Improving Early Life Survival of Priority Grazers – Phase II

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Final Report

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Management Summary

This work was designed to advance scalable production of two reef grazers, *Diadema antillarum* and *Maguimithrax spinosissimus*, and to maintain a living archive of the federally Endangered Atlantic pillar coral, *Dendrogyra cylindrus*, in support of active restoration on Florida's Coral Reef. For *D. antillarum* land-based production, results suggest that supplemental benthic diets developed at research scale (an initial feeding with *Navicula* sp. followed by a transition to flocculated *Rhodomonas salina* and *Chaetoceros muelleri*) should not be applied at the first observation of settlement, but rather delayed until a satisfactory proportion of the cohort has settled, as early application showed a potential negative effect on continued settlement success. Reduced among-vessel variance in survival in supplemented vessels indicates that standardized diets can improve production predictability by mitigating variation in naturally derived biofilm development across replicate vessels, and continued refinement of delivery methods is recommended. Offshore in situ nursery systems supported continued survivorship and growth of juvenile *D. antillarum* under two cage configurations evaluated, and cage partitioning may improve survival and growth during juvenile grow-out. These findings support the use of offshore nurseries as an intermediate phase between land-based aquaculture and direct reef stocking. Preliminary observations from the integrated coral-urchin experiment suggest that low to moderate urchin densities may provide grazing benefits without substantial negative impacts to co-cultured coral fragments, but continued monitoring is needed to identify optimal stocking densities. For *M. spinosissimus*, growth and survival data from reciprocal transfers between recirculating and flow-through seawater systems will inform a growth model predicting time to target stocking size, with details to be provided in the final report. Husbandry of all 145 *D. cylindrus* colonies at The Florida Aquarium continued without mortality through the project period, with a subset of 85 colonies maintained in conditions promoting post-award spawning to support genotype banking.

Executive Summary
This report contains information on experiments conducted in FY 25-26 intended to advance scalable production of two reef grazers, *Diadema antillarum* and *Maguimithrax spinosissimus*, and to maintain a living archive of the federally Endangered Atlantic pillar coral, *Dendrogyra cylindrus*, in support of coral reef restoration on Florida's Coral Reef. Building on Phase I (FY 24-25) research-scale work that identified candidate live diets, settlement substrates, and culture methods for these grazer species, Phase II shifts focus toward application of those findings within larger, production-oriented culture systems and toward in situ rearing as a complement to land-based aquaculture. The sea urchin *D. antillarum* is a Western Atlantic reef herbivore important in maintaining productive reef community structure, but numbers remain exceedingly low on Florida's Coral Reef following the 1980s die-off and may be supplemented with aquaculture individuals. Two sequential experiments were conducted by the University of Florida evaluating the effect of a supplemental benthic diet on settlement success and on growth and survival of newly settled juveniles within a purpose-built ten-vessel recirculating culture system. The supplemental diet showed no statistically significant effect on settlement, survival, or growth at the production scale tested, in contrast to positive effects observed at research scale during Phase I, but reduced among-vessel variance in survival suggests a benefit for production predictability. An incidental observation that vessels with greater natural sunlight exposure developed more robust biofilms and produced higher juvenile growth and survival highlights the value of standardized supplemental diets in mitigating among-vessel variability. Complementary in situ work conducted by Sustainable Ocean and Reefs in an offshore Florida Keys nursery evaluated two experimental approaches: a cage configuration experiment that compared divided and undivided cages for juvenile lab-reared *D. antillarum* over ~150 days, and a density-dependent experiment evaluating interactions between *D. antillarum* and *Siderastrea siderea* and *Porites astreoides* coral fragments at four urchin densities. Juveniles maintained relatively high survivorship and continued positive growth trajectories. Preliminary observations from the density experiment, which remains ongoing, suggest that low to moderate urchin densities may provide grazing benefits without substantial negative impacts to coral fragments. The Caribbean king crab, *M. spinosissimus*, is the largest crab naturally occurring in the Western Atlantic and is an effective grazer of macroalgae on coral reefs, but its naturally low abundance throughout its range necessitates aquaculture production to support stocking efforts. Reciprocal transfer experiments conducted by Mote Marine Laboratory evaluated the effect of two seawater system types on growth and survival of juvenile crabs: a recirculating system at the Sarasota hatchery and a flow-through system on Summerland Key. These data will support development of a growth model to predict time to target stocking size. Data collection and analysis for this task remain ongoing. Finally, husbandry and veterinary care for 145 *D. cylindrus* colonies held at The Florida Aquarium's Coral Conservation and Research Center continued without mortality from January through June 2026, with quarterly health assessments completed and a subset of 85 colonies maintained in two greenhouse systems exposed to annual photoperiod, lunar, and temperature variation to promote post-award spawning. By advancing scalable reef grazer production methods and securing the largest living archive of *D. cylindrus*, this work directly supports active restoration of Florida's Coral Reef.

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1. DESCRIPTION

Project objectives fall under the broader goals of improving the ability to propagate priority invertebrate grazers for use in coral reef restoration and maintaining a living archive of Atlantic pillar coral, *Dendrogyra cylindrus*, to support gene banking and future restoration. The outcomes of this project will be incorporated into ongoing coral disease response and reef recovery efforts that seek to identify management actions, remediate disturbance impacts, and restore affected resources.

Florida's Coral Reef continues to suffer from compounding disturbances. Stony Coral Tissue Loss Disease (SCTLD), the heatwave of 2023, and threats from increasing hurricane frequency and severity have all driven loss of coral cover. Even with rapid and successful action to address the stressors causing coral reef decline, the current state of the ecosystem will inevitably require active restoration to regain lost ecosystem function. Coral propagation and outplanting represent an important facet of active restoration and have the potential to return genetic diversity and biomass to reefs. However, outplanting corals alone does not address the stressors that cause their decline. To reduce the widely acknowledged impacts of macroalgal overgrowth, culture techniques for invertebrate species that provide important grazing services have been developed. Both the long-spined sea urchin *Diadema antillarum* and the Caribbean king crab *Maguimithrax spinosissimus* have been documented in the refereed literature as having positive impacts on macroalgal cover and reef health.

This project builds upon results of a successful collaboration between the University of Florida (UF) and Mote Marine Laboratory established under FY 2022-23 DEP CPR funding (PO# C061D7) and continued in FY 2023-24 (PO# C2096D) and FY 2024-25 (PO# C44417). Phase I (FY 24-25) focused on lab-scale experimentation to address early life stage bottlenecks in two grazers of recognized reef rehabilitation importance. Phase II shifts toward application of those findings to improve scalable production. A third partner, Sustainable Ocean and Reefs (SOAR), has been added to incorporate an ocean-based growout component, expanding the project from purely land-based aquaculture into in situ rearing and integrated multi-trophic aquaculture with corals. Phase II also adds a fifth task led by The Florida Aquarium (TFA) covering husbandry of TFA's living *D. cylindrus* archive. This species was recently uplisted to Endangered under the US Endangered Species Act, the first coral species to reach this status, and is listed as Critically Endangered by the International Union for Conservation of Nature (IUCN). With wild colonies on Florida's Coral Reef having declined by 97% from 2013 to 2025 (Neely et al. 2025), ex situ holding of rescued colonies and their progeny is essential to the future survival of the species.

1.1. Goal: Enhance settlement and/or early post-settlement survival in two important grazer species

Objective 1 - Evaluate the application of prepared live diets for newly settled *D. antillarum* at a production scale.

Rationale: Phase I work identified candidate live diets that improved growth and survival of newly settled D. antillarum at research scale. Verifying that the best-performing diet can be applied at production scale, and quantifying its improvement over current biofilm-based practice, is a necessary step toward reliable mass culture of this priority grazer.

Objective 2 - Evaluate in situ rearing methodologies and integrated multi-trophic aquaculture of *D. antillarum* and Scleractinian corals.

Rationale: A scalable D. antillarum production pipeline will require grow-out and acclimation of hatchery-propagated urchins beyond what land-based systems can accommodate. Offshore caged nurseries offer opportunities for increased efficiency and co-culture with corals.

Objective 3 - Develop early life history growth model describing growth and survival of cultured *M. spinosissimus* and evaluate the effect of seawater source/system type on growth and survival.

Rationale: Strategic implementation of grazers in restoration efforts will require the integration of production timelines in planning stages. A model predicting time-to-target size will offer resource managers and restoration practitioners a tool for quantitatively estimating production timelines for M. spinosissimus *in restoration plans.*

1.2. Maintain a living archive of Atlantic pillar coral (*Dendrogyra cylindrus*) to support gene banking and future restoration

Objective 4 - Provide husbandry and veterinary care for the 145 *D. cylindrus* colonies held at The Florida Aquarium, including maintenance of a subset under conditions that promote spawning to support genotype banking.

Rationale: D. cylindrus was recently uplisted to Endangered under the US Endangered Species Act, the first coral species to reach this status, and is listed as Critically Endangered by the IUCN. With wild colonies on Florida's Coral Reef having declined by 97% from 2013 to 2025 (Neely et al. 2025), ex situ holding of rescued colonies and their progeny is essential to preserving the remaining genetic diversity of the species.

1.3. Reef Management Application

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

- Improved natural sexual coral recruitment in reef areas that have received grazer augmentation.
- Increased success of SCTLD-susceptible and other coral species outplants due to reduced algal competition.
- Algal mitigation at existing or future restoration sites where traditional outplanting or natural/induced spawning is targeted or 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 (e.g., cold snaps, bleaching events, coral disease) to avoid algal dominance.
- Preservation of *D. cylindrus* genetic diversity through ex situ holding of rescued colonies and their progeny, with a subset maintained under conditions that promote spawning, supporting future restoration of this species.

2. METHODS

2.1. Task 1 - Quality Assurance Plan and Reporting

The project team worked with Florida DEP project managers to develop a research Quality Assurance (QA) Plan document. An initial draft of the QA plan was submitted on 31 July 2025. Following collaborative revisions, the final document was approved on 28 August 2025. This plan outlined the purpose of the project, provided a brief historical overview and literature background, and provided a detailed description of the work to be accomplished and appropriate quality control methods. It also outlined planned methods for data acquisition, generation, and evaluation as well as documentation and data curation/storage.

2.2. Task 2 - Evaluate the application of prepared live diets for newly settled *Diadema antillarum* at production scale

This task made use of a custom-engineered recirculating aquaculture system co-operated by the University of Florida and The Florida Aquarium, configured with ten 40-L pseudo-kreisel culture vessels of clear acrylic construction. All vessels were stocked with an equal number of *D. antillarum* larvae from a single spawn on 23 January 2026 at 0.58 larvae mL^{-1} and fed equal concentrations of *Rhodomonas salina* and *Chaetoceros muelleri* microalgae throughout the larval phase. Timed aeration was used to generate water movement sufficient to suspend the weakly swimming planktonic larvae in the water column. First settlement was observed on 14 March 2026 at 53 days post fertilization.

Phase I of this project identified a candidate live feed for newly settled, benthic *D. antillarum* juveniles consisting of the benthic diatom *Navicula* sp., followed by a transition to flocculated microalgae once juveniles reached approximately 1 mm test diameter. That work was conducted at research scale to allow rigorous tracking of individual urchin growth and survival. The objective of Phase II Task 2 was to evaluate the effect of this supplemental benthic diet on growth and survival of newly settled juveniles within a larger, production-oriented culture system. The initial study design called for assigning culture

vessels to either the supplemental diet treatment or a no-supplement control treatment at a single, distinct timepoint after a minimum settler density had been reached in each vessel.

Settlement of *D. antillarum* in pseudo-kreisel culture is typically protracted, with metamorphosis of a cohort occurring over a period of weeks rather than days. In practice, this resulted in an extended period during which vessels contained large numbers of pre-competent and metamorphically competent larvae alongside relatively few newly settled juveniles. From a production standpoint, it is useful to know whether the benthic juvenile diet should be applied early in this protracted settlement phase, i.e., as soon as first settlement is observed, or delayed until a higher proportion of the cohort has settled. To address this, the work was restructured as two sequential experiments. The first (Experiment 1) evaluated treatment effects on cumulative settlement success during the mixed larval-juvenile phase. The second (Experiment 2) evaluated treatment effects on growth and survival of settled juveniles.

A revised feeding protocol was developed for application of the supplemental benthic diet. A 60mL syringe was modified to deliver measured volumes of the benthic diets directly to the bottom of each vessel. Aeration and water flow to each vessel were suspended for two hours following each feeding, allowing the diet to drop out of suspension and accumulate on the vessel bottom where newly settled benthic juveniles could encounter and graze on it. The same aeration cycle was applied to both diet and control vessels regardless of whether supplemental food was added, to standardize hydrodynamic conditions across treatments.

Experiment 1: Effects of supplemental diet on settlement success

The eight best-performing culture vessels (highest larval survival and visual health condition) were selected from the original ten to be used as experimental units. Competent larvae were redistributed among the eight vessels to equalize larval density to approximately 0.05 larvae mL^{-1}. Vessels were then randomly assigned to one of two treatments (n=4 per treatment): a supplemental diet treatment receiving 15 mL of *Navicula* sp. (a benthic diatom) per feeding, or a control treatment receiving no supplemental diet. A standardized settlement cue, consisting of ceramic tiles conditioned in seawater to develop crustose coralline algae, was added to each vessel. Feeding was initiated on 20 March 2026, after positive settlement had been observed but early in this protracted phase. Initial data collection occurred on 31 March 2026 after settlement was positively identified in all vessels. Two observers periodically counted the number of settled juveniles in each vessel via visual census. Per-vessel settler counts were calculated as the average of the two observers' counts. Average settler count was tracked four additional times across 13 days to quantify and compare settlement trajectories between treatments.

Experiment 2: Effects of supplemental diet on growth and survival of settled juveniles

After 13 days, total settler counts had increased across all vessels but with substantial variation among replicates. To begin the post-settlement growth and survival experiment under standardized conditions, settled juveniles were redistributed across the eight vessels to equalize stocking to a target of 35-40 juveniles per vessel. This occurred over an eight day period during which supplemental feeding was withheld. No urchins were moved among vessels during the last two days of this period. The eight vessels were then re-randomized into two treatment groups (n=4 per treatment): the supplemental diet treatment and the no-supplemental-diet control. Counts of surviving juveniles were conducted weekly in each vessel over 28 days using the same two-observer protocol described above. Test diameter was measured every other week from a subset of 10 haphazardly selected individuals per vessel using image analysis software, and used to compare growth rates between treatments.

The supplemental diet composition changed over the course of Experiment 2 to mirror the best practices that were suggested by experiments completed in the FY 25-26 project. From day 1 through day 14 (21 April 2026 - 4 May 2026), the supplemental diet consisted of *Navicula* sp. only, applied at 15 mL per feeding. From day 15 onward (5 May 2026 - end of experiment), the supplemental diet was changed to a flocculated preparation of *Rhodomonas salina* (10 mL) and *Chaetoceros muelleri* (5 mL) per feeding. The aeration suspension protocol described above was maintained throughout.

2.3. Task 3 - Experimental evaluations of rearing methodologies for juvenile lab-reared *D. antillarum* and density-dependent effects on coral fragments in an offshore in situ caged nursery

Experiments for Task 3 were conducted within Sustainable Ocean and Reefs (SOAR) offshore Tavernier nursery in the Florida Keys. Experimental systems were deployed mid-water and positioned approximately 20 ft in depth over sandy substrate. Cages were tethered to anchors and maintained in the water column using flotation attached to the upper cage framework to facilitate natural flow-through conditions while minimizing benthic sediment accumulation.

Experiment 1: Effects of cage spatial configuration on juvenile Diadema antillarum growth and survivorship

The first experiment evaluated the effects of cage spatial configuration on the survivorship and growth of juvenile lab-reared *D. antillarum*. Experimental cages measured approximately 2 ft × 2 ft × 2 ft and were constructed from recycled plastic framing and 1/4-inch diamond plastic mesh. Two cage configurations were evaluated with four replicate cages per treatment: divided cages and undivided cages. Divided cages incorporated internal partitions constructed from HDPE plastic sheets sandwiched between egg crate panels, creating three isolated sections within each cage. Each divided section housed three juvenile urchins, while undivided cages housed nine juvenile urchins collectively.

Juvenile *D. antillarum* were monitored approximately monthly throughout the experimental period from April through September 2025. Survivorship and test diameter were recorded during each monitoring event using scaled photographic analysis quantified in ImageJ software. Observational notes regarding fouling communities, associated fauna, and general cage condition were also documented throughout the monitoring period.

Experiment 2: Density-dependent interactions between Diadema antillarum and coral fragments

The second experiment evaluated preliminary density-dependent interactions between *D. antillarum* and coral fragments under integrated multi-trophic aquaculture conditions. Fully enclosed cages measured approximately 2 ft × 1 ft × 8 in and were constructed from sheets of 5052 marine-grade aluminum with pre-pressed 1/4-inch holes that were drilled and riveted together. Four replicate cages were utilized for each treatment.

Each cage contained two *Siderastrea siderea* and two *Porites astreoides* fragments along with one of four *D. antillarum* density treatments consisting of 0, 4, 6, or 8 individuals. The spatial configuration experiment utilized juvenile lab-reared *D. antillarum*, while the integrated coral–urchin density experiment utilized wild-collected individuals to evaluate grazing interactions under more ecologically representative field conditions.

Coral fragments and urchins were monitored approximately monthly using scaled photographic analysis to document changes in coral condition, survivorship, and urchin growth trajectories. Monitoring and data collection for this experiment remain ongoing.

2.4. Task 4 - Effect of seawater source and system type on growth and survival of cultured *Maguimithrax spinosissimus*

Experimental systems - Mote Marine Laboratory currently is able to produce juvenile *M. spinosissimus* in two geographically disparate locations with different system designs. Mote's Florida Coral Reef Restoration Crab Hatchery Research Center is located in Sarasota, FL at Mote's Aquaculture Research Park approximately 20 miles inland of the West Coast of FL. The 6,000 ft^2 facility is designed specifically for the mass production of juvenile Caribbean king crabs with a production design specification of ~250,000 juvenile crabs/year. The facility features a series of independent recirculating aquaculture systems collectively holding ~100,000 gallons of seawater. None of this water is discharged with all waste seawater being batch processed via aggressive ozonation, degassing/aeration, and activated carbon recirculation prior to testing and re-use. While nutrients, trace elements, and minerals are dosed into the system, there is concern about the consistent and repeated batch processing of seawater for the purpose of mass aquaculture production in an integrated multi-trophic aquaculture system.

Mote's Elizabeth Moore International Center for Coral Reef Research & Restoration (IC2R3) is located on Summerland Key, FL and features an open/flow-through seawater system drawing constantly from natural nearshore surface water. The system is also designed to disinfect incoming seawater with ozonation, but at a fraction of the dose rate

and contact time. All waste seawater is discharged into an engineered swale and percolates through the carbonate platform.

Both seawater systems have identical targets for temperature control and flow dynamics in crab culture systems. One focus of this study was to compare crab survival and growth with respect to system design with replicate cohorts hatched in each system type being reared in both systems. To accomplish this, larvae were hatched in both systems, allowed to develop undisturbed for 10 calendar days at which point, a subset of settled juvenile crabs (~100) were collected. Half of the animals were immediately placed into individual rearing chambers in a seawater raceway in the hatching facility and half were packaged in seawater and shipped overnight to the other hatching facility where the animals were placed into an identical rearing system. These reciprocal transfers of juvenile crabs allowed for a direct comparison of the effect of the hatching and rearing system on crab growth and survival.

Crab growth and survival - Crabs were tracked individually through the experiment, in each project location, with maternal clutch ID and hatching date being recorded for each focal animal. For a subset of animals (n = 10) within each clutch, a wet weight was measured at the beginning of the experiment and at each observed molt event. Each individual crab was checked daily for mortality, which was recorded as a binary response along with date - survival was calculated as the number of days that elapsed between hatching and mortality. Each individual crab was also checked, daily, for molting. When a molt was observed, the animal was removed from the individual culture unit, positioned on a dissection tray in the laboratory and photographed from above with a photo scale in frame to allow for morphometric measurements to be taken digitally to minimize physical/handling stress on the animals (see **Figure 8**). We quantified growth in three response variables: molt interval (days), wet weight (g), and molt increment (mm) in two morphometric dimensions – Carapace Width (CW), and Carapace Length (CL). Molt interval was recorded as the number of days elapsed between successive observed molt (ecdysis) events. Molt interval was calculated as the difference in each dimension (CW & CL) between post-molt and pre-molt measurements. Only crabs with at least one recorded molt event were used in the growth analysis, but all subjects were included in survival analyses.

Statistical analyses - All analyses were conducted in R studio (R version 4.6; R Core Team) with lme4, lmerTest, emmeans, MuMIn, car, survival, broom, ggplot2, and patchwork primary packages. We fitted three general linear mixed effects models to characterize the effects of natal location and rearing location on growth of cultured juvenile Caribbean king crabs. All three models shared the following fixed effects structure:
Log(response) ~ Rearing Location x Natal Location + log(previous CW) + Molt Stage + (1|Trial) + (1|Crab ID)

The location interaction term (Rearing x Natal) evaluated the effect of rearing system on growth with respect to natal system. Previous CW (crab size prior to each recorded molt event) was included (log-transformed) as a continuous covariate to account for conspicuous allometric scaling of growth with size/stage. Molt stage, here simply the iterative molt

event following the 10-day settlement period prior to the start of each trial, was included as a linear fixed effect. Two random intercepts were specified: Trial accounted for cohort/trial-level variation across the eight trials and Crab ID accounted for repeated measures within individual crabs/subjects.

Models were fit using restricted maximum likelihood via the lme4 package in R (version 4.6; R Core Team). Denominator degrees of freedom and p values were calculated using Satterthwaite approximations in lmerTest . The significance of fixed effects were evaluated in Type III Wald F-tests (car package). Marginal (R^2m) and conditional (R^2c) coefficients of determination were calculated for fixed and total model variance, respectively, using the MuMIn package .

Estimated marginal means for each rearing x natal treatment combination were calculated using the emmeans package. They were evaluated at the grand mean of log-transformed previous CW values and the mean molt stage across all observations. Because each model was fit with a manually log-transformed response (*i.e.*, a Gaussian model on log-scale data rather than a generalized linear mixed effects model with a formal log link), emmeans returns estimates on the log scale. Thus, back transformations were carried out exponentially on the estimated marginal mean and its 95% confidence interval bounds, producing geometric means. Pairwise contrasts among the four orthogonally crossed treatment groups were evaluated as ratios of geometric means and adjusted for multiple comparisons using the Tukey method.

To estimate time to target stocking size for cultured *M. spinosissimus* based on both natal and rearing location, we used an iterative molt stage-driven growth simulation using population mean (fixed effect only) predictions from Model A and Model B (molt interval & molt increment CW, respectively). Growth model simulations were initiated using the mean initial CW of crabs in our experiment (CW measured at beginning of each trial for each treatment group). At each simulated molt, the model predicted the expected molt interval and increment (CW) conditionally based on model results derived from the observed data by treatment group while holding all random effects to zero. The CW of each simulated crab was updated additively and accumulated time continued until the simulated CW met or exceeded the target CW of 30 mm. Parametric bootstrap 95% confidence intervals (n = 500 iterations) were calculated by sampling fixed effect coefficient vectors from their joint multivariate normal distribution (mean = maximum likelihood estimates; variance-covariance = asymptomatic fixed effects variance matrix) using MASS::mvrnorm, re-running the full simulation for each bootstrap draw, and computing the 2.5th and 97.5th percentiles of the resulting distribution of days-to-stage.

Survival status at the end of each trial was recorded for all 647 individuals across the 8 trials as one of three outcomes: living (alive at the end of the project period), observed/confirmed mortality, or missing (treated as censored). The probability of a confirmed mortality as a function of treatment group was modeled using a binary logistic regression with a rearing x natal location interaction (blm, binomial family). Type III Wald tests assessed the significance of fixed effects. Predicted group-level mortality probabilities and odds ratios with 95% profile-likelihood confidence intervals were derived from the fitted model. To assess the effect of selective differential mortality bias on growth

estimates, we added eventual mortality status ("fate" – alive, dead, missing) as a covariate in each linear mixed effects model and evaluated significance via Type III F-test. Kaplan-Meier survival curves were estimated for each of the four orthogonally-crossed treatment groups using the survival package , and differences among survival distributions were assessed by log-rank tests for the all treatment groups comparison and for each main effect separately.

Wet weight was collected from a subsample of individuals within each trial. We assessed how representative the subsamples were by comparing CW and molt number distributions between crabs that were subsampled for wet weight and those that were not in an independent samples t-test stratified by treatment group. The proportion of individuals with at least one wet weight observation was calculated for each treatment group. Allometric scaling of wet weight with respect to CW was characterized by fitting a simple linear regression model of log transformed we weight and CW measurements and extracting the scaling exponent b with 95% CI; b = 3 corresponds to isometric allometric scaling, b > 3 indicated positive allometric relationships and b < 3 indicated negative allometry. Treatment effects on weight by size were then evaluated in fourth linear mixed effects model (Model D)

Log(WetWeight) ~ log(CW) + Rearing location x Natal location + Molt stage + (1|Trial) + (1|Crab ID)

The model is intended to evaluate the effect of treatment group on crab wet weight (~biomass) with respect to CW and molt stage while accounting for allometric scaling and random variance among trials and individuals.We evaluated any potential effects of system location on carapace geometry (*e.g.*, potential differences in allometric exponent between rearing systems) by fitting a separate model that included interaction terms between CW and both rearing and natal location. We assessed these interaction terms using Type III Wald F-tests.

We generated a general condition index using the residuals from a trial adjusted allometric model: log(WetWeight) ~ log(CW) + (1|Trial). Positive residuals indicate heavier than expected crabs (*e.g.*, increasingly healthy crabs) whereas negative residuals were an indication of lighter than expected crabs (*e.g.*, potential emaciation). We used successive wet weight measurements to calculate a wet weight-based molt increment (g). Treatment effects on log-transformed wet weight increments were modeled parallel to CW and CL increments (*i.e.*, Models B & C) using a fifth model, Model E:

Log (Weight Increment) ~ Rearing location x Natal location + log(PreviousCW) + Molt stage + (1|Trial) + (1|Crab ID)

We evaluated the prospective effect of body condition at a molt on growth in the subsequent molt by matching the condition index to the CW molt increment and molt interval records at Molt stage $n + 1$ within each individual. We fitted two models to test the hypothesis that previous condition index value predicts: 1. Next molt increment (CW) and 2. Next molt interval (days). Each model controlled for treatment group, previous CW,

molt stage, and included Trial and Crab ID as random effects. The significance of the previous condition coefficient was evaluated using a Type III F-test. The back-transformed coefficient exp(b) represents the multiplicative increase in molt increment (CW) per unitary increase in condition index. We also evaluated the effect of mean individual condition index (*i.e.*, mean condition index for an individual across all molt events for which a wet weight measurement was taken) on survival probability in a logistic regression model (Survival ~ Mean Condition Index + Rearing location x Natal Location, binomial family) with the condition index odds ratio and 95% profile-likelihood confidence interval extracted from the fitted model.

2.5. Task 5 - Maintain Atlantic pillar coral (*Dendrogyra cylindrus*) living archive for gene banking

Pillar coral colonies were held in up to seven different recirculating aquarium life support systems in the Coral Conservation and Research Center. Each life support system consisted of a tank, sump, protein skimmer, media reactor containing activated carbon and polyfilter, and temperature control through heating and chilling. Additional flow was provided to each system with submerged wave devices providing a minimum of 50x the tank volume per hour in additional water movement (Octo Pulse pumps or Maxspect Gyres). Three systems received natural sunlight in greenhouses and four systems were illuminated with LED lighting (Ecotech Radion Pro Gen5). Detritus and algae were cleaned and siphoned from each aquarium weekly, and a minimum of 20% of the water was exchanged and replaced with filtered seawater. Corals were fed three times weekly with a mixture of dried pelleted foods (Reef Roids, Golden Pearls) and frozen foods including mysis shrimp, krill, copepods, and rotifers. Each coral was target-fed with a pipette or turkey baster with water circulation turned off while food was ingested. Temperature was recorded and life support system function assessed twice daily. Water quality was tested weekly at minimum in each aquarium system and recorded in Tracks. Systems C4.2 and C4.3 are exposed to annual photoperiod, lunar, and temperature changes and hold 85 pillar coral individuals in order to promote spawning which is anticipated to occur after the award period.

Quarterly health assessments were conducted in February and May 2026 following the standard protocols for the Florida Coral Rescue and Propagation Project, which include a visual assessment of each individual coral and a record of any recent medical treatments or tissue loss. The maximum straight length, width, and height of each coral was recorded in centimeters to the nearest 0.25 cm using a measurement tape in February 2026. Photographs of select corals were taken quarterly, and full photographs of each coral were taken in February of 2026.

3. RESULTS

3.1. Task 1 - Quality Assurance Plan and Reporting

The Quality Assurance plan successfully facilitated a rigorous scientific process and ensured usable data. While some setbacks occurred and not all results were as anticipated, the multiple experiments within the project went according to the plan and it was not

necessary to modify the QA plan at any point during the project. All data will be made available as part of the final deliverables for this effort.

3.2. Task 2 - Evaluate the application of prepared live diets for newly settled *Diadema antillarum* at production scale

Experiment 1: Effects of supplemental diet on settlement success

Cumulative settlement was compared between treatments at the final census (n = 4 vessels per treatment) using a Mann-Whitney U test. Mean settler counts increased over the 13-day census period in both treatments, from 12.0 ± 4.2 to 53.0 ± 17.2 settlers per vessel in the control and from 6.3 ± 2.8 to 23.3 ± 13.5 in the supplemented treatment (mean ± SE) (Figure 1). At the final census, settler counts did not differ significantly between treatments (W = 13, exact p = 0.20), although control vessels trended numerically higher (median 53.0 [range 15–91] vs 17.5 [range 1–57]). The supplemental benthic diet therefore showed no detectable effect on settlement success during the mixed larval–juvenile phase.

Experiment 2: Effects of supplemental diet on growth and survival of settled juveniles

Both treatments began the experiment at comparable juvenile densities (30.5 ± 5.5 control vs 30.9 ± 2.2 supplemented; mean ± SE). Survival, indexed by the number of juveniles per vessel at the final census (day 42), did not differ between treatments (Mann-Whitney U: W = 11.5, p = 0.38; 30.1 ± 8.8 [median 31.75, range 7.5–49.5] control vs 22.4.0 ± 2.4 [median 23.0, range 16.0–27.5] supplemented; n = 4) (Figure 2). Test diameter was analyzed with a linear mixed-effects model (log scale) with treatment, date, and their interaction as fixed effects and random intercepts for vessel and vessel × date. Juveniles grew strongly over the 42-day period, from 0.81 ± 0.09 and 0.75 ± 0.09 mm (control and supplemented; mean ± SE) to 2.97 ± 0.25 and 3.29 ± 0.60 mm (Figure 3). Respective growth rates were 51 and 61 μm d^{-1} (control and supplemented). Neither the supplemental diet ($F_{1,6.0} = 0.07$, p = 0.80) nor the diet × date interaction ($F_{3,17.9} = 0.33$, p = 0.80) significantly affected growth. The diet switch to flocculated *Rhodomonas/Chaetoceros* on 5 May produced no detectable divergence between treatments although supplemented vessels were numerically larger by 2 June (3.29 vs 2.97 mm); this divergence was not statistically significant.

3.3. Task 3 - Experimental evaluations of rearing methodologies for juvenile lab-reared *D. antillarum* and density-dependent effects on coral fragments in an offshore in situ caged nursery

Experiment 1: *Effects of cage spatial configuration on juvenile Diadema antillarum growth and survivorship*

Juvenile lab-reared D. antillarum maintained decent survivorship throughout the approximately 150-day monitoring period in both treatments. Final survivorship remained approximately 55% in divided cages and 45% in undivided cages by the conclusion of monitoring. Survivorship declined most rapidly during the initial acclimation period before

stabilizing through later monitoring events. Divided cages maintained slightly greater survivorship trajectories relative to undivided cages throughout the monitoring period, although treatment differences remained modest overall (Figure 4).

Juveniles in both treatments exhibited continued positive growth throughout the monitoring period. Mean test diameter increased from 2.12 ± 0.26 cm to 4.21 ± 0.46 cm (mean ± SD) in divided cages and from 2.46 ± 0.19 cm to 4.24 ± 0.21 cm in undivided cages between April and September 2025. Although juveniles stocked within divided cages began the experiment at smaller mean test diameters relative to those in undivided cages, individuals within divided cages exhibited comparable growth trajectories throughout the monitoring period and approached similar final mean test diameters by the conclusion of monitoring (Figure 5).

Experiment 2: Density-dependent interactions between Diadema antillarum and coral fragments

Monitoring indicates that *D. antillarum* maintained positive growth across all density treatments (0, 4, 6, and 8 urchins per cage) during the reporting period. Mean test diameter generally increased between sampling events in the 4-, 6-, and 8-urchin treatments, demonstrating that offshore in situ cages supported continued urchin growth under all evaluated stocking densities.

Coral fragment response varied among cages and species. *Siderastrea siderea* exhibited variable growth among density treatments, with the greatest increases generally occurring at intermediate urchin densities (Figure 6). *Porites astreoides* similarly exhibited variable responses among treatments, with positive growth observed across most density treatments during the monitoring period (Figure 7). Mean coral fragment size increased from 1.45 to 1.58 cm in the 4-urchin treatment, from 1.63 to 1.89 cm in the 6-urchin treatment, and from 1.69 to 1.81 cm in the 8-urchin treatment between March and May. In contrast, mean coral size in control cages remained relatively unchanged over the same period (1.60 to 1.61 cm). Across all fragments, the 6-urchin treatment exhibited the greatest average net growth (+0.26 cm), followed by the 4-urchin (+0.13 cm) and 8-urchin (+0.12 cm) treatments.

Although individual coral fragments displayed variable responses and some exhibited minor declines in measured dimensions, preliminary results suggest that the presence of *D. antillarum* may provide benefits to coral growth relative to control conditions (Figures 6 and 7). Among the densities evaluated to date, the intermediate density treatment (6 urchins per cage) produced the strongest positive coral growth response.

3.4 Task 4 - Effect of seawater source and system type on growth and survival of cultured *Maguimithrax spinosissimus*

The project team tracked a total of 634 individual crabs across 8 independent clutches with 5 hatched at MAP and 3 hatched at IC2R3 during the project period. Additional clutches (i.e., trials) are underway, but were not hatched in time to be included in this analysis. After filtering observer errors and missing data due to equipment failures (e.g., camera failures),

the team observed a total of 1,270 molt events from 333 individual juvenile crabs across eight trials (i.e., independent maternal clutches). Molt interval and molt increment (CW & CL) varied with respect to natal and rearing location (**Table A**).

We evaluated the effect of rearing location, natal location, their interaction, molt stage, and previous body size (CW) on molt interval and molt increment (CW & CL) in three linear mixed effects models. Previous body size (log(previous CW)) was the only parameter with strong predictive power in all three models suggesting, unsurprisingly, that molt interval and absolute increment (mm CW & CL) increase with with respect to molt stage using initial body size (CW) as a proxy for molt stage (**Table B**).

Juveniles hatched at IC2R3 and reared at MAP had significantly longer (11.2% longer) molt intervals than crabs that were hatched and reared at IC2R3. All other pairwise contrasts for molt interval, molt increment CW, and molt increment CL were not significantly different with respect to treatment group (Estimated marginal means: **Table C**; pairwise contrasts: **Table D**; Interaction plots: **Figure 9**).

We simulated the growth of cultured juvenile crabs to target stocking size (30 mm CW) with respect to treatment group and estimated time-to-target-size for juveniles using a treatment-specific initial body size (CW). All treatment groups required 12 molts to reach target stocking size in the simulation. Crabs hatched and reared at IC2R3 reached target size the earliest in an estimated 187 days (95% CI: 154 – 224 days) and crabs hatched and reared at MAP taking the longest with an estimated 216 days (95% CI: 186 – 271 days; **Table E**, **Figure 10**).

The project team tracked a total of 634 individual crabs for survival. The ultimate fate of these crabs, at the time of this report was 305 (48.1%) were recorded as confirmed mortalities, 204 (32.2%) were living at the time of writing this report, and 125 (19.7%) were classified as "missing" during the course of the experiment. We evaluated the effect of treatment group on the probability of mortality in a Logistic regression model. There was no significant difference in probability of mortality with respect to rearing location, natal location, nor their interaction. Predicted mortality was approximately 46 – 51% over the course of the experiment regardless of treatment group or trial.

To evaluate the effect of ultimate mortality fate on our growth model predictions, we added ultimate mortality fate as a covariate in each of the growth linear mixed models. Crabs that ultimately died had significantly different molt intervals ($F_{2,468} = 5.00$, $p = 0.007$) and molt increments (CW: $F_{2,370} = 16.74$, $p < 0.0001$) prior to death than crabs that ultimately survived. Crabs that ultimately died showed significantly different growth trajectories than crabs that survived, suggesting a variable mortality bias in growth models. We modeled survival in a Kaplan-Meier analysis which, likewise, found no significant difference in survival with respect to trial or treatment group (**Figures 11 & 12**).

For wet weight, we subsampled a total of 85 individual crabs accounting for 297 discrete molt events across all eight trials, which accounted for 17-34% of animals per treatment group. Crabs haphazardly selected for the weighed subsample group did not significantly

differ in mean CW from the other crabs in the experiment (t = -0.404, df = 528.7, p = 0.686), which suggested a representative subsample with respect to body size.

The grand allometric scaling exponent was calculated as b = 2.57 (95% CI: 2.47-2.67; R^2 = 0.903) which was significantly lower than isometry (*i.e.*, b = 3 – cube law) which indicated a negative allometric relationship between biomass and crab size (CW) – as crabs increased in size (CW), they tended to become proportionally lighter than expected based on geometric isometry. After controlling for allometric scaling and developmental stage, there was a significant difference in wet weight of crabs with respect to rearing location ($F_{1,134.5}$ = 14.15, p = 0.0003; Model D; **Table F**). Crabs that were reared at IC2R3 were heavier at the same carapace width as crabs reared at MAP (log-scale coefficient = 0.382 or ~46% wet weight advantage). Natal location, nor the interaction of rearing and natal location, had no effect on wet weight, but there were differences in estimated weight at size (**Table G**). There was a significant difference in scaling exponent with respect to rearing system (log(CW) x rearing: $F_{1,288.7}$ = 5.09, p = 0.025) however, there was no significant difference in scaling exponent by natal location ($F_{1,288.4}$ = 0.97, p = 0.324).

Body condition index (*i.e.*, size-corrected residual wet weight) showed a marked difference between treatment groups. Crabs that were both hatched and reared at MAP showed the lowest mean condition index value (mean Condition Index = -0.316 ± 0.639) and crabs that were both hatched and reared at IC2R3 had the highest (0.087 ± 0.417). Rearing location had the strongest and significant predictive effect on condition ($F_{1,\ 140.5}$ = 15.59, p = 0.0001); natal location had a marginally significant effect on condition ($F_{1,\ 24.8}$ = 3.63, p = 0.068); but there was no significant interactive effect ($F_{1,\ 63.5}$ = 2.44, p = 0.123; **Table F**).

We recorded weight increments for 193 molt events from 58 crabs. Crab size (CW) prior to molting (*e.g.*, previous CW) was the only factor with a significant effect on log-transformed weight increment ($F_{1,\ 42.9}$ = 136.96, $p < 0.001$, β = 2.60) suggesting that larger crabs gained proportionally more mass per molt, which agrees with allometric scaling (R^2m = 0.815; **Table F**). There were no significant differences in weight increment with respect to treatment group (all $p > 0.79$). However, body condition was an excellent predictor of molt increment (CW) at molt n + 1 ($F_{1,127.7}$ = 9.32, p = 0.003, β = 0.433; 136 molts from 49 crabs; **Table H**). With each unit increase in body condition index predicting a 54.2% greater CW molt increment in the next molt (**Figure 13**). However, molt interval in subsequent molts did not differ significantly with respect to body condition index ($F_{1,\ 128.7}$ = 0.154, p = 0.696) or survival outcome (Odds ratio = 0.680, 95% CI: 0.175 – 1.781, p = 0.50; **Table H**).

3.4. Task 5 - Maintain Atlantic pillar coral (*Dendrogyra cylindrus*) living archive for gene banking

Pillar coral colonies (145 total) were successfully maintained with no mortalities from January through June 2026. Figure 14 depicts an example colony. Six individuals received medical treatment in the first quarter of 2026 and no additional corals received medical

treatments in the final quarter. Health assessments and measurements were provided in the deliverables package on March 16, 2026 and will be provided again on June 16, 2026.

4. DISCUSSION

4.1. *Diadema antillarum* production scale-up and in situ grow-out

The supplemental benthic diet provided in Task 2 showed no statistical effect on settlement success in Experiment 1, but the numerically lower settler counts in supplemented vessels relative to controls (median 17.5 vs. 53.0) suggest a potential negative effect on continued larval development and settlement when the diet was applied during the protracted settlement phase. Providing a diet formulated for newly settled benthic juveniles during a period when the cohort is still largely composed of pre-competent and metamorphically competent larvae may interfere with planktonic development of those individuals not yet settled, and the two-hour aeration suspension protocol may further reduce water column conditions favorable for late-stage larvae. We therefore recommend that the supplemental diet not be applied until a satisfactory proportion of the cohort has settled, rather than at first observation of settlement.

Task 2 experiment 2 detected no significant effect of the supplemental diet on juvenile growth or survival over 42 days, in contrast to positive effects of comparable diets observed in Phase I research-scale experiments. This discrepancy likely reflects differences in delivery method and culture scale rather than a lack of underlying diet efficacy. Although mean survival did not differ between treatments, the supplemented treatment showed notably tighter among-vessel variance in survival than the control. Reduced variance is itself a meaningful benefit for production because it produces more consistent yields across vessels, which simplifies planning for downstream growout and restoration use. One possible explanation is that the supplemental diet partially compensated for differences in naturally derived biofilm development among vessels, narrowing the performance gap between vessels with strong and weak biofilm growth. During the final 14-day interval, supplemented vessels grew roughly twice as fast as controls (~63 vs 27 $\mu m\ d^{-1}$) and ended numerically larger. This emerging trend suggests that supplemental diets can improve growth outcomes once juveniles reach approximately 30 days post-settlement. By this stage, as urchins grow and consumption increases, competition for naturally derived food resources such as biofilms inside the culture tanks likely intensifies, indicating a role for supplemental diets to sustain growth in the hatchery setting. This window is operationally important because juveniles of this size are still too small to transfer to other land or ocean-based aquaculture systems for continued growout, so optimizing growth within the production system prior to restocking is critical.

This interpretation is supported by an incidental observation: vessels with greater exposure to natural sunlight developed more robust biofilms and produced higher juvenile growth and survival than vessels with less sunlight exposure. Among-vessel variation in biofilm development is difficult to standardize across a production system and likely contributes to the variable outcomes characteristic of *D. antillarum* hatchery rearing. A reliable,

standardized supplemental diet, once delivery is optimized, could reduce dependence on this unpredictable natural food source and improve consistency. We recommend continued development of delivery methods for supplemental benthic diets in production-scale settings, with priority on identifying the optimal timepoint for initiating the diet relative to settlement progression and evaluating diet delivery protocols that improve diet encounter rates by newly settled juveniles.

Results from the offshore in situ grow-out experiments outlined in Task 3 demonstrate that suspended mid-water cage systems can support continued survivorship and growth of juvenile *D. antillarum* under natural environmental conditions. Juveniles maintained relatively high survivorship throughout the monitoring period while exhibiting continued increases in test diameter across both cage configurations. These findings support the use of offshore nursery systems as a potential intermediate grow-out phase between land-based aquaculture and direct reef stocking.

The spatial configuration experiment suggests that cage partitioning may improve survivorship and growth consistency during juvenile grow-out. Although juveniles stocked within divided cages generally began the experiment at slightly smaller mean test diameters relative to those in undivided cages, individuals within divided cages ultimately achieved slightly greater mean test diameters by later monitoring periods while also maintaining slightly greater survivorship overall. Partitioning may reduce aggregation, competition, or other density-related interactions among juveniles, although additional work is needed to determine the mechanisms underlying these trends and whether these effects persist across larger production scales.

Importantly, offshore cages supported the development of ecologically realistic environmental conditions and associated reef communities throughout deployment. Exposure to reef-associated fauna, natural flow regimes, environmental variability, and potential predator interactions may help promote ecologically relevant behaviors such as predator defense responses that are difficult to replicate in laboratory culture systems. At the same time, these natural interactions may also contribute to some mortality within offshore nursery environments. Acclimation to natural conditions prior to outplanting may nevertheless provide advantages relative to direct transfer from land-based systems by preparing juvenile urchins for environmental conditions encountered on restoration reefs.

Observations from the integrated coral–urchin experiment further suggest that offshore co-culture systems may provide a useful framework for maintaining coral fragments alongside *D. antillarum* in situ, allowing grazing services provided by urchins to potentially improve coral husbandry while supporting concurrent herbivore production. Coral fragments maintained in cages containing *D. antillarum* generally exhibited greater net growth than fragments maintained in control cages without urchins. Among the densities evaluated, the intermediate-density treatment (6 urchins per cage) produced the greatest average coral growth response, while both the 4- and 8-urchin treatments also exhibited positive growth relative to controls.

Continued monitoring will help determine whether the apparent advantages observed at intermediate urchin densities persist through time and whether an optimal stocking density exists that maximizes coral growth while maintaining urchin performance. SOAR intends to continue monitoring this experiment beyond the reporting period at no additional cost to further evaluate long-term density-dependent interactions and inform future integrated restoration strategies that combine coral propagation with herbivore augmentation.

4.2. *Maguimithrax spinosissimus*

The Caribbean king crab, *Maguimithrax spinosissimus*, is the largest crab naturally occurring in the Western Atlantic. Its large size, rapid growth, and high fecundity make it an excellent candidate for aquaculture. Its remarkable grazing rates and generalist diet have strong potential for facilitating improved outcomes in direct restoration efforts by mediating coral-algal interactions and relatively rapidly and significantly reducing the cover of macroalgae and algal turf on reef structures. However, while the potential benefits of the species to restoration outcomes are well-documented, the species occurs at naturally low abundance and density throughout its natural range.

Efficient production of Caribbean king crabs remains a major bottleneck to scaling the implementation of the species in supporting improved coral reef restoration outcomes. Our results suggest that growth is affected both by where a crab is hatched and where it is reared. As expected, our results suggest that handling and transport stress result in extended molt intervals. There was no effect of treatment group on survival or the probability of mortality – this is surprising given that two treatment groups involved shipping newly settled juvenile crabs overnight prior to the start of each trial which, given previous experience with many different crustacean species, is typically an extremely physiologically stressful endeavor.

The significant differences in growth observed in both morphometric dimensions and mass between treatment groups is interesting. While water quality is an interesting and likely factor affecting these parameters, there were also substantial differences between facilities in diet composition. MAP is designed to function as an IMTA capitalizing on the higher biological load on the system and the resulting nitrogenous waste and bio-available phosphorus to drive the production of cultured macroalgae. These macroalgae are, in turn, used to supplement the diet of crabs in culture along with collected wild algae, commercial dry feeds, and frozen animal protein supplements. IC2R3 is an open flow-through system with limited algae productivity and, thus, cultured crabs are typically fed a diet largely composed of wild algae collected from coral reefs and nearshore hardbottom habitats and then supplemented with commercial dry feed and frozen animal protein supplements. Given that crabs reared within the recirculating system consistently exhibited longer molt intervals and smaller molt increments as well as a negative allometric relationship between weight and size, it is possible that despite a standard culture practice of feeding all cultured animals *ad libitum*, the composition of the diets are suboptimal. The project team plans to address the effect of nutritional condition and the effect of diet composition on nutritional condition in the next phase of this work.

The estimated time to size in our growth model does not agree with previous measurements from either system wherein target size was reached reliably within 90 – 150 days in both systems. Here, time-to-size estimates ranged from 187 – 216 days with substantial variance in estimates between and among treatment groups. However, survival and mortality models suggest that variance in growth metrics with respect to mortality fate may substantially be biasing these results. Additionally, substantial delays in the start of the experiment due to a lack of viable clutches early on in the project period resulted in the experimental dataset being substantially skewed toward early life history stages with none of the experimental animals reaching or exceeding the target stocking size prior to the end of the performance period. Over the molt stages that are well represented in the dataset, the variance in growth and survival metrics is much lower than in the later molt stages with relatively low sample size and thus a likely greater influence of pre-mortality bias on growth responses.

The project team plans to continue adding to the sample size of clutches as well as increasing our sample size in later molt stages to continue refining the accuracy and predictive power of the growth and survival models. As the accuracy of model predictions increases, the model will offer resource managers and practitioners an important tool for restoration planning with respect to Caribbean king crab production for stocking efforts. To expand the generality of the model or its application potential, the project team suggests replicating these studies in other areas throughout the region (*e.g.*, the Mesoamerican Barrier Reef, eastern Caribbean region) to parameterize the model for target restoration locations.

4.3. *Dendrogyra cylindrus*

Consistent health, growth, and survival of pillar coral gene bank colonies as well as sexual recruits previously produced at TFA indicates the long-term success of the ex-situ gene banking strategy for this Endangered coral species. Consistent funding to support this critical living collection and their annual reproduction cycles allows us to advance current methods in cryopreservation for this species, which will eventually help reduce the cost of long-term gene banking. This funding allowed us to fill a gap between a prior CORDAP project and a new DEP CPR grant (with Nova Southeastern University) that will support additional spawning and fertilization work later in 2026, and a possible new CORDAP award that will support additional cryopreservation work. We will continue to work with FWC to assist with Section 10 permit applications for pillar coral outplanting and research under the ESA.

5. FIGURES AND TABLES

FIGURES

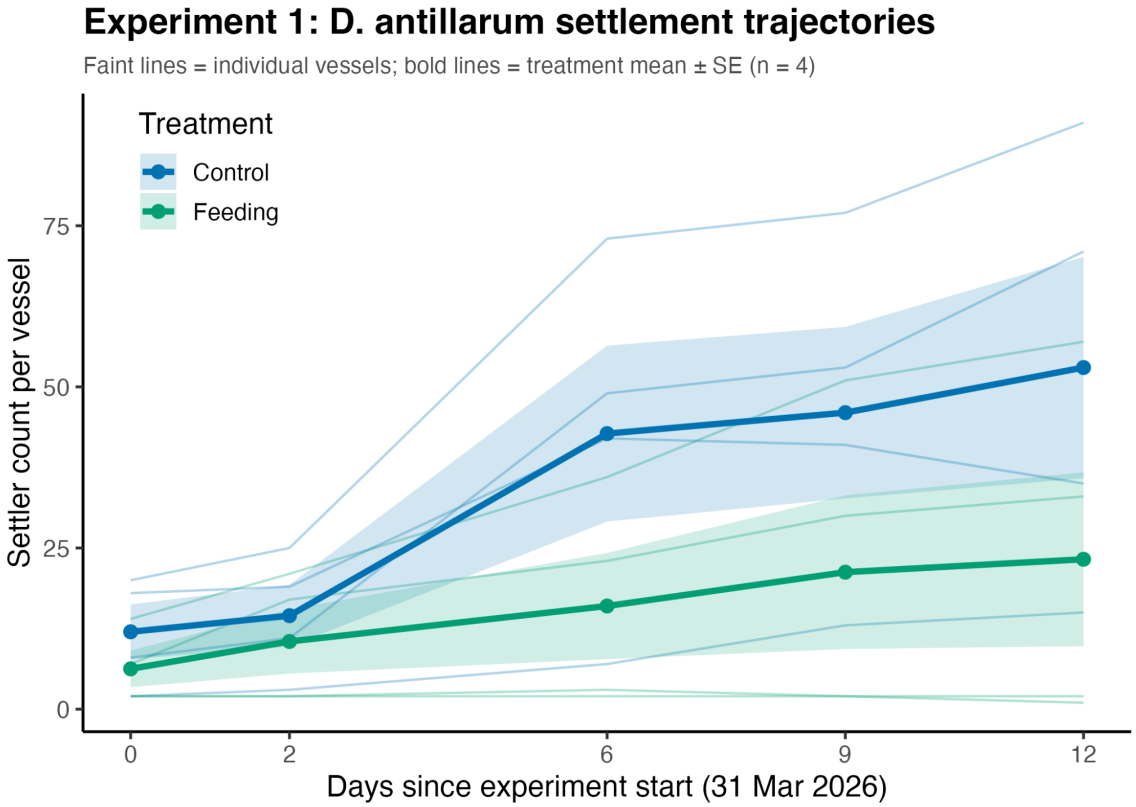

Figure 1. *Diadema antillarum* settlement trajectories during Experiment 1. Settler counts per culture vessel plotted against days since experiment start (31 Mar 2026) for the supplemented (Feeding) and control treatments. Faint lines show individual vessels (n = 4 per treatment); bold lines and points show treatment means, and shaded ribbons denote ± 1 SE. Settlement did not differ significantly between treatments at the final census (Mann-Whitney U, p = 0.20).

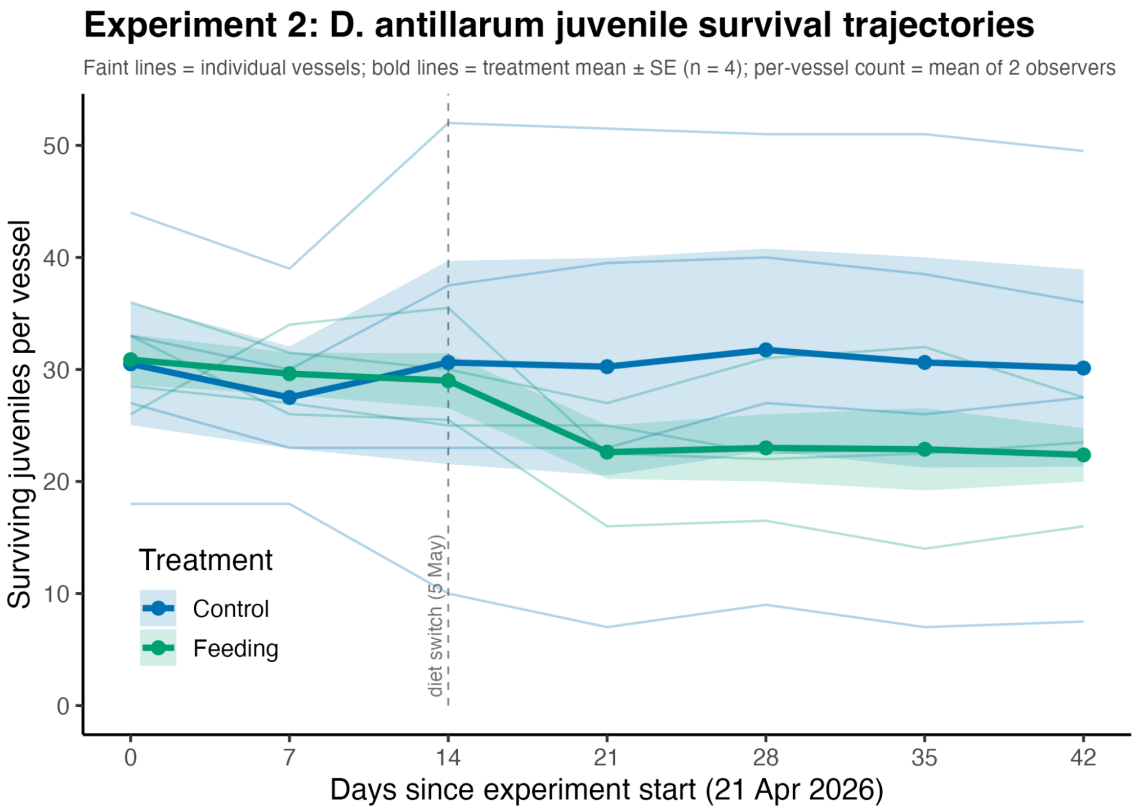

Figure 2. *Diadema antillarum* juvenile survival trajectories during Experiment 2. Number of surviving juveniles per vessel plotted against days since experiment start over five weekly censuses. Faint lines show individual vessels (n = 4 per treatment); bold lines and points show treatment means, with shaded ribbons denoting ± 1 SE. The dashed vertical line marks the day-14 supplemental-diet switch from *Navicula* sp. to flocculated *Rhodomonas/Chaetoceros*. Survival did not differ significantly between treatments at the final census (Mann-Whitney U, p = 0.49).

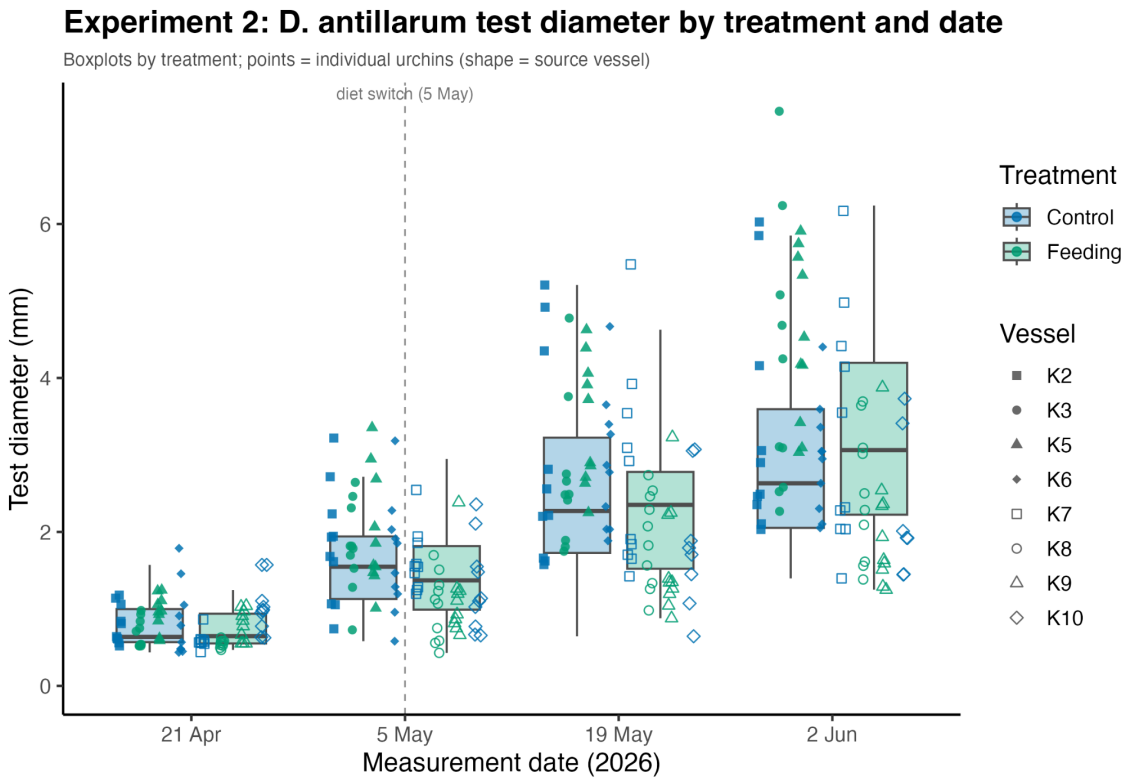

Figure 3. *Diadema antillarum* test diameter by treatment and date during Experiment 2. Boxplots reveal test diameter for the supplemented and control treatments at each measurement date (21 Apr, 5 May, 19 May 2026); overlaid points are individual urchins (~10 per vessel per date). The dashed vertical line marks the day-14 diet switch. Diameter increased significantly over time (linear mixed-effects model, $p < 0.001$), but neither treatment ($p = 0.62$) nor the treatment × date interaction ($p = 0.98$) was significant.

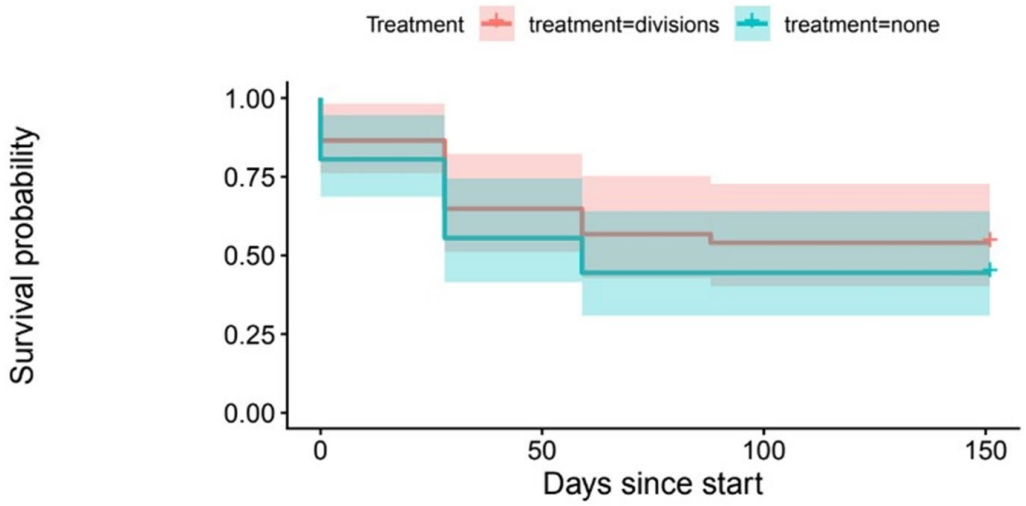

Figure 4. Kaplan-Meier survivorship trajectories of juvenile lab-reared *Diadema antillarum* maintained in divided and undivided offshore in situ nursery cages over an approximately 150-day monitoring period. Divided cages contained three isolated sections housing three juveniles per section, while undivided cages housed nine juveniles collectively (n = 4 cages per treatment). Shaded regions represent 95% confidence intervals surrounding survivorship estimates. Survivorship declined most rapidly during the initial acclimation period before stabilizing through later monitoring events. Divided cages maintained slightly greater survivorship probabilities throughout the monitoring period relative to undivided cages.

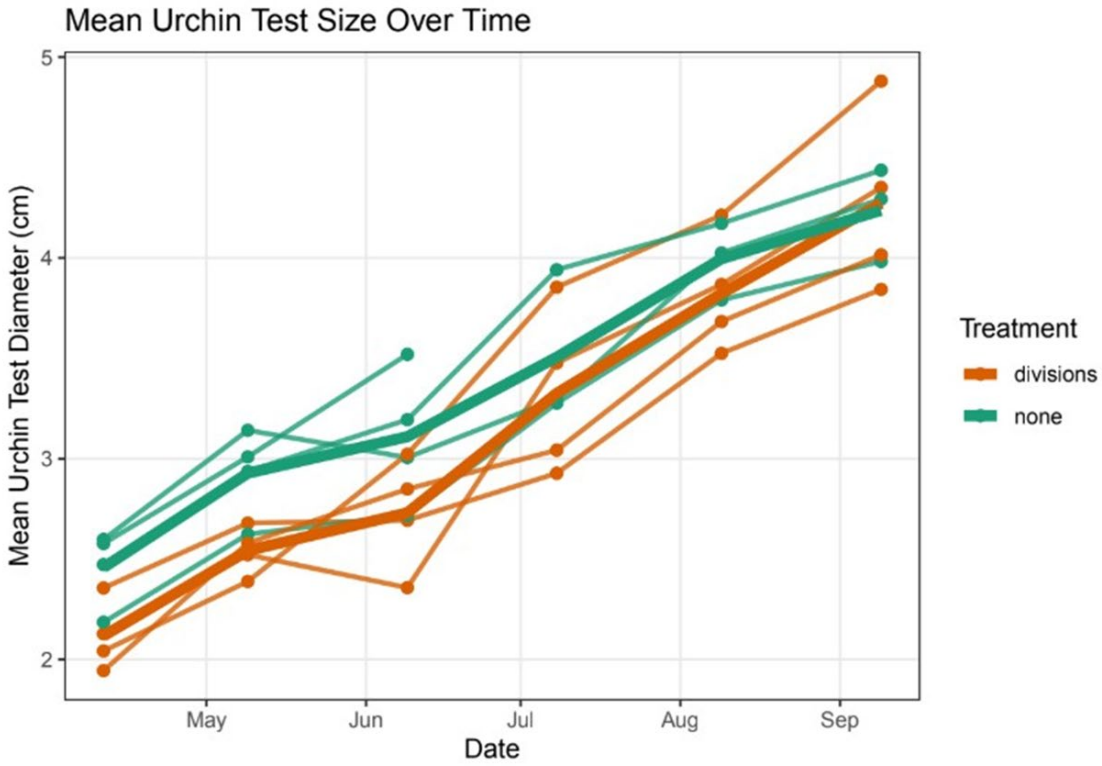

Figure 5. Mean test diameter trajectories of juvenile lab-reared *Diadema antillarum* maintained in divided and undivided offshore in situ nursery cages from April through September 2025. Thin lines represent individual cage means, while bold lines represent treatment means (n = 4 cages per treatment). Juveniles in both treatments exhibited continued positive growth throughout the monitoring period. Although juveniles stocked within divided cages began the experiment at smaller mean test diameters relative to those in undivided cages, individuals within divided cages exhibited comparable growth trajectories and approached similar final mean test diameters by the conclusion of monitoring.

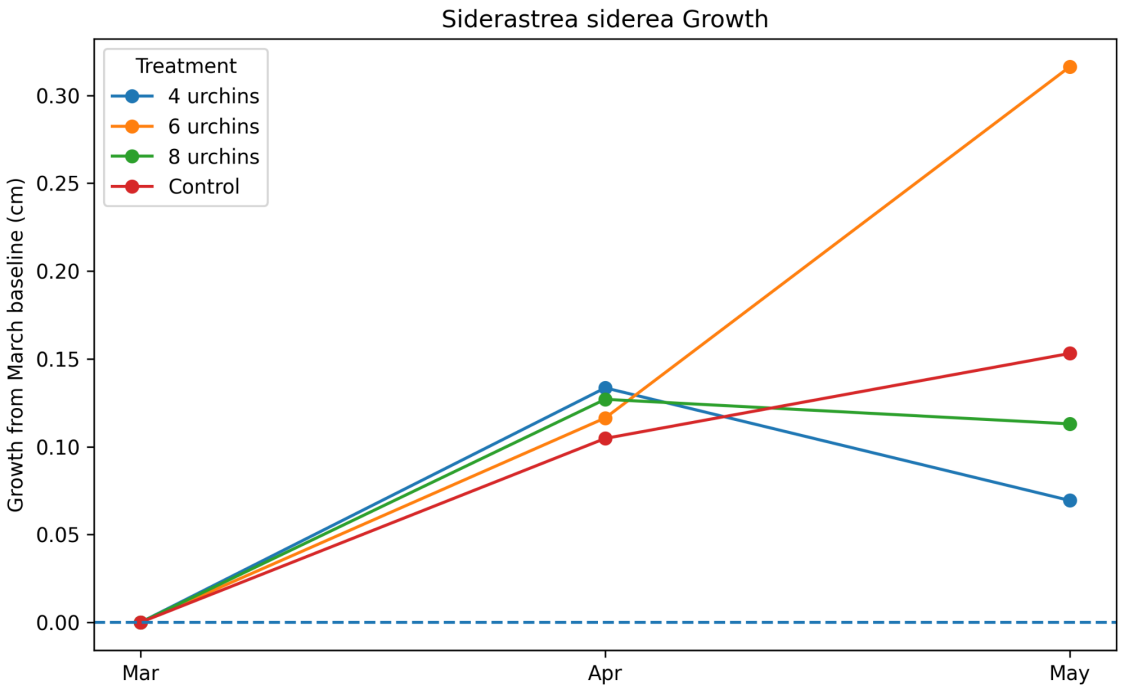

Figure 6: Results from Task 3, Experiment 2 showing growth of *Siderastrea siderea* fragments maintained with varying densities of *D.antillarum* in an offshore in situ coral–urchin polyculture cage.

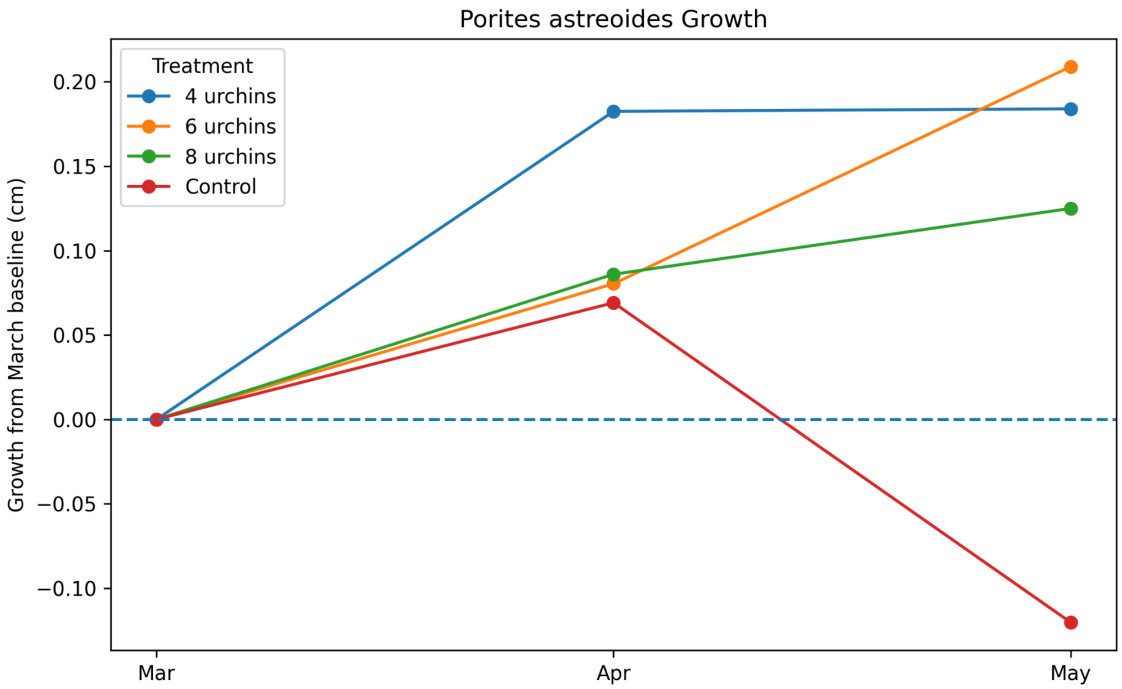

Figure 7: Results from Task 3, Experiment 2 showing growth of *Porites astreoides* fragments maintained with varying densities of *D.antillarum* in an offshore in situ coral–urchin polyculture cage.

Figure 8. Digital photograph of a cultured juvenile Caribbean king crab, *Maguimithrax spinosissimus*, with forensic photo scale in frame. White lines denote the morphometric dimensions (CW & CL) measured using digital images of each juvenile after each observed molt event.

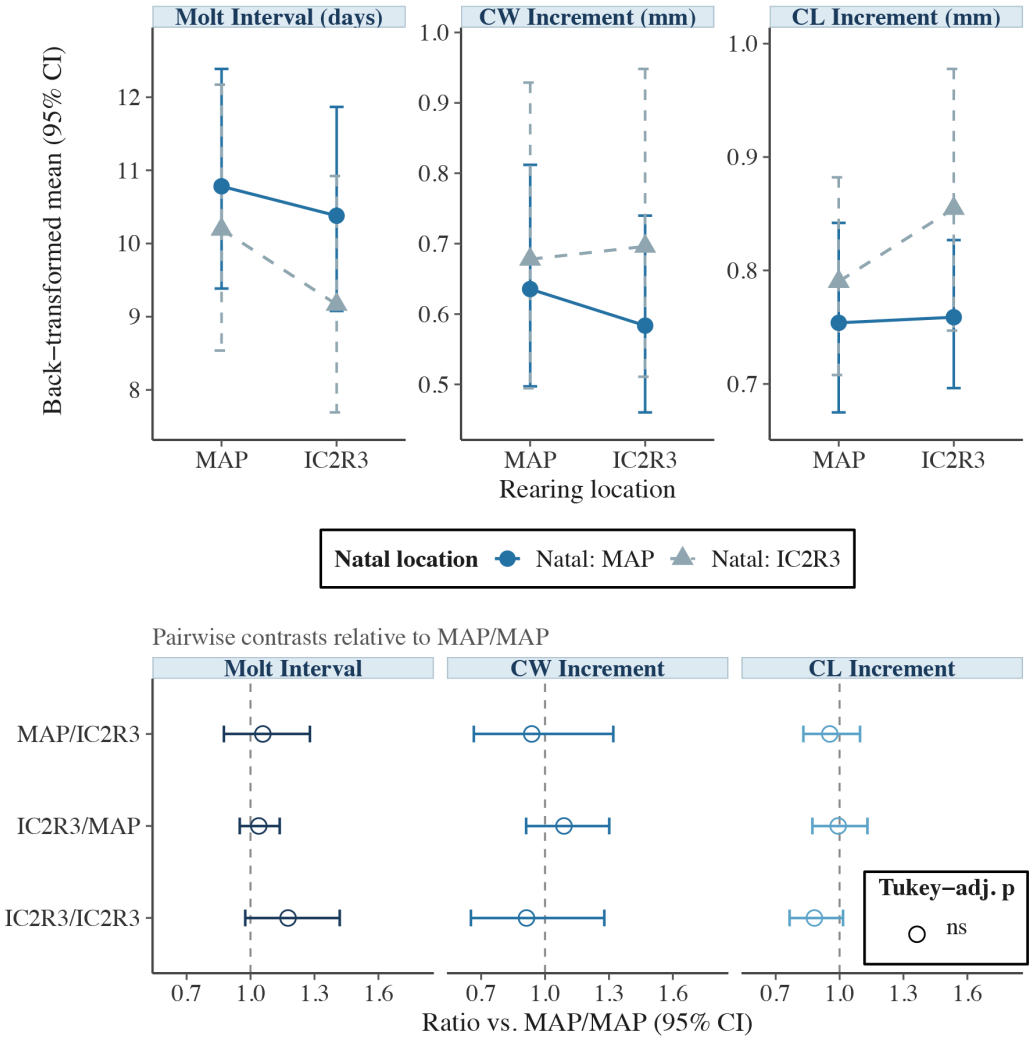

Figure 9. Interaction plots evaluating effect of treatment group combinations on molt interval (top left), molt increment CW (top middle), and molt increment CL (top right). Pairwise contrasts for each treatment group relative to MAP/MAP treatment (bottom).

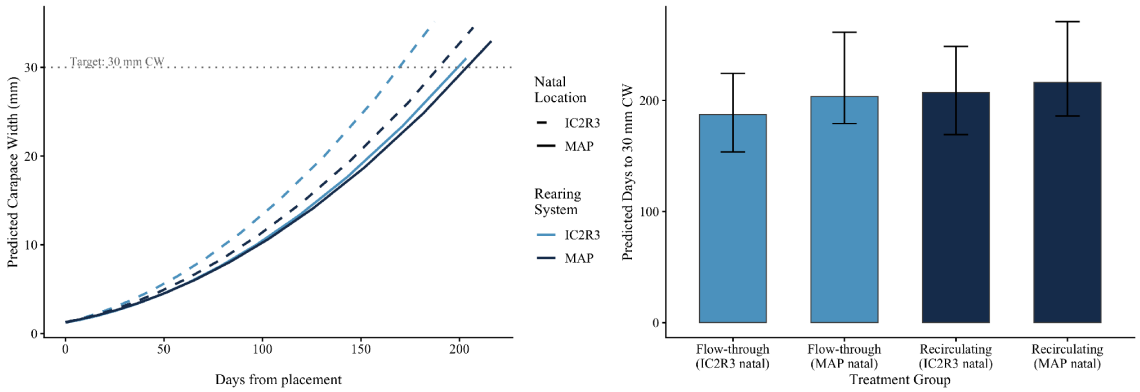

Figure 10. Simulated growth trajectories to target stocking size (30 mm CW; Left) and estimated time-to-target stocking size (days + 95% CI bars; Right).

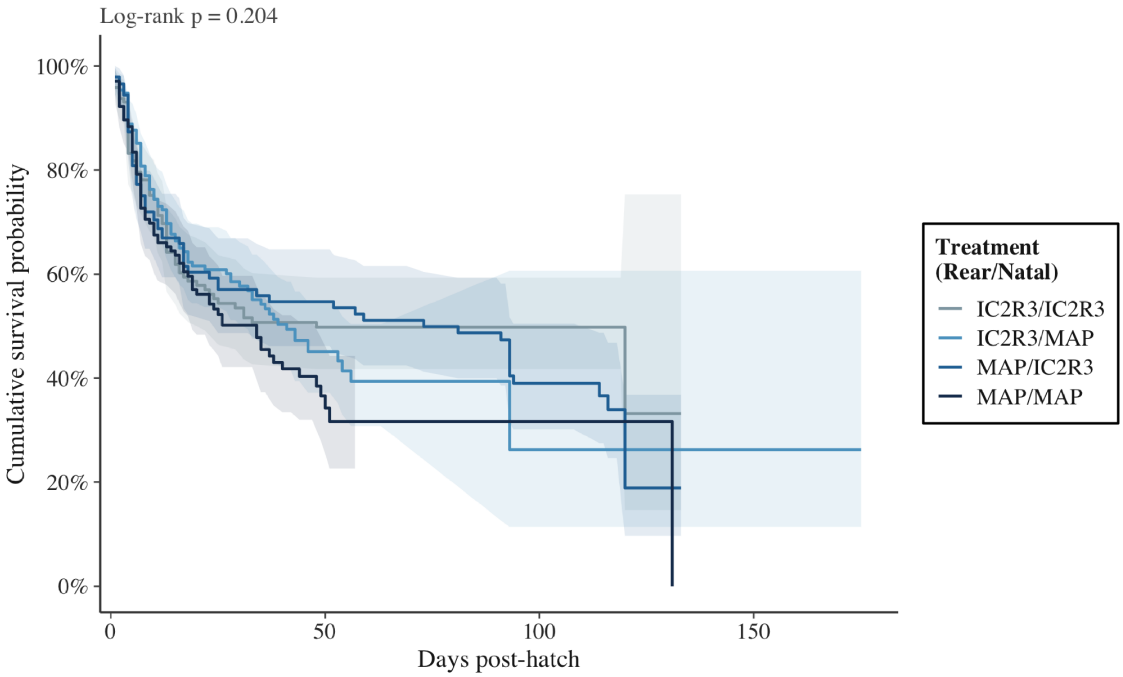

Figure 11. Kaplan-Meier survival curves for each treatment group.

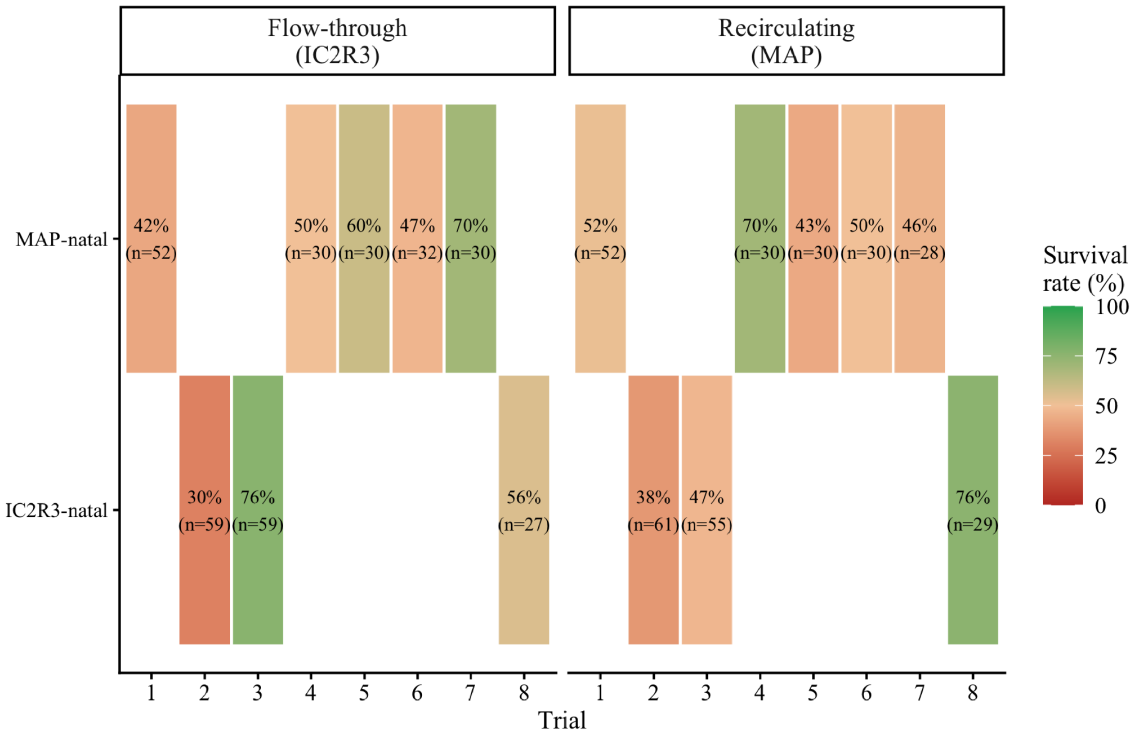

Figure 12. Survival rate heatmap by trial (e.g., maternal clutch) and treatment group.

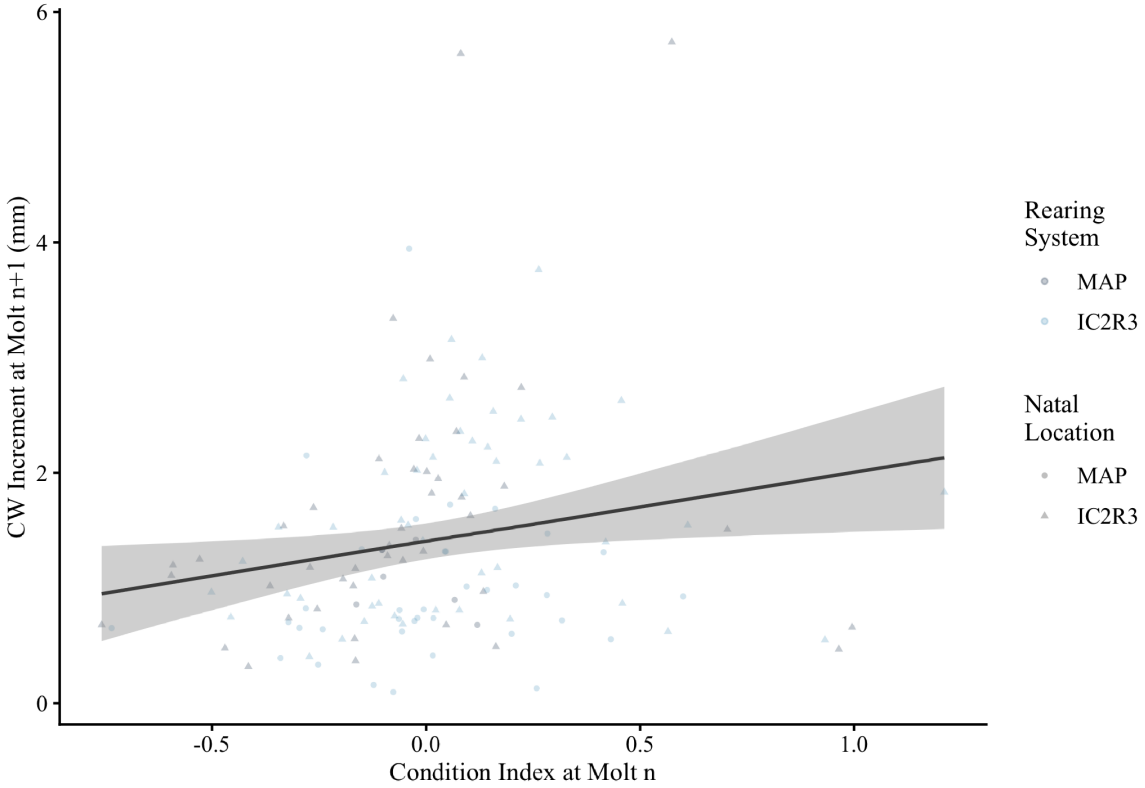

Figure 13. Model prediction of subsequent molt interval (mm CW) with Body Condition Index.

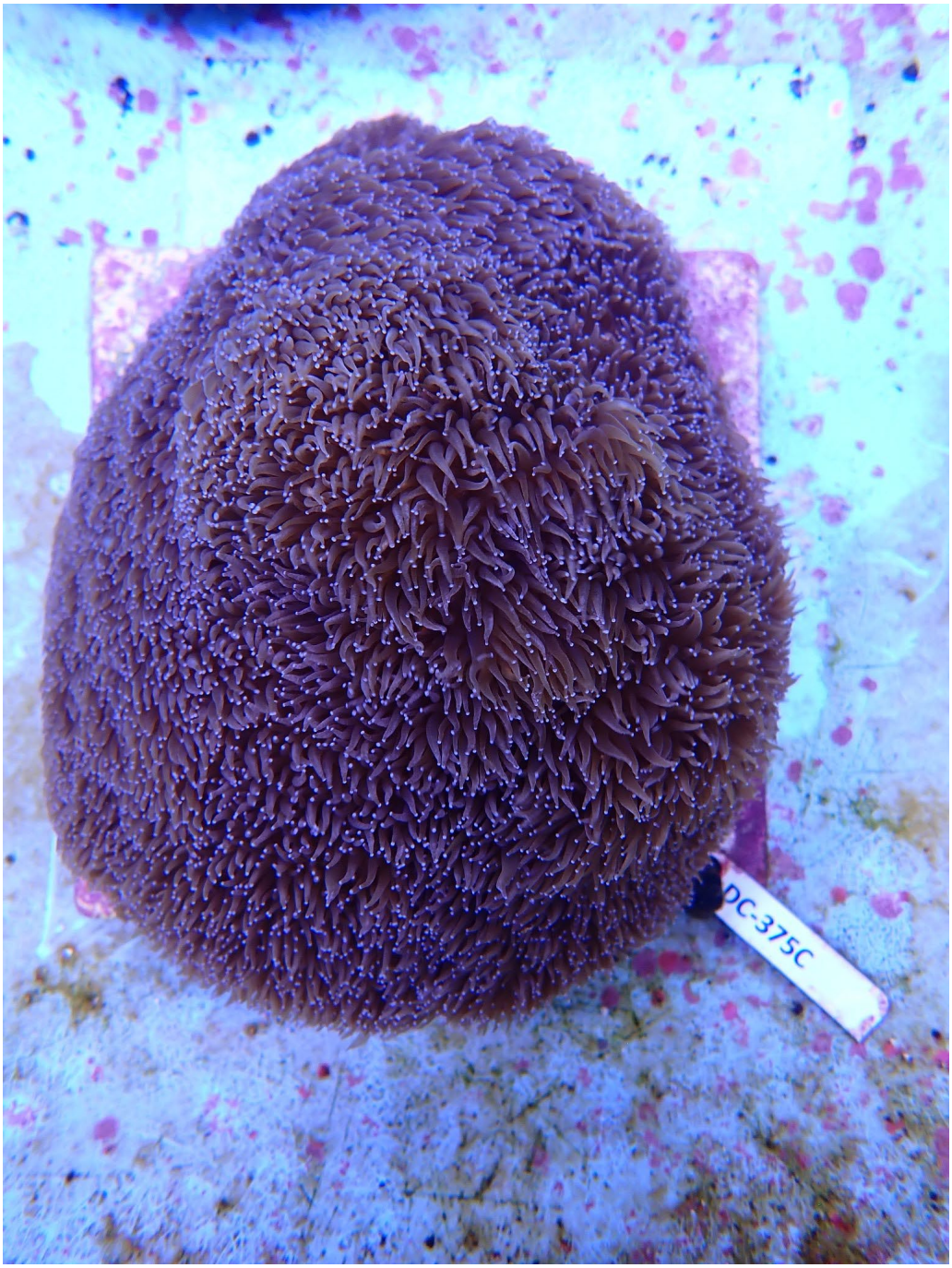

Figure 14. Representative photo of one of the 125 *D. cylindrus* that were maintained at The Florida Aquarium.

TABLES

Table A. *Descriptive statistics summarizing the effect of treatment group on molt interval and molt increment (CW & CL).*

Natal System	**Rearing System**	**Crabs** (*n*)	**Molts** (*n*)	**Molt Interval** (days; mean ± SD)	**Molt Increment CW** (mm; mean +/- SD)	**Molt Increment CL** (mm; mean +/- SD)	**Mean Initial CW** (mm)
MAP	MAP	87	183	11.5 ± 6.91	0.633 ± 0.525	0.759 ± 0.688	1.30
IC2R3	MAP	65	330	12.6 ± 6.48	1.10 ± 0.886	1.27 ±1.10	1.24
MAP	IC2R3	109	325	10.5 ± 5.58	0.701 ± 0.598	0.821 ± 0.711	1.24
IC2R3	IC2R3	72	432	11.5 ± 5.39	1.38 ± 1.07	1.52 ± 1.16	1.24

Table B. *Random effect variance components and model fit for three linear mixed models. R^2m = mariginal R^2 (variance explained by fixed effects only); R^2c = conditional R^2 (fixed + random effects), following Nakagawa & Schielzeth (2013). σ^2 = estimated variance component on the log scale.*

Model	***R^2m***	***R^2c***	**σ^2 Trial**	**σ^2 Crab ID**	**σ^2 Residual**
Molt Interval	0.248	0.285	0.01220	0.0000	0.24120
Increment CW	0.384	0.611	0.03560	0.1956	0.39610
Increment CL	0.441	0.441	0.00042	0.0000	0.50024

Table C. *Back-transformed estimated marginal means (EMM) for each treatment group combination evaluated at grand mean log(PreviousCW) and mean molt stage. EMM are geometric means on the original response scale, calculated by exponential back transformation of log-scale estimates and 95% confidence intervals.*

Response	**Rearing Location**	**Natal Location**	**EMM**	**Lower 95% CI**	**Upper 95% CI**
Molt Interval (days)	MAP	MAP	10.78	9.39	12.39
Molt Interval (days)	MAP	IC2R3	10.19	8.54	12.17
Molt Interval (days)	IC2R3	MAP	10.38	9.08	11.87
Molt Interval (days)	IC2R3	IC2R3	9.17	7.69	10.92
Increment CW (mm)	MAP	MAP	0.635	0.497	0.812
Increment CW (mm)	MAP	IC2R3	0.678	0.495	0.929
Increment CW (mm)	IC2R3	MAP	0.584	0.46	0.74
Increment CW (mm)	IC2R3	IC2R3	0.696	0.511	0.948
Increment CL (mm)	MAP	MAP	0.754	0.675	0.842
Increment CL (mm)	MAP	IC2R3	0.79	0.708	0.882
Increment CL (mm)	IC2R3	MAP	0.759	0.696	0.827
Increment CL (mm)	IC2R3	IC2R3	0.855	0.747	0.978

***Table D**. Pairwise contrasts among treatment groups for each response variable. Contrasts are expressed as ratios of geometric means. Ratio > 1 indicates first group > second group. SE is presented on the log ratio scale; all p values are Tukey adjusted.*

Model	Contrast (Rear/Natal)	Ratio	SE	*z*	*p* (adj.)
Molt Interval (days)	MAP/MAP vs. MAP/IC2R3	1.058	0.097	0.580	0.935
Molt Interval (days)	MAP/MAP vs. IC2R3/MAP	1.039	0.046	0.825	0.843
Molt Interval (days)	MAP/MAP vs. IC2R3/IC2R3	1.176	0.096	1.698	0.386
Molt Interval (days)	MAP/IC2R3 vs. IC2R3/MAP	0.982	0.093	-0.194	0.997
Molt Interval (days)	MAP/IC2R3 vs. IC2R3/IC2R3	1.112	0.038	2.793	0.030
Molt Interval (days)	IC2R3/MAP vs. IC2R3/IC2R3	1.132	0.092	1.353	0.564
Increment CW (mm)	MAP/MAP vs. MAP/IC2R3	0.937	0.175	-0.370	0.982
Increment CW (mm)	MAP/MAP vs. IC2R3/MAP	1.089	0.091	0.936	0.786
Increment CW (mm)	MAP/MAP vs. IC2R3/IC2R3	0.913	0.172	-0.531	0.949
Increment CW (mm)	MAP/IC2R3 vs. IC2R3/MAP	1.161	0.170	0.882	0.814
Increment CW (mm)	MAP/IC2R3 vs. IC2R3/IC2R3	0.974	0.096	-0.277	0.993
Increment CW (mm)	IC2R3/MAP vs. IC2R3/IC2R3	0.838	0.166	-1.060	0.722
Increment CL (mm)	MAP/MAP vs. MAP/IC2R3	0.954	0.071	-0.664	0.909
Increment CL (mm)	MAP/MAP vs. IC2R3/MAP	0.993	0.066	-0.099	1.000
Increment CL (mm)	MAP/MAP vs. IC2R3/IC2R3	0.882	0.072	-1.736	0.339
Increment CL (mm)	MAP/IC2R3 vs. IC2R3/MAP	1.041	0.061	0.669	0.906
Increment CL (mm)	MAP/IC2R3 vs. IC2R3/IC2R3	0.925	0.057	-1.377	0.516
Increment CL (mm)	IC2R3/MAP vs. IC2R3/IC2R3	0.888	0.061	-1.942	0.281

***Table E**. Growth simulation results for estimated time to stocking size (days) by treatment group.*

Natal System	Rearing System	Mean Time to size (days)	95% CI Time to size (days)
IC2R3	IC2R3	187	154 - 224
MAP	IC2R3	203	179 - 261
IC2R3	MAP	207	169 - 249
MAP	MAP	216	186 - 271

***Table F**. Type III Wald F - tests (Satterthwaite denominator df) for fixed effects in the wet weight linear mixed effects models (Model D, Condition LMM, and Model E). df_1 = numerator df; df_2 = denominator df (Satterthwaite). Reference level: Rearing = MAP, Natal = MAP. R^2m = marginal R^2 (fixed effects only); R^2c = conditional R^2 (fixed + random), following Nakagawa & Schielzeth (2013). All models include random intercepts for Trial and Crab ID.*

Model	Term	*F*	df_1	df_2	*p*	R^2m / R^2c
Model D (Weight-at-Size)	log(Current CW)	364.81	1	148.3	< 0.001	0.903 / 0.913
Model D (Weight-at-Size)	Rearing Location	14.15	1	134.5	0.0003	0.903 / 0.913
Model D (Weight-at-Size)	Natal Location	1.97	1	10.7	0.189	0.903 / 0.913
Model D (Weight-at-Size)	Rearing × Natal	1.76	1	68.7	0.189	0.903 / 0.913
Model D (Weight-at-Size)	Molt Number	1.51	1	139.4	0.221	0.903 / 0.913
Allometric Slope Test	log(CW) × Rearing	5.09	1	288.7	0.025	
Allometric Slope Test	log(CW) × Natal	0.97	1	288.4	0.324	
Condition LMM	Rearing Location	15.59	1	140.5	0.0001	0.070 / 0.070
Condition LMM	Natal Location	3.63	1	24.8	0.068	0.070 / 0.070
Condition LMM	Rearing × Natal	2.44	1	63.5	0.123	0.070 / 0.070
Condition LMM	Molt Number	0.88	1	289.3	0.349	0.070 / 0.070
Model E (Weight Increment)	Rearing Location	0.01	1	116.4	0.919	0.815 / 0.818
Model E (Weight Increment)	Natal Location	0.07	1	22.4	0.792	0.815 / 0.818
Model E (Weight Increment)	Rearing × Natal	0.02	1	74.3	0.878	0.815 / 0.818
Model E (Weight Increment)	log(Previous CW)	136.96	1	42.9	< 0.001	0.815 / 0.818
Model E (Weight Increment)	Molt Number	0.13	1	41.6	0.721	0.815 / 0.818

***Table G**. Estimated marginal means for each treatment group from wet weight models, all evaluated at grand-mean log(CW) and mean molt stage. Model D and Model E estimated marginal means are geometric means on the original scale, obtained by exponentiating log-scale emmeans estimates and 95% confidence limits. Condition LMM estimated marginal means are on the residual scale (no back-transformation required).*

Model	Rearing	Natal	EMM	95% CI Lower	95% CI Upper	Units
Model D (Weight-at-Size)	MAP	MAP	0.0292	0.0229	0.0373	g
Model D (Weight-at-Size)	MAP	IC2R3	0.037	0.0277	0.0495	g
Model D (Weight-at-Size)	IC2R3	MAP	0.0428	0.0347	0.0528	g
Model D (Weight-at-Size)	IC2R3	IC2R3	0.0455	0.0342	0.0604	g
Condition LMM (Body Condition Index)	MAP	MAP	-0.305	-0.483	-0.127	residual
Condition LMM (Body Condition Index)	MAP	IC2R3	-0.098	-0.259	0.063	residual
Condition LMM (Body Condition Index)	IC2R3	MAP	0.086	-0.016	0.188	residual
Condition LMM (Body Condition Index)	IC2R3	IC2R3	0.079	-0.153	0.311	residual
Model E (Weight Increment)	MAP	MAP	0.0238	0.0159	0.0355	g
Model E (Weight Increment)	MAP	IC2R3	0.0253	0.0175	0.0366	g
Model E (Weight Increment)	IC2R3	MAP	0.0243	0.0195	0.0302	g
Model E (Weight Increment)	IC2R3	IC2R3	0.0249	0.0168	0.0369	g

***Table H**. Body condition as a forward predictor of growth in subsequent molts and of survival outcome. β = fixed-effect coefficient on the log scale. Effect column: for CW increment and molt interval, reports (exp[β] − 1) × 100 as % change per unit increase in condition index; for survival, reports odds ratio with 95% profile-likelihood CI. CIdx = condition index.*

Outcome	**Predictor**	***β***	***F* / Wald χ^2**	df_1	df_2	***p***	**Effect** (back-transformed)
CW Increment (molt n + 1)	Condition (molt *n*)	0.433	9.32	1	127.7	0.003	+54.2% per unit CIdx
Molt Interval (molt n + 1)	Condition (molt *n*)	0.028	0.154	1	128.7	0.696	+2.8% per unit CIdx
Survival (logistic)	Mean Condition	-0.385	0.454	1	—	0.5	Odds Ratio = 0.680 (95% CI: 0.175–1.781)