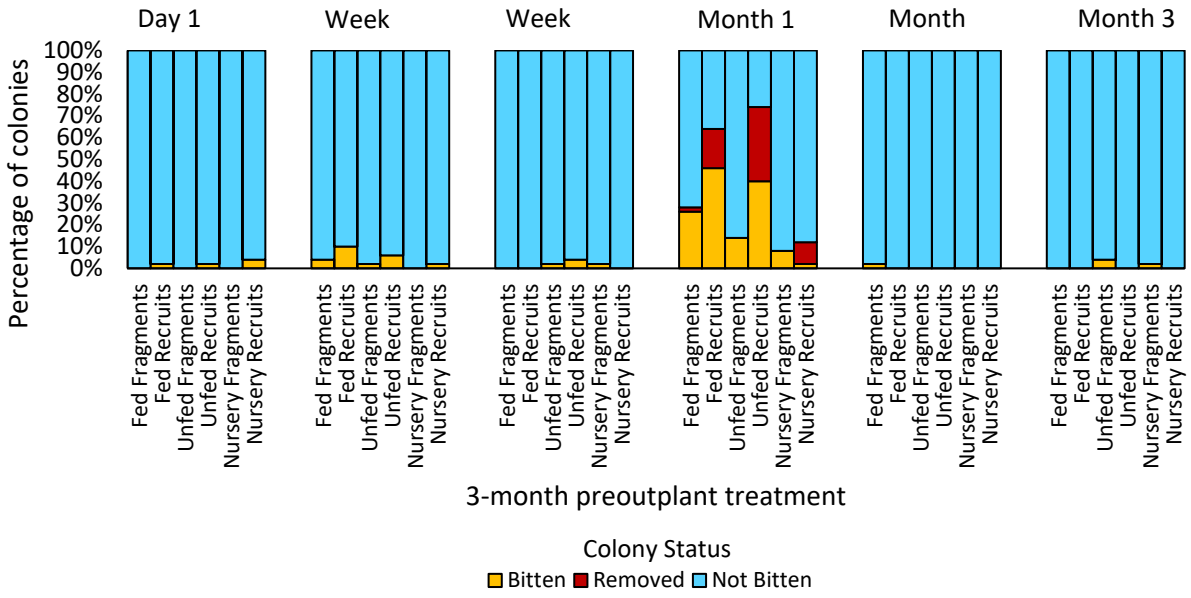

Figure 13. Percent of *M. cavernosa* colonies showing signs of predation after outplanting. Recruits exhibited higher predation rates than fragments, with unfed recruits having the highest predation rates.

Task 6 (NOAA and UM): Predation Mitigation and Predation Deterrents

Predation by fishes on outplanted colonies of massive coral species has emerged as a major bottleneck to restoration efforts. Efforts to restore colonies of SCTLD-susceptible species have recorded alarming rates of predation, with >70% of colonies being removed in some instances. Consequently, developing effective strategies to reduce predation on outplanted colonies is

essential to make outplanting massive species a feasible restoration strategy. Here, we explored the use of cement bases with physical protection (Task 6.1), bases that incorporated chemically defended reef taxa (*Palythoa* and *Amphimedon*) in proximity to coral outplants (Task 6.3), and deployed coral recruits in different orientations to examine the role of microhabitat on predation impacts (Task 6.2).

Task 6.1: We built, in partnership with Reef Cells, cement bases to be used for predation mitigation. A total of 224 Pcli fragments from a single genotype were outplanted onto cement bases. Each base received 3-4 Pcli (depending on base type) attached to Coral Loks. The 7 treatments used were: control (no protection), umbrella, embedded (coals deployed 1 cm int the base), cones, ridges, live and dead staghorn colonies (attached to the top of the bases). Bases were deployed as clusters of 7 (one per treatment), with each base separated by ~ 20 cm, and each cluster separated by at least 1 m. Corals were monitored 1 week and 1 month after deployment.

Results

The prevalence of predation was influenced by both time and the outplanting structures (Figure 14). Cones, ridges, live *A. cervicornis*, and dead *A. cervicornis* canopy treatments were the least effective at reducing predation. Corals under these protections showed very high predation rates that were similar to the control. Across these four treatments, 74% of *P. clivosa* were observed to have some degree of predation during the experiment with 27% being completely removed in the first week and 39% being removed after 1 month. The control (unprotected) group had the highest prevalence of predation with 88% of *P. clivosa* experiencing some predation and 28% completely removed after 1 month. The embedded plugs and spike umbrellas were the most effective at reducing predation. Under these treatments, predation only affected 25% and 34% of *P. clivosa*, respectively and no corals were completely removed under either treatment. There were no differences in predation between corals under live and dead *A. cervicornis* canopies. Finally, sediment was observed accumulating around the embedded *P. clivosa*. While this did not cause any partial mortality after 1 month, it may be a hindrance to embedded corals' performance in the long term. Instances of "fresh" predation were much less prevalent at 1 month compared to 1 week. After 1 month, corals that had experienced partial predation had fully healed their wounds and begun to encrust back over lost tissue.

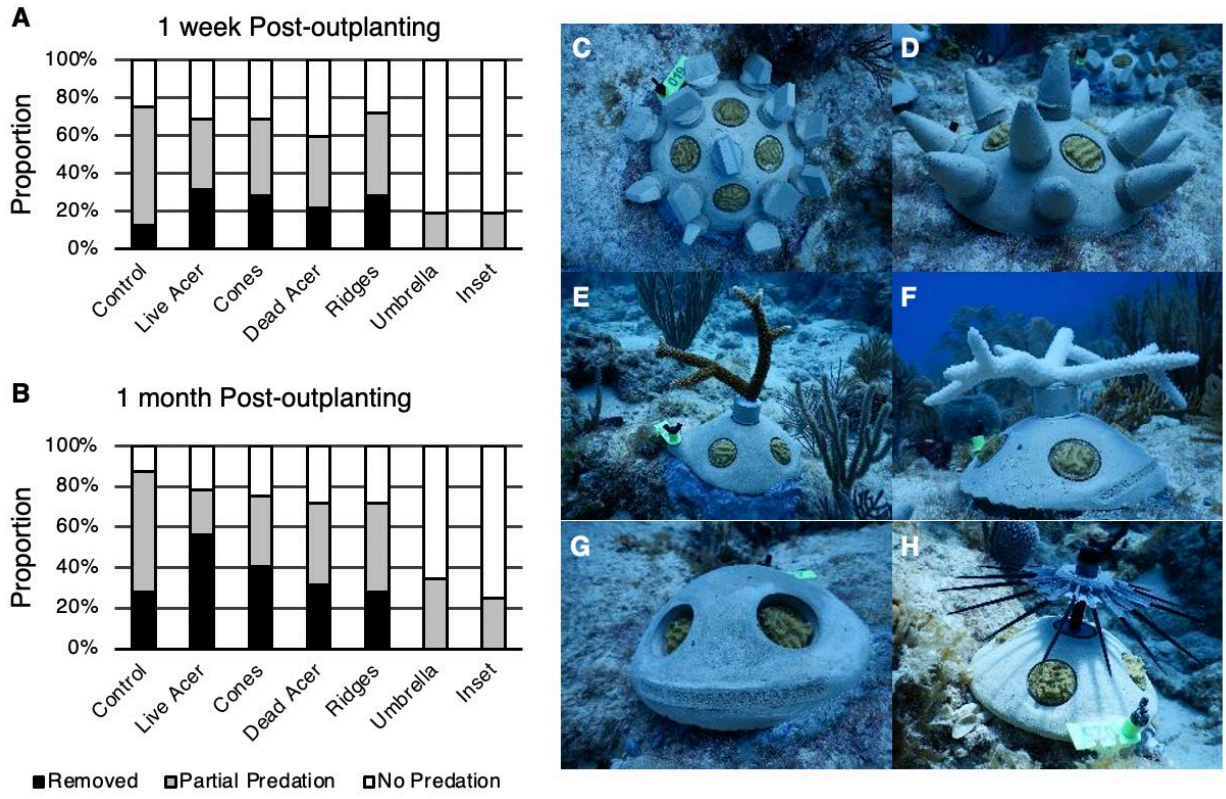

Figure 14. (Left) Proportions of *P. clivosa* under different physical predator deterrent treatments after 1 week (A) and 1 month (B) post-outplanting. (Right) Images showing physical predator deterrent treatments: ridges (C), cones, (D), live *A. cervicornis* (E), dead *A. cervicornis* (F), inset/embedded (G), and umbrella (H). Controls consisted of bases without any added physical protection.

Task 6.2: Sexual recruits (n = 99) of *D. labyrinthiformis* (2021 cohort) were provide by FLAQ, with each coral individually mounted on a small ceramic tile. All corals were outplanted on 4/15 to a single reef site (North North Dry Rocks) in the Upper Florida Keys. Corals were outplanted in one of three orientation treatments to examine the role of microhabitat and coral orientation on predation and survivorship. One third of tiles with *D. labyrinthiformis* recruits (n = 33) were outplanted in exposed locations on the reef, mimicking how corals are currently outplanted for restoration. Another third (n = 33) were outplanted in exposed locations in a vertical orientation to eliminate the effects of sedimentation on recruits but remained exposed to predation and grazing. The final third (n = 33) of these plugs were outplanted to sheltered locations on the reef; specifically on the underside or back of overhangs or structural features. Corals were photographed and surveyed on day 3, week 2, and week 4 to assess predation and survivorship.

Results

Colonies of *Diploria labyrinthiformis* outplanted to different substrate orientations (horizontal vs. vertical vs. sheltered) experienced similar levels of predation during our 4-week study (Figure 15). These findings suggest that placing corals in vertical or sheltered locations does not reduce predation on outplanted *D. labyrinthiformis* colonies. However, substrate orientation did affect

coral survivorship. Colonies outplanted in a horizontal orientation had the highest survivorship, with 72% of corals alive after four weeks. Corals outplanted in a vertical orientation had intermediate survivorship (52%), and corals outplanted to sheltered locations had the lowest survivorship (40%) after four weeks. The proportion of corals bitten was highest on Day 3 and Week 2 and then dropped off significantly by the Week 4 survey. The only coral completely removed was outplanted in a horizontal orientation. The colonies used in this study were raised in ex situ facilities before outplanting. Thus, colonies acclimated in in situ nurseries may have performed differently at the various orientations.

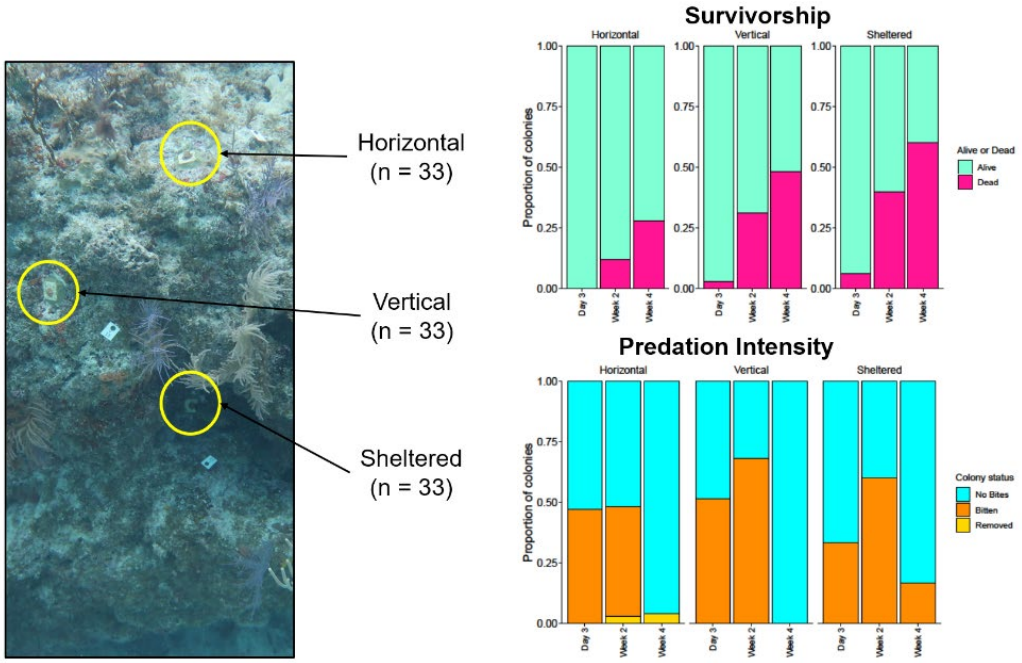

Figure 15. (Left) Photograph of *Diploria labyrinthiformis* colonies deployed to different orientations at North North Dry Rocks, Key Largo, FL. (Top Right) Survivorship and (Top Left) Predation intensity of *D. labyrinthiformis* colonies at Day 3, Week 2, and Week 4 surveys. For survivorship surveys, colonies were recorded as alive (teal) or dead (pink); for predation intensity surveys, colonies were recorded as either having no bites (blue), at least one new bite from a predator (orange), or completely removed (yellow) at each outplant site.

Task 6.3: We designed, in partnership with Reef Cells, cement bases used to test the potential role of the presence of predation deterrent taxa (*Palythoa* and *Amphimedon*) in proximity to coral colonies at the time of outplanting. These bases were used to deploy 500 Cnat corals from FLAQ (250 in Miami and 250 in Key Largo) in close proximity to *Palythoa* and *Amphimedon*. The bases were deployed in sets of 3 (control, + *Palythoa*, + *Amphimedon*) on 4/8 (Miami) and 4/15 (Key Largo). Bases within a cluster were separated by ~ 20 cm and each cluster was tagged and separated from each other by at least 1 m. Each base received 3 Cnat juveniles that were attached to coral plugs. Colonies of *Palythoa* and *Amphimedon* 4-5 cm in diameter were collected at each site and attached to the bases using wire (Figure 16).

Figure 16. Images of the bases used to test the influence of deterrent species on fish predation.

Results

Survival of *C. natans* colonies was higher at our reef site in Key Largo compared to Miami during our 4-week study. Predation intensity on Cnat colonies deployed to bases varied by time and location. However, the deterrent taxa tested, *Palythoa caribaoerum* and *Amphimedon compressa*, did not deter predation on outplanted colonies of *C. natans* (Figure 17). The proportion of Cnat colonies recorded as "bitten" (at least 1 bite visible) in Miami after 1 week of deployment was similar to that observed at the Key Largo site on Day 3 and Week 2. However, in Miami the proportion of corals recorded as bitten increased from Week 1 (range: 31 to 40%) to Week 4 (range: 56 to 75%). In contrast, at the Key Largo site the proportion of corals recorded as bitten decreased from Week 2 (range: 35 to 49%) to Week 4 (range: 16 to 25%). After 4 weeks of deployment, a total of 9 corals were completely removed at the Miami site and 8 corals were completely removed at the Key Largo site.

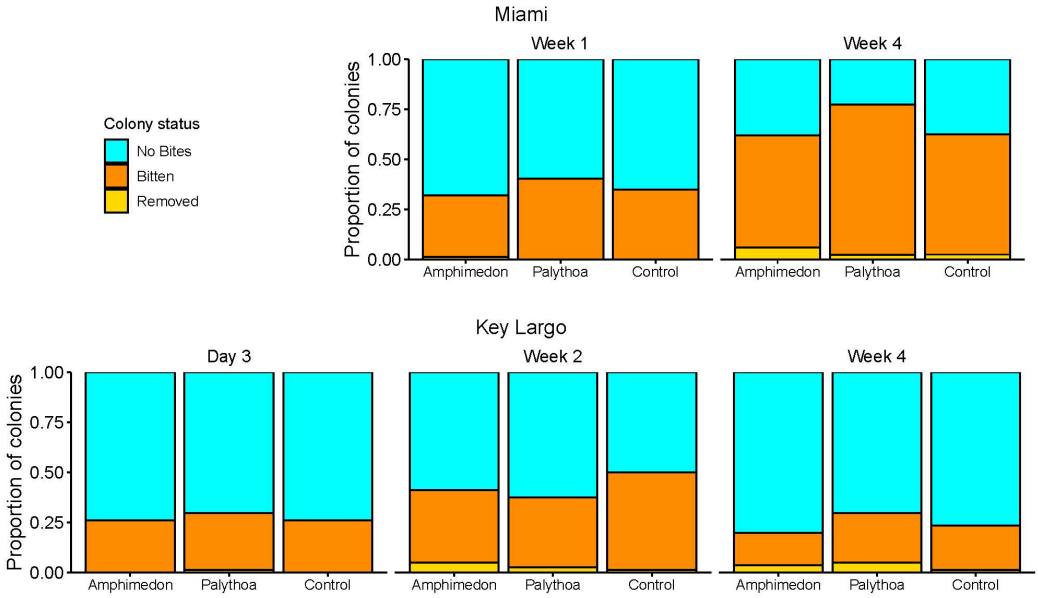

Figure 17. Proportion of *Colpophyllia natans* colonies recorded as either having no bites (blue), at least one new bite from a predator (orange), or completely removed (yellow) during each survey at the sites in Miami (top row) and Key Largo (bottom row). The bases were surveyed after 1 week and 1 month in Miami and after 3 days, 2 weeks, and 1 month in Key Largo due to weather.

The survivorship of Cnat colonies outplanted on bases differed substantially between Miami and Key Largo after 4 weeks (Figure 17). In Miami, survivorship was highest for corals outplanted on control bases (71%) and lowest for coral outplanted on + *Palythoa* bases (52%) after 4 weeks. Survivorship was higher in Key Largo, with 100% of Cnat colonies outplanted on + *Amphimedon* bases recorded as alive at the Week 4 survey, followed by 99% of colonies on + *Palythoa* bases, and 95% of Cnat colonies on control bases.

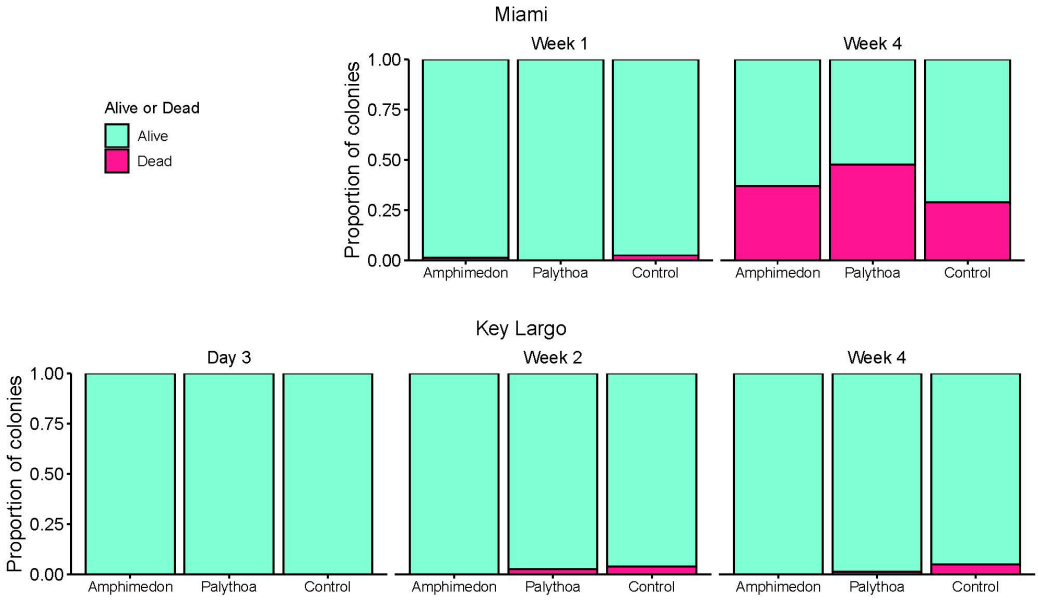

Figure 18. Proportion of *Colpophyllia natans* colonies recorded as alive (teal) or dead (pink) during each survey at the sites in Miami (top row) and Key Largo (bottom row).

Summary Findings

1) Coral rearing and conditioning

Survivorship of corals during ex situ conditioning at FLAQ was very high, with 100% survivorship for 2020 corals. For 2021 corals, survivorship was highest for the Fed (95%), followed by in situ (93%), and Unfed (90%) treatments.

Larger corals (2020 cohort) had higher ex situ survivorship and also higher growth rates during conditioning (3 months). However, smaller corals (2021) grew proportionally (% change in size) faster than larger (2020) corals.

The largest % increase in growth was recorded for the smaller, fed corals (2021). Feeding small corals is recommended for ex situ rearing.

Growth of in situ corals varied by location with the fastest growth recorded at MML, followed by UM, and then NSU. NSU in situ corals lost surface area due to predation while at the in situ nursery, which was not recorded at any of the other two nurseries.

Dlab reared ex situ (FLAQ) had higher protein content than corals reared in situ. No differences in lipids were found based on conditioning for the 2020 corals. Lipid content was higher in the 2021 corals reared ex situ compared to in situ. In general, corals kept in situ at NSU had lower protein and lipid content compared to UM and MML nurseries.

Dlab and Mcav can be grown equally well in situ and ex situ without any significant differences in survivorship. Mcav microfragments had higher survivorship than juveniles once outplanted.

Dlab sexual recruits had the lowest survivorship when placed on plugs as a single recruit or placed at the highest density treatment (+11 recruits per plug) in both *in*- and ex situ nurseries.

Feeding ex situ did not consistently enhance non-predation mortality once outplanted. However, corals conditioned ex situ had generally lower non-predation mortality compared to corals conditioned in situ prior to outplanting.

2) Predation patterns

Predation impacts on outplanted Dlab varied widely among regions. In Miami-Dade County (UM), predation was extremely high, with 50-75% of corals removed or bitten within the 24 hours.

Predation impacts declined over time, with the largest impacts recorded during the first few weeks after outplanting. Bitten corals that were not removed completely showed good recovery in Miami after 2 months.

Both predation and non-predation mortality were influenced by size; a higher proportion of 2021 corals were completely removed by fish predators compared to 2020 corals. Across all three regions, 2021 corals had a higher proportion of dead colonies compared to the 2020 corals, with the exception of 2020 in situ Dlab in Broward County (NSU), which had the highest mortality out of any cohort x treatment x region combination.

Contrary to expectations, predation impacts were higher on in situ and unfed corals compared to fed corals after 3 months.

Feeding corals, while enhancing growth ex situ, did not significantly increase predation impacts once outplanted for both Dlab and Mcav. Moreover, no clear relationships between tissue metrics and predation impacts were documented.

3) Predation mitigation strategies

The physical protection strategies used had very limited impacts on reducing predation on Pcli (77% of *P. clivosa* were observed to have some degree of predation). Cone, ridge, live *A. cervicornis*, and dead *A. cervicornis* canopy treatments were the least effective at reducing predation.

The embedded plugs and spike umbrellas were the most effective at reducing predation. Under these treatments, predation only affected 25% and 34% of Pcli, respectively and no corals were completely removed under either treatment.

Dlab colonies outplanted in a horizontal orientation had the highest survivorship, with 72% of corals alive after four weeks. Corals outplanted in a vertical orientation had intermediate survivorship (52%), and corals outplanted to sheltered locations had the lowest survivorship (40%) after four weeks. Contrary to expectations, there were no clear differences in predation intensity among the three orientation treatments.

Adding predation deterrent taxa (i.e., the zoanthid *Palythoa* and the sponge *Amphimedon*) to the same bases as outplanted corals did not offer any significant protection from predation. Predation intensity on Cnat colonies deployed to bases varied by time and location, but was not influenced by the presence or absence of deterrent taxa.

Management Recommendations

Feeding corals during ex situ rearing is highly recommended. Feeding corals does not seem to affect predation patterns but feeding enhances ex situ growth (reducing time at nursery) and survivorship once outplanted.

Size is a key driver of survivorship and predation patterns. Corals should be kept within ex situ and in situ nurseries (if protected from predation) for as long as logistically possible to maximize performance once outplanted.

Smaller corals benefit more from feeding in terms of increases in live area than larger corals, suggesting feeding may be more important for smaller/younger corals.

Unless needing to propagate and track single genets as brood stock within restoration gene pools, which is a common practice, we recommend settling larvae with a target density of 2-10 corals per plug and rear Dlab recruits in situ before outplanting.

For species like Mcav and Dlab that do well within in both nursery types, rearing in in situ nurseries prior to outplanting is a viable option if space is limited within ex situ facilities. Nevertheless

genotype redundancy is always recommended so that genotypes are represented in both types of nurseries to mitigate the impacts of acute mortality events.

We recommend outplanting colonies of *D. labyrinthiformis* to the reef in exposed habitats in a horizontal orientation. Outplanting this species colonies in vertical and sheltered locations (e.g., under overhangs) is not recommended given the substantially lower survivorship we recorded.

We recommend providing Mcav with adequate nutrition and to acclimate colonies at an in situ nursery prior to outplant on reefs until they reach a size (> 4 cm) that can potentially withstand algal overgrowth.

Outplanting *Palythoa caribaoerum* and *Amphimedon compressa* next to *C. natans* does not effectively deter fishes from preying on these corals. We suggest corals get outplanted at some distance from these taxa as they do not provide predation mitigation and may eventually outcompete small corals.

Most physical deterrents provide limited protection to outplanted massive species of corals, suggesting that their minimal benefit to corals is likely not worth the time and resources required to deploy and remove these structures.

Because in situ nursery success is influenced by nursery site location, nursery structure, stony coral species, and colony size (age), individual in situ nurseries need to determine best management practices for their nursery. For example, the NSU nursery will need to determine the best structures to hold small Dlab and other STLD-susceptible species colonies grown from lab spawned larvae to reduce potential predation and possible sedimentation stress.