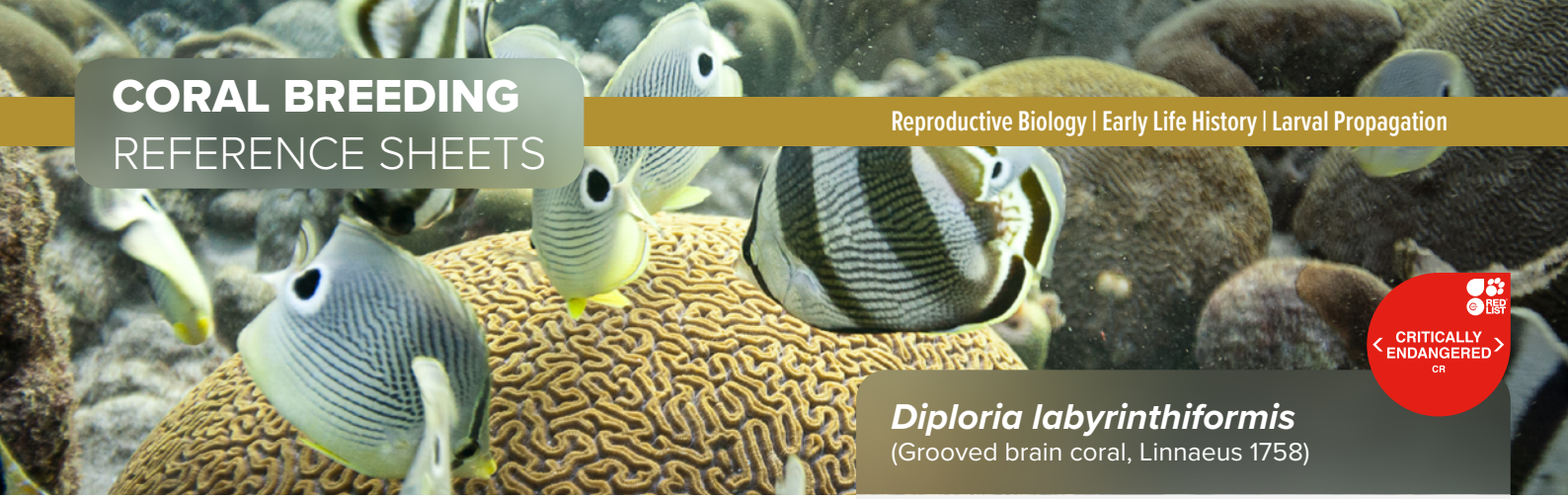


CRITICALLY ENDANGERED
CR

Diploria labyrinthiformis
(Grooved brain coral, Linnaeus 1758)

Chamberland VF, Latijnhouwers KRW, Marhaver KL, O'Neil K, Vermeij MJA, Geertsma RC, Bennett M-J, Doblado Speck T, Flores D, Le Trocquer N, Pizarro V, Ramirez M, Tichy L

Reproductive biology [1,2,3,4]

Reproductive mode:	**broadcast spawning**
Sexual system:	**hermaphroditic**
Reproductive events per year	
at the population level:	**up to 7**
at the individual level:	**up to 3**
Gamete bundle size:	**2380 ± 279 (11) (Ø)**
Egg size:	**298 ± 24 (319) (Ø)**
Eggs per bundle:	**88 ± 45 (34) (Ø)**
Eggs per mL of intact bundles:	**not yet available**
Eggs per mL of broken-up bundles:	**~15000–18000**
Sperm per bundle:	**not yet available**
Sperm to egg ratio per bundle:	**not yet available**

Values = average ± SD (n)

1. Adult colony

Distribution: 1–30 m depth in the Caribbean, Bahamas, Bermuda, Gulf of Mexico, Atlantic coast of Central Africa, and the western Atlantic coast.

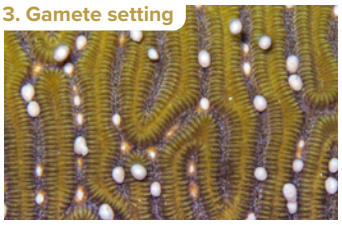
Reproductive timing and gamete collection [1,2,3,4]

Months	April	May	June	July	Aug	Sept	Oct	Nov
Days after full moon	9	10		11-12	13		14	
Minutes after sunset	-90		-60-0	15				
Duration of setting (min)	0	5	10	20				

possible
likely
very likely

2. Gamete setting close-up

3. Gamete setting



4. Colony spawning

5. Gamete collection

Considerations for spawning observations and gamete collection: This species is known to reproduce monthly from April to October in the Southern Caribbean islands of Bonaire and Curaçao, with peaks in spring (May–June) and in autumn (Aug–Sept). Spring- and autumn-spawning colonies represent distinct genetic lineages. Spawning records in other parts of the Caribbean revealed that the spring lineage occurs in Colombia, Saint Croix, Puerto Rico, Saint Thomas, Dominican Republic, Cuba, The Bahamas and Florida, whereas the autumn lineage has so far only been observed in Mexico, Dominican Republic and Puerto Rico. This species spawns before nightfall, in the hour preceding sunset. In some locations, schools of butterfly fish swarming around a colony indicate imminent or ongoing spawning of this colony. Setting is often very brief or unapparent (hence easy to miss). It can therefore be worth placing collectors on colonies shortly before spawning is expected.

Fertilization

Time to gamete bundle break-up:	**≤60 min PS**
Gamete longevity until fertilization:	**240 min PS**
Suggested duration of fertilization:	**30–45 min**
Optimal sperm concentration during co-incubation:	**10^6–10^8 cell mL $^{-1}$**

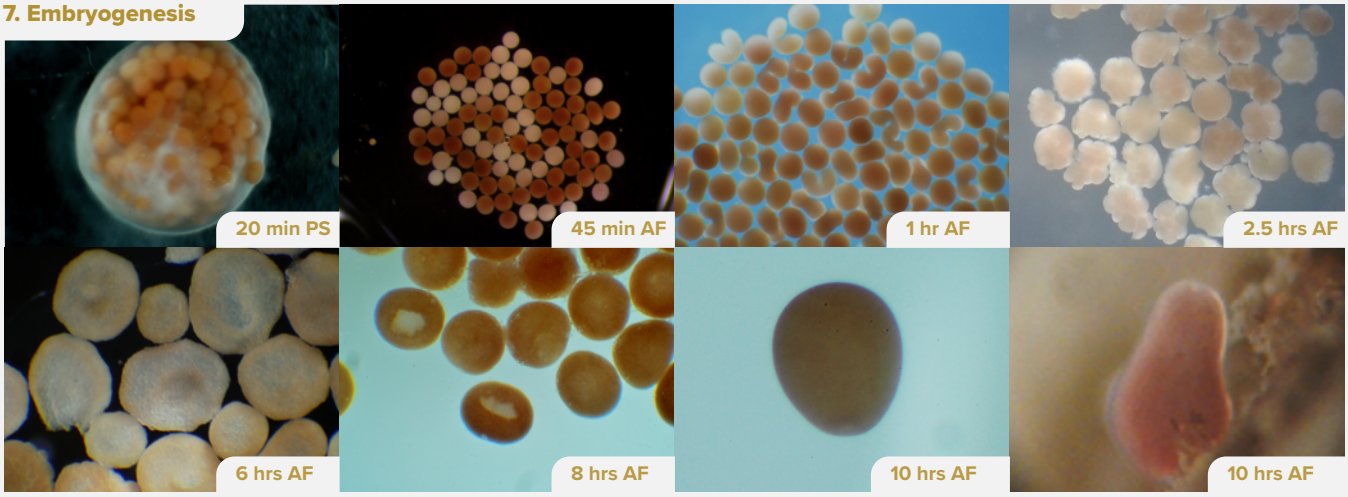
PS = post-spawning

Considerations for fertilization: Sperm-egg bundles break up shortly after release, typically during a collection effort. Eggs from different colonies can be pigmented in different shades of brown, pink and yellow. Gametes remain viable for at least 4 hours after spawning (possibly longer), but fertilization success approaches zero 6 hours after spawning. Fertilization is likely to fail at sperm concentrations below 10^5 cell mL^{-1}. When conducted at ~10^6 cell mL^{-1}, fertilization success is high even when gametes were co-incubated for a short period of time (15 min).

6. Fertilization

Embryogenesis [3]

7. Embryogenesis



Time to first cleavage: **60-90 min AF**
Cleavage mode: **holoblastic**
Rearing conditions: **Rearing conditions: 28°C, still water containers, filtered seawater (0.45 µm, 0.5 µm, or GF/F)**

PS = post-spawning
AF = after fertilization

Considerations during embryogenesis: This species has a very short time to first cleavage relative to other species. Rinsing eggs 30–45 min AF is important to avoid handling zygotes/embryos during the first cell divisions. This species is particularly prone to polyembryony (embryo fission) which results in viable but smaller embryos. While these undersized embryos develop normally, this results in a large variation in larvae and settler size and it is suspected that these smaller larvae and settlers are at higher risk of mortality. Exceptionally delicate handling of embryos is therefore recommended until gastrulation is completed. Note that embryos can be oddly shaped during the blastula stage.

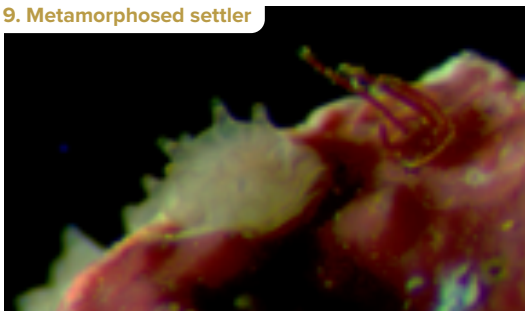
Larval behavior, settlement, and metamorphosis [3]

Larval size:	**306 ± 84 µm (56) (longest axis)**
Symbiont transfer mode:	**horizontal**
Larval feeding mode:	**lecithotrophic**
Onset of bright green fluorescence:	**0 hrs AF (eggs fluoresce)**
Time to motility:	**12–15 hrs AF**
Time to directed swimming:	**15–25 hrs AF**
Time to negative buoyancy:	**25-40 hrs AF**
Onset of settlement:	**≥4 days AF***
Peak window of competency:	**4–10 days AF***
Substrate preference:	**not yet available**
	cryptic undersides
	of settlement surfaces

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

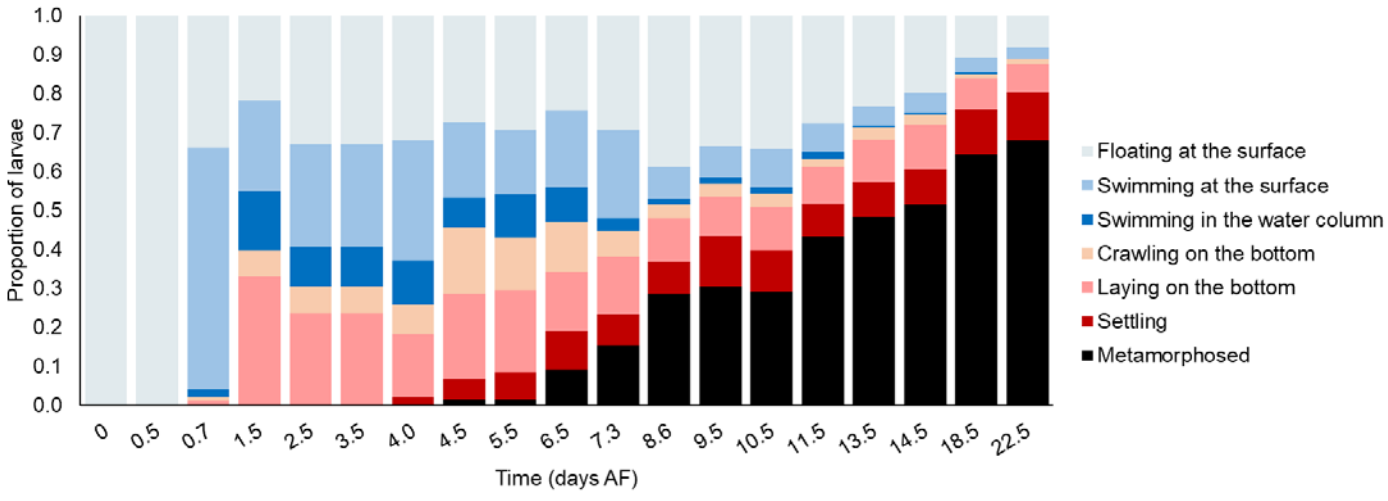
8. Fully developed larva

9. Metamorphosed settler



Considerations for larval rearing: Larvae become motile within 12–15 hours AF, after which it will only be possible to do full water changes using a sieve or a pipette, and not with a fat separating pitcher which is less stressful for developing embryos. It is therefore recommended to do a complete water change using a fat separating pitcher once embryos have completed gastrulation (i.e., have become rounded), but before they are motile and no longer float at the surface. Larvae quickly reach competency and can start settling and metamorphosing within 4 days AF and settlement rates peak between 4 and 10 days AF. It is therefore recommended to provide larvae with settlement surfaces during that period to maximize settlement. Gentle water movement or aeration further enhance settlement success.

Larval behavior, settlement, and metamorphosis through time



Post-metamorphosis development and ecology [2,3,5]

Initial primary polyp size:	**200–500 µm (18)**
Onset of calcification:	**6 days PM**
Skeleton morphology:	**not yet available**
Time to first polyp budding:	**6–12 months PM**
Budding mode:	**extratentacular**
Age to sexual maturity:	**not yet available**
Minimum size at sexual maturity:	**≥110 cm²**

Values = range (n)
PM = post-metamorphosis

10. Post-metamorphosis development

Considerations for early post-metamorphosis rearing:
Settlers are very small, grow slowly, and tend to suffer high post-settlement mortality. Providing them with extra sources of nutrients as well as controlling the proliferation of benthic competitors and other pests are key to improving early growth rates.

Primary polyps can capture and ingest *Artemia* nauplii as early as 4 days PM. Providing young settlers with *Artemia* nauplii during a two-week nursery period was found to double their growth and survival rates in the following 3 months. Settlers establishing symbiosis early after metamorphosis (≤3 weeks PM) further benefit from increased growth (2-fold) and survival (4-fold) rates during the following 6 months. At first, primary polyps invest in growth rather than budding, with the first polyp division typically occurring between 6 and 12 months PM.

Long-term ex situ rearing [5]

Known threats: Outbreaks of tissue loss have been observed in young recruits, especially when settled at high densities. Recruits can be successfully treated with ampicillin at 100 mg L^{-1} for 10 days, with daily antibiotic administration and water changes. Other threats include algal mats, *Aiptasia*, coral-eating flatworms, ciliates, and crabs.

Optimal light availability: Maintaining PAR levels between 30–50 µmol m^{-2} s^{-1} shortly after metamorphosis has been successful. PAR should be increased to 100–150 µmol m^{-2} s^{-1} after primary polyps have established symbiosis with Symbiodiniaceae. To encourage growth, PAR should then be gradually increased to ≥300 µmol m^{-2} s^{-1} in the following weeks.

Optimal water flow: Primary polyps exhibit high prey capture rates (~10 prey/hr^{-1}) when provided with live *Artemia* spp. nauplii feed under a water flow rate of 15 cm s^{-1} .

Onset of heterotrophy: This species is able to capture and ingest *Artemia* spp. nauplii starting at the age of 4 days following metamorphosis.

Optimal diet: Although an optimal diet is yet to be determined for this species, young recruits will readily feed on commercially available aquarium coral diets such as Reef Roids and Coral Frenzy 30–200 µm pellet feeds, and on freshly hatched *Artemia* spp. nauplii.

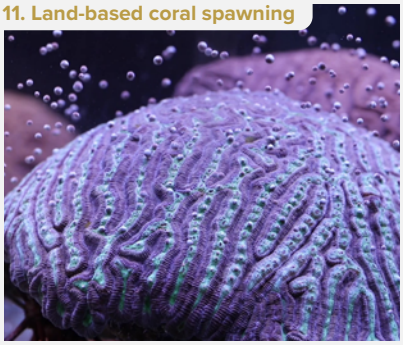
Land-based coral spawning [6]

Main cues for ex situ spawning: not yet available

Specific settings for abiotic parameters: This species successfully spawned over repeated years in an aquarium setting at The Florida Aquarium's Center for Conservation, using the same artificial light and temperature settings as described for *Meandrina meandrites* in O'Neil et al. (2021). This species has also been observed to spawn in greenhouses under natural light, with seasonal temperature changes replicated manually by adjusting tank temperature settings each month.

Research groups that have attempted ex situ spawning of this species:
Center for Conservation at The Florida Aquarium, USA (Keri O'Neil)
Cape Eleuthera Institute, The Bahamas (Valeria Pizarro)

11. Land-based coral spawning



Progress in captive rearing: In The Bahamas, five colonies exceeding 20 cm in diameter were collected one week before spawning was expected and placed in an open circulation system at 26.5–27.3°C, with some light attenuation provided by shading cloth. One colony spawned 12 days AFM in May 2019 resulting in ~30% egg (self)fertilization. Colonies were kept in this system for two months before signs of stress became apparent and they were returned to the reef.

In Florida, colonies kept in an aquarium programmed to Key Largo seasonal environmental parameters with artificial lighting spawned on nights 12 and 13 AFM in May 2020, while colonies kept in a greenhouse under natural light spawned on nights 10 and 11 AFM in May 2020 at Apollo Beach. Land-based gamete release occurred between 117 and 83 minutes before sunset.

Sources

References

[1] Alvarado EM et al. (2004) http://www.ncbi.nlm.nih.gov/pubmed/17354395

[2] Weil E and Vargas WL (2010) https://doi.org/10.1007/s00227-009-1328-5

[3] Chamberland VF et al. (2016) https://doi.org/10.1007/s00338-016-1504-2

[4] Muller E and Vermeij MJA (2011) https://doi.org/10.1007/s00338-011-0814-7

[5] Geertsma RC et al. (2022) https://doi.org/10.1007/s00338-022-02310-2

[6] O'Neil K et al. (2021) https://doi.org/10.3389/fmars.2021.669976

Unpublished data

Reproductive biology: Chamberland VF[1,2,3], Vermeij MJA[2,3], Marhaver KL[2], Latijnhouwers KRW[1,2,3], Geertsma RC[1,4], Le Trocquer N[1], Bennett M-J[1], Doblado Speck T[1], Ramirez M[1], Tichy L[2], Flores D[2]

Fertilization: Chamberland VF[1,2,3], Bennett M-J[1], Doblado Speck T[1], Latijnhouwers KRW[1,2,3]

Larval behavior, settlement and metamorphosis: Chamberland VF[1,2,3], Latijnhouwers KRW[1,2,3], Bennett M-J[1], Doblado Speck T[1], Geertsma RC[1,4]

Post-metamorphosis development and ecology: Chamberland VF[1,2,3], Geertsma RC[1,4], Latijnhouwers KRW[1,2,3]

Long-term ex situ rearing: Chamberland VF[1,2,3] , Geertsma RC[1,4], Latijnhouwers KRW[1,2,3], O'Neil K[5]

Captive coral spawning: O'Neil K[5], Pizarro V[6]

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[7], Figueiredo J[8], Fogarty ND[9], Latijnhouwers KRW[1,2,3], Marhaver KL[2], Miller MW[1], O'Neil K[5], Stephenson C[10]

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Photo credits: Bennett M-J & Doblado Speck T, Chamberland V, Mendoza Quiroz S, Muller E, O'Neil K, Selvaggio P, Snowden S, Villaverde R

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