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Development of methods for spawning, fertilization, larval husbandry, and settlement in the temperate coral *Astrangia poculata*

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The temperate coral *Astrangia poculata* has recently emerged as a useful experimental organism for studying symbiosis, organism-environment interactions, and embryonic development. Crucial to the success of establishing any species as a laboratory animal are reliable husbandry protocols and the ability to culture the full life cycle in captivity. Here, we detail efforts resulting from several years (2016–2025) of collaboration by a multi-institutional group of researchers toward standardized and reproducible methods for *A. poculata* spawning, fertilization, larval husbandry, and settlement in the laboratory. Spawning in *A. poculata* can be reliably induced in the laboratory via thermal treatment, and the methods presented here yielded laboratory fertilization rates of up to 93%. Unfed larvae exhibit rates of development comparable to previous reports, and larvae supplemented with commercially available organic matter sources, including Reef Roids and Reef Snow, exhibited signs of accelerated development. Settlement (attachment and metamorphosis) of *A. poculata* larvae was achieved using tetrabromopyrrole (TBP), a small molecule previously shown to induce settlement and/or metamorphosis in several tropical coral species. The methods described here, including augmentation of *A. poculata* larval culture with feed, and TBP-

triggered settlement, represent a necessary first step for culturing *A. poculata* individuals throughout the life cycle, which can be scaled and adapted to enable new lines of inquiry dependent on experimental manipulation of specific genetic host lineages, and/or early life stages.

KEYWORDS

Astrangia poculata, coral husbandry, coral spawning, larval ecology, larval settlement, tetrabromopyrrole

Introduction

There has never been a more urgent time to understand the fundamentals of coral biology. Increased frequency, severity, and duration of sea surface warming events, due to human-induced climate change, has triggered massive disease outbreaks and coral bleaching events, threatening the survival of these ecosystems and their associated biodiversity (Van Hooideonk et al., 2016; Hughes et al., 2017; Alvarez-Filip et al., 2022; Zeng et al., 2025; Spady et al., 2026). Because coral bleaching and disease disrupt essential coral-microbe symbioses, successful restoration and management of reef ecosystems in the face of global climate change, strategies that are rooted in a deep mechanistic understanding of coral reproduction, physiology and symbiosis are required. Despite the intensive research funding and attention dedicated to the physiology and ecology of coral-microbe symbioses (Hoegh-Guldberg, 1999; Douglas, 2003), it has been hard to assess the dynamics of the complex, multi-domain animal-microbe and microbe-microbe interactions within the coral holobiont using tropical corals because of their obligate interdependence on symbionts (Bove et al., 2022). There are many tropical coral species being spawned and propagated in captivity for both research and restoration (Craggs et al., 2017; Randall et al., 2020; Hodges and Hallock, 2025). However, there remains no tractable calcifying model system capable of completing its full life cycle in captivity while enabling experimental investigation of multiple developmental stages in both symbiotic and aposymbiotic states. This has limited the capacity to perform controlled laboratory experiments on the physiology of symbiosis in scleractinian corals, and the response of the holobiont to environmental change throughout life history. As such, there remains a critical need for tractable experimental cnidarian systems to improve our ability to understand the factors that promote resilience of the holobiont to global change.

This need has resulted in the development of several model Anthozoan species that can be successfully reared in the laboratory. These systems include non-coral cnidarians like the sea anemones *Exaiptasia diaphana* (Grawunder et al., 2015) and *Nematostella vectensis* (Stefanik et al., 2013) and the upside down jellyfish *Cassiopeia xamachana* (Domin et al., 2018; Puntin et al., 2022). These animals have been used successfully for years to identify the mechanistic underpinnings of coral-microbe symbioses and coral bleaching (Weis, 2019), though they are non-calcifying. A handful of tropical calcifying (scleractinian) corals have now been established as experimental systems for studying coral biology (Puntin et al., 2022, Puntin et al., 2023). Scleractinians that have emerged as useful experimental organisms include *Galaxea fascicularis* (Al-

Horani et al., 2005; Schutter et al., 2008, Schutter et al., 2010; van Os et al., 2012; Evensen and Edmunds, 2018; Puntin et al., 2023; Yu et al., 2023) and Acroporid species (Ball et al., 2021) such as *A. millepora* (Pernice et al., 2011; Ramos-Silva et al., 2014; Pollock et al., 2017; Cleves et al., 2018, Cleves et al., 2020; Ying et al., 2019) and *A. digitifera* (Nakamura et al., 2005; Iguchi et al., 2009; Shinzato et al., 2011). Scleractinian corals that are amenable to laboratory manipulation will continue to enable experimentation within the context of a holobiont energy investment modeling that includes calcification. However, both of these scleractinian corals have obligate symbioses with intracellular, single-celled, photosynthetic protists of the family Symbiodiniaceae (LaJeunesse et al., 2018), making it difficult to study the impact of the dynamism, including onset, fluctuation, and/or disruption, of this symbiosis without adding confounding external stressors such as light or thermal stress in experimental treatments.

The scleractinian coral *Astrangia poculata* has recently been recognized as a useful organism for addressing specific questions about microbial (Puntin et al., 2022) and Symbiodiniaceae (Neff, 2020) contributions to cnidarian holobiont fitness and survival (reviewed by Ashey et al., 2025). Unlike most tropical corals, which require Symbiodiniaceae photosymbionts for their survival (Muscattine and Porter, 1977; Muscattine et al., 1984; Muscattine, 1990), *A. poculata* is facultatively symbiotic and naturally exhibits large variability in photosymbiont density (Dimond and Carrington, 2008; Sharp et al., 2017). While tropical coral species can display some degree of variation in Symbiodiniaceae densities (Cunning and Baker, 2014), low Symbiodiniaceae density is most often observed in response to stress (e.g., “coral bleaching”), making it difficult to link photosymbiont density to other aspects of host physiology. Consequently, *A. poculata* is a powerful system that can support hypothesis-testing regarding the influence of photosymbionts on physiology of the coral animal and its microbial associates, without requiring exposure to confounding stressors for laboratory manipulation of Symbiodiniaceae abundance. Though there may be multiple lineages, the natural range of *A. poculata* extends across the Atlantic coast of the United States from Massachusetts to the Gulf of Mexico (Peters et al., 1988), and further south through the Caribbean to northeastern Brazil (Dimond et al., 2013), with an IUCN Red List designation of Least Concern. This coral is abundant in near coastal ecosystems along accessible regions of the east coast of the United States and can be sampled via SCUBA in shallow waters. The small, compact colonies, which are relatively hardy to handling in the laboratory can be maintained long-term in laboratory conditions, making it a tractable study system (Puntin et al., 2022). Additionally, 16S rRNA

sequences from *A. poculata* specimens from two populations in the United States (Jamestown, RI, and Woods Hole, MA) have been deposited and are publicly available on the Coral Microbiome Portal (<https://www2.whoi.edu/site/amy-apprill/coral-microbiome-portal/>). Its unusual characteristics, including its facultative photosymbiosis and its large geographic range, coupled with its tractability in the laboratory and accessibility in the field, position it to be an excellent experimental system for studying physiological, ecological, and genetic basis of multi-partner symbioses in cnidarians. Here, we report methods for sexual propagation - spawning, larval rearing, and settlement (attachment and metamorphosis) - of the scleractinian coral *A. poculata* in the laboratory, in support of establishing this animal as a model system for continued studies of scleractinian biology.

Methods

Colony collection

Decades of research in tropical coral species have identified that gonochoric (= dioecious, separate sexes are segregated by colony) corals release their gametes in synchronous broadcast spawns, according to a number of cues, most notably according to the lunar cycle, at specific times of the day (Moritz et al., 2025) or night (Szmant, 1991), depending on the coral species. The timing of wild spawning in *A. poculata* remains unknown for a number of reasons. The high turbidity of seawater in Narragansett Bay – and most of the habitats where colonies occur in high enough density to monitor for spawning – combined with the small size and light pigmentation of *A. poculata* gamete bundles, have hampered productive, consistent results for *in situ* monitoring of *A. poculata* colonies, even in daytime. *Astrangia poculata* is gonochoric (= dioecious, separate sexes are segregated by colony) and releases gametes via broadcast spawning (Szmant-Froelich et al., 1980). Although observations of *A. poculata* spawning in the wild have not been recorded, previous research indicates that gametogenesis begins in early spring (March–April) and proceeds through summer (June–July) with peak spawning season in southern New England (Jamestown, Rhode Island) occurring in August–September (Szmant-Froelich et al., 1980), and laboratory-induced gamete release was reported via holding of colonies in still, slightly warmed seawater (Szmant-Froelich et al., 1980). Therefore, colony collection, laboratory spawning, fertilization, and larval rearing efforts were focused on the period of late July through early August each year during 2016–2025, to target a timeframe when colonies are most likely to possess mature gametes that are likely to be released if the colonies are disturbed. Additionally, these gametes would be most likely to result in successful fertilization, and subsequent development and growth.

Symbiotic, aposymbiotic, and mixed *A. poculata* colonies were collected from Fort Wetherill State Park in Jamestown, RI (41° 28' 40" N, 71° 21' 34" W) by SCUBA using hammers and chisels (RIDEM Permits). Any obvious burrowing/fouling organisms, including sponges, algae, and annelids, were removed from colonies in the field. Colonies were transported in coolers (in bulk or isolated into

separate containers) from the collection site back to the lab in seawater collected from the site and filtered through a 1 µm filter sock.

Laboratory spawning/fertilization

After collection, *A. poculata* colonies were held in filtered wild-fed seawater aquarium systems (19°C) in preparation for spawning. Multiple *A. poculata* colonies were then placed in small (<4L) containers with seawater from the systems. Additional seawater was heated to 27–28°C and was used to replace water in the benchtop containers, during which time air temperature was ~24–26°C. Within 1–1.25 h after colonies were transferred from tank to benchtop with heated water, the polyp morphology changed visibly from typical appearance with extended polyps (Figure 1a) to a swollen appearance (Figure 1b). Within an additional 5–30 min after the display of the morphological change, spawning typically occurred and required no additional stimulus. Gamete release also was observed at this stage after gentle disturbance of the water column above via pipette or turkey baster (Supplementary Video 1), though agitation of the seawater was not necessary for successful gamete release. Temperature induction was used with colonies that were collected within 24–72 h, and it was most reliable to perform this within the first 24 h after colony collection – mostly because most colonies collected during this time period typically released gametes within the first 24–36 hours after collection, whether or not they were purposefully induced and in containers. However, there was no apparent variable correlated with release, other than the apparent stress of collection and transportation to the laboratory. Therefore, temperature induction enabled a synchronized, predictable timing of gamete release after colony collection for purposeful experiments and monitoring. The described methods took place in a laboratory during daytime hours, most often falling between 11:00 am to 3:00 pm, under a variety of uncontrolled artificial lighting conditions. During spawning, observations included proportion of colonies releasing in each container. The order of sperm and egg release was also recorded to evaluate success of methodology and identify potential synchronization cues.

Once eggs and sperm were released, they were combined and monitored for fertilization. Fertilization was successful whether eggs and sperm were pooled from multiple colonies or from a single male and a single female housed in separate containers. Time of fertilization was estimated to be at the time of combination of sperm and egg into the same container.

Fertilization success rates were calculated by subsampling pooled gametes into a petri dish within 0.5–1 hours post fertilization (hpf) and counting via hemocytometer. Eggs that had undergone cell division 1h later were counted as fertilized and those that remained at one cell were counted as unfertilized. Fertilization success was assessed again at 1h intervals for the subsequent 6h of development to ensure an accurate representation of fertilization success for each spawn.

Larval husbandry

Approximately 1h after gamete release, fertilized embryos were transferred directly to a variety of plastic containers (1L beakers, 5

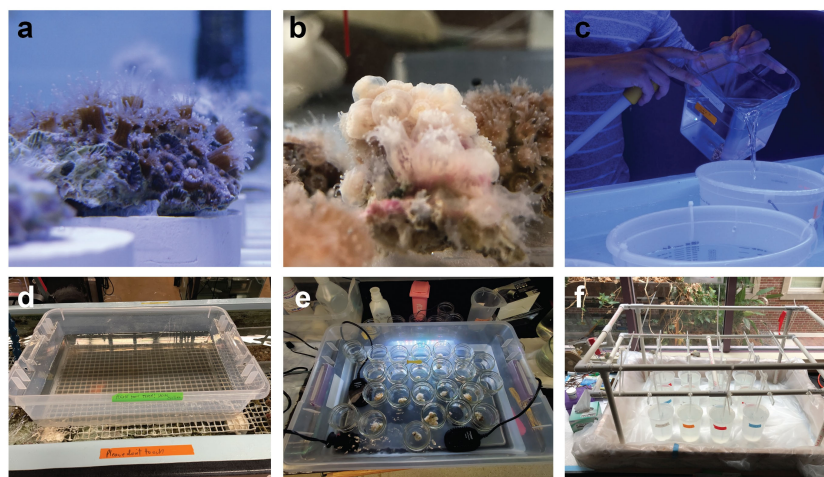


FIGURE 1

Astrangia poculata colonies, showing (a) typical polyp morphology, and (b) signature morphology of a polyp in the hour leading up to spawning. Embryos and larvae were held in a variety of containers including (c) 5L buckets, lined with Nitex mesh on the bottoms and (d) long, shallow plastic tubs with Nitex mesh on the bottom, in temperature-controlled recirculating seawater raceways, (e) in small-volume containers (glass jars; plastic beakers) in temperature-controlled waterbaths on the benchtop, or with paddles (f) to move seawater in the larval cultures, per Strathmann, 1987.

gal buckets, 32L rectangular plastic bins), all lined with 53 μ m Nitex mesh, across the bottom in the RWU Wet Lab coral aquarium systems (Figures 1c, d). Larvae were also maintained in benchtop closed containers (Figures 1e, f). In the case of the mesh-bottomed containers, to preserve high water flow and minimize fouling/overgrowth, the plastic containers housing the larvae were changed out once a day by placing a new container in the water directly next to the old one, slowly pulling out the old one at a slight angle, and rinsing down the sides with a turkey baster and system water. Once there was only a small amount of water pooled at the bottom corner of the bucket a turkey baster was used to gently skim the top layers of the water to remove all the healthy larvae swimming at the surface. The larvae were then gently dispensed into the new clean container. This process of rinsing the sides of the container and skimming off the top layers of water was repeated multiple times until it was established that no larvae remained in the container. Starting 72 hpf, larval cultures in the aquarium systems were fed daily with Reef Snow (5ml of full-strength solution into 80 gal recirculating tank; Brightwell Aquatics) or Reef Roids (5 ml of 0.05g/mL per day 80 gal recirculating tank; Polyp Lab).

In addition to aquarium systems, embryonic and larval cultures can also be maintained in closed, small volume benchtop containers without recirculation (Figure 1e). These closed systems required daily full or partial seawater changes and were maintained at temperatures ranging from 18–23°C, and in some cases included paddles for moving seawater (per Strathmann, Figure 1f).

Characterization of embryogenesis and early development

Morphological correlates of early embryogenesis were assessed in fixed embryos using immunofluorescence. Embryos and larvae were extracted from cultures at regular intervals during early development, collected in 2ml plastic screw-cap tubes, and fixed/stained using a procedure adapted from studies of *Nematostella*

vectensis. Briefly, embryos were first concentrated in a small volume of seawater and immobilized with a 5 min treatment in 100uM Ciliobrevin A (Tocris Bioscience 4529/5) diluted in filtered sea water, which blocks ciliary action. Excess seawater was removed and replaced with 4% paraformaldehyde made in phosphate-buffered saline with 0.1% Tween (PTw) and 0.25% glutaraldehyde; embryos were gently rocked in this solution for 1 min at room temperature (approximately 25 °C). This fixative was then removed and replaced with 4% paraformaldehyde (in PTw) and embryos were incubated in this solution for 1h at 4 °C. Following fixation, excess paraformaldehyde was removed with 3 10-min washes in PTw and embryos were stored at 4 °C in clean PTw for several weeks prior to analysis. For immunolabeling, fixed embryos were permeabilized with 3 20-min washes in PBS with 0.2% TritonX (PTx), blocked for 1h in 5% normal goat serum (diluted in PTx), and labeled overnight at 4 °C with anti-acetylated tubulin antibody (Sigma T6743) diluted 1/500 in normal goat serum (Sigma 9023) with PTx. Primary antibody was removed and embryos were rinsed in 3 20-min washes with PTx before being incubated in Alexa-568 secondary antibody (Invitrogen A11004) diluted 1/500 in PTx for 1h at room temperature. Secondary antibody was removed with extensive washing in PTx and embryos were counterstained with 1uM DAPI (D9542) diluted in PTw for 30 min at room temperature. Embryos were cleared in glycerol, mounted on glass slides, and imaged on a Zeiss 710 LSM confocal microscope at the Cornell Institute of Biotechnology.

Attachment and settlement assays

Astrangia poculata larvae (48 hpf) were shipped in filter-sterilized seawater (approximately 3000 larvae in 1L) overnight and kept in polystyrene containers in filter-sterilized seawater at room temperature until settlement experiments were performed with fresh tetrabromopyrrole (TBP) at the Smithsonian Marine Station, Fort Pierce, FL.

Pure, freshly synthesized TBP (see [Supplementary Figure 1](#)) was dissolved in dimethyl sulfoxide (DMSO) to 100 mM and stored at -20 °C until use, as TBP can degrade rapidly at room temperature. Settlement assays were performed in sterile, polystyrene 6-well plates with 10 mL FSW. TBP was diluted in DMSO to 0.25 mM and 0.5 mM and 10 µL was added to each assay well for a final concentration of 0.25 µM and 0.5 µM. In addition to TBP treatments, the experiment included a solvent control that was prepared by adding 10 µL DMSO, a FSW control with nothing added, and a CCA treatment, which was a small piece of unidentified crustose coralline algae (CCA). Immediately after treatments were added, ten 72 hpf larvae were counted into each well in a minimal amount of seawater. Five replicate wells were used per treatment and were randomized among the well plates using a random number generator. Settlement was evaluated via microscope and scored as the total number attached and metamorphosed after 24 hours. Survival was scored as the total number of live larvae after 24 hours. Mean proportion settlement or survival was arcsine square root transformed and subjected to statistical analysis using the Kruskal-Wallis Rank Sum Test followed by a Dunn's posthoc test using the Benjamini-Hochberg correction for multiple comparisons to identify differences between treatments. Alpha was set at $p = 0.05$ and the null hypothesis rejected when $p < \alpha/2$, according to the Benjamini-Hochberg method. Statistical analyses were run in R. All results are reported as mean proportion $\pm$ standard error.

Results

Laboratory-based spawning of *A. poculata* colonies

Consistent with previous reports ([Szmant-Froelich et al., 1980](#)), throughout collections in Rhode Island from 2016–2025, we anecdotally found that spawning yield (total number of gametes, proportion of colonies that released gametes) was highest and most consistent when colonies were collected in late July through late August. A wide range of spawning and husbandry system types supported successful larval husbandry, including closed, recirculating flow seawater tanks, and benchtop containers ([Figure 1](#)). Spawning could be reliably induced in lab-held colonies, usually within 24–72 hours of colony collection. Holding *A. poculata* colonies after collection in 19 °C seawater, which was approximately 3–5 °C cooler than average surface seawater temperature during time of collection and then moving them into small containers (0.5 L to 4 L capacity) at room temperature (25 °C) was enough to induce spawn in some colonies. In other cases, when shifting the temperature to 25 °C did not induce gamete release, adding heated seawater (27–28 °C) triggered gamete release. Addition of gentle agitation of seawater above *A. poculata* polyps exhibiting the swollen pre-spawn morphology ([Figure 1b](#)) with a glass pipet or turkey baster also helped to trigger gamete release in 25 °C and 27–28 °C treatments. Spawning was also reliably induced by moving individual colonies from cool seawater (19 °C) to ~150ml of seawater preheated to 28 °C in pint-sized jelly jars

([Figure 1](#)). This system allowed for tracking of spawning behavior of individual colonies, for controlled crosses, and it demonstrated unequivocally that individual colonies (both male and female) can be induced to spawn on multiple days in succession. After gamete collection, spawned colonies were transferred back to cool water and fed thawed mysid shrimp before returning the colonies to a flow through system to rest overnight. Several colonies were observed to spawn on four consecutive days; the maximum number of consecutive spawns was not determined. The proportion of colonies that released was not recorded in each year; however, in 2021, this temperature induction resulted in synchronous gamete release (start time of release, within 5–30 minutes of one another) in a range of 80–100% of colonies in batches of 30–40 colonies per bucket.

Sperm release was commonly observed prior to egg release, which occurred from 5–30 min after sperm release. Gamete release was also observed in colonies that had been in holding in the Roger Williams University (RWU) Wet Lab *A. poculata* system, a recirculating seawater system fed with filtered, wild seawater from Mount Hope Bay (Bristol, RI), for up to 10 months prior to collection. These colonies were held in the system as described above, at a constant temperature of 19°C, and fed live *Artemia* three times weekly. Colonies collected in September and October exhibited a lower proportion of spawning individuals relative to collections in July and August, and the colonies that did spawn at that time released fewer gametes. Throughout each year (2016–2025), lab-based spawning efforts were successful, independent of the phases of the lunar cycle.

Successful fertilization, embryonic development, and larval husbandry

Astrangia poculata colonies released either eggs or sperm into the water column, and fertilization occurred within 1 h after release. Eggs were spherical and approximately 100–120 µm in diameter ([Figure 2a](#), 0 hpf), and the sperm were about 15–17 µm in length. There was a significant difference in egg size across symbiotic states, where eggs from aposymbiotic colonies = 115 ± 1.1 µm in major-axis diameter (0.78 ± 0.0 nl volume), $n = 8$; and eggs from symbiotic colonies = 108 ± 1.2 µm in major-axis diameter (0.64 ± 0.0 nl volume); $n=8$ (Welch-corrected $t = 4.300$; $df = 13.9$; $p = 0.0007$). Major and minor axes were comparable as the eggs of *A. poculata* are largely spherical. Gametes collected from individuals in separate containers were combined into the same container to perform *in vitro* fertilization. The different institutional research groups report high fertilization success using varied concentrations of sperm; polyspermy was not detected via microscopy even at high sperm densities. Sperm concentration in combined/pooled gamete mixtures averaged 9.8×10^8 sperm/L. Fertilization success typically varied from 70% to 93%; the highest fertilization rate was recorded during peak spawning season (early August) when synchronous gamete release among colonies was induced in the laboratory.

Cell division and embryonic development were monitored via microscopy ([Figure 2](#)). Consistent with previously described results ([Szmant-Froelich et al., 1980](#)), cell division began at about 1 hpf. Each subsequent cell division, up to the blastula stage, took between

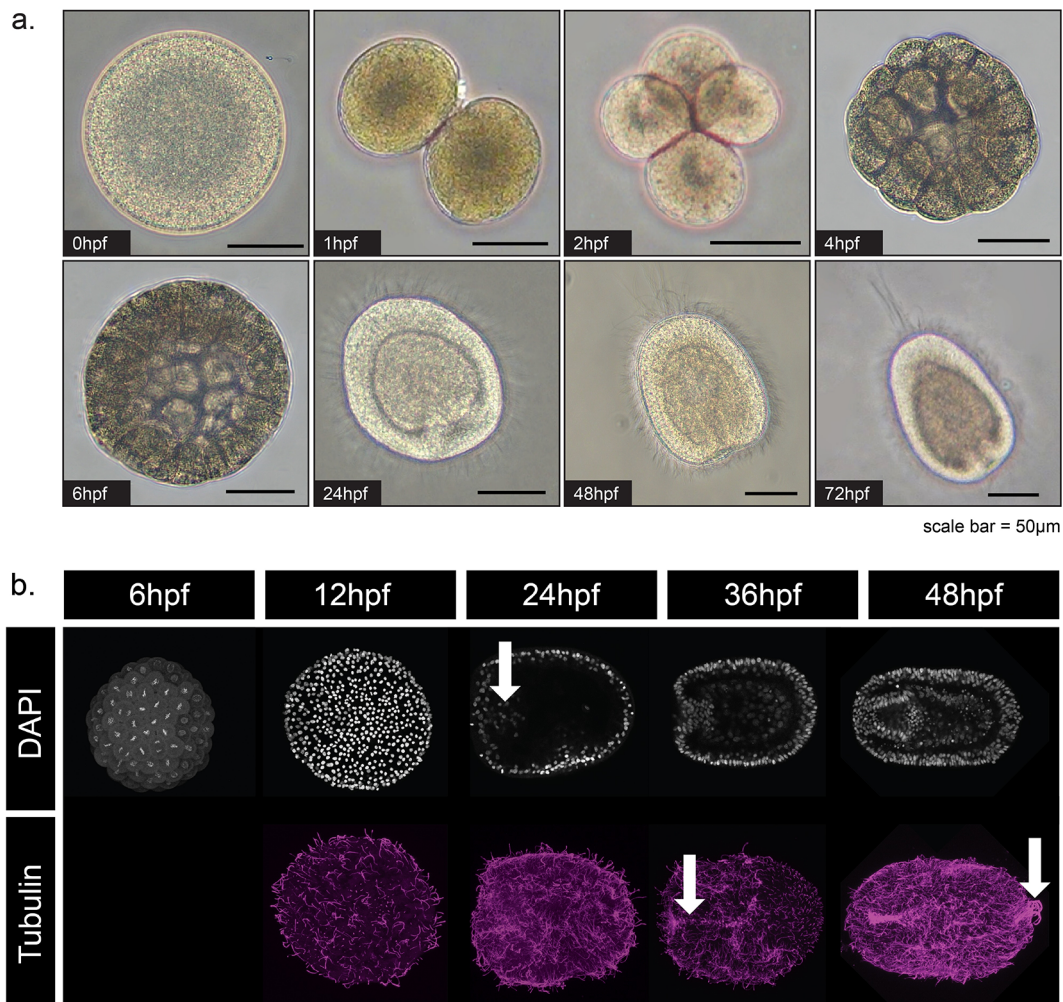


FIGURE 2

Astrangia poculata gametes and early development, showing (a) brightfield images of early development from egg to planula larvae with timepoints in hours post fertilization (hpf) and (b) confocal images of blastula and planula stages stained with DAPI (white) and anti-tubulin antibody (fuschia). Arrows indicate developmental milestones including appearance of invaginating endoderm (24 hpf), formation of the ciliated pharynx (36 hpf, tubulin), and appearance of the apical ciliary tuft (48 hpf, tubulin). Bar = 50µm.

0.5–1 hpf at 25 °C (Figure 2a). Several subsequent rounds of chaotic cleavage resulted in the development of a blastula stage embryo by 8 hpf. By 12 hpf, the ciliated embryos formed a temporary indentation that appeared like a blastopore, but no endoderm was internalized at this stage. Swimming was initiated as early as 15 hpf. A true blastopore was visible by 24 hpf, signifying the onset of gastrulation and by 36 hpf gastrulation was complete and a ciliated pharynx was visible internally (Figure 2b). By 48 hpf, elongated cilia were apparent on the aboral pole of the larva and an elongated, organized apical ciliary tuft was present by 72 hpf.

In *A. poculata* larvae that were fed Reef Snow (Brightwell Aquatics) or Reef Roids (Polyp Lab) every 24 h, and starting at 72 hpf, qualitative observations suggest a reduced apical ciliary tuft at 96 hpf, relative to unfed larvae raised under similar conditions (Figure 3). Although unfed *A. poculata* larvae did not survive longer than 12 days, fed larvae continued to survive and swim in laboratory culture for up to 40 days. In both unfed and fed larval cultures, larval settlement was not observed without chemical induction, even when larvae were presented with multiple types of hard

substrates (aragonite, ceramic, untreated concrete, unglazed terra cotta, wild shell) that had been conditioned in wild local seawater for several weeks.

Chemical induction of settlement (attachment and metamorphosis)

Astrangia poculata larval settlement was achieved in the presence of tetrabromopyrrole (TBP) at two concentrations (0.25 µM and 0.5 µM), in larvae as early as 96 hpf. Settlement was significantly higher when treated with 0.25 µM TBP ($52.5 \pm 4.8\%$) compared to crustose coralline algae (CCA) treatment ($2 \pm 2\%$, $p = 0.005$), FSW control (0%, $p = 0.001$) and DMSO control ($6 \pm 4\%$, $p = 0.019$; Figure 4a). While settlement was significantly different between the two TBP concentrations ($p = 0.039$), the larvae treated with 0.5 µM TBP were not significantly different from any other treatments ($p = 0.300$, 0.196 and 0.402 for CCA, FSW and DMSO respectively). Significantly fewer larvae survived treatment with the higher 0.5 µM TBP concentration ($80.0 \pm 7.1\%$) compared to the FSW controls

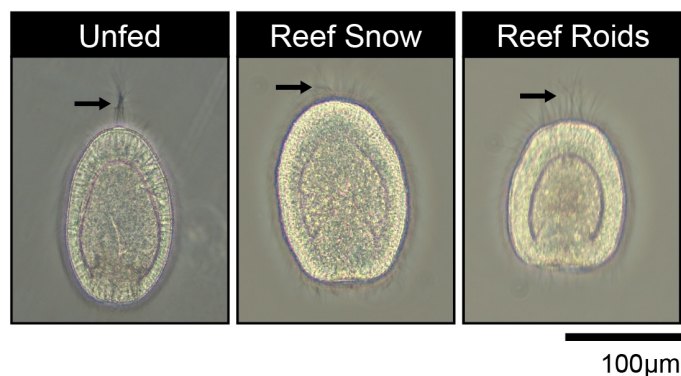


FIGURE 3

Astrangia poculata larvae at 96 hours post-fertilization from unfed larval cultures (left), and larval cultures fed with Reef Snow (middle) or Reef Roids (right). The larval apical ciliary tuft (arrow) was notably reduced in larvae supplemented with Reef Snow and with ReefRoids. Bar = 100 μ m.

(100%, $p = 0.024$; Figure 4b). There were no other significant differences in survival among treatments.

Discussion

New experimental systems are urgently needed to enable experimental manipulation of the coral holobiont (Neff, 2020). Toward that aim, we have described tools, equipment, and methods necessary for laboratory-based *A. poculata* spawning, larval husbandry, and settlement. Previous work achieved inducible spawning in the laboratory by transferring small pieces of gonadal tissue among colonies; here we establish methods for laboratory broadcast spawning without colony-transferred cues (Szmant-Froelich et al., 1980). It is not yet known if *A. poculata* spawning in the wild is linked to lunar cycles, as in many tropical corals (Sorek and Levy, 2014), or at what time of day *A. poculata* spawns, but our findings suggest that thermal induction of spawning in the laboratory of colonies collected during the period of late July-early September is a

reliable method for sourcing gametes, even when colonies are isolated from one another (i.e. not relying on triggers from one another to induce release). While we did periodically record unintended gamete release overnight, most of the spawning recorded in this study occurred during daytime hours. Considering the success of laboratory induction of spawning during daylight hours, future work should explore whether *A. poculata* spawns during daytime hours in the wild, similar to *Porites rus*, another highly abundant, successful coral species (Moritz et al., 2025). We further demonstrate that individual colonies can spawn on multiple consecutive days, providing access to zygotes for at least four days post collection reliably, though a maximum interval of repeated spawning in individual colonies was not specifically determined. We report here standardized methods for culturing the larvae of *A. poculata* and demonstrate that feeding larvae within the first week after fertilization induces morphological changes consistent with competency to settle. Finally, we describe methods for chemical induction of settlement in lab-reared larvae. Importantly, the spawning and husbandry methods can be adapted/scaled as

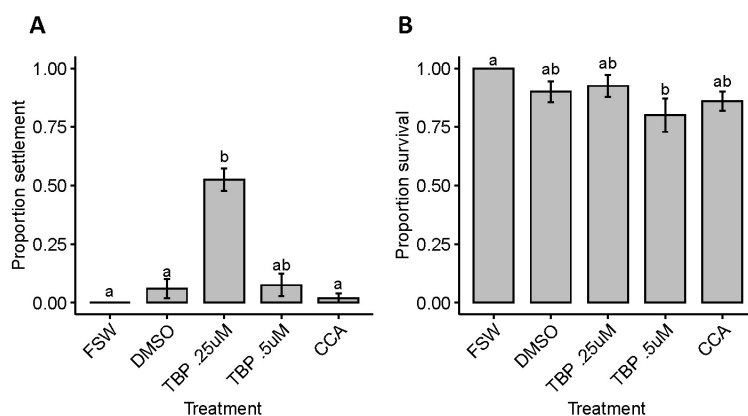


FIGURE 4

Induction of settlement in 72 hpf *A. poculata* larvae, by the chemical cue tetrabromopyrrole (TBP). (a) Mean proportion settlement (attachment + metamorphosis) of *A. poculata* larvae in response to filter-sterilized seawater control (FSW), solvent control (DMSO), 0.25 μ M and 0.5 μ M tetrabromopyrrole (TBP) and an unidentified crustose coralline alga (CCA) after 24 hours. (b) Mean proportion survival of *A. poculata* larvae in response to filter-sterilized seawater control (FSW), solvent control (DMSO), 0.25 μ M and 0.5 μ M tetrabromopyrrole (TBP) and an unidentified crustose coralline alga (CCA) after 24 hours. Error bars represent standard error. Different letters above bars represent significant differences among treatments ($p < 0.025$, Dunn's Test using Benjamini-Hochberg correction). N = 5. 10 larvae per replicate.

needed, depending on institutional capacity/size of facility, and seawater availability. Together, these protocols make it possible to acquire early life stages of *A. poculata* across a wide range of laboratories, sites, and budgets.

The *A. poculata* symbiont, *Breviolum psygmophilum*, is culturable (Parkinson et al., 2016; Aichelman et al., 2024), further contributing to the tractability of this experimental system. In the years reported here (2016–2025), there were multiple attempts at exposure of *A. poculata* to *B. psygmophilum*, including the addition of *B. psygmophilum* liquid cultures to *A. poculata* larval cultures, larval cultures in locally sourced wild seawater, or larval culture in containers with high densities of adult *A. poculata* colonies. None of the protocols yielded a method for predictable *B. psygmophilum* uptake or colonization. Therefore, the source, timing, and method of *B. psygmophilum* acquisition by *A. poculata* larvae remains unknown. The majority of tropical reef-building scleractinian coral species are broadcast spawners and acquire Symbiodiniaceae horizontally, via seawater or sediment, but brooding coral species transmit Symbiodiniaceae vertically to their larvae (reviewed in Mies et al., 2017). In corals of the genera *Acropora* and *Fungia*, larvae have been documented to acquire Symbiodiniaceae horizontally at 5–6 days post fertilization, corresponding to the formation of the oral pore and coelenteron (reviewed in Mies et al., 2017). Most tropical scleractinian coral larvae are lecithotrophic, and acquisition of photosymbionts during the larval phase appears to support longer survival in planula stages, which in turn allows them to disperse farther and increases likelihood of encounters of suitable settlement habitat (reviewed in Mies et al., 2017). Although symbiotic photosynthate appears to support the energetically costly processes associated with metamorphosis and skeleton deposition, resulting in accelerated metamorphosis, higher post-settlement survival rates, larger juvenile size, and higher polyp numbers during early development (Hazraty-Kari et al., 2022; reviewed in Mies et al., 2017), there is little evidence suggesting that establishment of symbiosis is a critical cue for metamorphosis in tropical scleractinians. However, it has been identified as an obligate cue for metamorphosis in other cnidarians: multiple genera of Symbiodiniaceae, including *Breviolum*, are capable of inducing strobilation in the upside-down jellyfish *Cassiopeia xamachana* (Sharp et al., 2024). *A. poculata* is unlike tropical scleractinian corals in some key aspects: it engages in a facultative symbiosis with its photosymbiont, and the larvae appear to be planktotrophic. Ongoing laboratory culture efforts should therefore model methods that have been successful across a wider range of marine invertebrates.

Because the developmental timing of onset of symbiosis in this species is still unknown, it is an important consideration that without *B. psygmophilum*, laboratory-produced larvae may exhibit different developmental patterns and behaviors, especially including settlement, than in the wild. However, *A. poculata* larval husbandry without uptake of symbionts also provides the potential for flexibility and controlled experiments on symbiosis onset in later stages of development, as long as uptake of Symbiodiniaceae is not critical for successful settlement and/or post-settlement growth and survival. Future efforts focused on innovating methods for inoculation of *A. poculata* early stages with *B. psygmophilum* – and other heterologous species of Symbiodiniaceae – will allow further

investigation of the role of *B. psygmophilum* in larval development, settlement, growth, and mechanistic and physiological studies of this symbiosis. This should include varying sources of *B. psygmophilum*, and culture conditions under which they are added, including considerations of nutrient content in the seawater and augmentation of settlement substrate with photosymbionts (per Hagedorn et al., 2015; Harii et al., 2009).

Our experiments demonstrate that *A. poculata* larvae feed as early as 72 hpf, indicating that planktotrophy may be an important aspect to support larval husbandry. Larvae of the temperate scleractinian coral *Caryophyllia smithii* are planktotrophic (Tranter et al., 1982), and this is a characteristic that often correlates with extended larval duration – weeks to months – in the wild, in contrast to lecithotrophic larvae that often settle within days (Miller and Mundy, 2003). In the case of *C. smithii*, planulae have been documented to remain planktonic for 4 weeks prior to settlement and exhibit a reduced apical ciliary tuft just prior to settling (Tranter et al., 1982). The apical ciliary tuft is a structure conserved across many invertebrate larvae and thought to be involved in chemodetection and motility associated with detection of settlement substrate (Bezares-Calderón et al., 2020). Following feeding with Reef Roids and Reef Snow, the apical ciliary tuft of 72 hpf *A. poculata* larvae appeared substantially reduced. While it is unknown if *A. poculata* exhibits development and settlement similar to *C. smithii*, the reduction of the ciliary tuft points to a hypothesis that feeding may be an important support for the advancement to larval competency. To date, wild *A. poculata* larval duration is undocumented, and wild larval diet is not yet known. In larvae of the sea anemone *Aiptasia* (*Exaiptasia diaphana*), carnivory of nauplii of the copepod genus *Tisbe* supported larval husbandry and provided the necessary nutrition and/or cue for successful attachment, metamorphosis, and post-settlement growth, even in the absence of Symbiodiniaceae uptake (Maegle et al., 2023). Initial attempts to settle *A. poculata* larvae with other food sources, including homogenized frozen copepods (Cyclop-eeze, JEHMCO), microalgal cultures (*Rhodomonas* sp., *Tisochrysis* sp., and *Pavlova pinguis*), and copepod nauplii (*Tisbe* sp.) had no impact on larval behavior or development. Future studies testing combinations of food sources with chemical settlement cues will be important for method development of culturing *A. poculata* into post-settlement stages.

Larval settlement in this study was achieved on a small scale via addition of TBP, a chemical documented to induce tropical coral larval settlement (Sneed et al., 2024), into the seawater with swimming *A. poculata* planulae. In *A. poculata* larvae, 0.25 μ M TBP resulted in high settlement success rates (~60%). These settlement experiments were performed with larvae that were ~72 hpf when TBP was added, and therefore settlement was scored as early as ~96 hpf. While the 96 hpf larvae settled, post-settlement survival of the individuals was not monitored or measured. Our results demonstrate the capacity for TBP to be used for inducing settlement in this species, but optimal timing of TBP-induced settlement in support of metamorphosis and post-settlement survival, for the goal of grow-out of new colonies, has not yet been established and needs to be a focus of future studies. This chemical induction method has shown promise in multiple tropical coral

species (Sneed et al., 2024), and in addition to *A. poculata* larval culture can result in successful metamorphosis and development, scaling of this method for culturing efforts may be valuable for supporting purposeful settlement and propagation of known genetic lines, and/or transgenic individuals of *A. poculata*.

New resources that bolster *A. poculata* research efforts have been developed by the Temperate Coral Research Group (<https://sites.tufts.edu/astrangia/>), which was founded in 2012. This collaborative research network – which has convened seven conferences – facilitates the sharing of data, protocols, and laboratory tools, in addition to collaboration and identification of collective research priorities and outcomes. Among the resources generated by group efforts are curated culture collections of *Breviolum* strains, recently published *Breviolum* transcriptomes (Parkinson et al., 2016), and an *A. poculata* host genome sequence (Stankiewicz et al., 2025). The new tools and refined methods for spawning, larval culture, and settlement presented here, together with the community's emerging resources and continued research findings, position this coral as a tractable and accessible experimental organism that can enhance the capacity for scleractinian biological research and inform solutions to the critically urgent threats facing corals and coral reefs around the globe.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Ethics statement

Ethical approval was not required for the study involving animals in accordance with the local legislation and institutional requirements because the work was done on invertebrate animals (coral *Astrangia poculata*).

Author contributions

EB: Methodology, Data curation, Software, Project administration, Conceptualization, Validation, Visualization, Writing – original draft, Investigation, Funding acquisition, Supervision, Formal analysis, Writing – review & editing, Resources. IC: Conceptualization, Methodology, Writing – review & editing, Formal analysis, Resources, Writing – original draft. JM: Visualization, Formal analysis, Methodology, Writing – original draft, Data curation, Validation, Investigation, Resources, Supervision, Funding acquisition, Software, Project administration, Writing – review & editing, Conceptualization. TD: Methodology, Investigation, Validation, Writing – review & editing. SD: Formal analysis, Project administration, Data curation, Writing – original draft, Visualization, Methodology, Validation, Investigation,

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Conflict of interest

The author(s) declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Generative AI statement

The author(s) declared that generative AI was not used in the creation of this manuscript.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fmars.2026.1901305/full#supplementary-material>

SUPPLEMENTARY VIDEO 1

Benttop *A. poculata* spawning in a 4L bucket, showing swollen polyps, disturbance of water column via baster, and gamete release.

SUPPLEMENTARY FIGURE 1

Synthesis of tetrabromopyrrole. Pyrrole-2-carboxylic acid (200 mg, 1.8 mmol, 1 eq.) and *N*-bromosuccinimide (1.54 g, 8.64 mmol, 4.8 eq.) were dissolved in 11 mL 10:1 anhydrous THF/DMF at 0 °C. The reaction was stirred at 0 °C for 2 h before quenched with water (10 mL) and diluted with EtOAc (10 mL). The layers were separated and the aqueous phase was extracted with EtOAc (10 mL, 3X). The organic layers were combined, dried with anhydrous Na₂SO₄, and concentrated under vacuum. Purification by silica flash column (hexane to 12% EtOAc/hexane) afforded tetrabromopyrrole (141.9 mg, 24%) as dark grey solid.

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