Reproductive Biology
Early Life History
Larval Propagation

CORAL SPECIES FACT SHEET

Diploria labyrinthiformis
(grooved brain coral, Linnaeus 1758)

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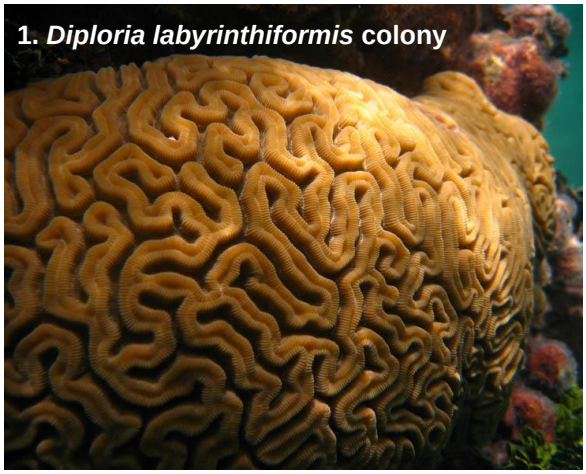
Reproductive biology

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 (Ø, µm):	2380 ± 279 (11)
Egg size (Ø, µm):	298 ± 24 (319)
Egg per bundle:	88 ± 45 (34)
Egg per mL of intact bundles:	*tbd*
Egg per mL of broken-up bundles:	~15000-18000
Sperm per bundle:	*tbd*
Sperm to egg ratio per bundle:	*tbd*

Errors are SD and numbers in brackets are sample sizes


1. *Diploria labyrinthiformis* colony

Distribution: 1-30 m depth in the Caribbean, Bahamas, Southern Florida, Bermuda, Golf of Mexico, Atlantic Central America

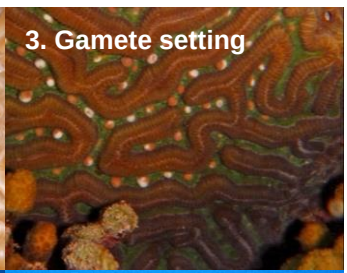
Reproductive timing and gamete collection

month	April	May	June	July	Aug	Sept	Oct	Nov
day AFM	9	10	11-12	13	14			
min RS	-75	-60-0		15				
duration of setting (min)	0	5	10	30				

AFM stands for after full moon
RS stands for relative to sunset

possible | likely | very likely


2. Close-up of gamete setting


3. Gamete setting


4. Colony spawning


5. Gamete collection

Considerations for spawning observations and gamete collection: This species spawns before nightfall, in the hour preceding sunset. In some locations, schools of butterflyfish swarming around a colony indicate imminent or ongoing spawning of this colony. Colonies often spawn in up to three consecutive pulses that last on average 5 min and are spaced by intervals of 3 to 20 min. This species is known to reproduce monthly from April to November in the Southern Caribbean islands of Bonaire and Curaçao, with a peak in the spring (May-June) and a peak in the fall (Aug-Sept). It is however unknown if this species spawns both in the spring and fall in other parts of the Caribbean.

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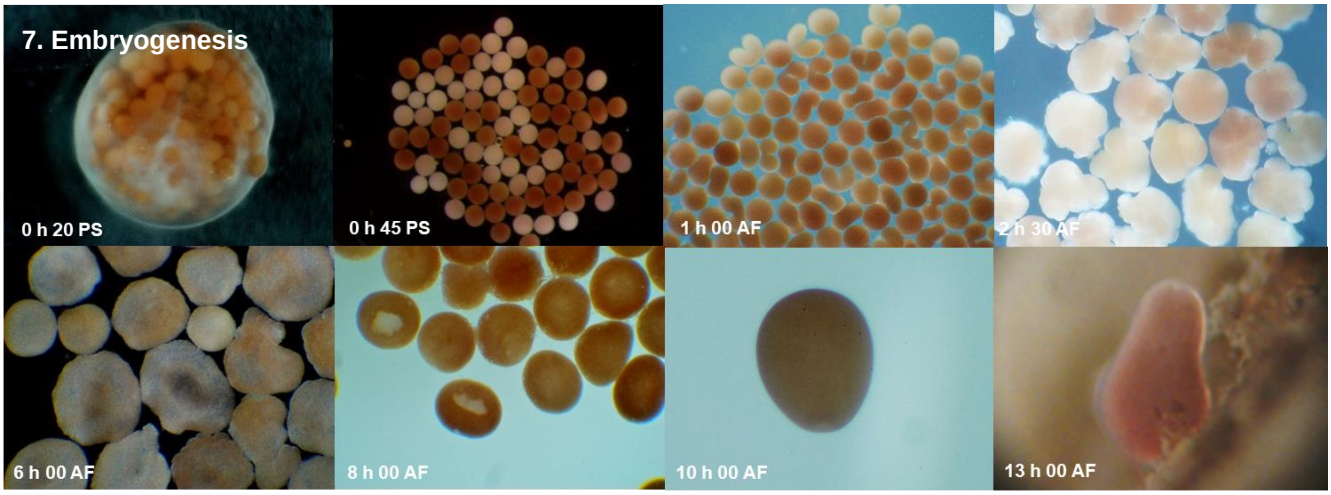
Fertilization

Time to gamete bundle break-up (min PS):	≤ 60
Sperm viability (min PS):	*tbd*
Egg viability (min PS):	*tbd*
Maximum gamete age until fertilization (min PS):	180
Suggested duration of fertilization (min):	30-45
Optimal sperm concentration for fertilization (sperm mL^{-1}):	10^6-10^8

PS stands for post-spawning

Considerations for fertilization: Sperm-egg bundles typically break-up during the collection dive. Eggs from different colonies contain pigments of different colors (e.g., brown, beige, pink, yellow). Gametes remain viable for up to three hours but it is recommended to proceed with fertilization as early as possible after spawning. There is a near complete breakdown of fertilization at sperm concentrations below 10^6 sperm mL^{-1} and a slow decrease in fertilization success above 10^8 sperm mL^{-1}. At a concentration of 10^6 sperm mL^{-1}, fertilization success is high even when gametes were in contact for a short period of time (i.e.,15 min). It is suggested to start rinsing eggs 30-45 min after introducing gametes together and once bundles have mostly broken-up.

Embryogenesis



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

AF stands for after fertilization
PS stands for post-spawning

Considerations during embryogenesis: Rinsing eggs/embryos 30-45 min AF avoids handling embryos while they are undergoing first cell divisions. This species is particularity prone to polyembryony (i.e., embryo fission) which results into smaller embryos that are viable and develop normally. This results in a large variation in larvae and settler size. It is however unknown if these smaller 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.

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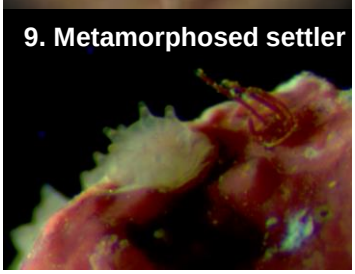
Larval behavior, settlement and metamorphosis

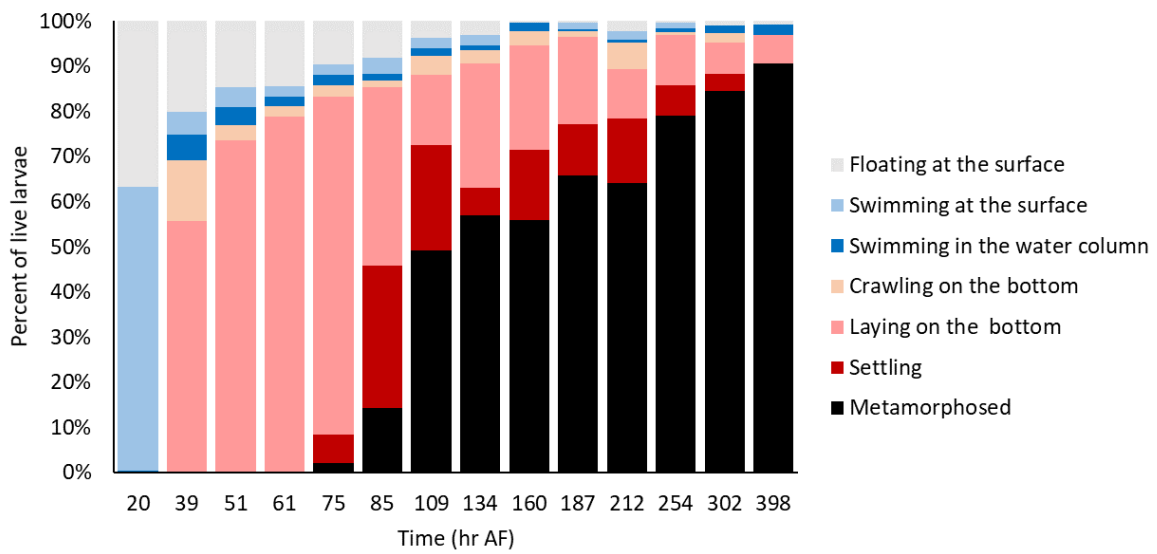
Larval size (longest axis, µm):	306 ± 84 (56)
Symbiont transfer mode:	horizontal
Larval feeding mode:	lecithotrophic
Onset of bright green fluorescence (hr PS):	0 (eggs fluoresce)
Time to motility (hr AF):	12-15
Time to directed swimming (hr AF):	15-25
Time to negative buoyancy (hr AF):	25-40
Onset of settlement (hr AF)*:	≥75
Onset of metamorphosis (hr AF)*:	≥75
Substrate preference:	*tbd*
Habitat preference:	cryptic undersides of settlement surfaces

PS stands for post-spawning
AF stands for after fertilization
** In the presence of settlement cues (*crustose coralline algae, *Hydrolithon boergesenii)*



Considerations for larval rearing: Larvae will be motile within 10-15 hr AF, after which it will only be possible to do full water changes using a sieve or a pipette, in contrast to using fat separators (less stressful handling for embryos). It is therefore recommended to do a complete water change using fat separators once embryos are past gastrulation (i.e., have become rounded), but before they are motile and no longer floating at the surface. Larvae quickly reach competency and can start settling and metamorphosing within 75 hr AF. Settlement rates peak between 3 and 6 days after spawning. It is therefore recommended to provide larvae with settlement surfaces during that period to maximize settlement. Gentle water movement further enhances settlement rates.

Larval behavior, settlement and metamorphosis through time*



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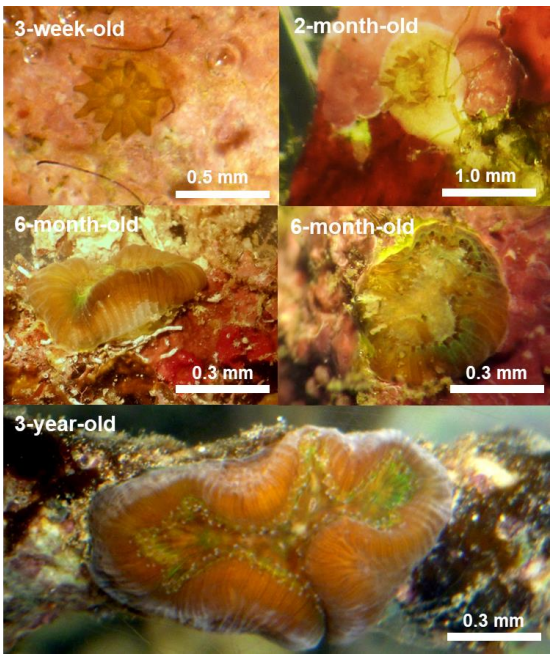
Post-metamorphosis development & ecology

Initial primary polyp size (Ø, µm):	200-500
Time to calcification (days PM):	6
Skeleton morphology:	*tbd*
Time to first polyp budding (months PM):	6-12
Budding mode:	extratentacular
Age to sexual maturity (year):	*tbd*
Minimum size at sexual maturity (cm^2):	110

PM stands for post-metamorphosis

Considerations for early post-metamorphosis rearing: Four-day-old primary polyps are able to capture and ingest *Artemia* nauplii, likely increasing settler growth and survival. The establishment of symbiosis early after metamorphosis (≤ 3 weeks PM) was further shown to increase survival 4-fold and growth 2-fold during the 6 months post-outplanting on the reef. During the months following metamorphosis, primary polyps invest in polyp growth rather than in polyp divisions.

10. Post-metamorphosis development



Long-term *ex situ* rearing

Species susceptibilities:	*tbd*
Known threats:	*tbd*
Optimal light availability:	*tbd*
Optimal water flow (cm s^{-1}):	15 (during *Artemia* nauplii feeding to increase capture rates of primary polyps)
Onset of heterotrophy:	known to feed on *Artemia* nauplii starting at the age of 4 days PM
Optimal diet:	*tbd*

Captive coral spawning

Main cues for *ex situ* spawning:	*tbd*
Specific settings for abiotic parameters:	*tbd*
Research groups that have attempted *ex situ* spawning of this species:	The Florida Aquarium, FL, USA (Keri O'Neil) Cape Eleuthera Institute, The Bahamas (Dr. Valeria Pizarro)

Additional considerations: In The Bahamas, five >20 cm Ø colonies were collected one week prior spawning and placed in an open system at 26.5-27.3°C with some light attenuation provided by shading material. One colony spawned 12 days AFM in May 2019 resulting in ~30% larvae issued from self-fertilization. Colonies were kept in the system for two months before signs of stress became apparent and they were returned to the reef.

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Sources

Photo credits
Nemcok J (1), Muller E (2,4), Snowden S (3,7), Mendoza Quiroz S (5), Selvaggio P (6), Villaverde R (7), Chamberland VF (7-8, 10), Bennett JM & Doblado Speck T (9)

Unpublished data
Reproductive biology: Chamberland VF[1,2,3], Vermeij MJA[2,3], Marhaver KL[2], Latijnhouwers KRW[1,2], Geertsma RC[1,4], Le Trocquer N[1], Bennett MJ[1], Doblado Speck T[1], Ramirez M[1], Tichy L[2], Flores D[2]
Fertilization: Chamberland VF[1,2,3], Bennett MJ[1], Doblado Speck T[1], Latijnhouwers KRW[1,2]
Larval behavior, settlement and metamorphosis: Chamberland VF[1,2,3], Latijnhouwers KRW[1,2], Bennett MJ[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]
Long-term *ex situ* rearing: Chamberland VF[1,2,3] , Geertsma RC[1,4], Latijnhouwers KRW[1,2]
Captive coral spawning: O'Neil K[5], Pizarro V[6]

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[6]Cape Eleuthera Institute, Rock Sound, Eleuthera, The Bahamas

References
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Alvarado EM et al. (2004) https://www.ncbi.nlm.nih.gov/pubmed/17354395
Chamberland VF et al. (2016) doi 10.1007/s00338-016-1504-2

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