CORAL BREEDING
REFERENCE SHEETS

Reproductive Biology | Early Life History | Larval Propagation

CRITICALLY ENDANGERED
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Acropora palmata
(Elkhorn coral, Lamarck 1816)

Chamberland VF, Mendoza Quiroz S, Bennett M-J, Banaszak AT, Miller MW, Marhaver KL, O'Neil K, Latijnhouwers KRW, Geertsma RC, Culbertson E, Fogarty ND, Doblado Speck T

Reproductive biology [1,2]

Reproductive mode:	**broadcast spawning**
Sexual system:	**hermaphroditic**
Reproductive events per year	
at the population level:	**1 to 2**
at the individual level:	**1 to 2**
Gamete bundle size:	**1352 ± 338 µm (10) (Ø)**
Egg size:	**561 ± 64 µm (1489) (Ø)**
Eggs per bundle:	**5.9 ± 2.4 (157)**
Eggs per mL of intact bundles:	**not yet available**
Eggs per mL of broken-up bundles:	**~4600**
Sperm per bundle:	**2–8 × 10^6 (13)**
Sperm to egg ratio per bundle:	**4–19 × 10^5 : 1 (5)**

Values = average ± SD (n)

1. Adult colony

Distribution: Typically found between 0–5 m depth in the Caribbean, Bahamas, Bermuda, Gulf of Mexico, and the tropical western Atlantic coast.

Reproductive timing and gamete collection [1,3,4]

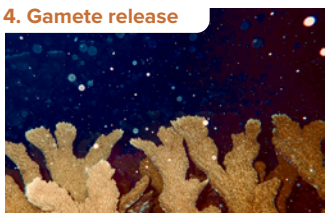
Months (Northern Caribbean)	July	August	September
Months (Southern Caribbean)	July	August	September
Days after full moon	0-2	3-7	8-14
Minutes after sunset	90	140-190	210
Duration of setting (min)	10	20	30

possible
likely
very likely

2. Gamete setting close-up

3. Gamete setting

4. Gamete release



5. Gamete collection

Considerations for spawning observations and gamete collection: This species typically occurs in wave exposed habitats which often presents extra challenges during gamete collection dives. It is recommended to cancel collection efforts during rough sea conditions. The reproductive activity of this species is notoriously unpredictable in terms of lunar calendar days. More nights of diving effort are likely required to increase chances of observing spawning and collecting gametes. Unless collecting from small-sized colonies, collection nets should be placed on individual branches where setting is visible. Polyps producing gametes on a given night are often patchy across the colony surface, including undersides of branches. Gametes are positively buoyant and can be collected in conical nets fitted with removable collection tubes at the top. However, gamete bundles are relatively small and therefore only slightly buoyant; bundles make slow (or, in case of surge, very slow) progress toward apex collection tubes. Collectors made of transparent material are helpful for monitoring the progression of spawn collection and may require a finer mesh given the small size of the bundles.

A. palmata is a highly clonal species in which robust thickets may be composed of few genotypes, which can lead to poor fertilization. Thus, is it particularly important to collect from multiple genets; this can be maximized by spreading collection effort out across the reef and/or at different sites.

Fertilization [2,5]

Time to gamete bundle break-up:	**30-120 min PS**
Gamete viability:	**at least 6 hrs PS**
Suggested duration of fertilization:	**60-90 min**
Optimal sperm concentration for fertilization:	**10^7 cell mL^{-1}**

PS = post-spawning

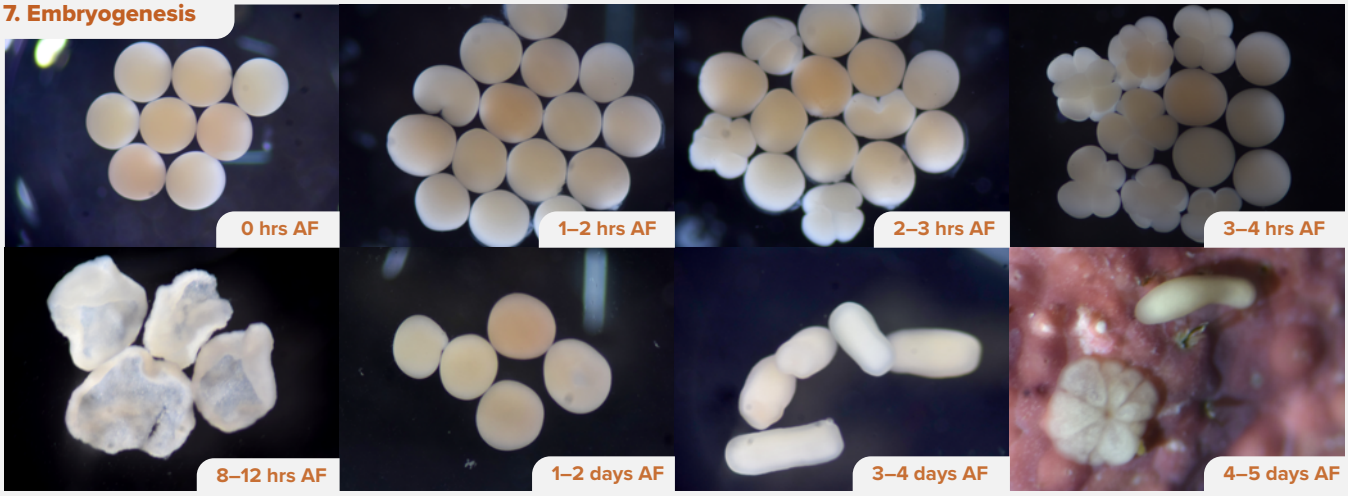
6. Fertilization

Considerations for fertilization: Gamete bundles break up quite slowly in this species with most remaining intact for over an hour after spawning. Collection tubes can be gently swirled on land to encourage egg and sperm separation. Once gamete bundles from various parental colonies are mixed together, it is recommended to gently stir the culture regularly to promote fertilization. *A. palmata* eggs tend to agglomerate together, making this species particularly prone to culture failures if reared at high densities. A complete fertilization failure may also be the result of clonality (for example, if gametes were collected from one or very few unique genets due to a highly clonal population). Self-fertilization is common in this species. Single-genet crosses have yielded up to 70% fertilization success in Florida and Curaçao and resulted in embryos that developed normally. Therefore, if conducting specific genetic crosses, self-fertilization controls are crucial to determine if embryos indeed result from outcrossing.

Embryogenesis [6]

AF = after fertilization

7. Embryogenesis



Because the developmental process for *Acropora* spp. is relatively slow, variations in development rates caused by temperature are more pronounced and noticeable. For example, larval competency will be noticeably delayed at lower temperatures.

Time to first cleavage: **60–120 min AF** | Cleavage mode: **holoblastic**
Rearing conditions: **28°C, still water containers, filtered seawater (0.45 µm, 0.5 µm, or GF/F)**

Considerations during embryogenesis: Gastrulation progresses very slowly in this species. During this period, embryos are extremely fragile and should not be handled unless absolutely necessary. Eight to twelve hours AF, embryos will have reached the prawn chip stage which can be seen with the naked eye due to their flattened, translucent appearance. At this stage, healthy embryos can be mistaken for dead embryos. However, unfertilized eggs will be easily distinguished from embryos because they will remain round throughout this time. These can thus be removed from the culture, gently, using a transfer pipette before they start degrading. Once embryogenesis is complete, indicated by rounded embryos, a full water change using a fat separating pitcher is recommended. This will help to maintain water quality.

Larval behavior, settlement, and metamorphosis [7,8,9]

Larval size:	**950 ± 253 µm (37) (longest axis)**
Symbiont transfer mode:	**horizontal**
Larval feeding mode:	**lecithotrophic**
Onset of bright green fluorescence:	**4–6 days AF (elongated larva)**
Time to motility:	**3–4 days AF**
Time to directed swimming:	**3–4 days AF**
Time to negative buoyancy:	**3.5–5.5 days AF**
Onset of settlement:	**4.5–5.5 days AF***
Onset of metamorphosis:	**4.5–5.5 days AF***
Peak widow of competency:	**7–15 days AF***

AF = after fertilization
* In the presence of settlement cues (crustose coralline algae, *Hydrolithon boergesenii)*

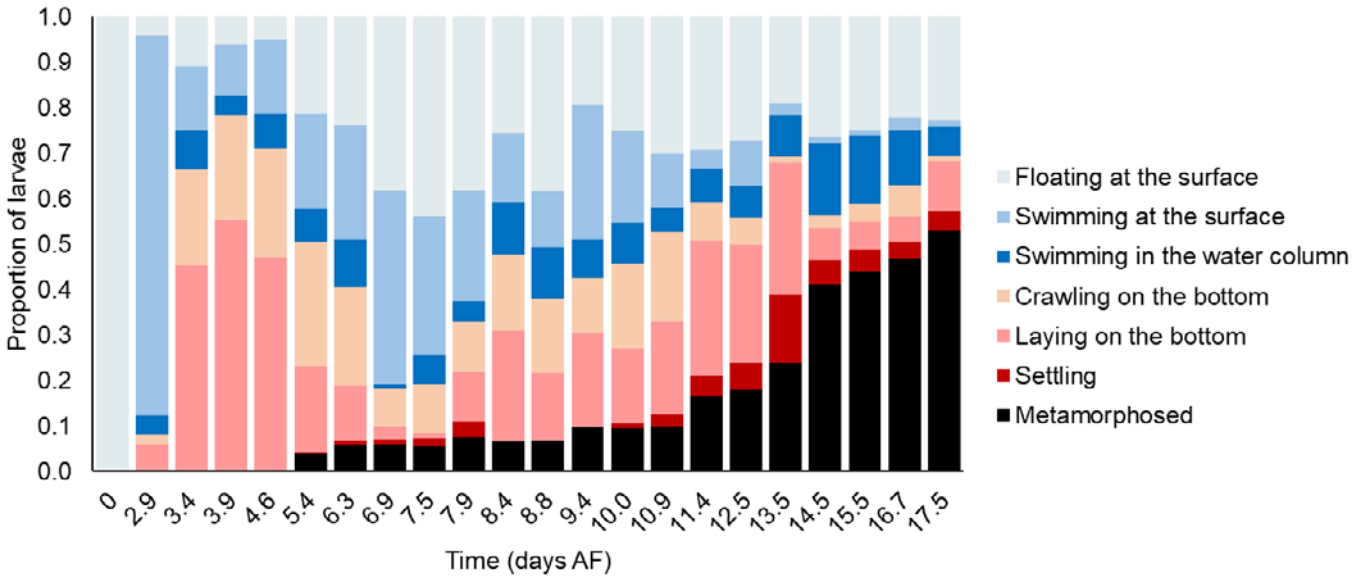
Substrate preference: Well-conditioned substrates colonized by thin crustose coralline algae (*Titanoderma prototypum* and *Hydrolithon boergesenii*). High settlement success can be achieved on concrete substrates relative to ceramic.

Habitat preference: Larvae disproportionally settle on the cryptic undersides of settlement surfaces but may also settle on exposed surfaces.

8. Competent larva and metamorphosed settler

Considerations for larval rearing: Larvae become motile 3 to 4 days AF, after which it will only be possible to do full water changes using a sieve or a pipette. Prior to this point, water changes are possible with a fat separating pitcher, which is less stressful for developing embryos, thus early water changes during days 1 to 2 are important for overall water quality in the culture. Larvae will elongate as they develop and may retain green fluorescence until after metamorphosis is complete. Settlement can be gradual and take more than a week after spawning. It is recommended to wait until day 5 or 6 before providing them with settlement substrates. Gentle water movement and aeration is highly recommended as it helps maintain water quality and enhances settlement rates.

Larval behavior, settlement, and metamorphosis through time

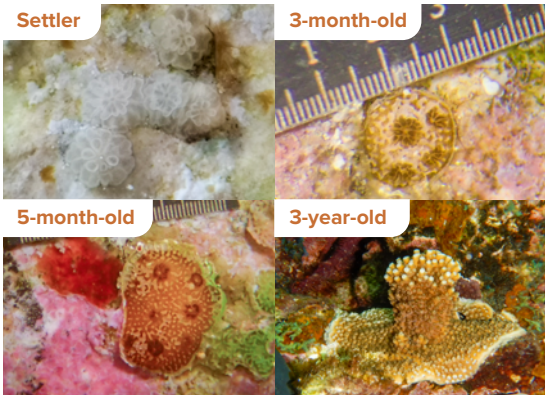


Post-metamorphosis development and ecology [10,11]

Initial primary polyp size:	**1077 ± 100 µm (38)**
Onset of calcification:	**4–10 days PM**
Skeleton morphology:	**no columella, poorly developed septae**
Time to first polyp budding:	**2–4 weeks PM**
Budding mode:	**symmetrical**
Age to sexual maturity:	**≥4 years**
Minimum size at sexual maturity:	**30–40 cm (Ø)**

PM = post-metamorphosis

9. Post-metamorphosis development



Considerations for early post-metamorphosis rearing: Symbiont (Symbiodiniaceae) acquisition early on is critical and can sometimes be challenging. Symbionts are generally acquired after metamorphosis, once the mouth is formed. To date, the symbiont of A. *palmata (Symbiodinium fitti)* has not been successfully cultured. Options for providing symbionts to settlers include: 1) extracting them from small tissue fragments, and adding the freshly isolated symbionts to the settlers; 2) placing fragments of adult colonies near settlers; 3) adding sediments taken from a reef where A. *palmata* is present; or 4) allowing the settlers to uptake free-living symbionts in a flow-through system or by placing them on a reef close to adult colonies. Once symbionts can be seen around the mouth and in the tentacles, the settlers need access to light, preferably natural solar radiation that is attenuated to low light levels (~ 100 µmol photons m^{-2} s^{-1}) and gradually increased as the symbionts populate the settlers and the settlers grow.

A. palmata settlers are particularly sensitive to variations in environmental factors such as temperature or salinity.

Long-term ex situ rearing [12]

Known threats: This species is particularly susceptible to thermal stress, infections by ciliates, predation (before settlement: bivalve mollusks and serpulids; after settlement: planarians and copepods), competition with *Aiptasia*, and overgrowth by algal mats.
Tissue loss can occur rapidly in small recruits, but can be successfully resolved with Lugol's iodine dip at 0.5-1.0 mg L^{-1} or a 10-day immersion in amoxicillin or ampicillin at 100 mg L^{-1} with daily water changes and antibiotic replenishments.

Optimal light availability: Settlers will require light as soon as they as soon as they acquire Symbiodiniacea, preferably natural solar radiation that is attenuated to low light levels (~100 µmol photons m^{-2} s^{-1}) and gradually increased as the symbionts populate the settlers.

Optimal water flow: This species thrives under high water flow rates.

Onset of heterotrophy: This species does not prey on *Artemia* spp. nauplii during the first 3 weeks following metamorphosis (<20 days) and it is unclear when this species initiates zooplanktivory.

Optimal diet: Settlers readily accept commercial diets such as ReefRoids, Kent Marine and Brightwell Aquatics products, smaller sized Golden Pearls (<500 µm), as well as frozen copepods.

Land-based coral spawning

Main cues for ex situ spawning:
Likely (but still uncertain) the period of darkness between sunset and moonrise. Light pollution during this time may therefore impact spawning.

Specific settings for abiotic parameters:
Sunset and moonrise must be finely controlled with no errors or light pollution around spawning time. Programming using the 'Season Table' function in Neptune Apex may not have sufficient precision in moonrise timing to induce spawning in this species. Programming with a full 365-day custom program in the EcoTech Marine Mobius application 'Insolation Table' function resulted in a higher percentage of spawning colonies.

Research groups that have attempted ex situ spawning of this species:
This species has spawned successfully after up to 3 years of holding ex situ at The Florida Aquarium Center for Conservation. However, only 20% of the colonies spawned in 2022 compared to 100% of other species receiving identical cues and in the same systems, including the closely related species *A. cervicornis*. Further work is required to increase the ex situ spawning output in this species.

10. Land-based coral spawning

Additional considerations:
It is very challenging to maintain healthy adult colonies in ex situ care. Ex situ spawning of A. *palmata* is significantly more difficult than in other species.

Sources

References

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Unpublished data

Reproductive biology: Chamberland VF[1,2,3], Marhaver KL[2], Latijnhouwers KRW[1,2,3], Bennett M-J[1], Geertsma RC[1]
Fertilization: Chamberland VF[1,2,3], Marhaver KL[2], O'Neil K[5]
Embryogenesis: Chamberland VF[1,2,3], Culbertson E[7]
Larval behavior, settlement and metamorphosis: Chamberland VF[1,2,3], Mendoza Quiroz S[1,4], Bennett M-J[1], Doblado Speck T[1], Geertsma RC[1,6], Miller MW[1], Banaszak AT[4]
Post-metamorphosis development and ecology: Chamberland VF[1,2,3], Banaszak AT[4], Mendoza Quiroz S[1,4], Fogarty ND[8]
Long-term ex situ rearing: Banaszak AT[4], Mendoza Quiroz S[1,4], O'Neil K[5]
Captive coral spawning: O'Neil K[5]

Conceptual idea Chamberland VF[1,2,3] | **Layout and graphic design** Ney L[1]

Sources *(continued)*

Concept development Chamberland VF[1,2,3], Banaszak AT[4], Figueiredo J[9], Fogarty ND[8], Latijnhouwers KRW[1,2,3], O'Neil K[5], Marhaver KL[2], Miller MW[1], Stephenson C[10]

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