Improving and expanding the portfolio of grazers available for coral co-culture and reef restoration – Phase II

FLORIDA'S CORAL REEF

Improving and expanding the portfolio of grazers available for coral co-culture and reef restoration – Phase II

Final Report

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Management Summary and Recommendations

Recommendations from this work are to transfer information on sea urchin growth rates and crab grazing rates into practical utility for coral co-culture. Based on resource availability, small juveniles of *T. ventricosus* and *D. antillarum* should take ~2 months to reach a test diameter of ~1 mm. Due to intraspecific variability in growth rates, especially in *T. ventricosus*, coral culturists may need to monitor tanks containing urchins and remove larger individuals as they are identified to prevent inadvertent grazing. Results obtained in the early growth stages of *D. antillarum* and one cohort of *T. ventricosus* also suggest that it may be possible to control growth rate and reduce intra-cohort size variability through resource limitation. This could have practical utility by limiting grazer size in instances where it is difficult to match timing of coral settlement with availability of newly settled juvenile urchins. Availability of small *L. americanum* juveniles for coral co-culture remains limited due to an inability to control spawning in this species. The project team plans to continue working in this area. Similarly, the project team plans to continue developing hatching and settlement techniques for *M. coryphe*, which has particular promise in facilitating restoration of branching corals in low relief habitat types. Capacity for eventual use of this species and immediate use of *M. spinosissimus* in reef stocking will be greatly enhanced by development and approval of a health certification process, which the project team will continue working closely with resource managers to generate.

Executive Summary

This report contains information on activities conducted in FY 23-24 intended to build on past work developing a suite of invertebrate grazers that can be employed in coral co-culture, active reef restoration, or both. Specifically, we investigated the snail *Lithopoma americanum*, the sea urchins *Diadema antillarum* and *Tripnuestes ventricosus*, and the crabs *Mithraculus coryphe* and *Maguimithrax spinosissimus*. While *L. americanum* and *M. coryphe* were identified as having significant impediments to culture, specifically reproductive dysfunction at various points in the pre-larval life cycle, important progress was made and spawns of both species were eventually obtained. Previous limitations to spawning in *T. ventricosus* were overcome during this project, which allowed for insightful comparisons of growth rate between cohorts of both species. Due to their dual utility in coral co-culture as small juveniles and for direct stocking as reef grazers as larger individuals, both urchins are promising candidates. The same is true for *M. spinosissimus*. Grazing rates for both crab species were measured individually and in tandem, with *M. spinosissimus* in particular demonstrating great promise in algal control. While the project team failed to observe any successful hatches in *M. coryphe* until shortly before the end of the study, the observation of successful and near complete embryonic development is encouraging. The project team plans to continue working on the development of hatching and settlement techniques for the species beyond the life of the project. Further, the results of grazing assays indicate that cultured *M. spinosissimus* and wild *M. coryphe* are complementary in function rather than redundant and represent strong candidates for both co-culture with corals and for stocking onto Florida's Coral Reef to support and facilitate improved restoration outcomes. Ultimately, the results obtained during this project represent a positive contribution to coral reef conservation and restoration generally as well as SCTLD response specifically. Sexual propagation in land-based systems will be a vital component of SCTLD recovery in terms of rebuilding lost genetic diversity, and all of the species for which knowledge was generated in this project have demonstrated utility in co-culture of small coral recruits. Concurrently, production capacity is increasing for some of the grazer species that are of interest in direct stocking on the reef, specifically *D. antillarum* and *M. spinosissimus*. These species and others may eventually aid in addressing the macroalgal overgrowth that is hampering recovery of Florida's Coral Reef.

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Table of Contents

List of Figures

Figure 11. Growth curves with 95% C.I. bands for newly settled *Diadema antillarum* cultured from gametes. Two separate cohorts (A and B) were maintained from the same spawn and cultured as described in the text.

Figure 12. Growth curves with 95% C.I. bands for newly settled *Tripnuestes ventricosus* cultured from gametes. Two separate cohorts (A and B) were maintained from the same spawn and cultured as described in the text.

Figure 13. Boxplots depicting median, first and third quartiles, and minimum/maximum values along with any outliers for *D. antillarum* test diameters across time. Culture conditions for Cohorts A and B are described in the text.

Figure 14. Boxplots depicting median, first and third quartiles, and minimum/maximum values along with any outliers for *T. ventricosus* test diameters across time. Culture conditions for Cohorts A and B are described in the text.. Cohorts are depicted in separate figures as sampling took place on different days within the same timeframe.

Figure 15. Photo panel presenting embryonic development observations in the 12 adult female *M. coryphe* used during the study. The crab labelled C1 featured an abnormal tumor-like growth in the abdomen that apparently precluded the brooding of embryos but otherwise did not overtly affect crab behavior. Photos: Shelly Lancaster/Mote

Figure 16. Box plot summarizing individual grazing rates of cultured juvenile *Maguimithrax spinosissimus* (blue) and wild adult *Mithraculus coryphe* (green). Note the substantially higher mean grazing rate of juvenile *M. spinosissimus* than adult *M. coryphe*.

Figure 17. Box plot summarizing intraspecific (blue and green) and interspecific (gray) grazing rates between cultured juvenile *M. spinosissimus* and wild adult *M. coryphe*. Note a similar pattern in relative rates, but lower magnitude, in both intraspecific rates, than individual grazing rates, but a substantially greater rate of interspefic pairs than any other assay treatment (individual or intraspecific pairs).

List of Acronyms

CPRP – Coral Protection and Restoration Program

DEP – Florida Department of Environmental Protection

FKNMS – Florida Keys National Marine Sanctuary

IC2R3 – Mote's Elizabeth Moore International Center for Coral Reef Research & Restoration

SCTLD – Stony Coral Tissue Loss Disease

SGR - Specific growth rate

1. DESCRIPTION

Project objectives fall under the goal of improved ability to propagate a suite of mobile invertebrate grazers and employ them in coral recruit co-culture. The outcomes of this project will be incorporated into an on-going coral disease response effort that seeks to identify management actions, remediate disease impacts, and restore affected resources among other outcomes.

This project is fulfilling priority recommendations highlighted in the "State of Florida Restoration Priorities for Florida's Coral Reef: 2021-2026" report. This project addresses both priority 3.5 '*Research and Development in Support of Land-based and In-water Coral Propagation and Rearing*' as well as priority 6.1 '*Propagation of Reef-Associated Species*'.

Sexually propagating Caribbean corals, especially SCTLD-susceptible species, is a rapidly developing capability that offers the only opportunity to enhance lost genetic diversity in restored populations. A major limitation for sexual production of coral colonies is overgrowth by macroalgae. Co-culturing coral sexual recruits with various invertebrate grazers is an emerging practice that is showing promise in addressing algal overgrowth limitations while reducing labor inputs. Further, there is growing interest in using cultured invertebrate grazers for reef enhancement. These species are generally large-bodied and so adults are not suitable for coral recruit co-culture. However, newly settled or small juveniles of larger grazer species may be viable. Given these frontiers in active coral reef restoration, we propose a goal that will build upon information obtained in an initial project conducted during FY22-23 to benefit resource management of Florida's Coral Reef by improving the ability to propagate and employ mobile invertebrate grazers.

1.1. Goal : Build knowledge to develop scaled propagation of grazers and better employ juveniles in coral co-culture

Objective 1 – Develop culture techniques for *Lithopoma americanum* snails and *Mithraculus coryphe* crabs

Rationale: Small *Lithopoma* snails are proven as valuable grazers and despite improved understanding of reproductive biology, their spawning induction remains a challenge. The nodulose spider crab *M. coryphe* holds promise for coral co-culture and reliable hatching and settlement techniques do not yet exist.

Objective 2 – Generate information for better utilization of juvenile sea urchins and crabs in coral co-culture

Rationale: Newly settled sea urchins can have high intra- and inter-specific variability in growth rates, which affects their compatibility with juvenile corals in co-culture scenarios. Understanding the grazing rates of cultured early life stage *M. coryphe* and *Maguimithrax spinosissimus* crabs would improve the efficiency of their coral co-culture application.

1.2. Reef Management Application

Outcomes of this project have multiple potential applications for improved reef management. New knowledge, techniques, and capabilities generated by this project may aid restoration efforts and/or be applied to increase coral resilience through:

- Improved ability to propagate invertebrate grazers for *ex situ* production of coral recruits.
- Increased future availability of SCTLD-susceptible coral species sexual recruits to restoration practitioners.
- Algal mitigation at existing or future restoration sites where traditional outplanting or natural/induced spawning is targeted/anticipated.
- Methods that could be applied in the field to areas affected by acute disturbances that shift the phase dynamics of a reef in favor of algae - i.e., 2010 cold snap(s), bleaching events, coral disease - to avoid algal dominance.

2. METHODS

The purpose and intended use of the data generated by the proposed activities are to inform regional and local management, specifically active restoration activities, aimed at improving the health and resilience of Florida's Coral Reef. Activities detailed herein will be conducted under the advisement of relevant groups associated with, and staff of, the Florida DEP Coral Protection and Restoration Program. This will ensure that methodologies are not duplicated, best practices are employed, and project results are effectively communicated to all stakeholders. All required state and federal permits were obtained prior to the work beginning.

2.1. Task 1 –*Lithopoma* snail gametogenesis and attempted spawning

Reproductive stage analysis via histology - Continuing work that was mostly done in Phase I (FY 22-23), additional wild-collected *L. americanum* snails were obtained from the Florida Keys and processed for histology by partners at FWRI in St. Petersburg in September 2024. Combined with the snails obtained and histology slides created in late May of the previous project period (which were not available in time to include in the final report for Phase I), we generated a total of 10 months of histological data from 219 individual snails. Missing months are February and July. Histology slides for each animal processed in both Phase I and Phase II were analyzed and quantified according to the scoring system for gonad maturity that was developed in Phase I. These individual scores were converted to proportion data and plotted by month in a stacked bar graph to visualize relative abundance of the various reproductive stages across time in wild *L. americanum* from the Florida Keys.

Historical volitional spawning and targeted observations - To date, volitional spawning in *L. americanum* held in land based systems represents our only opportunity to obtain gametes and larvae. We worked with partners at The Florida Aquarium to review and curate detailed system records dating back to early 2019 that included observations of volitional spawning in *L. americanum*. A cloud-based spreadsheet was also created and disseminated among UF and Aquarium staff to improve opportunistic reporting of *L. americanum* volitional spawning moving forward. Based on these data and because spawning often occurs near or after sunset and is often only apparent the following morning as aggregated egg proteins collected in foam fractionators and sock filters, we also made ten targeted observations of systems containing large numbers of *L. americanum* in May 2024. Targeted observations were generally made following a water change, with UF staff staying to near or past sunset and closely observing each system for two hours or until a spawn occurred (Figure 1). Data collected on each system included temperature, salinity, an estimate of the number of snails in the system, and notes on recent water changes. When a spawn was collected, the number of embryos was estimated by triplicate volumetric counts and fertilization rate was determined.

Induced spawning attempts - While Phase I of this project showed that inducing spawning in *L. americanum* is difficult, we did not want to completely abandon this idea especially given new information generated on likely spawning seasons. Additional attempts at induced spawning occurred on November 1, 2023 and March 20, 2024 using elevated temperature, osmotic stress via reduced salinity, desiccation, and/or hydrogen peroxide application. These treatments were based on a review of gastropod spawning literature. Each was applied to groups of snails individually and three replicate buckets of each treatment were used in both trials (Figure 2).

2.2. Task 2 – Generate growth curves and measure intra- and inter-specific variability in this metric for newly settled sea urchins

Larviculture remains a resource and labor intensive aspect of the sea urchin culture process, especially for *D. antillarum*. Animals of this species used to generate growth data were spawned from broodstock we maintain on 09.12.23. Larvae were fed daily with a mixture of *Rhodomonas salina* and *Chaetocerous mulleri* microalgae, water quality was meticulously maintained, and husbandry techniques our group has developed over multiple years were applied. This culture run was very successful, eventually producing over 1,000 juvenile *D. antillarum*. Small, newly settled urchins were obtained from larviculture vessels and separated into two cohorts on 12.14.23. They were moved into petri dishes to facilitate close observation and photography for repeated measurements. From 12.14.23 to 01.03.24, Cohort A was fed *Rhodomonas salina* and *Chaetocerous mulleri* that was flocculated with a chitin-based product to aggregate and stabilize the cells (Figure 3) while Cohort B was fed with a naturally occurring biofilm that was scraped from the larviculture vessels. Each petri dish received a 50% water change daily.

On 01.04.24, both cohorts were moved into separate plastic buckets with outflow screens that were integrated into a larger recirculating system and received water from a header tank (Figure 4). This improved the consistency of water quality and eliminated the need for daily water changes. Diets provided to each cohort remained the same as in petri dishes. Finally, on 02.15.24 animals from both cohorts were moved into two different tanks in the same greenhouse recirculating system. For this portion of the growth data, we are not able to fully characterize the differences in resource availability between the two cohorts but data obtained do still demonstrate a level of intraspecific variability in growth. Scaled images of haphazardly collected animals from both cohorts were measured for test diameter at 11 time points throughout the growth period either using Moticam software (for the petri dish and plastic bucket stages) or ImageJ (for the greenhouse system stage).

Previous experience in our lab indicated that larviculture of *Tripnuestes ventricosus* is difficult, but less so than *D. antillarium*. However, we struggled initially to spawn this species. Three consecutive attempts using thermal induction were finally successful on 01.29.24. Larvae were rearing in the same recirculating system and fed the same microalgae as *D. antillarum*. Due to a shorter larval phase and settlement/metamorphosis period, newly settled *T. ventricosis* were available to begin the growth trial just a month later, on 02.29.24. To more fully elucidate intraspecific variability in growth, Cohort A was moved as soon as possible from their settlement location in the larviculture kriesels to the same plastic bucket system used for *D. antillarum*, where they had access to only a sparse biofilm for the ~2 month growth period. This cohort represented growth under a resource-limited condition, which we have anecdotally observed can lead to very limited growth. Conversely, Cohort B remained in the highly biofilmed larviculture kriesels where they settled before being transferred on 03.28.24 to a greenhouse recirculating system that also contained high levels of primary productivity. Scaled images of haphazardly collected animals from both cohorts were measured for test diameter at >10 time points throughout the growth period using ImageJ.

2.3. Task 3 – Develop hatching and settlement techniques for the nodulose spider crab *Mithraculus coryphe*

The nodulose spider crab, *Mithraculus coryphe* (Figure 5), is an excellent candidate for grazing enhancement to support and facilitate improved coral reef restoration interventions on Florida's Coral Reef. While the utility of *Maguimithrax spinnosissimus* (Figure 6), the Caribbean king crab, for this purpose is apparent, the diminutive *M. coryphe* is even more cryptic in its function. In other parts of the region where branching corals (*i.e.*, *Acropora* spp) are still common and, in some cases, abundant, *Mithraculus* spp. crabs, including *M. coryphe* are common and, arguably, obligate associates of these branching coral colonies. In colonies with *Mithraculus* crab associates, substantially fewer incidences of damsel fish damage (*e.g.*, tufts of algae on branch tips) were observed and a conspicuous halo of bare substrate was observed about the base of the colony. In those colonies devoid of *Mithraculus* associates, damsel fish damage was prevalent and benthic algae were abundant and dense about the base of the colony and often extended into the colony among the branches. In Florida, *Acropora cervicornis* (ACER), a major focus of restoration efforts, is typically outplanted onto the forereef terrace habitat type where the architectural complexity of the reef matrix is not sufficiently rugose to support high densities of *M. spinosissimus*. However, the ACER colonies potentially represent sufficient structure to serve as shelter for *M. coryphe* and *M. coryphe* grazing rates likely are sufficient to conduct colony-scale algae mitigation of coral-algal interactions/competition. Thus, the production of this new species of interest in facilitating coral reef restoration efforts may be an important component of restoration site maintenance along Florida's Coral Reef.

The project team collected a total of 39 *M. coryphe* from backreef rubble zones in the Lower Florida Keys to serve as broodstock for the current study. The relative paucity of adult *M. coryphe* at sites that have, previously, been productive for collecting the species opens its own research question regarding the natural density and population dynamics of the species. Broodstock were transported to Mote's Florida Coral Reef Restoration Crab Hatchery Research Center where they were placed into a purpose-built recirculating aquaculture system supplied with synthetic seawater. Broodstock crabs were fed a mixed diet of wild and cultured algae, fresh frozen animal protein (*e.g.* minced shrimp and squid), and a shelf-stable gelatin feed product (*i.e.*, Mazuri, inc) to satiation three times per week. Feeding was increased to daily after observing several clutches of eggs progress to late stage (*e.g.*, visible eyespots in the embryos) with no apparent hatching. The project team observed a total of 21 clutches of eggs from 11 adult female crabs over the project duration. 1 crab produced three consecutive clutches of eggs, three crabs produced a single clutch of eggs, and seven crabs produced two clutches through the duration of the study. No successful hatches were observed in the course of the study at the facility in Sarasota, but a single successful clutch was observed with apparently healthy and active larvae just prior to the writing of this report at Mote's Elizabeth Moore International Center for Coral Reef Research & Restoration (IC2R3) on Summerland Key, FL. No larval settlement has been observed at the time of this writing, but the larvae will be maintained beyond the duration of the project to continue this work.

2.4. Task 4 – Measure grazing rates of cultured early life stage *Maguimithrax spinosissimus* and *Mithraculus coryphe*

The competitive interactions between and among corals and algae are a substantial bottleneck in the restoration and recovery of Caribbean coral reef ecosystems. As corals are lost and more 'real estate' is opened on coral reefs, grazing intensity is reduced with the same community of herbivores acting on an ever growing 'field' of algae. One solution to the proliferation of upright fleshy macroalgae on reefs in the region has been the production and stocking of native invertebrate grazers to enhance grazing intensity on degraded reefs. These efforts have been successful at experimental scales with single species and in laboratory experiments with multiple species. However, recent efforts are almost entirely focused on two species, the long-spined sea urchin, *Diadema antillarum*, and the Caribbean king crab, *Maguimithrax spinosissimus.* While recent work suggests that the species are complementary rather than redundant as many have assumed, with each species using algal and spatial resources in slightly different ways. While the focus on evaluating multiple species for restoration of critical ecological functions is commendable, the richness of species available to resource managers and restoration practitioners should be maximized to spread risk and increase resilience of the function in the face of marine diseases and changing environmental conditions in the region.

Here, we compared the grazing rates of cultured Caribbean king crabs to those of a new species, *Mithraculus coryphe*, of interest in facilitating coral reef restoration efforts. We measured individual grazing rates of each species using cultured algae on small (3 cm x 3 cm) ceramic tiles in a 24 hour laboratory grazing assay (Figure 7). Tiles with algae were weighed using a microbalance prior to each trial and placed into small aquaria along with a single piece of calcium carbonate "shelter" (*e.g.*, limestone rock, bivalve shell) before a single individual of each species was haphazardly added to each the experimental tanks. Crabs were allowed to graze undisturbed for 24 hours at which point the crabs were removed and the algae tile was again weighed. The difference in mass between initial and final tile measurements was used as a measure of the mass of algae consumed over the trial. Next, measurements of grazing rates for intraspecific and interspecific pairs of crabs were conducted in the same fashion to evaluate interactions between the species and individuals.

3. RESULTS

3.1. Task 1 – *Lithopoma* snail gametogenesis and attempted spawning

Reproductive stage analysis via histology - Proportional data generated from histological images is contained in Figure 8. Gonad maturation appears to occur year round and animals with mature gametes can be found in all months of the year for which data are available. Post spawn (spent/recovering) animals were only found in the 7 months from April - October. However they were found in low proportions and not in all months, suggesting that this may be a short-lived stage and probability of detection is low. Further supporting a summer spawning season, no undeveloped or early developing animals were obtained from May - August. Visualizing reproductive stage by shell length (Figure 9) showed that reproductive activity occurs across the range of sizes collected for this Task. Further, animals as large as 16mm shell length were observed to be in the undeveloped stage, indicating that this species is iteroparous, meaning it goes through multiple spawning cycles during a lifetime.

Historical volitional spawning and targeted observations - Observations of volitional spawning dating back to January 2019 suggest that such spawning is more likely to occur following a water change. Well over half of spawns occurred immediately following a water change, with more occurring later that evening or the next day. With water changes taking place at most weekly, this represents a disproportionate frequency that strongly suggests some aspect of the activity triggers spawning. Very little if any lunar periodicity was detected in the data (Figure 10a). Just over 30% of spawns occurred between 3 and 6 days prior to the full moon of a given month, but significant spawning activity happened throughout the lunar cycle. In contrast to lunar periodicity, a relatively strong annual cycle was observed in the data (Figure 10b). The majority of spawns occurred from April to July, with a gap in June that likely represents a lack of observed/reported spawns rather than an actual drop off in spawning activity. Ultimately, *L. americanum* spawning was only recorded on a total of 23 dates ranging back to January of 2019, with many observations coming in the last two years. This likely reflects an increased awareness of the importance of *L. americanum* spawning following the initiation of Phase I of this project in 2022.

Induced spawning attempts -

3.2. Task 2 – Generate growth curves and measure intra- and inter-specific variability in this metric for newly settled sea urchins

Urchin test diameter data were used to generate growth curves with 95% confidence intervals for *D. antillarum* (Figure 11) and *T. ventricosus* (Figure 12) as well as boxplots for each cohort at each sampling interval for both species (Figures 13 and 14, respectively). It was not possible to ensure that the same individuals were measured each time, so animals were haphazardly selected to be representative of the cohort. For *D. antillarum*, 5 to 10 individuals were measured from each cohort at each timepoint and for *T. ventricosus*, 4 to 20 individuals were measured from each cohort at each timepoint.

Specific growth rate (SGR) can be calculated to determine percentage growth per unit time and offers an opportunity to account for differences in growth period. In this trial, *D. antillarum* growth occurred over more than twice as many days as *T. ventricosus*, so SGR was calculated as follows:

$$SGR = \frac{ln(f) - ln(i)}{t} \times 100$$

where f is the final test diameter in mm and i is the initial test diameter in mm for a given cohort in a given time period and t is the duration of the time period in days.

Calculations revealed that *D. antillarum* cohorts A and B grew at 1.47%/day and 1.57 %/day, respectively while *T. ventricosus* cohort A and B grew at 1.99%/day and 4.78%/day, respectively.

3.3. Task 3 – Develop hatching and settlement techniques for the nodulose spider crab *Mithraculus coryphe*

While the lack of observed hatching precluded any settlement technique development, the project team did determine that daily feeding and a varied diet was effective in driving egg production and brood development. The single hatch of extremely small and fast moving larvae at IC2R3 has led the project team to suspect that tank volume, flow, water chemistry, and diet composition may play a strong role in hatching success. Efforts will continue to develop hatching and settlement techniques for the species to support coral reef restoration efforts along Florida's Coral Reef and herbivore coral co-culture.

The team observed a total of 21 clutches, 17 of which were extruded during the project period. Three crabs produced only one clutch, seven crabs produced two clutches, and one crab produced three clutches of eggs during the study. Clutch and embryo development was monitored with digital photographs (Figure 15). The successful progression of clutches through the same general developmental stages (*e.g.*, early, middle, and late stage eggs) as those observed in Caribbean king crabs, is encouraging and suggests that the project team is close to success in hatching and, presumably, subsequently settling larvae of the species.

3.4. Task 4 – Measure grazing rates of cultured early life stage *Maguimithrax spinosissimus* and *Mithraculus coryphe*

The nodulose spider crab, *M. coryphe*, consumed less algae per individual than the Caribbean king crab (Figure 16) which agrees with previous data on wild crabs. Intraspecific grazing rates were similarly lower in the *M. coryphe* treatment than in *M. spinosissimus* but the magnitude of algae consumed by both species was lower than in individual grazing rates assays (Figure 17). The mean interspecific grazing rate was substantially greater than either of the intraspecific rates or the individual rates (Figure 17) suggesting some form of facilitation.

4. DISCUSSION

4.1. *Lithopoma* gametogenesis and spawning

Analysis of the large volumes of histological data generated between the current project and Phase I in FY 23-24 provide previously unavailable insight in *L. americanum* spawning. The spawning periodicity described in section 3.1 corroborates well with the more limited data available from observations of volitional spawns made in recirculating systems described. This suggests that lab based facilities, especially those in greenhouses subjected to a natural photoperiod, may well replicate the natural spawning seasonality of this species. This information is especially important as additional attempts at induced spawning during this project period continued to be unsuccessful. Until this capability is developed, we will have to rely on volitional spawn collection to produce larvae and subsequently the small juvenile snails that are so valuable for co-culture with newly settled coral recruits. While barriers do remain once larvae are obtained, the high fertilization rate obtained in the one collected spawn during the project period suggests that larval production should be relatively efficient given gamete availability. We still have demand from coral spawning practitioners for small juvenile *L. americanum* and our team plans to continue efforts to develop methodologies to reliably spawn this species. Other gastropods (e.g. abalone) are often spawned with a combination of the stressors that we have applied in isolation (e.g. temperature and hydrogen peroxide). Our plan is to continue evaluating these stressors in a less replicated and rigorous manner during what we have identified as a late spring/summer spawning season in this species.

4.2. Juvenile sea urchin growth

This project has allowed us to generate detailed information on test diameter and growth rate from very early post settlement for two sea urchin species of interest for the first time. Most previous work on sea urchin growth has started with much larger juveniles and not collected test diameter data as frequently as we were able to with the focused intent of this project. Early post settlement sea urchins are potentially of high utility and understanding their growth rates is important as we have anecdotally observed that they can rapidly outgrow coral recruits, potentially causing damage through inadvertent grazing. A top end growth rate of almost 5% per day in *T. ventricosus* is extremely rapid growth for any animal in an aquaculture setting. Conversely, we also observed that resource limitation can lead to stunted growth, as the other cohort of *T. ventricosus* grew at only ~2% per day. This represents a large difference as daily growth rates compound and results in vastly different final sizes as seen when comparing *T. venricosus* growth curves and boxplots between cohorts. The slower overall daily % growth of *D. antillarum* is likely due to 1) the fact that initial growth occurred in petri dishes and the plastic bucket system, and 2) growth occurred over a longer period of time resulting in a larger denominator in the SGR calculation. Resource limitation appears to reduce intra-cohort variability in size, which is a desirable outcome. However, in practicality, systems in which juvenile urchins would be employed for co-culture with juvenile corals would be more similar to the later phase of growth for both *D. antillarum* cohorts and Cohort B of *T. ventricosus*. These are typically not resource-limited environments due to the light and feeding regimes required for coral growth. Based on our data, *T. ventricosus* appear to have higher levels in intraspecific variability in growth than *D. antillarum*, a trait which we have previously observed anecdotally. Ultimately, the animals used to generate data for this trial were employed by coral propagation practitioners at The Florida Aquarium for control of algae in land-based systems, demonstrating the immediate practical application of small juvenile urchins. Further, because larger individuals of both *T. ventricosus* and *D. antillarum* are candidates for direct stocking , these species have the potential for dual utility in the reef restoration enterprise.

4.3. *Mithraculus coryphe* hatching and settlement

This project, as with many new scientific endeavors, has led to more questions than answers in a number of dimensions. While the team was able to collect a modest number of broodstock animals of *M. coryphe*, the paucity of the species in natural habitats that have, in the past, been particularly productive (in both the Upper and Lower Florida Keys) has raised substantial questions regarding the species natural abundance and distribution along Florida’s Coral Reef. The team has, in the past, been able to collect hundreds of adult crabs in a single day at any of multiple backreef rubble zone sites throughout the FKNMS. However, the modest number of broodstock that the team was able to collect took substantial time and effort in multiple locations. The team has added exploratory surveys of the species' natural abundance, size structure, sex ratio, and distribution across the FKNMS to its research priorities over the next several years.

Further, the observation of complete, or near complete, embryonic development of multiple existing and new clutches of eggs in a closed system, but only a single clutch of larvae hatching has brought up questions surrounding both broodstock maintenance (*e.g.*, effect of water quality, diet composition, feeding amount and frequency, flow dynamics, shelter) and embryo/larval conditions that are suitable for production in closed systems. The team is dedicated to continuing this project beyond the life of the funding, but will seek additional funding to support investigating the production and use of the species in both coral co-culture and direct restoration efforts along Florida's Coral Reef.

The fact that the species' larvae are substantially smaller and more 'energetic' than that of their larger cousins, the Caribbean king crab, has led the team to consider potential design changes in the hatching and rearing systems for the species - another ongoing research objective for the project team. However, the similarities in the embryonic development cycle, from gross morphology to brood duration to interval between clutches indicates that the production of the species is tractable in systems similar to those used in the production of *M. spinosissimus* and the potential utility of the species in mitigating coral/algal interactions (see task 4) make the pursuit of producing the species at scale a worthwhile research and development avenue, however, the species' natural density being so low across Florida's Coral Reef presents both a justification (and potential urgency) for continued study and a challenge in terms of broodstock collection.

4.4. Crab grazing rates

The substantially higher grazing rates of juvenile cultured Caribbean king crabs is encouraging and supports a previous report of an ontogenetic shift in the gut chemistry of *M. spinosissimus* from a mostly herbivorous diet to one proportionally higher in animal protein. This suggests that the current strategy of using relatively early juvenile Caribbean king crabs to support coral reef restoration efforts along Florida's Coral Reef is both sound and likely efficient with smaller crabs likely exhibiting similar or higher grazing rates than larger crabs. The similar pattern but different magnitude of *M. coryphe* grazing rates being lower than those of *M. spinosissimus* in intraspecific assays suggests that individuals compete with conspecifics for the same resources again, as expected. The substantially greater grazing rates observed in interspecific pairs of the species suggests that they are complementary rather than redundant in function. These results support the use of both species in supporting direct restoration efforts and bears further investigation to determine how and if the species differ in diet preference for common reef algae. Further, the lack of cultured *M. coryphe* juveniles precluded a comparison of wild and cultured crabs in terms of grazing rates and interspecific interactions. This should remain a research focus moving forward to determine if there are functional or behavioral differences between wild and cultured crabs helping to inform their potential use in facilitating coral reef restoration efforts.

5. FIGURES

Figure 1. Example photograph of UF biologist Jessica Smith observing a system containing a large number of *L. americanum* snails. Photos were not taken during the observations for which data are reported because a single person was present.

Figure 2. UF biologist Jessica Smith observing replicate buckets during the 03.20.24 *L. americanum* induced spawning attempt.

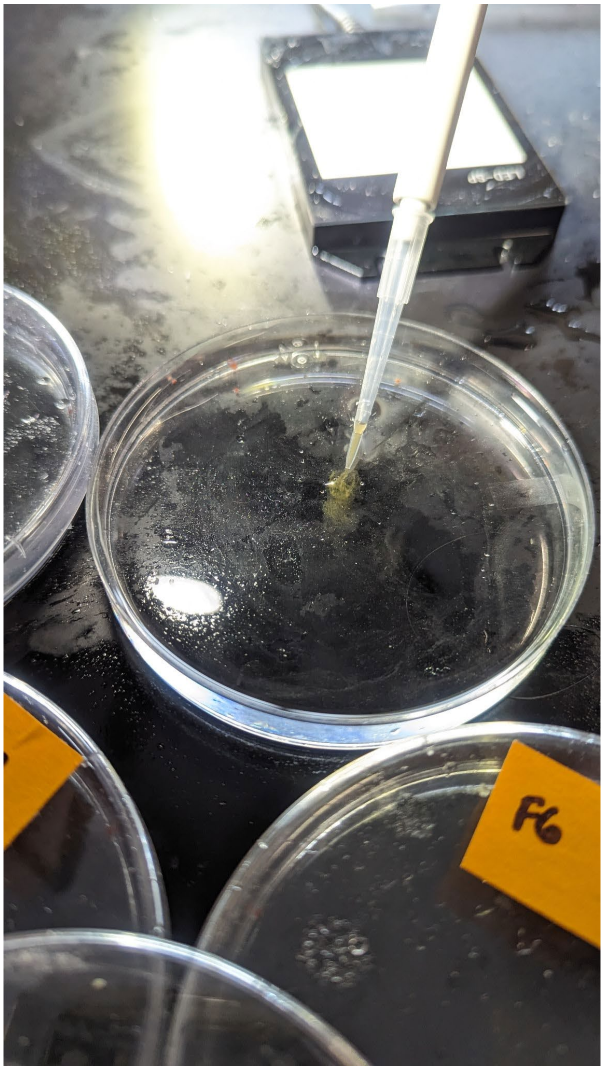

Figure 3. A micropipette being used to deliver a specific volume of flocculated *Rhodomonas salina* and *Chaetocerous mulleri* to a petri dish containing newly settled *Diadema antillarum*. The urchins are visible as small, pinkish specs in the most distant part of the petri dish.

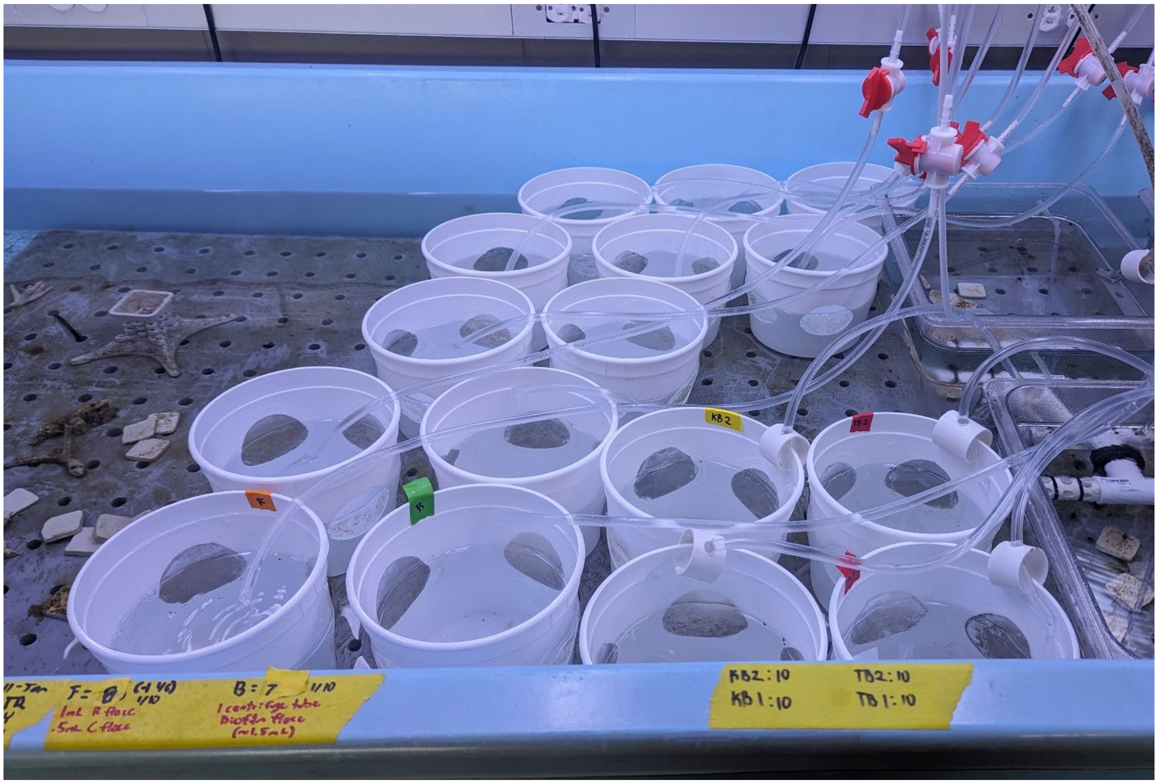

Figure 4. The plastic bucket system that was used to culture Cohorts A and B of *Diadema antillarium* and Cohort A of *Tripnuestes ventricosus*. Water coming from a header tank is delivered through the clear lines with red valves for flow control, passes through micron mesh windows in the buckets into the larger tray, and then flows back to the sump and system life support before returning to the header tank.

Figure 5. Representative photo of an adult *Mithraculus coryphe*, the nodulose spider crab, used in the recirculating aquaculture systems at Mote's Florida Coral Reef Restoration Crab Hatchery Research Center in Sarasota, FL. Photo: Jason Spadaro/Mote

Figure 6. Representative photo of a juvenile cultured *Maguimithrax spinosissimus*, the Caribbean king crab, produced in the recirculating aquaculture systems at Mote's Florida Coral Reef Restoration Crab Hatchery Research Center in Sarasota, FL. Photo: Jason Spadaro/Mote

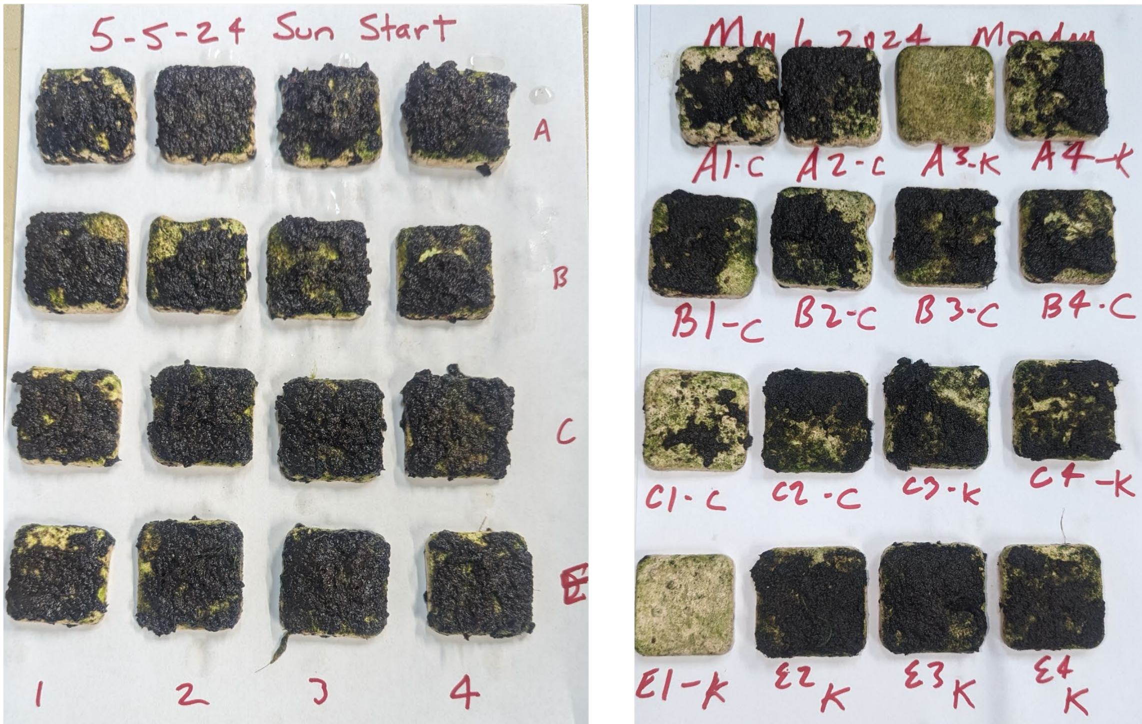

Figure 7. Representative photos of cultured algal turf on 3 cm x 3 cm ceramic tiles prior to (left) and after (right) use in a 24 hour individual grazing rates assay. Photos: Tom Waldrop/Mote.

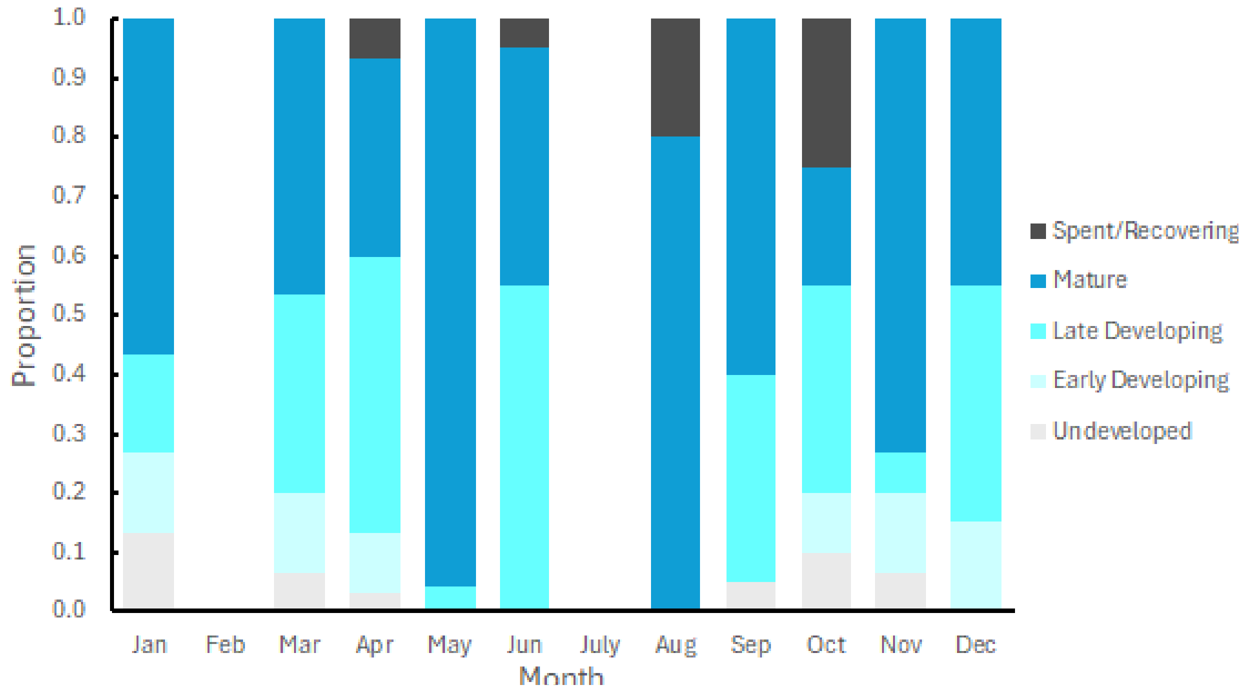

Figure 8. Ten months of wild-collected *Lithopoma americanum* gonad histology scored by reproductive stage (n = 10-30 animals per month).

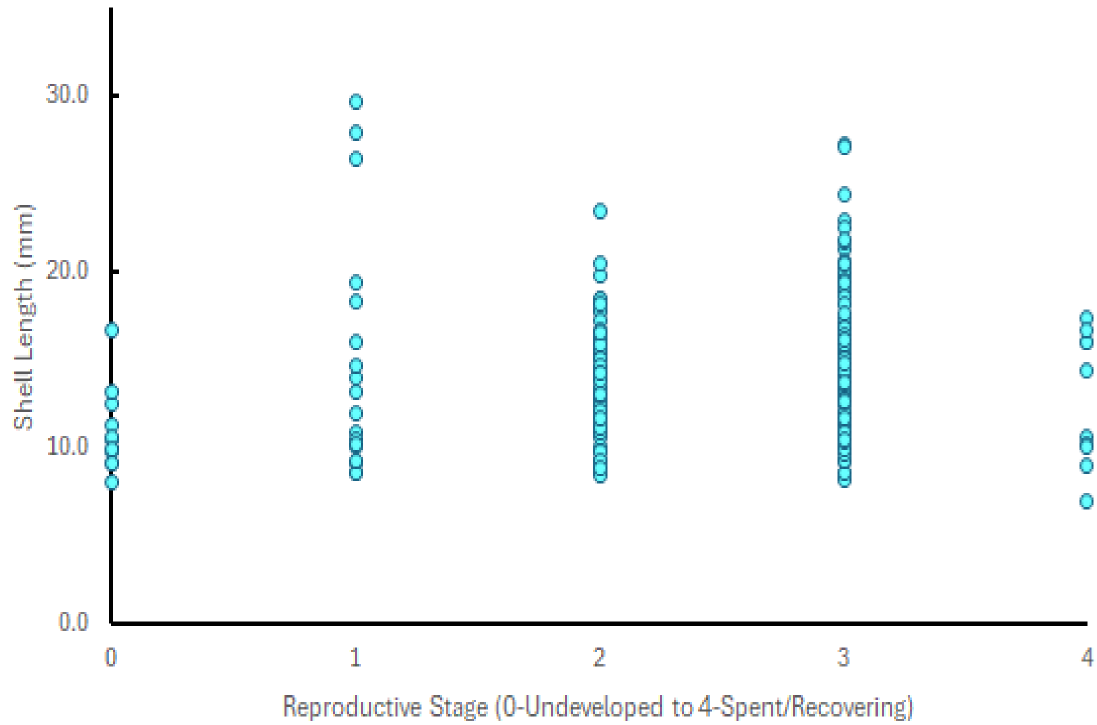

Figure 9. Reproductive stage as determined by gonad histology plotted against shell length for all 219 wild-collected *Lithopoma americanum*.

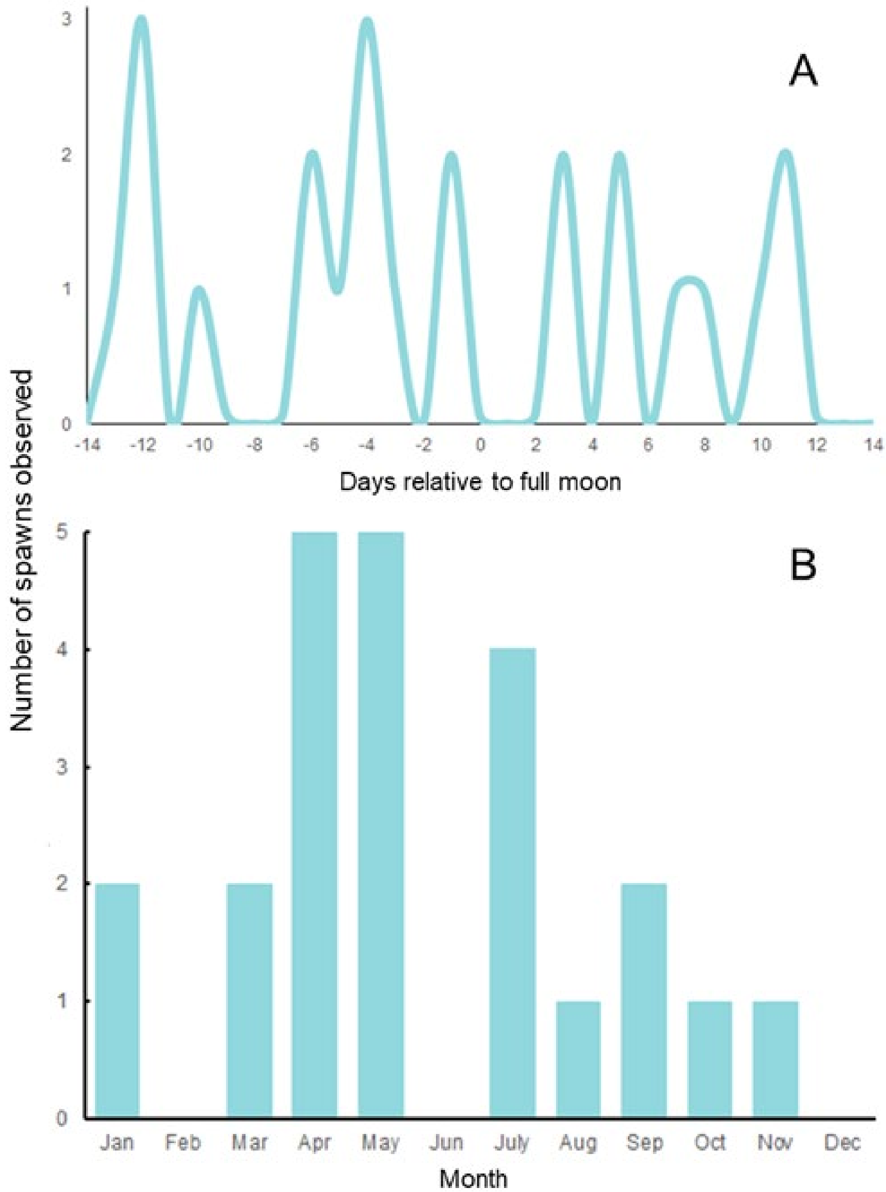

Figure 10. Observations of *Lithopoma americanum* volitional spawning in recirculating systems (n = 23) dating back to January 2019 and plotted with days relative to full moon (A) and month (B).

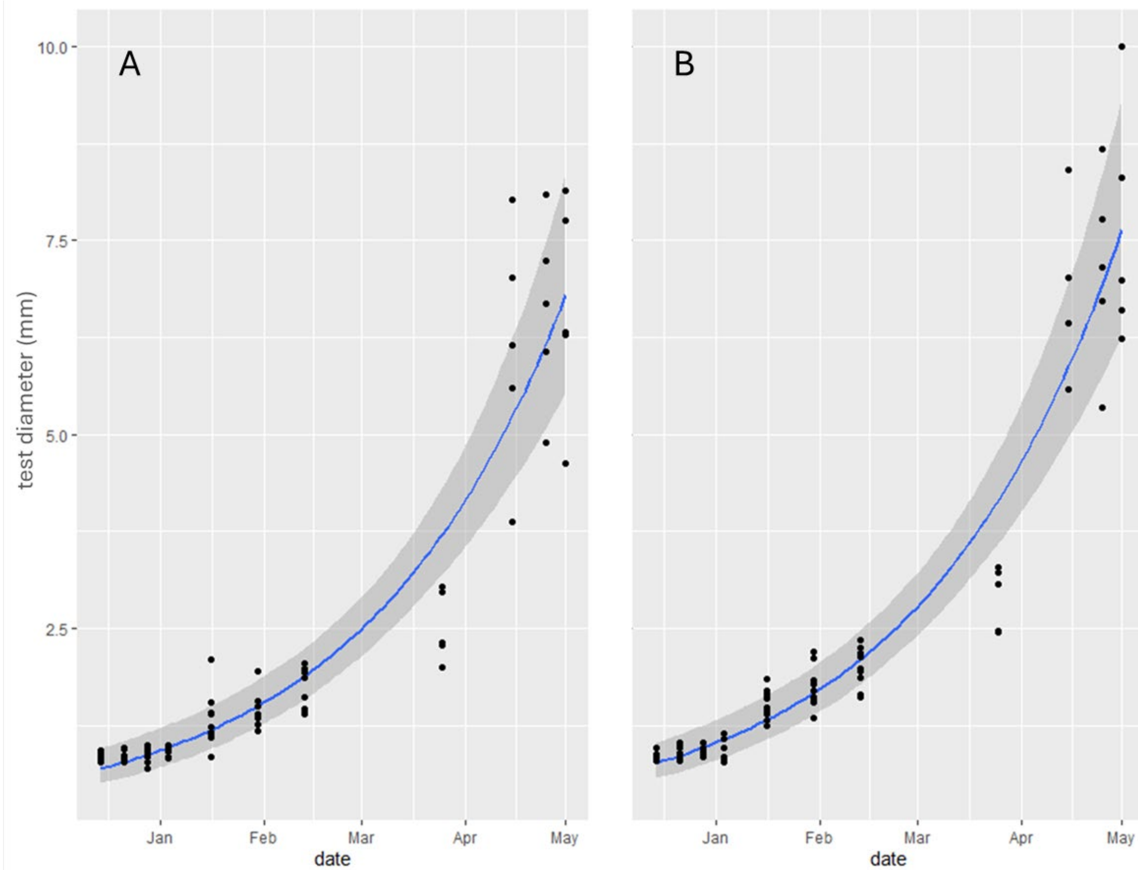

Figure 11. Growth curves with 95% C.I. bands for newly settled *Diadema antillarum* cultured from gametes. Two separate cohorts (A and B) were maintained from the same spawn and cultured as described in the text.

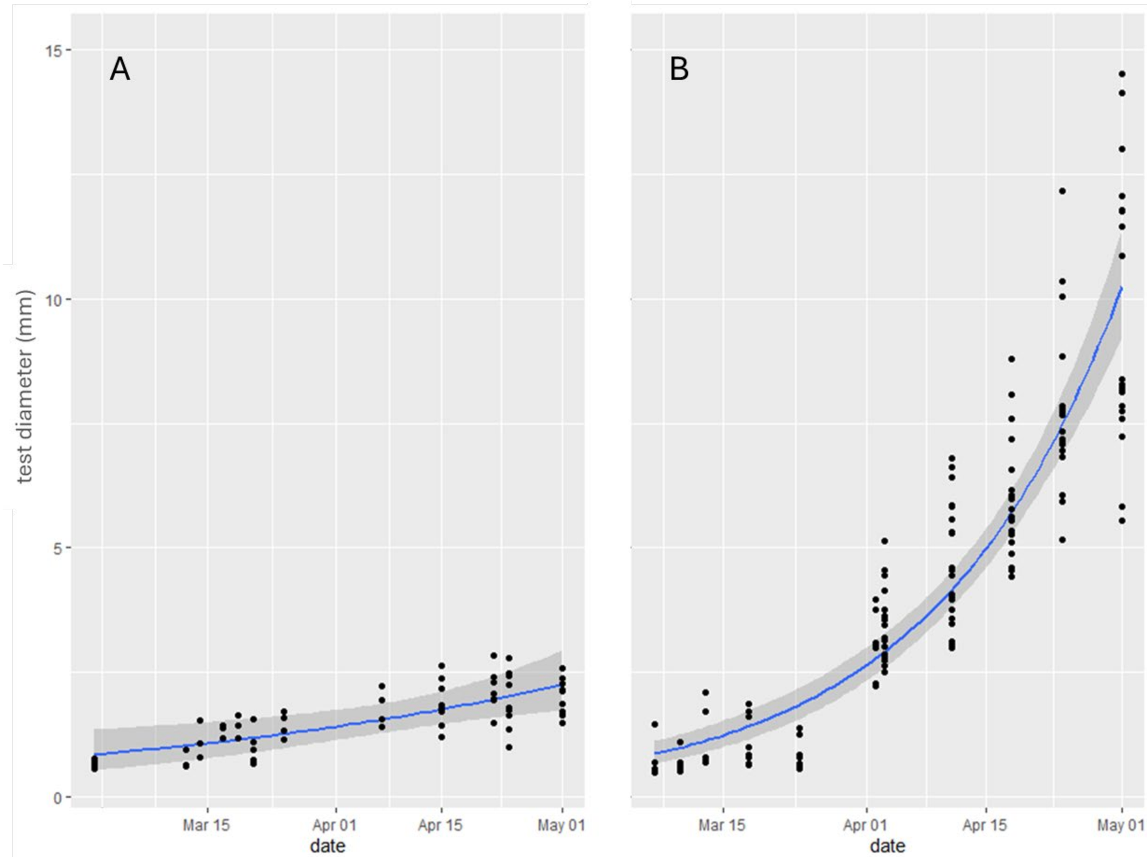

Figure 12. Growth curves with 95% C.I. bands for newly settled *Tripnuestes ventricosus* cultured from gametes. Two separate cohorts (A and B) were maintained from the same spawn and cultured as described in the text.

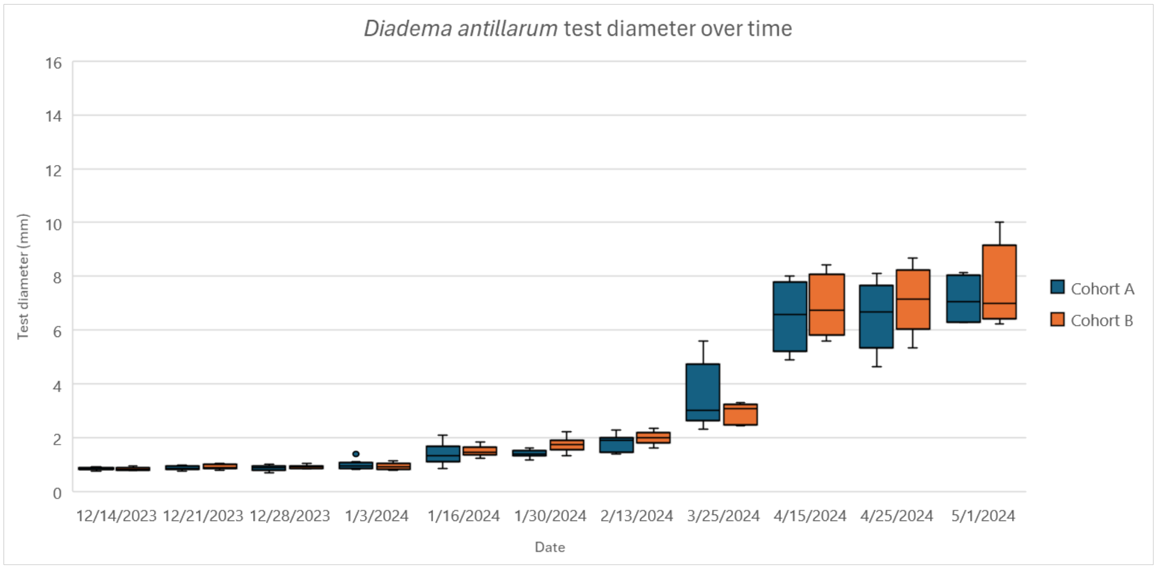

Figure 13. Boxplots depicting median, first and third quartiles, and minimum/maximum values along with any outliers for *D. antillarum* test diameters across time. Culture conditions for Cohorts A and B are described in the text.

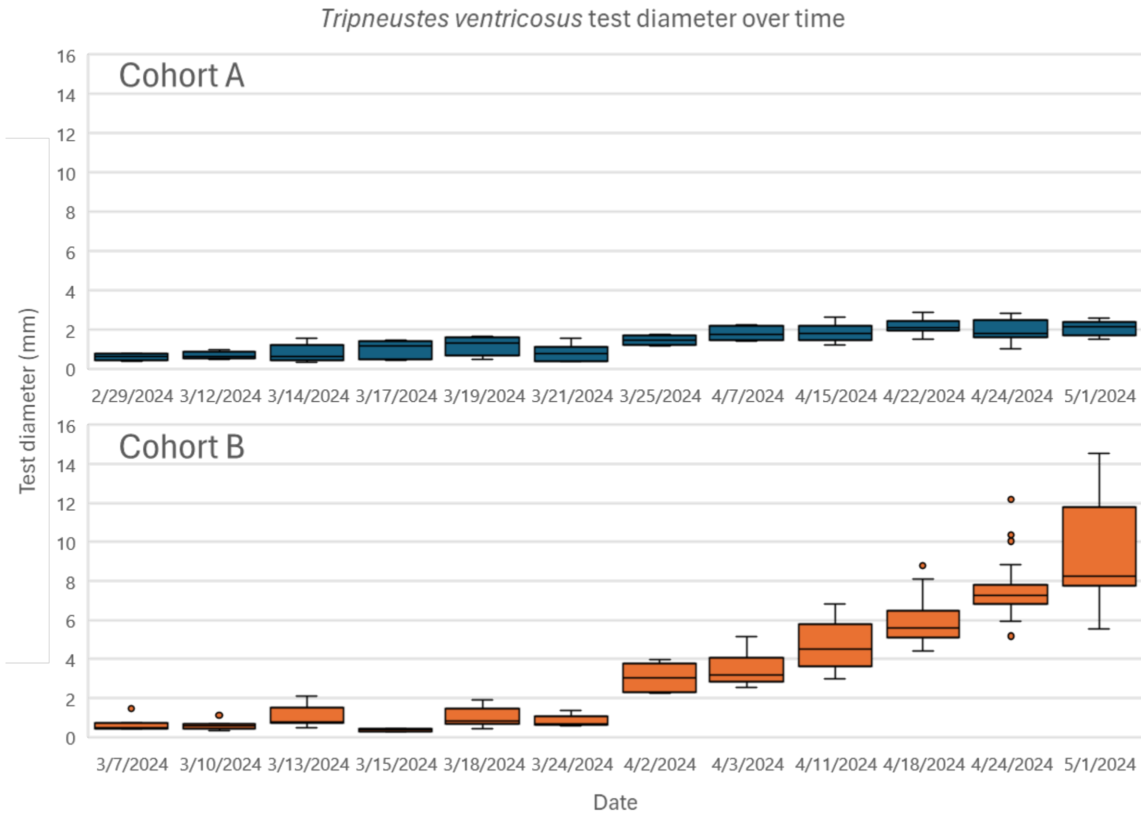

Figure 14. Boxplots depicting median, first and third quartiles, and minimum/maximum values along with any outliers for *T. ventricosus* test diameters across time. Culture conditions for Cohorts A and B are described in the text.. Cohorts are depicted in separate figures as sampling took place on different days within the same timeframe.

Figure 15. Photo panel presenting embryonic development observations in the 12 adult female *M. coryphe* used during the study. The crab labelled C1 featured an abnormal tumor-like growth in the abdomen that apparently precluded the brooding of embryos but otherwise did not overtly affect crab behavior. Photos: Shelly Lancaster/Mote

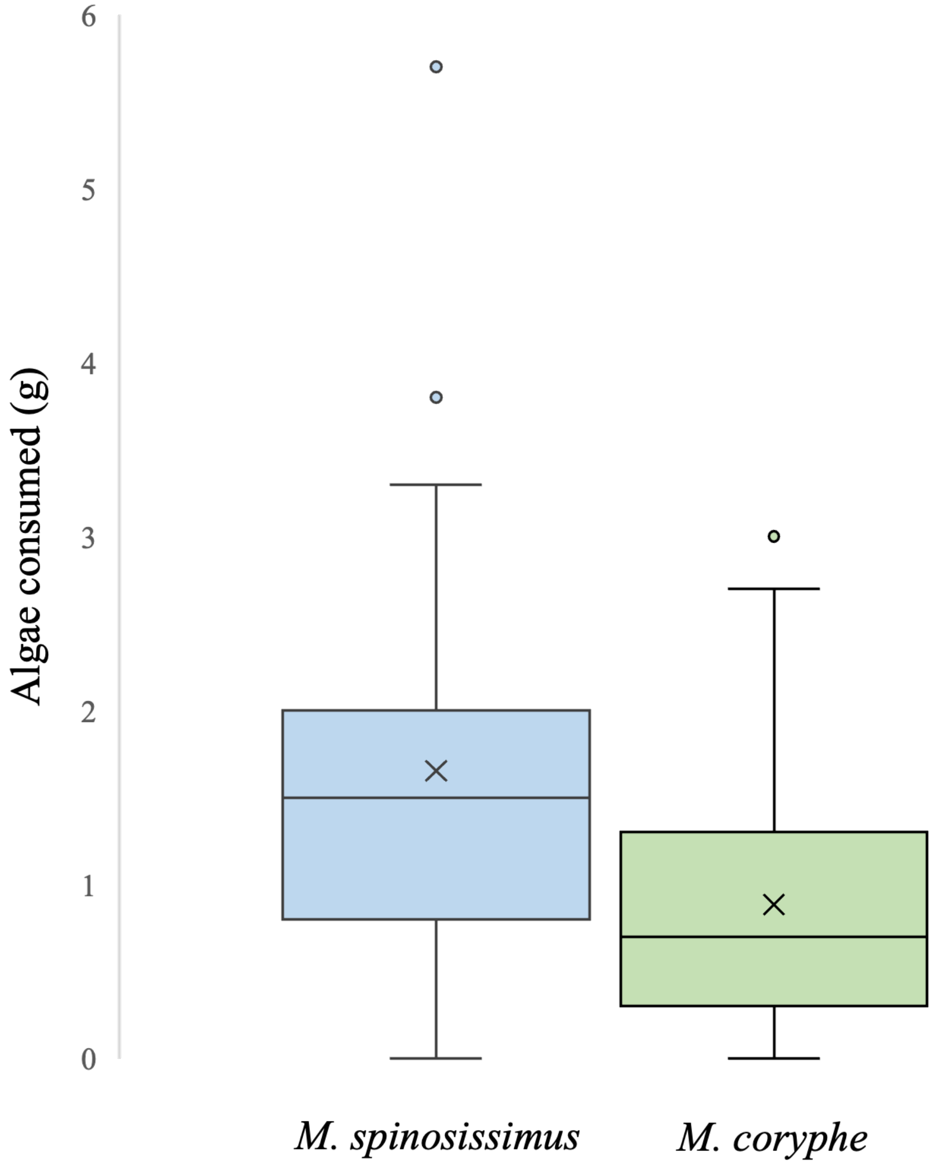

Figure 16. Box plot summarizing individual grazing rates of cultured juvenile *Maguimithrax spinosissimus* (blue) and wild adult *Mithraculus coryphe* (green). Note the substantially higher mean grazing rate of juvenile *M. spinosissimus* than adult *M. coryphe*.

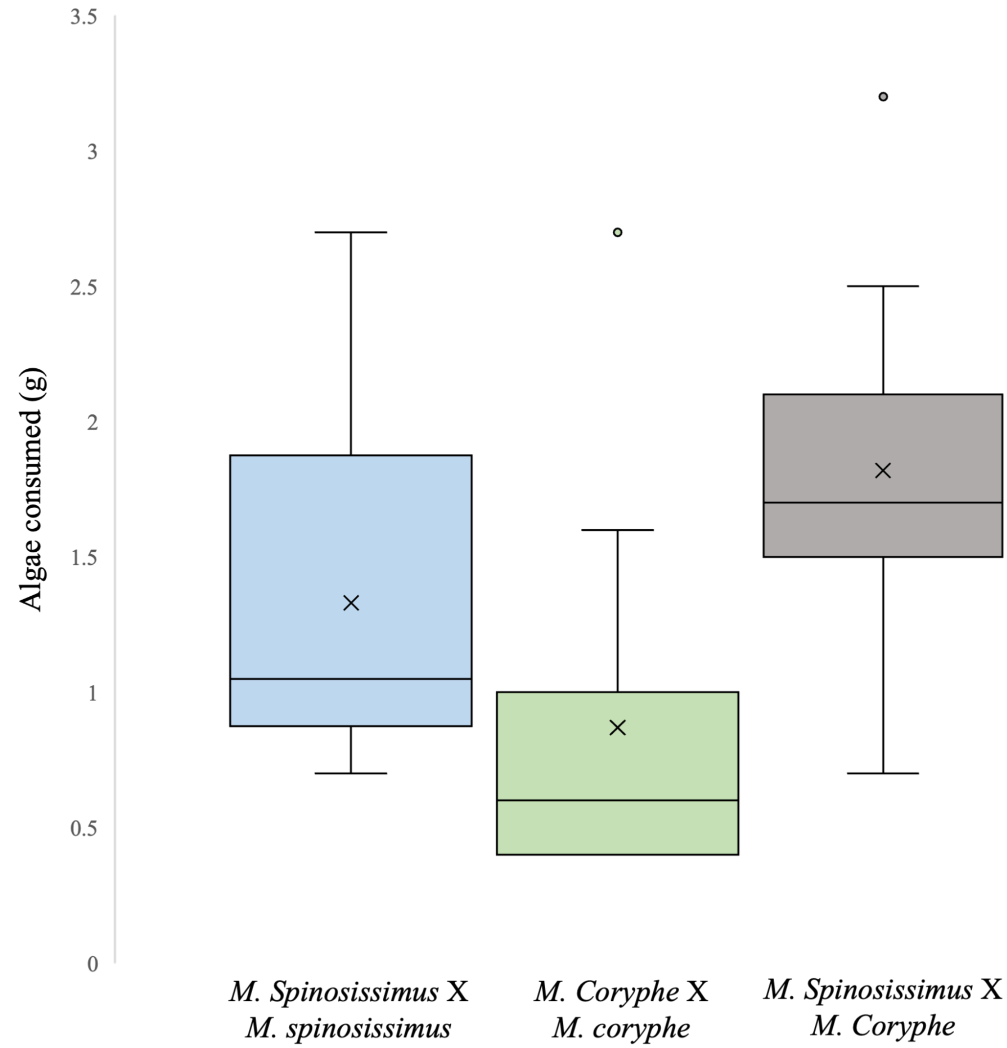

Figure 17. Box plot summarizing intraspecific (blue and green) and interspecific (gray) grazing rates between cultured juvenile *M. spinosissimus* and wild adult *M. coryphe*. Note a similar pattern in relative rates, but lower magnitude, in both intraspecific rates, than individual grazing rates, but a substantially greater rate of interspefic pairs than any other assay treatment (individual or intraspecific pairs).