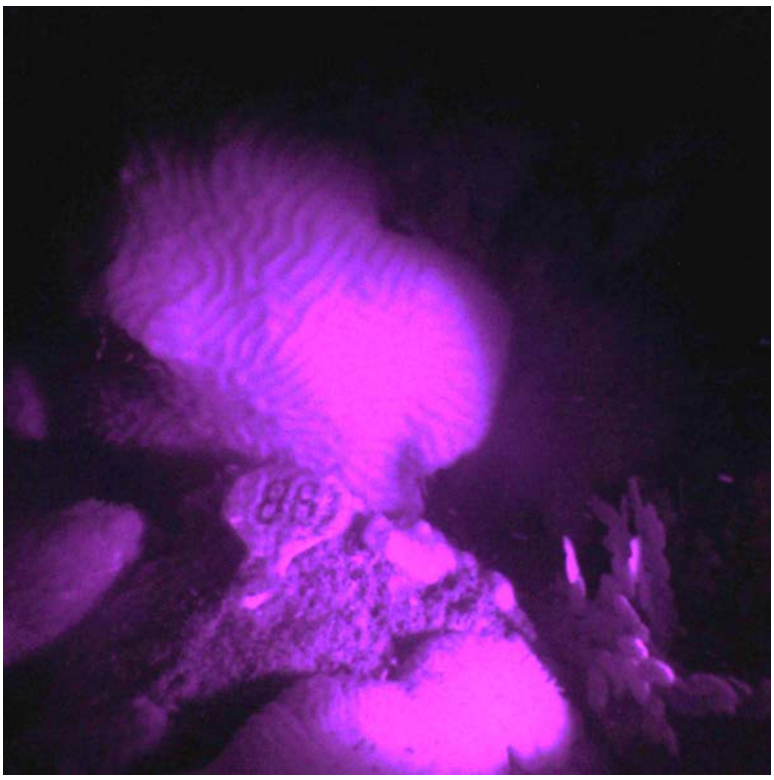
Restoration potential of corals from urbanized and marginal habitats in Florida



Restoration potential of corals from urbanized and marginal habitats in Florida

Final Report

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Management Summary

Given decades of population declines on Florida's Coral Reef and recent large-scale disturbance events such as the stony coral tissue loss disease (SCTLD) epidemic and the 2023 marine heatwave, it is critical to identify priority coral habitats and populations for targeted conservation and restoration. 'Urban corals' have been discovered on artificial substrates in urbanized habitats such as the Port of Miami, and research by the Coral Program at the University of Miami CIMAS and NOAA-AOML since 2018 has shown these corals are more resilient to environmental stressors than their reef counterparts. This project evaluated the spawning activity of urban corals through field-based and land-based observations and fecundity assessments, while also incorporating urban coral genotypes into land-based restoration pipelines with partners at the University of Miami Rosenstiel School, NOAA-SEFSC, FWC, and Nova Southeastern University. While spawning activity was not observed in urban corals in their native Port of Miami habitats, fecundity analysis confirmed that viable gametes were produced in sampled corals, which were likely released sometime during the overall spawning season for each species. Further, we induced one urban *Diploria labyrinthiformis* colony to spawn in a land-based aquaculture system at the University of Miami Rosenstiel School and successfully conducted a gamete cross with a Miami reef conspecific, resulting in ~2,676 surviving hybrid urban x reef recruits. There were, however, challenges related to incorporating urban corals of opportunity (COOs) into land-based propagation pipelines, likely stemming from sedimentation stress and potential pathogen introduction during collection and fragmentation. Urban corals remain a conservation priority for their potential to supplement restoration efforts, but perhaps may be best suited for sexual reproduction initiatives to increase genetic diversity of gene banks. Taken together, these outcomes add to growing knowledge on the ecological benefits of urban coral populations in the persistence and recovery of Florida's Coral Reef.

Executive Summary

Florida's Coral Reef has experienced severe declines due to disease outbreaks, thermal stress, and degraded water quality, with notable recent impacts from stony coral tissue loss disease (SCTLD) and the 2023 marine heatwave. This project investigated the restoration potential of "urban corals"—those persisting in highly urbanized environments such as the Port of Miami, as a potential means to aid in the recovery of Florida's Coral Reef. Studied since 2018, these corals have shown resilience to environmental stressors that have devastated reef populations, and therefore serve as valuable genotypes for restoration initiatives.

The study focused on three primary objectives: 1) identifying spawning windows of urban coral populations, 2) quantifying their reproductive capability and gamete viability, and 3) incorporating urban genotypes into land-based propagation and restoration pipelines at the University of Miami's Cooperative Institute for Marine and Atmospheric Studies (CIMAS), NOAA's Southeast Fisheries Science Center (SEFSC), the University of Miami's Rosenstiel School, the Florida Fish and Wildlife Conservation Commission (FWC), and Nova Southeastern University (NSU). Field-based monitoring efforts consisted of 17 total field days across summer 2024 and spring 2025 spawning seasons in the Port of Miami and Port of Palm Beach, tenting of 40 colonies of the species *Colpophyllia natans*, *Diploria labyrinthiformis*, *Orbicella faveolata*, and *Pseudodiploria strigosa* with gamete collection tents, extended observation of 22 colonies of the same species with time lapse cameras, and sampling of 67 colonies of the same species but also including *P. clivosa*, before and after their respective spawning seasons (127 samples total) for fecundity analysis. No spawning was observed in the wild, likely due to asynchronous timing driven by environmental factors such as artificial light pollution. However, fecundity analysis revealed that urban corals are reproductively viable and produce gametes comparable in size and fecundity to reef corals, and post-spawning sampling shows these corals released gametes during the predicted spawning season. Transfer of 29 corals of opportunity (COOs) to a land-based aquaculture system also did not result in observed spawning activity, with the exception of one urban *D. labyrinthiformis* colony that released gamete bundles 11 days after the full moon (AFM) on April 23, 2025. Despite over 2 hours in time delay between urban and reef corals spawning, we conducted an urban x reef parental cross with a Miami reef conspecific, resulting in successful fertilization and settlement of approximately 2,676 surviving recruits. This novel finding demonstrates the potential for integration of urban corals into sexual reproduction programs.

Efforts to propagate urban corals through conventional asexual fragmentation practices faced challenges, particularly low survivorship due to ciliate infections. It is likely that factors unique to their native habitats, such as pre-existing stress from sedimentation and burial, as well as the potential for harmful microbes being transferred with the corals, heavily impacted their survival in a land-based system. Nevertheless, several urban coral genotypes were preserved and transferred to multiple facilities across Florida, expanding the network of live gene banks and restoration partners. This work will continue in FY26 through additional support from FDEP, and is expected to further expand the network of urban coral gene banking and land-based sexual reproduction with partners at the Reef Institute and Mote Marine Laboratory.

These findings underscore the ecological importance of urban coral populations and support their inclusion in conservation and restoration strategies. Despite existing challenges, urban corals offer a promising source of resilient genotypes. Continued research and targeted conservation of these corals are essential, particularly as urban development intensifies along Florida's coastlines. Ultimately, these efforts will improve restoration success, efficiency, and cost-effectiveness in the recovery of Florida's Coral Reef.

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1. BACKGROUND

Populations of reef corals have been declining on Florida's Coral Reef over the past four decades due to disease outbreaks, thermal stress and coral bleaching events, and coastal development leading to declining water quality. Most recently, the devastating effects of stony coral tissue loss disease (SCTLD; Papke et al., 2024) and the 2023 global coral bleaching event (Hoegh-Guldberg et al., 2023) on Florida's coral populations underscore the urgent need to identify and integrate resilient genotypes into restoration pipelines. Consequently, it is paramount to identify priority habitats for conservation and to consider alternative sources of broodstock to improve local restoration efforts. Despite these myriad stressors, diverse coral populations have been discovered on artificial substrates (e.g., seawalls and rip-rap) in highly urbanized habitats throughout southeast Florida, including the Port of Miami (Miami-Dade County), north Biscayne Bay (Miami-Dade County), Government Cut (Broward County), and Peanut Island (Palm Beach County).

Since 2018, the Coral Program at the University of Miami CIMAS and NOAA-AOML has sought to characterize the environmental variability and benthic communities of 'urban coral' habitats in the Port of Miami (Enochs et al., 2023), as well as to examine the molecular signatures of resilience in urban versus reef corals (Rubin et al., 2021; Studivan and Enochs, 2022). Through these efforts, we have shown that Port of Miami corals are remarkably persistent in the face of thermal stress, sedimentation, and disease events (Enochs et al., 2023). Molecular assessments have determined that these urban corals demonstrate unique patterns of gene expression, including front-loading of genes associated with immunity and heterotrophy (Rubin et al., 2021), and exhibit increased resilience to environmental stressors compared to reef corals (Studivan and Enochs, 2022). Ongoing efforts by our group are also identifying the genetic relatedness of urban corals relative to reef populations across southeast Florida with population genetics approaches, as well as characterizing the algal symbiont assemblages that may confer stress tolerance to urban corals (Ing, 2024). Most recently, spawning observations in summer 2023 of *Colpophyllia natans* (CNAT), *Pseudodiploria strigosa* (PSTR), and *Pseudodiploria clivosa* (PCLI) in the Port of Miami revealed that these corals are reproductively viable (Rossin et al., 2025 in prep), though outstanding questions remain pertaining to their spawning periodicity and settlement success in the wild. Species present and thriving in these urban habitats include *Orbicella faveolata* (OFAV), CNAT, *Diploria labyrinthiformis* (DLAB), PSTR, and PCLI, which are now largely absent on nearby reefs due to the SCTLD epidemic and are priority species for restoration efforts.

Given their environmental tolerances, unique molecular makeup, and reproductive potential, ***urban corals are excellent candidates for targeted restoration efforts of resilient genotypes.*** In this project, we tested this hypothesis by addressing the following goals:

1. To determine when urban corals spawn in the wild, for targeted collection of gametes to support land-based sexual reproduction.
2. To incorporate urban corals of opportunity (COOs) into ongoing restoration efforts for downstream propagation, outplanting, and experimentation.
3. To propagate any sexual recruits of wild-collected spawn for downstream propagation, outplanting, and experimentation.

2. METHODS

2.1. Establish spawning windows for wild populations of urban corals

This task had two objectives: 1) to identify potential spawning windows of urban corals in the Port of Miami with field-based observations, and 2) to quantify gamete production, development, and potential release (i.e., fecundity) in urban corals. To accomplish these objectives, we had to initially rely on knowledge regarding spawning and fecundity of corals on natural reefs, since these data did not exist for urban corals in Florida. Spawning observations of wild corals throughout the Caribbean have been collected for several decades, resulting in the publication of region-specific spawning calendars that predict the most likely nights to observe peak spawning activity based on previous years' observations. For example, perhaps one of the most comprehensive spawning guides is developed by CARMABI for Curaçao and the southern Caribbean (https://www.agrra.org/wp-content/uploads/2024/08/2024-CARMABI-Coral-Spawning-Predictions-FINAL-for-Printing-3-Page-A4-HR-compressed.pdf). While a similar hard-copy guide does not exist for Florida's Coral Reef, researchers here often use it as a guide, modifying the predicted spawn windows based on local sunset times. For this project, we adopted a modified version of the CARMABI spawning calendar that was developed by NOAA-SEFSC, which is tailored to Florida-specific patterns such as sunset times and previous years' observations (Bright et al., 2024). We then used the Florida spawning prediction calendar to determine which peak dates to target for field-based spawning observations in the Port of Miami. For our priority species (CNAT, DLAB, OFAV, PCLI, and PSTR), the peak spawning calendars can be found in Table 1.

To quantify fecundity, tissue sampling was conducted before and after the predicted peak spawning windows in order to quantify gamete production, development, and potential release. Oocytes take ~9–12 months to develop, whereas spermatocysts take ~1–2 months (Leinbach et al., 2021; Rossin et al., 2025 in prep). To quantify oocytes and determine size and stage of oocytes prior to spawning, samples were taken as close to the peak spawning window as possible. Corals do not have true gonads, however, oocytes and spermatocysts develop within mesenterial filaments in bundles. During spawning, the entire bundle is released from the polyp, leaving the mesenteries empty. Tropical corals spawn in peak summer months following the full moon, when water temperatures are at their peak. This high temperature can result in bleaching or thermal stress for the corals, which can affect reproductive capacity. Corals and other invertebrates are able to reabsorb their oocytes and redistribute the lipids to essential biological processes (Rossin et al., 2019). This process takes several months and is evident in tissues before and after spawning events. Given the

more extreme environments in the Port of Miami, histology samples from before and after the spawning window were analyzed for fecundity, oocyte diameter and stage, presence of reabsorption and degradation, and sperm stage.

2.1.1. Field-based spawning observations and sampling

We conducted field-based spawning observations at an urban coral site in the Port of Miami, where long-term monitoring has occurred since 2018, and spawning observations have been conducted since summer 2023 (MacArthur Causeway North: 25.77293, -80.15263; Figure 1). Field observations using small boats or shore-based snorkeling targeted extended timeframes beyond peak spawning windows established for target species CNAT, DLAB, OFAV, and PSTR (Table 1), given previous observations by our group suggesting that gamete release in Port of Miami corals does not follow expected timeframes and may be influenced by water quality and light pollution. PCLI was not actively monitored for spawning activity, as this species has not been reliably observed to spawn in Florida. Gamete collection tents were deployed on fate-tracked colonies during peak spawning windows while observers were on site, in an attempt to collect gametes in case any colonies spawned.

During spawning observations conducted in summer 2023 and spring 2024, we deployed gamete collection tents designed by Dana Williams of the University of Miami CIMAS and NOAA-SEFSC. These tents were primarily designed for deployment on typical reef environments (Figure 2), constructed of lightweight, fine mesh, with relatively small base diameters and taller heights. While these tents work well on a reef environment with relatively little sedimentation and weaker currents, we found them to be inadequate for deployment at urban coral sites. Specifically, these tents were 1) prone to collecting fine silt in the mesh, 2) easily dislodged due to strong tides, 3) too tall for the shallow water (<1 m), and 4) often too small for the large size (>1 m diameter) of urban coral colonies. In collaboration with Dana, we redesigned these tents for urban corals, using a heavier-duty, more rigid window screen mesh, larger diameter base, and shorter height (Figure 2). Both tent designs were deployed in similar fashions, with less sediment buildup and reduced impacts to the coral colonies (i.e., extension of mesenterial filaments) in the urban coral gamete collection tents.

In addition to in-water observations by personnel, we invested in efficient, low-cost technologies to greatly expand upon our observation periods. GoPros are cost-effective, rugged, and natively support time lapse photography modes. With the addition of external battery packs, deployment times can theoretically be greatly enhanced (Figure 3). We purchased six GoPro HERO11 cameras for long-term time lapse deployments, due in part to their advertised night vision capabilities. Initial testing of these cameras prior to deployment resulted in lower-than-expected battery duration, on the order of approximately 6–9 hr per deployment. This was largely due to the GoPro remaining on

during the entire duration, where most of the battery was drained when the camera was idling in between photo intervals. Instead, we loaded experimental firmware developed by GoPro Labs (https://gopro.github.io/labs/; https://gopro.github.io/labs/control/longtimelapse/) onto the cameras, which allowed the cameras to shut off in between photos. This firmware allowed the user to input the total duration wanted for the time lapse, and a custom QR code was created with the desired parameters (Figure 3). Then, in the field, the user only had to hold the QR code in front of the camera, and it would automatically begin the time lapse/interval shutdown process. For spawning deployments, cameras were set to take photos every 96 sec, for a total target duration of 12 hr. During September 2024, two deployments per day were attempted to achieve nearly continuous, 24-hr time lapse coverage, however, this was later abandoned as a strategy due to personnel and boating safety limitations.

Finally, as the Coral Program had several older-model GoPro HERO6 cameras already in-house, we identified ways to modify them to be more successful in nighttime deployments. Aftermarket lenses are available for this model camera, allowing it to capture infrared light. Coupled with the external battery packs, and a separate infrared emitter costing approximately $11, we were able to deploy a limited number of infrared cameras per peak spawning window, targeting one colony per species (Figure 4). The experimental firmware was not available for this model camera, however, battery life was often sufficient for deployments up to 12 hr, which roughly corresponded with sunrise the next morning. In summer 2024, we deployed the infrared emitters in pre-made Ikelite housings designed for cameras, but following flooding of one system due to silt in the o-ring, we transitioned to custom-made PVC and acrylic enclosures for spring 2025 (Figure 5), which were easier to deploy and replace batteries during field operations.

2.1.2. Fecundity analyses

Complementing in-water observations of urban corals for spawning activity, histology was used to quantify the number and size of gametes produced (i.e., fecundity). Tissue cores were collected using a Nemo underwater drill and 2.5 cm diamond-tipped core bits by SCUBA divers, held in separate zip-top bags for the duration of the dive, and returned to the boat for processing. Pre- and post-spawning tissue cores were taken from the center of colonies to avoid non-reproductive tissue areas as the colony margins.

Tissue cores were then preserved in buffered formalin (Anatech Z-fix) diluted with seawater on the boat, with unique identifier tags in each sample container. Preserved samples were returned to the lab at room temperature for further processing: 1) tissue cores were removed from buffered formalin after a minimum of at least 24 hr, 2) soaked in seawater for 24 hr, and then 3) transferred to 70% ethanol for shipment to Louisiana State University (LSU). Once there, samples were processed and analyzed per the protocol

described in Leinbach et al. (2021). In summary, samples were first decalcified (i.e., the skeleton was dissolved away, leaving only the coral tissue) with a 1% HCl EDTA solution that was changed every 24 hr, and resulting tissues of three polyps or an area of approximately 1 cm^2 were processed in serial dilutions of ethanol to remove all water from the samples, and then serial changes of xylenes as a clearing agent to prepare the tissues for wax infiltration and make the tissues more transparent for visualization. Samples were then embedded in paraffin wax blocks in cross section and longitudinal orientations, which allowed tissue layers to be cut on a microtome and then mounted on slides for visualization and data collection. Tissue sections were 5 μm thick, then stained with hematoxylin and eosin stain. Hematoxylin and eosin are standard stains for viewing various tissue structure types, making oocytes and spermatocytes (cells that become eggs and sperm, respectively) easy to identify and determine developmental stages. Fecundity was evaluated through the quantification of oocyte diameter/stage per polyp averaged over three polyps per coral for OFAV, and per cm^2 for CNAT, PSTR, and PCLI (Leinbach et al., 2021; Rossin et al., 2025 in prep). Spermatocyst stage was evaluated qualitatively.

2.2. Conduct fragmentation and rearing of corals of opportunity (COOs) from urbanized and marginal habitats

This task focused on incorporating urban coral genotypes into existing land-based propagation and restoration programs, leveraging the combined decades' worth of experience related to coral spawning and rearing practices at NOAA-SEFSC, the University of Miami CIMAS, and the University of Miami Rosenstiel School. In short, these facilities combine personnel expertise and state-of-the-art aquaculture infrastructure with innovative technologies to improve and scale up land-based coral restoration. This task also prioritized preserving genetic diversity of urban corals, as these corals are threatened by the very habitats that house them. Nearshore environments in the Port of Miami are degraded by continued coastal construction, declining water quality, and warmer temperatures due to relative shallow depths compared to reef environments. Therefore, urban corals are often prone to dislodgment, burial, or bleaching as a direct result of these stressors. By collecting these corals that would otherwise succumb to multiple co-occurring stressors, we not only preserved valuable genotypes, but also generated coral tissue for restoration efforts. These efforts also expanded the emphasis on sexual reproduction in land-based systems to supplement asexual reproduction (fragmentation) and enhance genetic diversity.

2.2.1. Field-based coral of opportunity collections

Corals of opportunity (COOs) were collected from the Port of Miami and collaborators (Lindsay Spiers, FWC) and incorporated into existing fragmentation/grow-out operations at the University of Miami CIMAS and NOAA-SEFSC (Ladd and Enochs, 2024). During spawning observation dives, loose coral colonies and those suffering from partial mortality due to excessive sedimentation were identified and collected using a hammer and chisel and/or crowbar. Collected coral colonies were returned to the boat and maintained in

coolers with local seawater and wet bubble wrap for the duration of transit back to the lab. COOs were then transferred into existing propagation and restoration infrastructure following established quarantine and acclimation protocols at University of Miami and NOAA-SEFSC facilities as described below.

2.2.2. Land-based fragmentation and propagation

Collected COOs underwent an acclimation process that slowly brought the water conditions the corals arrived in to match the water conditions in the quarantine system to reduce stress. Corals were then placed on a 45-day hold in an isolated quarantine system, where they were monitored for signs of disease or other health issues such as tissue loss and paling. Corals were treated with Lugol's iodine and/or antibiotics to facilitate the healing process if stress signs occurred, with extended quarantine if the coral was showing signs of health issues. Extreme cases, such as rapid tissue loss due to ciliate infection (i.e., brown jelly syndrome), were treated with amputation of affected tissue followed by successive iodine/antibiotic applications. Corals were only moved out of the quarantine system once it was confirmed that they were in good health and acceptable to integrate into general growout stock.

Corals were then cut using a diamond blade bandsaw into smaller fragments that were affixed to ceramic grow out substrates using cyanoacrylate superglue. For grow-out, lighting, flow, and feeding regimes were implemented based on coral husbandry standards (Sprung and Delbeek, 1997; Holmes-Farley, 2004; FishLore, 2013), with modifications to maximize growth and survivorship for the different species. This entailed leveraging years of experience from NOAA-SEFSC (unpublished) regarding optimal conditions for different species/life history stages, as well as communicating with other nursery practitioners if they had more experience growing particular species/life history stages of corals. All ceramic tiles/plugs (3 cm on a side/diameter) were labeled with unique identification codes that were recorded in a spreadsheet to allow for tracking individual survivorship and parentage.

Coral survivorship was assessed throughout the project duration by recording the identity and timing of individual fragment mortality. We maintained records of coral health including changes in coloration, bleaching, or other observations per standard operating procedures (Bright et al., 2024).

2.2.3. Land-based gene banking and broodstock

Collection of COOs also allowed us to conduct additional spawning observations of urban corals in a land-based aquaculture system at the University of Miami Rosenstiel School (UM Hatchery). COOs were generally collected 2–3 months prior to the beginning of the species' respective spawning seasons, and were maintained in flow-through quarantine as described previously. As high mortality rates in microfragments were observed following the summer 2024 spawning season, we transitioned to keeping larger colony fragments of each COO, not only to reduce potential colony mortality, but also to facilitate land-based

spawning observations and cross-institute transfers for gene banking. Broodstock fragments of at least 10 cm in diameter from the center of the colony were targeted to maximize the chance of gamete release during spawning season.
During peak spawning windows, land-based spawning observations were conducted at the same time and fashion as field-based observations described in section 2.1.1, as personnel availability permitted. In addition to in-person observations, we deployed time lapse GoPro cameras as available to conduct photo time lapses in the flow-through aquarium systems. A combination of GoPro HERO11 cameras with experimental firmware and infrared GoPro HERO6 cameras were used, with external battery packs for both setups (see section 2.1.1 for full details).

Finally, starting in spring 2025, we placed *Diploria labyrinthiformis* broodstock fragments into individual buckets in the flow-through aquarium system. Each bucket maintained fresh seawater flow with the surrounding aquarium raceway via fine mesh-screened holes, as well as supplemental seawater sources with manifolds and tubing (Figure 6). This setup allowed us to capture any potential gametes released during spawning without requiring constant personnel supervision, but at the same time maintaining suitable husbandry conditions.

2.2.4. Cross-agency collaborations

Throughout the project, we developed collaborations to transfer COOs in the form of microfragments and larger broodstock fragments. The primary goal of this task was to preserve genetic diversity of urban coral genotypes across as many facilities as possible, and to expand the network of urban coral restoration and land-based spawning. To date, we have transferred urban coral fragments to FWC (Lindsay Spiers) and NSU (Joana Figueiredo) in support of land-based propagation efforts and spawning observations. Corals were transported by van in insulated coolers containing minimal seawater, with fragments wrapped in bubble wrap according to parent colony identity. Care was taken to minimize transport time and coral stress, with standard acclimation and quarantine periods as previously described in section 2.2.2.

2.3. Create histopathology disease identifier GitHub

This task is cross-listed with the project led by Lauren Fuess at Texas State University (FDEP PO C1E0A5). Quantitative histopathology data were obtained for SCTLD-affected coral samples collected from Florida, the U.S. Caribbean, and Gulf of America. These data had already been collected and analyzed as part of the associated project, and were uploaded into a GitHub repository for future comparative analyses.

Disease measurements were done using the same methodology as previous studies (Meiling et al., 2021; Studivan et al., 2022b, 2022a; Beavers et al., 2023; Rossin et al., 2025 in review) to compare tissue signs between coral diseases. Based on the histopathology data collected as part of the associated project, analysis of SCTLD in Dry

Tortugas National Park was compared to SCTLD in St. Thomas and St. Croix, U.S. Virgin Islands, white plague from St. Thomas and St. John, U.S. Virgin Islands, and the unknown disease outbreak in Flower Garden Banks National Marine Sanctuary.

3. RESULTS

3.1. Establish spawning windows for wild populations of urban corals

3.1.1. Field-based spawning observations and sampling

During summer 2024 and spring 2025 spawning seasons, we conducted 17 field operations with 4 daytime SCUBA and 13 night snorkel days. In total, gamete collection tents were deployed nightly during the respective peak spawning windows on 10 colonies each for the species CNAT, DLAB, OFAV, and PSTR. During these same date ranges, 15 colonies were monitored for spawning activity using time lapse cameras throughout the night: in summer 2024, 3 CNAT, 2 OFAV, and 3 PSTR were monitored; in spring 2025, 7 DLAB were monitored (Table 2). In summer 2024, there were 3 per species for time lapses using GoPro HERO11 cameras with experimental firmware (except for OFAV which had 2), and 1 per species for infrared GoPro HERO6 time lapses. In spring 2025, there were 5 GoPro HERO11 cameras with experimental firmware and 2 infrared GoPro HERO6 cameras deployed for DLAB. As of the completion of the May 2025 spawning window, a total of 58,834 photographs were taken during field deployments between summer 2024 and spring 2025 with the two time lapse camera approaches. Average field-based camera time lapses were 283 photos per deployment, or 7.1 hours. Despite these efforts, no spawning activity of wild urban corals was observed for any species over the course of the project.

3.1.2. Fecundity analyses

Initial assessments of urban coral fecundity were conducted for summer 2023 and spring 2024 samples collected in the Port of Miami: CNAT (n = 10 pre/post), PCLI (n = 10 pre/post), and PSTR (n = 11 pre/post) in summer 2023, and DLAB (n = 10 pre/post) in spring 2024. During this project, OFAV samples (n = 11 pre, 10 post) were collected from the MacArthur Causeway North site in the Port of Miami (Figure 1) in summer 2024, and CNAT (n = 3 pre/post), OFAV (n = 1 pre/post), PCLI (n = 7 pre, 3 post), and PSTR (n = 4 pre, 2 post) samples were collected from Peanut Island in the Port of Palm Beach (26.771530°, -80.044210°; Figure 7) in summer 2024. In total, 127 samples were collected for fecundity analyses (Table 3), though only summer 2023 and spring 2024 data have been analyzed in FY25. Remaining samples will be analyzed in FY26.

Pre-spawning samples from all species had oocytes and spermatocysts with no signs of oosorption (i.e., sign of coral reabsorbing gametes to redistribute energy to biological processes; Rossin et al., 2019). Post-spawning samples had empty mesenteries, also with no signs of oosorption, indicating gamete bundles had been released from all species (Figure 8). To assess gamete viability, we measured oocyte diameter and fecundity, and assessed spermatocyst development for all species (CNAT, DLAB, PCLI, and PSTR).

Average oocyte diameter for CNAT was 158.84 μm, DLAB was 249.58 μm, PCLI was 284.21 μm, and PSTR was 226.64 μm (Figure 9). Average fecundity per cm^2 for CNAT was 0.00154, DLAB was 0.0238, PCLI was 0.00611, and PSTR was 0.00141 (Table 4; Figure 10). Spermatocysts from all four developmental stages were seen in the pre-spawning cores.

3.2. Conduct fragmentation and rearing of corals of opportunity (COOs) from urbanized and marginal habitats

3.3. Field-based coral of opportunity collections

A total of 31 corals of opportunity (COOs) were collected from the MacArthur Causeway North site in the Port of Miami (Figure 1): 13 (3 CNAT, 3 OFAV, and 7 PSTR) in July 2024, 12 (8 CNAT, 1 OFAV, 1 PCLI and 2 DLAB) in October 2024, and 7 DLAB in February 2025. The majority of these colonies were dislodged or loose, suffering from partial mortality due to sedimentation, or a combination of both.

3.4. Land-based fragmentation and propagation

Seventeen of the 31 colonies (4 CNAT, 2 DLAB, 6 OFAV, 1 PCLI, and 4 PSTR) were fragmented into 270 microfragments and 42 larger broodstock fragments. Survivorship among all fragment sizes was low due to consistent water quality issues, particularly resulting in rapid tissue loss via ciliate infection of the microfragments. Combined average survivorship across all coral fragments was 47%: 50% for CNAT, 100% for DLAB, 44% for OFAV, 0% for PCLI, and 38% for PSTR (Figure 11). Of this, average survivorship of microfragments was 40%: 41% for CNAT, 100% for DLAB, 32% for OFAV, 0% for PCLI, and 25% for PSTR (Table 5; Figure 12). Survivorship of broodstock fragments was higher but still relatively low: 57%: 91% for CNAT, 100% for DLAB, 58% for OFAV, 0% for PCLI, and 33% for PSTR (Table 6; Figure 13). DLAB colonies collected in February 2025 have not yet been fragmented since the spawning season is not over until late June, however, we may not fragment them in order to preserve them as larger broodstock for future cross-institute transfers (see section 3.6 below).

3.5. Land-based gene banking and broodstock

We monitored 21 broodstock fragments (12 CNAT, 7 OFAV, and 3 PSTR) through the summer 2024 spawning season in land-based aquarium systems at the UM Hatchery (Table 7). A total of 8,641 photos were taken during land-based deployments of the time lapse cameras in summer 2024, however, no observations of spawning were noted for CNAT, OFAV, and PSTR. Average land-based camera time lapses were 31 photos per deployment, or 0.78 hours. In spring 2025, 8 DLAB colonies were monitored in the passive gamete collection bucket system (see section 2.2.3 for full details). No field spawning was reported for April (see section 3.1.1), however, one DLAB released gamete bundles on the 11th day after the full moon (AFM) on April 23, 2025. Approximately 10 mL of gamete bundles were collected at 1923 local time, while Miami reef DLAB spawned on the same day in a

land-based system at 1720. Despite a >2-hr delay between reef and urban coral spawning, we were able to cross gametes between two parents (1 urban x 1 reef), and successful fertilization was observed. Larvae were moved to kreisels in a flow-through aquarium system at the University of Miami CIMAS/NOAA-SEFSC approximately 18 hr after spawning, and settlement on tile substrates began to occur after ~36 hr (Figure 14). Since initial settlement, approximately 2,676 recruits have survived at the time of writing this report.

3.6. Cross-agency collaborations

A total of 43 urban coral microfragments (16 CNAT, 8 DLAB, 15 PSTR, and 4 OFAV) and 1 OFAV broodstock fragment were transferred to FWC in December 2024. A total of 12 broodstock fragments (9 CNAT, 1 DLAB, and 2 PSTR) were transferred to NSU in December 2024 (Table 8). To date, none of the transferred broodstock fragments have been reported to have spawned during their respective peak spawning windows. Additional coral transfers are anticipated to occur in summer and fall 2025 to the Reef Institute (Leneita Fix) and Mote Marine Laboratory (Erinn Muller; Table 8), with a specific emphasis on larger broodstock fragments for land-based spawning and sexual reproduction programs.

4. DISCUSSION

4.1. We have established mis-timing of spawning between urban and reef corals

From summer 2023 to present, we have not observed spawning of urban corals from the Port of Miami in their natural habitat. However, we have strong evidence to suggest that these urban corals are both producing viable gametes (see also section 4.2) as well as releasing them sometime during the overall spawning season based on histological analysis. We also have further evidence that urban corals can spawn in a land-based aquaculture system with little modifications to husbandry conditions beyond removal of light pollution that is common in the Port of Miami. While this is limited to a single colony at this time, perhaps additional time in a land-based system that mimics natural reef conditions will induce further spawning activity in urban corals during predicted windows established for reef corals.

Throughout Florida and the Caribbean, there are well-documented spawning windows for scleractinian corals on natural reef environments. There is, however, a dearth of knowledge regarding spawning cycles for corals from urbanized habitats in Florida. Studies conducted in other urbanized environments globally have identified impacts of urbanization on natural coral processes, particularly when artificial light pollution is involved. For example, in the Gulf of Aqaba, light pollution has been shown to alter coral transcriptomic patterns, symbiont and microbiome communities, seasonal physiology, and gametogenic cycles (Rosenberg et al., 2022). In a lab-based experiment with Indo-Pacific acroporids, exposure to artificial light at night (ALAN) resulted in delayed gametogenesis and a breakdown in gamete release synchronization among colonies (Ayalon et al., 2021). Indeed, a meta-analysis of global spawning observations from 2000-2019 revealed significantly early

release of gametes by approximately 1–3 days in corals exposed to ALAN (Davies et al., 2023). This has profound implications for spawning of urban corals on two fronts: 1) gametogenesis cycles can be disrupted by ALAN commonly found in urbanized habitats, resulting in potential poor fitness, fertilization rates, and larval survival, and 2) gamete release may be unsynchronized between urban and reef corals, causing a breakdown in gene flow among populations.

The extension of our spawning observations through time lapse camera technologies suggests that urban corals in the Port of Miami are not spawning within hours (or possibly even days to weeks) of their reef counterparts. Even under 'natural' conditions in the land-based aquaculture facility at the UM Hatchery, the one DLAB colony that spawned released its gametes over 2 hr after reef conspecifics in a similar setting. Successful fertilization and settlement was observed despite this relatively long time gap (particularly for DLAB, which develops more rapidly than most other scleractinians), but once again, this was an isolated observation and may not be applicable to all Port of Miami urban corals. This asynchronous timing of gamete release may result in a lack of gene flow among these populations, though this needs to be confirmed with population genetics approaches. These efforts are underway as part of a leveraged, NOAA 'Omics-funded project, and we expect to have results in the upcoming year.

While the time lapse GoPro cameras greatly increased the amount of time we were able to observe urban corals in the field, there are several limitations of these platforms. Despite using experimental firmware designed to prolong battery life and external battery packs, deployment of the GoPro HERO11 cameras was consistently problematic. A full 12-hr time lapse was rarely attained for unexplained reasons, but was likely related to unequal battery consumption rates among individual cameras. Even when a particular camera successfully captured photos throughout the night, their advertised "enhanced low light capability" did not live up to expectations underwater. Most photos taken during the night appeared totally black, even in the presence of ALAN. While the infrared GoPro HERO6 cameras with infrared emitters fared much better in terms of image quality (Figure 4), battery life was still inconsistent, and these cameras rarely lasted until morning. Battery life of the infrared emitters (2 D-cell rechargeable batteries wired in parallel) was similarly short. Finally, one infrared GoPro HERO6 and its infrared emitter were stolen from the MacArthur Causeway North site during the May DLAB spawning window. Based on these experiences, we have concluded that GoPro camera systems are not appropriate for the needs of this project.

Fortunately, we have identified a low-cost, purpose-built alternative that we are pursuing for the FY26 project cycle. The KiloCam platform was initially designed to be a low-cost, efficient time lapse camera system with incredibly long deployment times (weeks to months), due to its cost-effective, low power-consumption components and custom-coded circuit boards (Besson et al., 2022; Boulais et al., 2023). This system was designed by Ecologis Consulting, and has been further refined as a spawning camera platform (https://www.ecologisconsulting.com/custom-camera-systems) that is specifically designed for no-light, in-water photography of coral spawn. These cameras can be

deployed for 2–3 days at a time and are pre-programmed to take photos with a supplemental light source at a customized interval and quantity. This will greatly increase our spawning observations through longer and more consistent deployments, and decrease the personnel hours and field costs required for deployments. The system is also built from relatively inexpensive and easily procured components, as well as 3D printed mounts and brackets to allow easy and cost-effective maintenance.

4.2. Urban corals produce viable gametes at or above average sizes established for Caribbean reef corals

Oocyte diameter and stage are standard metrics to quantify gamete viability and to predict the time to spawning (Szmant, 1986). Prior to this study, there were no records of oocyte diameter for urban corals in Florida or the Caribbean. Gamete production is an area of research that demands more attention, particularly considering the overall failure of coral reproduction and recruitment across Florida and the Caribbean (Holstein et al., 2015). Most tropical coral reproduction studies are from Puerto Rico, the U.S. Virgin Islands, and Mexico. We found that urban corals in the Port of Miami are producing oocytes similar in size to those among Caribbean reefs (Alvarado Ch et al., 2004; Weil and Vargas, 2010; Graham and van Woesik, 2013; Holstein et al., 2015; Bloomberg and Holstein, 2021). Under sustained periods of stress, however, corals can also reabsorb their eggs to transfer the lipids, break them down, and redistribute to life-sustaining processes. Oosorption is apparent in corals as well-defined egg structures (Figure 8) to speckled oocytes where the lipids are condensing to be packaged and the outer lining breaks down (Rossin et al., 2019). No oosorption was seen in the tissue samples from these colonies. This was surprising as the pre-spawning cores were collected in August 2023 during the fourth global bleaching event (Hoegh-Guldberg et al., 2023). Taken together, these lines of evidence indicate that despite the harsh environmental conditions observed in the Port of Miami (Enochs et al., 2023), these corals are able to expend energy to produce gametes, possibly due to enhanced heterotrophic feeding (Rubin et al., 2021; Macartney et al., in prep).

4.3. Challenges exist for land-based propagation of corals from urban environments

The majority of corals of opportunity (COOs) collected from the Port of Miami for this project were in imminent risk of death due to sedimentation/smothering or dislodgement. Interestingly, several of the collected colonies displayed old signs of mortality, and appeared as lobed areas of skeleton and tissue, suggestive of repeated partial mortality events due to burial. While our efforts ultimately preserved these genotypes which would have otherwise been lost, we suspect that initial signs of stress may have contributed to mortality once they were in our land-based aquaculture systems. First, recent mortality due to sedimentation and partial burial manifested as stark lines of tissue loss. It is therefore likely that many of the COOs entered the land-based system at a severe level of stress, with potential ramifications for wound healing and immune processes. Second, much of the sediment surrounding these colonies in their native urban habitat is extremely fine, and showed signs of anoxia. While efforts were undertaken to remove these sediments prior to the corals' transfer into the land-based systems, it is possible that potentially harmful

microbes and/or pathogens may have been retained on the colonies. Proliferation of such microbes in the aquaculture system may have then resulted in the observed tissue loss, and in some cases, colony mortality.

Aside from their source environment, additional considerations may be needed for particular corals regarding their life history characteristics and adaptability to land-based systems. For example, fragments regardless of size (microfragments versus larger broodstock fragments) of the brain coral species CNAT, PCLI and PSTR experienced the most mortality in this project, relative to the star coral OFAV. These species were largely affected by ciliate infections commonly referred to as 'brown jelly syndrome,' which progresses rapidly and often results in total mortality despite repeated treatments and amputations. Once ciliates are exposed to a tank system, it is incredibly difficult to remove them, even with complete transfers of all corals to a new, clean system. As a result, quarantine systems were prone to ciliates 'leap-frogging' among colonies, and there were limited additional systems available for quarantining individual colonies.

In order to accommodate biological differences among coral species, and potential unique challenges of urbanized habitats on propagation success of urban corals, advancements to land-based aquaculture systems are needed to handle the scale of coral restoration proposed by 2050 in Florida (Florida's Coral Reef Restoration and Recovery Initiative). This includes greatly increased tank space, improvements to water quality controls, and further refinement of husbandry practices based on past experiences. Many of these advancements are already underway at Florida-based coral aquaculture facilities, including the UM Hatchery where land-based restoration occurred for this project. It is likely that corals from stressful environments such as urbanized habitats in the Port of Miami will require extra care and/or treatments relative to natural reef corals in order to become efficient and cost-effective sources of biomass for restoration pipelines.

4.4. Histopathology identifies consistency among disease signs across regions

Preliminary analyses indicate the disease seen at Dry Tortugas was consistent with SCTLD. Vacuolization of the symbiosome (increase in size of the coral vacuole containing Symbiodiniaceae cells relative to the algal cells themselves) is a predictive disease sign for SCTLD-affected colonies (Meiling et al., 2021; Studivan et al., 2022b, 2022a; Beavers et al., 2023; Rossin et al., 2025); indeed, disease-affected corals in Dry Tortugas exhibited higher rates of vacuolization. Whereas diseased corals also typically demonstrate higher proportion of exocytosis (ejection of resident Symbiodiniaceae in their symbiosomes), we observed healthy samples from Dry Tortugas to have higher rates of exocytosis than diseased samples, although this could be indicative of a higher symbiont turnover in health corals. An open-source GitHub repository (https://github.com/ashleyrossin/dry_tortugas) was created for continued use of the two metrics (vacuolization and exocytosis) to facilitate comparisons among other regions and disease outbreaks, particularly white plague disease.

4.5. Management recommendations

While we have not observed spawning of urban corals in the Port of Miami in their native habitats, evidence of their viable gamete development and release, as well as successful spawning, fertilization, and settlement of an urban x reef DLAB cross gives us promise that urban corals remain a valuable resource for restoration efforts. Field observations of spawning in wild urban corals remains one of our top priorities, as it is critical to their full-scale incorporation in restoration pipelines. Confirmation of spawning activity and timing may not only indicate that urban coral populations may be self-sustaining from a recruitment perspective, but in combination with the leveraged population genetics analyses that are ongoing, will resolve patterns of gene flow from urban to reef populations, and vice versa. Land-based spawning observations and larval crosses among urban and reef parents also represent a valuable opportunity to better understand the biological processes contributing to the resilience of urban corals, and perhaps provide a means of maintaining and increasing genetic diversity of restoration stocks beyond conventional asexual reproduction through fragmentation. This is particularly important as urban habitats likely contain resilient genotypes, owing to their observed proliferation in adverse conditions such as those found in commercial ports in Florida. Further, if urban parental genotypes confer resilience to environmental stressors, this knowledge could be operationalized into informed parental crosses with reef corals. Especially as coastal construction and urbanization increases in Florida, more urban coral populations may be at risk of destruction, and therefore should be prioritized for conservation and restoration initiatives.

Given their remarkable resilience and resistance to a host of stressors, incorporating corals from marginal and extreme habitats like commercial ports into live gene banks should be a high priority. Similar to ongoing efforts for corals sourced from reef habitats, representative fragments of these corals should be spread across multiple live gene banks to ensure these valuable genotypes are not lost in the event that parent colonies die at their native sites. Incorporating these corals into coral production pipelines for restoration can also support outplanting efforts, allowing the introduction of local resilient genotypes to nearby reefs with diminished populations.

Beyond their reproductive capabilities and potential for supplementing land-based asexual and sexual reproduction efforts, urban corals represent a critical resource for the future of Florida's Coral Reef. Their observed resilience despite constant exposure to adverse conditions is not yet fully understood. Continued research into their mechanisms of resilience, particularly at a genetic level, will help us better understand what processes enhance (or conversely, impede) stress tolerance in corals. With this knowledge, we could incorporate stress-hardening techniques in land-based aquaculture systems in order to increase resilience of outplanted coral fragments, such as repeated exposures to sublethal environmental stressors or supplemental feeding to enhance growth (Ladd and Enochs, 2024). Combined, these strategies will increase the success and efficiency of coral propagation and restoration efforts, while simultaneously reducing cost and time investment, and ultimately promoting the recovery of Florida's Coral Reef.

5. REFERENCES

Alvarado Ch, E. M., García U, R., and Acosta, A. (2004). Sexual reproduction of the reef-building coral *Diploria labyrinthiformis* (Scleractinia: Faviidae), in the Colombian Caribbean. *Revista de biología tropical* 52, 859–868.

Ayalon, I., Rosenberg, Y., Benichou, J. I. C., Campos, C. L. D., Sayco, S. L. G., Nada, M. A. L., et al. (2021). Coral gametogenesis collapse under artificial light pollution. *Current Biology* 31, 413-419.e3. doi: 10.1016/j.cub.2020.10.039

Beavers, K. M., Van Buren, E. W., Rossin, A. M., Emery, M. A., Veglia, A. J., Karrick, C. E., et al. (2023). Stony coral tissue loss disease induces transcriptional signatures of in situ degradation of dysfunctional Symbiodiniaceae. *Nat Commun* 14, 2915. doi: 10.1038/s41467-023-38612-4

Besson, M., Alison, J., Bjerge, K., Gorochowski, T. E., Høye, T. T., Jucker, T., et al. (2022). Towards the fully automated monitoring of ecological communities. *Ecology Letters* 25, 2753–2775. doi: 10.1111/ele.14123

Bloomberg, J., and Holstein, D. M. (2021). Mesophotic coral refuges following multiple disturbances. *Coral Reefs* 40, 821–834. doi: 10.1007/s00338-021-02087-w

Boulais, O., Schar, D., Levy, J., Kim, K., Levy, N., Reichert, J., et al. (2023). A demonstration of acoustic enrichment in Hawai'i using autonomous cameras for reef fish larval detection. *Proc. Mtgs. Acoust.* 52. doi: 10.1121/2.0001870

Bright, A. J., Williams, D. E., Grazioso, K. N., Ippolito, S., Studivan, M. S., and Ladd, M. C. (2024). Quicklook Report. Coral Spawning 2023: Activities and Observations. NOAA Southeast Fisheries Science Center, Miami, FL. 15 pp.

Davies, T. W., Levy, O., Tidau, S., de Barros Marangoni, L. F., Wiedenmann, J., D'Angelo, C., et al. (2023). Global disruption of coral broadcast spawning associated with artificial light at night. *Nat Commun* 14, 2511. doi: 10.1038/s41467-023-38070-y

Enochs, I. C., Studivan, M. S., Kolodziej, G., Foord, C., Basden, I., Boyd, A., et al. (2023). Coral persistence despite marginal conditions in the Port of Miami. *Scientific Reports* 13, 6759. doi: 10.1038/s41598-023-33467-7

FishLore (2013). Saltwater Aquarium & Reef Tank Book, FishLore.com. 628 pp. Available at: https://www.fishlore.com/saltwater-aquarium-reef-tank-book.pdf (Accessed May 29, 2025).

Graham, J. E., and van Woesik, R. (2013). The effects of partial mortality on the fecundity of three common Caribbean corals. *Mar Biol* 160, 2561–2565. doi: 10.1007/s00227-013-2248-y

Hoegh-Guldberg, O., Skirving, W., Dove, S. G., Spady, B. L., Norrie, A., Geiger, E. F., et al. (2023). Coral reefs in peril in a record-breaking year. *Science* 382, 1238–1240. doi: 10.1126/science.adk4532

Holmes-Farley, R. (2004). Reef Alchemy: Reef Aquarium Water Parameters. Website. Available at: https://reefkeeping.com/issues/2004-05/rhf/index.php (Accessed May 29, 2025).

Holstein, D. M., Smith, T. B., Gyory, J., and Paris, C. B. (2015). Fertile fathoms: deep reproductive refugia for threatened shallow corals. *Scientific Reports* 5, 12407. doi: 10.1038/srep12407

Ing, L. (2024). Can Miami Corals Beat the Heat? Insights into the Algal Symbiont Assemblages of Highly Resilient 'Urban Corals' in Southeast Florida. Undergraduate Honors Thesis. Smith College, Northampton, MA. 91 pp. Available at: https://scholarworks.smith.edu/theses/2616

Ladd, M. C., and Enochs, I. C. (2024). A coral production pipeline to achieve effective reef restoration at scale in Florida (abstract). Ocean Sciences Meeting 2024, New Orleans, LA.

Leinbach, S. E., Speare, K. E., Rossin, A. M., Holstein, D. M., and Strader, M. E. (2021). Energetic and reproductive costs of coral recovery in divergent bleaching responses. *Sci Rep* 11, 23546. doi: 10.1038/s41598-021-02807-w

Macartney, K. J., Studivan, M. S., Karp, R. F., Baker, A. C., Hicks, D., and Enochs, I. C. (in prep). Eat to live: the increasingly important role of heterotrophic inputs in urban coral persistence.

Meiling, S. S., Muller, E. M., Lasseigne, D., Rossin, A., Veglia, A. J., MacKnight, N., et al. (2021). Variable species responses to experimental stony coral tissue loss disease (SCTLD) exposure. *Frontiers in Marine Science* 8, 670829. doi: 10.3389/fmars.2021.670829

Papke, E., Carreiro, A., Dennison, C., Deutsch, J. M., Isma, L. M., Meiling, S. S., et al. (2024). Stony coral tissue loss disease: a review of emergence, impacts, etiology, diagnostics, and intervention. *Frontiers in Marine Science* 10, 1321271.

Rosenberg, Y., Simon Blecher, N., Lalzar, M., Yam, R., Shemesh, A., Alon, S., et al. (2022). Urbanization comprehensively impairs biological rhythms in coral holobionts. *Global Change Biology*. doi: 10.1111/GCB.16144

Rossin, A. M., Beavers, K. M., Bloomberg, J., Carpenter, G. E., Farmer, B. H., Green, B., et al. (2025). Runaway coral-algal dysbiosis may be responsible for rapid coral tissue loss. *in review*.

Rossin, A. M., Coleman, M., Coleman, G., Ippolito, S., Kolodzeij, G., Gill, T., et al. (2025). Sex on the beach: mistiming of coral spawning in urbanized habitats in the Port of Miami. *in prep*.

Rossin, A. M., Waller, R. G., and Stone, R. P. (2019). The effects of in-vitro pH decrease on the gametogenesis of the red tree coral, *Primnoa pacifica*. *PLOS ONE* 14, e0203976. doi: 10.1371/journal.pone.0203976

Rubin, E. T., Enochs, I. C., Foord, C., Mayfield, A. B., Kolodziej, G., Basden, I., et al. (2021). Molecular mechanisms of coral persistence within highly urbanized locations in the Port of Miami, Florida. *Frontiers in Marine Science* 8, 695236.

Sprung, J., and Delbeek, J. C. (1997). *The Reef Aquarium: A Comprehensive Guide to the Identification and Care of Tropical Marine Invertebrates. Vol. 2*. Two Little Fishies Inc., Miami Gardens, FL. pp 544.

Studivan, M. S., Baptist, M., Molina, V., Riley, S., First, M., Soderberg, N., et al. (2022a). Transmission of stony coral tissue loss disease (SCTLD) in simulated ballast water confirms the potential for ship-born spread. *Sci Rep* 12, 19248. doi: 10.1038/s41598-022-21868-z

Studivan, M. S., and Enochs, I. C. (2022). Final Report: Urban coral resilience and relocation. National Marine Fisheries Service, Miami, FL. pp. 19.

Studivan, M. S., Rossin, A. M., Rubin, E., Soderberg, N., Holstein, D. M., and Enochs, I. C. (2022b). Reef sediments can act as a stony coral tissue loss disease vector. *Frontiers in Marine Science* 8, 815698. doi: 10.3389/fmars.2021.815698

Szmant, A. M. (1986). Reproductive ecology of Caribbean reef corals. *Coral Reefs* 5, 43–53. doi: 10.1007/BF00302170

Weil, E., and Vargas, W. L. (2010). Comparative aspects of sexual reproduction in the Caribbean coral genus *Diploria* (Scleractinia: Faviidae). *Mar Biol* 157, 413–426. doi: 10.1007/s00227-009-1328-5

6. TABLES

Table 1. Predicted spawning windows for project priority species, modified from CARMABI's coral spawning calendar by NOAA-SEFSC. AFM = days after full moon, BS = minutes before sunset, AS = minutes after sunset.

Species	**Month 1**	**Month 1**	**Month 3**
Colpophyllia natans	August 6-8 days AFM 35-110 min AS	September 6-8 days AFM 35-110 min AS	October (possible) 6-8 days AFM 35-110 min AS
Diploria labyrinthiformis	April 9-11 days AFM 70 BS-10 min AS	May 9-11 days AFM 70 BS-10 min AS	June (possible) 9-11 days AFM 70 BS-10 min AS
Orbicella faveolata	July 6-7 days AFM 285-370 min AS	August 6-7 days AFM 285-370 min AS	September 6-7 days AFM 285-370 min AS
Pseudodiploria clivosa	August 7-8 days AFM 210-255 and 280-350 min AS	September 7-8 days AFM 210-255 and 280-350 min AS	October (possible) 7-8 days AFM 210-255 and 280-350 min AS
Pseudodiploria strigosa	August 6-8 days AFM 30-70 and 220-270 min AS	September 6-8 days AFM 30-70 and 220-270 min AS	October (possible) 6-8 days AFM 30-70 and 220-270 min AS

Alt text: Table showing predicted peak spawning windows for five coral species based on lunar timing and local sunset times, adapted from NOAA-SEFSC and CARMABI. Columns include species name and spawning windows across up to three months. Timing is given as days after the full moon (AFM) and minutes before or after sunset (BS or AS). For example, *Colpophyllia natans* is predicted to spawn 6–8 days after the full moon and 35–110 minutes after sunset from August through October. *Diploria labyrinthiformis* spawns 9–11 days AFM, between 70 minutes before and 10 minutes after sunset, from April to June. *Orbicella faveolata* spawns July through September, 6–7 days AFM, 285–370 minutes AS. *Pseudodiploria clivosa* and *P. strigosa* have dual spawning windows in August through October, with overlapping ranges of minutes AS.

Table 2. Field-based spawning observations. Date ranges indicate monitoring windows. Timestamps correspond to time lapse camera deployments and brackets indicate gamete collection tent deployments.

Species	**Month 1**	**Month 1**	**Month 3**
Colpophyllia natans	August 25-27 1950 [2020-2135]	September 23-25 1950 [2020-2135]	Field operations not possible due to inclement weather
Diploria labyrinthiformis	April 21-23 1845 [1800-2000]	May 21-23 1845 [1800-2000]	June 20-22 (anticipated)
Orbicella faveolata	July 27-28 2200 [2315-0000]	August 25-26 2200 [2315-0000]	September 23-24 2200 [2315-0000]
Pseudodiploria clivosa	Colonies not tented, observations noted during snorkel	Colonies not tented, observations noted during snorkel	Colonies not tented, observations noted during snorkel
Pseudodiploria strigosa	August 25-27 1950 [2020-0015]	September 23-25 1950 [2020-0015]	Field operations not possible due to inclement weather

Alt text: Table summarizing field-based coral spawning observations for five species across three lunar months. Columns include monitoring date ranges, time lapse camera deployment timestamps, and gamete collection tent deployment times (in brackets) across three lunar months for each species. For *Colpophyllia natans*, spawning was observed on August 25–27 and September 23–25, with cameras deployed at 1950 and tenting from 2020–2135; October observations were canceled due to weather. *Diploria labyrinthiformis* was observed April 21–23 and May 21–23 (camera: 1845; tent: 1800–2000), with June anticipated. *Orbicella faveolata* was observed July–September (camera: 2200; tent: 2315–0000). *Pseudodiploria clivosa* was noted visually during snorkel surveys only. *Pseudodiploria strigosa* was monitored in August and September with camera and tent times matching *C. natans*; October observations were canceled due to weather.

Table 3. Coral sample metadata of tissue cores collected for fecundity analysis.

Site	Season	Species	Pre-spawning	Post-spawning	Status
MacArthur Causeway North	Summer 2023	*Colpophyllia natans*	10	10	Processed; analyzed in FY25
MacArthur Causeway North	Spring 2024	*Diploria labyrinthiformis*	10	10	Processed; analyzed in FY25
MacArthur Causeway North	Summer 2024	*Orbicella faveolata*	11	10	Processed; analysis to be completed in FY26
MacArthur Causeway North	Summer 2023	*Pseudodiploria clivosa*	10	10	Processed; analyzed in FY25
MacArthur Causeway North	Summer 2023	*Pseudodiploria strigosa*	11	11	Processed; analyzed in FY25
Peanut Island	Summer 2024	*Colpophyllia natans*	3	3	Processed; analysis to be completed in FY26
Peanut Island	Summer 2024	*Orbicella faveolata*	1	1	Processed; analysis to be completed in FY26
Peanut Island	Summer 2024	*Pseudodiploria clivosa*	7	3	Processed; analysis to be completed in FY26
Peanut Island	Summer 2024	*Pseudodiploria strigosa*	4	2	Processed; analysis to be completed in FY26

Alt text: Table showing coral tissue core sample metadata used for fecundity analysis across two sites and multiple spawning seasons. Columns list site, season, species, number of pre- and post-spawning samples, and processing status. At MacArthur Causeway North, samples were collected across Summer 2023, Spring 2024, and Summer 2024 for five species, with 10–11 pre- and post-spawning samples per species, all processed and analyzed in FY25 or scheduled for analysis in FY26. At Peanut Island, Summer 2024 collections were made for four species, with smaller sample sizes (1–7 pre-spawning and 1–3 post-spawning), also processed with analysis scheduled in FY26.

Table 4. Mean reproductive capacity metrics. Fecundity was assessed per area versus per polyp since there are inherent anatomical differences among star and brain corals. Numbers in parentheses correspond to the proportion of coral tissue area.

Species	**Diameter (µm)**	**Fecundity (oocytes/cm^2)**	**Qualitative observations**
Colpophyllia natans	158.84	0.00154 (0.154%)	Mesenteries were full pre-spawning but empty post-spawning, with no signs of reabsorption
Diploria labyrinthiformis	249.58	0.0238 (2.38%)	Mesenteries were full pre-spawning but empty post-spawning, with no signs of reabsorption
Orbicella faveolata	Results anticipated in FY26	Results anticipated in FY26	Results anticipated in FY26
Pseudodiploria clivosa	284.21	0.00611 (0.611%)	Mesenteries were full pre-spawning but empty post-spawning, with no signs of reabsorption
Pseudodiploria strigosa	226.64	0.00141 0.141%)	Mesenteries were full pre-spawning but empty post-spawning, with no signs of reabsorption

Alt text: Table presenting mean reproductive metrics for five coral species based on tissue area, including oocyte diameter, fecundity (oocytes per cm²), and qualitative reproductive observations. *Colpophyllia natans* had a mean oocyte diameter of 158.84 µm and fecundity of 0.00154 oocytes/cm² (0.154% tissue area). *Diploria labyrinthiformis* showed higher values: 249.58 µm diameter and 0.0238 oocytes/cm² (2.38%). *Orbicella faveolata* results are pending for FY26. *Pseudodiploria clivosa* had a diameter of 284.21 µm and fecundity of 0.00611 (0.611%), and *Pseudodiploria strigosa* had 226.64 µm and 0.00141 (0.141%). In all species with data, mesenteries were full pre-spawning and empty post-spawning with no signs of reabsorption.

Table 5. Survivorship statistics for microfragments. Survivorship percentage was recorded through May 30, 2025.

Species	Colony	Starting Microfragments	Surviving Microfragments	Survivorship (%)
CNAT	1	10	0	0
CNAT	2	47	31	66
CNAT	3	10	0	0
CNAT	4	23	23	100
DLAB	1	7	7	100
DLAB	2	19	19	100
OFAV	1	3	1	33
OFAV	2	6	0	0
OFAV	3	7	0	0
OFAV	4	23	3	13
OFAV	5	18	1	6
OFAV	6	28	28	100
PCLI	1	5	0	0
PSTR	1	6	0	0
PSTR	2	7	0	0
PSTR	3	6	0	0
PSTR	4	45	45	100

Alt text: Table summarizing survivorship statistics for coral microfragments as of May 30, 2025. Columns include species, colony number, number of starting fragments, number of surviving fragments, and survivorship percentage. Several colonies had 0% survivorship, including CNAT colonies 1 and 3, OFAV colonies 2 and 3, PCLI colony 1, and PSTR colonies 1–3. OFAV colony 4 had low survivorship (13%) and colony 5 had 6%, while colony 6 was 100%. CNAT colony 2 showed 66% survivorship, while colony 4 maintained 100%. All DLAB colonies (1 and 2) had 100% survivorship. PSTR colony 4 also had 100% survivorship.

Table 6. Survivorship statistics for broodstock. Survivorship percentage was recorded through May 30, 2025.

Species	Colony	Starting Broodstock	Surviving Broodstock	Survivorship (%)
CNAT	2	10	7	70
CNAT	4	5	5	100
CNAT	5	1	0	0
CNAT	6	1	0	0
CNAT	7	1	0	0
CNAT	8	1	0	0
CNAT	9	1	0	0
CNAT	10	1	0	0
CNAT	11	1	0	0
CNAT	12	1	0	0
DLAB	2	2	2	100
DLAB	3	1	1	100
DLAB	4	1	1	100
DLAB	5	1	1	100
DLAB	6	1	1	100
DLAB	7	1	1	100
DLAB	8	1	1	100
OFAV	2	2	0	0
OFAV	4	3	1	33
OFAV	5	5	4	80
OFAV	6	5	4	80
OFAV	7	1	0	0
PCLI	1	1	0	0
PSTR	1	1	0	0
PSTR	2	1	0	0
PSTR	4	3	3	100

Alt text: Table showing survivorship statistics for coral broodstock colonies as of May 30, 2025. The table includes columns for species, colony number, number of starting fragments, number of surviving fragments, and percent survivorship. Colonies with 0% survivorship include CNAT colonies 5 through 12, OFAV colonies 2 and 7, PCLI colony 1, and PSTR colonies 1 and 2. Several DLAB colonies (2–8) and PSTR colony 4 had 100% survivorship. OFAV colonies 4, 5, and 6 showed variable survivorship (33% to 80%). CNAT colonies 2 and 4 had 70% and 100% survivorship, respectively.

Table 7. Land-based spawning observations. Date ranges indicate monitoring windows. Timestamps correspond to time lapse camera deployments and brackets indicate in-person observations. Asterisk denotes an additional window added following successful spawning the night before.

Species	**Month 1**	**Month 1**	**Month 3**
Colpophyllia natans	August 25-27 No camera deployments [2020-2135]	September 19-29 1700 September 23-25 [2020-2135]	October 21-28 1700 No in-person observations
Diploria labyrinthiformis	April 13-20 [1700-1930] April 24* [1700-2000]	May 21-23 No camera deployments [1715-2015]	June 20-22 Anticipated [1715-2015]
Orbicella faveolata	August 25-26 No camera deployments [2315-0000]	September 19-29 1700 September 23-24 [2315-0000]	October 21-28 1700 No in-person observations
Pseudodiploria clivosa	August 25-27 No camera deployments [2020-0015]	September 19-29 1700 September 23-25 [2020-0015]	October 21-28 1700 No in-person observations
Pseudodiploria strigosa	August 25-27 No camera deployments [2020-0015]	September 19-29 1700 September 23-25 [2020-0015]	October 21-28 1700 No in-person observations

Alt text: Table summarizing land-based coral spawning observations for five species across three lunar months. Columns include monitoring date ranges, time lapse camera deployment times (if any), and in-person observation periods in brackets across three lunar months for each species. An asterisk indicates extended monitoring after a successful spawning event was observed. For *Colpophyllia natans*, August and September included in-person observations (2020–2135), with cameras deployed starting September 19. October had only camera deployment at 1700. *Diploria labyrinthiformis* was observed April 13–20 and April 24 (in-person 1700–1930/2000), and again in May with in-person observations only. June monitoring is anticipated. *Orbicella faveolata* and *Pseudodiploria* spp. had August–October coverage, with in-person observations in August and September but none in October. Cameras were deployed at 1700 in September and October for all species.

Table 8. Colony metadata for cross-agency transfers of microfragments and broodstock. Corals listed as 'Reef Institute' are marked for transfer in FY26.

Species	Colony	Location	Microfragments	Broodstock
CNAT	2	UM Rosenstiel	1	0
CNAT	2	FWC	10	0
CNAT	2	NSU	0	5
CNAT	2	Reef Institute	0	2
CNAT	2	UM Rosenstiel	20	0
CNAT	4	UM Rosenstiel	1	0
CNAT	4	FWC	7	0
CNAT	4	NSU	0	4
CNAT	4	Reef Institute	0	1
CNAT	4	UM Rosenstiel	15	0
DLAB	1	FWC	2	0
DLAB	1	UM Rosenstiel	5	0
DLAB	2	FWC	6	0
DLAB	2	NSU	0	1
DLAB	2	Reef Institute	0	1
DLAB	2	UM Rosenstiel	12	0
OFAV	1	NOAA-SEFSC	1	0
OFAV	4	NOAA-SEFSC	3	1
OFAV	5	NOAA-SEFSC	1	4
OFAV	6	UM Rosenstiel	22	0
PSTR	4	UM Rosenstiel	1	0
PSTR	4	FWC	15	0
PSTR	4	NSU	0	2
PSTR	4	Reef Institute	0	1
PSTR	4	UM Rosenstiel	29	0

Alt text: Table listing partner institutions involved in the maintenance of coral broodstock and microfragments. Columns include species, colony number, location, and the number of surviving broodstock and microfragments at each site. Locations include UM Rosenstiel, FWC, NSU, Reef Institute, and NOAA-SEFSC. Additional microfragments and broodstock are expected to be transferred to the Reef Institute and Mote Marine Laboratory during FY26.

7. FIGURES

Figure 1. The MacArthur Causeway North urban coral site in the Port of Miami (Miami-Dade County), where environmental monitoring and benthic community characterization has been ongoing since 2018, and spawning observations and fecundity sampling have been conducted since 2023.

Figure 2. Left: Reef gamete collection tents designed by Dana Williams of the University of Miami CIMAS/NOAA-SEFSC. Right: Modified urban coral gamete collection tents constructed by Ashley Rossin, with an akita mix (Oskar) for scale.

Figure 3. Left: Custom QR code to start a 12-hr time lapse, taking photos every 94 sec. Right: GoPro HERO11 with external battery pack during deployment of experimental time lapse firmware.

Figure 4. Left: GoPro HERO6 with infrared lens. Right: *Pseudodiploria strigosa* colony illuminated by infrared light during time lapse deployment.

Figure 5. Left: Infrared emitter in Ikelite camera housing. Right: Infrared emitter in custom-built PVC and acrylic enclosure.

Figure 6. Passive gamete collection apparatus incorporated into land-based aquaculture system at the UM Hatchery, allowing the collection of released gametes from individual broodstock fragments while still maintaining water quality.

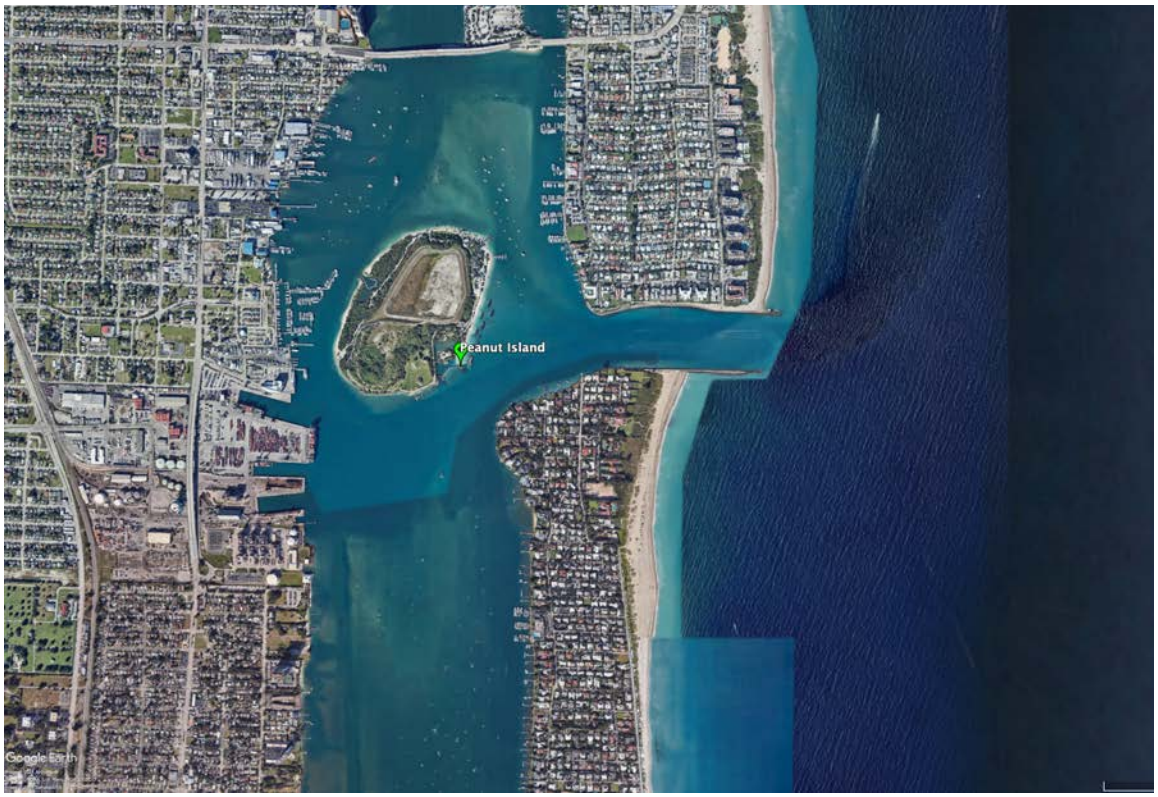

Figure 7. The Peanut Island urban coral site in the Port of Palm Beach (Palm Beach County), where benthic community characterization and spawning observations have been underway by the Reef Institute since 2022.

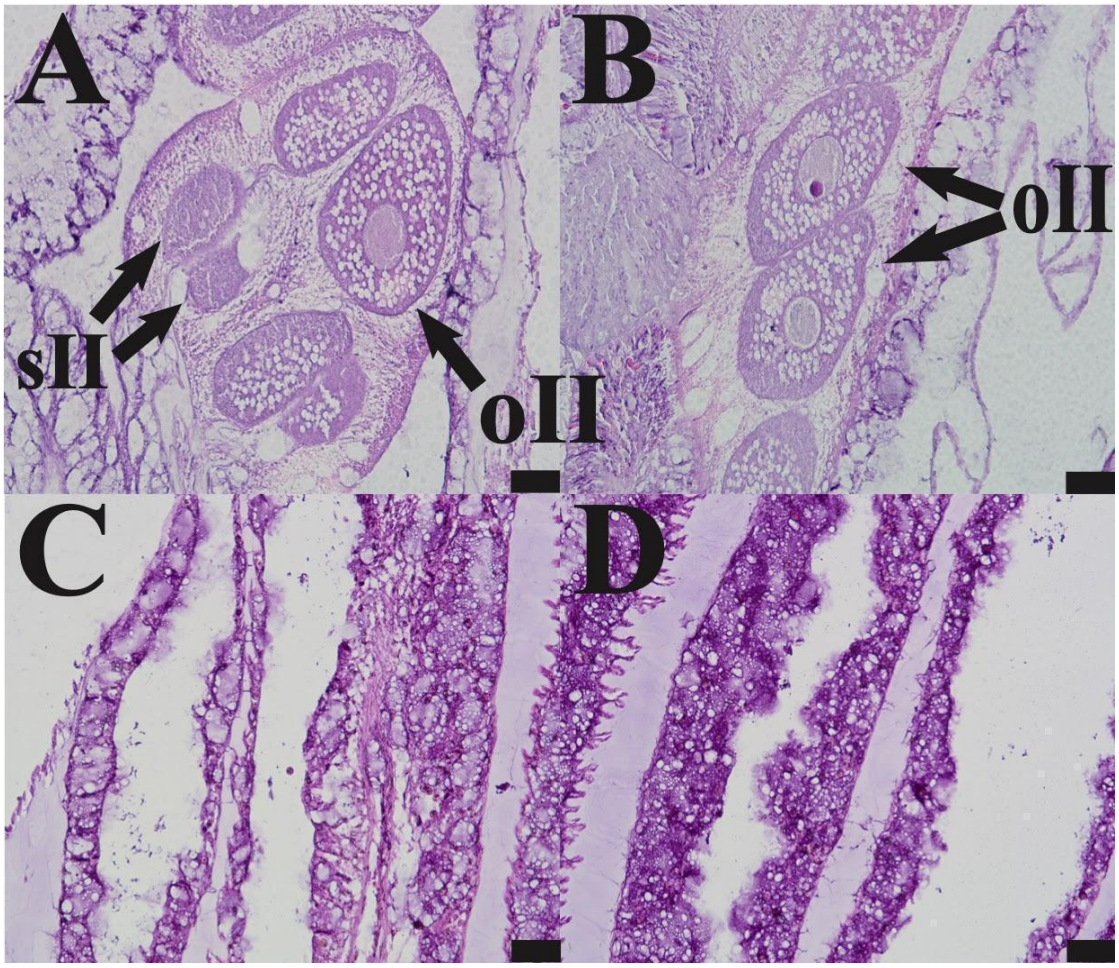

Figure 8. A & B: Histological slides of PSTR from before spawning in summer 2023; sII indicates second-stage sperm and oII indicates second-stage oocytes. C & D: PSTR post-spawning, where mesenteries no longer have gametes.

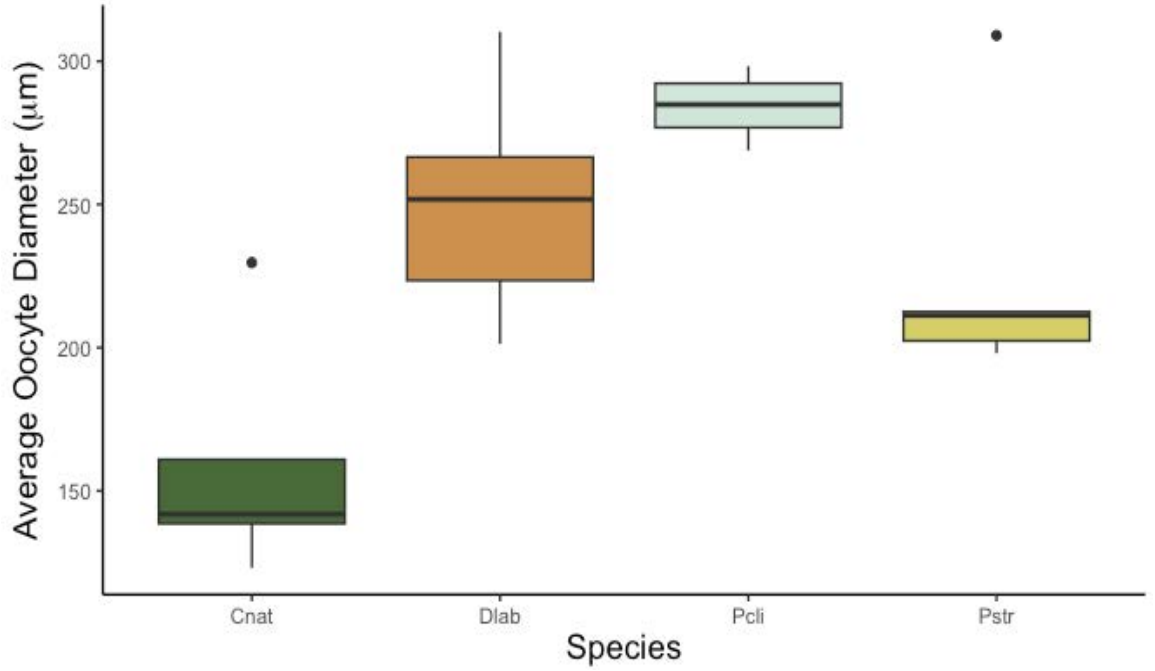

Figure 9. Oocyte diameter for CNAT, DLAB, PCLI, and PSTR from 2023 before the spawning window. Diameter reflects Feret diameter and is measured in microns.

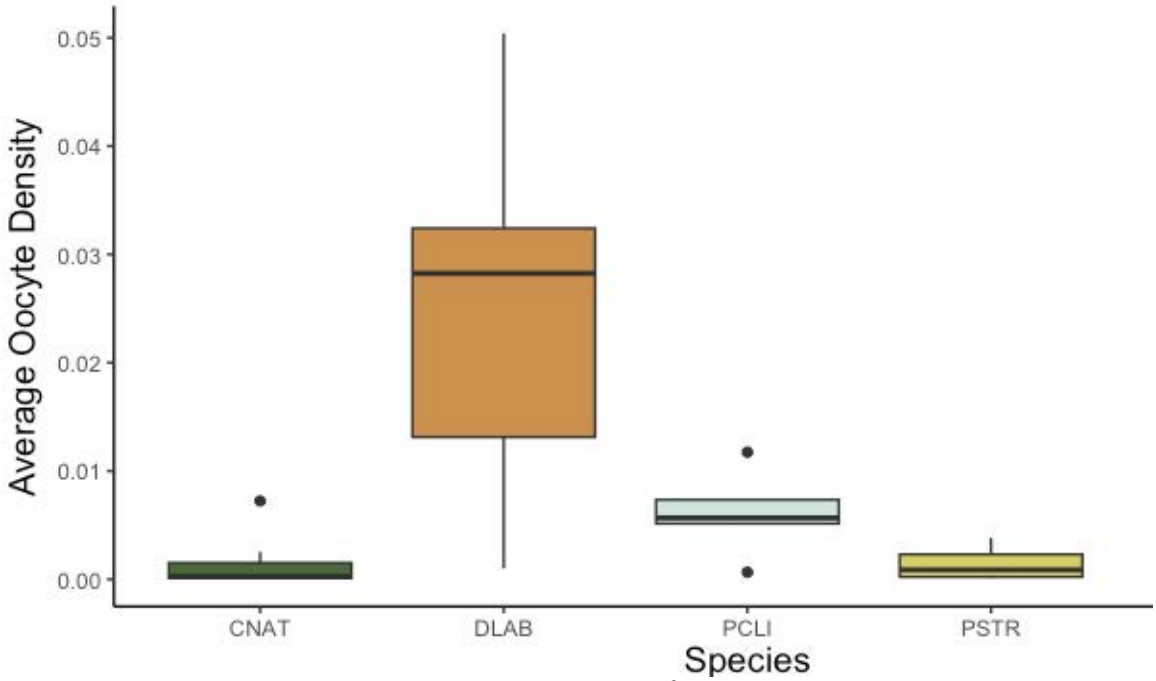

Figure 10. Average fecundity per mesentery per cm^2 of coral tissue.

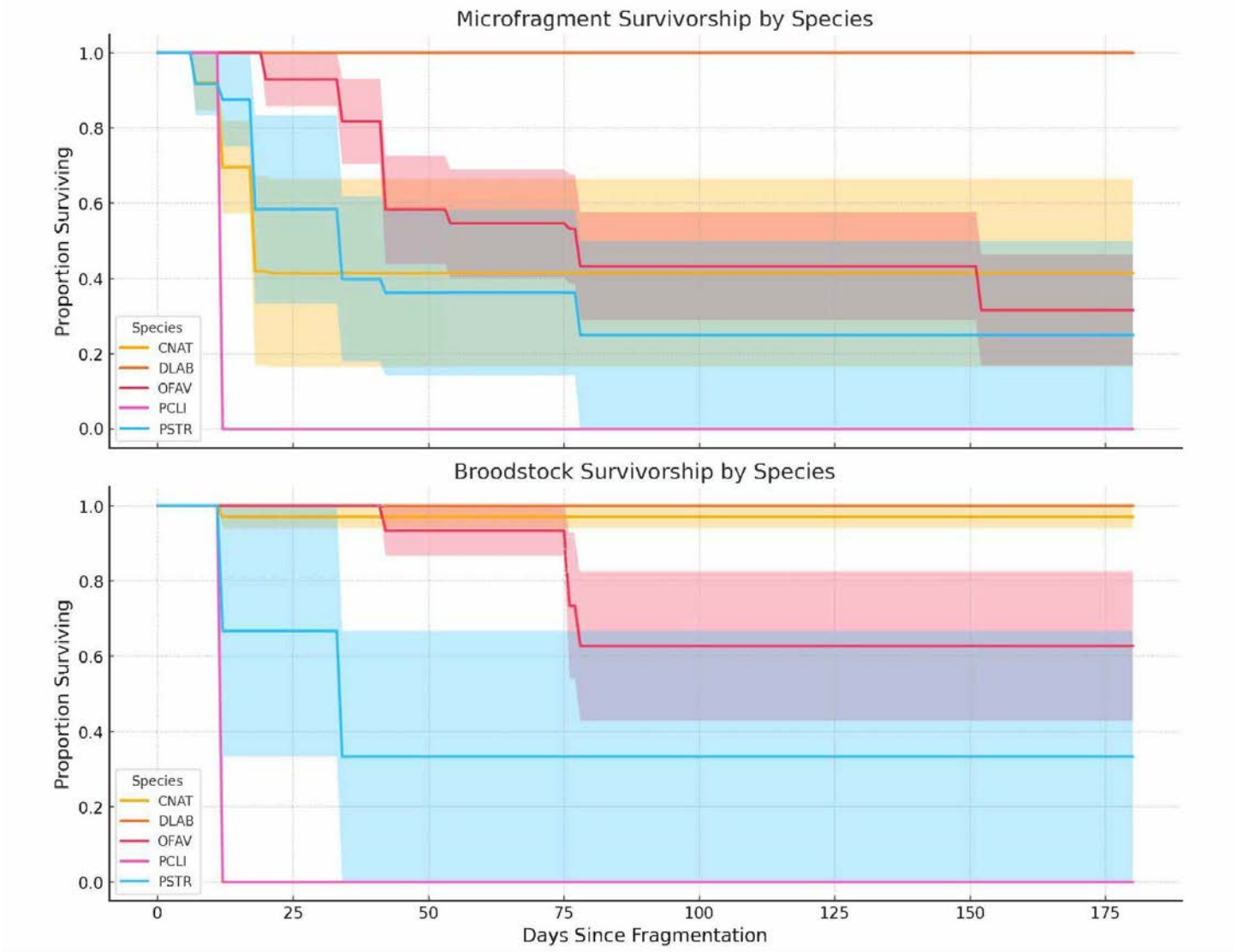

Figure 11. Survivorship curves of microfragments and broodstock grouped by species. Colors denote species and shaded areas mark standard error.

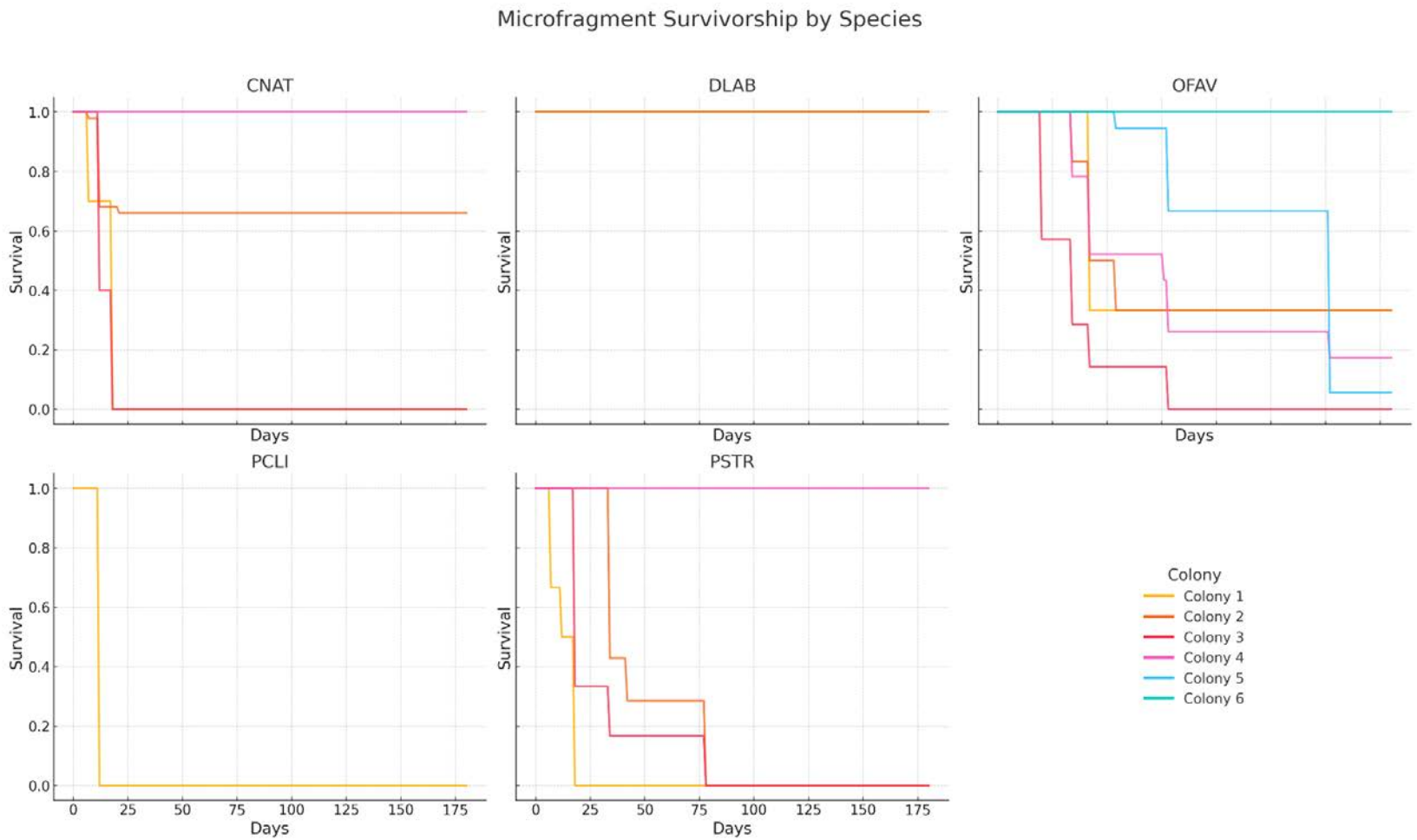

Figure 12. Microfragment survivorship over time. Color in each denotes parent donor colony.

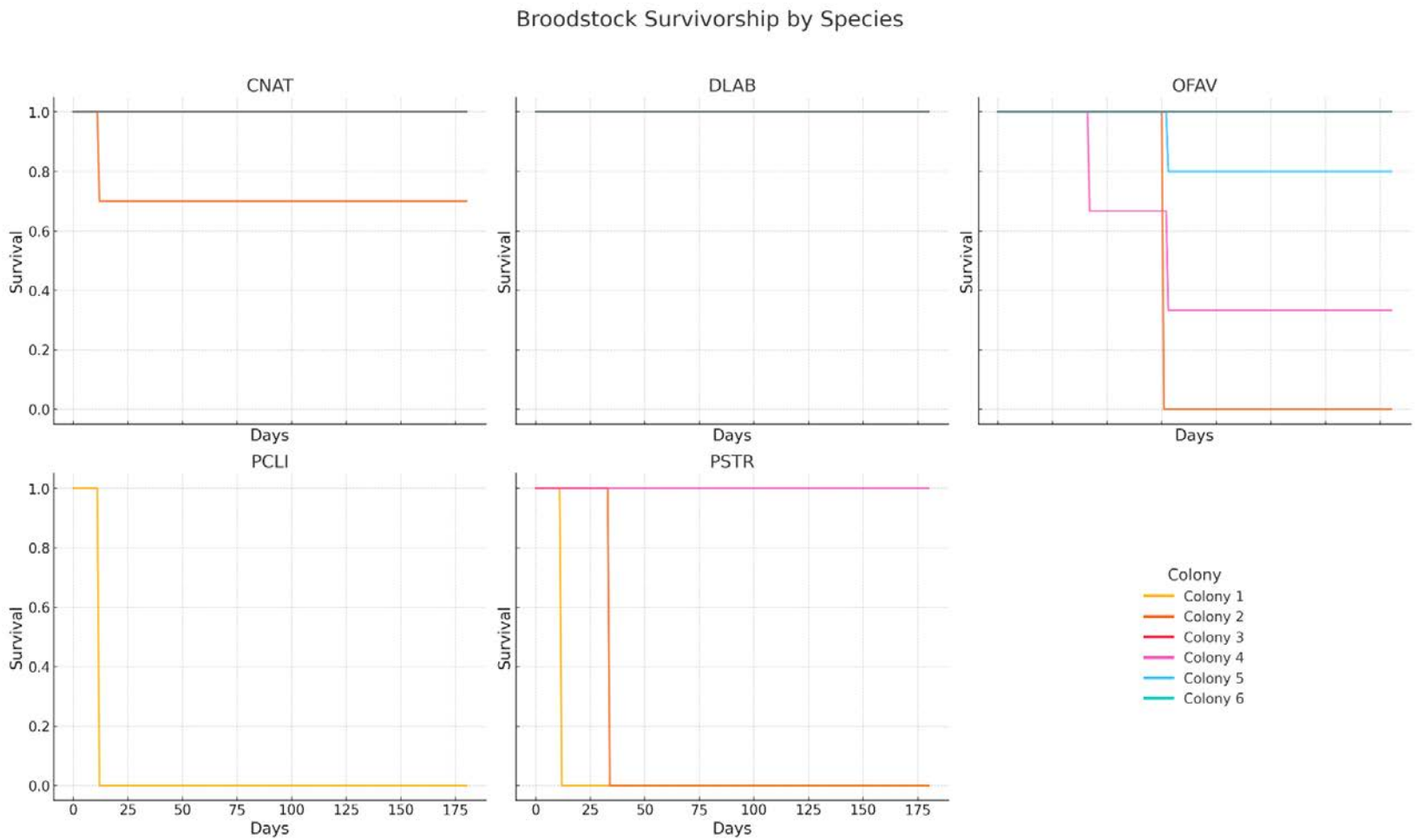

Figure 13. Broodstock survivorship over time. Color denotes parent colony.

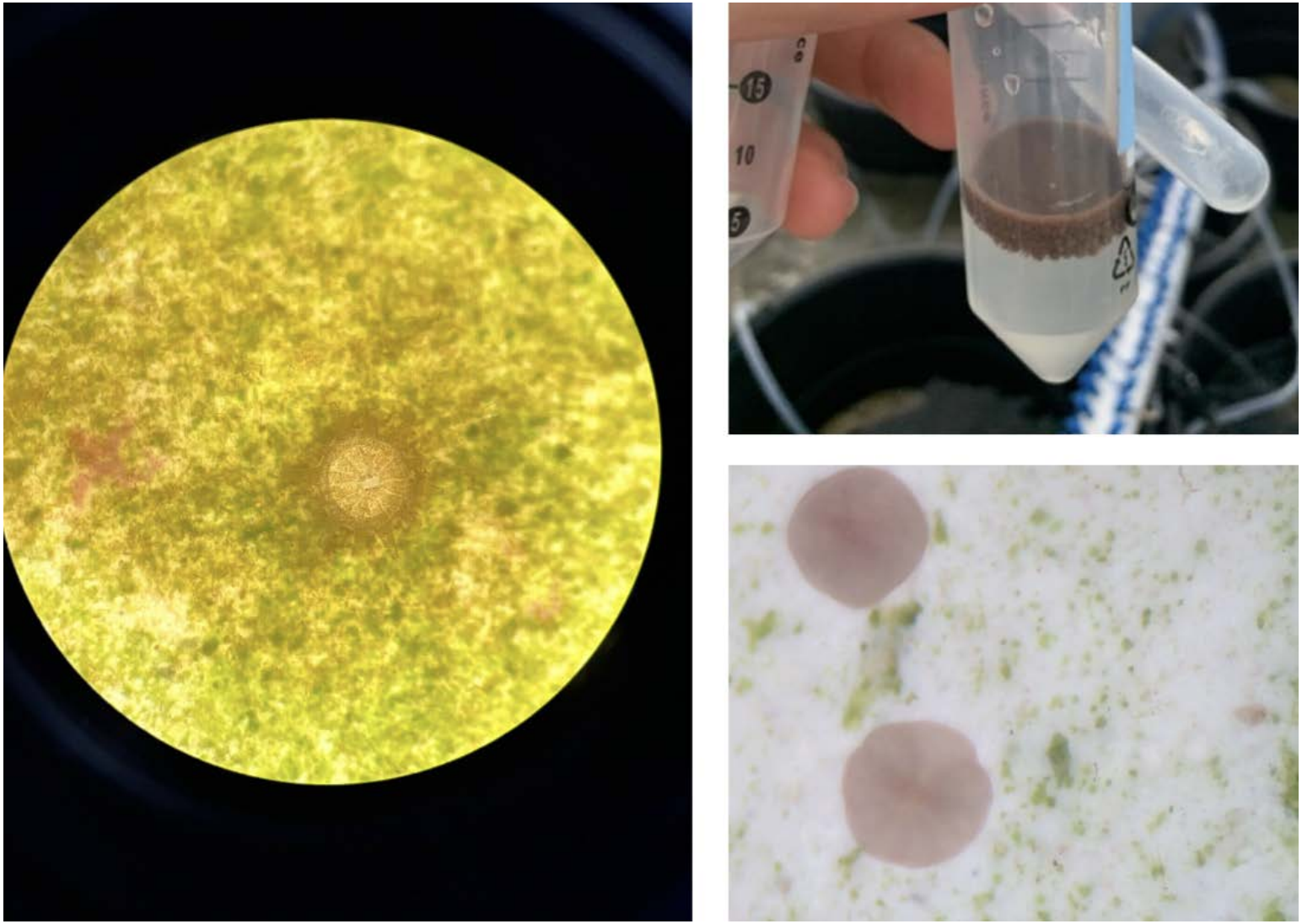

Figure 14. Left: Settled DLAB larvae containing Symbiodiniaceae from urban x reef gamete cross conducted on April 23, 2025. Top Right: Gamete bundles collected at 1923 local time that were crossed with a reef colony that spawned at 1720. Bottom Right: Initial settlement of larvae on ceramic tiles.