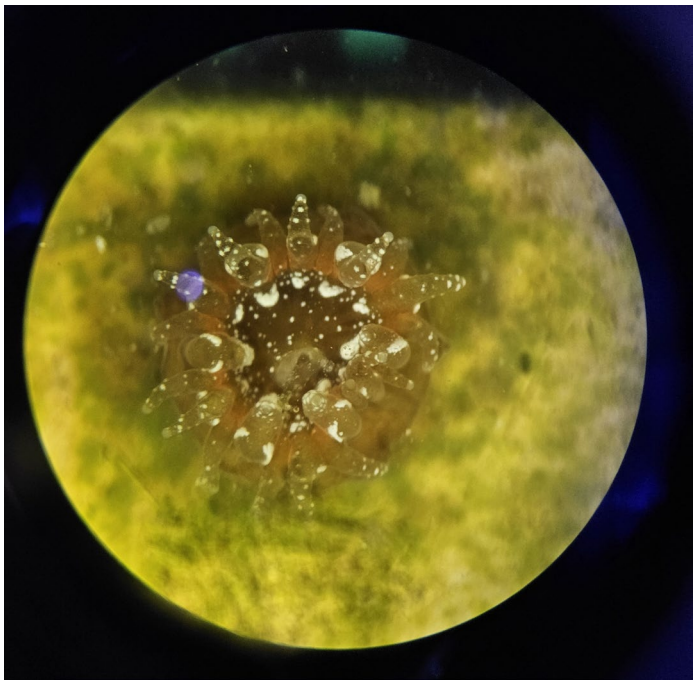
Optimizing reproductive success of corals spawned in land-based nurseries (phased)



Juvenile *Orbicella faveolata* produced in the Coral REEF Lab in August 2025.

Optimizing reproductive success of corals spawned in land-based nurseries (phased)

Final Report

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Management Summary (300 words or less)

Within the UNCW Coral REEF Lab, our research aims to improve the efficiency, predictability, and success of coral restoration and conservation through advances in coral propagation, settlement ecology, and reef substrate characterization. Over the past five years, we have focused on optimizing the *ex situ* spawning of our four Caribbean coral species, producing a high biomass of coral to support restoration and experimentation. The AI coral area sizing tool (COAST program) has the potential to decrease labor associated with manually sizing corals from photographs, reducing processing time to 3–5 seconds per image (24–140 times faster than manual measurements) and increasing efficiency in tracking coral growth over time. Using CASA software, we have improved our understanding of sperm motility and velocity in five Caribbean coral species, helping refine fertilization techniques during spawning. Long-term datasets tracking the timing of coral spawning at both day and minute scales will improve the ability of researchers to forecast spawning events *in situ* and identify species who are shifting spawning suboptimally that may benefit from targeted for assisted evolution approaches. We are also investigating biofilm communities conditioned for different durations of time to determine which may better enhance coral larval settlement and improve propagation success within our *ex situ,* sexual propagation facility. In parallel, our work on crustose coralline algae (CCA) identification on Florida's reefs has highlighted that substrate communities are more diverse, spatially variable, and ecologically unresolved than previously recognized. Finally, preliminary results suggest that *ex situ* thermal preconditioning of *Pseudodiploria clivosa* does not universally improve thermal resilience and resistance *in situ*, with environmental conditions contributing more to the observed differences in health and metabolism over time, highlighting the importance of site selection for outplanting success.

Executive Summary (1 page max)

The UNCW Coral REEF Lab focuses on the optimization of production in the earliest life history stages in our *ex situ* facility and corals' physiological response to stressors both in situ and ex situ. We have successfully spawned four Caribbean coral species, *ex situ*, for five years, producing a high biomass of coral to aid in coral reef restoration and experimentation. In addition, we have conducted a suite of experiments to increase efficiency and success for *ex situ* coral sexual propagation and *in situ* restoration. Broodstock has been improved through adjustments of the light intensity (PAR), light spectrum, feeding regimes, flow, and water quality. Long-term datasets are critical in determining whether historical shifts are occurring in the timing of coral spawning at both day and minute scales. Such information helps researchers to forecast spawning *in situ* and therefore know when to monitor and can also help to detect species who are shifting spawning suboptimally to be targeted for assisted evolution. Differences in spawning times based on observation methods indicate that lighting plays a critical role in synchrony. It is also likely that observed differences in timing between *in situ* and *ex situ* spawning events are explained, at least in part, by temperature differences. Sperm quality and longevity have been monitored for the past 2 years. Using CASA software, we have improved our knowledge of sperm motility and velocity over time for five species of Caribbean coral. We hypothesize that interspecific variation in sperm tail length, motility, and velocity may be driven by historic adult colony densities; species with historically high population densities would be subjected to higher sperm competition for fertilization and therefore could have evolved longer sperm tails in order to be more motile and faster. Such information allows us to continue improving fertilization techniques during spawning. Coral settlement continues to be a large bottleneck when producing large numbers of coral recruits. We are working to understand biofilm communities on settlement substrates when scrubbed, under varying light environments, and different crustose coralline algae (CCA) species to determine which may better enhance coral larval settlement. The CCA specimens were collected from the lower Florida Keys. Molecular analysis highlighted that substrate communities are more diverse, spatially variable, and ecologically unresolved than thought. Overall, 25 potentially novel species of CCA and peyssonnelid algal crusts (PAC) have been identified across Newfound Harbor and Looe Key. While diversity between sites was similar, community composition and abundance were different. Once coral larvae settle and metamorphose, colleagues at UNCW and our lab created a tool to determine settler size using artificial intelligence (AI). The AI coral area sizing tool (the COAST program) has decreased labor in sizing corals from photographs manually, reducing processing time to 3–5 seconds per image (24–140 times faster than doing so manually). This will ultimately increase efficiency in tracking the growth of corals over time. Finally, our lab conducted the first *ex situ* thermal preconditioning of *Pseudodiploria clivosa* paired with *in situ* physiological monitoring during typical summer temperatures. Preliminary results suggest that preconditioning did not improve performance, while site contributed more to the observed differences in health and metabolism over time. This highlights the importance of site selection for outplanting success.

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1. BACKGROUND

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. Over 20 species of coral, including both Endangered Species Act-listed and the primary reef-building species, have displayed tissue loss lesions that often result in whole colony mortality. First observed near Virginia Key in late 2014, the disease has since spread throughout the Florida Reef Tract and most regions of the Caribbean. In addition, thermal stress events resulting from changing climate and increasing sea surface temperatures are adding to this mortality and loss. Collecting corals for gene banks and sexual coral reproduction efforts are being utilized to help protect genetic diversity and restore coral reefs in Florida.

Innovative and radical measures are needed to assist with the recovery of Florida's Coral Reef. Among the suggested transformative research endeavors are land-based coral spawning, assisted fertilization, settlement, and subsequent rearing for experimentation and restoration. 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. Coral fertilization, larval development, and recruitment are the most vulnerable life history stages, and therefore the most important to understanding coral reef resilience. However, the logistical difficulties of collecting coral gametes in the field and transporting them to a laboratory for fertilization within a limited window has limited utility for research in this area. Additionally, rearing larvae and settling corals in the field without adequate control of light and temperature has limited the number of healthy coral recruits produced for restoration.

Through the support of Florida DEP and UNCW, my laboratory has established an impressive Spawning and Experimentation of Anthropogenic Stressors (SEAS) facility. This includes: four tanks that mimic *in situ* temperature and light regimes (solar and lunar), three nursery raceways for growing recruits, 12 larval rearing tanks, a 36-tank stressor experimentation system, two raceways for experimentation, a quarantine tank, and a crustose coralline algae (CCA) propagation tank. Notable findings from Florida DEP funded research, thus far:

1. We have repeatedly spawned four species *Orbicella faveolata* (OFAV), *Pseudodiploria clivosa* (PCLI), *Diploria labyrinthiformis* (DLAB), and *Acropora cervicornis* (ACER), and previously planulated one species Porites asteroids (PAST). We were the first to spawn PCLI and OFAV in an *ex situ* laboratory. https://www.pbsnc.org/watch/sci-nc/
2. Over the past 5 years we have contributed to optimizing *ex situ* spawning, larval rearing, settlement, and juvenile grow-out (Figueiredo et al. in late prep for publication).
3. We established and continue to organize and facilitate the Land-based Assisted Sexual Reproduction (LASR) group, which is dedicated to sharing knowledge, troubleshooting, and training new facilities interested in *ex situ* spawning.
4. We have quantified sperm size, morphology, and motility of PCLI, OFAV, DLAB and continue to optimize efforts to record sperm velocity (Laforest et al. dissertation chapter).

5. Our laboratory was the first to develop probiotics for the purpose of enhancing post-metamorphosis survival and growth in a Western Atlantic coral species (Weeks thesis publication in prep).
6. Collaborating with a UNCW professor that specializes in artificial intelligence (AI), a program (beta stage) has been developed to measure the size of coral recruits using AI instead of time intensive manual tracing. The model has been published in the journal Sensors; see Zhao et al. 2026.
7. Our team continues to compile coral spawning data from Florida to compare differences among species, years, regions, and observational methods (Laforest et al. in late prep for publication).
8. A light experiment examining the effect of blue- versus green-shifted spectra on recruit survival and growth demonstrated tradeoffs where less handling and cleaning occurred in those under the green-shifted spectra, but those under the blue-shifted spectra grew quicker during the first 14 weeks post-settlement. This led to additional funding through NOAA. (Van horn et al. in review the journal Coral Reefs.
9. A project examining the use of chimeras for restoration led to the discovery that increased temperatures can increase chimeric formation in species that are less aggressive (ACER), but not in combative species (OFAV). Additionally, during this study we documented the first evidence of cannibalism in corals (Shackelford thesis in progress).
10. Many other projects have been indirectly supported such as physiological effects of corals after exposure to ocean warming, ocean acidification, microplastics, and low dissolved oxygen (hypoxic) conditions. Findings have been published in Frontiers in Marine Science (See Sugiersk et al. 2025).

With recent Florida DEP funding, project goals included:

- Improving upon aquaria induced coral spawning, larval survival, settlement, and grow out using innovative methods (Task 2a).
- Identifying crustose coralline algae (CCA) and peyssonnelid algal crusts (PAC) found on Florida's reefs to species level, as well as their associated microbiome communities, using advanced molecular tools (Task 2b).
- Determining whether morphogenic compounds increase settlement, metamorphosis, and benefit in post-metamorphosis survivorship (Task 2c).
- Identifying sperm traits across species and individuals within our facility, using specialized sperm analysis software (CEROS II formerly CASA) recently purchased by UNCW's Center for Marine Science (Task 2d).
- Finalizing the coral area sizing tool (COAST) that uses AI to measure coral recruits photographed under a microscope (Task 2e).
- Better understand why corals under artificial light spawning are shifted in days after the full moon by comparing spawning times of corals in the wild versus under artificial light (Task 3).
- Determining if preconditioning improves this species' resistance and resilience to ocean warming compared to controls by exposing 1 year old *P. clivosa* recruits produced in our laboratory to thermal stress in our experimental systems prior to outplanting (Task 4).

1.1. Task 2a: Coral husbandry and *ex situ* spawning

UNCW's land-based spawning and nursery facility, Dr. Fogarty's Coral REEF Lab, has successfully housed broodstock of priority Florida coral species and sexually reproduced them *ex situ* since 2020. We maintain our broodstock corals in spawning tanks that mimic the environmental cues for spawning and provide husbandry and care for those individuals. We shifted sunset, and therefore spawning time, by 4 hours to help with researcher fatigue during experimental setup following spawning events. Gametes were collected and fertilized in gravy separators, reared in larval cones/bins and settlement bins, and settled on pre-conditioned tiles with CCA dust. Based on experiments conducted in 2024, colored tiles and plugs improved settlement and were subsequently used. All recruits produced were grown in dedicated nursery raceways and/or shared with our partners and receive continual care and supplemental feedings. Dr. Fogarty continues to organize at least a pre-spawning and post-spawning LASR (Land-based Assisted Sexual Reproduction) meeting with all interested parties.

1.2. Task 2b: Crustose coralline algae species identification

Larval settlement is one of the most critical recruitment bottlenecks (Arnold & Steneck, 2011). Settlement decisions directly influence post-settlement survival, growth, and spatial patterns of reef recovery. Successful settlement depends strongly on benthic substrate quality, particularly the presence of crustose coralline algae (CCA), which stabilize reef frameworks and release chemical cues that induce coral larval settlement and metamorphosis (McCoy & Kamenos, 2015; Giorgi et al., 2024; Vermeij 2004). Importantly, not all CCA promote coral larval settlement equally (Giorgi et al., 2024); coral settlement responses vary widely among CCA taxa, meaning that species composition of benthic substrates can strongly influence coral recruitment potential. At the same time, spatially aggressive peyssonnelid algal crusts (PAC) have expanded rapidly across many tropical Western Atlantic reefs (Edmunds et al., 2023; Wilson et al., 2020) and host distinct microbial communities that are hypothesized to inhibit coral larval settlement (Wilson et al., 2020; Stockton & Edmunds, 2021), thus potentially reinforcing recruitment failure and reduced reef resilience. While earlier studies have examined CCA species settlement preferences in Western Atlantic corals (Ritson-Williams et al. 2010, 2014), CCA species identification was based solely on morphology which has since been shown to be unreliable due to the presence of multiple cryptic CCA species (Twist et al., 2020). We collected CCA/PAC specimens from Florida reefs (Broward County and Florida Keys National Marine Sanctury) and identify them to species level using Sanger sequencing. This work begins to establish locations at which PAC are present in Florida and lay the groundwork for future studies testing coral settlement outcomes with specific substrates. Because CCA and PAC microbiomes contribute to coral larval settlement cues, we are characterizing their *in situ* microbiome community composition using (16S amplicon sequencing). Understanding the microbial communities associated with both beneficial (CCA) and potentially inhibitory (PAC) substrates in Florida will support more informed site selection (e.g., avoiding areas with PAC dominance) and highlight the potential need for intervention strategies (e.g., PAC removal) in Florida reef restoration efforts.

1.3. Task 2c: Settlement enhancement

Coral settlement is one of the greatest bottlenecks in coral restoration (Banaszak et al., 2023). By increasing both settlement and metamorphosis, we can drastically increase the production of coral recruits for outplanting. Typically, conditioning ceramic tiles with both crustose coralline algae (CCA) and biofilms is used to induce larval settlement. However, settlement induction is often species-dependent (Abdul Wahab et al., 2023; Randall et al., 2024; Whitman et al., 2020). There is also discourse on whether the active cue in CCA settlement is a result of physical contact with CCA (Harrington et al., 2004; A. N. C. Morse et al., 1996; D. E. Morse & Morse, 1991), biochemical cues from CCA (Lei et al., 2021; Quinlan et al., 2023; Tebben et al., 2015; Whitman et al., 2020), biochemical cues from CCA-associated microbes (Giorgi et al., 2024; Jorissen et al., 2021; Negri et al., 2001; Siboni et al., 2020), or various combinations of these potential cues (Dobretsov & Rittschof, 2020; Gómez-Lemos et al., 2018; Padayhag et al., 2023). Some practices include the use of either tetrabromopyrrole (TBP) or cycloprodigiosin (CYPRO), hydrophobic, pyrrole- containing compounds that are isolated from CCA biofilm-associated Gammaproteobacteria, to induce larvae settlement (Fiegel et al., 2025; Petersen et al., 2023; Petersen et al., 2021; Sneed et al., 2014, 2024; Tebben et al., 2011). Furthermore, some coral larvae settle on marine biofilms without the presence of CCA (Webster et al., 2004).

Marine mono-films have been studied on coral larvae settlement (Negri et al., 2001; Petersen, Moeller, et al., 2021; Sharp et al., 2015; Sneed et al., 2014; Tran & Hadfield, 2011), yet there is no comprehensive data regarding biofilms. Recently, sequencing experiments on marine biofilm communities show that they are rapidly transforming over time across various environmental conditions (Bech et al., 2024; Remple et al., 2021; Sajid et al., 2024) and can have various effects on larval settlement (Padayhag et al., 2023; Turnlund et al., 2023; Yanovski et al., 2024). More specifically, it has been shown that biofilm conditioning, and presence or absence of light, plays a key role in developing settlement-inducing biofilms (O'Brien et al., 2025).

To expand this research, we investigated the effects of both biofilm conditioning (temporal) and light intensity on influencing ceramic tile biofilm composition, and the subsequent effect on larval settlement success. This research will enhance efforts to rear corals for outplanting and experimentation by providing information on the ideal biofilm propagation strategies for coral larval settlement.

1.4. Task 2d: Sperm traits

During the 2025 spawning seasons, we learned an incredible amount about how to video record sperm traits, including: the proper techniques, microscopy setup, temperature for ideal video recording, and the appropriate chamber to record small, fast-moving sperm. With the purchase of the necessary supplies and equipment and utilizing the air-stabilization table at the microscopy facility at UNCW, we minimized microscope vibration while conducting this sensitive work. While this allowed us to collect data to assess sperm size and motility, the OpenCASA software struggled to accurately and consistently track individual sperm to assess velocity. After several conversations with Dr. Mary Hagedorn (Smithsonian) and her team and local IVF clinics, we aim to improve our

research efforts with the purchase of CEROS II software by UNCW's Center for Marine Science. This software has previously recorded both coral sperm motility and velocity in Pacific coral species. We continue to make progress on this objective by utilizing better software, incorporating other priority species, and assessing how additional factors, such as sperm age and temperature, impact sperm fitness.

1.5. Task 2e: Finalize AI coral area sizing tool (COAST) program

Over the past year, the Coral REEF Lab has worked with UNCW collaborator, Dr. Cuixian Chen, and her team to develop a program that applies AI technology to measure recruits through microscopy images. The program was tested with photos taken at UNCW and Mote Marine Laboratory and then tested across multiple facilities (UNCW, NSU, Mote, TFA) and species to fine-tuned to increase accuracy. This program has saved facilities countless hours of manual recruit measuring and/or image analyses, which ultimately allows funding to be applied to other restoration activities.

1.6. Task 3: Spawning calendar

Following the compilation of thirty years of historical spawning observation data, we were able to create a spawning prediction calendar for several priority species, noting whether historical shifts and regional differences in spawning times have occurred. With advances in spawning induction systems, we have seen an increase in *ex situ* observation data over the last few years, particularly in species with deficient data. Inversely, there was a notable decline in *in situ* spawning observations, particularly in 2024, which was likely a result of intense stress following the 2023 bleaching event and thermal stress of 2024. While anecdotal evidence suggests that *ex situ* corals spawn later than *in situ* corals, additional data is needed to accurately assess whether *in situ* and *ex situ* spawning occurs synchronously.

1.7. Task 4: Preconditioning and outplanting

We thermally preconditioned juvenile corals prior to deploying onto Florida's reefs to increase resistance and resilience to ocean warming events. *Pseudodiploria clivosa* recruits (from *ex situ* spawning in the lab) were employed to assess whether pre-exposure to increased temperatures enhances resistance (i.e., preconditioned corals are less likely to have pale/bleach during the warmest months) and/or resilience (i.e., pre-conditioned corals may still pale/bleach but recover faster than non-conditioned counterparts), ultimately improving survival and recovery rates for outplanted corals. Juvenile *P. clivosa* produced from our 2023 spawning event were exposed to thermal stress treatments (i.e., acute thermal stress of 32°C) and outplanted at Newfound Harbor and Looe Key in the Florida Keys. We collaborated with FWC and Seacamp staff to monitor corals periodically between UNCW monitoring visits. The resulting data will be used to make recommendations to FL DEP, FKNMS, and other restoration practitioners on whether preconditioning is a viable option to enhance coral resistance and resilience to fluctuating

temperatures. This project also collaborated with Seacamp staff to incorporate restoration research curriculum into their grade school program.

2. METHODS

2.1. Task 2a: Coral husbandry and *ex situ* spawning

At UNCW's land-based spawning and nursery facility, we have maintained the health of all broodstock and housed juvenile colonies over the last year. We have worked to optimize coral husbandry, standard operating procedures, and methodology to increase spawning production of our broodstock colonies and survivability of juvenile recruits.

We collected water quality data from each recirculating aquarium system every weekday to ensure that these parameters were within accepted ranges: temperature (74–84.5°F seasonally), salinity (34–36 ppt), pH (7.9–8.4), and alkalinity (7.7 dKh). Additionally, we collected nitrate (1.0–3.0 ppm), phosphate (<0.1 ppm), calcium (380–450 ppm), and magnesium (1300–1450 ppm) readings two to three times weekly. Water changes were conducted twice weekly, totaling an average of ~20% water volume replaced per week. Broodstock colonies were fed four times weekly to maximize energy available for gametogenesis and continued growth, while juvenile recruits were fed twice weekly to limit the risk of algal overgrowth. All juvenile recruits have been sexually propagated from our land-based spawning efforts or from the efforts of Florida partner facilities. These juvenile recruits are settled and grown in dedicated nursery raceways and regular aquarium maintenance conducted (described above).

All broodstock colonies originated from Broward County and have been housed in designated aquarium systems in black cubicles to block light pollution. All light cues (solar and lunar) and temperature parameters to induce spawning were programmed via Neptune Apex aquarium controllers to mimic South Florida seasonal conditions. *Acropora cervicornis* (n = 11), *Orbicella faveolata* (n = 9), and *Pseudodiploria clivosa* (n = 14) colonies were added to our collection in 2020–2021 and have been held in our land-based facility for 5–6 years. *Diploria labyrinthiformis* (n = 13) colonies were collected in June 2024 and have been held in our system for 2 years. These four species have been identified by the State of Florida as priority species for rescue efforts, including land-based propagation.

As done yearly, in March 2026 the sunset time of all aquarium systems was shifted four hours earlier by adjusting the programmed local time zone via Neptune Apex. This practice allows for spawning times to occur four hours earlier than their wild predicted spawning times, reducing researcher fatigue. For the spawning window of July–October 2025, *A. cervicornis*, *O. faveolata*, and *P. clivosa* colonies were monitored for spawning activity beginning five days after the full moon (DAFM) and 90 minutes after sunset (MAS). For the spawning window of April–June 2026, *D. labyrinthiformi*s colonies were monitored for spawning activity beginning eight days after the full moon (DAFM) and 240 minutes prior to sunset.

All colonies were initially monitored every 5–10 minutes until setting in at least one colony was observed, at which time the coral species, tag ID number, time of setting, and spawn time were recorded. Gamete bundles were collected via pipette into designated

urine cups labeled with tag ID number(s) of parent colonies. Ratios of 1 bundle per ~1 ml of seawater were maintained from bundle collection through fertilization to encourage greatest possible fertilization with sperm concentrations at approximately 10^6 sperm ml^{-1}. Bundles were added to gravy separators in multi-parent (ideally >3) batch crosses in equal proportions and left for one hour in a pre-heated seawater bath to maintain appropriate temperatures at 79–81°F. Embryos were rinsed five times (or until clear) with filtered (to 1-micron) seawater to remove excess sperm solution and prevent polyspermy. Gravy separators were placed in the pre-heated seawater baths for 1–2 hours until cell division was clearly visible in most embryos. Once counts were collected to estimate fertilization success and therefore initial production, batch crosses were added to larval cones (<100,000 embryos) or larval rearing bins (<35,000 embryos) to continue cell division and development.

We continue to use the larval rearing bin recirculating system (designed and built in 2024) consisting of four conical tanks and ten 38 L flat-bottom containers tied together with driplines via a common sump tank. In 2025, we tested a different type of outflow system on half of the flat bins with a central standpipe to encourage additional circulation within each bin. Additionally, a longer inflow drip line was added to encourage more flow throughout the depth of the bin rather than only on the surface. In May 2026, we utilized both the central outflow and side outflow bin designs to determine if one might broadly improve survivability or health of larvae. Once larvae reached competency determined by directional swimming behavior, they were transferred to settlement bins with nitex windows and seawater driplines to allow for limited water flow (≤20 mL per minute) with ceramic settlement tiles lining the bottom and a small amount of crushed crustose coralline algae pipetted onto each tile. Ceramic tiles with one or more settled recruits after the allotted settlement window (48–72 hours) were added to our nursery growout raceways with reduced waterflow and low light (25 PAR) for the first four weeks post settlement an incrementally increased throughout development.

Dr. Fogarty has continued to organize and facilitate the Land-based Assisted Sexual Reproduction (LASR) working group to information-share about optimizing land-based spawning systems and protocols. Over the last year, this group has doubled in membership with several facilities joining from across the country (i.e., the Association of Zoos and Aquariums Florida Reef Tract Rescue Project) and several international facilities that are working towards land-based spawning initiatives. Our next meeting time will be scheduled for late June to recap *D. labyrinthiformis* spawning observations across all facilities.

2.2. Task 2b: Crustose coralline algae species identification

Crustose coralline algae (CCA) and peyssonnelid algal crust (PAC) specimens were collected from Newfound Harbor (n = 81) and Looe Key (n = 40) Sanctuary Preservation Areas (SPA) in the Florida Keys (permit FKNMS-2025-012-A1). Specimens were photographed *in situ* before being sampled with hammer and chisel. For a subsample of the specimens collected (~25), a voucher was placed into DNA-RNA shield for microbiome analysis.

Specimen DNA was extracted using Qiagen DNeasy Blood and Tissue kit following standard protocols and cleaned using Zymo Clean and Concentrator Kit.

Cleaned, concentrated DNA was sequenced on a Sanger sequencer (ABI 3500 Genetic Analyzer) for both *rbc*L and *psbA* for identification to species level, where possible.

To account for unequal sampling effort between sites, diversity was estimated using coverage-based rarefaction/extrapolation in the R package iNEXT. Hill numbers representing richness, Shannon diversity, and Simpson diversity were standardized to equivalent sample coverage and expressed as effective taxa.

Microbiome DNA was extracted using Qiagen DNeasy PowerSoil Pro kit following standard protocols and cleaned using Zymo Clean and Concentrator Kit. Cleaned, concentrated DNA was sent to Zymo for 16S sequencing. Microbiome samples were extracted in the Ushijima lab and sent to Zymo for 16S microbiome sequencing.

2.3. Task 2c: Settlement enhancement

Six-weeks prior to the May 2026 *D. labyrinthiformis* spawning window (9–15 May), 45 ceramic settlement tiles were placed in 50 PAR light treatments (n = 3) across two established aquaculture raceways. Three weeks prior to the spawning window, 45 additional ceramic settlement tiles were placed across the same treatments, as well as 45 more into a separate established aquaculture tank with crustose coralline algae (CCA) at 50 PAR.

Experimental design consisted of two types of settlement assays. Both non-choice (one tile) and choice (two tiles) assays were utilized to assess coral larval survival and settlement. Half of the tiles in each treatment were left non-scrubbed, and the other half scrubbed uniformly with a mascara brush. All treatments were tested in non-choice assays (n = 3), and all combinations of treatments were assessed with choice assays (n = 3).

On 14 May 2026, seven *D. labyrinthiformis* colonies spawned at ~50–90% coverage. Gametes from a subset of these colonies were mixed and fertilized. After an hour of fertilization, sperm was removed by rinsing, and an estimated 139,000 embryos remained.

On 16 May 2026, 48 hours post-fertilization, both unconditioned (controls) and conditioned settlement tiles were separated and assigned to Tupperware settlement containers. Approximately 6,600 larvae were hand-counted using borosilicate pipettes and 100 were put into each container. Additionally, subsets of tiles from non-scrubbed and 6-week conditioned tiles had their biofilms scraped with sterile scalpel blades, placed in a microcentrifuge tube with DNA/RNA shield, and stored in a –80ºC freezer awaiting DNA extraction and analysis. Tiles in other treatments had both inaccessible and low biomass that were below the weight requirements for the QIAGEN DNeasy Power Biofilm Kit. A sonicated water bath will be used in the future to dislodge biofilms from these treatments.

On 18 May 2026, 48 hours after placing the larvae into the settlement assays, all settlement tiles and containers were scored for both larval settlement and overall survival. On 20 May 2026, 96 hours post-scoring, non-choice settlement containers were scored again for larval settlement only. Visual assessment of larval survivorship in choice assays between 48 and 96 hours after the settlement experiment was initiated was minimal, and as a result they were not scored again at 96 hours.

A generalized linear mixed model (GLMM) with a beta distribution was used to assess any differences in the proportion settled over time among treatments. The main effects were treatment (tile conditioning location and scrubbed) and time point (48hrs vs. 96 hrs) and the random factor was the coral tile identifier. There was no interaction between treatment and time; therefore, it was removed from the model.

2.4. Task 2d: Sperm traits

In July, August, and September 2025, and May 2026, we successfully recorded videos for sperm velocity and motility utilizing Computer Assisted Sperm Analysis (CASA) software to evaluate sperm fitness in *Montastraea cavernosa, A. cervicornis, O. faveolata, P. clivosa,* and *D. labyrinthiformis*.

To measure sperm velocity and motility, labeled Eppendorf tubes of sperm (~$1x10^4$ cells mL^{-1}) were held in a dry bath to maintain optimal temperature (26.7ºC). 4 µL of sperm were pipetted into a 20-micron fixed-depth slide and held on a slide warmer to maintain optimal temperature. A minimum of 5 videos containing 200 sperm were recorded for each slide using the CASA software, which automatically detects sperm movement and calculates the motility and velocity of the sample. This process was repeated hourly for up to 11-hours post bundle breakage, or until 90% of the sample was immotile.

To assess sperm tail morphology, 4 µL of fixed sperm were placed in a 20-micron fixed-depth slide and viewed under 40x magnification using phase contrast on an Olympus IX73 inverted microscope. Using Cellsens software, a minimum of five photos, containing ~200 sperm, per spawning colony, were captured to assess sperm quality. In each photo, an individual sperm was categorized as having normal or abnormal morphology, including discrepancies in sperm head (i.e., free head) and sperm tail (i.e., bent, coiled, tail droplets, shortened, or disconnected tail). Sperm were also analyzed using Cellsens software to measure tail length.

A generalized linear mixed model (GLMM) with a beta distribution was used to assess differences in percent motility over time between species. Similarly, a GLMM with a gamma distribution was used to determine whether there were significant differences in sperm velocity over time and across species. Morphological differences in tail length between colonies were analyzed using a Kruskal Wallis test. A non-parametric one-tailed Mann-Whitney Wilcoxon test was utilized to determine whether there were interspecific differences in sperm tail length.

2.5. Task 2e: Finalize AI coral area sizing tool (COAST) program

We present a hierarchical deep learning pipeline that automates surface area measurements of corals by integrating YOLO-based (You Only Look Once) detection with Segment Anything Model (SAM) segmentation (Zhao et al., 2026). YOLO localizes recruits and classifies them by developmental stage; stage-specific fine-tuned SAM models then segment live tissue using bounding box and background point prompts to suppress segmentation leakage and improve boundary precision (Zhao et al., 2026). Surface area is computed directly from the segmented masks using pixel size extracted from image metadata (Zhao et al., 2026). The tool was evaluated on 3,668 microscopy images from two national coral research facilities (Zhao et al., 2026).

2.6. Task 3: Spawning calendar

Over 2,300 spawning observations have been compiled from various sources including peer-reviewed journals, data from NOAA's Coral Health and Monitoring Program Coral ListServer, posts from the Coral Spawning Research Facebook page, and unpublished data contributions from researchers throughout Florida. This thirty-year dataset (1993–2026) represents spawning information for twenty-seven species, including Florida priority species *O. faveolata, P. strigosa, D. labryrinthiformis*, and *Montastraea cavernosa*. Additionally, through a combination of field and aquaria observations, we have obtained spawning information about lesser understood species, including *Mussa angulosa, Mycetophyllia lamarckiana*, *Meandrina meandrites,* and *Eusmilia fastigiata*. However, only analyses for species with sufficient spawning data (i.e., *A. cervicornis, A. palmata*, and *O. faveolata*) have been conducted to determine whether there are historical shifts and/or regional differences among *in situ* spawning sites (at the scale of days and hour) using a Kruskal Wallis test. Similarly, where data was sufficient (i.e., *A. cervicornis, D. labyrinthiformis, O. faveolata,* and *P. strigosa*), differences in spawning times between *in situ*, induced (i.e., in captivity for a full gametogenic cycle under artificial lighting), and *ex situ* (i.e., temporarily in captivity under natural lighting) data were determined via Kruskal Wallis analyses.

2.7. Task 4: Preconditioning and outplanting

Juvenile *P. clivosa,* reared in our lab in 2023 (~1.5 years old at time of experiment), were randomly assigned to one of two treatment groups: a control group (n = 144) maintained at ambient temperature (27°C), or a preconditioned group (n = 144) exposed to 32°C for increasing durations of 4-, 12-, and 24-hours, each followed by a week of recovery, over an eight-week period. Each treatment was replicated across 6 experimental tanks. For the preconditioned group, each temperature increase occurred from ambient (27°C) to 32°C occurred over a ~12-hour ramp period, was held for the allotted time, and then returned to ambient over a ~12-hour ramp-down period. The target temperature of 32°C was selected to reflect mean temperatures recorded during the 2023 Florida Keys bleaching event. These thermal exposures were designed to simulate acute, sublethal thermal stress events that allow for recovery and minimize mortality. This strategy was informed by methods outlined in the Coral Bleaching Automated Stress System (CBASS) protocol. Throughout the preconditioning period, corals were monitored daily for visible signs of stress, including paling, bleaching, tissue loss, or mortality. No experimental cycles were terminated early, as bleaching severity and mortality remained low across treatments.

Following laboratory treatments and an additional week of recovery, all corals were transported down to the Florida Keys in May 2025. After 7 days of acclimating to local conditions, the corals were outplanted at two NOAA Mission Iconic Reefs (MIR) sites: Newfound Harbor (NEWF) and Looe Key (LOOE). Each site varies in offshore distance,

structure, depth, and water quality. Our site in the Newfound Harbor Special Protection area (SPA) is a flat hard bottom site with intermittent coral heads that averages a depth of ~15ft, and experiences more sediment and murky water. Looe Key is a more traditional, complex spur-and-groove reef site, with depths ranging from 7–30 feet, and experiences clearer water with higher light intensity.

Monitoring was conducted in July and October 2025 and May 2026. During each monitoring phase, all fragments will be assessed for survival, bleaching severity, growth, and overall condition. Standardized CoralWatch health charts, high-resolution photographs, and metric rulers were used for color and size calibration. Growth was evaluated using planar area measurements in ImageJ, while height was derived from side-profile images using image-specific scaling. Bleaching, tissue loss, and signs of predation were documented using visual assessment rubric.

To evaluate physiological function, a subset of corals (n = 96; 24 controls and 24 preconditioned corals from each site) were measured using the Community In Situ MEtabolism (CISME) device, a non-invasive, self-contained, flow-through chamber system that quantifies coral metabolic rates (net photosynthesis and respiration) *in situ*. All values were normalized to coral surface area. From these values, gross photosynthesis is calculated as:

Gross Photosynthesis = Net Photosynthesis + Dark Respiration

3. RESULTS

3.1. Task 2a: Coral husbandry and *ex situ* spawning

In 2025, 8 of 9 broodstock of *O. faveolata*, 12 of 13 broodstock of *P. clivosa*, 9 of 13 broodstock f *D. labyrinthiformis*, and 6 of 11 broodstock of *A. cervicornis* successfully spawned (Table 1). Though *P. clivosa* had a high percentage in number of colonies spawned (12 of 13 colonies), the magnitude and observed percentage of each individual colony releasing gametes was reduced from previous years. In 2025 on average, *O. faveolata* spawned at around 224 minutes after sunset (MAS) (Figure 1, Table 2), *P. clivosa* spawned at 398 MAS (Figure 2, Table 2), *A. cervicornis* at 200 MAS, and *D. labyrinthiformis* at –176 MAS (Figure 3, Table 2). These spawning observation times for 2025 are within predicted peak spawning windows, except for *D. labyrinthiformis* colonies which spawned earlier than anticipated at –176 minutes after sunset (MAS) (Table 2). Between April, May, and June 2026, 11 out of 13 broodstock *D. labyrinthiformis* successfully spawned (Table 1) with an average time of –144 minutes after sunset (MAS) which is much closer to the anticipated spawning window than observed in the previous year (Figure 3, Table 2). We have not yet determined the reason for this earlier average spawning time; however, we have adjusted our yearly spawning observation times for *D. labyrinthiformis* to begin at approximately 240 minutes before sunset to ensure that observers are present for any potential early spawning activity.

Table 1. Number of genotypes for broodstock species that successfully spawned each year. ^indicates years that colonies were in spawning systems for <3 months; "" indicates colonies that were diseased or recovering from tissue loss; "?" indicates years where asynchronous spawning events occurred outside of monitoring windows (in DAFM).*

Species	2020	2021	2022	2023	2024	2025	2026
OFAV	*3/ 4^*	*3/11*^*	*4/11**	*7/10*	*5/9**	*8/9*	*TBD*
PCLI	*4/ 6^*	*12/15^*	*7/15*	*13?/15*	*8/13*	*12/13*	*TBD*
DLAB	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*9/13*	*11/13*
ACER	*N/A*	*4 / 11*	*4/11*	*2?/11*	*0/11**	*6/11*	*TBD*

Table 2. Average spawning times across all colonies observed in UNCW's land-based facility across all spawning years. Predicted peak minutes after sunset (MAS) in column 1 recorded from Coral Restoration Consortium (CRC) Coral Breeding Reference Sheets (Chamberland et al 2023).

Predicted peak MAS	Species	2020 Avg. MAS	2021 Avg. MAS	2022 Avg. MAS	2023 Avg. MAS	2024 Avg. MAS	2025 Avg. MAS	2026 Avg. MAS
-60 *(-90-15)*	*DLAB*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*-176*	*-144*
174-192 *(145-210)*	*ACER*	*N/A*	*182*	*183*	*N/A*	*N/A*	*200*	*TBD*
220-245 *(190-275)*	*OFAV*	*233*	*235*	*217*	*245*	*232*	*224*	*TBD*
340-410 *(300-460)*	*PCLI*	*362*	*354*	*359*	*374*	*303*	*398*	*TBD*

On average across all years, *P. clivosa* has spawned on day 8–9 after the full moon (Figure 2) and *O. faveolata* has spawned on day 8 after the full moon. However, in 2025, *O. faveolata* colonies spawned on days 6 and 7 after the August and September full moons (Figure 1). Additionally, in 2026 *D. labyrinthiformis* spawned on days 13 and 14 after the May full moon when the anticipated peak would be 12 days after the full moon (Figure 3). We are not certain why this difference in spawning days occurred in these two species, but we hypothesize that it may have to do with the differences in the moon calendar as calculated by the Neptune Apex season table from the natural moon calendar. In some months, the Neptune Apex season table moon calendar may be up to two days offset from the natural moon calendar as Neptune Apex calculates this schedule in a linear model, and the natural moon does not follow a strict linear track. Our *D. labyrinthiformis* broodstock have successfully spawned both seasons thus far (2025 & 2026) since their collection in late June 2024. Their reduced time in our land-based spawning systems (as compared to our other broodstock) may also be contributing to this minor asynchrony. To account for these potential shifts, we typically extend our spawn watch windows beyond peak predicted days to 8–15 days after the full moon in April through June and 5–12 days after the full moon from July through October.

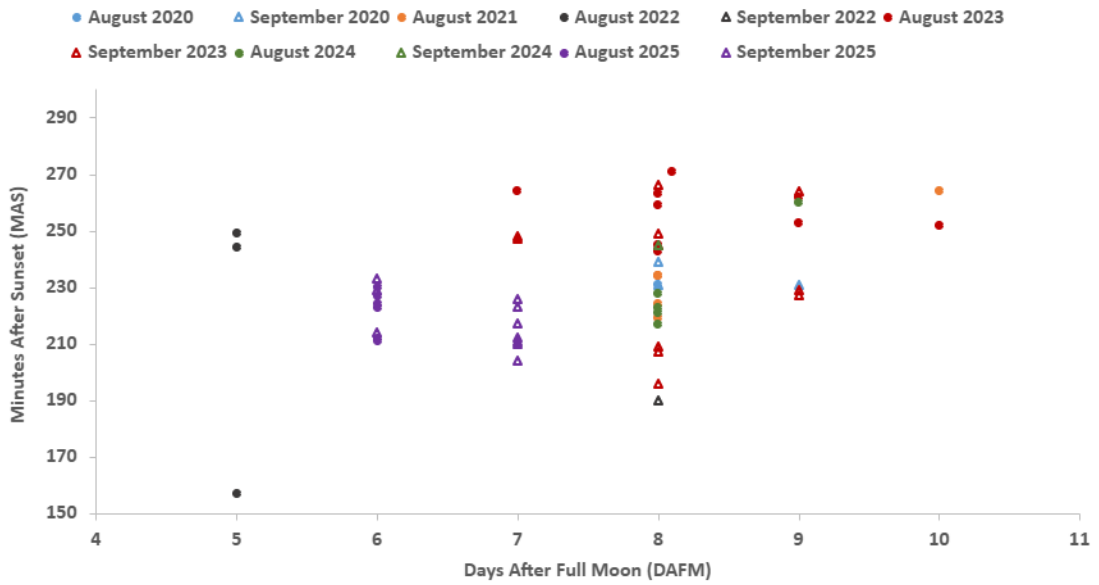

Figure 1. Observed Orbicella faveolata spawning times (minutes after sunset; MAS, and days after full moon; DAFM) observed in UNCW's spawning systems from August 2020 through September 2025. Circles denote August spawning months and triangles denote September spawning months.

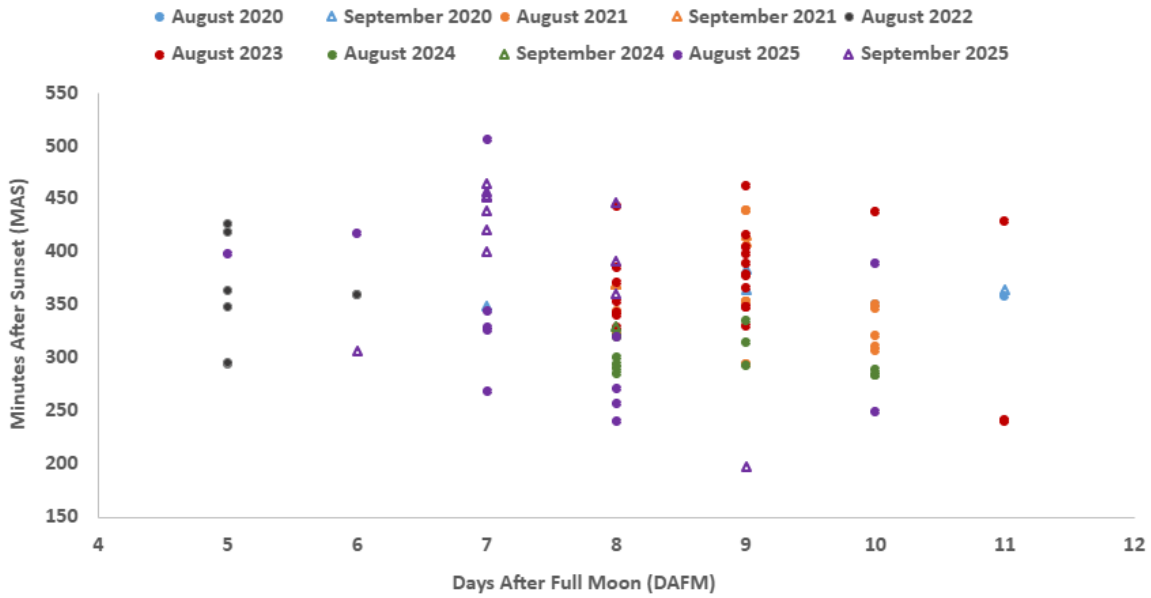

Figure 2. Observed Pseudodiploria clivosa spawning times (minutes after sunset; MAS, and days after full moon; DAFM) observed in UNCW's spawning systems from August 2020 through September 2025. Circles denote August spawning months and triangles denote September spawning months.

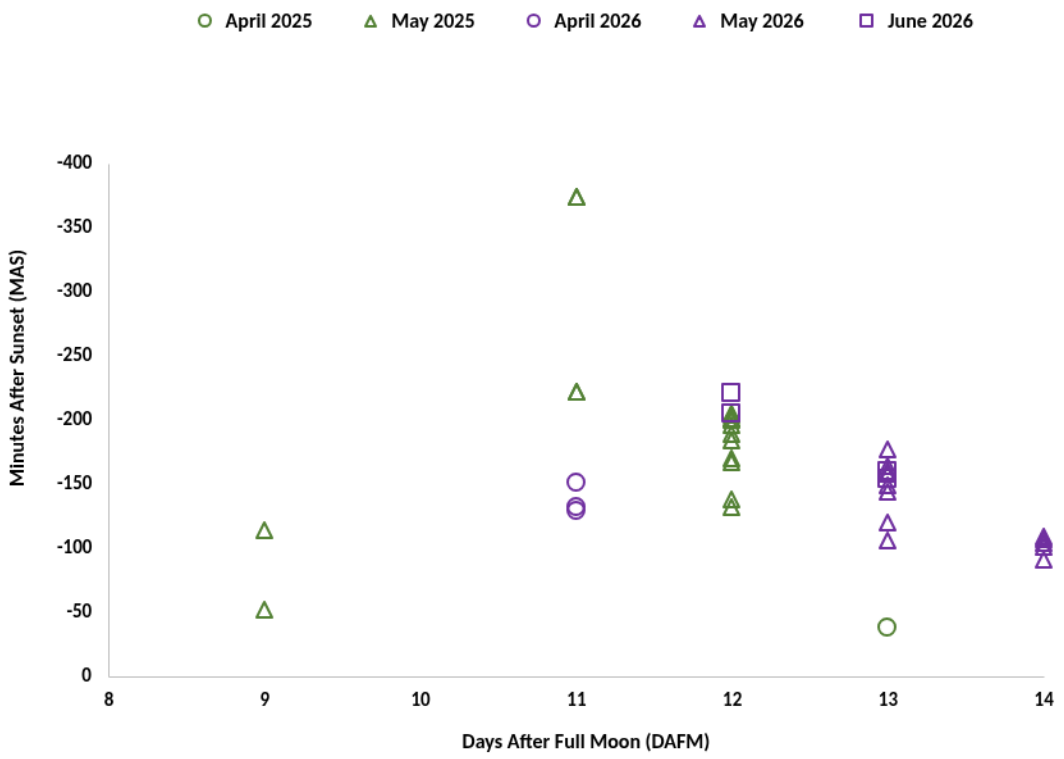

Figure 3. Observed Diploria labyrinthiformis spawning times (minutes after sunset; MAS, and days after full moon; DAFM) observed in UNCW's spawning systems from April of 2025 through May of 2026. Circles denote April spawning months, triangles denote May spawning months, and squares denote June spawning months.

In 2025, we observed high settlement success in *D. labyrinthiformis* and *O. faveolata* recruits compared to prior spawning years. For *D. labyrinthiformis,* we produced 295,891 larvae initially from six multi-parent batch crosses. Of the larvae that were not shipped to partners, we added approximately 173,575 larvae to our settlement bins and produced 9,587 settlers (18–28.1% in some settlement bins). In *A. cervicornis,* 2,600 larvae were initially produced with two multi-parent batch crosses, and 5 settled recruits. For *O. faveolata* across August and September, we produced 584,598 larvae in ten multi-parent batch crosses. Of what was not shipped to partners or used in experiments, we added 44,000 larvae to settlement bins resulting in 10,237 settlers. . In some settlement bins for *O. faveolata,* we observed 16.5–23.3% total settlement rates, which is higher than anticipated as *O. faveolata* typically has low settlement success. For *P. clivosa* across August and September, we produced an estimated 54,650 larvae in six multi-parent batch crosses resulting in 99 settled recruits. Many of these crosses were also utilized in a thermal stress settlement experiment and were exposed to a temperature hold at 32°C within 24-hours post-fertilization, so this stress likely caused high larval morality. As a result of the temperature increase and the reduced quality of *P. clivosa* spawning, many batch crosses showed less than 1% settlement rates. Thus far in 2026, we produced approximately 592,000 *D. labyrinthiformis* larvae from seven multi-parent batch crosses. In May, 74,790

larvae were added to settlement bins following shipping and experimental use, resulting in approximately 3,753 settlers. The June 2026 settler data have yet to be quantified.

In 2025 and thus far in 2026, we were able to provide gamete bundles and larvae of multiple species to various partners/collaborators. In 2025, we were able to provide an estimated 50 total gamete bundles (2 per spawning colony) for sperm data collection for task 2d (described below), 50 *O. faveolata* gamete bundles for on-site collaborators from University of Texas at Austin, and approximately 50 *O. faveolata* gamete bundles (resulting in an estimated 75,000 total larvae) for on-site UNCW collaborators across August and September. In the 2025 spawning season, we were able to send 20,000 *D. labyrinthiformis* larvae (University of Massachusetts) as well as 91,000 *O. faveolata* larvae to partners at The Reef Institute (25,000), NOAA Hollings Marine Lab (25,000), University of Miami (25,000) University of Texas Austin (1,000), Boston University (5,000), Texas A&M University (5,000), and the University of Rhode Island (5,000). In May 2026, we were able to provide 1,200 collected gamete bundles to UNCW undergraduate student research and 30 gamete bundles to on-site visiting collaborators from Duke University. We were able to ship an estimated 188,000 total *D. labyrinthiformis* larvae to partners at the University of Miami (100,000), The Reef Institute (50,000), The Florida Aquarium (30,000), and the University of Massachusetts (8,000). In June 2026, we were able to send an estimated 105,000 *D. labyrinthiformis* larvae to the Nova Southeastern University (80,000), NOAA Hollings Marine Lab (5,000), and Florida International University (20,000).

In April of 2026, an updated count was conducted to collect the number of juvenile coral recruits of various species and ages housed in our nursery raceways (Table 3). As recruits grow over time, they require larger tile sizes to support their tissue sheeting. Most *P. clivosa* recruits reared in our lab between 2021 and 2023 are now large enough to require a 7–10cm tile size and this is a limiting factor for nursery tank space available. Within the last year, we have been able to transfer many juvenile coral recruits to partner facilities to expand our available nursery growout space. For Task 4, 288 *P. clivosa* recruits produced in 2023 and 24 *P. clivosa* recruits produced in 2021 were utilized for in situ experiments. We provided 170 *M. cavernosa* (2024), 80 *O. faveolata* (2024), and 50 *P. strigosa* (2023) recruits to UM. We provided 90 *M. cavernosa* (2024) recruits and 180 *P. clivosa* (2022, 2023) recruits to NSU though 90 of these (2023) were lost by the shipping company. Lastly, we provided 119 *D. labyrinthiformis* settlement tiles with an estimated 532 total recruits and 70 *P. clivosa* recruits (2021) to the Reef Institute.

*Table 3. Current number of juvenile coral recruits held in our facility by the year they were produced. *indicates corals settled from donated larvae from Florida partners; ^ indicates lower counts than previous years due to transfer of coral recruits to partners or internal experiments/outplanting; ! indicates May settlement data only. Parenthesis indicate where the recruits were produced.*

Species	**2021**	**2022**	**2023**	**2024**	**2025**	**2026**
Orbicella faveolata (UNCW)	N/A	N/A	11	136	3680 ^	TBD
Pseudodiploria clivosa (UNCW)	169^	284^	142^	30	46	TBD
Pseudodiploria strigosa (TFA)	N/A	N/A	95*^	N/A	N/A	N/A
Acropora cervicornis (UNCW)	N/A	N/A	2*	N/A	3	TBD
Colpophyllia natans (TFA)	N/A	N/A	45*	N/A	N/A	N/A
Montastrea cavernosa (NSU)	N/A	N/A	N/A	303*^	N/A	N/A
Diploria labyrinthiformis (UNCW)	N/A	N/A	N/A	N/A	605^	3,753!

3.2. Task 2b: Crustose coralline algae species identification

Thus far, 78 algal specimens from Newfound Harbor SPA and 37 algal specimens from Looe Key SPA have been successfully sequenced and identified to the lowest taxonomic level (note, not all to species level). Across the two sites (n = 115), a total of 22 CCA species and 11 PAC species have been identified (Figure 4). This includes 17 potentially novel CCA species and 8 potentially novel PAC species (note, these potentially novel specimens are those which could not be identified to species level).

Coverage-standardized Hill diversity estimates revealed differences in assemblage structure between the two sites. Richness was higher at Newfound Harbor (25.0 effective taxa) than at Looe Key (13.6 effective taxa), whereas Shannon diversity was similar between sites (Newfound Harbor: 9.0; Looe Key: 9.3 effective taxa). Simpson diversity was higher at Looe Key (6.7 effective taxa) than at Newfound Harbor (3.8 effective taxa).

Microbiome data from a subset of the specimens collected have been received from Zymo and awaiting data analysis with Dr. Ushijima's laboratory.

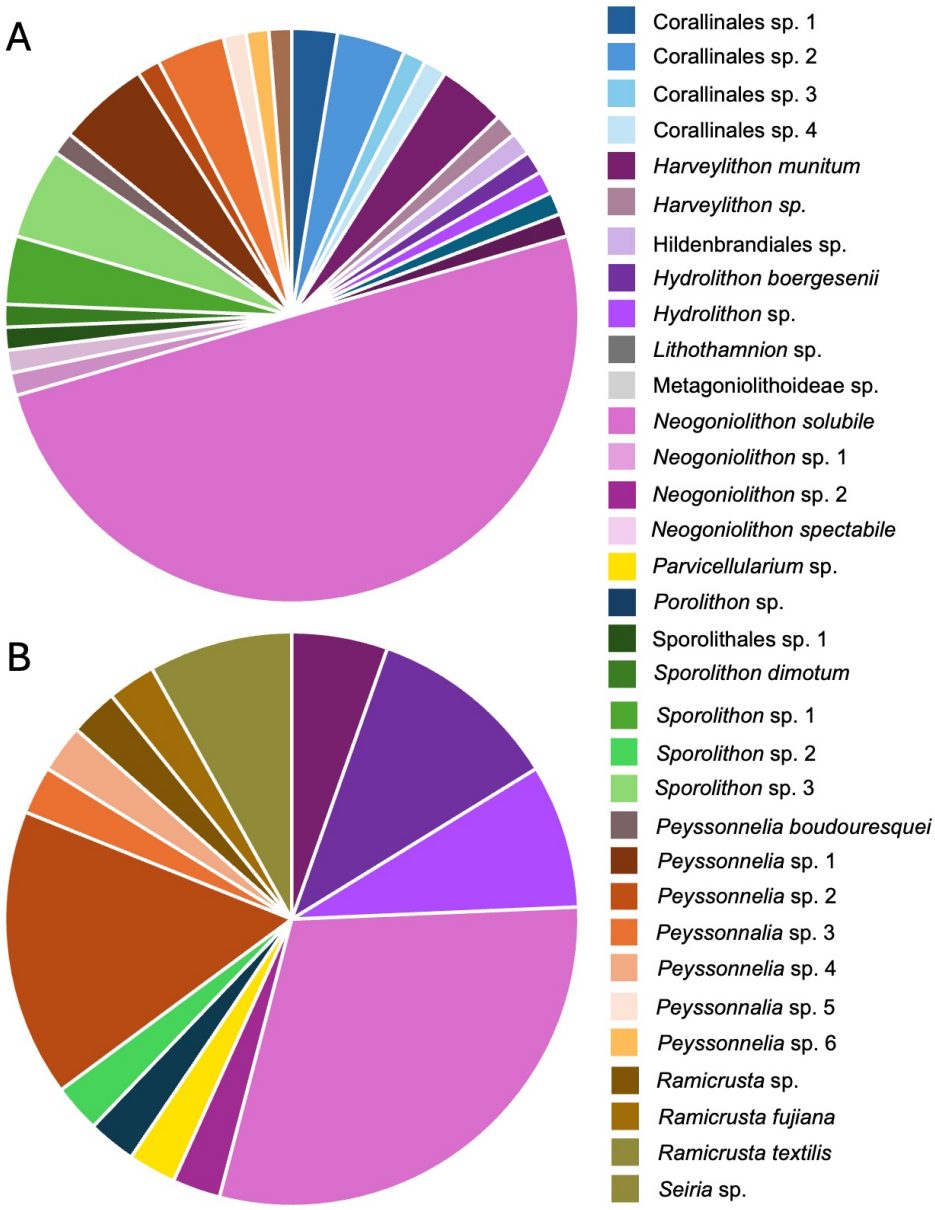

Figure 4. Relative composition of molecularly identified crustose coralline algae (CCA) and peyssonnelid algal crust (PAC) taxa at: (A) Newfound Harbor Sanctuary Preservation Area (n = 78), and (B) Looe Key Sanctuary Preservation Area (n = 37).

3.3. Task 2c: Settlement enhancement

At 48 hours, *D. labyrinthiformis* larvae survivorship was at least 45% across all non-choice larval settlement assays (Figure 5). There was no significant difference in the survival counts among treatment groups at 48 hrs (Kruskal-Wallis p= 0.1593), likely in part due to the low sample size of this pilot experiment. There were no clear trends in survival after 48 hrs for tile preparation or conditioning duration. However, when using a t-test between each pair, there were statistical differences. Tiles conditioned for 3 weeks in

our CCA tank that were lightly scrubbed prior to the experiment had higher survival than tiles that were scrubbed after being conditioned for 6 weeks (t-test, p=0.0046). The tiles that were conditioned for 6 weeks had higher survival when not scrubbed (t-test, p=0.0227). Between the tiles that were conditioned for 3 weeks, those conditioned in the CCA tank had higher survival than those that were not scrubbed and conditioned in raceways (t-test, p=0.0317). Finally tiles that were scrubbed but conditioned for only 3 weeks had higher survival than those conditioned for 6 weeks (t-test, p=0.0464).

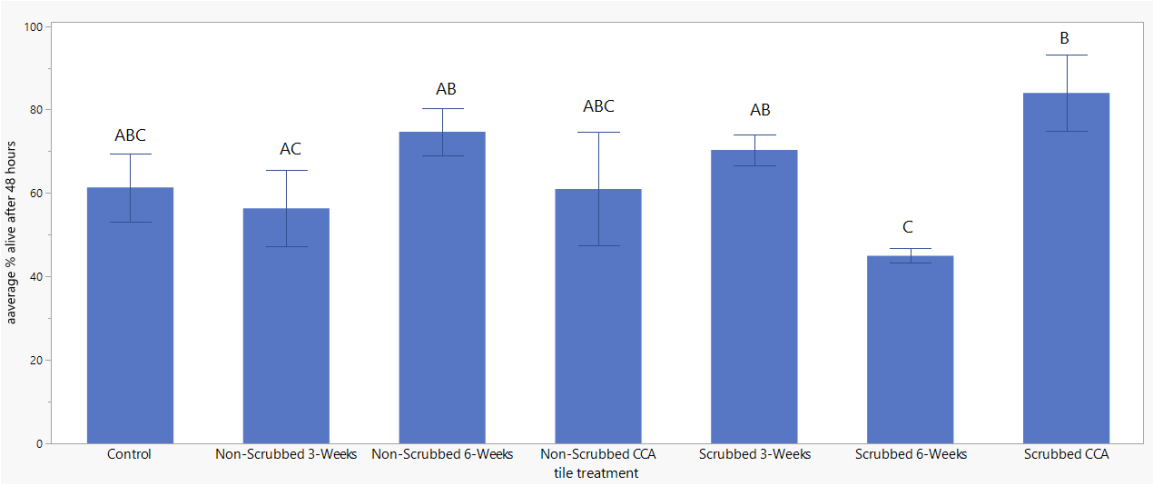

Figure 5. Diploria labyrinthiformis larval no-choice survivorship across scrubbed and non-scrubbed tiles with various conditioning durations (n = 3 for each treatment). Assays were scored for survivorship 48 hours after larvae were introduced to treatments. All error bars represent mean ± standard error.

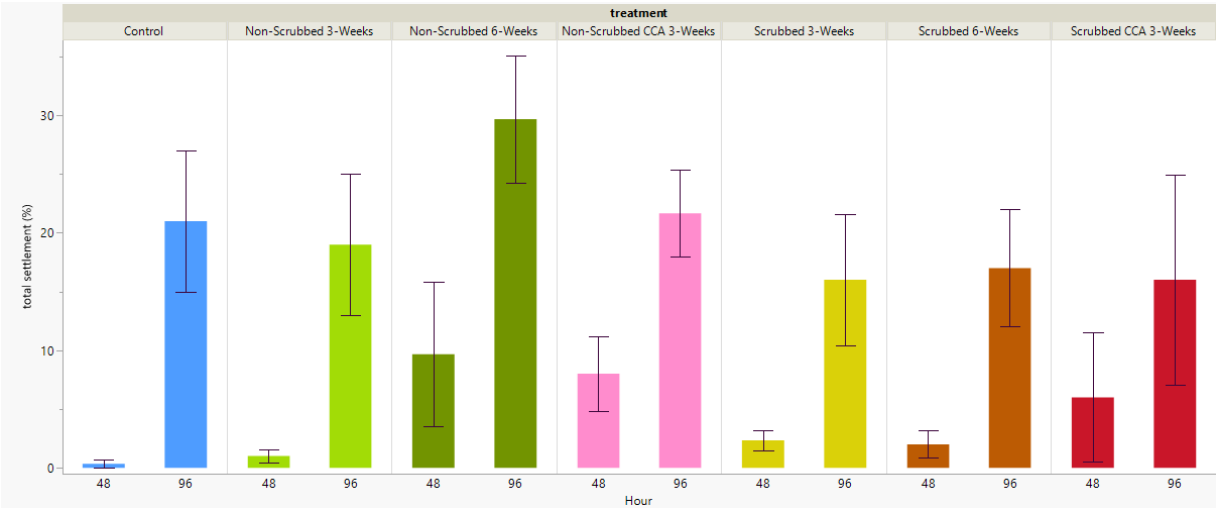

Figure 6. Diploria labyrinthiformis larval no-choice settlement across scrubbed and non-scrubbed tiles with various conditioning durations (n = 3). Assays were scored at 48 hours and 96 hours after larvae were introduced to treatments. All error bars represent mean ± standard error.

Larval settlement increased between 48 and 96 hours across all treatments (Figure 6). All treatments had at least 16% settlement overall, with the highest at 29.7 for non-scrubbed 6-week tiles. While all treatments had higher settlement than the control at 48 hours, the only treatment to outperform the control in settlement at 96 hours were the non-scrubbed 6-week tiles. A trend to focus on in the future is both scrubbed and non-scrubbed tiles and time of assay scoring.

At 48 hours, *D. labyrinthiformis* averaged 50.0 ± 13.1% survivorship across all choice larval settlement assays (Figure 7) There was little to no variation between treatments, likely in part to the low level of replication in this pilot experiment.

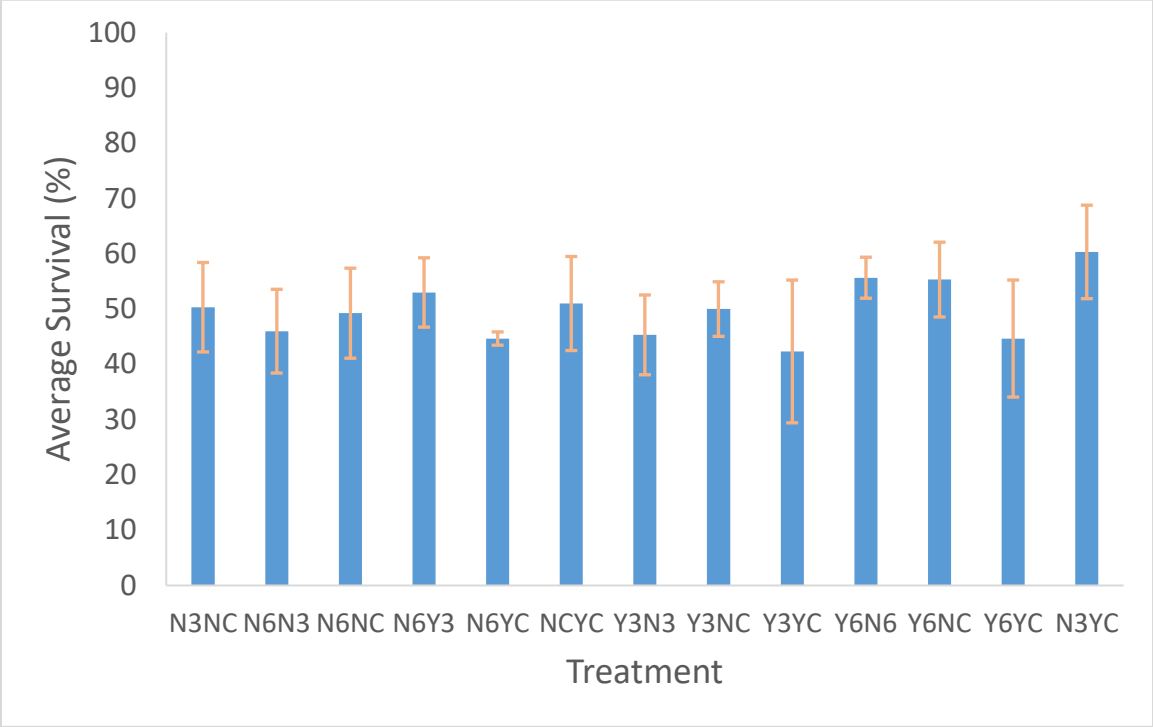

Figure 7. Diploria labyrinthiformis larval choice-assay survivorship across scrubbed and non-scrubbed tiles with various conditioning durations (n = 3 for each treatment; N6NC and N6Y3 are n = 4). Entire assay containers and both tiles within were scored for survivorship 48 hours after larvae were introduced to treatments. Treatment names were derived from combinations of the following: Y3 = scrubbed three-weeks, N3 = non-scrubbed three-weeks, Y6 = scrubbed six-weeks, N6 = non-scrubbed six-weeks, YC = scrubbed CCA-tiles 3-Weeks, and NC = non-scrubbed CCA-tiles 3-Weeks. All error bars represent mean ± standard error.

A majority of the larvae in all choice-assay treatments settled on the plastic Tupperware container instead of the tiles and is depicted below (Figure 8). Consistent with the low variation with survival, larval settlement was also low overall, potentially a consequence of poor water quality or smaller container size.

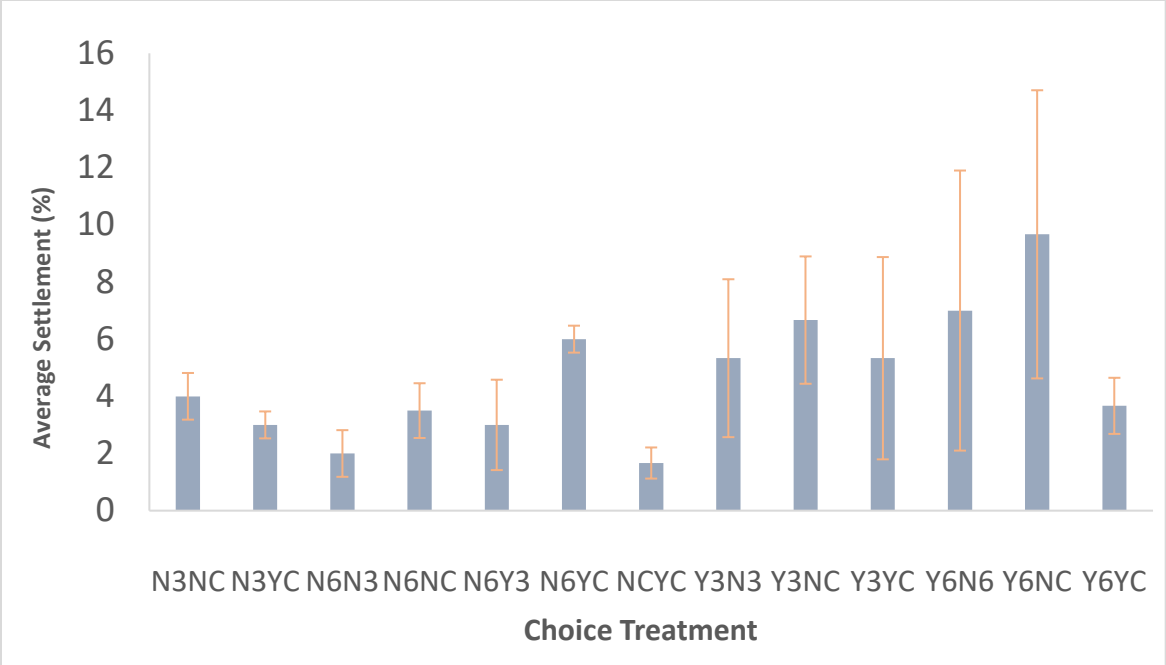

Figure 8. Diploria labyrinthiformis larval choice assay average settlement across scrubbed and non-scrubbed tiles with various conditioning durations (n = 3 for each treatment; N6NC and N6Y3 are n = 4). Entire assay containers and both tiles were scored for settlement 48 hours after larvae were introduced to treatments. Individual tiles were scored for settlement but are not depicted here. Treatment names were derived from combinations of the following: Y3 = scrubbed three-weeks, N3 = non-scrubbed three-weeks, Y6 = scrubbed six-weeks, N6 = non-scrubbed six-weeks, YC = scrubbed CCA-tiles 3-Weeks, and NC = non-scrubbed CCA-tiles 3-Weeks. All error bars represent mean ± standard error.

3.4. Task 2d: Sperm traits

Orbicella faveolata average sperm motility was initially 51.8% and declined to 23.3% after 11 hours of sampling (Figure 9). Initial velocity of *O. faveolata* sperm was 164.8 ± 56.2 µm s^{-1} and increased to 196.4 ± 61.3 µm s^{-1} after 11 hours (Figure 10). *Pseudodiploria clivosa* average sperm motility was initially 23.3% and declined to 7.5% after 6 hours of sampling (Figure 9). Initial velocity of *P. clivosa* sperm was 123.4 ± 34.7 µm s^{-1} and decreased to 90.8 ± 23.3 µm s^{-1} after 6 hours (Figure 10). *Acropora cervicornis* average sperm motility was initially 12.8% and increased to 16.6% after 4 hours of sampling (Figure 9). Initial velocity of *A. cervicornis* sperm was 107.8 ± 37.9 µm s^{-1} and decreased slightly to 105.8 ± 55.5 µm s^{-1} after 4 hours (Figure 10). *Montastraea cavernosa* had very limited spawn; additionally collecting sperm from gonochores at high enough concentration to measure sperm velocity and motility proved quite difficult. Therefore, there is limited data available (n = 2) from this species. Average sperm motility was initially 16% and increased to 3.6% after 2 hours of sampling (Figure 9). *Diploria labyrinthiformis* average sperm motility was initially 42.1% and decreased to 13.4% after

8 hours of sampling (Figure 9). Initial velocity of *D. labyrinthiformis* sperm was 142.1 ± 33.3 μm s^{-1} and decreased to 76.6 ± 23.1 μm s^{-1} after 8 hours (Figure 10).

There were significant differences in sperm motility across species ($p < 0.001$); *O. faveolata* had significantly higher motility than *P. clivosa, A. cerviconis, M. cavenosa,* and *D. labyrinthiformis* (Figure 9; $p < 0.0001$). There were marginally significant differences in motility over time ($p = 0.0534$). Specifically, motility significantly decreased between 0 and 11 hours after bundle breakage ($p < 0.01$) and 1 and 11 hours after bundle breakage ($p < 0.01$). There were significant differences in sperm velocity across species (Figure 10; $p < 0.0001$). *Orbicella faveolata* had significantly faster sperm than *P. clivosa* and *D. labyrinthiformis* ($p < 0.0001$). There were also significant differences in velocity over time ($p < 0.05$), but the velocity trends vary by species.

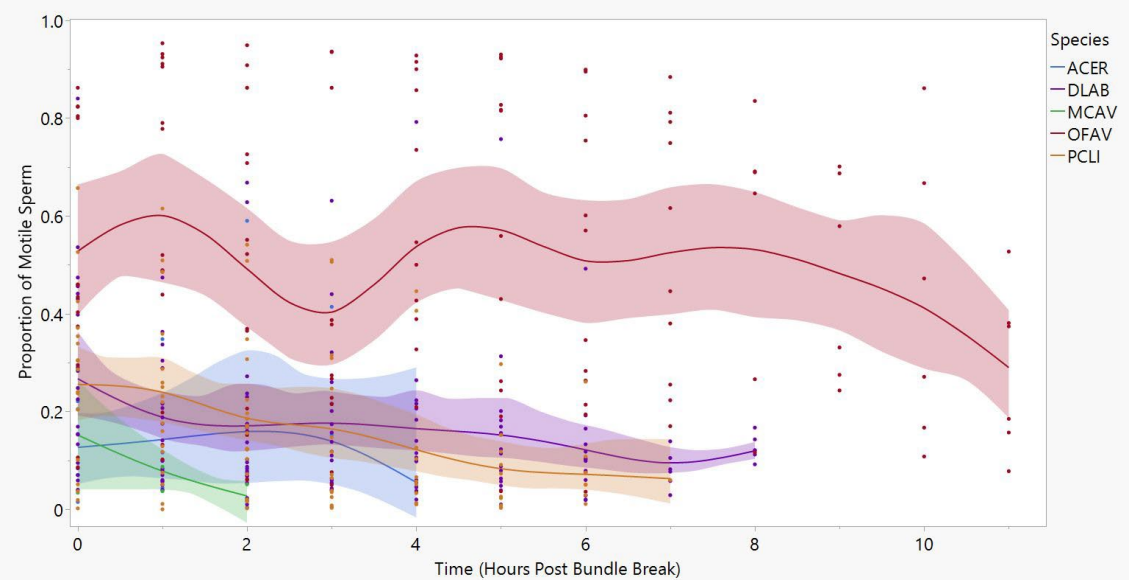

Figure 9. Sperm motility over time for O. faveolata (OFAV), P. clivosa (PCLI), A. cervicornis, (ACER) M. cavernosa (MCAV), and D. labyrinthiformis (DLAB). Motility was assessed hourly for up to 8 hours after bundle breakage or until motility was equal or less than 10%. Circles represent samples from each colony, whereas lines represent the line of best fit for the data and shaded areas indicate the 95% confidence interval.

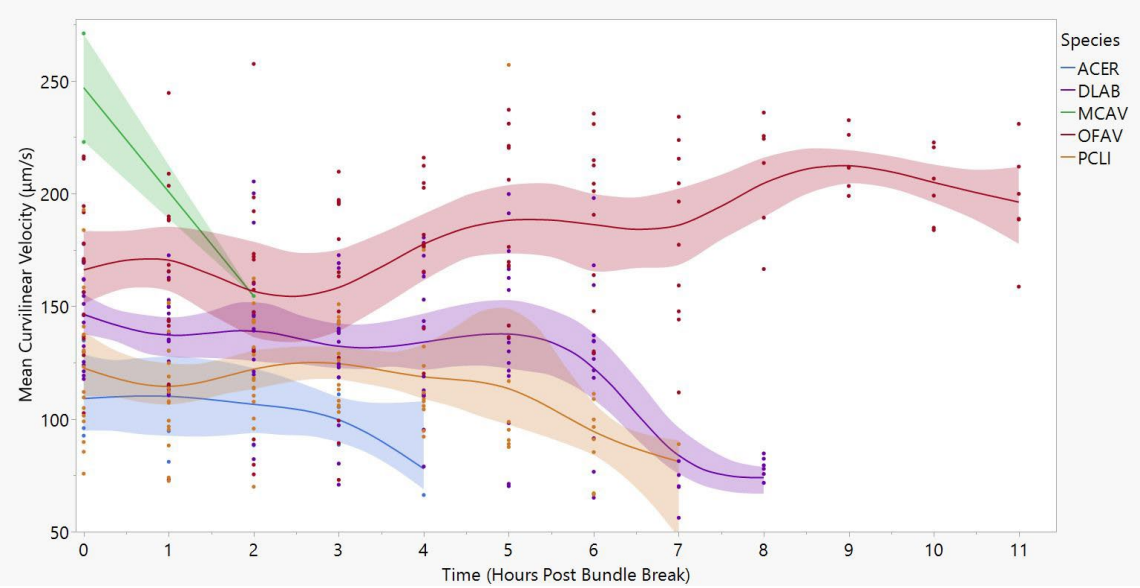

Figure 10. Sperm velocity over time for O. faveolata (OFAV), P. clivosa (PCLI), A. cervicornis (ACER), M. cavernosa (MCAV), and D. labyrinthiformis (DLAB). Circles represent mean velocity from individual colonies, lines represent the line of best fit for the data, and shaded areas indicate the 95% confidence interval.

Average *A. cervicornis* sperm tail length was 4.32 ± 0.659 µm (n = 55) and ranged from 2.32–5.93 µm (Figure 11). Of the tails measured 42% had abnormalities, most commonly bent and coiled tails. Due to low spawning magnitude, *M. cavernosa* sperm was limited to assessing morphology only. The average *M. cavernosa* sperm tail length was 4.01 ± 1.27 µm (n = 10) and ranged from 1.59–5.25 µm (Figure 11). Average *O. faveolata* sperm tail length was 5.09 ± 0.44 µm (n = 1,795) and ranged from 0.71–6.44 µm (Figure 11). Of the tails measured 29.3% had abnormalities, most commonly bent and coiled tails. Average *P. clivosa* sperm tail length was 4.05 ± 0.48 µm (n = 2,335) and ranged from 1.4–6.10 µm (Figure 11). Of the tails measured 20.9% had abnormalities, most commonly coiled tails. Average *D. labyrinthiformis* sperm tail length was 4.28 ± 0.50 µm (n = 1,453) and ranged from 0.11–5.35 µm (Figure 11). Of the tails measured 8.3% had abnormalities; most commonly tails contained droplets. There were significant interspecific differences in sperm tail length (Figure 11; $\chi 2(4) = 3264.5753$, $p < .0001$). Specifically, *P. clivosa* had significantly shorter tails than *A. cervicornis, D. labyrinthiformis,* and *O. faveolata* ($p < 0.001$), while *O. faveolata* had significantly longer tails than *A. cervicornis, D. labyrinthiformis,* and *M. cavernosa* ($p < 0.001$).

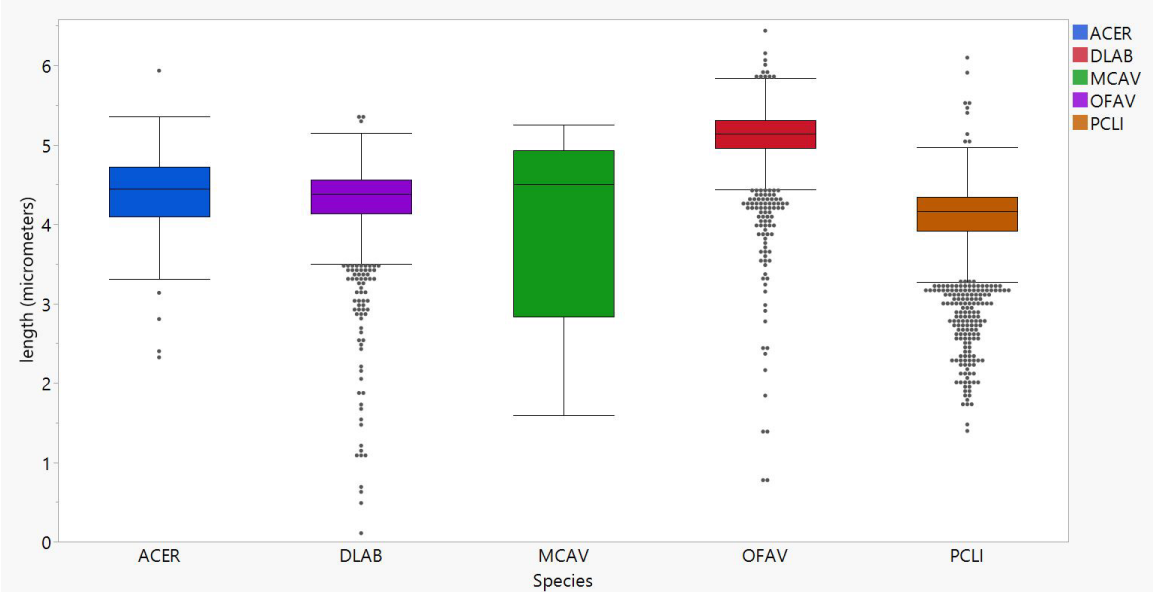

Figure 11. Sperm tail length in O. faveolata (OFAV), P. clivosa (PCLI), A. cervicornis (ACER), D. labyrinthiformis (DLAB), and *M. cavernosa (MCAV). Error bars are standard deviations; circles represent outliers, and solid lines represent the median tail length.*

3.5. Task 2e: Finalize AI coral area sizing tool (COAST) program

The COAST system achieved a mean IoU exceeding 95% and an auto-acceptance rate (AAR) of 71.5%, where predicted-to-ground-truth area ratios fall within a ±5% tolerance of expert annotation, substantially reducing manual workload while maintaining measurement reliability across species, developmental stages, and imaging conditions (Zhao et al., 2026). Overall, the pipeline reduces processing time to approximately 3–5 s per image—a 24–140× speedup over manual tracing (Zhao et al., 2026). Additional results of this work have been published in Zhao et al. (2026; https://doi.org/10.3390/s26082291). This program can be applied to any photograph that has pixel size data embedded in the image.

3.6. Task 3: Spawning calendar

Spawning time observations were collected for eleven species (Table 4; Table 5) and spans 24 years (2001–2025) for *A. cervicornis*. Spawning for this species occurred from -7–15 days after the full moon (DAFM), between 30–255 minutes after sunset (MAS). There were significant differences in regional spawning times, at both the DAFM (Figure 12A; chi-sq = 7.8335, df =3, $p < 0.05$) and MAS (Figure 12B; chi-sq = 25.9958, df =3, $p < 0.0001$) scale. The Upper Florida Keys spawned significantly earlier in terms of DAFM compared to the Middle Keys ($p < 0.05$), but not significantly earlier than Southeast Florida, Upper and Lower Florida Keys ($p > 0.05$). All other regions did not have significantly different spawning days ($p > 0.05$). Similarly, the Upper Florida Keys spawned significantly earlier in terms of MAS than Southeast Florida ($p < 0.0001$), Middle Florida Keys ($p < 0.05$), and the Lower Florida Keys ($p < 0.001$). Corals in the Middle

Florida Keys spawned significantly earlier than those in Southeast Florida ($p < 0,05$). To assess historical shifts in spawning, observations were placed into bins spanning ~5 years. There were no significant differences between days (chi-sq = 0.9266, df = 4, $p = 0.19207$) of spawning, however there were significant differences in the minutes of spawning (Figure 12c; chi-sq = 17.7410, df = 4, $p < 0.01$). Specifically, corals in 2021–2025 spawned significantly later than corals in 2006–2010 ($p < 0.01$) and 2011–2015 ($p < 0.01$), corals in 2016–2020 spawned significantly later than corals in 2006–2010 ($p < 0.01$) and 2011–2015 ($p < 0.01$), and corals in 2001–2005 spawned significantly later than corals in 2006–2010 ($p < 0.05$) and 2011–2015 ($p < 0.05$). There were no significant differences in the day of spawning based on observation method (chi-sq = 4.9504, df = 2, $p > 0.05$). However, there was a significant difference in spawning time in MAS depending on observation method (Figure 12D; chi-sq = 32.28, df = 2, $p < 0.001$). Specifically, corals observed spawning *in situ* spawned significantly earlier in MAS than corals observed spawning ex *situ* ($p < 0.0001$) and in induction systems ($p < 0.0001$).

Spawning data spans 30 years (1995–2025) for *A. palmata*. Spawning for this species occurred from -5–15 days after the full moon (DAFM), between 55–171 minutes after sunset (MAS). There were no significant differences in regional spawning times in terms of DAFM (chi-sq = 4.5256, df = 2, $p = 0.1041$). However, there were significant differences in spawning in terms of MAS by region (Figure 13A; chi-sq = 9.4549, df = 2, $p < 0.01$). Similar to patterns observed in *A. cervicornis*, *A. palmata* in the Upper Florida Keys spawned significantly earlier in terms of MAS than Southeast Florida ($p < 0.01$). Additionally, *A. palmata* in the Lower Florida Keys spawned significantly earlier than those in Southeast Florida at the MAS scale ($p < 0.05$). There were no significant differences between the Lower and Upper Keys spawning times in terms of MAS ($p > 0.05$) and there was insufficient data from the Middle Florida Keys to assess synchrony at the MAS level. To assess historical shifts in spawning, observations were placed into bins spanning ~5 years. There were significant differences between days (Figure 13B; chi-sq = 14.2304, df = 5; $p < 0.05$). Corals in 2020–2025 spawned significantly closer to the full moon than corals in 1995–1999 and 2000–2004 ($p < 0.05$). Corals in 2015–2019 spawned closer to the full moon than corals in 1995–1999 ($p < 0.05$), 2000–2004 ($p < 0.01$), 2010–2014 ($p < 0.05$), and marginal significance was found from 2005–2009 (p = 0.0538). Corals in 2000–2004, 2005–2009, and 2010–2014 spawned significantly closer to the full moon than those in 1995–1999 ($p < 0.05$). There were no significant differences in spawning time in minutes after sunset (chi-sq = 8.1847, df = 4, $p = 0.085$). There were insufficient data to determine whether there were significant differences in spawning times based on observation method, as a majority of data compiled were from *in situ* observations.

*Table 4. Spawning times for Florida coral species based on in situ data. * indicates minutes before sunset. DAFM = days after full moon. MAS = minutes after sunset.*

Species	Spawning Month(s)	DAFM	MAS
Acropora cervicornis	July–August	1–15	30–192
Acropora palmata	July–September	-5–12	55–171
Dendrogyra cylindrus	August	2–4	54–110
Diploria labyrinthiformis	April–June	10–13	54*–120*
Montastraea cavernosa	August–September	4–8	19*–96
Orbicella annularis	August–September	6–8	185–216
Orbicella faveolata	August–September	5–11	171–255
Pseudodiploria strigosa	August–September	6–8	55* – 5

*Table 5. Spawning times for Florida coral species based on ex situ data. * indicates minutes before sunset. DAFM = days after full moon. MAS = minutes after sunset.*

Species	Spawning Month(s)	DAFM	MAS
Acropora cervicornis	July–September	-7–9	0–255
Acropora palmata	July–August	0-9	79–153
Colpophyllia natans	August–October	5–10	188*–8; 98–214
Dendrogyra cylindrus	July–August	1–5	87–138
Diploria labyrinthiformis	April–July	9–15	38*–374*
Montastraea cavernosa	July–October	1–11	46*–714
Orbicella faveolata	July–September	6–11	97–275
Orbicella franksi	August–September	7–10	86–259
Pseudodiploria clivosa	August–October	5–11	182–701
Pseudodiploria strigosa	August–October	5–13	86*–73; 168–284

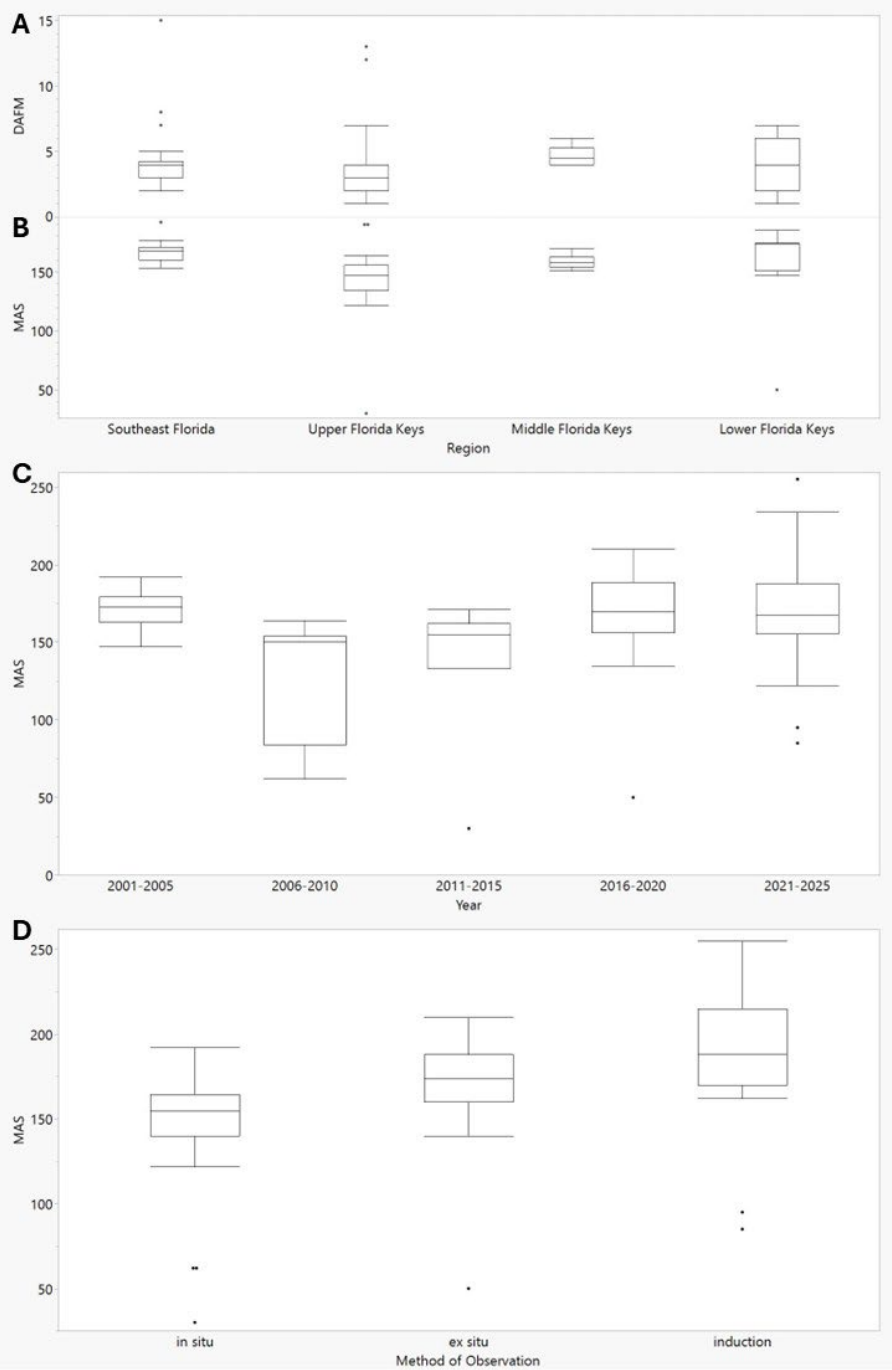

Figure 12. A) Regional differences in spawning times (days after the full moon; DAFM) of A. cervicornis. Circles indicate outliers and solid lines indicate mean spawning time (DAFM). B) Regional differences in spawning times (minutes after the sunset; MAS) of A. cervicornis. Circles indicate outliers and solid lines indicate mean spawning time (DAFM). C) Historical spawning times (minutes after sunset; MAS) of A. cervicornis. Circles indicate outliers and solid lines indicate mean spawning time (MAS). D) Spawning times (minutes after sunset; MAS) of A. cervicornis by observation method. Circles indicate outliers and solid lines indicate mean spawning time (MAS).

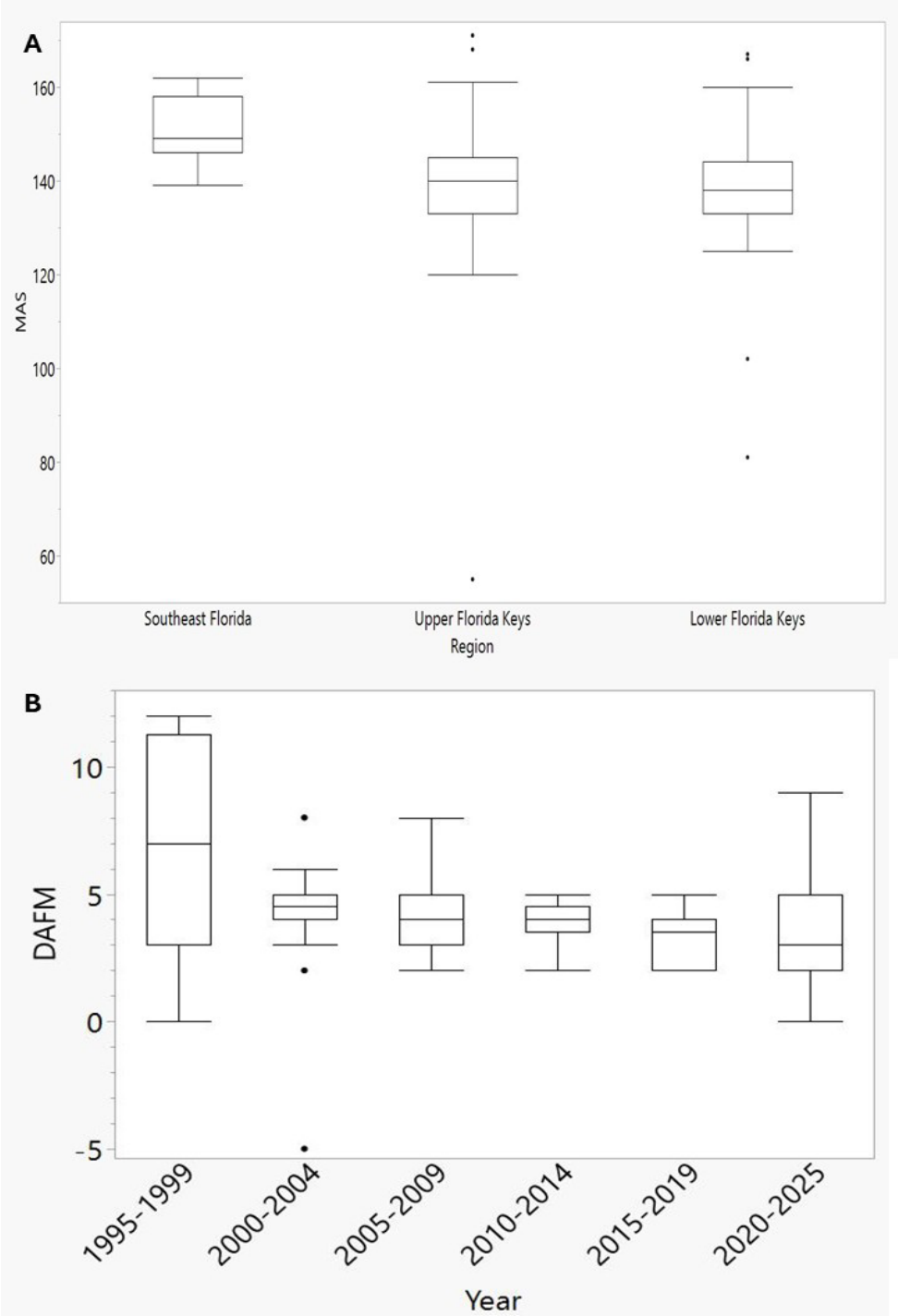

Figure 13. A) Regional differences in spawning times (minutes after the sunset; MAS) and B) historical differences in spawning times (days after full moon; DAFM) of A. palmata. Circles indicate outliers and solid lines indicate mean spawning time.

Spawning data spans 32 years (1993–2025) for *O. faveolata*. Spawning for this species occurred from 4–13 DAFM, between 97–275 MAS. There were significant differences in regional spawning times, at the DAFM scale (Figure 14A; chi-sq = 9.573, df = 3, $p < 0.05$). The Middle Florida Keys spawned significantly later in terms of DAFM compared to the Upper Keys and Southeast Florida ($p < 0.01$). All other regions did not have significantly different spawning days ($p > 0.05$). There were no significant differences in spawning time in terms of MAS between Southeast Florida and the Upper Florida Keys (chi-sq = 2.2073, df = 1, $p = 0.1374$). There was insufficient data from the Middle and Lower Florida Keys to assess synchrony at the MAS level. To assess historical shifts in spawning, observations were placed into bins spanning 5 years. There were significant differences in historic timing in terms of DAFM (Figure 14B; chi-sq = 29.5415, df = 6, $p < 0.0001$). Specifically, corals between 2023–2025 spawned significantly later in the lunar cycle than corals that spawned between 1998–2002 ($p < 0.05$), 2003–2007 ($p < 0.001$), 2008–2012 ($p < 0.01$), and 2018–2022 ($p < 0.001$). There were marginally significant differences with corals spawning later in terms of DAFM between 2023–2025 and 1993–1997 ($p = 0.0787$) and 2013–2017 ($p = 0.0549$). However, it is unclear at this time whether this is due to increased observations in recent years and/or environmental changes. There were insufficient data to assess historical shifts in MAS from 1993–2017; there were no significant differences in spawning times in MAS between 2018–2022 and 2023–2025 ($p = 0.6191$). There were significant differences in the day of spawning based on whether observations were conducted *in situ,* in an induction system, or *ex situ* (Figure 14C; chi-sq = 62.9326, df = 2, $p < 0.0001$), with *in situ* corals spawning significantly closer to the full moon than corals in induction systems ($p < 0.0001$). There was insufficient data to assess MAS-scale differences using *ex situ* data, however no significant difference in spawning times in terms of MAS between *in situ* and induction system observations were documented ($p = 0.1226$).

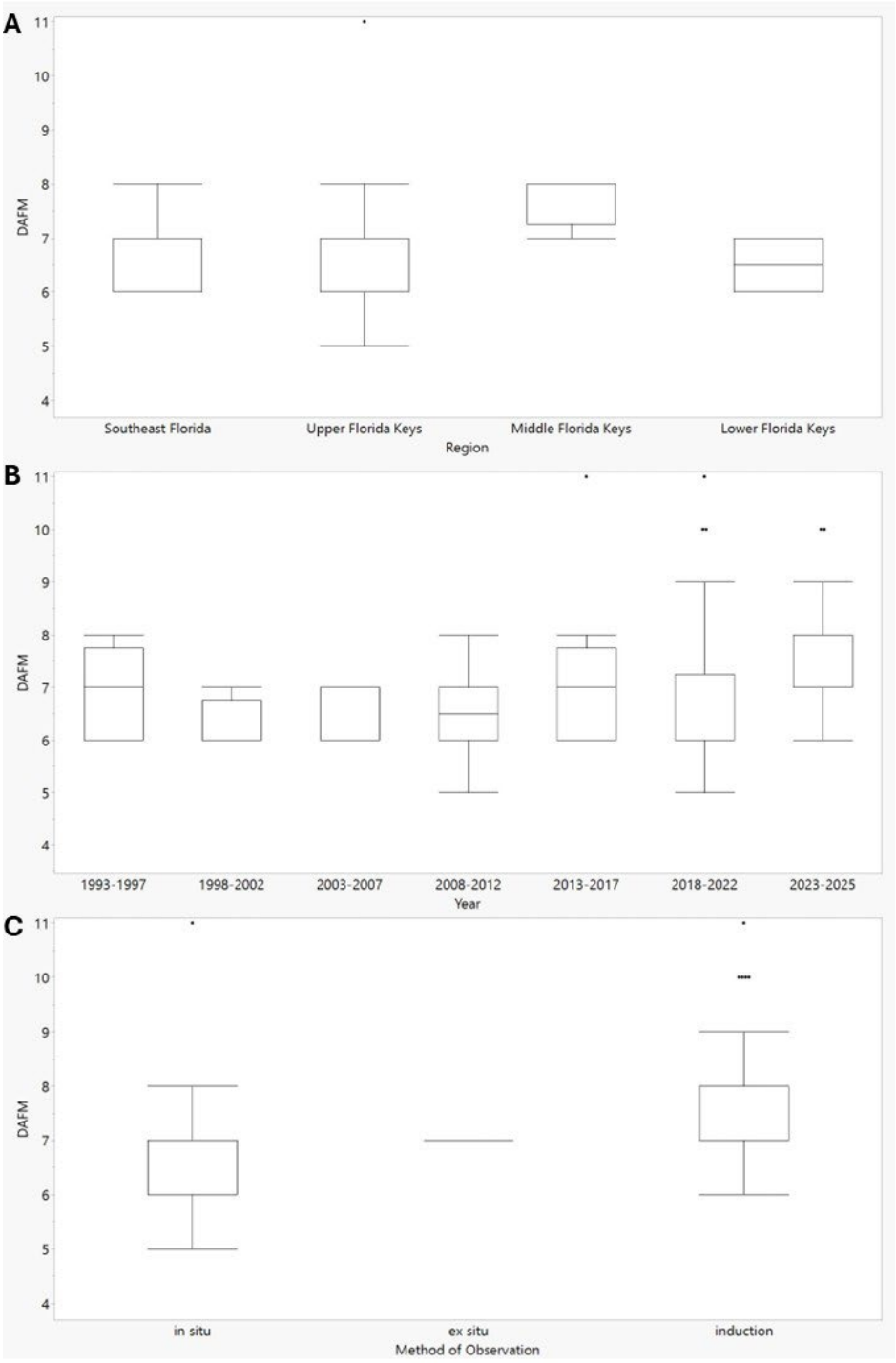

Figure 14. A) Regional differences in spawning times (days after the full moon; DAFM) of O. faveolata. B) Historical shifts in spawning times (days after the full moon; DAFM) of O. faveolata. C) Spawning times (days after the full moon; DAFM) of O. faveolata by observation method. Circles indicate outliers, and solid lines indicate mean spawning time (DAFM).

We have collected 5 years of data for *D. labyrinthiformis* and *P. strigosa* across i*n situ*, *ex situ*, and induction system observations. While there was insufficient data to assess regional and historical shifts in spawning for these two species, enough data was available to assess significant differences between observation methods. There were significant differences in spawning times in terms of DAFM (Figure 15; chi-sq = 136.3827, df = 2, $p < 0.0001$), with both *in situ* and *ex situ D. labyrinthiformis* spawning occurring significantly closer to the full moon than induction system spawning ($p < 0.0001$). However, there were no significant differences in spawning times in terms of MAS across observation methods for *D. labyrinthiformis* (chi-sq = 2.39, df = 2, $p = 0.302$). There were significant differences in spawning times in terms of DAFM (Figure 16a; chi-sq = 95.604, df = 2, $p < 0.0001$), with *in situ P. strigosa* spawning occurring significantly closer to the full moon than induction system spawning ($p < 0.0001$). Additionally, there were significant differences in spawning times in terms of MAS across observation methods for *P. strigosa* (Figure 16b; chi-sq = 64.2609, df = 2, $p < 0.0001$). Specifically, induction corals spawn significantly later than *in situ* corals ($p < 0.0001$). This may be a result of sampling bias due to logistical constraints making monitoring the "early group" spawn (86 MBS – 68 MAS) of *P. strigosa in situ* easier. However, the "early" group spawn *in situ* peaks between 30–10 minutes before sunset, whereas the "early group" spawn in induction systems peaks between 41–70 minutes after sunset. There was insufficient data to assess differences with *ex situ* observations.

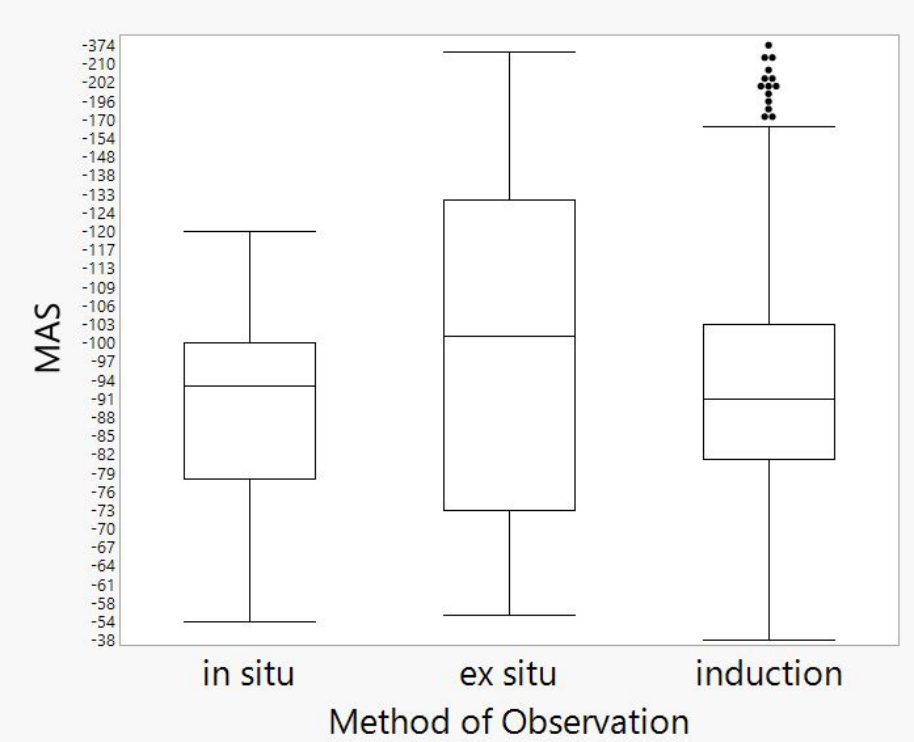

Figure 15. Spawning times (minutes after sunset; MAS) of D. labyrinthiformis by observation method. Circles indicate outliers, and solid lines indicate mean spawning time (MAS).

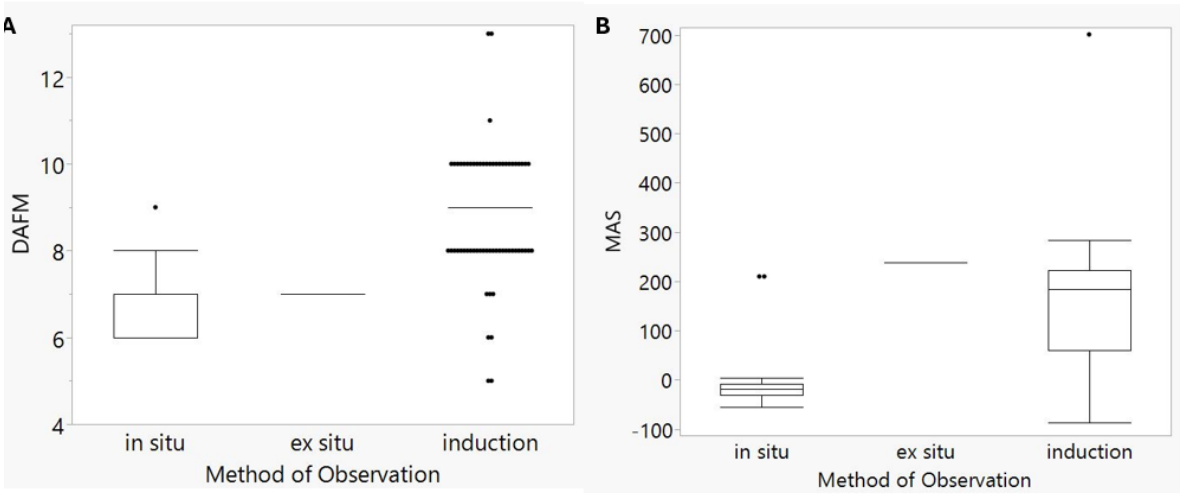

Figure 16. A) Spawning times (days after the full moon; DAFM) of P. strigosa by observation method. Circles indicate outliers and solid lines indicate mean spawning time (DAFM). B) Spawning times (minutes after sunset; MAS) of P. strigosa by observation method. Circles indicate outliers, and solid lines indicate mean spawning time (MAS).

3.7. Task 4: Preconditioning and outplanting

Survival remained high during the first 5 months across both sites, with only five corals reported to be lost or dead by October 2025 (4 from Looe Key and one from Newfound Harbor) (Figure 17).

Two weeks after outplanting the corals in May 2025, paling and predation were site-dependent, with significantly more paling and predation at Looe Key, potentially resulting from the shuffling of symbionts as corals acclimate local conditions. Increased predation is also common within the first couple of weeks following outplanting.

In July 2025, paling was widespread at both sites, with nearly all corals showing paling resulting from thermal stress. Corals at Looe Key exhibited higher algal overgrowth and tissue recession, likely due to environmental conditions at the site (e.g., higher light intensity, lower turbidity, increased depth on one transect).

In October 2025, paling remained widespread at both sites with nearly all corals exhibiting some paling. Corals at Newfound Harbor showed significantly more bleaching compared to those at Looe Key, while corals at Looe Key continued to have more paling and significantly more algal overgrowth.

At this time, coral outplants have undergone their full cycle of thermal stress *in situ* (July–October 2025) and should begin to show recovery in our final monitoring trip (one-year; May 2026).

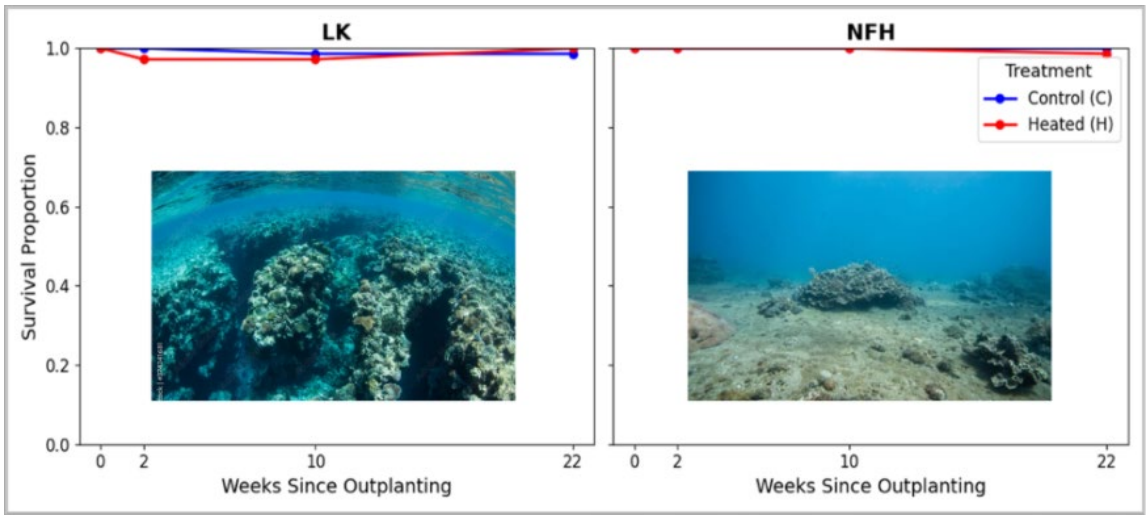

Figure 17. Survival of control (blue) and preconditioned (red) P. clivosa over time from original outplanting in May 2025, at Looe Key (n = 144) and Newfound Harbor (n = 144).

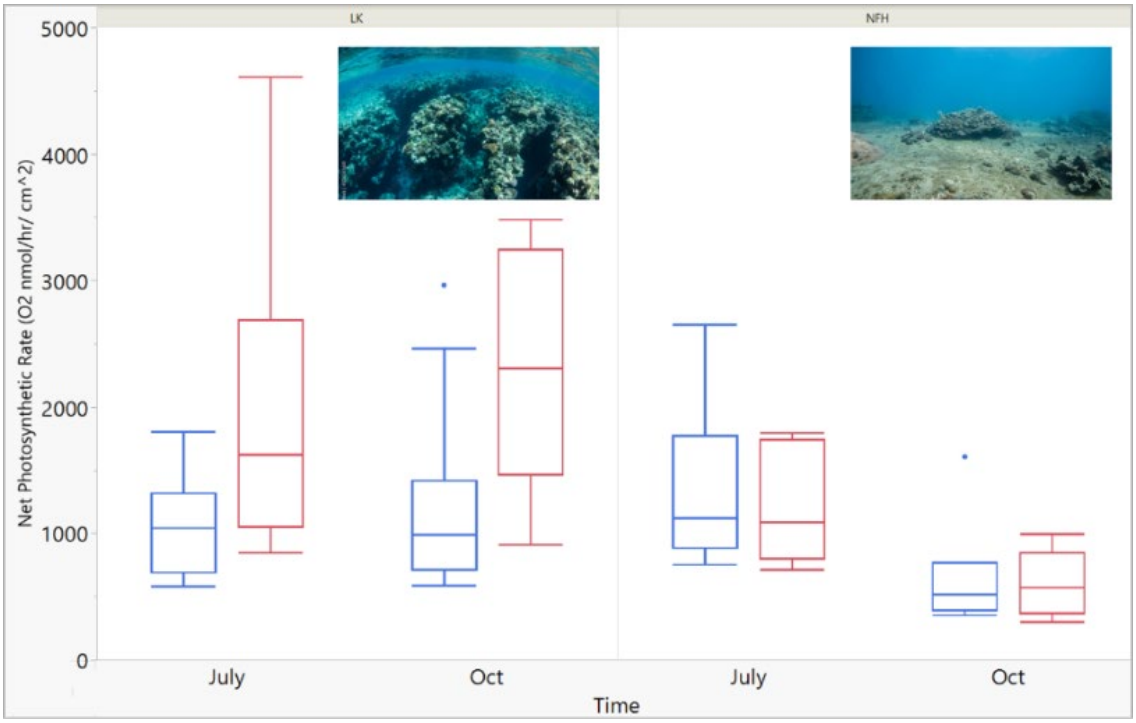

Figure 18. Net photosynthetic rates (O_2 $nmol^{-1}$ hr^{-1} cm^{-2}) from control (blue) and preconditioned (red) corals from Looe Key (LK; n = 144) and Newfound Harbor (NFH; n = 144) in July and October 2025.

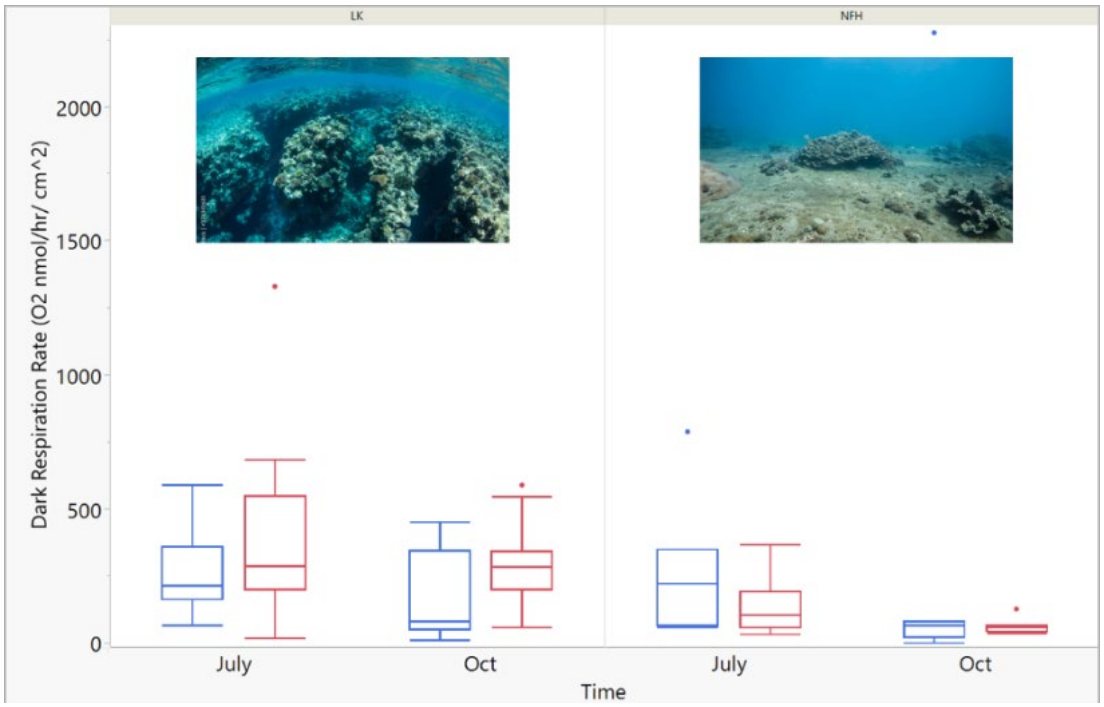

Figure 19. Dark respiration rates (O_2 $nmol^{-1}$ hr^{-1} cm^{-2}) from control (blue) and preconditioned (red) corals from Looe Key (LK; n = 144) and Newfound Harbor (NFH; n = 144) in July and October 2025. While the values are positive, this is the oxygen which is being used (not produced) during respiration.

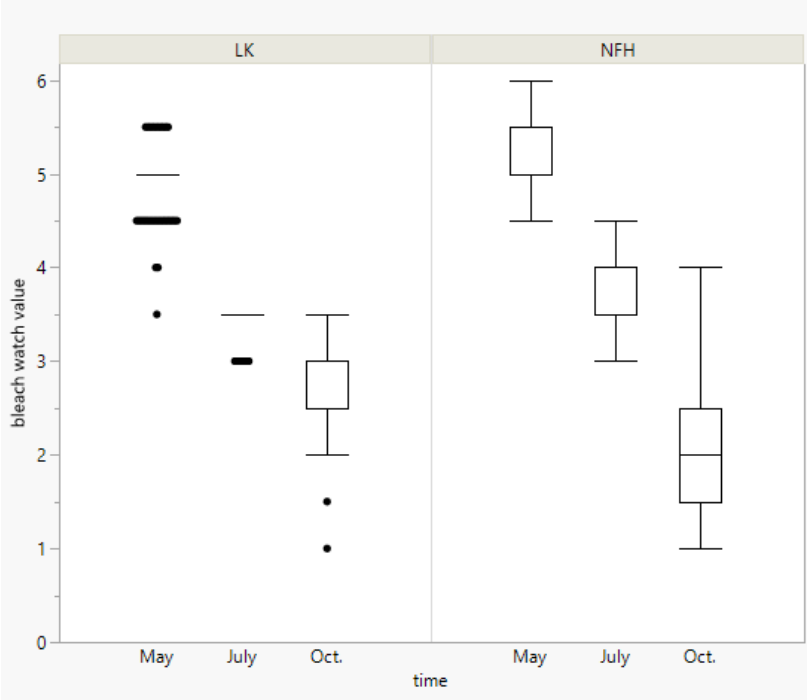

Figure 20. Bleaching watch pigmentation values between sites across the 3 timepoints. Lower values indicate pale and bleached corals. LK= Looe Key; NFH= Newfound Harbor.

Preliminary results suggest no significant difference in respiration between control and preconditioned corals in July, nor October, at either site (Figures 18 & 19). However, these results are pending baseline calibration of CISME measurements that will be gathered in June 2026.

Bleaching/paling varied among site and month. Using a GLMM with time, treatment, and site as the fixed factors, there was a significant interaction with site and time (GLMM, $p<0.001$). Treatment was not significantly different (GLMM, $p=0.3376$); however Loe Key had higher bleaching/paling compared to Newfound Harbor (GLMM, $p=0.0002$) and all months were significantly different from each other with bleaching/paling increasing over time ($p<0.001$).

4. DISCUSSION AND MANAGEMENT RECOMMENDATIONS

4.1. Task 2b: Crustose coralline algae species identification

Results suggest that Newfound Harbor is substantially richer in total taxa (CCA and PAC) with higher cryptic diversity (i.e., more rare taxa) than Looe Key, but that overall diversity (accounting for relative abundance) between the sites is similar. However, Newfound Harbor is dominated by fewer dominant taxa (i.e., *Neogoniolithon solubile*), while Looe Key has a more even distribution among dominant taxa. Overall, these results contribute to the growing body of literature indicating that there is substantial hidden diversity among both CCA (Giorgi et al., 2024) and PAC on Florida's coral reefs and provide baseline CCA and PAC diversity for Newfound Harbor and Looe Key. This information begins to fill a major taxonomic and ecological knowledge gap; clearly, reef substrate communities are far more diverse, spatially variable, and ecologically unresolved than current monitoring frameworks recognize. Therefore, we caution against prescribing management actions focused on specific CCA or PAC species at this stage. Instead, our findings emphasize the need to better characterize the diversity, functional roles, and ecological significance of these taxa before species-specific management recommendations can be developed.

4.2. Task 2c: Settlement enhancement

Understanding larval settlement preferences is essential for increasing coral recruit biomass and grow-out in marine aquaculture. Marine biofilms are reflective of their environment, and their diversity can impact coral settlement. Revealing the composition and diversity of biofilms in our systems will help inform our settlement efforts in the future. Investigation of the infraspecific settlement differences and preferences between coral larvae will help inform researchers on coral larval diversity and guide settlement tile conditioning for various coral species.

4.3. Task 2d: Sperm traits

With the use of the CASA software, we were able to improve our knowledge of sperm motility and velocity over time for five species of Caribbean coral. Interspecific variation in sperm tail length, motility, and velocity may be driven by historic adult colony densities. Species that historically had high densities would be subject to increased sperm competition for fertilization and therefore could have evolved longer sperm tails to be highly motile and swim faster.

As CASA can provide instantaneous information about sample motility and velocity, we can improve our fertilization techniques during annual spawning events. While it is ideal to utilize fast and highly motile sperm for fertilization, colonies that produce slower and/or lower sperm motility can still be utilized and have successful fertilization with prolonged egg contact time. Furthermore, understanding intraspecific differences in sperm velocity and motility may highlight the need for colony specific, rather than batch crosses in the future to maximize the potential genetic variation in offspring, as faster and more motile sperm may have higher fertilization potential than colonies that produce slower sperm with lower motility.

4.4. Task 2e: Finalize AI coral area sizing tool (COAST) program

The automated tool presented, COAST, will reduce manual labor while maintaining accuracy by allowing the quick quantification of settler size to assist with various optimizing projects focusing on coral growth. Increasing efficiency and reducing effort in this way will be increasingly important as reef restoration continues to expand globally (Zhao et al., 2026). It is suggested that such a modular design is adaptable for additional tasks and could be expanded further for use in large-scale coral monitoring (Zhao et al., 2026). The quicker the settlers can reach a size threshold that increases the probability of post-outplanting survival, the more cost-efficient coral rearing will be.

4.5. Task 3: Spawning calendar

Long-term monitoring datasets are critical to determining whether there are historical shifts in the timing of spawning at both the day and minute scale. Understanding these changes and their underlying drivers can help scientists and restoration practitioners to better prepare for monitoring *in situ.* Furthermore, species that may be shifting spawning to suboptimal times for larval development and recruitment should be managed through assisted fertilization efforts. Regional differences in spawning times may also indicate that micro-environmental factors impact the timing of coral reproduction. Regional differences in spawning times may result in genetic bottlenecks that could impact reef system recovery. *Ex situ* spawning induction systems containing broodstock from throughout Florida's Coral Reef would maximize the chances of fertilization success between genetically diverse parents that would not normally create offspring in the wild due to both temporal and geographic restraints. Finally, differences in spawning times based on observation methods

indicate that lighting plays an important role in the synchrony of coral spawning. It has been demonstrated in other studies that *ex situ* coral spawning occurs later in the evening than *in situ* likely due to artificial night light pollution. Furthermore, while induction systems can reliably induce gametogenesis and spawn corals in captivity, it is not perfectly synchronous with spawning times *in situ*. Current *in situ* thermal conditions are not optimal for long-term captivity. Therefore, most induction systems are programmed to utilize historic temperature cycles that do not exceed the optimal temperature threshold for coral health, which may result in asynchrony with *in situ* spawning in present day. Alternatively, asynchrony may indicate that improvements in the delivery of lighting cues, either through more precise programming or through additional fine-scale environmental factors (e.g., twilight and spectral changes in light), are necessary to mimic *in situ* conditions and have tighter synchrony between observation methods.

4.6. Task 4: Preconditioning and outplanting

As this is the first study pairing *ex situ* preconditioning of *P. clivosa* with *in situ* physiological monitoring, results provide key insights into how thermally preconditioned *P. clivosa* responds to natural thermal stress *in situ*. Thus far, preliminary results suggest that preconditioning does not universally improve performance for *P. clivosa*, with environmental conditions likely contributing more to differences in health and metabolism across time than preconditioning. Importantly, however, preconditioning did not cause detriment to the coral. Therefore, future studies could test increasing the preconditioning temperature (i.e., >32ºC), or lengthening the duration of each pre-exposure (i.e., >24 hours and/or >8-weeks). Results also highlight that restoration efforts should consider upon which reefs outplants are being placed, as environmental conditions likely contributed to the health of our outplants.