

The Role of Outplant Density on Coral Survivorship, Growth, Predation Impacts, and Disease Spread



The Role of Outplant Density on Coral Survivorship, Growth, Predation Impacts, and Disease Spread

Final Report

Prepared By:

D. Lirman, M. D'Alessandro, A. Gleason (UM), J. Spadaro, G. Klinges (Mote Marine Laboratory, MML), M. Ladd (NOAA/NMFS), D. Gilliam (Nova Southeastern University, NSU), A. Bourque (Biscayne National Park, BISC), K. O'Neil (Florida Aquarium, FLAQ)

June 15, 2024

Completed in Fulfillment of PO# C21412 for

**Florida Department of Environmental Protection
Coral Protection and Restoration Program
1277 N.E. 79th Street Causeway
Miami, FL 33138**

This report should be cited as follows:

Lirman, D., M. D'Alessandro, M. Ladd, J. Spadaro, D. Gilliam, A. Bourque, K. O'Neil, A. Gleason, G. Klinges. 2023. The role of outplant density on coral survivorship, growth, predation impacts, and disease spread. Florida Department of Environmental Protection Report. 29 pages.

This report was prepared for the Florida Department of Environmental Protection, Coral Protection and Restoration Program by University of Miami. Funding was provided by the Florida Department of Environmental Protection. The views, statements, findings, conclusions, and recommendations expressed herein are those of the authors and do not necessarily reflect the views of the State of Florida or any of its sub-agencies.



AWD-C21412
June 2024

Acknowledgements

This project would not have been possible without funding from DEP or the combined efforts of the students, interns, faculty, and staff dedicated to this project who planned out the study design, outplanted the corals, and participated in data collection and analysis. We are grateful for the diving and boating office staff who supplied cylinders, boats and other field equipment and supported all field activities.

Management Summary

Our project has demonstrated that offspring from parent colonies originating from Monroe County can be successfully restored in Miami-Dade and Broward Counties to increase the genetic and genotypic diversity of this species that is found in critically low densities throughout the Florida Coral Reef. The high survivorship of *A. palmata* clusters grown at northern sites in Miami (97.5%) and Broward (83.9%) following the Summer 2023 bleaching event indicates the viability of the assisted migration of this keystone species.

In the absence of source populations outside the Keys, only the ex situ husbandry of this species will provide the tissue needed for successful restoration. We strongly encourage management agencies to provide financial resources to expand these coral transfers to preserve and restore connectivity.

Fish predation continues to be the main driver of tissue losses for outplanted massive corals. Differences in predation impacts were found among coral species, site, and outplant density. Outplanting corals in high-density arrangements appears to provide benefits to corals through predation dilution. Fish predation impacts appear to be consistently most severe within the first week to month following coral outplanting, so additional measures should be explored to reduced fish predation of newly outplanted corals, which reached an average of >30% tissue removal for the most susceptible species (*C. natans* and *O. faveolata*) after just one week.

We encourage management agencies to continue to allocate resources to understand the drivers of fish predation and design mitigation strategies so that massive corals can be effectively incorporated into large-scale restoration efforts. Until these bottlenecks have been explored, we suggest focusing restoration on species like *D. labyrinthiformis* that maintained high survivorship over the 1-year monitoring period (97.5%) and are significantly less susceptible to predation (other species still need to be propagated and outplanted to help understand drivers of species susceptibility to predation).

Executive Summary

In February 2023, Restoration partners from The University of Miami, Nova Southeastern University, Mote Marine Laboratory, Biscayne National Park, NOAA Southeast Fisheries Center, and the Florida Aquarium collaborated to evaluate the role of coral outplant density on

the survivorship and growth of corals with branching (*Acropora palmata*) and massive (*Colpophyllia natans*, *Diploria labyrinthiformis*, and *Orbicella faveolata*) colony morphologies. This project was extended to allow us to continue monitoring corals up to 1 year after outplanting.

Fish predation was highest during initial surveys and was the main driver of tissue losses for massive corals. No evidence of disease was observed on these coral outplants. Predation impacts varied between sites, by coral species and density treatment (for one species, *C. natans*). Predation on *C. natans* was significantly lower when outplanted in high-density arrays (12 corals per m⁻²) compared to corals outplanted in low- (3 corals m⁻²) and medium- (9 corals per m⁻²) density arrays, suggesting that predator dilution can limit predation impacts on a per-coral basis and that predation-susceptible corals may benefit from high-density deployments on reefs with high fish predation. In contrast, outplant density did not influence the impacts of fish predation on *O. faveolata* and *D. labyrinthiformis*. Fish predation was highest on *C. natans*, followed by *O. faveolata*, and *D. labyrinthiformis*. As in prior studies, fish-predation impacts were concentrated during the first week after outplanting and declined over time.

A total of 4,140 *A. palmata* fragments from 50 parent genotypes were provided by Mote Marine Laboratory for this project. These fragments were deployed onto 5 sites throughout Florida's Coral Reef in arrangements of 3, 9, and 12 corals per cluster/base. These outplanted corals were monitored visually and photographically for one year after outplanting. The survivorship of the *A. palmata* clusters was high through August 2024 (6 months after outplanting), ranging from 86.3% (NSU) – 100% (NOAA). These high survivorship values clearly indicate that *A. palmata* corals grown from parents from the Florida Keys can be transferred to the northern counties (Miami-Dade and Broward) as part of an assisted gene flow project. The Summer 2023 bleaching event caused a significant amount of bleaching and mortality across sites with clear regional patterns. Coral outplants in the Lower Florida Keys, which experienced 24.8 Degree Heating Weeks (DHW), had 100% mortality, followed by corals in Key Largo (~ 50% mortality), Biscayne National Park (96% mortality), Miami (< 3 % mortality), and Broward (16% mortality caused by predation and not bleaching). The symbiont community structure was assessed at the time of outplanting and after 6 months (prior to the onset of the bleaching) and while the corals outplanted had 8% abundance of *Durusdinium* (*Symbiodinium* was present at > 72% average abundance), the abundance of *Durusdinium* declined to non-detectable levels before the onset of bleaching and thus did not provide any protective benefits during the thermal anomaly. Productivity was significantly higher in the low-density clusters compared to the high density treatments (p<0.05), suggesting that outplanting *A. palmata* in lower densities (e.g. 3 corals per base) is a preferred method to maximize growth in this species.

Table of Contents

Acknowledgements	1
Management Summary	1
Executive Summary	1
1. BACKGROUND.....	6
1.1 Acropora palmata	6
1.1.1 Methods.....	6
1.1.2 Results.....	7
1.1.1.1 Growth of A. palmata	7
1.1.1.2 A. palmata Bleaching and Mortality.....	13
1.1.1.3 Symbiont Community Analysis	17
1.1.1.1.1 Methodology	17
1.1.1.1.2 Results.....	18
1.1.1.4 Predator Tracking and Removal	22
1.1.1.1.3 Hermodice carunculata	22
1.1.1.1.4 Coralliophila abbreviata.....	22
1.1.1.5 Discussion.....	23
1.2 Massive Corals	24
1.1.3 Methods.....	24
1.1.4 Results.....	26
1.1.5 Discussion	32
Management Recommendations.....	32

List of Figures

Figure 1. Plug-sized A. palmata fragments were outplanted in low (N=3), medium (N=6) and high (N=12) density clusters to enhance growth and fusion.....	8
Figure 2. Between 6-12 month surveys, A. palmata continued to grow and fuse and several clusters exhibited vertical growth and branching.....	9
Figure 3. LAI varied across sites over the 1-year period of monitoring.	10
Figure 4. Change in LAI among A. palmata outplants between 1-week and 6-month surveys showed that corals in the lower density treatment had significantly higher productivity relative to the medium- and high-density treatments across sites.	11

Figure 5. Divers captured imagery at the baseline, 6- (August 2023), and 12- (February 2024) month monitoring timepoints. Mosaics (in order from top right image, then left to right and down) of Miami plot collected 12 month, NSU, NOAA, BNP, and MML..... 13

Figure 6. Diver sampling *A. palmata* to conduct symbiont community analysis..... 13

Figure 7. Temperatures were recorded in situ at 4 of the restoration sites throughout the duration of the project. 14

Figure 8. During the Summer of 2023, DHW varied by site, ranging from 7.3 DHW at the NSU Site to 24.8 DHW at the MML Site. 15

Figure 9. During 6-month surveys (August 2024) the majority of *A. palmata* clusters at the BNP and MML sites were completely blea. During 6-month surveys (August 2024) the majority of *A. palmata* clusters at the BNP and MML sites were completely bleached. 16

Figure 10. Probability of *A. palmata* survivorship across the 5 sites over the duration of the outplanting project..... 17

Figure 11. Relative abundance of the most abundant symbiont type profiles across two time points.in *Acropora palmata* fragments outplanted to different sites. Taxa are included in the plot if they had a relative abundance greater than 1% within a site. Symbiont profiles are colored according to genus within the family Symbiodiniaceae. Strains are identified in accordance with symbiont clade/genus abbreviations (e.g., A = Symbiodinium, C = Cladicopium, D = Durusdinium). Data are averaged across replicates of the same genotype. 19

Figure 12. Relative abundance of the most abundant symbiont type profiles in *A. palmata* fragments prior to outplanting. Taxa are included in the plot if they had a relative abundance greater than 1% within a site. Data are averaged across replicates of the same genotype. 20

Figure 13. Relative abundance of the most abundant symbiont type profiles in *A. palmata* six months after outplanting to different sites. Taxa are included in the plot if they had a relative abundance greater than 1% within a site. Data are averaged across replicates of the same genotype. 20

Figure 14. Principal components analysis of symbiont community data for *A. palmata* samples at two timepoints. Samples are plotted in the left panel and symbiont clades are plotted on the right. 21

Figure 15. Relative abundance of the most abundant symbiont type profiles in *A. palmata* genotype AP125 at five sites, showing an increase in symbionts assigned to strain F3w. Data are averaged across replicates of the same genotype. 21

Figure 16. Count of fireworms that were caught in baited traps across the 5 sites between August 2023 – February 2024. 22

Figure 17. Evidence of snail predation was documented and snails were removed from affected colonies (right) and fireworms were counted and removed from the *A. palmata* plots via baited trapping (left). 23

Figure 18. Count of *Coralliophila abbreviata* snails that were tracked and removed from the 5 site between 6-12 month surveys..... 23

Figure 19. Schematic of the design used to assess the role of coral outplant density on fish predation showing the site layout, the density arrangements, and the teepees used for coral protection..... 25

Figure 20. Location of outplant sites where massive corals were deployed in February 2023 and the partners that completed the outplanting and monitoring at each site..... 26

Figure 21. Probability of outplant survivorship between coral species and density treatment at the Miami Site.....27

Figure 22. Probability of outplant survivorship of *C. natans* outplants between density treatments at the Miami and Broward Sites.28

Figure 23. Proportion of *D. labyrinthiformis* outplants that experienced fish predation at the Miami Site.....29

Figure 24. Proportion *C. natans* outplants that experienced fish predation at the Miami Site.29

Figure 25. Proportion *C. natans* outplants that experienced fish predation at the Broward Site.30

Figure 26. Probability of surviving *O. faveolata* outplants between density treatments at the BNP Site.....31

Figure 27. Proportion of *O. faveolata* that experienced fish predation at the BNP Site.31

List of Tables

Table 1. Site locations of massive morphology coral species7

Table 2. Survivorship of *A. palmata* outplants across the 5 sites between 6-month and 12-month surveys.....13

Table 3. Survivorship of coral species of massive morphologies across the 2 sites from 1-week to 1-year monitoring.....30

List of Acronyms

- BNP: Biscayne National Park
- FLAQ: Florida Aquarium
- MML: Mote Marine Lab
- NSU: Nova Southeastern University
- NOAA: National Oceanic and Atmospheric Administration
- UM: University of Miami
- APAL: *Acropora palmata*
- DLAB: *Diploria labyrinthiformis*
- OFAV: *Orbicella faveolata*
- CNAT: *Colpophyllia natans*
- SCTLD: Stony Coral Tissue Loss Disease

1. BACKGROUND

The goal of this collaborative proposal (UM, NSU, MML, BNP, NOAA, FLAQ) was to expand continue assessing the role of outplant density in restoration success, measured by survivorship and growth, utilizing three coral species (*C. natans*, *D. labyrinthiformis*, and *O. faveolata*) that are susceptible to both SCTL D and fish predation impacts, major bottlenecks for the restoration of this coral species. Outplant density can drive changes in the growth and survivorship of outplanted corals and can impact exposure to predation and SCTL D.

A secondary objective of this project is to complete the assisted gene flow (or assisted relocation) of the threatened species *A. palmata* from the Florida Keys to Miami and Broward counties where only a handful of colonies of this keystone species survive today. The role of density in determining outplant success directly addresses a direct restoration activity priority and the coral species to be used in this project have been identified as priority species for propagation and restoration by the State of Florida in the “State of Florida Restoration Priorities for Florida’s Coral Reef: 2021-2026” report.

Goal 1: Expand the monitoring timeline to evaluate the role of outplant density on the survivorship, growth, predation impacts, and disease prevalence (if observed) of *A. palmata*, *C. natans*, *O. faveolata*, and *D. labyrinthiformis* outplanted in Feb 2023 to 1 year. In addition, we will build landscape mosaics of the outplanted *A. palmata* plots to measure changes in cover and provide a visual representation of elkhorn restoration progression.

Goal 2: Analyze the symbiont communities in outplanted *A. palmata* prior to outplanting and after 6 months to evaluate the role of symbiont identity on survivorship and growth.

1.1 *Acropora palmata*

1.1.1 Methods

In February 2023, 4,140 *A. palmata* of 50 genotypes were outplanted across 5 regions (Lower Keys, near Key West (MML), Upper Keys, near Key Largo (NOAA), Biscayne National Park (BNP), Miami Dade (UM), and Broward County (NSU). Corals were outplanted as single genotype clusters in various cluster size treatments: low (N=3), medium (N=6) and high (N=12) (Fig. 1 and 2). Each site received 23 sets of 36 corals for a total of 828 fragments per reef.

Table 1. Site locations of massive morphology coral species

Table 1. Site locations of massive morphology coral species

Site	Species Outplanted	Latitude (°N)	Longitude (°W)	Depth (ft)
Broward 1	APAL	26.2060	80.0856	15
Broward 2	CNAT	25.9768	80.1000	20
Miami	APAL, CNAT, DLAB	25.6441	80.0969	21
BNP	APAL, OFAV	25.4698	80.1317	15
Upper Keys	APAL	25.1541	80.2677	15-22
Lower Keys	APAL	24.4823	81.7042	12-15

1.1.2 Results

1.1.1.1 Growth of *A. palmata*

Live Area Index (LAI) was calculated for each individual *A. palmata* cluster by squaring the average dimension $((\text{Length} + \text{Width} + \text{Height})/3)^2$ and multiplying by the percent live tissue of the coral cluster (Fig 3).

The *A. palmata* outplanted at the 5 reef sites were monitored 6, 9, and 12 months after outplanting to assess survivorship, growth, predation impacts, and disease prevalence.

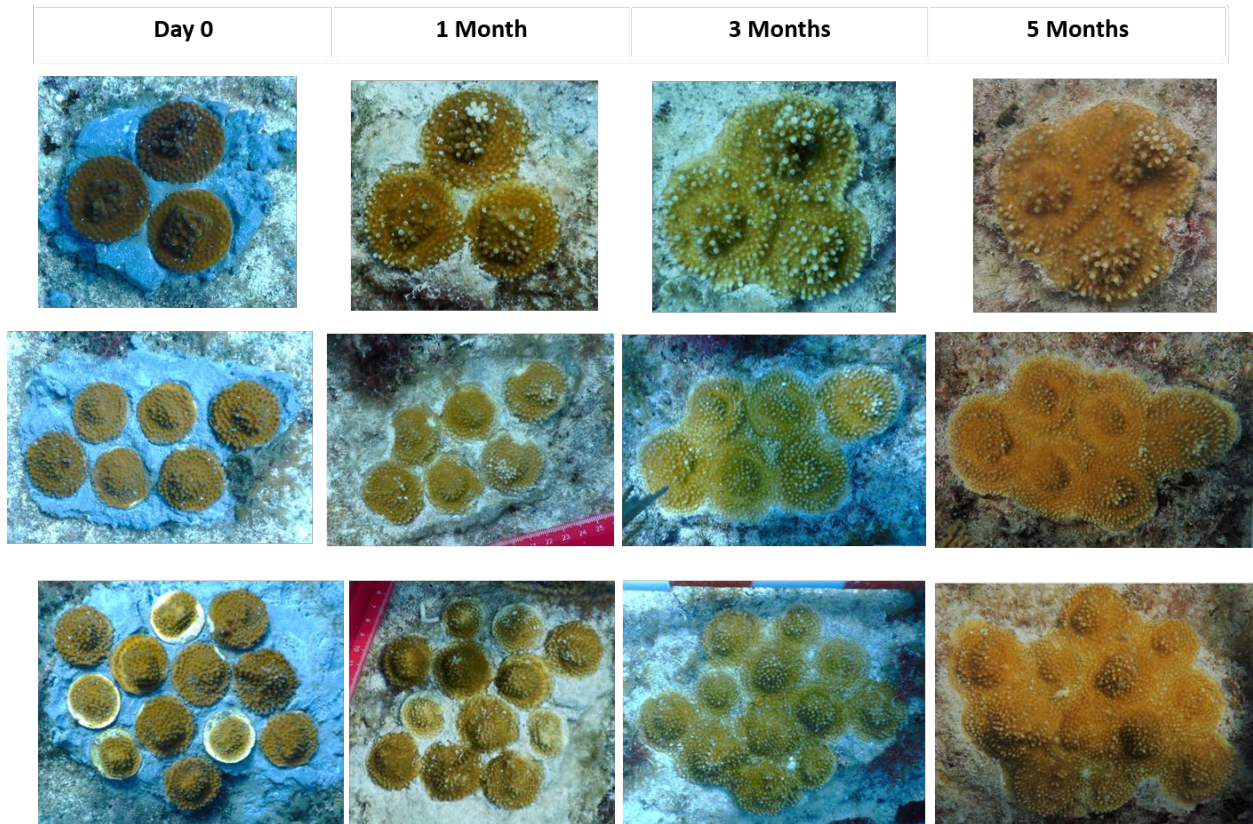


Figure 1. Plug-sized *A. palmata* fragments were outplanted in low (N=3), medium (N=6) and high (N=12) density clusters to enhance growth and fusion.

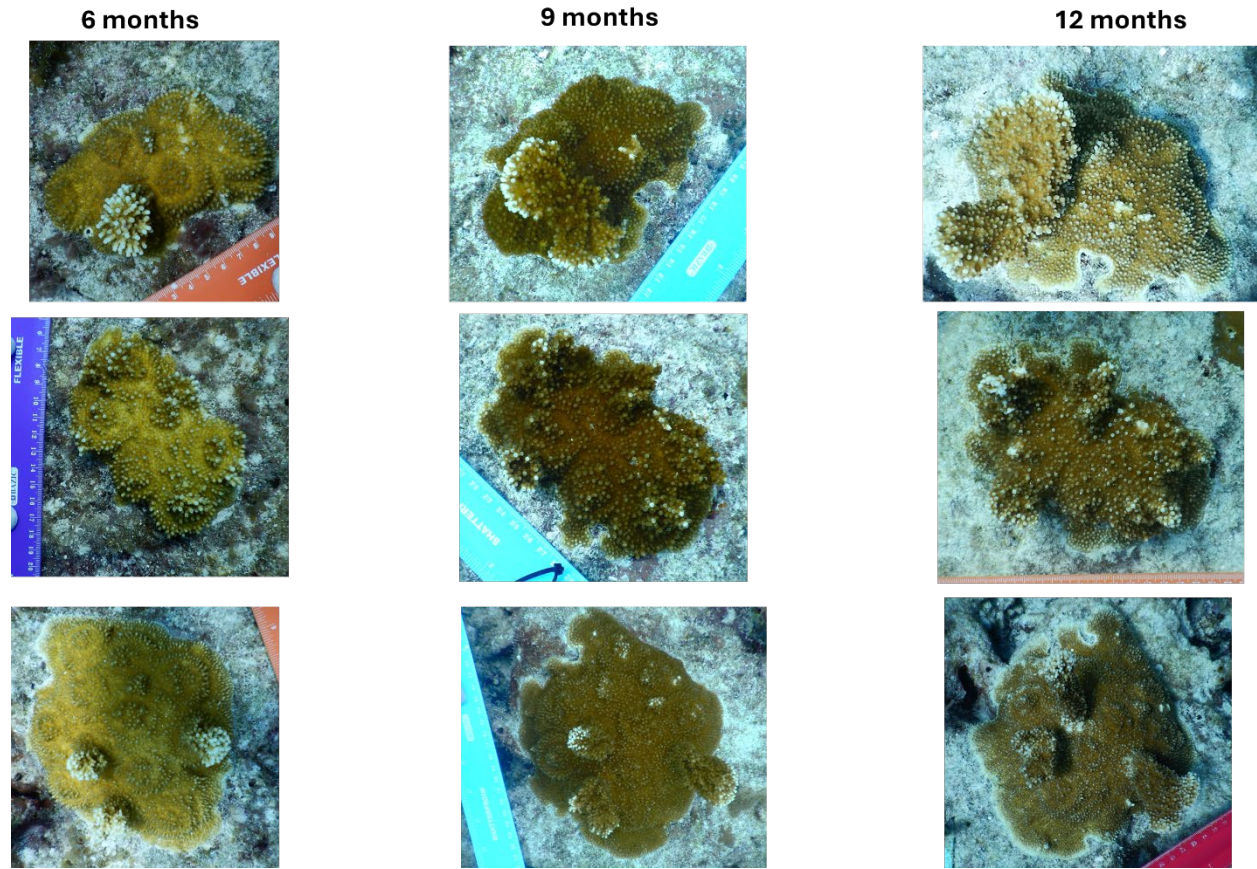


Figure 2. Between 6-12 month surveys, *A. palmata* continued to grow and fuse and several clusters exhibited vertical growth and branching.

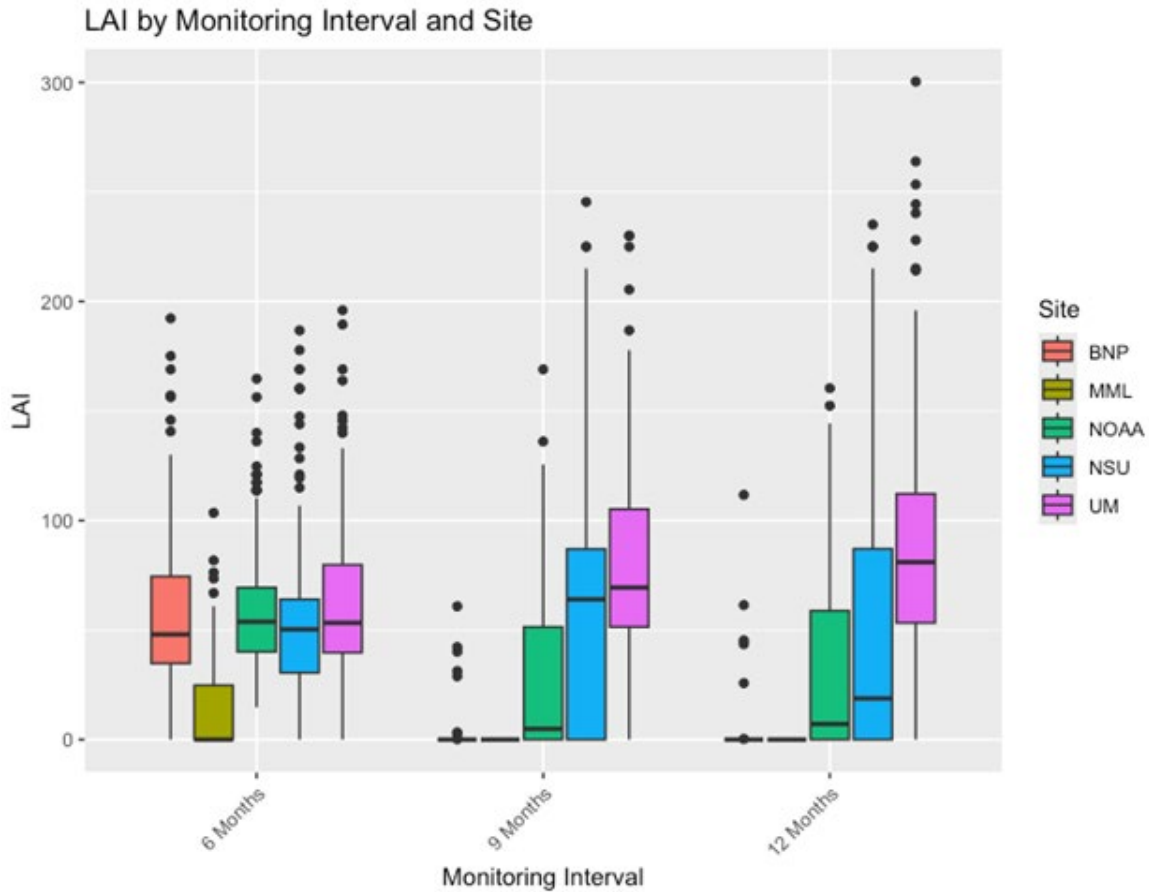


Figure 3. LAI varied across sites over the 1-year period of monitoring.

The LAI of the three density treatments, low (N=3), medium (N=6), and high (N=12) was compared between 1-week and 6 months post outplanting across sites to determine the proportional increase in growth within each density treatment (Fig 4). Fully dead and clusters whose LAI decreased between timepoints were excluded so that growth potential by density treatment could be evaluated. Productivity was highest in the low-density clusters and was significantly higher in the low-density compared to the high-density treatments ($p < 0.05$), suggesting that outplanting in lower densities of 3 corals per base is more effective at maximizing productivity compared to higher densities.

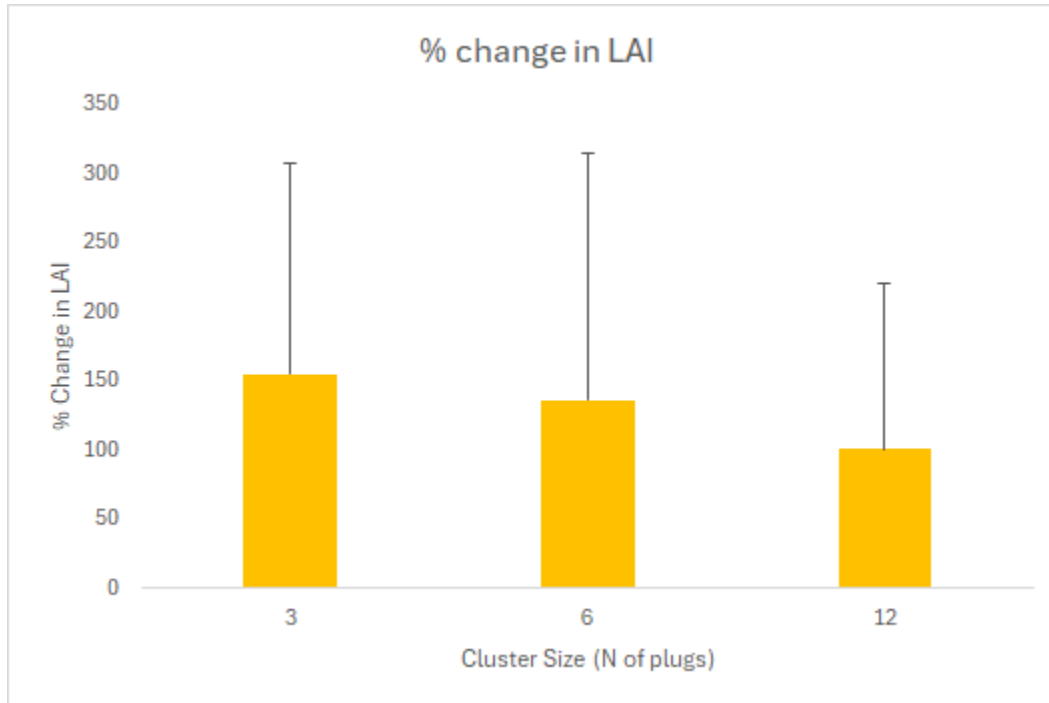
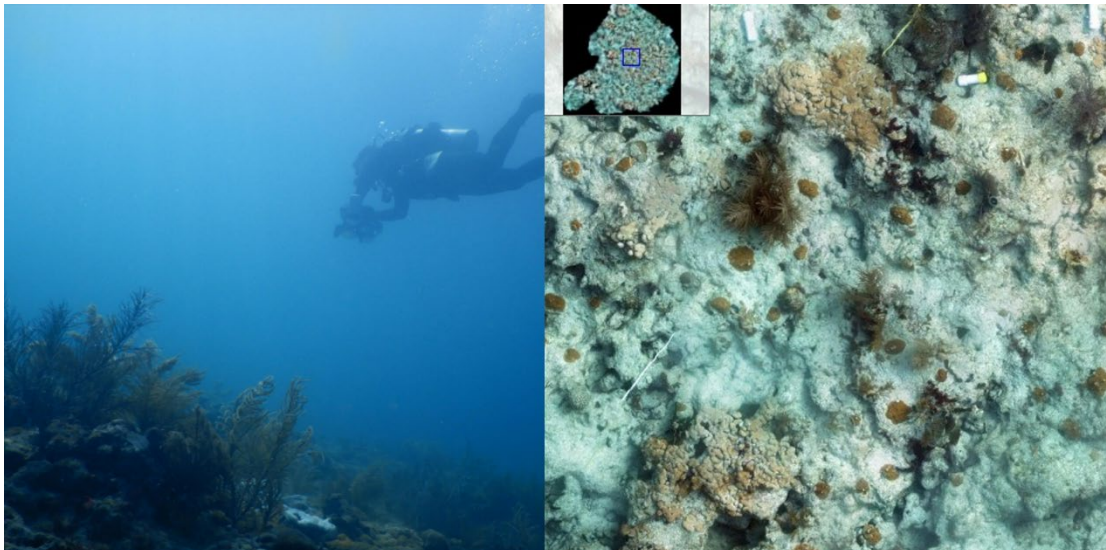


Figure 4. Change in LAI among *A. palmata* outplants between 1-week and 6-month surveys showed that corals in the lower density treatment had significantly higher productivity relative to the medium- and high-density treatments across sites.

Divers captured imagery to build landscape mosaics of the outplanted *A. palmata* plots to measure changes in cover and provide a visual representation of elkhorn restoration progression (Fig. 5).



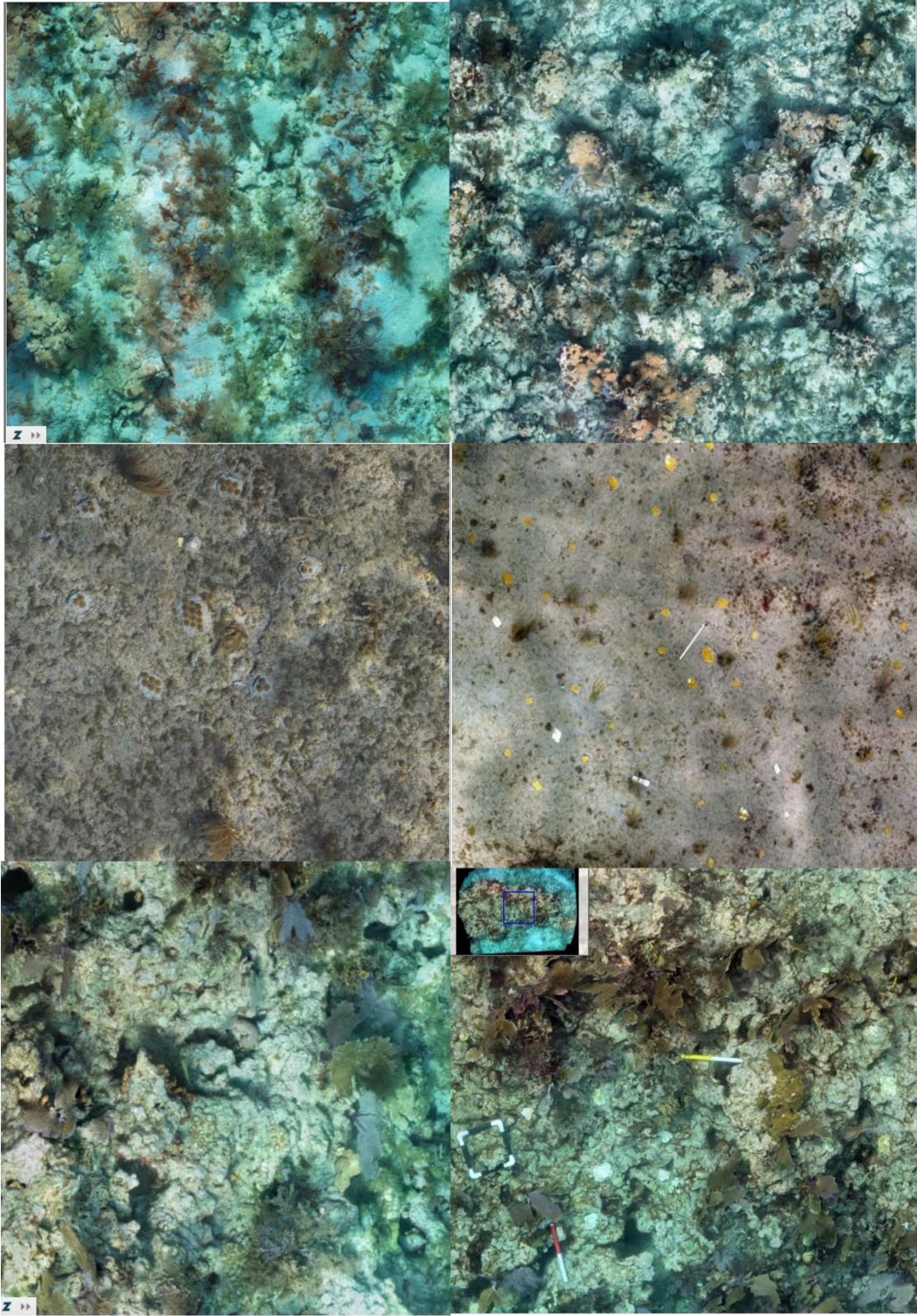


Figure 5. Divers captured imagery at the baseline, 6- (August 2023), and 12- (February 2024) month monitoring timepoints. Mosaics (in order from top right image, then left to right and down) of Miami plot collected 12 month, NSU, NOAA, BNP, and MML.

Tissue samples were collected prior to outplanting and again at the 6-month monitoring timepoint from *A. palmata* colonies to assess the symbiont communities and evaluate the role of symbiont identity on survivorship and growth (Fig. 6).



Figure 6. Diver sampling *A. palmata* to conduct symbiont community analysis.

1.1.1.2 *A. palmata* Bleaching and Mortality

In June 2023, South Florida entered a Bleaching Watch, which quickly progressed up to an Alert Level 2, meaning that ocean temperatures remained above a bleaching threshold of 8-12 degree heating weeks (DHW).

At each site, HOBO temperature loggers were deployed between January 2023-March 2024 to record in situ temperature data and found that the coral bleaching threshold (30.5°C) was exceeded across all sites in the summer of 2023 (Fig. 7). Onset of bleaching occurred in May at the most southern MML site, late June at the intermediate Miami Site, and in July at the NOAA and NSU Sites.

Table 2. Survivorship of *A. palmata* outplants across the 5 sites between 6-month and 12-month surveys.

Survey	MML Survivorship (%)	BNP Survivorship (%)	NOAA Survivorship (%)	UM Survivorship (%)	NSU Survivorship (%)
6-month	36.6	97.5	100	98.8	86.3
12-month	0	4.3	53.4	97.5	83.9

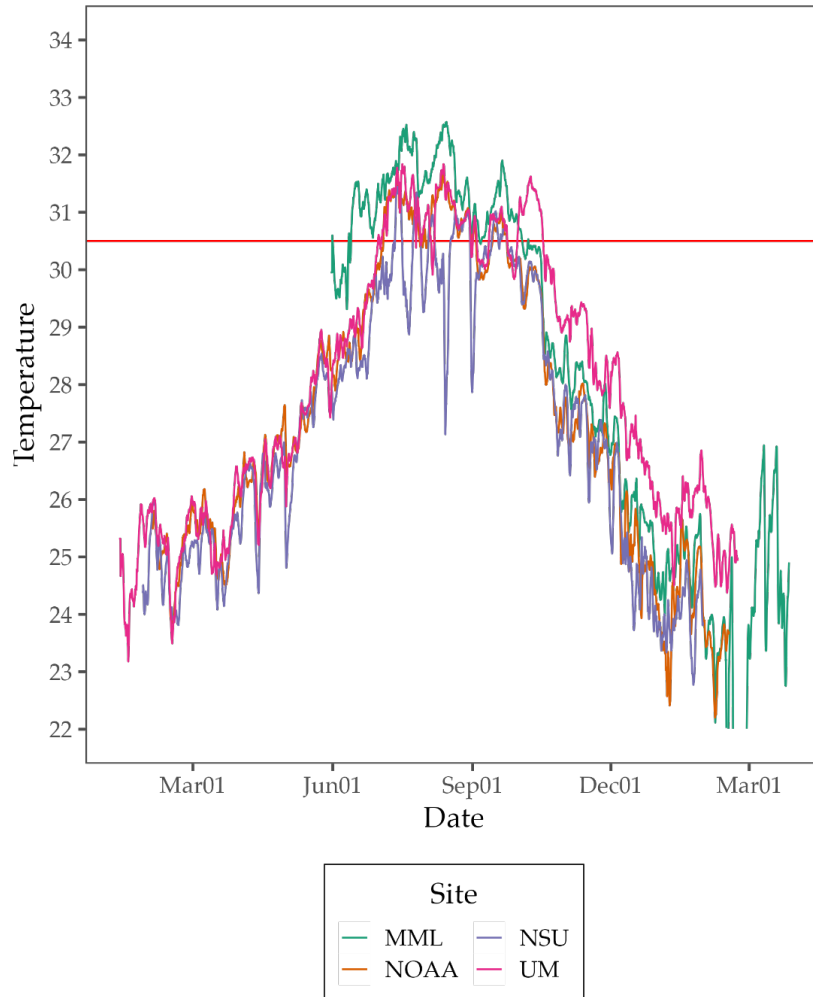


Figure 7. Temperatures were recorded in situ at 4 of the restoration sites throughout the duration of the project.

When ocean temperatures accumulate up to 4 DHW, significant coral bleaching is predicted and beyond 8 DHW severe, widespread bleaching and coral mortality can result due to starvation caused by bleaching stress.

Between June-December 2024, DHW varied by site and was the most severe at the Lower Keys Site which experienced a maximum of 24.8 DHW, followed by the Miami and Broward Sites with 16.2 and 14.3 DHW, respectively, and the NSU Site with 7.3 DHW (Fig. 8). *A. palmata* outplants south of Miami Dade experienced a significant amount of bleaching and thermal stress-related mortality (Fig. 9).

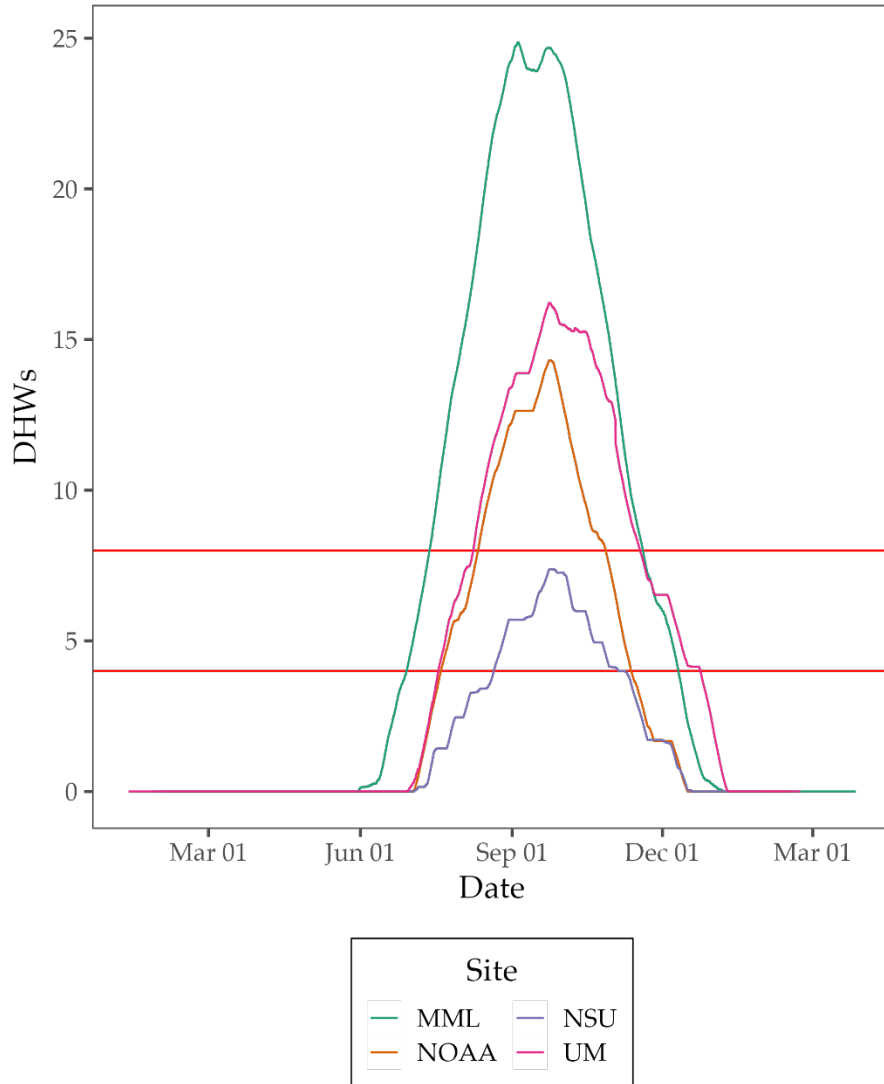


Figure 8. During the Summer of 2023, DHW varied by site, ranging from 7.3 DHW at the NSU Site to 24.8 DHW at the MML Site.

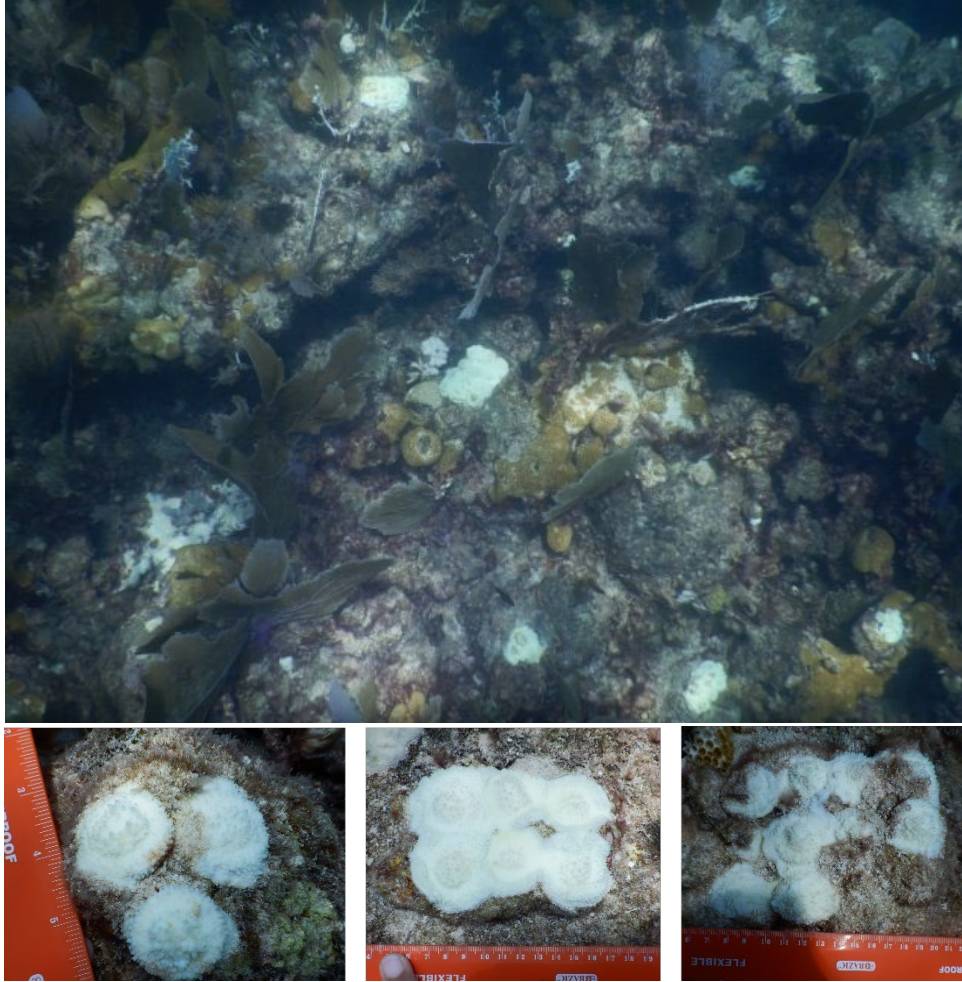


Figure 9. During 6-month surveys (August 2024) the majority of *A. palmata* clusters at the BNP and MML sites were completely blea. During 6-month surveys (August 2024) the majority of *A. palmata* clusters at the BNP and MML sites were completely bleached.

Survivorship of *A. palmata* outplants remained high prior to the Summer 2023 bleaching event, with overall values ranging from 86.3% (NSU) – 100% (NOAA) in August 2024 (Table 2, Fig. 10) with the exception of corals at the southernmost site, MML, which experienced higher temperatures and bleaching earlier than the other 4 sites and had 36.6% overall survivorship.

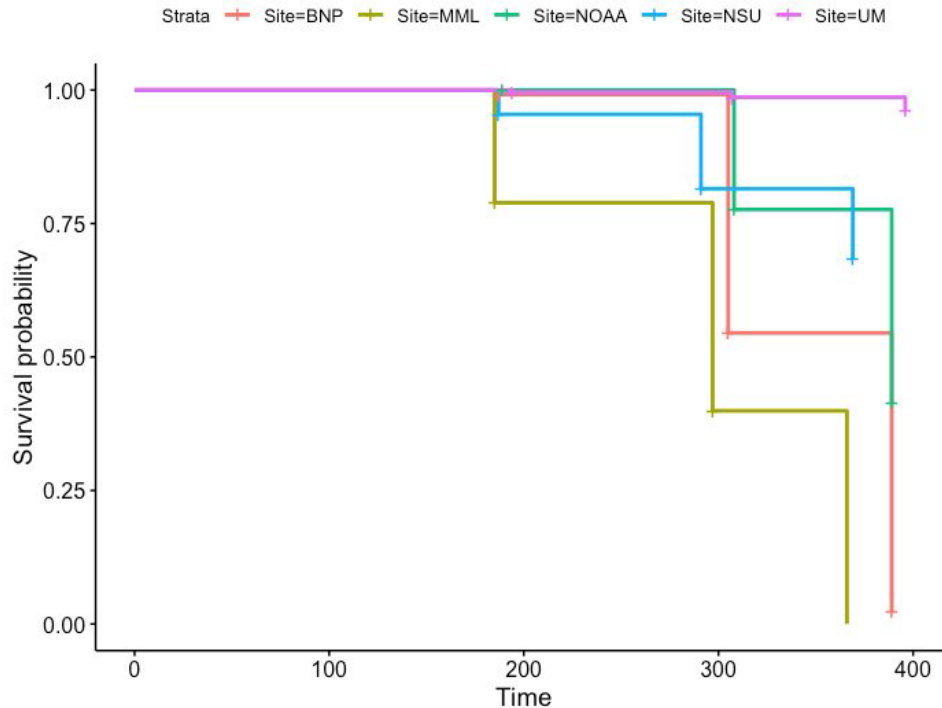


Figure 10. Probability of *A. palmata* survivorship across the 5 sites over the duration of the outplanting project.

Bleaching was the main cause of coral mortality across sites. During 6-month surveys, several *A. palmata* clusters were noted as being bleached-13% at the NSU Site, 4% at the Miami Site, 59% at the Upper Keys Site, 97% at the BNP Site, and 37% at the Lower Keys Site (an additional 61% were already dead) (Table 2).

1.1.1.3 Symbiont Community Analysis

1.1.1.1.1 Methodology

Prior to our first deployment of *Acropora palmata* in January 2023, five ramets per genet were sampled for all genets (30) outplanted at more than one site, while three ramets were sampled from genets (20) only outplanted to one site. Corals (210) were sampled before transport from MML to other organizations for outplanting. An additional sampling timepoint of all live corals was taken at 6 months post outplant (late Summer 2023) for a total of 379 samples. At each timepoint, coral polyps were sampled using bonecutters and promptly transferred to DNA/RNA Shield (a nucleic acid preservative). DNA was extracted using the E.Z.N.A.® Soil DNA Kit (Omega Bio-Tek, Norcross, GA, USA) and manufacturer’s protocol. We sequenced and analyzed samples from deployment 1 to determine whether symbiont composition variability exists between sites and genets, with sample archives retained for future analysis of bacterial symbiont variation. Algal community composition was assessed via sequencing of the algal ITS2

rRNA gene on an Illumina MiSeq high-throughput sequencing machine. ITS2 type profiles were called using Symportal (<https://symportal.org/>). Bioinformatic analysis was conducted as previously described (Klinges *et al.*, 2020, Klinges *et al.*, 2022) by Co-PI Klinges.

1.1.1.1.2 Results

Corals of 50 different genotypes were outplanted to sites in five regions: Biscayne National Park, Broward County, Miami, the upper FL Keys (near Key Largo) and the lower FL Keys (near Key West). We found that, regardless of genotype or timepoint, algal symbionts assigned to the species *Symbiodinium fitti* strain A3 were the most dominant strain across all samples of *A. palmata* (Figure 11). Abundance of this strain averaged $72.73\% \pm 21.87\%$ of the algal community prior to outplanting across all sites, and increased to $84.21 \pm 3.20\%$ after six months. Algal communities were more variable prior to outplanting (Figure 12), with some samples dominated by strains within the genus *Durusdinium*. The most abundant strain of *Durusdinium* was strain D1 at an average of $7.59 \pm 13.77\%$ with a maximum of 54.58% abundance in one sample. Very few ramets retained *Durusdinium* after outplanting (Figure 13). *Durusdinium* strain D1 was almost completely lost by six months after outplanting: abundance of strain D1 was reduced to $0.034 \pm 0.081\%$ with a maximum of 0.77% in any sample. Likewise, strain D4, which averaged $3.50 \pm 6.44\%$ with a maximum of 26.43% before outplanting, was significantly reduced by six months post-outplant and averaged $0.015 \pm 0.035\%$ with a maximum of 0.349% in any sample.

Community structure was most strongly driven by timepoint and coral genotype, rather than site. There were no significant differences in community structure by replicate within each genotype ($F_{4,374} = 0.4834$, $R^2 = 0.18521$, $p = 1.000$) and as such, data was averaged within genotype for ease of interpretation. Differences in symbiont community composition between samples were significantly influenced by timepoint ($F_{1,377} = 47.65$, $R^2 = 0.1122$, $p = 0.001$). Outplant site did not explain significant variation in community composition at six months post-outplant ($F_{4,164} = 1.09$, $R^2 = 0.0259$, $p = 0.348$). Coral genotype significantly influenced community composition at both timepoints ($F_{50,328} = 4.2142$, $R^2 = 0.39114$, $p = 0.001$), however, pairwise comparisons at six months post-outplant did not show any significant differences between genotypes. Further, when data was examined through ordination it was clear that timepoint was the strongest driver (Figure 14). Community dispersion (variability within groups) was strongly influenced by timepoint ($F_{1,377} = 48.059$, $p = 1.803e-11$) and by genotype ($F_{50,328} = 3.388$, $p = 2.389e-11$), though the strength of the model (as assessed through F values) was considerably higher for timepoint. Site did not significantly influence community dispersion at six months ($F_{4,164} = 1.3984$, $p = 0.2368$).

We determined that samples of one genotype of *A. palmata* (genotype AP125) demonstrated increases in symbionts belonging to clade F, while no other genotypes showed this increase. Interestingly, this strain (strain F3w) only increased in abundance in ramets outplanted in Broward County and near Miami (Figure 15). Nonetheless, this genotype was not found to be

significantly different in community composition from any other genotype by six months post-outplant.

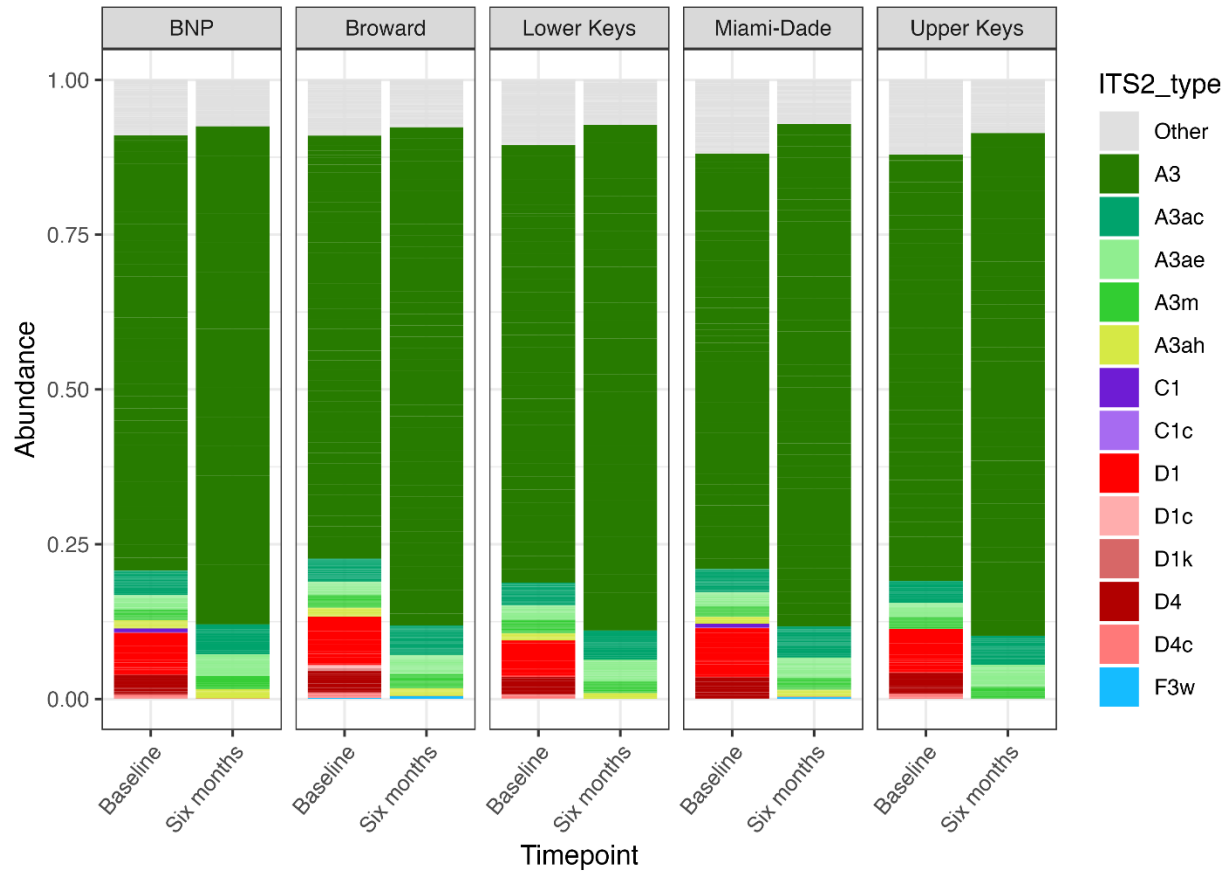


Figure 11. Relative abundance of the most abundant symbiont type profiles across two time points in *Acropora palmata* fragments outplanted to different sites. Taxa are included in the plot if they had a relative abundance greater than 1% within a site. Symbiont profiles are colored according to genus within the family Symbiodiniaceae. Strains are identified in accordance with symbiont clade/genus abbreviations (e.g., A = Symbiodinium, C = Cladocopium, D = Durusdinium). Data are averaged across replicates of the same genotype.

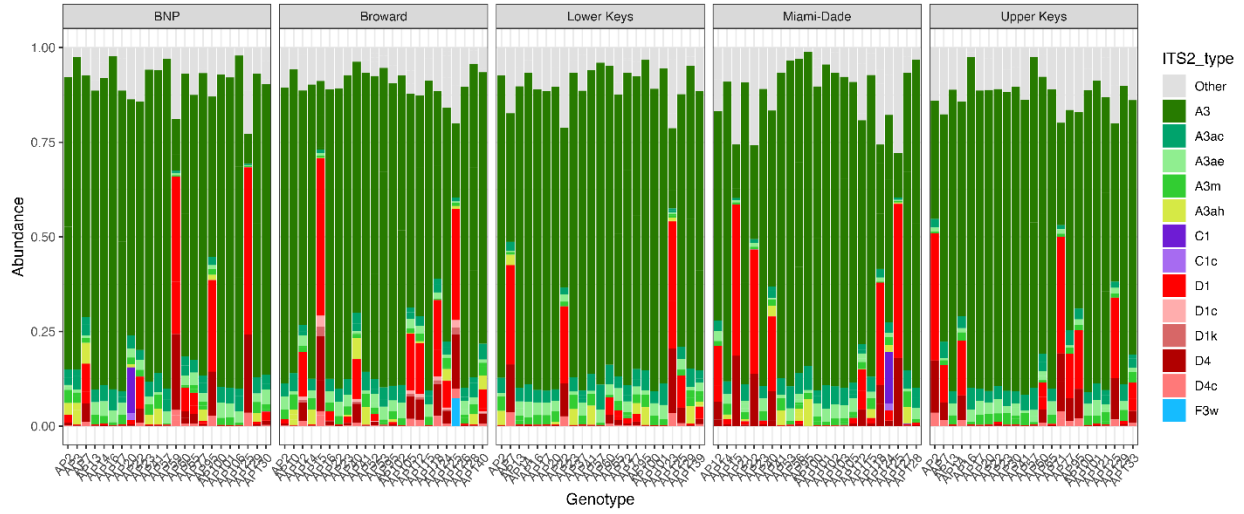


Figure 12. Relative abundance of the most abundant symbiont type profiles in *A. palmata* fragments prior to outplanting. Taxa are included in the plot if they had a relative abundance greater than 1% within a site. Data are averaged across replicates of the same genotype.

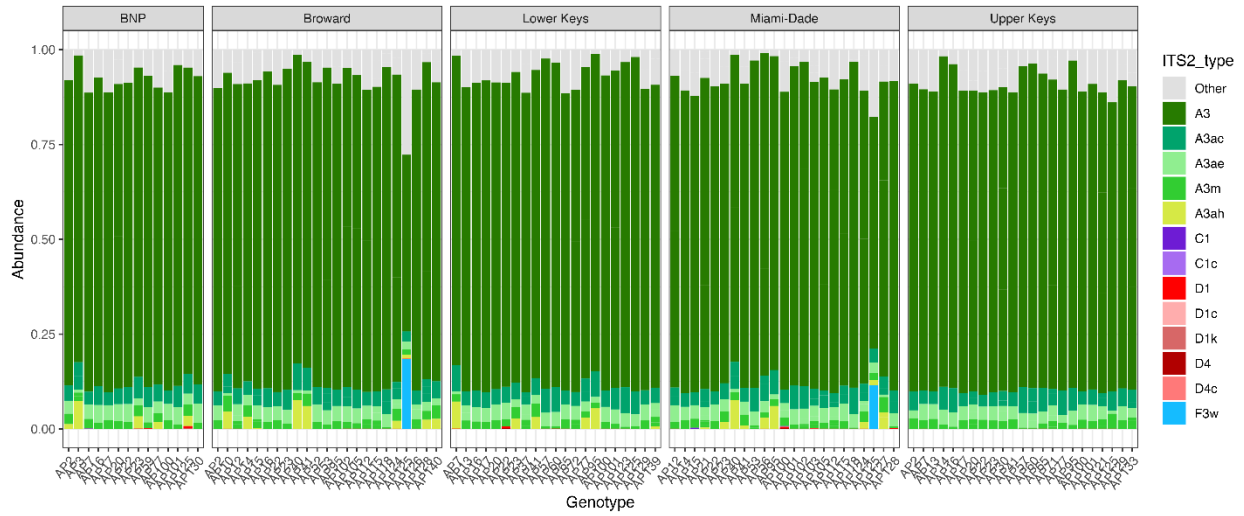


Figure 13. Relative abundance of the most abundant symbiont type profiles in *A. palmata* six months after outplanting to different sites. Taxa are included in the plot if they had a relative abundance greater than 1% within a site. Data are averaged across replicates of the same genotype.

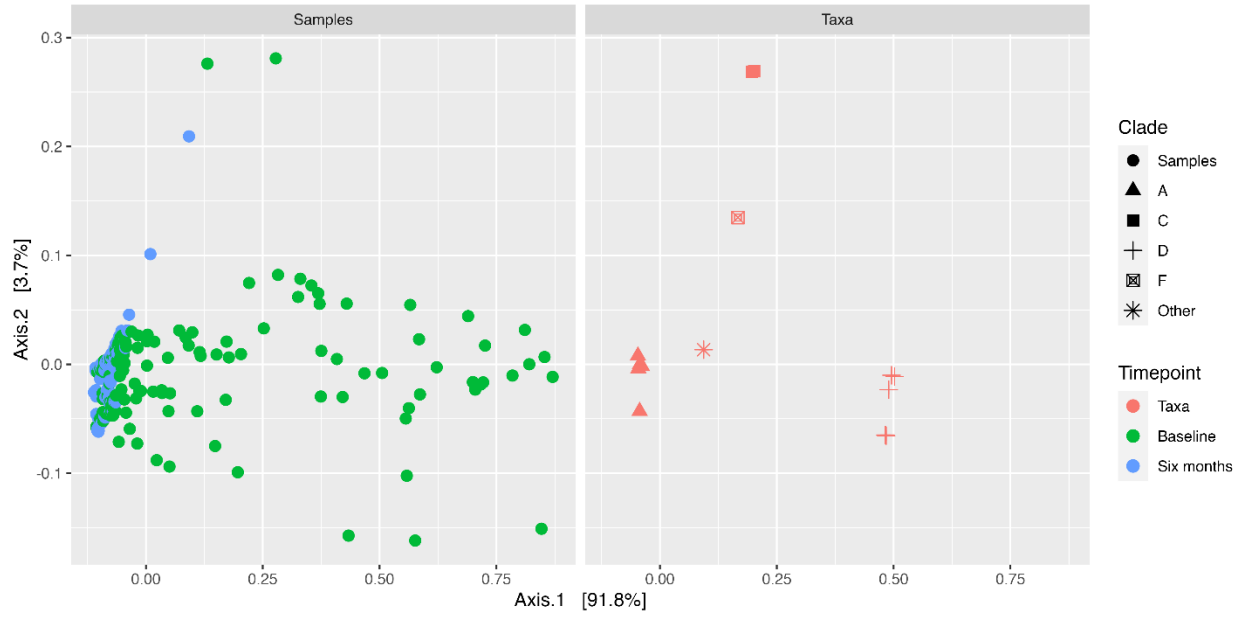


Figure 14. Principal components analysis of symbiont community data for *A. palmata* samples at two timepoints. Samples are plotted in the left panel and symbiont clades are plotted on the right.

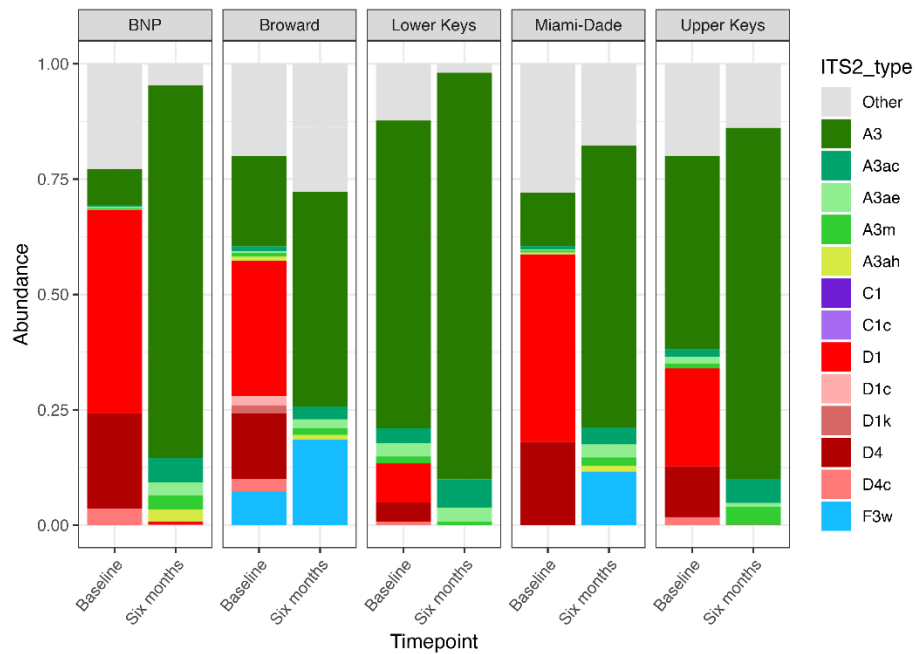


Figure 15. Relative abundance of the most abundant symbiont type profiles in *A. palmata* genotype AP125 at five sites, showing an increase in symbionts assigned to strain F3w. Data are averaged across replicates of the same genotype.

1.1.1.4 Predator Tracking and Removal

1.1.1.1.3 Hermodice carunculata

Throughout the duration of the 1-year monitoring, fireworm traps were deployed within restored *A. palmata* plots at each of the 5 sites to track fireworms, of the species *Hermodice carunculata*, abundance. Fireworm traps were constructed of pvc (n = 10 per plot) and baited with squid prior to each deployment (Fig. 17). Divers noted the date and time of deployment and retrieval, measured the size of the fireworms caught within the traps, and discarded fireworms on land. Between August 2023 - February 2024, a total of 1-2 fireworm trap deployments were completed at each site fireworm traps were deployed at each site. A total of 48 fireworms were trapped and removed from the sites (Fig. 16).

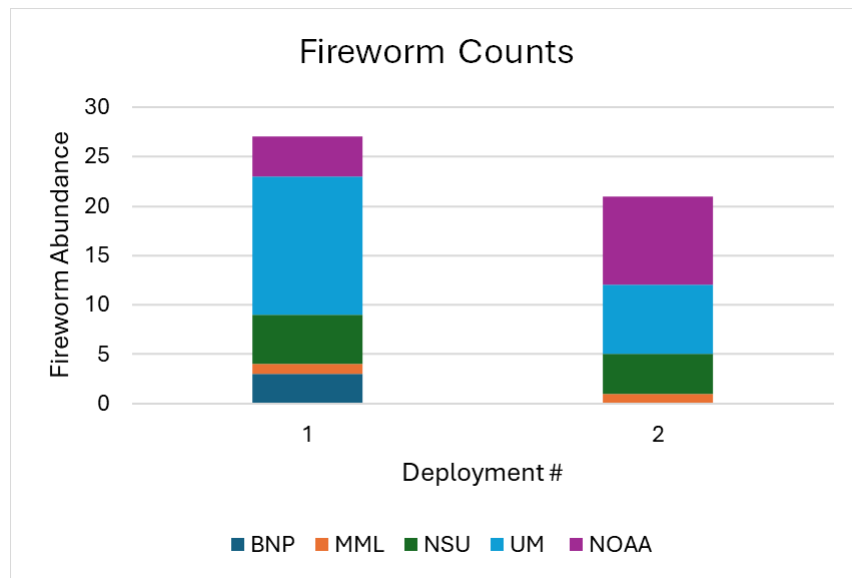


Figure 16. Count of fireworms that were caught in baited traps across the 5 sites between August 2023 – February 2024.

1.1.1.1.4 Coralliophila abbreviata

At each monitoring interval, snails of the species *Coralliophila abbreviata* were removed from *A. palmata* colonies and the plot, measured and discarded on land. Between 6-12 month surveys, a total of 56 snails were tracked and removed from affected colonies across the 5 sites (Fig. 18).



Figure 17. Evidence of snail predation was documented and snails were removed from affected colonies (right) and fireworms were counted and removed from the *A. palmata* plots via baited trapping (left).

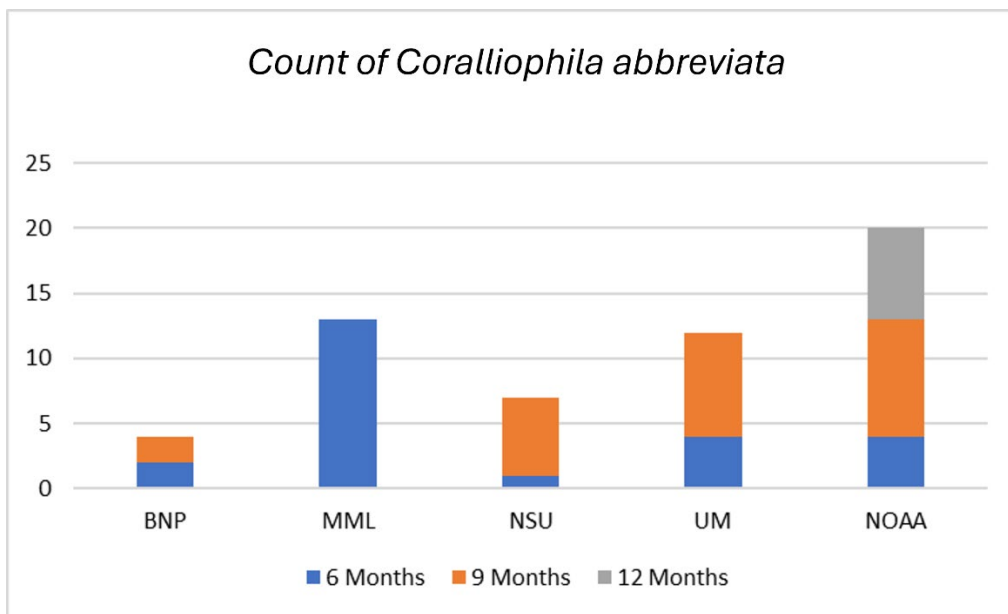


Figure 18. Count of *Coralliophila abbreviata* snails that were tracked and removed from the 5 site between 6-12 month surveys.

1.1.1.5 Discussion

Over the one year of monitoring *A. palmata* outplants across the 5 regions (Lower Keys, Upper Keys, BNP, Miami, and Broward), coral survivorship was high prior to the Summer 2023 bleaching event. Sites south of Miami Dade experienced a significant amount of bleaching and thermal stress related mortality, particularly at the Upper Keys, Lower Keys, and BNP Sites. By the time of 9-month surveys, the Lower Keys site experienced complete mortality, though

survivorship remained high at the two norther sites in Miami and Broward, confirming that the *A. palmata* offspring of Key Largo parent colonies can be successfully outplanted in northern counties.

Based on the live area index values calculated for each of the *A. palmata* clusters across the 5 sites, productivity was enhanced by outplanting corals in small (N=3) density arrangements. There were no reported incidents of disease and mortality did not appear to be influenced by density.

Coral tissue sampling of the *A. palmata* outplants showed that symbiont and microbial communities were significantly more variable prior to being outplanted. Baseline samples showed that across the 50 genotypes, the dominant strain was *Symbiodinium fitti* strain A3, though some samples had lower abundances of *Durusdiniam*, with one sample having as much as 57% proportion of D1. After 6 months, *A. palmata* outplants were resampled and it was determined that the proportion of *Symbiodinium fitti* strain A3 increased while the proportion of *Durusdiniam* across samples decreased. These *A. palmata* were reared at the MML facility and it appears that corals at this facility may carry the more thermally tolerant zooxanthellae strains, though the potential benefit is lost as corals spend time on the reef and proportions of *Durusdiniam* apparently decrease.

1.2 Massive Corals

1.1.3 Methods

In February 2023, divers from Broward, Miami and BNP outplanted 840 corals of massive morphologies (*C. natans*, *D. labyrinthiformis* outplants and *O. faveolata*) at 3 sites (Fig. 19). At each reef site, 15m x 25m plots were set up using nails and tags. Each treatment plot was 1m x 1m in size, spaced 5m apart. Corals were outplanted using cement in the following treatments:

- 1) High-density quadrats (6 plots; 15 corals m⁻²)
- 2) Medium-density quadrats (6 plots, 9 corals m⁻²)
- 3) Low-density quadrats (6 plots, 3 corals m⁻²)

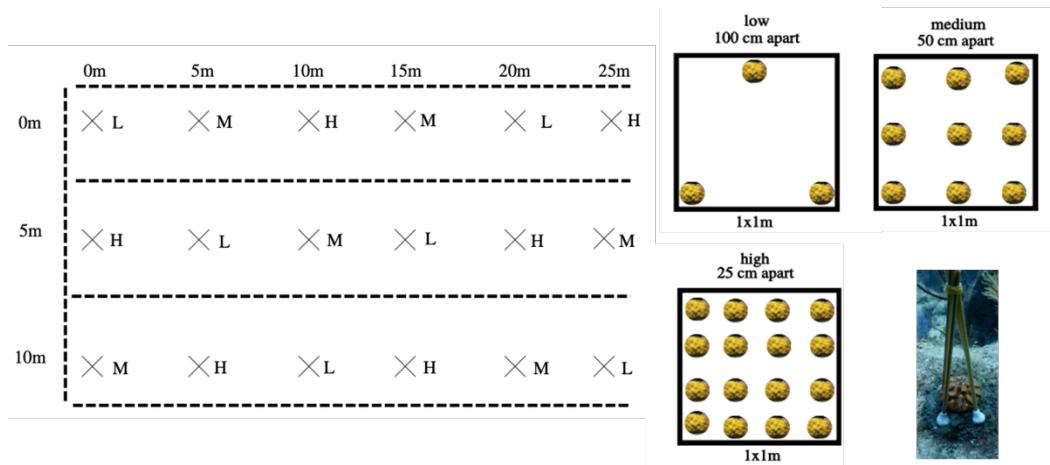


Figure 19. Schematic of the design used to assess the role of coral outplant density on fish predation showing the site layout, the density arrangements, and the teepees used for coral protection.

Divers from Broward outplanted 210 *C. natans* at their site, Miami outplanted 210 *C. natans* and 210 *D. labyrinthiformis* corals at their site, and BNP outplanted 210 *O. faveolata* at their site (Fig. 20). Monitoring for this project was expanded up to 1 year post outplanting, so surveys were completed after 1 week, then 1, 3, 6, 9 and 12 months post outplanting to conduct RVC fish surveys and coral condition surveys to assess for paling, disease, or causes of partial mortality. The *C. natans*, *O. faveolata*, and *D. labyrinthiformis* outplanted at the 3 reef sites by Miami, BNP, and Broward were monitored at the 6, 9, and 12 months timepoints to assess survivorship, growth, predation impacts, and disease prevalence.



Figure 20. Location of outplant sites where massive corals were deployed in February 2023 and the partners that completed the outplanting and monitoring at each site.

1.1.4 Results

A pairwise comparison using a log rank test determined that there were significant differences between species and density treatments at the Miami Site (Fig. 21). Specifically, *C. natans* in the high-density treatment had significantly higher survivorship compared to *C. natans* in the low-density treatment ($p=0.0026$). Survivorship of *C. natans* was significantly lower relative to *D. labyrinthiformis* outplants at the Miami Site ($p<0.01$).

D. labyrinthiformis survivorship remained high throughout the project (99.4%). Treatment density did not significantly influence susceptibility of *D. labyrinthiformis* to predation.

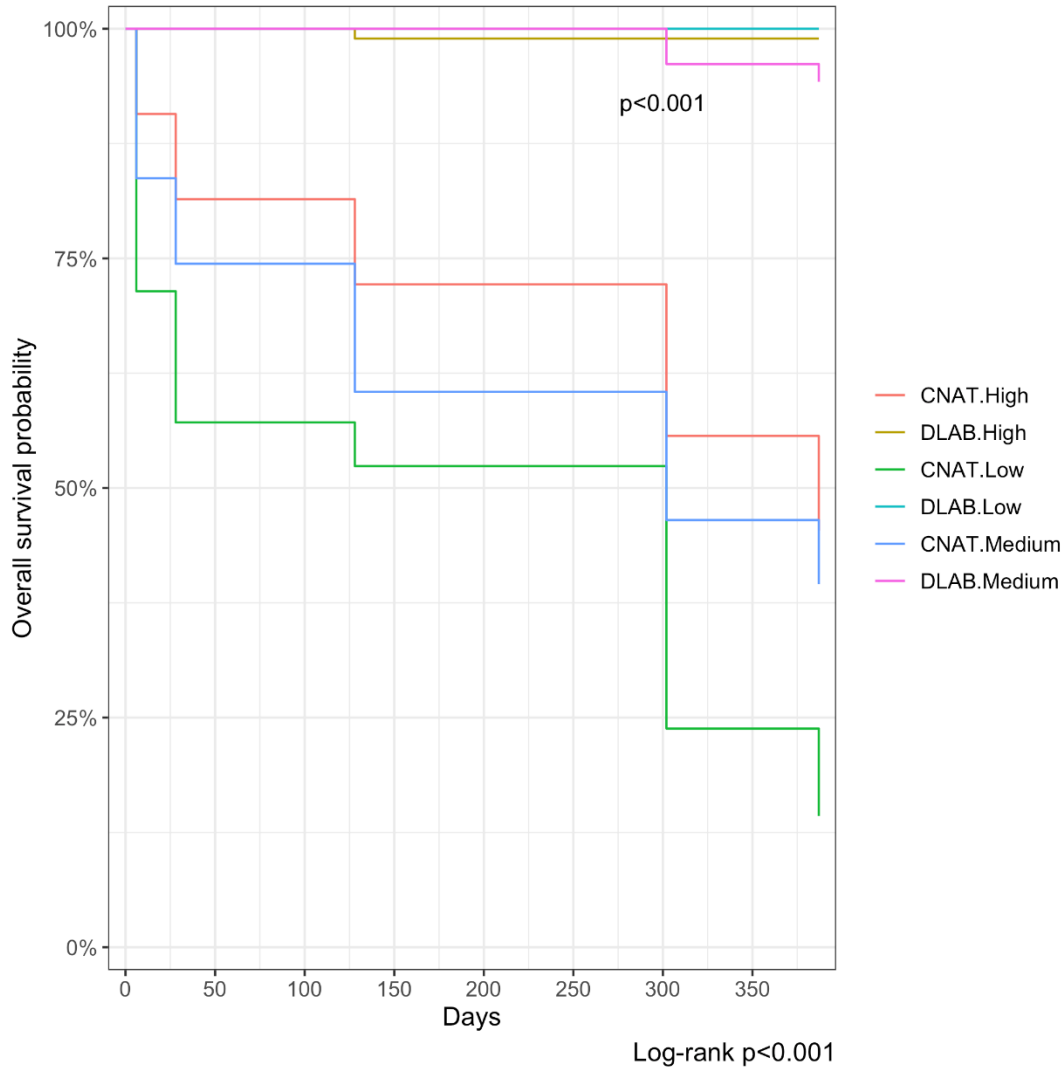


Figure 21. Probability of outplant survivorship between coral species and density treatment at the Miami Site.

The leading cause of partial mortality among coral outplants at the Miami Site was fish predation with the proportion of corals bitten or removed being highest during the first week following outplanting among *C. natans* (81% mortality in low-, 67% in medium-, 61% in high-density treatments) and the first month among *D. labyrinthiformis* (67% in low-, 68% in medium-, 76% in high- density treatments) (Fig. 23 and 24)

A pairwise comparison using a log rank test determined that site and treatment affected the survivorship of *C. natans* outplanted at the Miami and Broward Sites (Fig. 22. Between the Broward and Miami Sites and the three density treatments, *C. natans* outplants in the low-density treatment at the Miami Site had the lowest overall survivorship over the 1-year monitoring

period. *C. natans* in the low-density treatment at the Miami site had significantly lower survivorship compared to the high- density treatment at the Miami Site ($p=0.013$) and in the low- ($p=0.047$) and high- ($p= 0.0093$) density treatments at the Broward Site.

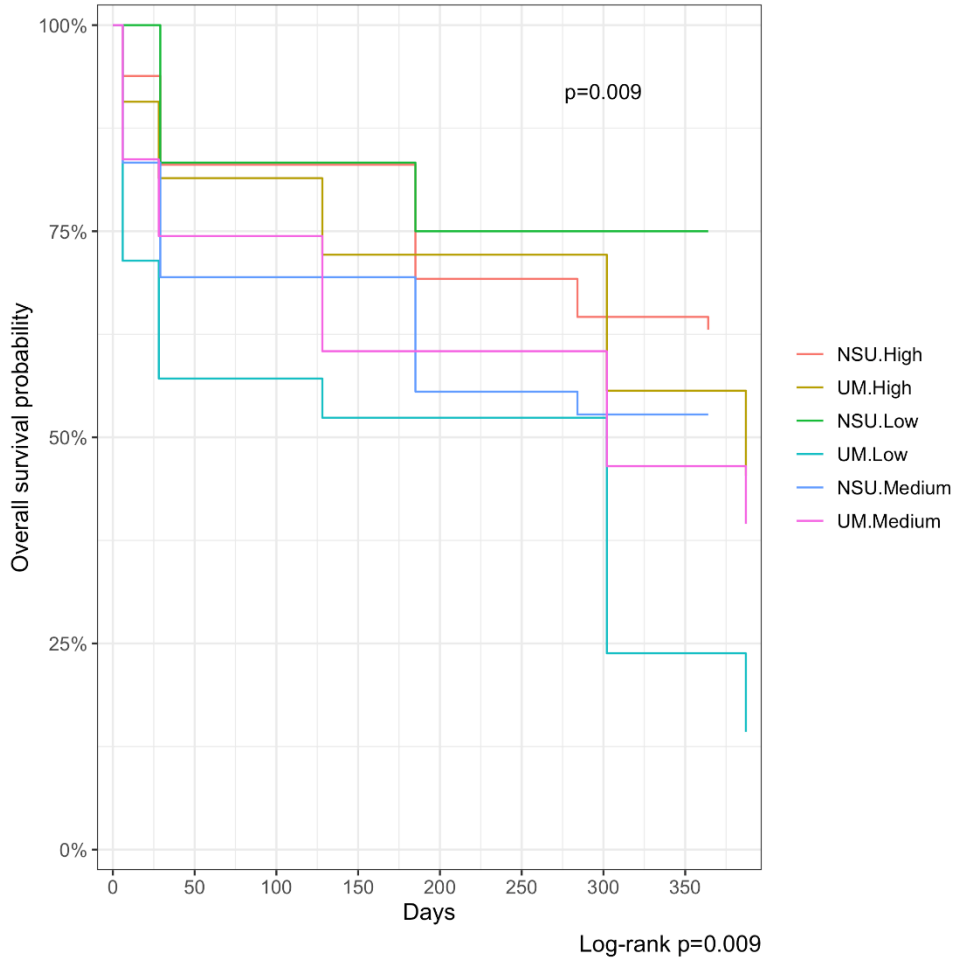


Figure 22. Probability of outplant survivorship of *C. natans* outplants between density treatments at the Miami and Broward Sites.

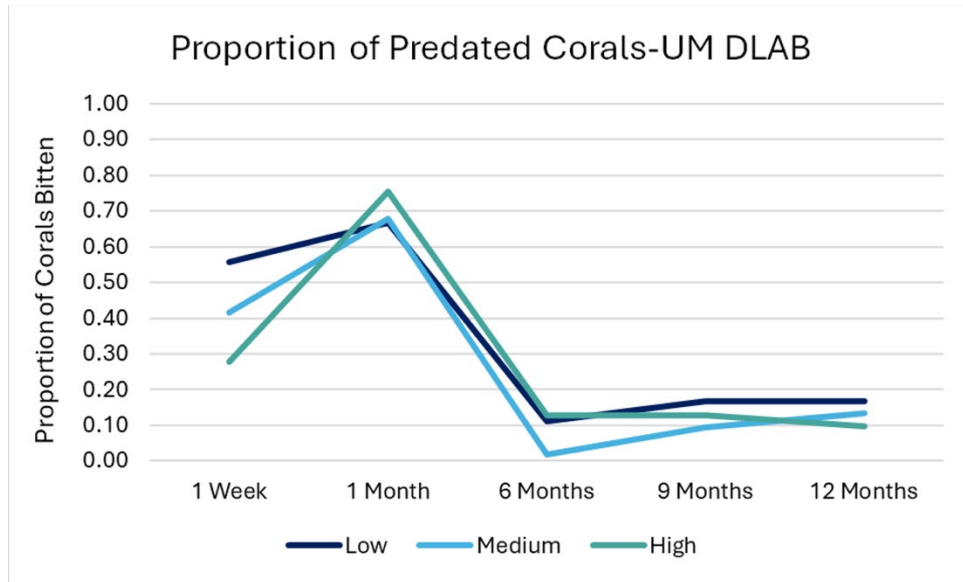


Figure 23. Proportion of *D. labyrinthiformis* outplants that experienced fish predation at the Miami Site.

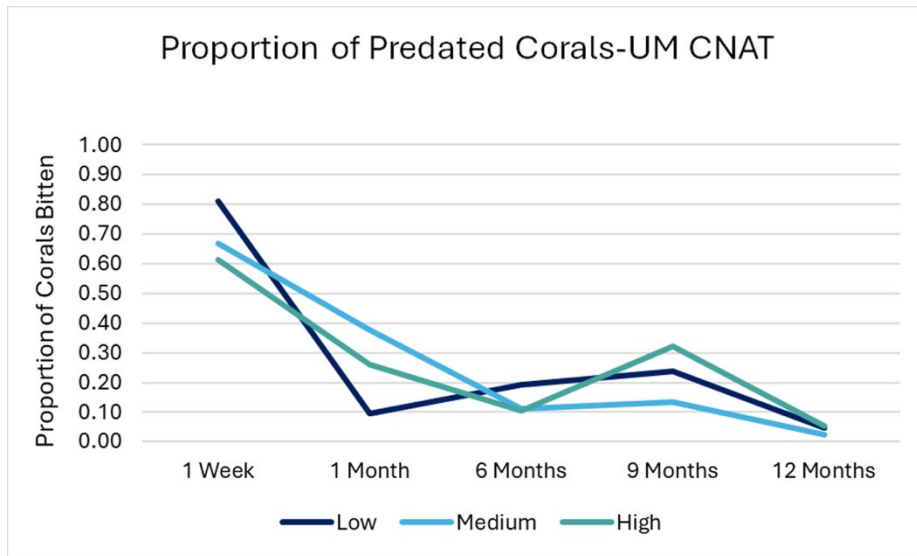


Figure 24. Proportion *C. natans* outplants that experienced fish predation at the Miami Site.

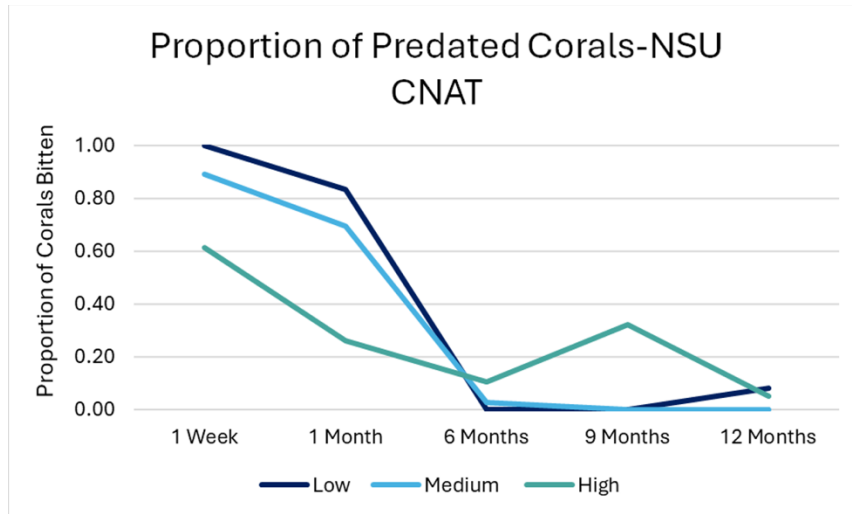


Figure 25. Proportion *C. natans* outplants that experienced fish predation at the Broward Site.

Over the 1-year monitoring period, *C. natans* at the Broward and Miami Sites experienced high initial predation and survivorship over the 1-year monitoring was similar at the two sites (41.8% and 38.8% respectively). *D. labyrinthiformis* appeared to be much less susceptible to fish predation and maintained high survivorship throughout the duration of the 1-year monitoring (97.5%). One year post outplanting, survivorship of *O. faveolata* at the BNP Site was (63%) (Table 3). Proportion *C. natans* outplants that experienced fish predation at the Broward Site was highest during the first week of monitoring (Fig. 25).

Table 3. Survivorship of coral species of massive morphologies across the 2 sites from 1-week to 1-year monitoring.

Site, Species	1 Week Survivorship (%)	1 Month Survivorship (%)	6 Months Survivorship (%)	9 Months Survivorship (%)	12 Months Survivorship (%)
BNP OFAV	89.1	80.6	69.7	68.5	63.0
Broward CNAT	61.8	53.3	44.2	42.4	41.8
Miami CNAT	84.8	75.2	66.1	47.9	38.8
Miami DLAB	100	100	99.4	98.2	97.5

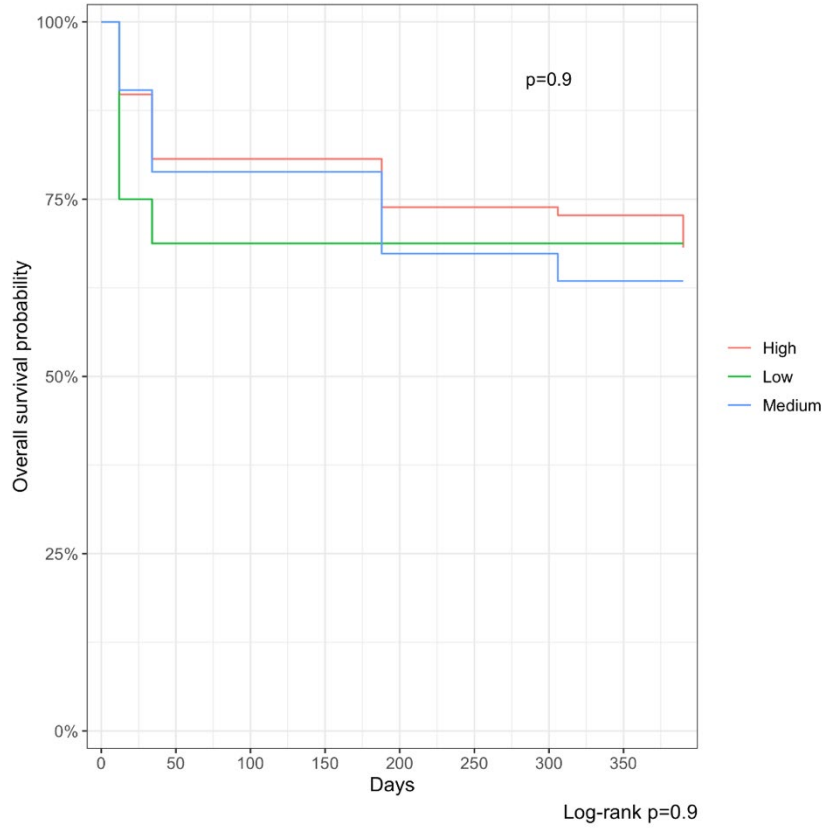


Figure 26. Probability of surviving *O. faveolata* outplants between density treatments at the BNP Site.

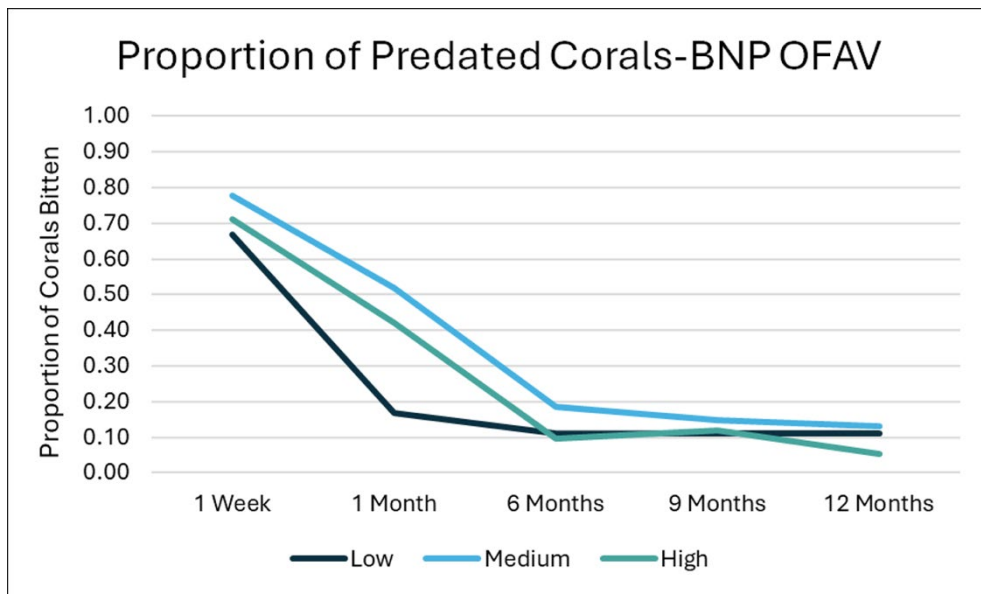


Figure 27. Proportion of *O. faveolata* that experienced fish predation at the BNP Site.

Fish predation on *O. faveolata* outplants was most severe in the first week following outplanting (Fig. 27). A pairwise comparison using a log rank test determined that survivorship of *O. faveolata* was not significantly affected by density treatment at the BNP Site (Fig. 26).

1.1.5 Discussion

Of the three SCTL D-susceptible species that were outplanted across the 3 regions (BNP, Miami, and Broward), there were clear differences in coral species and density treatments that impacted susceptibility to fish predation. *D. labyrinthiformis* experienced minimal mortality over the duration of the outplant project, with 97.5% survivorship after 1 year, while *O. faveolata* at the BNP Site and *C. natans* (63% ad ~40% respectively) at the Miami and Broward Sites experienced more predation, suggesting these corals may require more protection to mitigate predation pressure in order to enhance long term survivorship. Regardless of coral species, outplant location, and outplant density treatment, fish predation was most severe during the first week following outplanting. This suggests that it may be helpful to use temporary, mitigative measures to deter fish predation during the first weeks following outplanting to increase outplant survivorship. Among *C. natans* corals at the Miami and Broward Sites, those outplanted in the high-density treatment appeared to be less susceptible to fish predation compared to those in the low-density treatment. Given this observation, we would recommend that restoration efforts involving *C. natans* use mitigative measures, such as caging or similar methods to deter fish predation, in order to ensure long term survivorship. Finally, no disease was documented among any of the coral species at any of the sites, so there was no impact of density treatment on susceptibility to disease.

Management Recommendations

- Our project has demonstrated that offspring from parent colonies originating from Monroe County can be successfully restored in Miami-Dade and Broward Counties to increase the genetic and genotypic diversity of this species that is found in critically low densities throughout the Florida Coral Reef.
- The high survivorship of *A. palmata* clusters grown at northern sites UM (97.5%) and NSU (83.9%) following the Summer 2023 bleaching event indicates the viability of the assisted migration of this keystone species.
- In the absence of source populations outside the Keys, only the ex situ husbandry of this species will provide the tissue needed for successful restoration. We strongly encourage management agencies to provide the financial resources needed to expand these coral transfers to preserve and restore the connectivity of this depleted species along Florida's Coral Reef.
- Fish predation continues to be the main driver of tissue losses for outplanted massive corals. Differences in predation impacts were found among coral species, site, and outplant density. Outplanting corals in high-density arrangements appears to provide benefits to corals through predation dilution. Fish predation impacts appear to be

consistently most severe within the first week to month following coral outplanting, so additional measures should be explored to reduced fish predation of newly outplanted corals, which reached an average of >30% tissue removal for the most susceptible species (*C. natans* and *O. faveolata*) after just one week.

- We encourage management agencies to continue to allocate resources to understand the drivers of fish predation and design mitigation strategies so that massive corals can be effectively incorporated into large-scale restoration efforts. Until these bottlenecks have been explored, we suggest focusing restoration on species like *D. labyrinthiformis* that maintained high survivorship over the 1-year monitoring period (97.5%) and are significantly less susceptible to predation (other species still need to be propagated and outplanted to help understand drivers of species susceptibility to predation).