Examining novel techniques in lab-based spawning to upscale coral restoration efforts

Examining novel techniques in lab-based spawning to upscale coral restoration efforts

Final Report

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Management Summary
The innovative research and development work that the UNCW Coral REEF lab is pursing includes land-based coral spawning, methods of upscaling recruit rearing via light spectra and development of probiotics, and enhancing genetic diversity within a colony (i.e., chimeras). The initial findings of the light experiment suggested that *Pseudodiploria clivosa* recruits likely benefit from lower blue light than adult corals. To determine if and/or when recruits should be moved to higher blue light to maximize growth and survivorship before being outplanted to the wild, a light experiment was conducted. Use of probiotics has been proposed to enhance aquaculture, although very few facilities have attempted to use bacterial isolates for this application in corals. Previously, three isolate groups previously showing higher survival than controls were retested and broken into smaller subgroups of which several showed promise. Identifying probiotics takes a specialized microbiology skillset, along with experience in coral husbandry, land-based coral spawning, experimentation, and a substantial amount of time. Yet despite the challenges, if probiotics can be identified, this will greatly increase the number of coral recruits that can be supplied for restoration. Chimeric formation has been proposed as a novel technique to enhance coral genetic diversity. However, outside of fusion within the first six months post-settlement, there is little evidence that chimeras can form in adults. Using thermal stress for the purpose of lowering the allorecognition response threshold resulted in species-specific chimeric formation. *Orbicella faveolata* adult microfragments do not fuse even regardless of the temperature of the aquarium, while *Acropora cervicornis*, showing less interspecific aggression, had increased chimeric formation under thermal stress. With this considered, chimeric formation is recommended within six months of settlement to enhance genetic diversity instead of relying on thermal stress that also may have negative consequences on coral health.

Executive Summary
Florida's Coral Reef is currently experiencing a multi-year disease-related mortality event that has resulted in massive die-offs in multiple coral species. Sexual reproduction is critical to coral recovery by increasing genetic diversity and creating individuals that are potentially resistant to the factors that have led to coral mortality of Florida's Coral Reef. In addition, coral fertilization, larval development, and recruitment are the most vulnerable life history stages, and therefore the most important to understand for coral reef recovery and resilience. However, the logistical difficulties of collecting coral gametes in the field and transporting them to a laboratory has limited utility for research in this area. Innovative and radical measures are needed to assist with the recovery of Florida's Coral Reef. The primary objective of our research is optimizing and upscaling *ex-situ* coral sexual propagation techniques, which ultimately serve to increase the genetic diversity of coral populations used for restoration. Three promising areas of research were focused on to meet this objective: (1) enhancing methods in *ex-situ* coral spawning, larval rearing, and recruit grow out, (2) screening bacterial isolates to test for their potential use as probiotics in coral aquaculture, and (3) create multigenotypic individuals (chimeras) through use of thermal stress. This year two facilities were consolidated into one dedicated building which has improved every aspect of the *ex-situ* propagation methods. This includes identifying the optimal light spectrum (lower blue light) to grow *Pseudodiploria clivosa* recruits, which may also be tested on other species. Three bacterial groups isolated from adults were identified to have the potential of enhancing *Pseudodiploria clivosa* recruit survival and growth. These isolate groups are going to be further screened to identify if a single bacterial isolate is responsible for the enhanced health benefits. The final task focused on creating chimeras with microfragmented adult colonies exposed to heat stress. These three tasks furthered the knowledge and understanding of various aspects of *ex-situ* sexual reproduction and techniques to improve upscaling land-based restoration efforts and enhancing genetic diversity of wild populations in the coming years.

Acknowledgements
We thank the Florida Department of Environmental Protection for their financial support of these projects, UNCW's Center for Marine Science for providing the infrastructure for this research, and aquaculture specialists Jimmy White and Ron Moore for their assistance. We would also like to thank Dr. Blake Ushijima and his laboratory for their assistance with the probiotics experiment (task 2). This research could not have been completed without the dedication of the following UNCW students: Alissa Brennan, Allie Reno, Anna Edmundson, Annabel Burcham, Avry Regan, Carly Stines, Jena Myers, Jenna Pullarkat, Juanita Gonzalez, Julia Feeth, Katelin Cole, Kayleen Bello, Leah Hasstedt, Lucille Alluin, Maggie Fowler, Mark Leavitt Jr., Maureen Howard, Mazzy Morrison, Mia Lezenweger, Nicolo Cohen, Paige Shackelford, Phoebe Whitbeck, Reanna Jeanes, Ryan Besemer, Sam Heaton, Sidney Westfall, Tara Hernandez.

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MAS	Minutes after sunset
DAFM	Days after full moon
GLMM	Generalized linear mixed model

1. AQUARIUM-BASED CORAL SEXUAL REPRODUCTION

1.1 Overview

Efforts to maintain and spawn corals in the land-based nursery at UNCW were continued. As this technology has only recently been applied to Caribbean corals, the methodologies as well as infrastructure are continually optimized to maximize spawning success and recruit survival. For example, an experiment was conducted to determine the optimal light spectrum for rearing *Pseudodiploria clivosa* recruits. In general, algae and Symbiodiniaceae thrive under blue light, but there is anecdotal evidence that before coral recruits acquire photosynthetic symbionts, blue light may be harmful. Here, coral recruits were exposed to different levels of blue light to compare their survivorship and growth for eight months after settlement. Optimizing the light spectrum for recruit rearing has broad implications for the field of *ex-situ* coral reproductive restoration and will help inform managers and stakeholders on how to design their recruit grow out facilities. Additionally, Dr. Fogarty continued to coordinate and facilitate the land-based assisted sexual reproduction (LASR) group that consists of the experts in the field of artificial light and indoor cue mimics. This group shares important information on land-based spawning techniques that helps advance knowledge in this emerging field.

1.2. Methods

Spawning and fertilization

Corals collected off Broward County were added to our spawning system that mimics natural seasonal temperature and light (solar and lunar) cycles. The facility houses *Orbicella faveolata* (n=11), *Pseudodiploria clivosa* (n=15), *and P. strigosa* (n=2) that the State of Florida has identified as rescue priorities, in addition to 11 genotypes of *Acropora cervicornis*. These species were monitored for spawning in August and September. The corals were monitored starting three days after the full moon (DAFM) and 90 minutes after sunset (MAS). Corals were monitored every 10 minutes for setting and if setting occurred, corals were monitored continuously until spawning. The coral species, identifying number, time the colony set, proportion of colony setting, and spawn time were recorded. Gamete bundles were collected by pipetting or scooping bundles with a petri dish then adding the bundles to gravy separators or smaller cups if fewer than 200 gamete bundles were collected. Bundles from multiple colonies were mixed in approximately equal proportions and gently swirled to break apart using a shaker, which could take up to 90 minutes. Sperm concentrations were targeted at 10^6 by keeping a ratio of approximately 1 gamete bundle to 1 ml of seawater. Gravy separators were placed in water baths set at (27^{o}C) for 1 -2 hours until the first cell cleavage was evident. Eggs were rinsed a minimum of five times and gently added to larval cones or dish bins.

Larvae rearing and settlement

Embryos were added to larval cones (volume 70 l) at densities of at least 1 larva/ mL). Water was added to the cones at a flow of (1ml/sec). Air was added to cones after 24-48 hours depending on stage of embryological development. Saran wrap was used to remove lipids from the surface of the cones and dead, decaying embryos were pipetted from the cones. Cones were checked, cleaned, and banjo filters exchanged every 6 hours for the first 72 hours. Tiles that had been conditioned for 1-1.5 months in our spawning system were placed in dish bins with Nitex mesh sides in raceways, stagnant jars and dishes, and larval rearing cones. Crustose coralline algae (CCA) were scraped from the sides of our raceways, crushed into a fine powder with a mortar and pestle and placed on tiles to serve as a settlement cue. With limited CCA in our system settlement dust was therefore limited, which may have contributed to the lower settlement rate (~10%). This will hopefully be improved in the future by having a dedicated tank to grow CCA.

Recruit under varying light spectra

For the light experiment, 12 tanks were set up in our experimental rack system and divided equally across three light treatment groups using a 6,000 K, 10,000 K, and 20,000 K light color setting in the Ecotech Mobius app (Fig. 1). The distance of the Radion Gen 6 XR15 lights to the water's surface was 20.5 cm. The distance from the light to the tiles was 50.5 cm. At 14 weeks, tiles in all tanks were elevated to 17 cm from this height to reach the targeted PAR of 100 µmol m-2 s-1 for the 6,000 K treatment. Fourteen coral recruits at 2 weeks post-settlement were added to each tank for a total of 168 recruits (56 per treatment). Survivorship was quantified weekly (n=13) and photographed approximately every 3-5 weeks for growth analysis, except for weeks 15-20 when survival was not quantified, and weeks 15-24 when growth photographs were not taken. Light intensity (PAR) was ramped from 25 to 100 µmol m-2 s-1 over the experimental duration (Fig. 2). Once per week, tiles were cleaned, tank water was changed (25-30%), and recruits were quantified and fed a fixed volume of REEF-Roids, Golden Pearls and liquid SELCON (~1ml per coral).

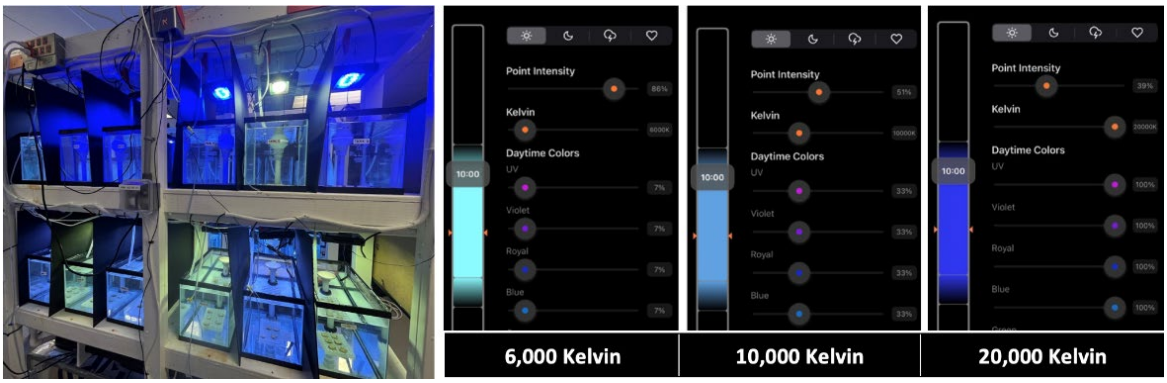

Figure 1 *Experimental setup and settings in Mobius app.*

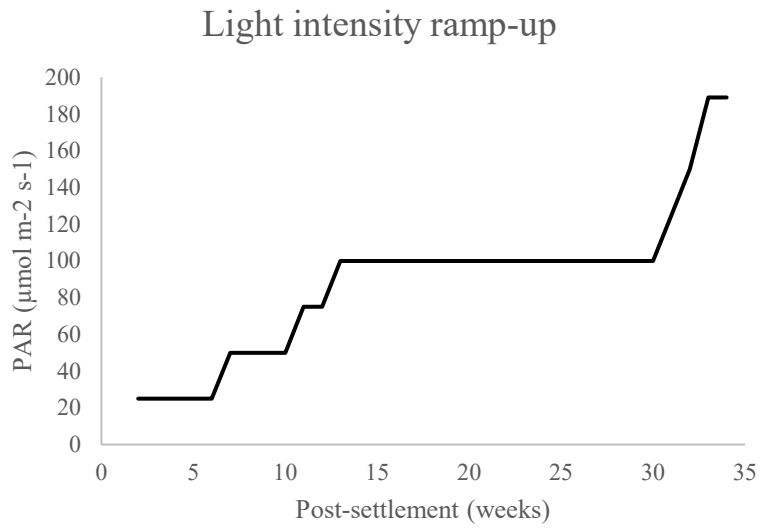

Figure 2 *Light intensity ramp-up over the course of the experiment.*

1.3. Results

Spawning and fertilization

The first year of this project, most of the colonies in our facility spawned (Table 1). In June 2021, more corals were acquired however many *O. faveolata* showed signs of disease shortly following their transfer and therefore only a few spawned. The disease portion was cut out of most of the new *O. faveolata* and only one of the surviving portions spawned in 2022. The hope is now that the formerly diseased colonies have recovered, they will spawn in 2023. At least half of the *P. clivosa* colonies spawned in 2021 and 2022 (Table 1).

Table 1 *Frequency of corals that spawned each year. ^ include colonies that were in our system for <3 months and *includes colonies that were diseased or recovering from disease.*

Species	**2020**	**2021**	**2022**
O. faveolata	3 of 4^	3 of 11*^	4 of 11*
P. clivosa	4 of 6^	12 of 15^	7 of 15

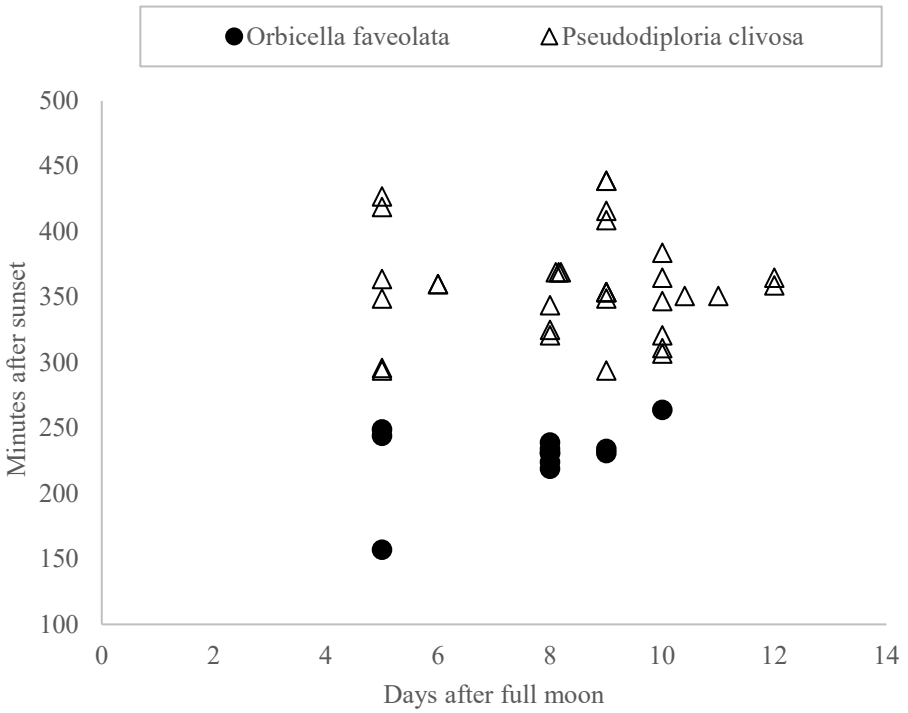

Figure 3*3 Orbicella faveolata and Pseudodiploria clivosa colonies that spawned in the UNCW SEAS facility from 2020-2022.*

From 2020-2022, *O. faveolata* and *P. clivosa* spawned between 5-12 DAFM and between 150-450 MAS (Fig. 3). In 2020 and 2021, these species spawned between 8-12 DAFM. In 2022, after all corals had been in the spawning system for at least a year, many colonies spawned earlier, 5 DAFM. Overall, more corals spawned in August than September (Fig. 4,5). Most *P. clivosa* spawned later in the evening between 290-370 MAS (Fig. 4). Most *O. faveolata* spawned between 210-250 MAS, which is consistent with field observations (Fig. 5).

Larvae rearing and settlement

O. faveolata (n=22,800) and *P. clivosa* (n=59,000) embryos were added in the four cones. 25,000 *P. clivosa* larvae were shipped to NSU. 1000s of *P. clivosa* were settled on tiles in dish bins and cones. Settling in stagnant dishes (Task 1: light optimization) or jars (Task 2: probiotic experiment) proved largely unsuccessful with these species. Settlement of *O. faveolata* was limited due to a majority being lost in attempts at stagnant settlement and a small number of remaining individuals being lost within the first weeks. Currently the lab has 184 and 490 *P. clivosa* recruits from 2021 and 2022, respectively.

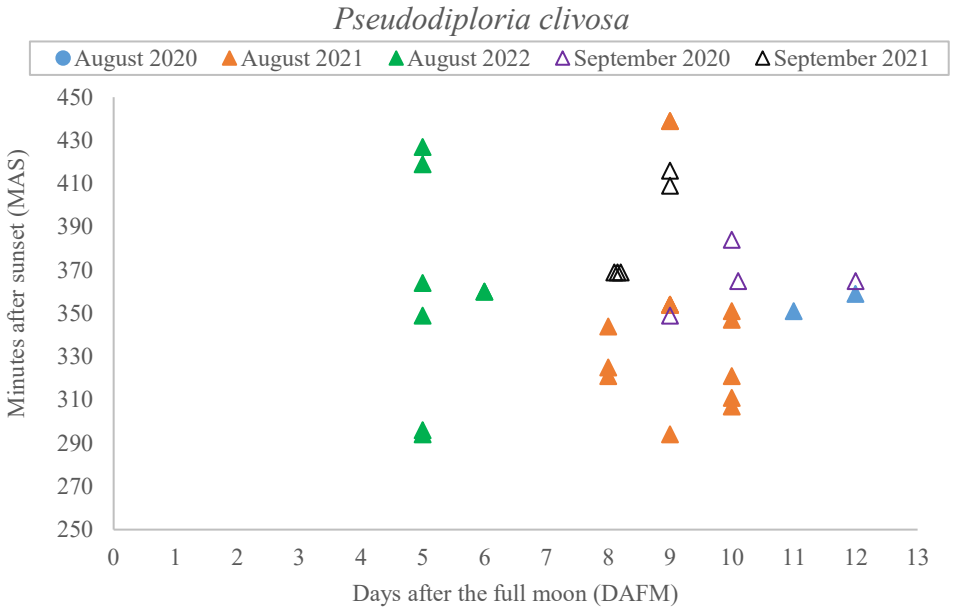

Figure 4 *Pseudodiploria clivosa spawning times in minutes after sunset (MAS) and days after the full moon (DAFM) in August and September from 2020-2022.*

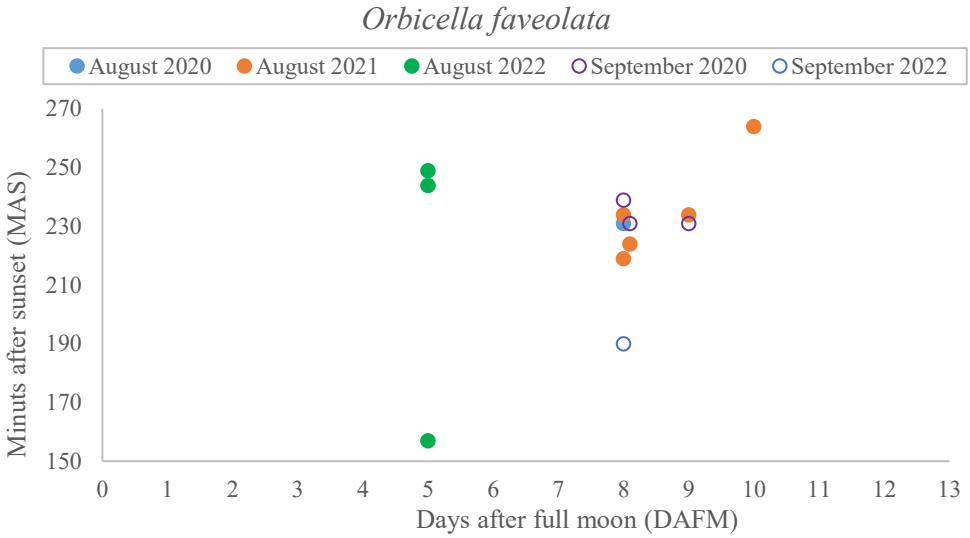

Figure 5 *Orbicella faveolata spawning times in minutes after sunset (MAS) and days after the full moon (DAFM) in August and September from 2020-2022.*

Recruit under varying light spectra

Survival was high over the course of the 8-month experiment with averages above 53%. After 34 weeks, survivorship in the three different light treatments was not statistically significant (log rank survival analysis, P>0.05; Fig. 6). Average recruit survivorship in the 6,000 K treatment was 69.6% by the end of the experiment, while the 20,000 K and 10,000 K treatments had 60.7% and 53.6% average

survival, respectively. From an upscaling perspective, using a wavelength equivalent to the Ecotech lights 6,000 K compared to 10,000 K, a 16% increase in average survivorship could substantially increase the number of coral recruits used for restoration efforts.

Recruit size did not significantly differ between light treatments throughout the experiment (GLMM, P=0.1881; Table 2). However, sampling week (GLMM, p<0.0001) and the interaction between sampling week and treatment were significant (GLMM, P<0.0001; Table 2). The interaction indicates that treatment differs across time points; therefore, differences were examined among light spectra at each time point. The average total growth of recruits was consistent across treatments for the first 6 weeks, but at weeks 10 (Wilcoxon, P=0.0346) and 14_(Wilcoxon, P=0.0113), recruits in the 20,000 K treatment were significantly larger compared to the 6,000 K and the 10,000 K treatments (Fig. 7). At week 25, there again was no significant difference among treatment (Wilcoxon, p>0.05). Then in weeks 29 (Wilcoxon, P=0.0045) Finally at week 34 (Wilcoxon, P=0.0304) the 10,000K treatments significantly differed with the 6,000 K treatment having higher growth and nearly doubling size at each of these timepoints (Fig. 7). Additionally, more macroalgae grew in the 10,000 and 20,000 K treatments; therefore, it was more labor intensive to maintain those tiles.

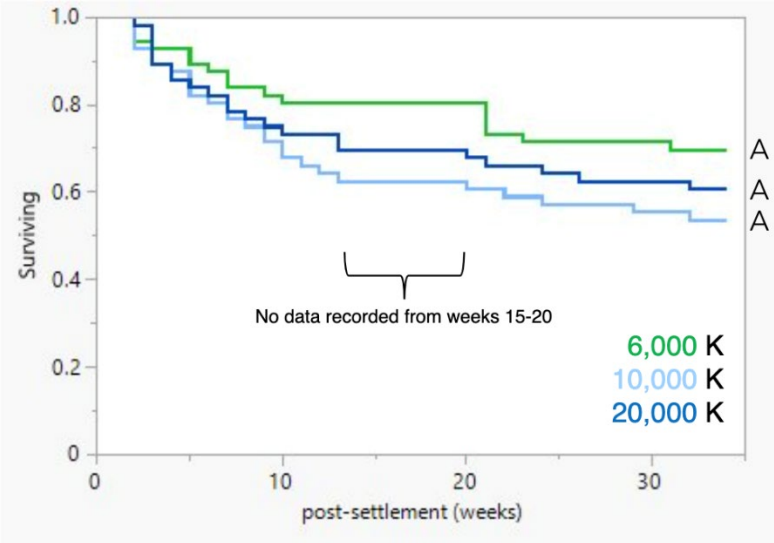

Figure 6 *Pseudodiploria clivosa recruit survival log-rank survival analysis (p>0.05) for the three different light spectrum treatments (6,000 K, 10,000 K, 20,000 K) over the 34-week experiment. Data was based on weekly survivorship counts, excluding weeks 15-20.*

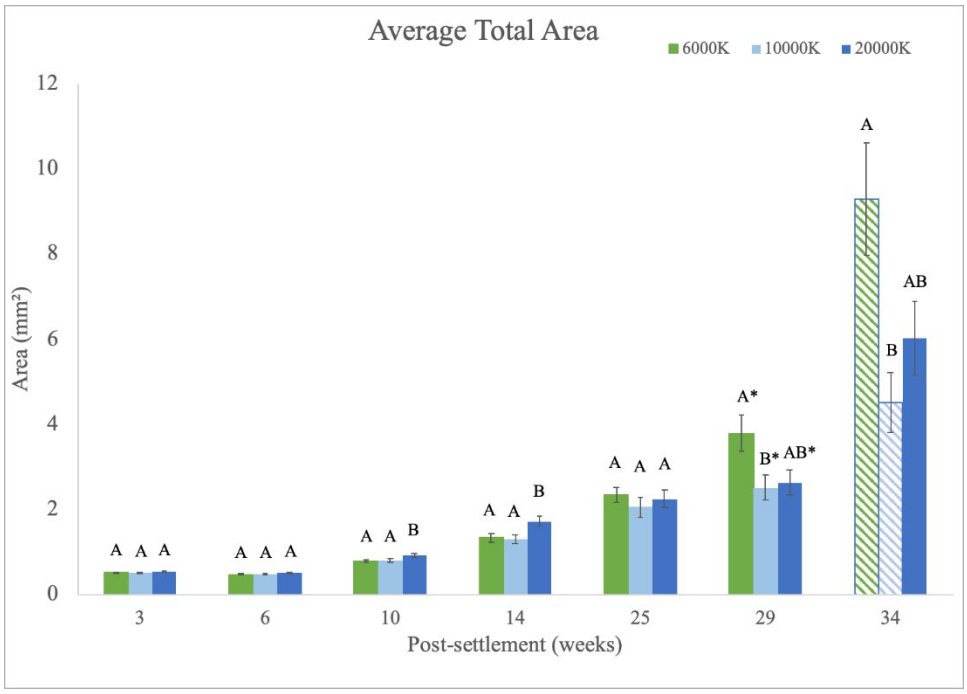

Figure 7 *Pseudodiploria clivosa recruits average total growth +/- SE for each treatment at each of the timepoints. A post-hoc connecting letters report was performed and the letters denote significant differences. No significant difference (Wilcoxon, p>0.05) in growth was observed at weeks 3, 6, and 25 post-settlement for the three different light treatments. * Indicates a marginal significance difference of (Wilcoxon, p<0.06) at week 29.*

Table 2 *GLMM with treatment (light spectra) as fixed effects, time as the continuous predictor and tank and coral as random effects to determine their effect on growth.*

Source	Nparm	DF	DFDen	F Ratio	Prob > F
treatment	2	2	9.635	1.9973	0.1881
week	1	1	710.6	489.7982	**<.0001***
week*treatment	2	2	709.9	22.8076	**<.0001***

1.4. Discussion

Spawning and fertilization

Within a given year, corals spawned consistently in terms of DAFM. The shift in 2022 to several days earlier may be an artifact of the corals being removed from light pollution and being on a consistent light and temperature schedule for at least a year. Because oogenesis takes at least 6 months and spermatogenesis at least two months, changes to coral reproductive cycles would likely lag one to two years. *Orbicella faveolata* spawning times occurs 5-8 DAFM so this shift is still within wild spawning observations. Field observations are limited for *P. clivosa* so it is difficult to compare. The land-based spawning times in terms of MAS have always

been consistent with the range of wild *O. faveolata* colonies. This consistency in spawning time and repeated spawning in some individuals across multiple years suggest these artificial spawning cues are reliable and the corals in the facility are healthy. As one of only a few laboratories in the country who are spawning Caribbean corals; therefore, these repeated spawning observations are important to understand the optimal techniques and to train other facilities on how to spawn corals in land-based nurseries using artificial light.

Larvae rearing and settlement

The conical tanks make rearing larvae much easier than older methods of rearing in buckets, coolers, and deli dishes, but our bottleneck is still settlement. The greatest settlement success has been in the dish bins with Nitex mesh sides placed in flowing raceways. It is clear that *P. clivosa* larvae need water flow to settle; therefore, stagnant dishes or trays should be avoided. The facility is limited in space and therefore has limited raceways for settlement. Although there was success settling in the conical tank in 2021, this method was not as successful in 2022. When space is limited, however, conditioned tiles can be added to egg crate wedged in the bottom of conical tanks. Understanding species specific rearing and settlement methods will help new spawning facilities maximize recruit production.

Recruit under varying light spectra

Overall, the 10,000 K treatment had lower survivorship and low growth on average compared to the other two treatments (albeit not always significantly lower at each time point); therefore, it is not recommended when rearing *P. clivosa* recruits. The 20,000 K treatment would only be recommended if the coral recruits were to be outplanted within the first three months and if larger size was the emphasis of project. If this is not the case, the 6,000 K treatment would be recommended for rearing *P. clivosa* recruits due to the higher average total area, high survivorship (albeit not statistically higher than the other treatments after 34 weeks) and less macroalgal growth on the tiles.

2. PROBIOTIC DOSING OF LARVAE & RECRUITS

2.1. Overview

Thus far, use of probiotics is an understudied area for coral biology, and little is known as to whether probiotics can enhance coral reproduction, survival, and growth at the earliest life history stages. Collaborating with Dr. Blake Ushijima, the Fogarty lab conducted follow-up experiments to determine if dosing *P. clivosa* larvae and/or recruits with promising probiotic isolates tested in 2021 can lead to enhanced survival, settlement, and growth. In 2021, 250 isolated bacteria were screened from mucus samples collected from *Pseudodiploria clivosa* colonies housed in our *ex-situ* coral spawning facility. Bacteria (n=250) were haphazardly placed into groups of 10 (n= 25 isolate groups) and screened for their effect on larval and recruit survival and growth.

Three isolate groups (15,17,19) showed promise as potential probiotics. These were further explored last year.

2.2. Methods

Larval dosing

The original isolate groups (15, 17, and 19) that showed promise were re-tested in 2022 and broken down into smaller groups of three or four isolates. These treatment groups and the seawater control were each replicated 6 times. To ensure the larvae were viable, the larval experiment commenced 2 days after spawning. The larvae were reared in glass jars with 99ml of filtered seawater (FSW) (0.2u FSW) plus 100ul of each bacterial strain (1ml total) or 1ml of FSW for the control. Thirty larvae were initially added to each jar followed by daily counts and a 50% FSW change. Jars were inoculated on the 1st and 4th experimental day; the experiment ran for 6 days.

Recruit dosing

After the August full moon, the recruit portion of the experiment began ten days after spawning (8/16/23) and continued over 8 months after spawning. Each isolate group and the control were tested six times. The larvae and recruits were kept at 27.5^{o}C. To use the same larvae and recruits throughout their life history stage, viable larvae (n=30) were also added to 78 individual jars containing a ceramic tile that had been conditioned for approximately six weeks prior to experiment start. Unfortunately, the larvae did not settle in the jars, and tiles with existing recruits (from the same cohort as larval experiment) were used instead. When the experiment commenced, recruits were approximately 1-week post-settlement (8/26/22) when the basal plate was well formed. Recruits were sacrificed from the tile until there were no more than eight remaining on one side with enough separation to ensure they did not grow into each other over the course of the experiment. Weekly inoculations occurred during the first 4 weeks by placing recruit tiles in jars for 24 hours then rinsed and placed in recirculating tanks. Survivorship was recorded weekly for the first 16 weeks then transitioned to biweekly until the end of the experiment at week 30. Growth photographs using cellSens began when the coral had completely formed their cup and septa (3 weeks post settlement-9/8/22), then occurred every 3-4 weeks for the first 15 weeks, and the last photographs were taken 8 weeks later at week 24.

2.3. Results

Larval dosing

Overall survivorship of control groups was high across treatments (>75% on average). No full or subset isolate group enhanced larval survival over control (log rank analysis, P>0.05; Fig. 8). A GLMM using isolate group as fixed factor and

time as continuous predictor and jar replicate as a random factor showed no significant differences between the isolate groups, but time was significant (Table 3).

Table 3 *GLMM examining isolate group (fixed factor), time (continuous predictor) and jar replicate as a random factor.*

Source	Nparm	DF	DFDen	F Ratio	Prob > F
week	1	1	455	940.5957	**<.0001***
treat	12	12	65	1.4690	0.1591
treat*week	12	12	455	3.9120	**<.0001***

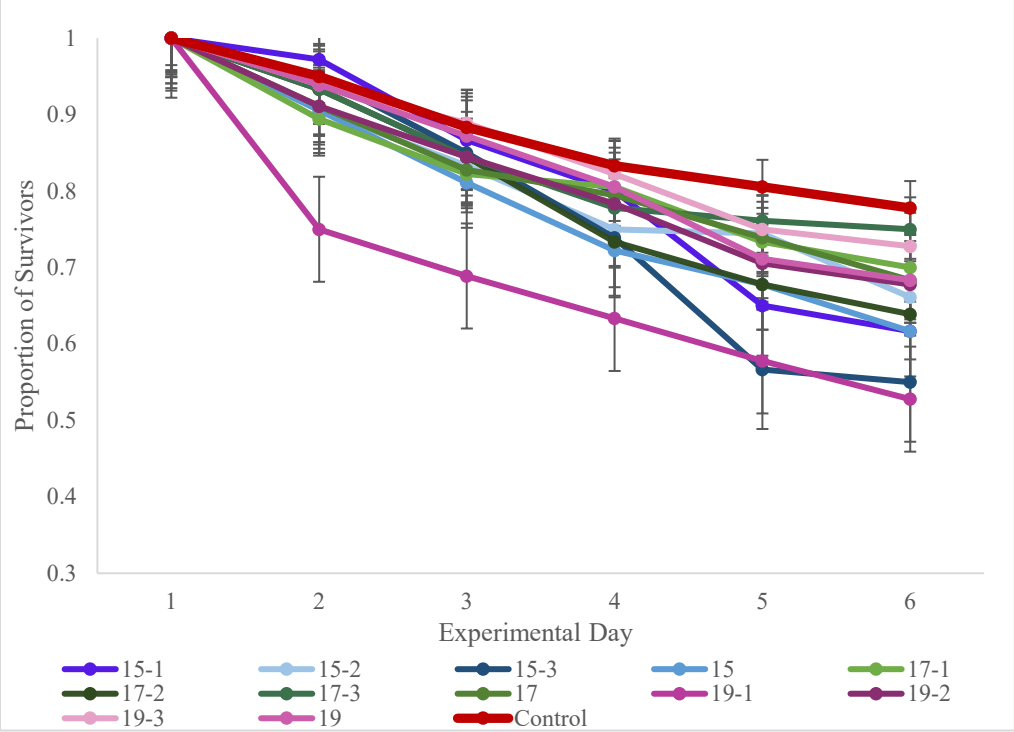

Figure 8 *Larval survival after exposed to bacterial inoculants on day 1 and 4.*

Recruit dosing

Over the 30-week recruit experiment, although 3 groups (17-1, 19-1, 19-2) showed promise with a 4-13% higher survival over controls, these increases were not statistically significant (log rank survival analysis, P>0.05, Fig. 9). Just examining the first 12 weeks post-settlement when recruits are most vulnerable as their skeletons are formed and their Symbiodiniaceae endosymbionts are acquired, 2 bacterial isolate subgroups (17-1, 19-2) had a marginal statistical significance increase (16-19%) over controls (log rank survival analysis, p<0.07, Fig. 10). Three groups (15, 17-1, 17-3) showed promise by increasing growth 9-23% over controls;

however, this increase was not statistically significant (t-test, P >0.05; Fig. 11). A GLMM using inoculum as the fixed factor, time as a continuous predictor, and tank and recruit as the random factors showed inoculant, time, and the interaction of time and inoculant was significant (Table 4). Lastly, one isolate group (17-1) has the potential to enhance both survival and growth.

Table 4 *GLMM using inoculum as a fixed factor, time as a continuous predictor, and tank and coral as random factors to determine their effect on growth.*

Source	Nparm	DF	DFDen	F Ratio	Prob > F
Inoculum	12	12	415.4	2.8833	**0.0008***
week	1	1	1537	2356.620	**<.0001***
week*Inoculum	12	12	1535	8.4519	**<.0001***

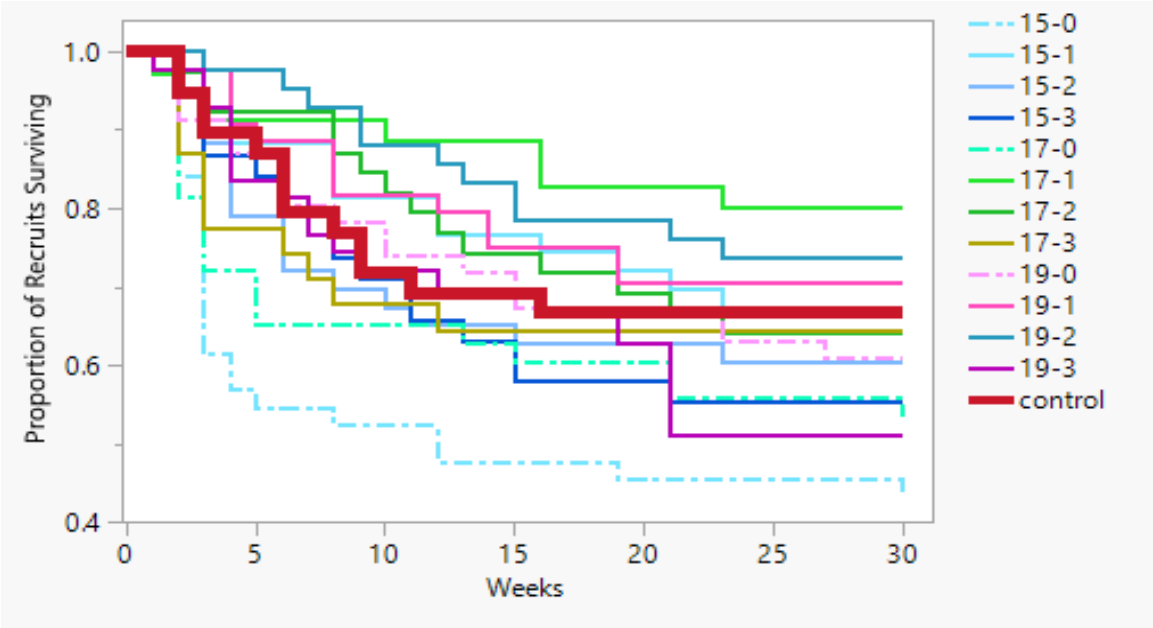

Figure 9 *Pseudodiploria clivosa recruit survival data over the entire 30-week experiment. Controls are designated by the red line.*

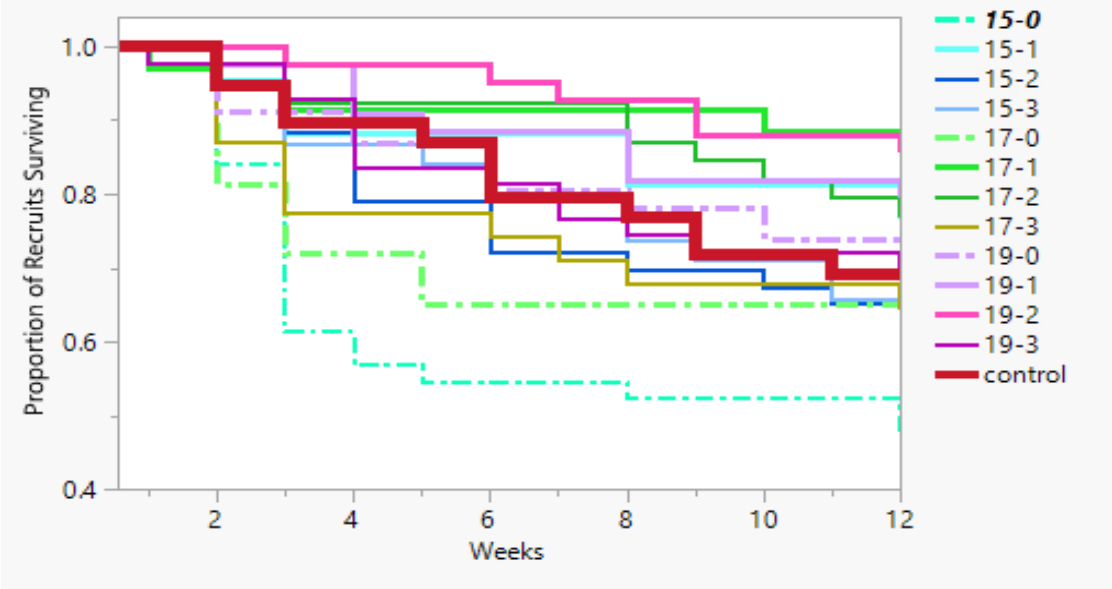

Figure 10 *Pseudodiploria clivosa recruit survival data over the first 12 weeks when recruits are acquiring their Symbiodiniaceae and are at a small, vulnerable size. Controls are designated in by the red line. Isolate groups 17-1 and 19-2 had marginally higher (log rank survival analysis P<0.07) survivorship than the controls.*

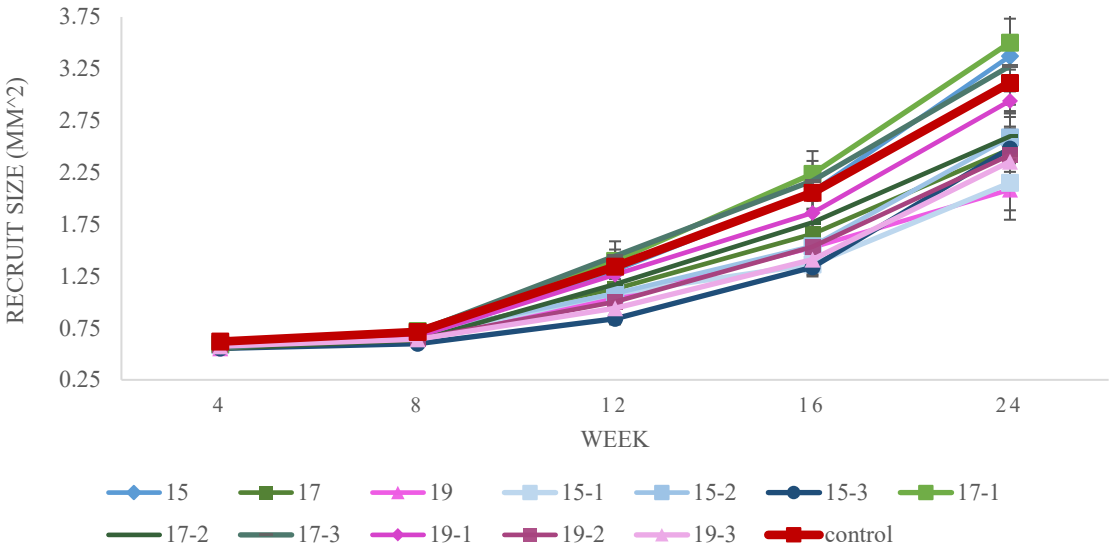

Figure 11 *Pseudodiploria clivosa recruit size over time for each inoculant test.*

2.1. Discussion

Larval dosing

Some isolate groups showed promise of up to 19% and 23% enhanced average survival and growth, respectively, which would substantially enhance coral biomass production for restoration efforts. It appears that no obvious health benefits

of these bacterial isolates were conferred to the larvae. This is surprising because in 2021 these isolates enhanced larval survival. It may be prudent to repeat probiotic dosing of *P. clivosa* larvae using the same 3 isolate groups (15,17,19) that showed promise for enhancing survival in the 2021 experiment to provide another time point for comparison.

Recruit dosing

The mechanism of how probiotics can enhance the health of coral larvae and recruits as well as any potential drawbacks of selectively introducing a microbiome, are unclear, and as such, this line of research needs to be further pursued. Some of the isolate groups showed promise of up to a 19% on average increase in survival over controls and increased average growth up to 23% over controls. Our future efforts will focus on testing each of the promising bacterial groups individually, as well as combining isolate groups that seemed to enhance growth and/or survival. Identification of isolate groups that exhibit potential for increased survival and growth could enhance coral biomass production for restoration efforts. Isolating, screening, and identifying probiotics that can enhance coral aquaculture efforts can be a slow and time-consuming process with a laboratory with a specialized skillset. However, if overall production and size of corals can be enhanced by at least 20% then it is a worthwhile endeavor.

3. CHIMERA FORMATION DURING ADULT STAGES

3.1. Overview

Chimeras have been suggested to aid coral resilience through a more varied response to stressors and offer a competitive edge. Multiple genotypes in a single colony through chimeric formation can be advantageous to combat future environmental changes and emerging disease, in addition to enhanced sexual reproductive success. For instance, if one of the genotypes within a chimera is resistant to a new disease or increased temperature, then it increases the likelihood that at least part of the colony will survive. Additionally, because most corals do not self, for sexual reproduction to be successful, more than one genotype is needed. Chimeras may enhance fertilization because unique genotypes are next to each, which increases the probability of egg-sperm interactions and fertilization success through outbreeding. Despite the potential benefits, there is much we do not know about chimeras. However, researchers are beginning to explore chimeric formation as an exciting restoration tool to enhance genetic diversity.

Based on the previous findings of strong intraspecific competition between adult microfragmented genotypes, it was hypothesized that exposing microfragments to stress might temper the allorecognition response. If chimeras can be formed after sublethal exposure to a stressor, then enhanced genetic diversity of the resulting chimeric colonies may prove to be more resilient to future stressors. In this experiment, a sublethal increase in temperature was used as our stressor.

3.2. Methods

This experiment was conducted for 6 weeks using leftover fragments from the disease excision that occurred in October 2021, as well as portions of adult colonies of *O. faveolata* and *A. cervicornis*. These fragments had not shown any signs of disease since being excised from the original diseased colony. There were two types of genotype pairings in this experiment: a monoclonal microfrag array and a bioclonal microfrag array (Fig. 12). Small microfrags (0.5cm^2, ~3 polyps) were glued direct;y abutting each other. Four *A. cervicornis* genotypes were used, and each genotype was paired with itself to serve as fusion control. The chimeric treatment groups paired two different genotypes (Fig. 12). One set of 10 arrays (4 monoclonal and 6 biclonal) were reared at ambient temperature (24ºC), while the second set of 10 arrays was reared at 3ºC above ambient (27ºC). The second set of arrays tested if the potential effect of sublethal thermal stress could facilitate chimeric fusion through a depression of the allorecognition response. After a 3-day recovery period from microfragmentation, temperature was gradually ramped up daily by 0.5ºC using Neptune Apex system aquaria controllers. Weekly surface area photo measurements were taken and analyzed in ImageJ to quantify tissue loss from interspecific competition.

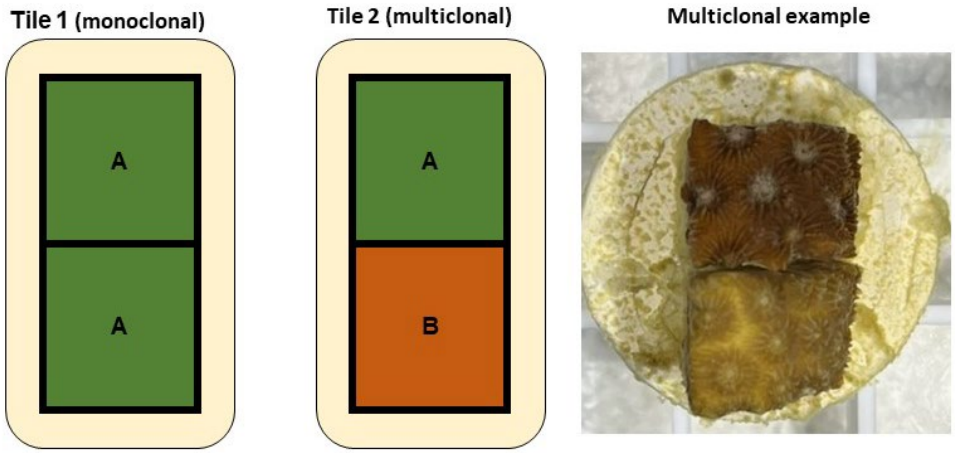

Figure 12 *Chimera setup with different letters and colors representing unique genotypes and a photograph of an Orbicella faveolata multiclonal array.*

3.3. Results

Acropora cervicornis showed signs of growth at their tissue margins, indicating that a 3ºC heating treatment is sublethal. All pairs with the same genotype (monoclonal) fused. All biclonal pairs in the heated treatment fused, but only half of the biclonal pairs fused in ambient tanks. There was no difference in tissue fusion between ambient and heated tanks for monoclonal controls (t-test, Fig. 13). Because fusion was high, delineating genotypes for growth data became difficult as no obvious morphotypic patterns existed.

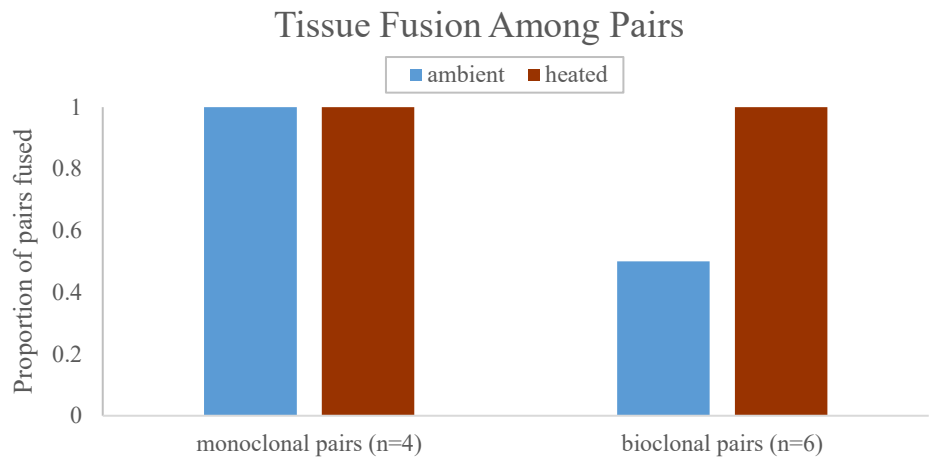

Figure 13 *The proportion of monoclonal and multiclonal pairs that fused in Acropora cervicornis.*

Like *A. cervicornis*, all of the monoclonal arrays in *Orbicella faveolata* fused over the 6-week experiment, regardless of temperature. However, unlike *A. cervicornis*, none of the *O. faveolata* bioclonal pairs fused (Fig. 14). This is likely due to the extremely aggressive nature of *O. faveolata*, where mesenterial filaments extended attacking the abutting genotype was often observed.

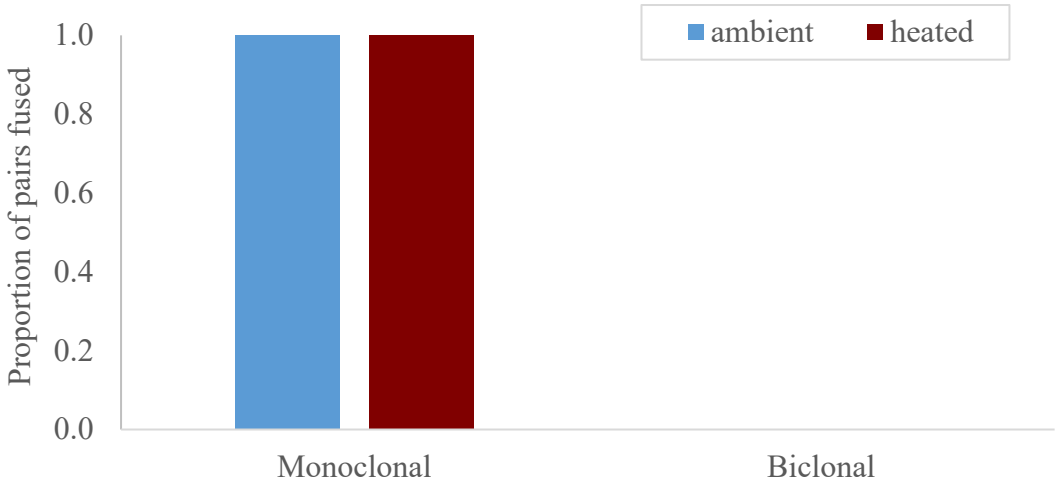

Figure 14 *The proportion of monoclonal and multiclonal pairs that fused in Orbicella faveolata* under ambient (24ºC) *and thermal stress conditions* (27ºC).

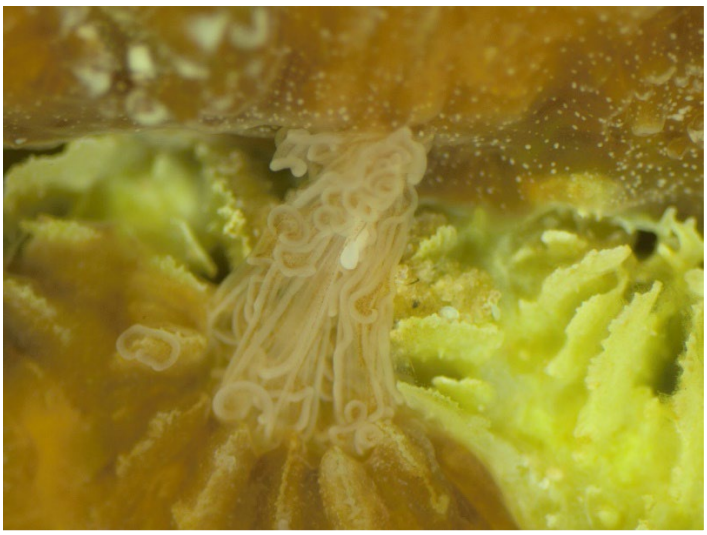

Figure 15 *Mesenterial filaments extended by an aggressive genotype. Additionally, it appears that the aggressor is consuming the tissue of the adjacent genotype.*

3.4. Discussion

There was little aggression observed (by the presence of mesenterial filaments and tissue regression) between genotypes in the biclonal arrays for both ambient and heated tanks, which allowed for fusion among genotypes. Based on these data it can be concluded that different genotypes of *A. cervicornis* will fuse when abutting, but heat stress appears to further subdue the allorecognition response allowing for more pairings to fuse. This provides possibilities for continued research not only for chimera formation, but the physiological effects thermal stress has on *Acropora cervicornis* immune response.

Studying chimeras in adults is challenging because of their slow growth and competitive nature. Based on our research, the ability to temper the allorecognition response of corals through thermal stress exposure seems to be species-specific. *Orbicella faveolata* allorecognition and aggression do not appear to be tempered by thermal stress. Anecdotal observations suggest the aggression was intensified in the heated tank. An unexpected result of this study occurred as aggression was captured between *O. faveolata* genotypes; cannibalism between genotypes also appeared to be observed (Fig. 15). If there is tissue being consumed by the aggressor, then this is the first time that cannibalism has been reported in corals. Chimeras are a useful tool but seem to only be able to form at the early recruit stage in *O. faveolata*. Because half of the *A. cervicornis* genotype pairs fused under ambient conditions, it is not advised to expose the corals to thermal stress unless managers want specific genotypic pairings, i.e., known thermal stress and disease resistant that will not fuse under ambient conditions.