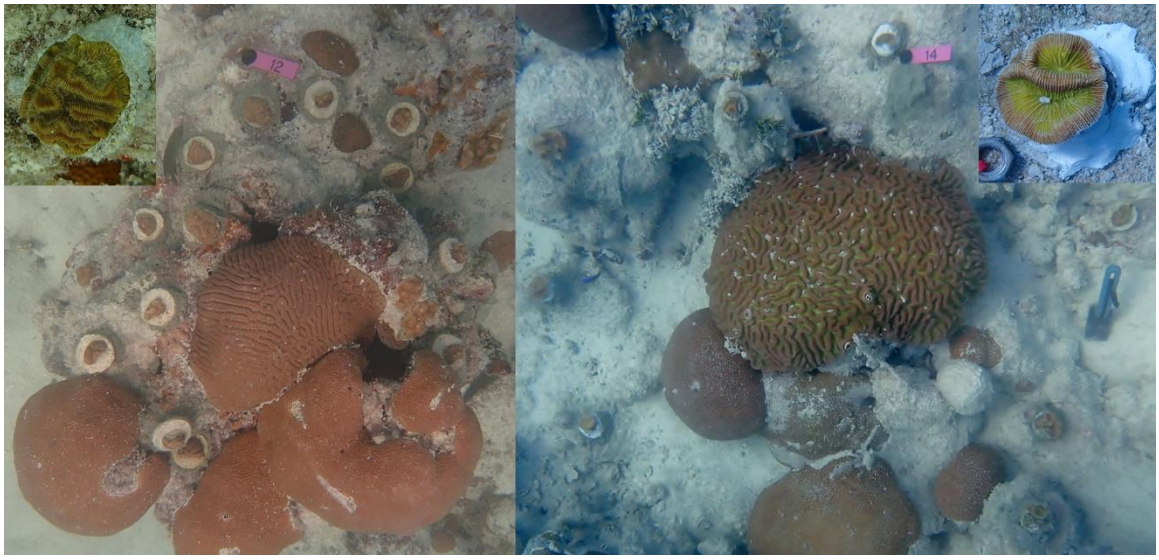
F5619-25-F

Harnessing Coral Resilience through Nursery-to-Reef Restoration Processes Phase 2



FLORIDA'S CORAL REEF

F5619-25-F

Harnessing Coral Resilience through Nursery-to-Reef Restoration Processes Phase 2

Final Report

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Management Summary (300 words or less)

This multi-phase project was designed to track corals through coral collection, propagation, nursery grow-out, and outplanting. This provides the foundation for developing optimal outplanting strategies for *Colpophyllia natans* and *Diploria labyrinthiformis* by evaluating whether coral that survived disease and thermal events maintain resilience during nursery grow-out and subsequent outplanting. Phase 2 of this project was divided into two sections: the continued monitoring of coral within the in-water coral nursery and post-outplanting monitoring. The outplanting was designed to evaluate the effects of shallow versus deep reef environments and proximity of a conspecific on post-outplant growth and survival.

Nursery monitoring found that the overall genotypic variation in Phase 1 survival persisted into Phase 2 with some changes within specific genotypes. Genetic variation was greater within *C. natans* compared to *D. labyrinthiformis*. Additionally, analysis of survival throughout Phase 1 and Phase 2 found differences in thermal sensitivity. The mortality of some genotypes was significantly affected by cumulative exposure to temperatures above 30°C whereas others showed little to no effect. Instead, some genotypes showed consistent higher mortality due to non-thermally-related factors.

Monitoring data suggest species specific effects of reef depth and proximity to conspecifics within the first month after outplanting. Specifically, percent living tissue of *D. labyrinthiformis* outplants was significantly affected by conspecific proximity and reef depth. Those corals outplanted at the shallow sites had higher percent living tissue compared to deep sites and within the corals at the shallow sites, corals outplanted closer to a conspecific had higher percent living tissue. Results represent immediate responses to the different outplant conditions rather than long-term restoration performance. Long term monitoring will allow us to make outplanting recommendations for these two species. Long-term monitoring will allow us to evaluate whether genotypic performance within the in-water nursery predicts post-outplant survival and condition.

Executive Summary (max 1 page)

The marine heatwave and mass coral bleaching event of 2023 highlighted the need to identify and propagate coral genotypes with increased tolerance to environmental stressors. To this end, the Florida Fish and Wildlife Conservation Commission (FWC), with the support of the Florida Department of Environmental Protection, designed a multi-phase experiment to track corals through coral collection, propagation, nursery grow-out, and outplanting. In the first phase, FWC collected *Colpophyllia natans* and *Diploria labyrinthiformis* from nearshore reefs in the Upper, Middle, and Lower Florida Keys. These corals were kept within FWC's in-water coral nursery and monitored monthly. Survival differed significantly by species, genotype, and phase. While the majority of *D. labyrinthiformis* demonstrated higher survival in Phase 2 than Phase 1, survival differences in *C. natans* between phases were less clear due to higher between phase variation relative to *D. labyrinthiformis. D. labyrinthiformis* survival was 90% in Phase 2, 96.4% when removing the single genotype that died, and 82.3% in Phase 1. For *C. natans,* some genotypes that showed high mortality (>35%) in Phase 1 had lower mortality in Phase 2 (<8%) while some had the opposite trend with mortality <5% in Phase 1 and mortality > 25% in Phase 2. Some genotypes did not show significant differences in mortality trends between Phase 1 and 2 with some showing persistent mortality >20% while others showed no mortality.

The next step of this project was to examine how coral resilience/resistance before collection and during in-water nursery grow-out is related to post-outplant performance. We also examined the effects of outplant reef depth and outplanted coral proximity to wild conspecifics on post-outplant survival. Significant differences in *D. labyrinthiformis* survival between reef depth and conspecific proximity were revealed one-month post-outplant; corals outplanted at shallow reef depths demonstrated 13% higher percent living tissue than corals outplanted on deeper reefs. Additionally, *D. labyrinthiformis* outplanted near conspecifics showed 2.4% higher percent living tissue compared to corals outplanted >1m from conspecifics. The mechanisms underlying these patterns remain unclear and warrant further investigation. Because temperatures at the outplant sites have not yet exceeded the 30.5°C threshold identified in FWC and FKNMS protocols for the stress management protocols (NOAA, FKNMS, FWC 2026), potential depth-related refugia effects cannot yet be assessed.

Continued monitoring both within the coral nursery and outplant sites will clarify the effect of genotypic differences on *D. labyrinthiformis* and *C. natans* survival and resilience to stressors, including disease and thermal conditions. Collection of corals of opportunity and integration into the restoration pipeline continues to be a vital tool used by restoration practitioners to preserve the genetic diversity of Florida's Coral Reef. The identification of traits and selection of genotypes associated with higher probabilities of survival across a gradient of reef environments holds potential for improving the success of reef restoration efforts. Integration of selection for species-specific reef environments and methodologies into outplanting strategies should be considered to enhance restoration success. For example, reef depth and outplant proximity to a conspecific are both characteristics that can be targeted when outplanting without site manipulation, thus traits associated with these factors are ideal to test. Results from this project will be disseminated to restoration practitioners to aid in the selection of outplanting locations and strategies for these priority species that may optimize growth and survival to achieve restoration goals.

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1. BACKGROUND

The increased prevalence and severity of thermal events, storms, and chronic disease outbreaks have accelerated the deterioration of Florida's Coral Reef (FCR). The summer of 2023 was marked by an unprecedented marine heatwave that resulted in a mass coral bleaching event throughout the Caribbean and Florida and caused extensive mortality of corals along the FCR and within in-water coral nurseries in the Florida Keys, including the nursery operated by the Florida Fish and Wildlife Conservation Commission (FWC).

The challenges of conducting coral restoration efforts under the prospect of continuing environmental stressors provided the impetus for the FWC to evaluate coral species and genotypes that may exhibit resistance and/or resilience to future environmental disturbances. Resilient genotypes might be incorporated into restoration efforts and improve the likelihood of positive restoration outcomes. To address that need, and with the support of the Florida Department of Environmental Protection, the FWC is conducting a multi-phase project focused on identifying, collecting, propagating, and outplanting corals and assessing both within-nursery and post-outplant survival.

This study is structured into two phases that reflect both temporal progression and experimental scope. Phase 1 encompassed the initial collection, fragmentation, and nursery-based grow-out and survival tracking from 2024 through mid-2025. Phase 2 represents the continuation of nursery monitoring beginning during July 2025, as well

as the initiation of the outplanting experiment and subsequent reef-based monitoring. Within Phase 2, nursery survival analyses and outplanting analyses represent distinct but complementary components of the broader restoration evaluation framework. Nursery-based analyses focus on continued genotype performance under in-water coral nursery conditions, whereas outplanting analyses evaluate genotype performance under field conditions across reef depth gradients and conspecific proximity treatments.

Phase 1 aimed to identify coral traits indicative of resistance and/or resilience to environmental conditions through the evaluation of coral microbiomes and metabolomics during colony collection, propagation, and grow-out within FWC's in-water coral nursery. The effort focused on two coral species, *Colpophyllia natans* and *Diploria labyrinthiformis*, both of which are reef-building species that are susceptible to Stony Coral Tissue Loss Disease (SCTLD) and exhibited significant bleaching during the summer of 2023 and subsequent declines in abundance. However, declines in abundance for both species were lower than those observed in many species commonly propagated within in-water nurseries, suggesting the presence of individuals of these species on reefs within the FCR with the potential for inherent resistance and/or resilience to thermal stress and disease impacts relative to other coral species.

In Phase 1 we collected colonies of *C. natans* and *D. labyrinthiformis* from three regions of the Florida Keys that survived both the 2023 thermal event and the continued presence of SCTLD. The survival of these colonies under concurrent thermal and disease stressors served as a proxy for potential genotypic resistance and/or resilience, making them particularly valuable candidates for propagation, restoration, and future restoration performance assessments.

C. natans and *D. labyrinthiformis* were maintained in the FWC's in-water coral nursery. These genotypes were fate-tracked, and their microbiomes and metabolomes were analyzed. Whereas some genotypes showed little to no mortality in the first year, others experienced mortality rates greater than 80%. Similarly, some genotypes grew sufficiently large for secondary propagation following initial fragmentation, whereas others exhibited little observable growth or experienced tissue recession. Survival analyses identified differences associated with genotype, collection location, and initial colony size.

Metabolomic profiles differed significantly by collection location with the Upper Keys separating from the Middle and Lower Keys sites initially. After two months within the coral nursery, metabolomic diversity increased relative to that of initial colonies, with Middle Keys corals separating from the Upper and Lower Keys corals. Investigations into specific components of the metabolome identified a suite of metabolites positively associated with survival.

Analyses revealed that microbial communities were significantly influenced by

genotypic differences and varied between the two sampling timepoints. In addition, specific bacterial families were associated with survival in both *C. natans* and *D. labyrinthiformis* in the months after transport to the coral nursery.

Although these results for nursery maintained corals give us insight into which genotypes may be resistant or resilient to nursery stressors, it is important to evaluate genotypic-specific growth and survival of outplanted corals to inform restoration practices that potentially enhance success. To this end, extensive effort has been directed at the development of restoration techniques that optimize outplant survival with the aim of increasing coral cover on degraded reefs. Accordingly, Phase 2 of this study sought to inform this effort by investigating 1) the effects of site depth and 2) the proximity to conspecifics on the growth and survival of outplanted *C. natans* and *D. labyrinthiformis.*

As the prevalence of thermal events and subsequent coral bleaching increases, deep reef habitats have been proposed as possible refugia (Riegl and Piller 2002, Bongaerts *et al*., 2010). Coral bleaching is a response to elevated water temperatures and/or elevated solar irradiance (Fitt *et al.*, 2001), therefore depth can act as a refuge from both. Importantly, however, depth effects have been demonstrated to vary between species (Montgomery *et al*., 2021, Pereira *et al.*, 2021) and genotype (Maneval *et al*. 2021). Although mesophotic reefs (greater than 30 meters) have been considered as potential refugia, this study focuses on shallower depth ranges more relevant to restoration efforts (less than 8 meters vs greater than 15 meters). These depths may still influence coral performance through variation in environmental conditions such as temperature and light attenuation (Riegl and Piller 2002, Pereira *et al.*, 2021).

In addition to the potential effects of depth, we also investigated the influence of outplanting corals in proximity to wild-occurring conspecifics. Coral microbiome and metabolomic characteristics may contribute to coral resilience and survival *via* pathogen defense, metabolic cycling, and thermal tolerance. Some coral species have been shown to shift their microbiome when transferred from the source location (i.e. reef) to a coral nursery, and shift yet again when outplanted (Strudwick *et al.*, 2022, 2023). Because corals acquire portions of their microbiome from their environment (Schul *et al.*, 2024), proximity to wild conspecifics that survived the recent environmental disturbances, may, at least in part, influence the microbial environment experienced by outplanted fragments and potentially affect their post-outplant performance. Although microbiome and metabolome were not directly tested during this phase of the project, our results may provide collective evidence of the benefits of outplanting in proximity of resilient coral colonies that may inform future research exploring these biological mechanisms underlying coral health and resilience. To evaluate this hypothesis in a restoration context, we designed a field experiment to test

whether reef depth and proximity to wild conspecific coral colonies influence post-outplant coral performance.

2. METHODS

Fragments of *Colpophyllia natans* and *Diploria labyrinthiformis* used in this study originated from Phase 1 of the project and were maintained in FWC's in-water coral nursery prior to outplanting and monitoring in Phase 2. Outplanting was conducted April 2026 following receipt of permits. As a result, post-outplant monitoring encompasses approximately one month of observations. Monitoring beyond this reporting period is ongoing and will be incorporated into subsequent analyses.

2.1. Nursery

A total of 30 coral genotypes from two species (15 *C. natans* and 15 *D. labyrinthiformis*) were collected and placed within FWC's in-water coral nursery in Phase 1 (2024-2025) of this project. Coral colonies unattached to the substrate and considered at-risk (i.e. corals of opportunity or COOs) were collected from three inshore patch reefs in the Upper, Middle, and Lower Florida Keys. Five individuals each of *Colpophyllia natans* and *Diploria labyrinthiformis* were collected from each reef. These corals were fragmented, placed within the in-water nursery, and monitored monthly to assess overall survival, disease prevalence, predation, and bleaching. For Phase 2 of this experiment, monitoring began July 8, 2025, and continued after outplanting occurred. In addition to monitoring corals, environmental parameters within the nursery were monitored throughout the duration of this project. Survival of each genotype was analyzed in R using Kaplan–Meier survival curves and Cox proportional hazards regression models. The relationship between temperature and survival was analyzed using generalized linear mixed-effects models. The temperature characteristics analyzed were mean temperature, max temperature, hours above 30°C, hours above 31°C, and hours above 32°C. One temperature metric was used per model due to each temperature metric not being independent of the others. In addition to a separate generalized linear mixed-effects model being run for each of these temperature factors, genotype and species was added to the best fit temperature model to assess if these metrics improve fit. Akaike Information Criterions (AIC) were compared for each model to determine the best fit.

2.2. Outplanting

The outplanting experiment evaluated the effects of reef depth (shallow vs deep) and the effects of proximity to wild conspecific colonies (near vs far) separately on post-outplant performance. Conspecific proximity, near (<0.5m) or far (>1m), was

tested at the shallow sites only. Only shallow sites had the required abundance of large conspecifics that survived SCTLD and the 2023 thermal event. Post-outplant coral condition was analyzed in R using generalized linear mixed models to test the effects of our treatments (i.e., reef depth (shallow or deep) or distance to conspecific (near or far) at shallow sites) on the survival of outplants.

Corals were outplanted across sites in the Middle Keys (6 sites): 3 shallow, nearshore patch reef environments <8m and 3 deep offshore reefs >15m (Figure 1). Shallow outplant sites were Washerwoman (24.664, -81.074), NOAA8 (24.694, -81.017), and RAWA3 (24.719, -80.933). The deep outplant sites were Washerwoman Deep (24.627, -81.096) NOAA8 Deep (24.657, -80.997) and East Turtle Deep (24.704, -80.871).

Figure 1. Google Earth Map of Colpophyllia natans and Diploria labyrinthiformis outplant sites comprising three shallow sites (Washerwoman, NOAA3, and RAWA-3) and three deep sites (Washerwoman Deep, NOAA8 Deep, and East Turtle Deep). All outplant sites are located off Marathon, Florida Keys.

At each site, 4 or 5 *C. natans* genotypes and 7 *D. labyrinthiformis* genotypes were outplanted. At the shallow sites, half of the fragments were placed adjacent (<50 cm) to the wild conspecifics and half of the fragments placed away from conspecifics (1-2 m) (Figure 2a). At the deep sites, each fragment was outplanted ~1.5 meters from the center point of the plot (Figure 2b). Individual genotypes were placed on newly cleaned substrate using a golf ball sized portion of cement (10:1 Portland Cement: Silica Fume).

Before outplanting the substrate was scrubbed clean of all algae using a wire brush so that bare substrate was visible, usually indicated by white rock.

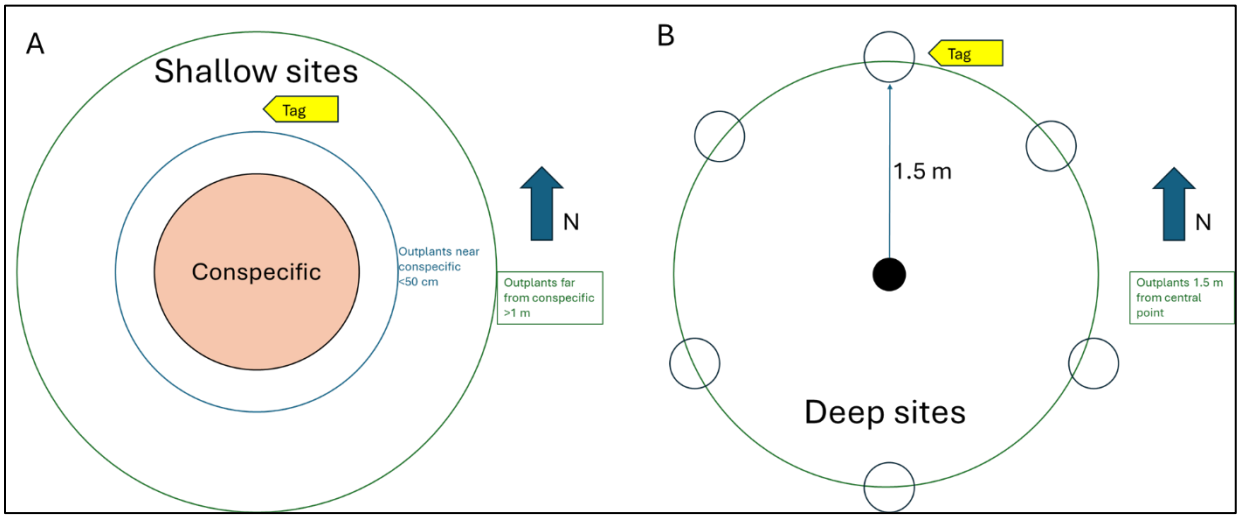

Figure 2. Layout for outplanting of corals at the Shallow (A) and Deep (B) outplant sites. Shallow sites were outplanted in two rings around a conspecific. Deep sites were outplanted in one ring around a central point.

Coral outplants were monitored monthly to examine the effects of depth and conspecific proximity on coral growth and survival. Each month, corals were visually assessed for overall percent living tissue and signs of disease, predation, and bleaching. The percent of the whole coral colony with live tissue was estimated and recorded by divers. In addition, the percent of the whole coral colony with old and new mortality was recorded. Old mortality is defined as non-living portions of the coral that have become completely overgrown by organisms such as algae, sponge, and encrusting octocorals. New mortality is defined as non-living parts of the coral where the white coral skeleton is visible, and can result from disease, predation, and abrasion. Together, the percent living tissue, percent old mortality, and percent new mortality must add up to 100%. Top-down images of outplants were collected for each coral fragment 1 week after outplanting and monthly thereafter using an OM SYSTEM Tough TG-series digital camera with a scale bar.

3. RESULTS

3.1. Nursery

Coral survival within the in-water nursery was analyzed in two ways; separately in Phase 1 and Phase 2 to analyze between phase differences and since placement in the nursery (combining Phase 1 and Phase 2) to analyze effect of temperature. For these analyses, Phase 1 was from placement in the nursery to May 2025 (approximately 290 days) and Phase 2 from July 2025 to April 2026 (approximately 295 days). In both

phases, survival differed significantly among genotypes of *C. natans* (Cox proportional hazards model; Phase 2 seen Figure 3A) and of *D. labyrinthiformis* (Cox proportional hazards model; Phase 2 Figure 3B). At the final monitoring interval, survival patterns were consistent across Phase 1 and Phase 2 for several genotypes. There were five genotypes (4 *C. natans* and 1 *D. labyrinthiformis*) that demonstrated 100% survival in both Phase 1 and Phase 2 of this experiment. One *C. natans* genotype, CN021, continued to experience the highest mortality in this species while the *D. labyrinthiformis* genotype DL064 demonstrated total mortality. When comparing Phase 1 and Phase 2 mortality, there were six C. natans and five *D. labyrinthiformis* genotypes that showed different survival patterns between phases. Four *C. natans* genotypes that showed <10% mortality in the first year, had >25% mortality in the second year while 2 genotypes that had >35% mortality in year one had <10% mortality in year two. Similarly, some *D. labyrinthiformis* genotypes with high mortality in Phase 1 had lower mortality in Phase 2. Specifically, four genotypes with 27-62% mortality in Phase 1 demonstrated 0-13% mortality in Phase 2. However, genotype DL064 experienced 100% mortality in year 2.

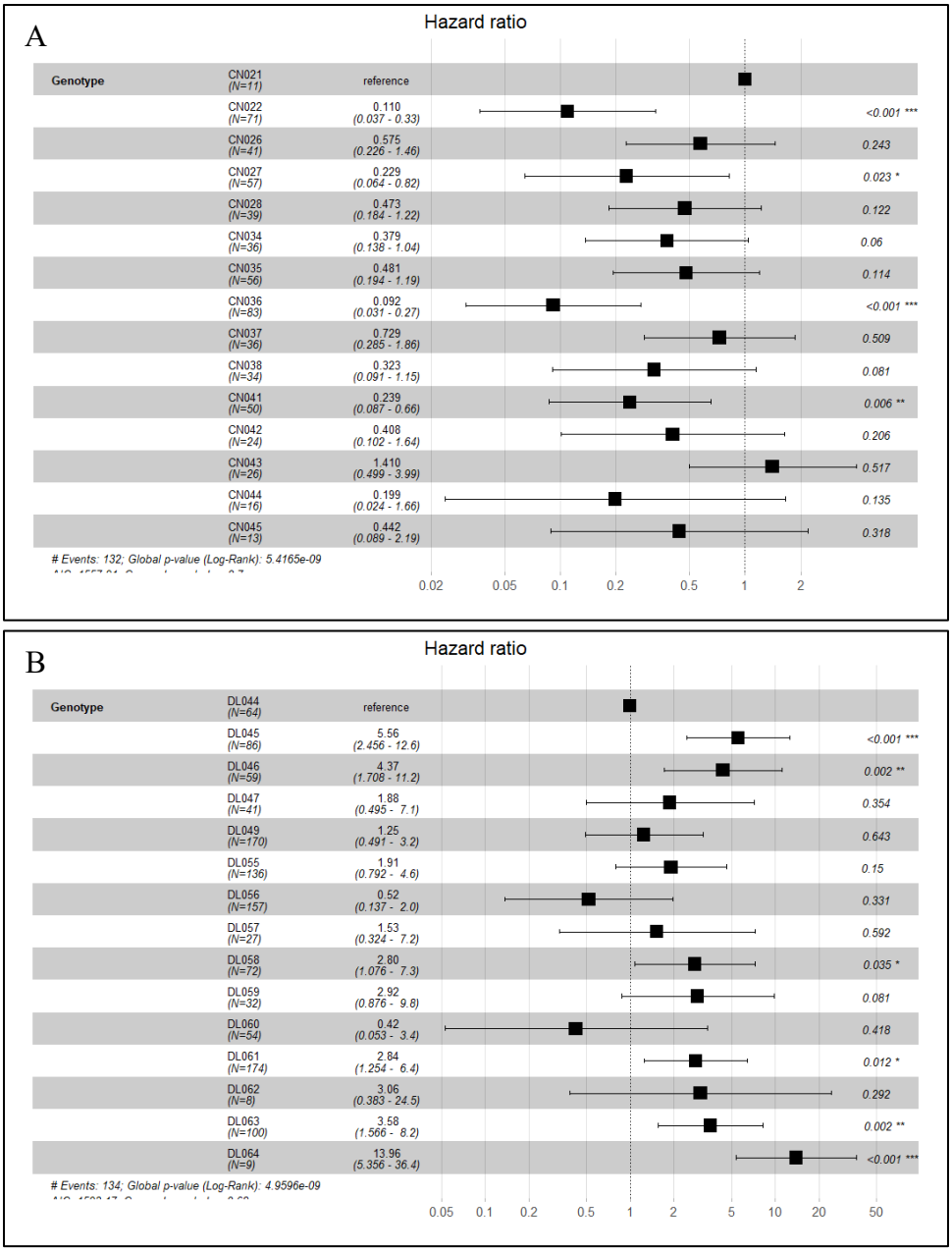

Figure 3. Forest plot of hazard ratios (HRs) and 95% confidence intervals for survival by genotype subgroup for Colpophyllia natans (A) and Diploria labyrinthiformis (B). Squares represent HR estimates and horizontal lines indicate 95% CIs. The vertical dashed line at HR = 1 denotes no difference in risk relative to the reference group. Genotypes with confidence intervals not crossing 1 were considered statistically significant. Overall differences among genotypes were significant based on the global log-rank tests.

During Phase 1, cox proportional hazards analyses indicated significant differences in survival among collection locations for both species, with corals from West Turtle showing reduced survival relative to the other two sites (Figure 4A–B). This

relationship was not observed during Phase 2 survival. Collection location did not significantly affect *C. natans* survival (Figure 5A). *D. labyrinthiformis* survival differed between Upper and Middle Keys collection locations with Pickles exhibiting 18% lower survival relative to the Looe corals (Figure 5B).

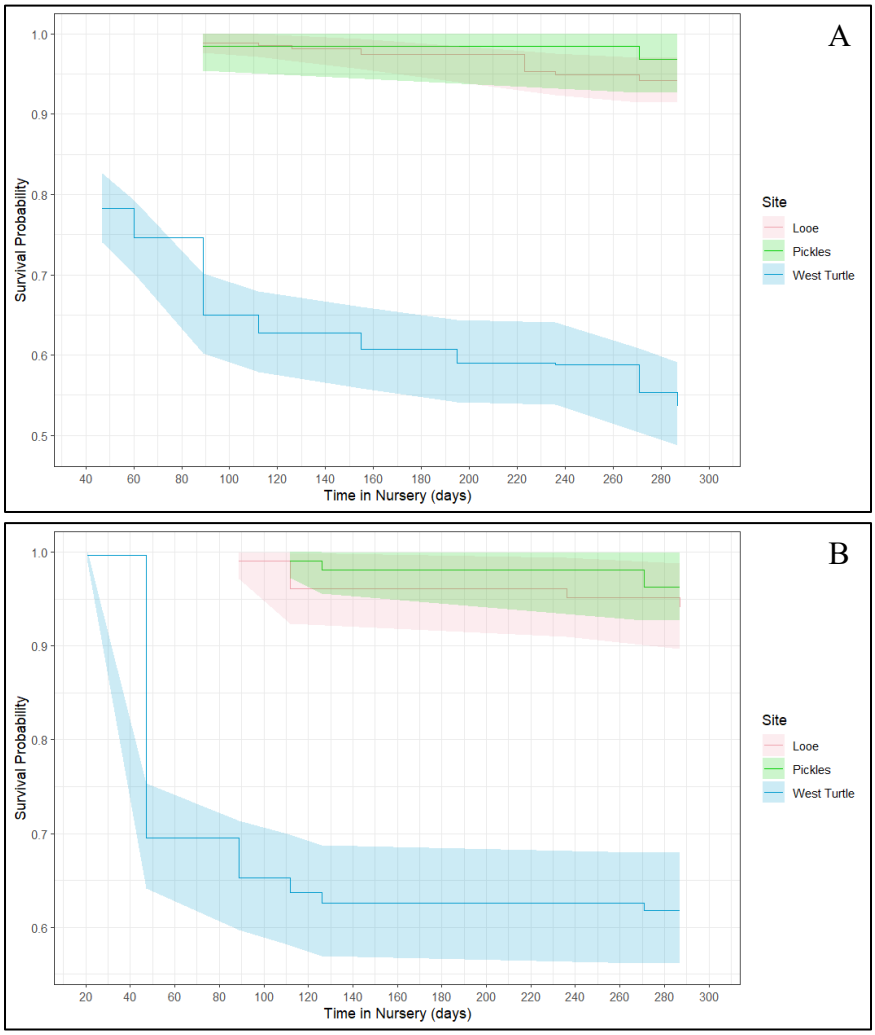

Figure 4. Kaplan-Meier survival curves for Colpophyllia natans (A) and Diploria labyrinthiformis (B) during Phase 1 grouped by collection site. Line plot displaying the estimated survival probabilities of corals by location over time (days) in an in-water nursery setting. Each colored line represents a location, with shaded ribbons indicating 95% confidence intervals. The y-axis shows survival probability from 0 to 1, and the x-axis depicts duration of time in the nursery up to 287 days. The legends on the right of each graph list locations, each color-coded to match the respective survival curve. Corals of both species sourced from West Turtle had significantly lower survival relative to Looe and Pickles corals.

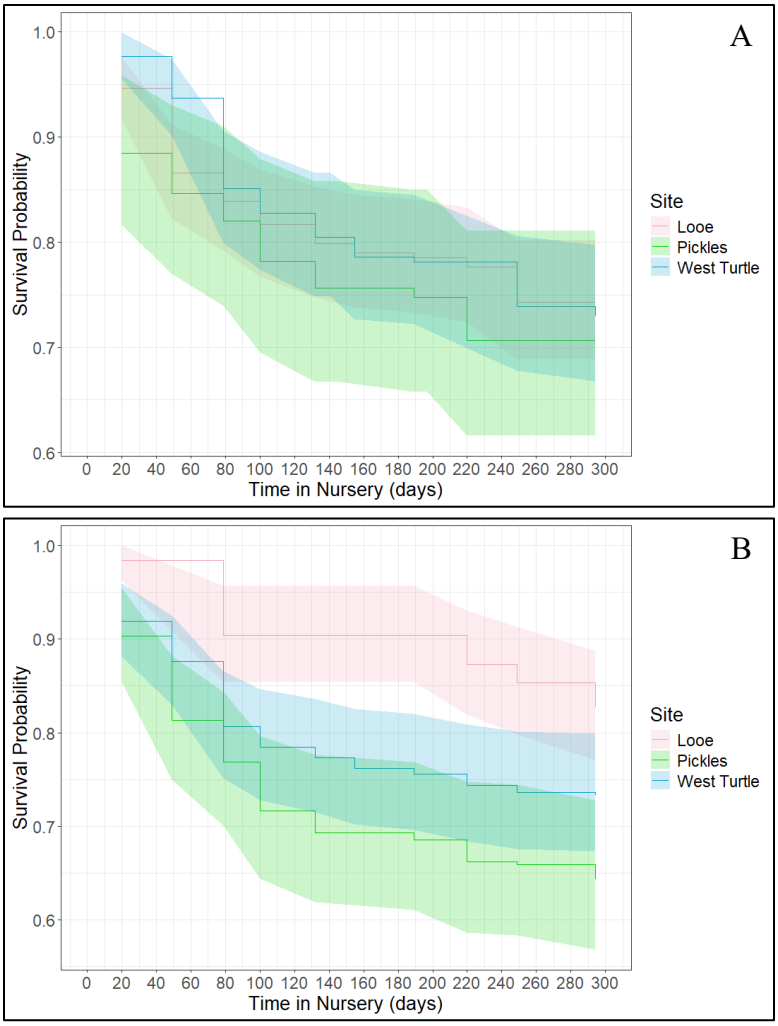

Figure 5. Kaplan-Meier survival curves for Colpophyllia natans (A) and Diploria labyrinthiformis (B) during Phase 2 grouped by collection site. Line plot displaying the estimated survival probabilities of corals by location over time (days) in an in-water nursery setting. Each colored line represents a location, with shaded ribbons indicating 95% confidence intervals. The y-axis shows survival probability from 0 to 1, and the x-axis depicts duration of time in the nursery up to 289 days. The legends on the right of each graph list locations, each color-coded to match the respective survival curve.

In addition to analyzing Phase 1 and Phase 2 survival separately to assess temporal differences, we evaluated species- and genotype-specific mortality responses to temperature across all monitoring intervals. A series of generalized linear models were used to assess mortality as a function of temperature metrics, genotype, and species. Temperature metrics used for models were mean and maximum temperature, and cumulative hours above 30 °C, 31 °C, and 32 °C. Table 1 shows these factors during the time between each monitoring interval in both Phase 1 and Phase 2 of this project. Cumulative hours above 30°C was the strongest predictor among all temperature

metrics with mortality increasing significantly with increasing hours above 30°C ($p < 0.001$). Species did not improve model fit. However, genotype significantly improved model fit ($\Delta AIC = 40.1$, $p < 0.001$) indicating substantial genotypic variation in survival responses to environmental conditions. Temperature variables explained 32.9% of the variation in mortality, while the full model including genotypes explained 86.1%, highlighting the strong influence of genotype-specific effects on thermal tolerance and mortality. All models, AIC values, R^2 marginal, R^2 conditional, and ΔAIC can be seen in Table 2. Based on model outputs, genotypes were divided into four categories reflecting their relative sensitivity to thermal stress and non-thermal sources of mortality: (I) coral with low (non-thermal) mortality and high thermal mortality, (II) coral with high (non-thermal) mortality and high thermal mortality, (III) coral with low (non-thermal) mortality and low thermal mortality, and (IV), corals with high (non-thermal) mortality and low thermal mortality (Figure 6).

Table 1. Summary of the temperature data collected from within FWC's in-water coral nursery. This table includes both monitoring dates and dates of last monitoring to indicate the time span of temperature data. Temperature data in this table includes mean temperature, max temperature, hours above 30°C, hours above 31°C, and hours above 32°C.

Monitoring Date	Date of previous monitoring	Mean Temp	Max Temp	Hours Above 30	Hours Above 31	Hours Above 32
9/5/2024	8/15/2024	31.13	32.81	504	277	53
10/1/2024	9/5/2024	30.96	32.14	552	353	7
10/14/2024	10/1/2024	29.70	31.27	123	8	0
11/12/2024	10/14/2024	27.79	29.35	0	0	0
12/5/2024	11/12/2024	25.81	28.10	0	0	0
12/19/2024	12/5/2024	23.87	25.50	0	0	0
1/17/2025	12/19/2024	23.34	25.84	0	0	0
2/26/2025	1/17/2025	23.81	26.39	0	0	0
3/26/2025	2/26/2025	24.20	25.50	0	0	0
4/8/2025	3/26/2025	26.44	27.96	0	0	0
5/13/2025	4/8/2025	26.35	28.00	0	0	0
5/29/2025	5/13/2025	29.00	30.85	42	0	0
7/28/2025	7/8/2025	30.48	31.41	439	34	0
8/26/2025	7/28/2025	31.42	32.72	696	494	102
9/25/2025	8/26/2025	30.91	32.87	716	206	97
10/16/2025	9/25/2025	29.91	31.13	200	6	0
11/17/2025	10/16/2025	27.16	29.36	0	0	0
12/10/2025	11/17/2025	25.81	26.79	0	0	0
1/13/2026	12/10/2025	24.57	26.38	0	0	0
2/13/2026	1/13/2026	21.66	25.02	0	0	0
3/14/2026	2/13/2026	23.67	26.50	0	0	0

Table 2. The table displays the model formula, AIC values, R^2 marginal, R^2 conditional, and ΔAIC for each model tested. Models are ordered by AIC scores with the best fit model listed first. This model with cumulative hours above 30°C and genotype received the strongest support (lowest AIC, highest AIC weight), making it a better fit than models based on mean or maximum temperature metrics or models incorporating higher thermal thresholds (31–32°C).

Model	Model formula	AIC	R^2m	R^2c	ΔAIC
m_tol	**glmer(cbind(Deaths, Survivors) ~ HoursAbove30_sc + Days_sc + (HoursAbove30_sc | Genotype), family = binomial)**	**1,823.15**	**0.329**	**0.861**	**0.00**
m_species_tol	glmer(cbind(Deaths, Survivors) ~ HoursAbove30_sc * Species + Days_sc + (HoursAbove30_sc | Genotype), family = binomial)	1,826.85	0.334	0.861	3.70
m_30	glmer(cbind(Deaths, Survivors) ~ HoursAbove30_sc + Days_sc + (1 | Genotype), family = binomial)	1,863.35	0.433	0.845	40.20
m_mean	glmer(cbind(Deaths, Survivors) ~ MeanTemp_sc + Days_sc + (1 | Genotype), family = binomial)	1,873.34	0.497	0.859	50.19
m_31	glmer(cbind(Deaths, Survivors) ~ HoursAbove31_sc + Days_sc + (1 | Genotype), family = binomial)	1,891.94	0.356	0.815	68.79
m_max	glmer(cbind(Deaths, Survivors) ~ MaxTemp_sc + Days_sc + (1 | Genotype), family = binomial)	1,902.51	0.451	0.846	79.36
m_32	glmer(cbind(Deaths, Survivors) ~ HoursAbove32_sc + Days_sc + (1 | Genotype), family = binomial)	2,056.42	0.095	0.769	233.27

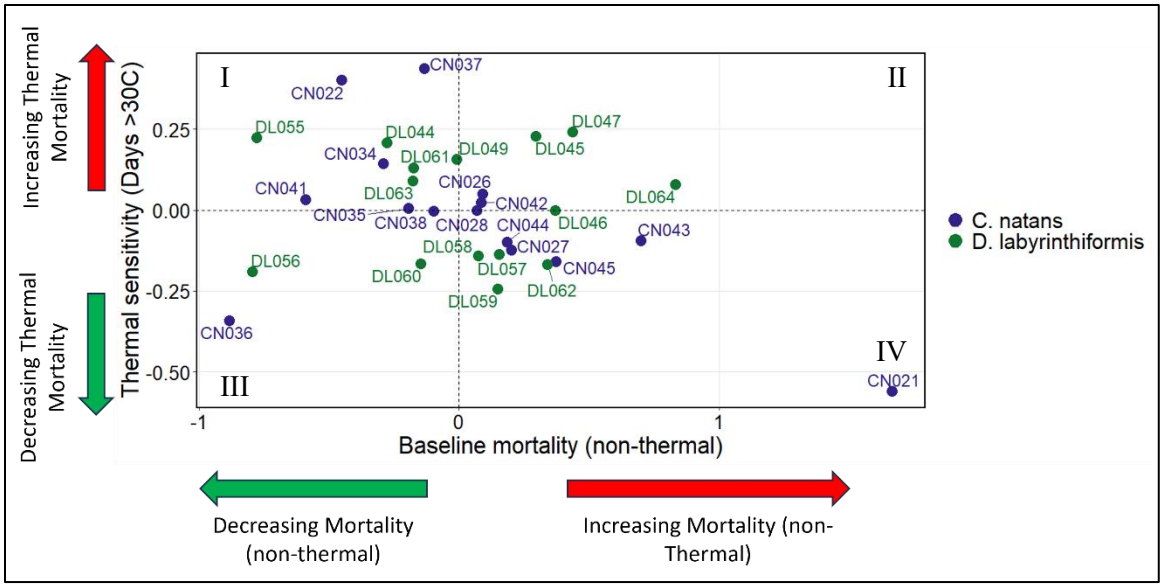

Figure 6. Relationship between baseline mortality and thermal sensitivity among coral genotypes. Each point represents a genotype of Colpophyllia natans (CN; purple circles) or Diploria labyrinthiformis (DL; green circles). The x-axis shows baseline mortality (non-thermal mortality), with values farther to the left indicating lower mortality and values farther to the right indicating higher mortality. The y-axis shows thermal sensitivity, measured as the effect of exposure to temperatures above 30°C on mortality, where higher values indicate greater mortality associated with thermal stress and lower values indicate lower mortality associated with thermal stress. Dashed horizontal and vertical lines indicate zero effect for thermal sensitivity and baseline mortality, respectively.

3.2. Outplanting

A total of 511 corals were outplanted across six sites for this experiment, 203 fragments of *C. natans* and 308 fragments of *D. labyrinthiformis*. 378 fragments were outplanted across the three shallow reef sites and 133 were outplanted at the deep reef sites.

Outplant monitoring occurred approximately one week after outplanting, then one month after outplanting, and monthly thereafter. Generalized linear mixed-effects models were used to examine the effect outplant reef site, proximity to a wild conspecific, and monitoring interval on the percent living tissue of corals. Analysis of conspecific proximity effects included only corals outplanted on shallow reef sites.

Analysis of *C. natans* at shallow sites found that percent living tissue differed among sites ($p=0.035$) and monitoring interval ($p=0.004$) (Figure 7A). Proximity to the conspecific did not affect tissue cover ($p=0.50$). Coral at Washerwoman had higher live tissue cover compared to colonies at RAWA3 (mean difference = 5.93%, Tukey-

adjusted p = 0.035), while NOAA8 did not differ significantly from either site. Across all sites, live tissue cover declined from approximately 100% at the outplanting to 94% at the 1-week monitoring interval (p = 0.004) and remained stable thereafter, with no difference between the 1-week and 1-month monitoring intervals (p = 0.82).

Analysis of *D. labyrinthiformis* at the shallow sites found that monitoring interval was significantly different as well as Near vs Far and Site (Figure 7B). For the *D. labyrinthiformis* the one-month monitoring was significantly different from the time of outplanting (mean difference = 4.9%, Tukey-adjusted p= 0.001) and the 1-week monitoring (mean difference = 3.3%, Tukey-adjusted p=0.017). In addition, those corals outplanted further from the conspecific (>1m) had significantly lower percent living tissue as compared to those close to the conspecific (mean difference = 2.35%, Tukey-adjusted p=0.05). Finally, RAWA3 had significantly lower percent living tissue as compared to NOAA8 (mean difference = 3.6%, Tukey-adjusted p=0.034).

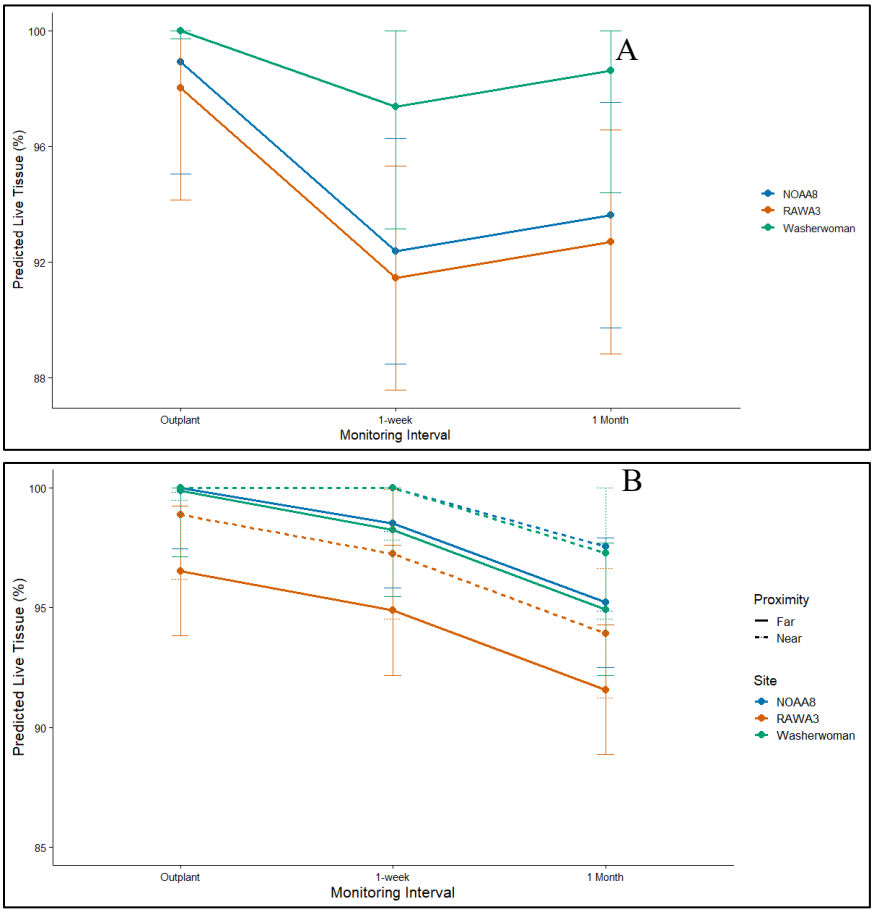

Figure 7. Predicted mean living tissue cover (%) for Colpophyllia natans (A) and Diploria labyrinthiformis (B) at NOAA8, RAWA3, and Washerwoman sites at outplanting, one week after outplanting, and one month after outplanting. D.

labyrinthiformis (B) also shows the predicted mean live tissue at two proximities to a conspecific (Near and Far) Points represent estimated marginal means from the mixed-effects model, and error bars indicate 95% confidence intervals.

Analysis of *C. natans* between the deep and shallow sites revealed significant differences between the monitoring intervals ($p < 0.001$) but not between depth treatments ($p = 0.176$). (Figure 8A). Percent living tissue declined to 92.0% during the 1-week monitoring interval and remained similar at the 1-month monitoring (93.2%).

Analysis of *D. labyrinthiformis* across the deep and shallow sites indicated significant differences by monitoring interval and a significant interaction between the depth and monitoring interval factors (Figure 8B). Live tissue cover did not differ between depth treatments at outplanting ($p = 1.00$) or the 1-week monitoring ($p = 0.53$). However, by the 1-month monitoring, corals at the deep sites had significantly less live tissue cover than shallow corals (79.9% vs. 92.8%; difference = 12.9%, $p < 0.0001$). This indicates that tissue loss through time was substantially greater for deep colonies.

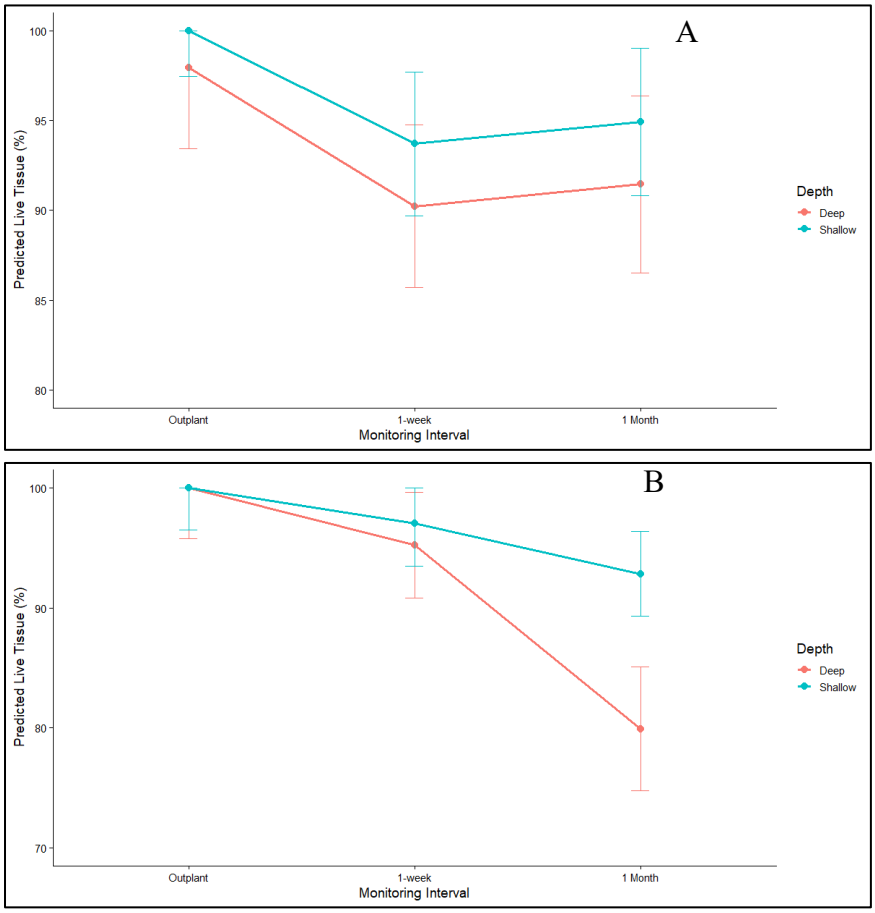

Figure 8. Predicted mean percent living tissue for Colpophyllia natans (A) and Diploria labyrinthiformis (B) at outplanting, one week after outplanting, and one month after

outplanting at shallow and deep sites. Each point represents the mean percent living at that monitoring interval ±95% CI.

4. DISCUSSION AND MANAGEMENT RECOMMENDATIONS

4.1. Discussion

Phase 2 of this project provides the foundation for optimizing outplanting strategies for *Colpophyllia natans* and *Diploria labyrinthiformis* by assessing how resilience/resistance to potential environmental stressors before collection and within in-water nurseries (Phase 1) is related to post-outplant performance (Phase 2). Both reef depth and proximity to wild conspecifics are outplanting parameters that restoration practitioners should consider when outplanting that do not require manipulation of the outplant site, thereby increasing the productivity of restoration efforts while potentially improving survival.

Routine monitoring within FWC's in-water coral nursery emphasized the genotypic differences in survival for both *C. natans* and *D. labyrinthiformis*. These differences were observed both in the Phase 1 and Phase 2 survival curves and in the figure showing thermal mortality versus mortality from other factors. These analyses highlighted that some genotypes, particularly CN021, showed continued mortality year round unrelated to temperature stress while other genotypes, such as CN036, CN022, and DL055, showed mortality when temperatures were consistently above 30°C but little to no mortality when not experiencing these higher temperatures. Although the temperatures within the nursery were less extreme than the Summer 2023, mortality increased for some genotypes with exposure to elevated temperatures, indicating genotype-specific sensitivity to thermal conditions. Bleaching was only observed in *C. natans* but genotypes of both *C. natans* and *D. labyrinthiformis* showed higher mortality in the summer indicating that thermal stress can manifest in ways other than visible bleaching.

Whereas collection location influenced survival during Phase 1, these effects were substantially reduced during Phase 2. However, genotype-specific differences in survival persisted throughout both phases of the project. This pattern suggests that factors associated with collection reef conditions may have influenced initial acclimation and early survival and fragmentation, whereas long-term performance within the nursery was more strongly associated with genotype. Continued monitoring both within the coral nursery and post-outplant will help determine whether genotype-specific performance remains a stronger predictor of restoration success than coral collection location.

Although our post-outplant observations remain limited, preliminary findings after one-month post-outplanting indicate that reef depth and conspecific proximity influenced outplant condition. Initial analysis suggests that *D. labyrinthiformis* outplanted at the shallow sites had higher percent living tissue than those outplanted at deep reef sites. As summer temperatures increase, the trend of shallow sites exhibiting higher percent living tissue may shift. Although deeper reef habitats may ultimately provide refuge from thermal stress, the present short-term observations indicate lower living tissue cover at deep sites during the first month after outplanting. This may be more evident in the genotypes that have been identified as more susceptible to cumulative exposure to temperatures over 30°C.

In addition, *D. labyrinthiformis* outplanted near wild conspecifics may experience greater survival than those further away. Although the mechanisms underlying the benefits of outplanting near conspecifics are unknown, structural sheltering of outplants from fish predation and the sharing of microbiomes between species may play important roles and warrant further investigation. As this project progresses we will gain more insight into the role of conspecifics and reef depth on outplant success.

We further note that because conspecific proximity treatments were only implemented at shallow sites, this study cannot determine whether proximity effects vary across reef depths. Future studies that evaluate the distance to conspecifics across depths would allow direct evaluation of potential depth-by-proximity interactions and help clarify whether the benefits associated with conspecific proximity are consistent across reef environments

The next objectives for this project are to continue to monitor corals both outplanted on the reef and held within the coral nursery to assess changes in responses to environmental conditions and to evaluate the utility of genotype tracking for predicting outplant success. Additionally, we will collect samples from each coral genotype at all outplant sites to investigate effects of reef depth and conspecific proximity on the microbiome and metabolome of outplanted corals.

4.2. Management Recommendations

- Initial mortality after a coral is placed within a nursery may not be indicative of long-term success. However, corals that continue to show mortality may be poor performers.
- Both depth and proximity to conspecifics significantly affect *D. labyrinthiformis* immediately after outplanting. However, recommendations are based on preliminary monitoring data collected within the first month post-

outplanting. Longer-term monitoring is ongoing and will inform more definitive species- and genotype-specific guidance for restoration practitioners.

- Multi-year survival data for genotypes can provide strategic guidance for restoration practitioners to enhance restoration success, and such data are needed by managers to inform species-specific outplant recommendations for improved restoration of Florida's Coral Reef.

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