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Restoration of Stony Coral Tissue Loss Disease Susceptible Species in the Arrecife de Puerto Morelos National Park, Mexico Using Colony Microfragmentation

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By

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Abstract:

The Arrecife de Puerto Morelos National Park (APMNP) has been a marine protected area in Mexico's Mesoamerican Reef since 1998 and includes ~90 km^2 of coral reef. Significant declines in stony coral cover have been recorded within the APMNP, primarily due to increasing ocean temperatures and disease events which precipitated the need for active restoration activities. This study addressed if current APMNP conditions, in relation to Stony Coral Tissue Loss Disease (SCTLD), are appropriate for the restoration of stony corals through outplanting SCTLD-susceptible species microfragments. In September 2022, three species (*Montastraea cavernosa, Orbicella annularis,* and *O. faveolata*) were cut into 1-4 cm^2 microfragments (n = 1,504) and secured onto plugs. Microfragments were kept at an *ex situ* nursery before being outplanted at six APMNP sites. At each site, cement bases with three or seven microfragments from one parent colony were outplanted in a random pattern within a 20 m^2 plot. Microfragment outplant success was assessed by survival, growth, and health conditions between species and site locations. Additionally, during each monitoring event, SCTLD prevalence was recorded at outplant and control sites to evaluate if outplanting SCTLD-susceptible species affected disease prevalence in the natural population. Although mean (±SE) survival for all species combined was 84.28% ± 3.28 nine months post-outplanting, the microfragments exhibited minimal relative net growth suggesting chronic pressures currently limit the long-term potential for restoration via microfragmentation. However, introducing SCTLD-susceptible stony coral species did not increase disease prevalence in the surrounding natural colonies at any sites, suggesting that other restoration activities could be implemented in the APMNP.

Keywords: Arrecife de Puerto Morelos National Park, Coral restoration, Mesoamerican Reefs, stony coral microfragmentation, Stony Coral Tissue Loss Disease (SCTLD).

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1. Introduction

Coral reefs are highly biodiverse ecosystems that play a critical role in providing essential ecological, social, and economic benefits to millions of people across the globe (Allen et al., 2021; McManus, 2001). Despite their remarkable significance, these ecosystems are facing degradation at unprecedented rates due to anthropogenic stressors as well as biotic and abiotic factors (Hughes et al., 2017). Escalating sea surface temperatures driven by climate change have caused unprecedented mass bleaching events as well as an increase in disease prevalence and severity (Bruno et al., 2007; Hughes et al., 2017). Disease outbreaks can trigger mass mortality events, rapidly and significantly reducing populations, thereby reshaping the entire ecosystem's structure and function (Alvarez-Filip et al., 2022).

The Mesoamerican Reef (MAR) system is the second largest barrier reef in the world, extending approximately 1,000 km along the Caribbean coast of four countries: Mexico, Belize, Guatemala, and Honduras (Almada-Villela et al., 2002; Ardisson et al., 2011; Kramer et al., 2002; McField et al., 2016). The MAR is considered a biodiversity hotspot with high ecological, cultural, and economic value (Gress et al., 2019). However, the MAR's coral reef communities are threatened by climate change, overfishing, pollution, and disease outbreaks (Gress et al., 2019; Caballero-Aragón et al., 2020; Reguero et al., 2019; McField et al., 2020; Weil, 2014). Stony Coral Tissue Loss Disease (SCTLD) was first reported in the MAR in June 2018, four years after being initially reported on reefs in southeast Florida (Brandt et al., 2021; Estrada-Saldívar et al., 2020; Guzmán-Urieta et al., 2021; Hayes et al., 2022; Precht et al., 2016; Walton et al., 2018). SCTLD is characterized by varying levels of recent tissue necrosis, exposing a colony's white skeleton, which can then become covered with algae in 3-7 days (Hayes et al., 2022; NOAA, 2018). The disease affects approximately 22 reef-building coral species and has high prevalence and rates of transmission, often leading to extensive colony mortality (Dobbelaere et al., 2020; FDEP, 2022; Precht et al., 2016; Sharp et al., 2020). After first being observed in June 2018, SCTLD quickly spread along the MAR and mortality rates ranged from <10% to 94% among the affected coral species (Alvarez-Filip et al., 2019; McField et al., 2020). Such widespread mortality leads to significant decreases in coral cover and abundance which can have dramatic ecological consequences including reduced biodiversity (Estrada-Saldívar et al., 2021; Gilliam et al., 2019; Heres et al., 2021; Muller et al., 2020; Thomé et al., 2021). The full impacts to the MAR are still

unknown, as the prevalence of SCTLD remained high throughout the region during 2020 (Alvarez-Filip et al., 2022).

The Arrecife de Puerto Morelos National Park (APMNP) is an MPA located at the northern extent of the MAR, in Quintana Roo, Mexico, a region of the Mexican Caribbean coast with an annual tourism economy of 10 billion USD (Reguero et al., 2019). Mexico designated APMNP as an MPA in 1998, and it is one of the most important resources for local communities and the tourism industry (Ardisson et al., 2011; Murray et al., 2005; Rodriguez-Martinez, 2008). Encompassing approximately 90 km^2 of coral reef, the APMNP contains an extended fringing reef with a well-developed backreef and a relatively flat forereef (Caballero-Aragón et al., 2020; Rioja-Nieto et al., 2019; Rodríguez-Martínez et al., 2010). Marine protected areas (MPAs) have been established as a key strategy to mitigate anthropogenic disturbances affecting coral reefs (Caballero-Aragón et al., 2020). Numerous disease outbreaks in the APMNP have also significantly reduced the numbers of essential reef-building coral species (Alvarez Filip et al., 2022; Jordán-Dahlgren et al., 2005). However, coral cover, and abundance have continued to decline in these regions from white band disease (Estrada-Saldívar et al., 2020) and SCTLD. From 1985 to 2016, the mean coral cover in the APMNP backreef declined by almost 50%, mainly driven by the loss of branching coral species, while the forereef remained relatively stable (Estada-Saldívar, 2019). However, by 2019, after SCTLD reached the APMNP, the disease was documented to have affected 43% of the APMNP susceptible species (Alvarez-Filip et al., 2019), and by 2022, 55% of the surveyed colonies were diseased (Estada-Saldívar et al., 2022), resulting in major coral cover loss of massive, boulder species.

In response to the disease-related decline of massive stony corals in the APMNP, the Mexican National Fishing Institute (INAPESCA), based in Quintana Roo, Mexico, established an action plan to restore SCTLD-susceptible species within the APMNP (Calderon, 2021). The initial step was to conduct a study assessing whether there is a persistence of SCTLD in the APMNP which would preclude successful reintroduction (outplanting) of SCTLD-susceptible corals and whether the introduction of additional SCTLD-susceptible corals will exacerbate SCTLD prevalence in natural colonies at the outplant sites. To address these concerns, microfragmentation has been proposed as a restoration method. Stony coral microfragmentation, an asexual restoration technique that involves cutting coral colonies into ~4cm^2 microfragments, was proposed because

it specifically targets massive coral species, which were most heavily impacted by the SCTLD outbreak (Forsman et al., 2015; Knapp et al., 2022; Koval et al., 2020; Page et al., 2018; Soper et al., 2022). Microfragmentation aims to propagate slow-growing, massive stony coral species to increase spatial distribution and maximize growth (Forsman et al., 2015). It is hypothesized that this process stimulates corals to allocate energy away from reproductive processes and towards growth, particularly in smaller colonies and some species, leading to a doubling or quadrupling in size within a few months (Forsman et al., 2015; Knapp et al., 2022). However, the majority of successful microfragmentation efforts have been achieved through *ex situ* methods, which offer the advantage of adjusting environmental parameters to enhance coral health, growth and survival highlighting the critical need to investigate the viability of this technique *in situ* (Merck et al., 2022).

While microfragmentation has been documented to be successful as a propagation technique, the potential for successful restoration using microfragments may be jeopardized by disease (particularly SCTLD), predation, sedimentation, and macroalgal overgrowth. Corallivorous fish predation has been identified as a significant inhibitor to restoration success in Florida and Hawaii by reducing survivorship of microfragments through severe tissue damage or complete microfragment removal (Knapp et al., 2022; Koval et al., 2020). Sedimentation can further increase partial mortality and suppress growth by reducing available light for the coral's photosynthetic symbiotic microalgae, smothering the coral, and increasing competitive macroalgae overgrowth (Nugues et al., 2003). Nonetheless, given SCTLD devastating effect on massive coral species and the continued decline of the MAR reefs, the implementation of active intervention strategies are critical in preventing further loss of susceptible species (Williamson et al., 2022).

This study aimed to assess the suitability of reintroducing SCTLD-susceptible stony coral species in the APMNP by evaluating the efficacy of microfragmentation as a tool to promote massive stony coral recovery by outplanting three coral species (*Montastraea cavernosa, Orbicella annularis,* and *O. faveolata*), and evaluating their health and relative net growth rate over 9 months at six sites in the APMNP. Specifically, I 1) determined whether outplanting microfragments of SCTLD-susceptible species increased the prevalence of SCTLD in the outplant and adjacent reef sites in the APMNP, 2) assessed the impact of coral species, outplanting density,

site location and, water temperature on microfragment outplant survival and relative net growth, and 3) evaluated the extent to which microfragment health and predation by corallivorous fish hinders the success of microfragmentation at outplant sites. Findings from this study will enhance our understanding of the factors that influence coral growth and survival and provide insight into potential limitations of microfragmentation as a restoration tool in the Mexican Caribbean, given that it has never been tested. Coral restoration in this area is essential because it safeguards biodiversity, supports the economy, enhances resilience to climate change, preserves culture, and contributes to global marine conservation efforts.

2. Methodology

2.1 Site selection

This study was conducted at the Arrecife de Puerto Morelos National Park, located in Puerto Morelos, Mexico, from September 2022 to August 2023 (Fig. 1). A reef assessment conducted by INAPESCA in February 2020 was used to inform site selection. INAPESCA's reef assessment identified 16 sites within the APMNP suitable for restoration efforts. Site selection was based on a scoring system that included feasibility of management, vulnerability, and ecological analysis. The feasibility of management analysis considered factors such as the history of restoration interventions at the site, technical, and logistical feasibility of conducting restoration at the site, and the value of the site to management and research activities. The vulnerability analysis section factored in aspects such as hurricane impacts, bleaching, and disease prevalence at the site, while the ecological analysis involved evaluating fish biomass, historical coral cover, and the rate of change of coral assemblages. INAPESCA's assessment also considered the past prevalence of SCTLD and the reef morphology within the APMNP. The six study sites with the highest Reef Restoration Assessment Index values, which represents the average scores across the three described categories (feasibility of management, vulnerability, and ecological analysis), were selected as outplant sites (INAPESCA, 2022).

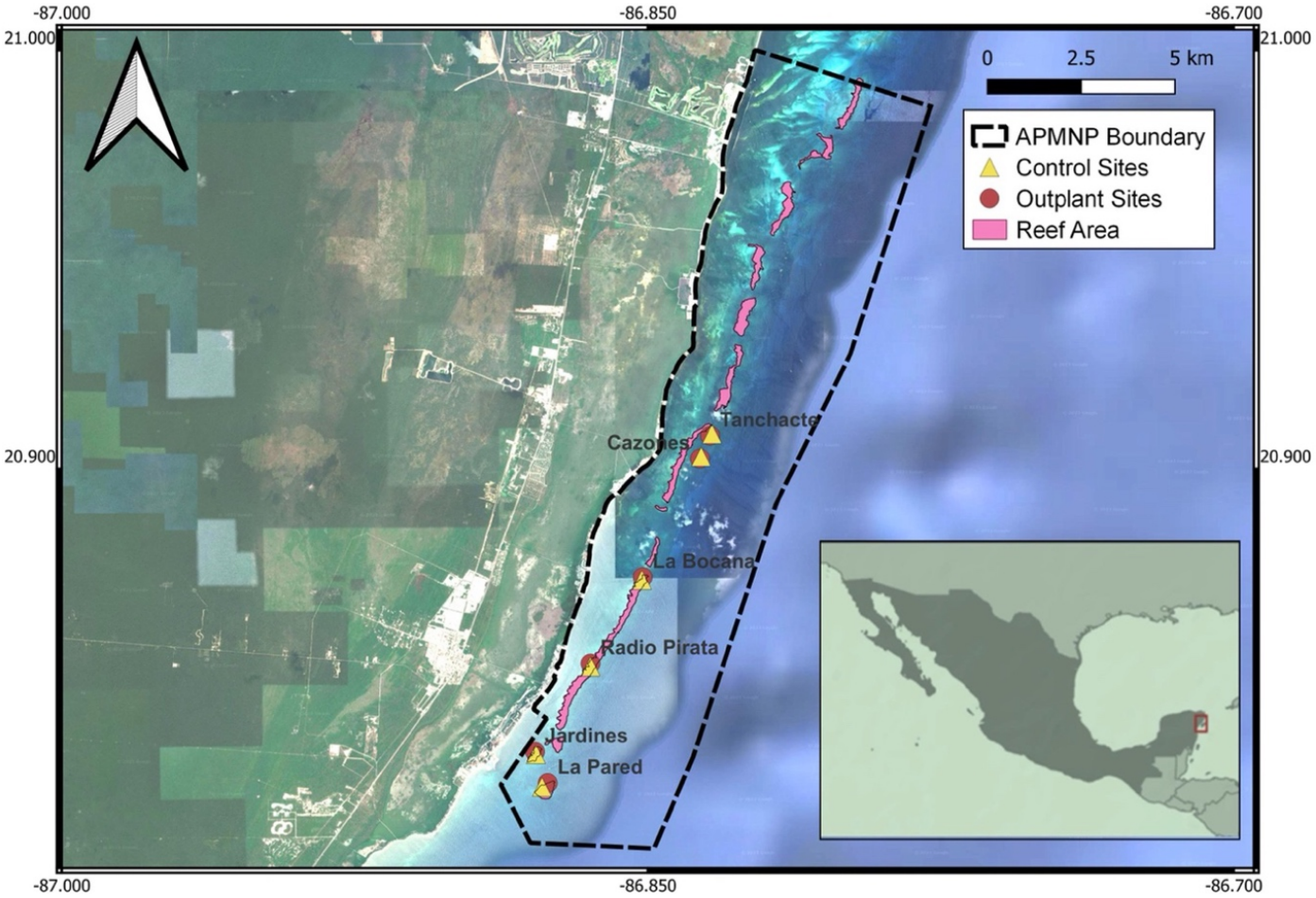

Figure 1. Arrecife de Puerto Morelos National Park (APMNP) with the outplant and control sites (Inset: Mexico with APMNP highlighted in red box).

The selected sites were categorized by depth and reef habitat. Shallow sites, Radio Pirata and Jardines, were located on the reef crest at 2-3 m depth and had 14% and 10.2% coral cover, respectively. Intermediate sites, La Bocana and La Pared, were situated in the backreef at 5-6 m depth, and had 14.3% and 9.8% coral cover, respectively. Deep sites were positioned on the forereef, with depths between 7-8 meters, and consisted of Tanchancte, which had a coral cover of 7.2%, and Cazones, the only site for which no coral cover data was available (Estrada-Saldívar et al., 2021; INAPESCA, 2022) (Table 1).

Table 1. Study site location, depth, depth classification, reef zone, and coral cover (Estrada-Saldívar et al., 2021).

Location	Depth (m)	Depth class	Reef Zone	Coral Cover
Jardines	2-3	Shallow	Reef crest	10.2%
Radio Pirata	2-3	Shallow	Reef crest	14%
La Bocana	5-6	Intermediate	Backreef	14.3%
La Pared	5-6	Intermediate	Backreef	9.8%
Cazones	7-8	Deep	Forereef	N/a
Tanchancte	7-8	Deep	Forereef	7.2%

2.3 Experimental Setup and Design

The stony coral species *Montastraea cavernosa*, *Orbicella annularis,* and *O. faveolata* were used. These species are historically predominant reef-building species in the APMNP, and they experienced significant losses from SCTLD throughout the Caribbean (Alvarez Filip et al., 2022). In August 2022, INAPESCA collected opportunistic coral microfragments from 2 or 3 genetically distinct sexually mature colonies of each target species in the APMNP (Table 2). Colonies were microfragmented using a C40 diamond band saw (Gryphon Corporation, Sylmar, CA, United States) into ~1-4 cm^2 diameter microfragments and secured onto circular ceramic plugs using epoxy. The microfragments were managed and held at the INAPESCA *ex situ* nursery in separate 340 L raceways for a 7-week attachment and recovery period. This was a flow-through system with a constant, uninterrupted flow of filtered seawater directly pumped from the ocean. Temperature in the raceway was regulated within the range of 25-26 °C, and the salinity was maintained at 36-37 ppt. Algae growth was controlled by daily siphoning and manual removal. The raceways were situated in an enclosed facility and covered with a transparent polyethylene film, permitting appropriate light to penetrate.

Table 2. Total number (n) of microfragments obtained from each species genotype.

Species	Genotype	n
O. faveolata	26	144
O. faveolata	298	293
O. annularis	222	317
O. annularis	299	237
M. cavernosa	224	133
M. cavernosa	225	219
M. cavernosa	300	161
Total microfragments		1,504

Some microfragments, mainly *O. faveolata*, died after microfragmentation, leaving 1,504 to outplant. The microfragments were outplanted in November 2022, using 303 cement bases which were distributed to six APMNP reef sites. The microfragments were outplanted using two density variations of cement bases, 3 and 7 plugs (Fig. 2).

Figure 2. Cement bases used for outplanting with each containing 3 or 7 microfragments. Produced by Reef Aquaculture Conservancy A.C.

At each site, 50 bases were haphazardly distributed around a central pin within a 10 m radius plot that was divided into four quadrants (Fig. 3). All base densities, species, and genotypes were equally represented within each plot (Table 3). Each base was tagged with a unique number that noted species, base density, and genotype information. After outplanting, the distance and bearing of each base from the central pin was recorded, using quadrants to aid in locating them during subsequent monitoring activities.

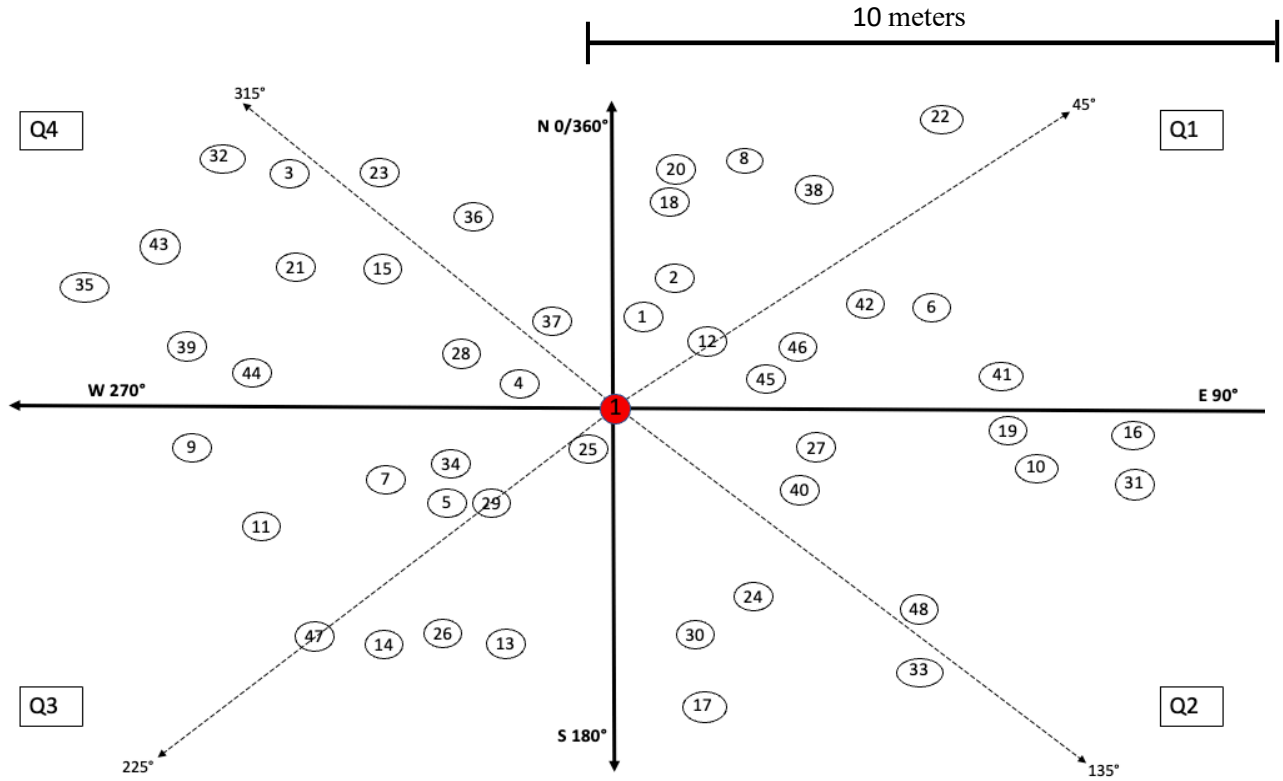

Figure 3. Schematic representation of outplant experimental design. A 20 m^2 plot divided into four quadrants with 50 bases (numbered ovals) haphazardly distributed around a central pin.

Table 3. The number of bases and microfragments for each species at each outplant site.

Location	Species	Bases with 3-microfragments	Bases with 7-microfragments	Total bases	Total microfragments
Cazones	*M. cavernosa*	9	8	19	83
	O. faveolata	8	7	15	73
	O. annularis	9	8	17	83
Cazones Total		**26**	**23**	**51**	**239**
Jardines	*M. cavernosa*	8	8	16	80
	O. faveolata	7	7	14	70
	O. annularis	10	10	20	100
Jardines Total		**25**	**25**	**50**	**250**
La Pared	*M. cavernosa*	8	11	19	101
	O. faveolata	7	7	14	70
	O. annularis	9	9	18	90
La Pared Total		**24**	**27**	**51**	**261**
La Bocana	*M. cavernosa*	8	8	16	80
	O. faveolata	7	8	15	77
	O. annularis	8	10	18	94
La Bocana Total		**23**	**26**	**49**	**251**
Radio Pirata	*M. cavernosa*	8	8	18	80
	O. faveolata	7	7	14	70
	O. annularis	10	9	19	93
Radio Pirata Total		**25**	**24**	**51**	**243**
Tanchancte	*M. cavernosa*	11	8	19	89
	O. faveolata	7	7	14	77
	O. annularis	8	10	18	94
Tanchancte Total		**26**	**25**	**51**	**260**
Total		**149**	**150**	**303**	**1,504**

2.4 Data collection

All corals were outplanted in November 2022, and the initial monitoring surveys were conducted one day after outplanting. Subsequent monitoring was carried out monthly for the first two months (December 2022 and January 2023), and then transitioned to a quarterly schedule, with monitoring occurring in May and August 2023. During each site visit, individual microfragment survival and health conditions (bleaching, disease, predation) were recorded. Specifically, the survey recorded the status of each microfragment (alive, dead, or missing),

instances and extent of predation, presence or absence of microfragment fusion, and any signs of disease. Additionally, during the last monitoring period in August 2023 the amount of sediment and macroalgae impacting the base were assessed on a scale of none (0), low (1), medium (2), or high (3). "Low" was defined as 5 mm or less of sediment accumulation and less than 10% of the base covered in macroalgae. "Medium" was characterized as 5 to 10 mm of sediment accumulation and macroalgae presence between 10-50%. "High" was used when sediment accumulation was greater than 10 mm and macroalgae cover was equal to or greater than 50% on the base. In the evaluation process for each base, all visible sedimentation, macroalgae, or overgrowth was removed using a small brush. This action served to facilitate a clear visual inspection of the coral tissue for the assessment of any health conditions and to allow further measurement of tissue growth.

To calculate microfragment growth, each base was photographed during each monitoring event using a camera mounted on a PVC frame with a set distance from the substrate. A standardized scale bar was included in the image for post-processing image analysis. Live tissue area (cm^2) was calculated from images for the initial and final monitoring period by tracing the outline of each microfragment in ImageJ (Schneider et al., 2012). From this, the net growth of each microfragment was calculated as the relative change in live tissue area (Eq. 1), which standardizes for initial size and captures increases or decreases in planar live tissue area as a result of growth and partial mortality. The relative net growth (% $\pm$ SE) was calculated as the net growth, standardized by the initial fragment size as per equation 1, where X_1 is the first time point (November 2022), X_2 is the last time point (August 2023). The mean relative change (% / month $\pm$ SE) was also calculated using the net growth as described, but using equation 2, where X_1 is the first time point (November 2022), X_2 is the last time point (August 2023) and t is time (9 months) (Eq. 2) The absolute growth rate ($cm^2 \pm$ SE) was then calculated as the change in live tissue area over the study period as per equation 3, where X_1 is the first time point (November 2022), X_2 is the last time point (August 2023).

$$\text{Relative Net Growth} = (X_2 - X_1) / X_1 * 100 \qquad \text{Equation 1}$$

$$\text{Mean Relative Change} = (((X_2 - X_1) /t/) X_1) *100) \qquad \text{Equation 2}$$

$$\text{Absolute Growth Rate} = X_2 - X_1 \qquad \text{Equation 3}$$

To assess whether outplanting exacerbated SCTLD prevalence, six control sites with comparable depths and coral assemblages, were established approximately 100 m from each outplant site. These control sites consisted of 20 x 20 m plots, each centered around a permanent pin. The control site area was the same as the outplant site area. All control sites contained SCTLD-susceptible species and served as reference sites to provide details on the natural assemblage of corals and the prevalence of SCTLD over time. Disease prevalence surveys were completed during each monitoring event to quantify disease prevalence in natural colonies at control and outplant sites. The divers recorded only species that were high or intermediate susceptibility to SCTLD according to the NOAA case definition established in 2018 (NOAA, 2018). They categorized these species based on their size class and noted their status with regards to presence or absence of SCTLD or other diseases, and bleaching. To further analyze the impact of environmental factors on microfragment survival, one pendant temperature logger (HOBO ProV2) was deployed at each of the six outplant sites. Water temperature was recorded every two hours over the course of this study.

2.5 Statistical analysis

All statistical analysis were performed in R version 4.3.1 (R Core Team 2020).

SCTLD prevalence in susceptible species at outplant sites in the APMNP

To investigate whether the addition of SCTLD-susceptible species influences disease prevalence and temporal changes in disease prevalence between outplant and control sites, an analysis of the percentage of disease natural colonies data was conducted using a binomial generalized linear model (GLM). The binomial response variable was denoted as the percentage of disease colonies, the continuous predictor was monitoring period (i.e., November, December, January, May, or August), and the categorical was type of outplant site (i.e., control and outplant). All predictors and their interactions were included in the full model, and model selection was determined using the Akaike Information Criterion (AIC) from all possible model combinations (Ruiz-Moreno et al., 2012).

Microfragment outplant survival and growth

Variation in the survival duration of each microfragment, up to 9 months post-outplanting, was analyzed using a survival analysis and Cox Regression Model (all monitoring periods, and only microfragments that survived were included in the analysis n=1,275). The survival status of the coral microfragment (alive = 0, dead = 1) was used as a categorical response variable, while location (Cazones, Jardines, La Bocana, La Pared, Radio Pirata, and Tanchacte), species (*M. cavernosa, O. annularis,* and *O. faveolata*), outplant densities (3 and 7 coral fragment bases), and depth (shallow, intermediate, and deep) were the categorical predictors. Parametric assumptions were not meet; therefore, a non-parametric Kruskal-Wallis test was used (Pinheiro et al., 2017).

To examine the effect of species, location, depth, and outplant density on the net growth on coral bases, a gaussian generalized linear mixed model (GLMM) was used. For the analysis, only the relative net growth of the initial vs. final monitoring periods was included. The continuous response variable was relative growth while the categorical predictors were species, location, and depth. Microfragment genotype was a random intercept and included in the full model using the function "glmmTMB" (Brooks et al., 2017). The minimum adequate model for each response variable was determined using the Akaike Information Criterion (AIC) from all possible model combinations. Model validation was performed on the minimum adequate model using the package "DHARMa" with residual diagnostics, including overdispersion, heterogeneity, and temporal autocorrelation conducted on the fitted model (Hartig, 2020). Post hoc, pairwise assessment of retained factors in the fitted models was conducted using the package "emmeans", where differences in the response variable were analyzed between levels of a factor (e.g., species) or interaction (e.g., species x location) based on model predictions using Tukey adjustment to control for type 1 error (Lenth, 2019).

A simple linear regression was employed to explore the influence of sedimentation and macroalgal levels specifically measured during the last monitoring period in August 2023, on the relative net growth on the bases over the past 3 months. For this analysis, the relative net growth used as X_1 (Equation 1) was May 2023 (6-months) and for X_2 was August 2023 (9-months). Furthermore, two factorial ANOVAs were performed. In the first one, the continuous response variable was macroalgae and in the second one, sedimentation level. Categorical predictors included location, and depth. Parametric assumptions were not met; therefore, a non-parametric

Kruskal-Wallis test was used (Pinheiro et al., 2017). For all the analyses conducted, only the sedimentation and macroalgal levels from the last monitoring period in August 2023 were utilized.

To assess the influence of the mean daily water temperature, including minimum, maximum, average, and range values recorded across all locations between the five monitoring events, on bleaching and disease prevalence in the coral microfragments, a binomial generalized linear model (GLM) was utilized (all monitoring periods were included in the analysis). The binomial response variable, denoting the prevalence of bleaching and disease in coral microfragments, was assessed against the continuous predictor (minimum, maximum, mean, and temperature range). All continuous predictors and their interactions were included in the full model, and model selection was determined using the Akaike Information Criterion (AIC) from all possible model combinations. However, the model suggested overdispersion in the data, so the model was refitted using a quasibinomial approach. Subsequently, the model was validated by examining the residuals against the fitted values.

To evaluate temperature variation between locations, time and depth throughout the study, a one-way ANOVA was used. The continuous response variable was average daily temperature, while the categorical predictors were locations, time, and depth, which were tested separately. Factors were tested for normal distribution using boxplots and a Shapiro-Wilk test. Parametric assumptions were not met; therefore, a non-parametric Kruskal-Wallis test was used (Pinheiro et al., 2017).

Bleaching, disease, and predation impacts

To evaluate microfragments health during each monitoring period, the presence or absence of predation, disease (due to the difficulty of determining SCTLD in microfragments was defined as any recent tissue loss), and bleaching (defined as any loss of color) of each microfragment was recorded. Notably, any coral microfragment that showed signs of disease, predation, or bleaching was marked as infected, even if it subsequently recovered. Thus, the health condition data of each coral microfragment by location, species, and depth was analyzed. Generalized linear mixed models (GLMM) were used to assess spatial and taxonomic variation in each of predation, bleaching, and disease prevalence. A binomial GLMM was fitted for each response variable, presence/absence of predation, bleaching or disease per coral microfragment per time point using

the function “glmmTMB” (Brooks et al., 2017). Species, location, and depth were fitted as categorical predictors in the full model. The microfragment genotype nested in the outplanted base ID as a random intercept. The minimum adequate model for each response variable was determined using the Akaike Information Criterion (AIC) from all possible model combinations. Model validation was performed on the minimum adequate model using the package “DHARMa”, with residual diagnostics, including overdispersion, heterogeneity and temporal autocorrelation, conducted on the fitted model (Hartig, 2020). Post hoc, pairwise assessment of retained factors in the fitted models was conducted using the package “emmeans”, where differences in the response variable are analyzed between levels of a factor (e.g., species) or interaction (e.g., species x location) based on model predictions using Tukey adjustment to control for type 1 error (Lenth, 2019).

3. Results

3.1 SCTLD prevalence in susceptible species at outplant sites in the APMNP

With all monitoring periods pulled, mean disease prevalence on natural colonies at control sites was 2.42% ± 0.64 SE while at outplant sites was 4.01% ± 0.55 SE; however, SCTLD prevalence throughout the monitoring periods on natural colonies did not significantly differ between outplant and control sites (p = 0.71; Fig. 4, and 5). All monitoring periods pulled, the natural colonies at the control site at La Bocana (intermediate site) exhibited the highest disease prevalence (7.06% ± 2.25 SE) while Radio Pirata (shallow site) had the highest disease prevalence (9.23% ± 0.78 SE) on natural colonies among outplant sites (Appendix Table 1, Appendix A).

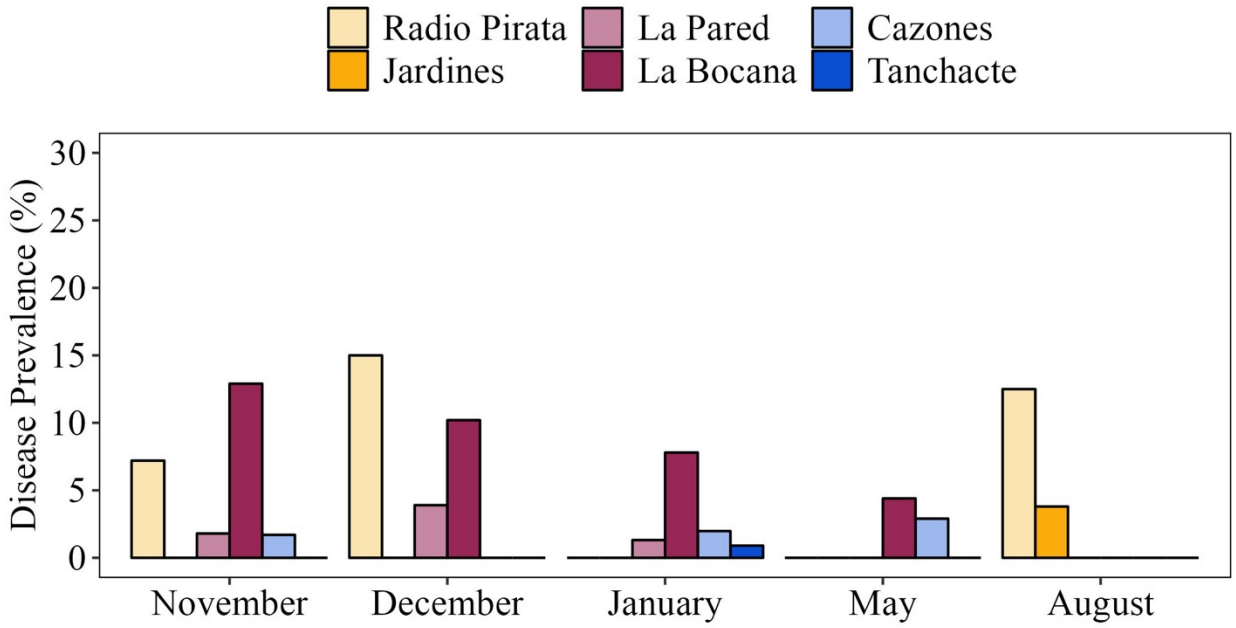

Figure 4. SCTLD disease prevalence (%) over time in natural colonies at the control sites. Disease prevalence is defined as the number of SCTLD infected colonies divided by the number of total colonies.

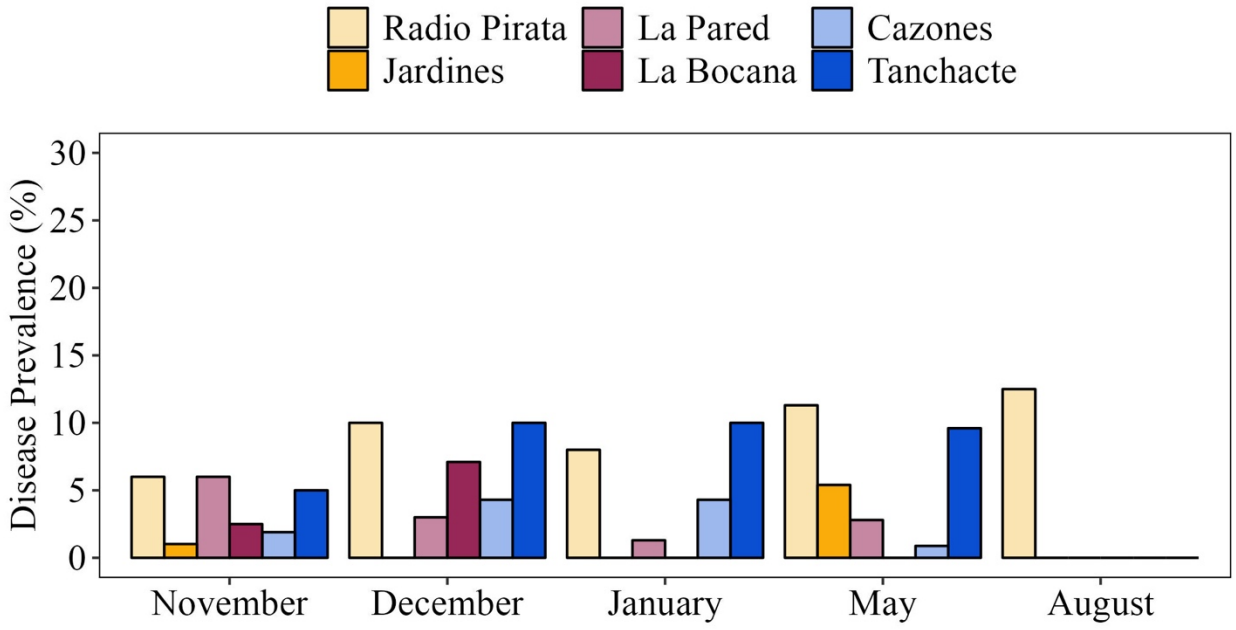

Figure 5. SCTLD disease prevalence (%) in natural colonies at the outplant sites. Disease prevalence is defined as the number of SCTLD infected colonies divided by the number of total colonies.

3.2 Microfragment outplant survival and growth

With all monitoring periods pulled, the overall microfragment survival was 84.28% ± 3.28 and survival probability significantly varied by coral species ($p < 0.0001$; Fig. 6), and location ($p < 0.0001$; Fig. 7). However, there was no significant difference between base densities ($p = 0.18$) and depth (p = 0.21). *Montastrea cavernosa* showed the highest survival (89.47% ± 4.63 SE) followed by *O. annularis* (85.18% ± 3.79 SE), and *O. faveolata* (78.16% ± 4.09 SE), although *M. cavernosa* was only significantly greater than *O. faveolata* (non-parametric Kruskal-Wallis test: $p < 0.0001$). Survival by location ranged from 70.80% ± 1.30 in Radio Pirata to 93.58 % ± 0.39 in Jardines. Therefore, at both shallow sites survival was significantly different from each other (non-parametric Kruskal-Wallis test: $p < 0.0001$). Intermediate sites, La Pared (86.97% ± 6.02 SE), and La Bocana (89.99% ± 3.86 SE), exhibited significantly greater survivial than Radio Pirata (shallow; non-parametric Kruskal-Wallis test: $p < 0.0001$). Deep sites, Cazones (80.73% ± 9.79 SE) and Tanchancte (85.37% ± 3.59 SE), showed lower suvivial than Jardines and intermediate sites.

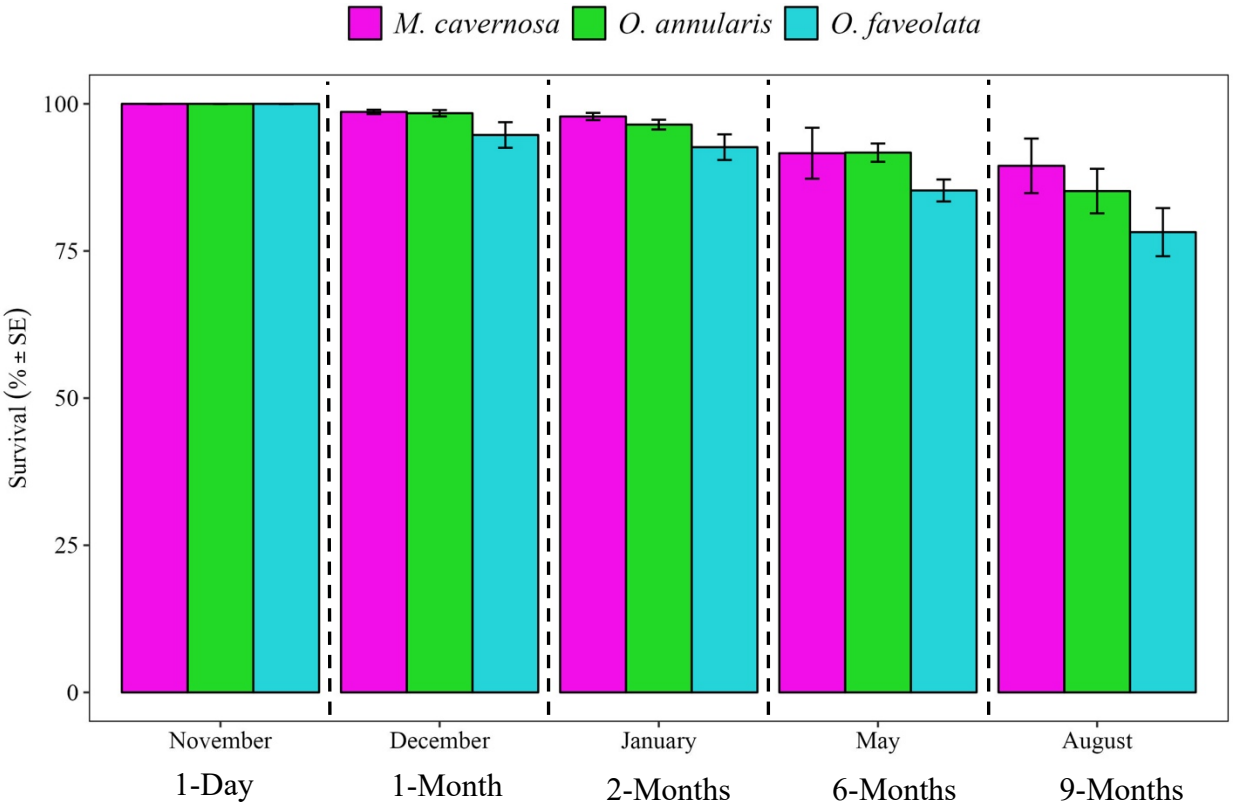

Figure 6. Mean percent microfragment survival 9-months post-outplanting for three coral species: *M. cavernosa*, *O. annularis,* and *O. faveolata* (with all locations combined).

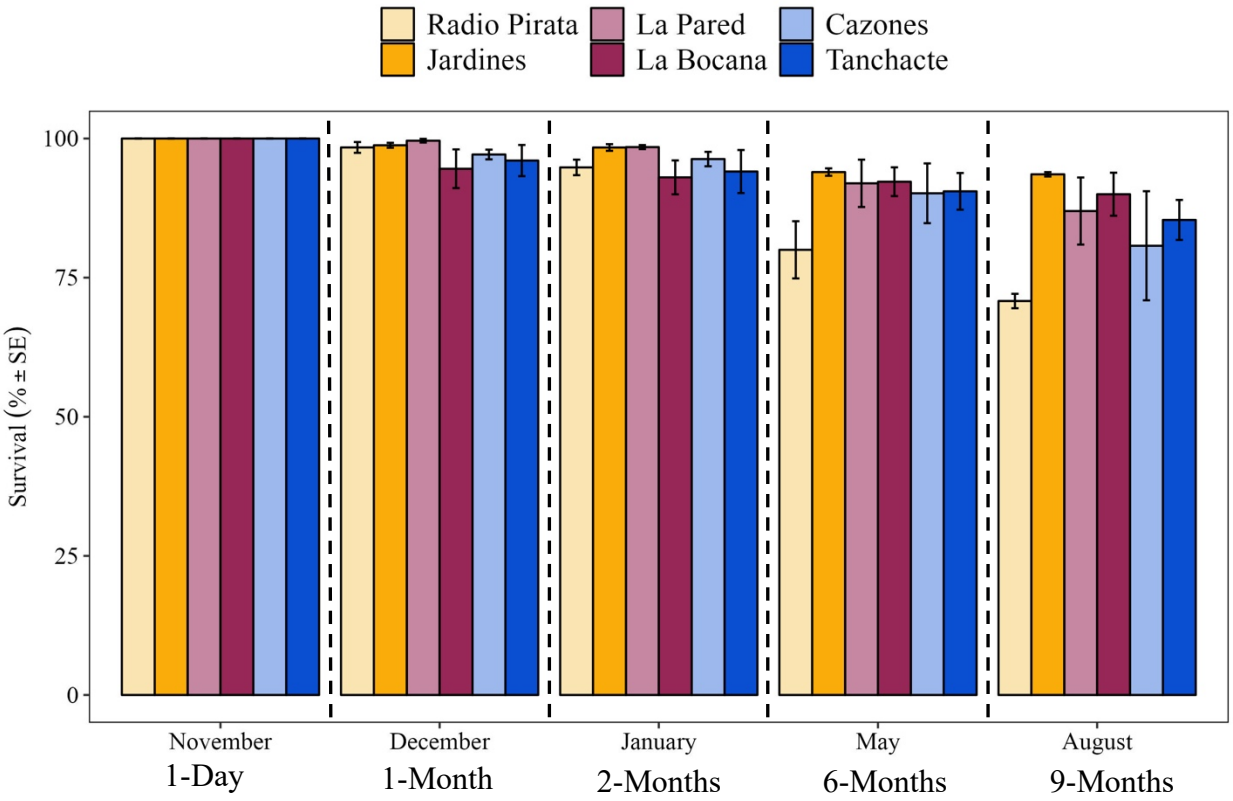

Figure 7. Mean percent microfragment survival 9-months post outplanting by location as a function of time since outplanting (with all species combined).

With all monitoring periods pulled, net microfragment bases growth was negligible throughout the AMPNP, but location significantly affected relative net growth. The minimum adequate model (fixed effect location and random effect genotype) explained only 17% of variation in the data (GLMM; Marginal R^2 (i.e., fixed effects only) = 0.155 while the Conditional R^2 (i.e., fixed and random effects) = 0.17). There was significant variability by location where shallow and intermediate sites had significantly higher relative net growth than deep sites (emmeans pairwise comparisons; $p < 0.0001$; Appendix Table 2, Appendix A). In the shallow sites, Jardines had significantly; higher, growth than Radio Pirata, while intermediate sites had significantly higher growth rate than Radio Pirata (shallow) and Tanchancte (deep) (emmeans pairwise comparisons; $p < 0.0001$); however, there was no significant difference between intermediate sites (Fig. 8). Absolute growth rate (cm^2), relative net growth (%), and mean relative change (% / month) had very similar findings (Fig. 8, 9, and 10).

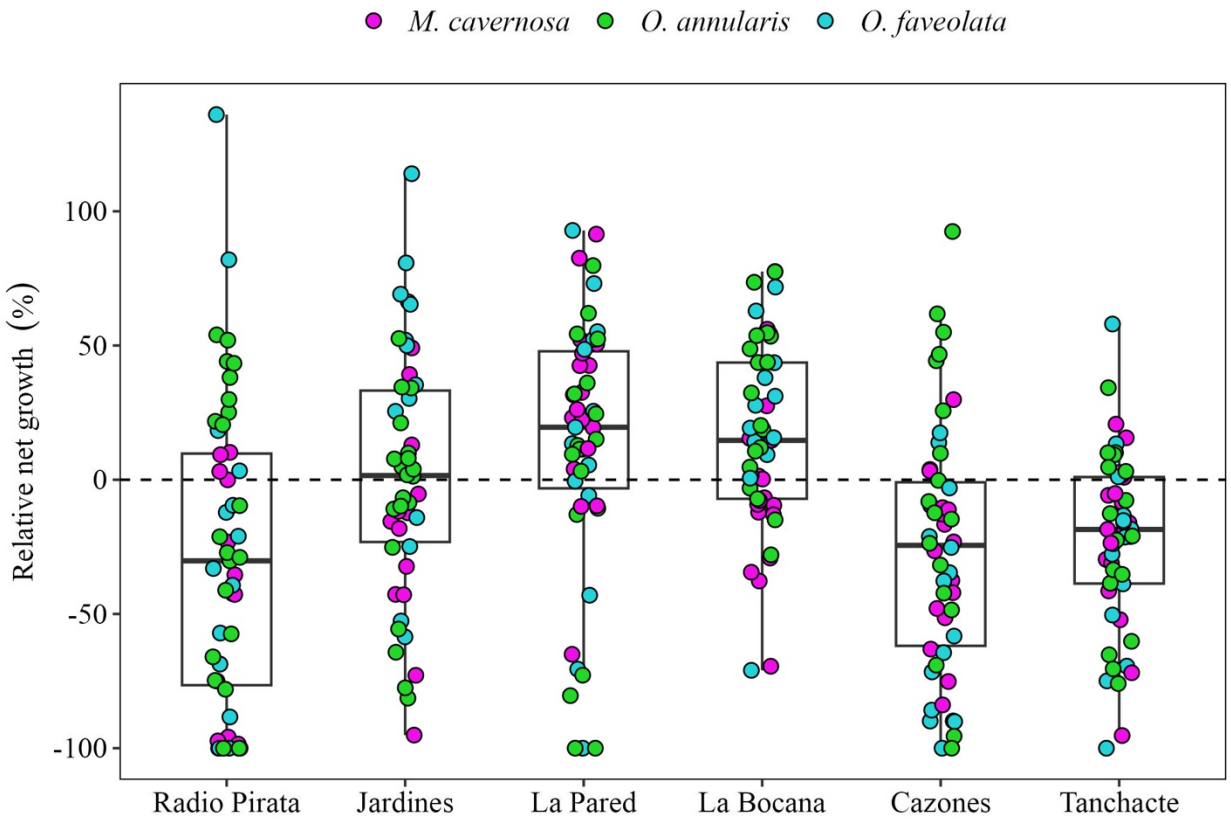

Figure 8. Distribution of relative net growth (%) by location. The central line inside the box represents the median relative net growth for each location. The dashed line across the plot represents the zero relative net growth threshold. Each data point represents one base with corresponding species. Points above the threshold indicate positive net growth rate, while those below represent species with net growth rate below zero.

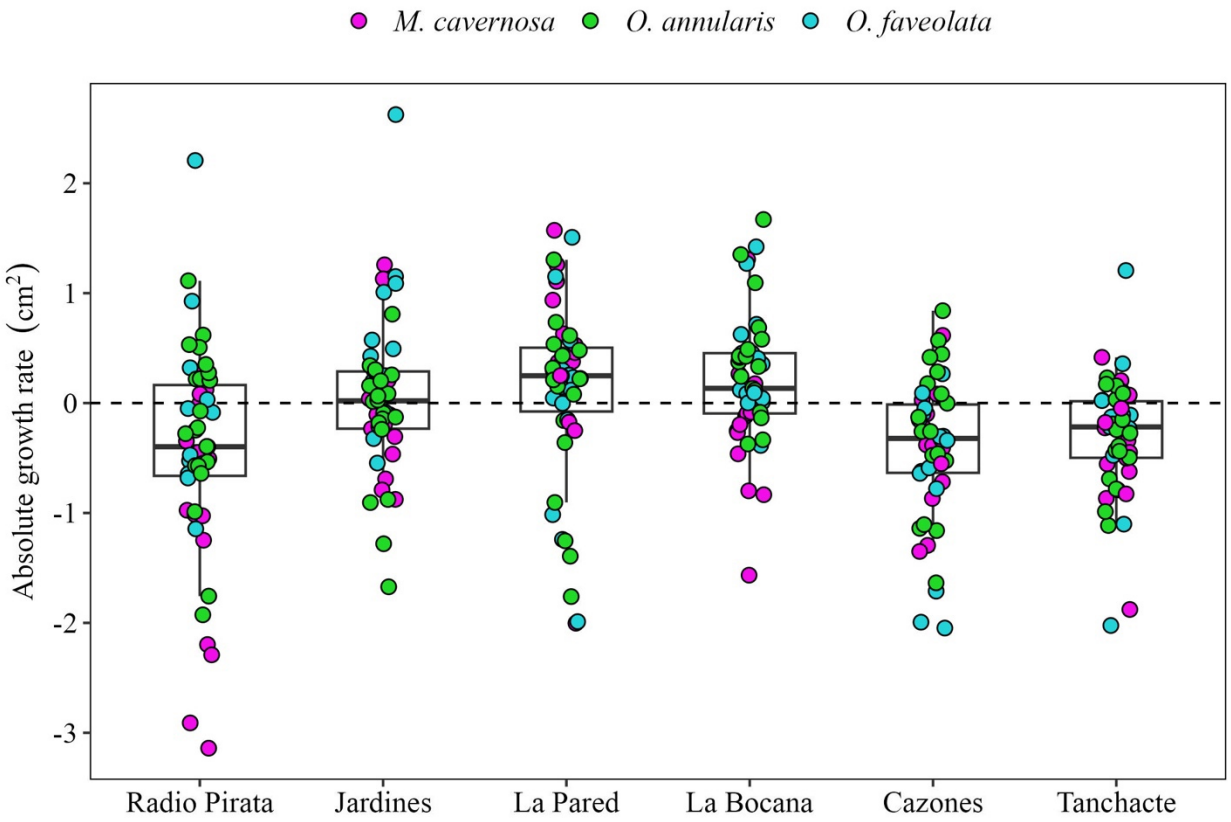

Figure 9. Distribution of absolute growth rate (cm^2) by location. The central line inside the box represents the median absolute growth rate for each location. The dashed line across the plot represents the zero absolute growth rate threshold. Each data point represents one base with corresponding species. Points above the threshold indicate a positive growth rate, while those below represent species a negative growth rate.

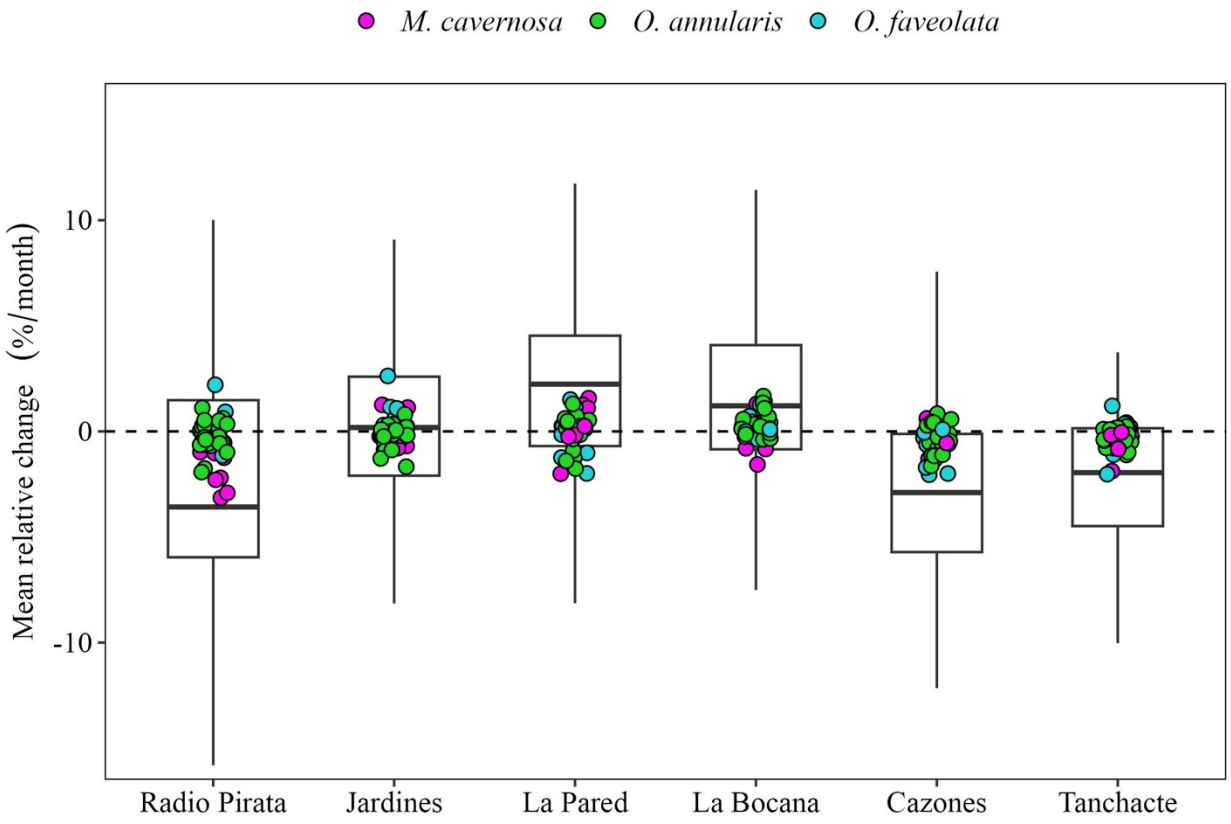

Figure 10. Distribution of mean relative change (% / month) by location. The central line inside the box represents the median mean relative change for each location. The dashed line across the plot represents the zero mean relative change threshold. Each data point represents one base with corresponding species. Points above the threshold indicate a positive growth rate, while those below represent species a negative growth rate.

With all monitoring periods pulled, there was negative relative net microfragment bases growth for all species (*O. annularis* -3.62 % ± 4.33 SE; *M. cavernosa* -15.68 % ± 4.11 SE; *O. faveolata* -5.76 % ± 6.09 SE; Fig. 8; Table 4), absolute growth rate (*O. annularis* -0.09 cm^2 ± 0.06 SE; *M. cavernosa* -0.25 cm^2 ± 0.08 SE; *O. faveolata* -0.51 cm^2 ± 0.09 SE; Fig. 9 ; Table 4), and mean relative change (*O. annularis* -0.40 % / month ± 0.48 SE; *M. cavernosa* -1.74 % / m ± 0.45 SE; *O. faveolata* -0.64 % / month ± 0.677 SE; Fig. 10; Table 4) (Appendix Table 3, Appendix A; Appendix Fig. 1 and 2, Appendix B).

Table 4. Total number of microfragments by species with calculated change in tissue area between initial and final monitoring periods (cm^2). Absolute growth rate ($cm^2 \pm SE$), mean relative change (% / month ± SE), and relative net growth (% ± SE) of microfragment bases.

Species	n	Initial cm^2	End cm^2	Absolute growth rate ($cm^2 \pm SE$)	Mean relative change (% / month ± SE)	Relative net growth (% ± SE)
O. faveolata	437	977.06	982.58	-0.51 ± 0.09	-0.64 ± 0.677	-5.76 ± 6.09
O. annularis	554	1,504.74	1,405.37	-0.09 ± 0.06	-0.40 ± 0.48	-3.62 ± 4.33
M. cavernosa	513	1,620.93	1,381.54	-0.25 ± 0.08	-1.74 ± 0.45	-15.68 ± 4.11

The last 3-months (May-August) relative net microfragment base growth (%) was not significantly affected by sedimentation or macroalgae levels recorded in the last (August) monitoring period (non-parametric Kruskal-Wallis test: sedimentation; 0.62 and macroalgae; 0.33 respectively). However, location significantly influenced sedimentation and macroalgae (Kruskal-Wallis; chi-squared = 25.81; 22.20, df = 5, $p < 0.0001$). Sedimentation was only significantly different across depth (Kruskal-Wallis; chi-squared = 17.16, df = 2, $p < 0.0001$), mainly shallow sites exhibit significantly greater sedimentation levels compared to intermediate or deep sites, while macroalgae was not significantly different by depth (Kruskal-Wallis chi-squared = 4.55, df = 2, $p = 0.10$). Over the last three months, sedimentation was higher in La Pared (1.05% ± 0.32 SE), and Radio Pirata (1.02 % ± 0.27 SE), while macroalgae was higher in La Bocana (0.47% ± 0.05 SE), and Tanchancte (0.38% ± 0.05 SE).

Across the five monitoring period intervals, there was a significant difference in temperature across time ($p < 0.0001$; Fig. 11) (Kruskal-Wallis; chi-squared = 1417, df = 9, $p < 0.0001$). However, there was no significant difference in temperature across locations ($p = 0.06$; Fig. 11) (Kruskal-Wallis; chi-squared = 10.36, df = 5, $p = 0.06$) or across depths (Kruskal-Wallis; chi-squared = 5.93, df = 2, $p = 0.05$). The overall average temperature for all locations combined was 28.45 °C ± 0.117 SE (Table 5).

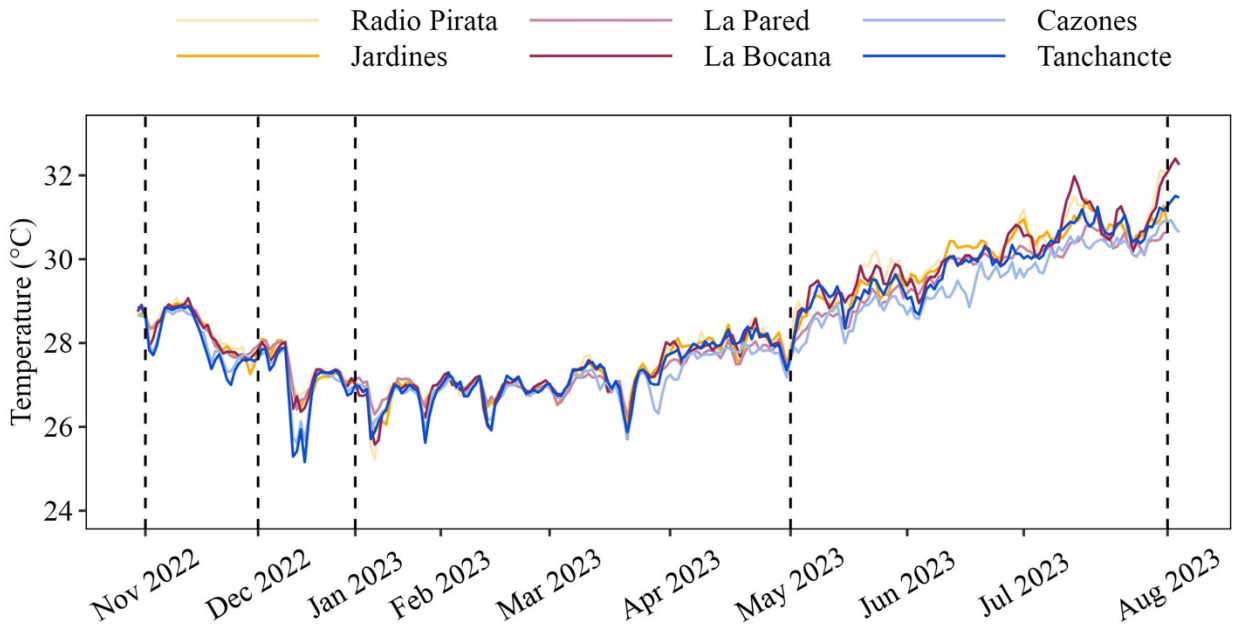

Figure 11. Average daily logger temperature (HOBO ProV2). The dash lines represent the five monitoring periods, and the different colors represent each location.

Table 5. The mean, minimum, maximum, and temperature range recorded at all locations.

Location	**Depth**	**Mean** °C	**Minimum** °C	**Maximum** °C	**Range** °C
Jardines	Shallow	28.39	24.99	31.79	6.80
Radio Pirata	Shallow	28.60	24.39	33.05	8.67
La Bocana	Intermediate	28.59	25.19	32.56	7.38
La Pared	Intermediate	28.42	25.74	31.28	5.54
Cazones	Deep	28.26	24.68	31.48	6.81
Tanchancte	Deep	28.44	24.12	32.10	7.98

With all monitoring periods pulled, microfragment bleaching prevalence significantly increased with mean temperature (R_2 = 0.27, p < 0.0001; Fig. 12, a)), and disease prevalence significantly increased with minimum temperature (R_2 = 0.17, p = 0.04; Fig. 12, b)).

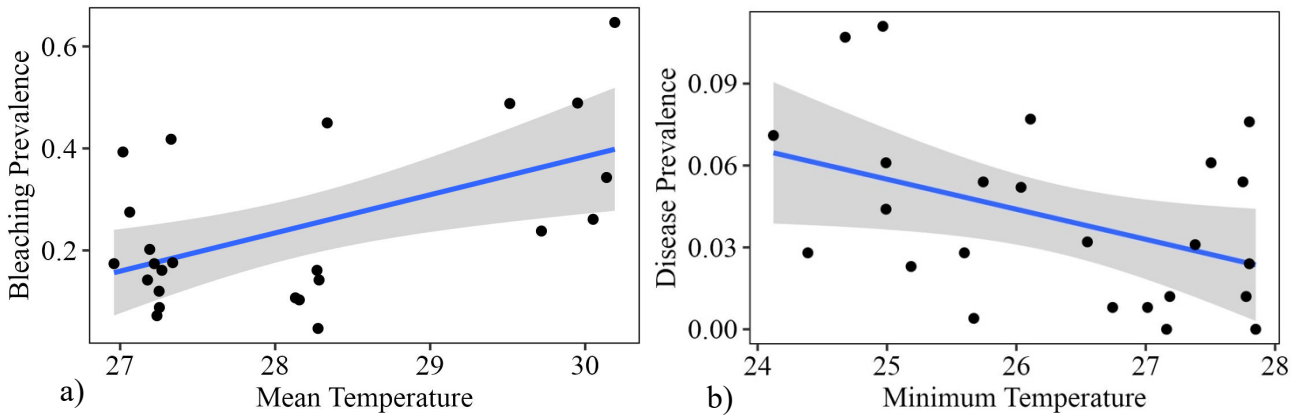

Figure 12. The relationship between a) mean temperature (°C), and bleaching prevalence (%) and b) minimum temperature and disease prevalence (%). Disease and bleaching prevalence are defined as the number of infected colonies divided by the number of total colonies. The gray area denotes the 95% confidence interval. Each data point represents disease or bleaching prevalence at each site at each time point.

3.3 Bleaching, disease, and predation impacts

After 9-months, microfragment bleaching prevalence, which peaked in August at 45.60% (Appendix Table 4, Appendix A), varied significantly by location. The minimum adequate model (fixed effect location and random effect genotype nested within base) explained 48% of variation in the data (GLMM; Marginal R^2 (i.e., fixed effects only) = 0.05 while the Conditional R^2 (i.e., fixed and random effects) = 0.48). Bleaching was most pronounced during the August monitoring period across all locations as observed in Jardines (27.26 % ± 8.17 SE), Radio Pirata (47.75 % ± 18.16 SE), La Bocana (69.76 % ± 13.54 SE), La Pared (24.73 % ± 14.06 SE), Cazones (57.82 % ± 6.73 SE), and Tanchancte (55.79 % ± 10.23 SE) (Fig. 13). Similarly, the August monitoring period showed higher bleaching prevalence across species, particularly in *O. annularis* (61.93 % ± 7.64 SE), as compared to *O. faveolata* (42.11% ± 12.99), and *M. cavernosa* (37.52% ± 7.14) (Fig. 14). Overall, significant variability in bleaching prevalence was observed across locations. Specifically, Cazones (deep site), exhibited significantly higher bleaching prevalence compared to Jardines (shallow site; emmeans pairwise comparisons; $p < 0.0001$). Furthermore, among the intermediate sites, La Bocana had significantly higher bleaching prevalence than La Pared (emmeans pairwise comparisons; $p < 0.0001$). Additionally, La Pared (intermediate site), had a significantly greater bleaching prevalence than Radio Pirata (shallow site; emmeans pairwise comparisons; $p < 0.0001$).

Post 9-months, disease prevalence in microfragments was generally low and varied significantly by depth ($p < 0.0001$). The minimum adequate model (fixed effect location and random effect genotype nested within base) explained 79% of variation in the data (GLMM; Marginal R^2 (i.e., fixed effects only) = 0.04 while the Conditional R2 (i.e., fixed and random effects) = 0.80). Notably, deep sites had significantly higher disease prevalence with a maximum of 11.99% ± 7.77 SE (January 2023) recorded in Cazones and Tanchancte 11.99% ± 3.50 SE (May 2023) compared to 6.23% ± 3.55 SE (Radio Pirata; January 2023) in shallow, and 7.25% ± 4.26 SE (La Pared; January 2023) intermediate sites (Appendix Table 5, Appendix A; $p < 0.05$). Particularly, mean disease prevalence remained consistently low, staying below 6% throughout the study (Appendix Table 4, Appendix A).

Microfragment predation was observed exclusively in the first two months of the study, but predation prevalence in microfragments was low (~1%) after which continuous monitoring revealed no further instances. Therefore, there was not a significant difference in predation prevalence with any of the tested factors. However, mean predation prevalence was observed highest in *M. cavernosa* (0.75%) and at La Bocana (1.411%).

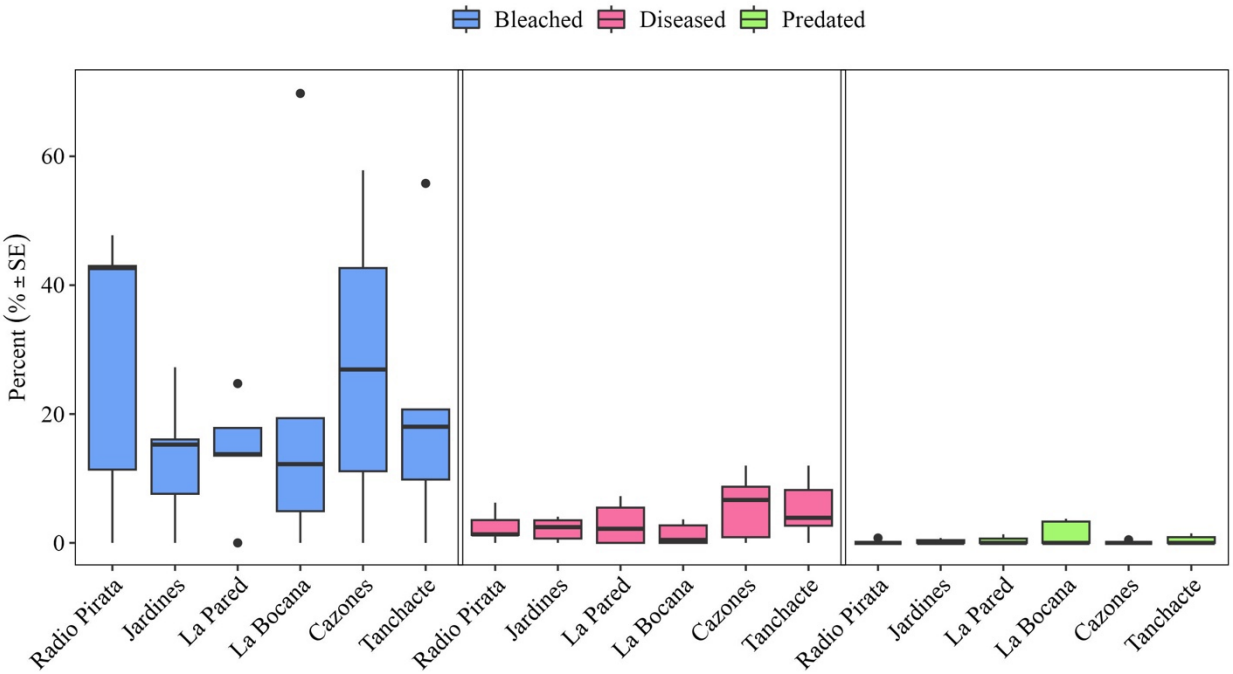

Figure 13. Mean percent of microfragments showing bleaching (blue), disease (pink), and predation (green) all monitoring events pulled by location: Radio Pirata and Jardines are the shallow sites, while La Pared and La Bocana, the intermediate sites and Cazones and Tanchancte, the deep sites. The 1-day post-outplanting monitoring (November) was removed because no conditions were observed.

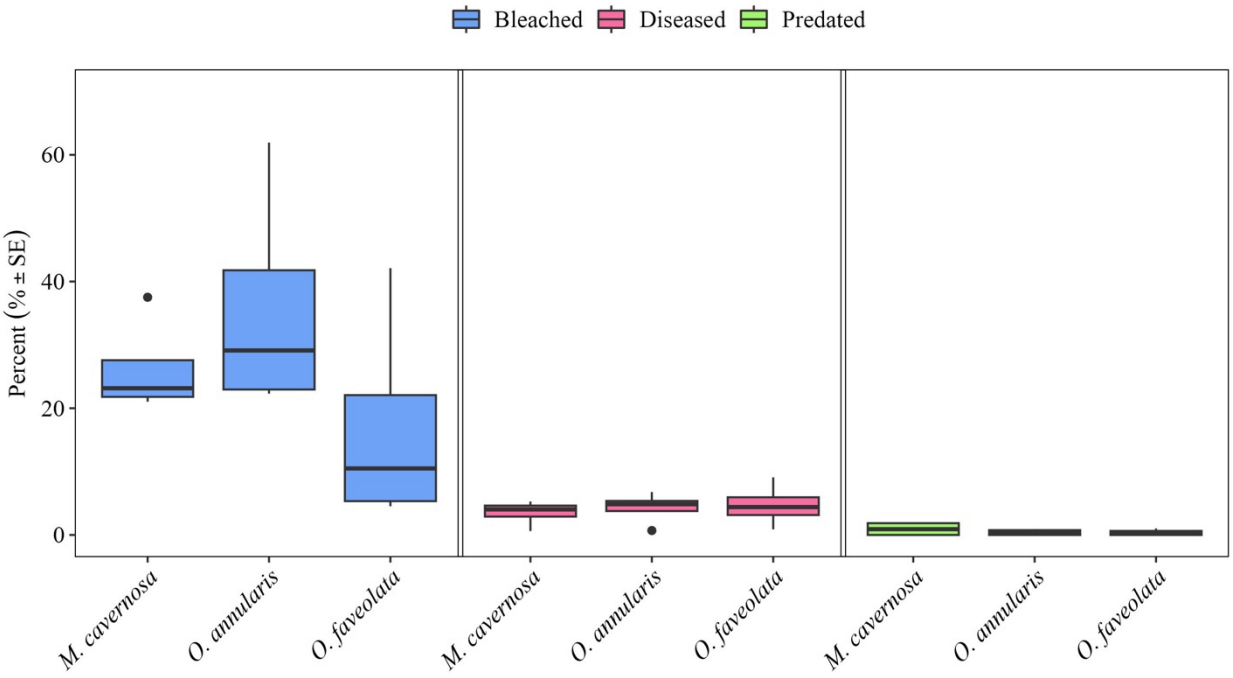

Figure 14. Mean percent of microfragments showing bleaching (blue), disease (pink), and predation (green) all monitoring events pulled by coral species: *M. cavernosa*, *O. annularis*, and *O. faveolata*. The 1-day post-outplanting monitoring (November) was removed because no conditions were observed.

4. Discussion

The introduction of SCTLD-susceptible stony coral species microfragments did not lead to an increase of disease in the surrounding natural colonies, suggesting that the start of restoration activities is feasible in the APMNP. After 9 months, overall microfragment survival including species and locations was high (84.28% ± 3.28), but relative net microfragment base growth was negligible in the APMNP. These results were primarily influenced by location differences. Notably, microfragments experienced high bleaching across all sites specially during the August period, yet they experienced relatively low disease and predation prevalence. During the last 3-months (May-August), there was a notable increase in macroalgal and sedimentation levels on the microfragment bases at the outplant sites.

With all monitoring periods pulled, I observed a slight increase in *O. faveolata* tissue area (+0.46% cm^2) and large declines in *O. annularis* and *M. cavernosa* (-6.60% and -14.76% cm^2, respectively). These declines in live tissue area were attributed to partial and whole microfragment mortality. When compared to studies that used similar restoration methods in different locations, such as Florida, *O. faveolata* and *M. cavernosa* yielded 262% increase in surface area after 2.5 years (Forsman et al., 2015). Site location significantly influenced growth, with intermediate sites; La Bocana (15.86% ± 5.03 SE) and La Pared (11.32% ± 7.00 SE) having a significant positive relative net growth (% / month). At both shallow sites (Jardines; 0.60 % ± 6.32 SE, Radio Pirata; -29.08 % ± 7.88 SE), and deeper sites (Cazones; -25.06 % ± 6.47 SE, Tanchancte; -22.84% ± 4.54 SE) in the APMNP, conditions did not appear to be conducive to microfragment growth. This is indicated by the predominantly negative relative net growth (% / month) observed in most of the microfragment bases at these sites. Despite sedimentation and macroalgae levels not significantly affecting relative net microfragment base growth, there was a significant increase in both macroalgal, and sedimentation levels observed across all locations during the last monitoring period. These drastic differences in growth among species may be attributed to factors such as chronic stressors, environmental conditions, and the different depth and outplant locations within the APMNP.

Although the mean water temperature across all monitoring events and locations was similar (28.45 °C ± 0.12 SE), the shallow site, Radio Pirata located in the reef crest (2-3 m) experienced a maximum temperature of 33.05 °C compared to the lowest maximum temperature value recorded

at the intermediate site, La Pared 31.28 °C. Even though temperature was not significantly different between location, the difference between the maximum and minimum was a 1.77°C which is high for a relatively small area. I observed some cases of bleaching prevalence in the beginning of the study, which is likely attributed to the need of a longer acclimatization period between outplanting from the *ex situ* nursery to the reef. In the final monitoring period (August 2023), the APMNP experienced the highest recorded temperatures of the study (33.05 °C), which many natural colonies in the outplant area were severely bleached and led to a mean bleaching value of 83.83% in the outplanted microfragments. This severe bleaching, varied significantly between locations despite similar water temperatures. Notably, it appeared that bleaching levels correlated with water depth and temperature, with deeper sites experiencing higher levels of bleaching during the highest temperature monitoring period (August 2023) compared to shallow sites. This depth-dependent gradient in bleaching has been observed in other regions such as Mo'orea (Penin et al., 2007). This pattern is likely driven by increased wave intensity and frequency characteristic of the reef crest where shallow sites are located, resulting in greater water movement compared to the deeper sites (Penin et al., 2007). However, this is not as clear in Radio Pirata since this site experienced high bleaching throughout the study compared to other sites that had higher bleaching during the last monitoring period.

My findings, which highlight location-specific effects, were consistent with the results of Knapp et al., (2022) who also found spatial variability in survivorship and growth. In the APMNP, shallow sites experienced significantly higher sedimentation levels and were subject to a high energy environment with increased wave action (Huston, 1985), and greatest light intensity (Hay, 1981), which could have negatively impacted the growth and survival of microfragments in Radio Pirata and Jardines. Deeper sites (7-8 m; Cazones and Tanchancte), situated in the northern extent of the APMNP, are prone to small-scale discharges of fresh or brackish wetland waters via submarine springs linked to the Yucatan Peninsula's aquifer system (Ruíz-Rentería, 1998). Additionally, overflow through small canals contribute to higher organic matter in the water column and increased turbidity (Ruíz-Rentería, 1998). Likely as a result, Cazones and Tanchancte experienced higher disease and bleaching prevalence, and less coral growth. High bleaching observed in the deep sites could also be attributed to the fact that deeper sites experience warm temperatures less often and thus have a lower thermal tolerance (Leinbach et al., 2019; Miller,

1995). Additionally, these sites exhibited high macroalgal overgrowth, potentially outcompeting microfragments for space and inhibiting growth (Soto et al. 2020).

Fish predation was low (~1%) and only observed during the first two months of the study. This contrasts to other locations (e.g., Florida and Hawaii) where up to 9% predation prevalence has been recorded (Koval et al., 2020). Although minimal predation was observed, it is worth noting that there was no effort to manage the land nursery water temperature to match the field conditions. Page et al. (2018) suggested that a temperature conditioning period might be critical to prevent excessive initial predation as microfragments change in color.

Disease prevalence in the outplanted microfragments remained relatively low, with an overall disease average of 3% and a maximum of 6.11% by location. Our findings align with those of another study conducted in Florida, which also reported an average of ~1-2% and a maximum of 4.3% of outplanted living colonies exhibiting active signs of SCTLD (FWC, 2021). While I observed higher SCTLD disease prevalence in natural colonies at outplant sites compared to control sites there was no significant increase in disease at these sites over time. SCTLD prevalence on natural colonies at control and outplant sites remained below 5%. Thus, the limited prevalence of SCTLD in the introduced microfragments and surrounding natural colonies may suggest that the restoration of susceptible species within the APMNP is not only feasible but also promising. This finding alleviates concerns about high mortality rates among outplanted corals and provides the possibility for large-scale restoration initiatives.

Various stressors were observed to be acting on the outplanted microfragments. As a result, it is not just a single pressure, but rather a combination of factors that are preventing the growth and survival (i.e, recovery) of microfragments. A similar study, which investigated factors affecting the growth of transplanted *M. cavernosa, O. faveolata,* and *O. annularis* colonies, found comparable results, which revealed high survivorship but limited growth in the prevalence of current disease, bleaching, sedimentation, and macroalgae pressures, without a clear trend indicating a specific stress factor (Lustic et al., 2020). Similarly, a study done on Florida's Coral Reef which examined spatiotemporal changes in benthic community structure over a 15-year period concluded that high disturbance frequency and chronic anthropogenic pressures have led to continuous declines in stony corals and corresponding proliferation of macroalgae (Jones et al., 2022). These findings suggest that the current environmental conditions are imposing substantial hurdles on the growth and survival of microfragments. Therefore, I recommend implementing an

experimental microfragmentation framework before outplanting to assess size-, species-, genotype-, and location- specific performance, to optimize active reef restoration activities. By doing so, future coral restoration projects can maximize their probability of success and alleviate some of the environmental pressures currently hindering growth and survival.

This study marks the first instance of microfragments being outplanted in the Mexican Caribbean. Therefore, I recommend additional experiments to better evaluate the technique's success. For example, Forsman et al. (2015) suggest that smaller microfragments tend to grow at slower rates than larger ones. Future studies could consider examining the impact of fragment size on massive coral restoration success within the APMNP. Further research could also explore acclimation to site conditions as a potential strategy before outplanting. Page et al. (2018) suggests that acclimating microfragments to their intended site conditions before outplanting may contribute to enhanced long-term survival rates. Despite relatively high survival, it was evident that microfragments struggled to grow and suggests chronic pressure likely limit the long-term potential for coral reef restoration via microfragmentation as a viable method to stimulate recovery in the APMNP. Despite this, introducing SCTLD-susceptible stony coral species did not contribute to an increase in disease in surrounding natural colonies, suggesting that SCTLD may no longer pose a significant threat to natural populations in the study area and thus restoration activities of SCTLD-susceptible species can be implemented in the APMNP.

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5. Appendices

Appendix A

Appendix Table 1. Overall (all monitoring periods) mean (± SE) disease prevalence in natural colonies at control and outplant sites.

Location	Type	Mean disease prevalence (%)
Cazones	Control	0.01 ± 0.01
Cazones	Outplant	0.02 ± 0.01
Jardines	Control	0.01 ± 0.01
Jardines	Outplant	0.01 ± 0.01
La Bocana	Control	0.07 ± 0.02
La Bocana	Outplant	0.02 ± 0.01
La Pared	Control	0.01 ± 0.01
La Pared	Outplant	0.03 ± 0.01
Radio Pirata	Control	0.04 ± 0.02
Radio Pirata	Outplant	0.09 ± 0.01
Tanchacte	Control	0.00 ± 0.00
Tanchacte	Outplant	0.07 ± 0.02

Appendix Table 2. After 9-months, microfragment base absolute growth rate (cm^2 ± SE), mean relative change (% / month ± SE), and relative net growth (% ± SE) by location.

Location	Absolute growth rate (cm^2 ± SE)	Mean relative change (% / month ± SE)	Relative net growth (% ± SE)
Radio Pirata	-0.43 ± 0.13	-3.23 ± 87.59	-29.08 ± 7.88
Jardines	0.05 ± 0.10	0.07 ± 70.58	0.60 ± 6.35
La Bocana	0.22 ± 0.09	1.76 ± 55.89	15.87 ± 5.03
La Pared	0.08 ± 0.12	1.26 ± 77.85	11.32 ± 7.01
Cazones	-0.39 ± 0.09	-2.78 ± 71.87	-25.06 ± 6.47
Tanchacte	-0.31 ± 0.07	-2.54 ± 50.47	-22.84 ± 4.54

Appendix Table 3. All microfragment base absolute growth rate ($cm^2 \pm SE$), mean relative change (% / month ± SE), and relative net growth (% ± SE) by species and location.

Specie	Location	Absolute growth rate ($cm^2 \pm SE$)	Mean relative change (% / month ± SE)	Relative net growth (% ± SE)
M. cavernosa	Cazones	-0.39 ± 0.12	-3.10 ± 0.81	-27.90 ± 7.29
M. cavernosa	Jardines	-0.09 ± 0.15	-0.37 ± 1.37	-15.52 ± 9.21
M. cavernosa	La Bocana	-0.15 ± 0.16	-5.48 ± 1.14	-7.11 ± 7.20
M. cavernosa	La Pared	0.34 ± 0.17	-1.72 ± 1.02	26.66 ± 7.96
M. cavernosa	Radio Pirata	-1.01 ± 0.27	-0.89 ± 0.91	-55.49 ± 11.24
M. cavernosa	Tanchacte	-0.33 ± 0.12	3.48 ± 1.52	-20.89 ± 6.62
O. annularis	Cazones	-0.21 ± 0.16	-0.79 ± 0.80	-3.34 ± 12.37
O. annularis	Jardines	-0.16 ± 0.13	3.05 ± 0.80	-8.00 ± 8.17
O. annularis	La Bocana	0.41 ± 0.13	2.94 ± 1.05	27.47 ± 7.21
O. annularis	La Pared	-0.03 ± 0.19	2.96 ± 0.88	0.91 ± 12.97
O. annularis	Radio Pirata	-0.20 ± 0.16	0.10 ± 1.44	-14.56 ± 11.22
O. annularis	Tanchacte	-0.30 ± 0.10	0.49 ± 1.71	-21.95 ± 7.45
O. faveolata	Cazones	-0.62 ± 0.19	-6.17 ± 1.25	-49.36 ± 10.23
O. faveolata	Jardines	0.50 ± 0.21	-1.62 ± 1.25	31.32 ± 13.72
O. faveolata	La Bocana	0.38 ± 0.13	-2.30 ± 2.00	26.44 ± 9.41
O. faveolata	La Pared	-0.14 ± 0.26	-2.32 ± 0.74	4.40 ± 15.42
O. faveolata	Radio Pirata	-0.12 ± 0.22	-2.44 ± 0.83	-20.69 ± 17.96
O. faveolata	Tanchacte	-0.30 ± 0.19	-2.96 ± 1.17	-26.63 ± 10.56

Appendix Table 4. Overall mean values for microfragment bleaching, disease, and predation throughout the five monitoring periods.

Type	Monitoring period	Mean % (± SE)
Diseased	November (1 day)	0 ± 0
	December (1 month)	1.51 ± 0.31
	January (2 months)	5.76 ± 0.61
	May (6 months)	5.37 ± 0.59
	August (9 months)	3.23 ± 0.48
Bleached	November (1 day)	0 ± 0
	December (1 month)	16.57 ±1.00
	January (2 months)	24.93 ± 1.13
	May (6 months)	16.51 ± 1.00
	August (9 months)	45.60 ± 1.35
Predated	November (1 day)	0 ± 0
	December (1 month)	1.00 ± 0.25
	January (2 months)	1.29 ± 0.29
	May (6 months)	0 ± 0
	August (9 months)	0 ± 0

Appendix Table 5. Summary data of bleaching, disease, and predation prevalence in microfragments at each location, throughout the monitoring periods. (November was taken out since there was no bleaching, disease, or predation observed at the initial timepoint).

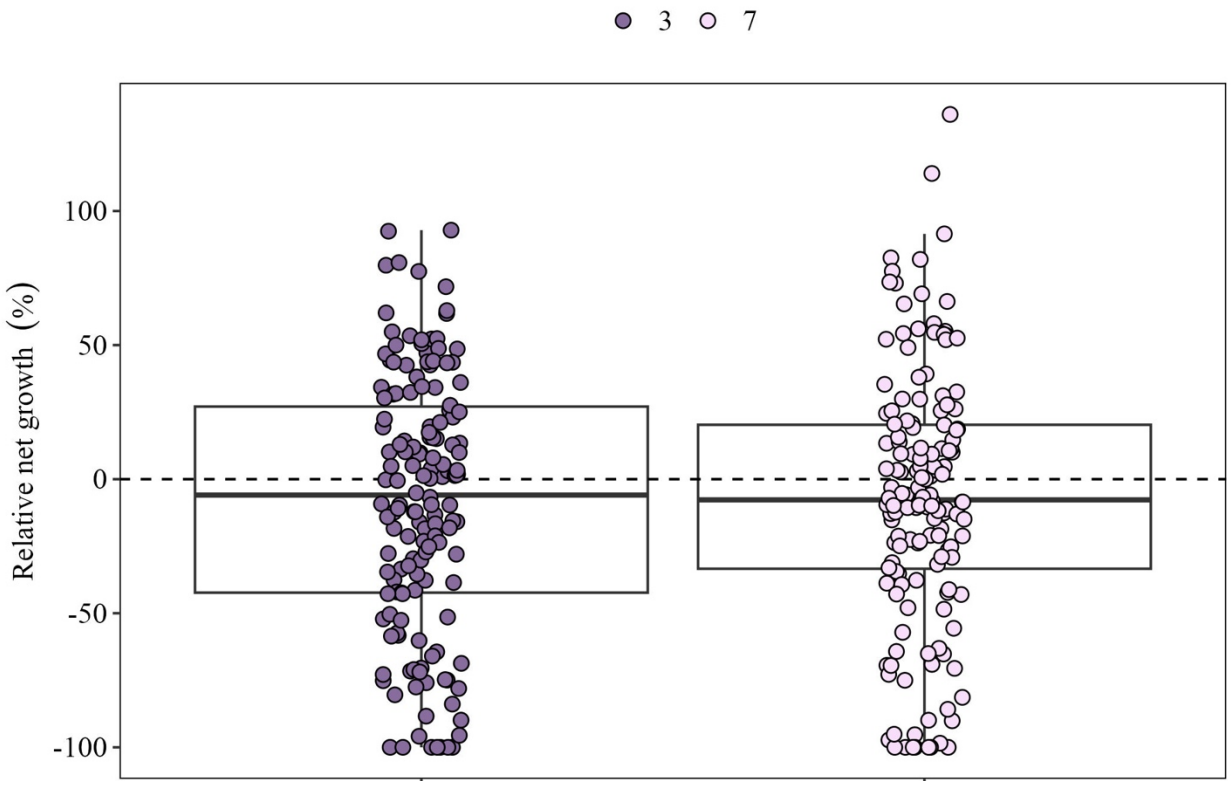
Location	Monitoring period	Type	Mean % (± SE)
Radio Pirata (Shallow)	December	Bleached	42.98 ± 18.34
		Diseased	1.32 ± 0.73
		Predated	0.76 ± 0.38
	January	Bleached	42.65 ± 8.25
		Diseased	6.24 ± 3.55
		Predated	0 ± 0
	May	Bleached	11.38 ± 4.29
		Diseased	3.54 ± 1.77
		Predated	0 ± 0
	August	Bleached	47.75 ± 18.16
		Diseased	1.23 ± 0.62
		Predated	0 ± 0
Jardines (Shallow)	December	Bleached	15.26 ± 7.92
		Diseased	0.67 ± 0.67
		Predated	0.42 ± 0.42
	January	Bleached	16.05 ± 4.38
		Diseased	2.44 ± 1.85
		Predated	0.76 ± 0.39
	May	Bleached	7.62 ± 1.81
		Diseased	3.51 ± 3.51
		Predated	0 ± 0
	August	Bleached	27.26 ± 8.17
		Diseased	4.07 ± 3.32
		Predated	0 ± 0
La Bocana (Intermediate)	December	Bleached	4.92 ± 3.44
		Diseased	3.65 ± 3.65
		Predated	3.31 ± 1.57
	January	Bleached	12.22 ± 6.29
		Diseased	0.46 ± 0.46
		Predated	3.75 ± 1.52
	May	Bleached	19.36 ± 8.32
		Diseased	2.72 ± 1.58
		Predated	0 ± 0
	August	Bleached	69.76 ± 13.54
		Diseased	0 ± 0
		Predated	0 ± 0

Appendix Table 5. Continue

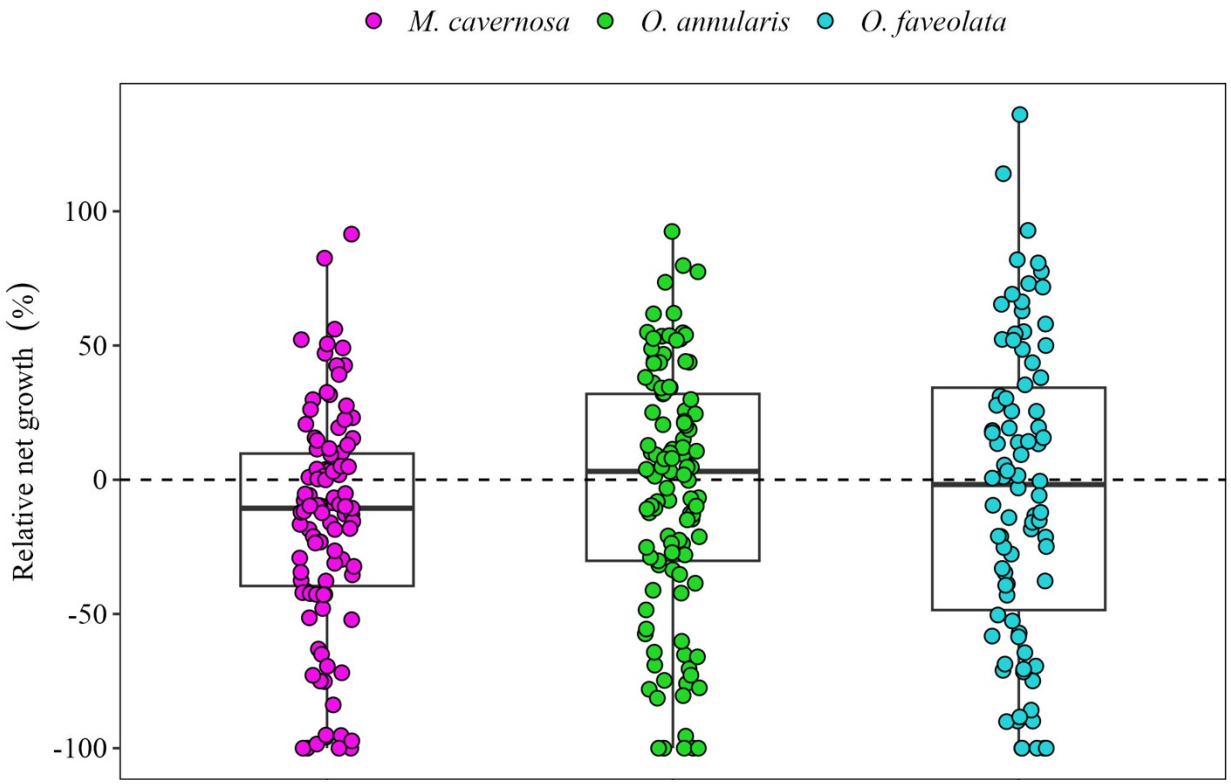
Location	Monitoring period	Type	Mean % (± SE)
La Pared (Intermediate)	December	Bleached	13.76 ± 8.16
		Diseased	0 ± 0
		Predated	0.67 ± 0.67
	January	Bleached	17.84 ± 9.31
		Diseased	7.26 ± 4.27
		Predated	1.33 ± 1.33
	May	Bleached	13.54 ± 6.80
		Diseased	5.47 ± 2.84
		Predated	0 ± 0
	August	Bleached	24.73 ± 14.06
		Diseased	2.19 ± 2.19
		Predated	0 ± 0
Cazones (Deep)	December	Bleached	11.12 ± 2.04
		Diseased	0.88 ± 0.45
		Predated	0 ± 0
	January	Bleached	42.66 ± 6.27
		Diseased	11.99 ± 7.78
		Predated	0.48 ± 0.48
	May	Bleached	26.91 ± 12.91
		Diseased	6.66 ± 1.61
		Predated	0 ± 0
	August	Bleached	57.83 ± 6.73
		Diseased	8.72 ± 4.53
		Predated	0 ± 0
Tanchacte (Deep)	December	Bleached	9.83 ± 7.58
		Diseased	3.89 ± 2.88
		Predated	0.89 ± 0.47
	January	Bleached	18.03 ± 10.16
		Diseased	8.21 ± 2.66
		Predated	1.48 ± 0.98
	May	Bleached	20.71 ± 14.86
		Diseased	11.99 ± 3.51
		Predated	0 ± 0
	August	Bleached	55.79 ± 10.23
		Diseased	2.67 ± 1.15
		Predated	0 ± 0

Appendix B

Additional net growth rate figures



Appendix Figure 1. Distribution of relative net growth (%) for 3 and 7 microfragment base densities. The central line inside the box represents the median net growth for each base. The dashed line across the plot represents the zero net growth threshold. Points above the threshold indicate positive net growth, while those below represents species with a net growth below zero.



Appendix Figure 2. Taxonomic variation in relative net growth (%) for three coral species. The central line inside the box represents the median net growth for each species. The dashed line across the plot represents the zero net growth threshold indicate positive net growth, while those below represents species with a net growth below zero, each data point represents one base.