

Running title: Onset of zooplanktivory in Caribbean corals

**Onset of zooplanktivory and optimal water flow rates for prey capture
in newly settled polyps of ten Caribbean coral species –
Supplementary Material**

Robbert C. Geertsma¹, Tim Wijgerde¹, Kelly R.W. Latijnhouwers^{2,3,4}, Valérie F. Chamberland^{2,3,4}

¹ Wageningen University & Research, Department of Marine Animal Ecology, Wageningen, The Netherlands

²SECORE International, Hilliard, OH, USA

³CARMABI Foundation, Piscaderabaai, Willemstad, Curaçao

⁴University of Amsterdam, Institute for Biodiversity and Ecosystem Dynamics, Department of Freshwater and Marine Ecology, Amsterdam, The Netherlands.

Corresponding author: Robbert C. Geertsma, robbert-jan.geertsma@wur.nl

ORCID: Robbert C. Geertsma (0000-0001-9467-6367), Tim Wijgerde (0000-0002-6698-9216), Kelly R.W. Latijnhouwers (0000-0002-4097-1093), Valérie F. Chamberland (0000-0002-1211-6575)

Supplementary information

Coral export to the Netherlands and husbandry in Wageningen

For transport by air to The Netherlands, *F. fragum* colonies were placed individually in 2-L plastic bags filled with 200 mL of 0.7- μm -filtered sea water (FSW; Whatman GF/F, GE Life Sciences, PA, USA) and transported in a coolbox as carry-on luggage following Petersen et al. (2005). During the flight, water temperature was monitored and bottles with warm water were added to the coolbox to maintain temperature at 28°C. Once in Wageningen, *F. fragum* colonies were kept in a 300-L closed circulation aquarium system with the following target parameters: a salinity of 36 g L^{-1} , a temperature of 28°C, a pH of 8.2, nitrate concentrations ranging from 0.25 to 1.0 mg L^{-1} , phosphate concentrations ranging from 0.02 to 0.10 mg L^{-1} and calcium levels of 420 mg L^{-1} . Light was provided by two LED light fixtures (CoralCare Philips) providing a peak photon flux density of 270 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (12 h/12 h parabolic light/dark regime). A water flow rate of $\sim 5 \text{ cm s}^{-1}$ was created at the bottom of the aquarium by a Tunze Turbelle E-jet powerfilter 3005 and a nanostream 6045 circulation pump (Tunze Aquarientechnik GmbH, Penzberg, Germany). Corals were fed with 300 nauplii L^{-1} daily. To reduce algal growth via grazing, 40 *Lithopoma tectum*, 30 *Clibanarius tricolor* and 4 *Diadema setosum* were added in the aquarium.

Gamete collections and fertilization

Hermaphroditic corals release positively buoyant sperm-egg bundles that float to the water surface after release. Just prior to spawning, colonies were 'tented' with cone-shaped nets made of nylon mesh. At the top of these nets, detachable 50-mL Falcon tubes were secured to an inverted funnel in which sperm-egg bundles would collect upon release. Once spawning ended, tubes containing spawn were removed from the nets, capped and brought back to the laboratory where gametes from different parents were pooled inside 0.95-L plastic fat separating pitchers (Norpro Inc, WA, USA) to allow fertilization. Sperm concentration was reduced to $\sim 10^6 \text{ cells mL}^{-1}$ by adding FSW following Hagedorn et al. (2009) to prevent polyspermy and maintain water quality. When cell divisions became visible, eggs were rinsed with FSW to remove excess sperm. This was done by pouring out seawater containing sperm through the spout of the fat separating

pitchers once buoyant eggs had concentrated at the surface, and by adding FSW into the fat separating pitcher via the spout. This process was repeated until the water column was clear.

In gonochoric species, eggs are either released in clumps or individually and are only slightly positively buoyant, while sperm is released in concentrated clouds that rapidly diffuse in the water column. Gametes released by gonochoric corals therefore cannot be collected using the nets described above. Instead, 150-mL syringes with wide openings (Becton, Dickinson and Company, NJ, USA) were used for collection, whereby sperm or eggs were gently drawn into the syringes immediately after release. In both *M. cavernosa* and *D. stokesii*, males typically spawn 15 to 45 min earlier than females, and when possible, syringes were first half filled with sperm released by one or more males before eggs were collected in the same syringes, allowing fertilization directly upon egg collection. When not possible to perform fertilization underwater, gametes were mixed in the laboratory similarly to hermaphroditic species. Regardless of the timing of fertilization (i.e., underwater or in the laboratory), fertilized eggs were rinsed in fat separating pitchers as described above.

Supplementary tables

Table S1. Reproductive characteristics for the ten coral species under study, including growth form (branching (Bra), massive (Mas) or plating (Pla)), reproductive mode (broadcast spawning (Spa) or brooding (Bro)), sexual system (Hermaphroditic (Her) or Gonochoric (Gon)), Symbiodiniacea transfer mode (horizontal (Hor) or vertical (Ver)). The table also includes predicted timing of spawning or larval release on Curaçao during which collections were performed. AFM is after the full moon, ANM is after the new moon, AS is after sunset and BS is before sunset

Species	Growth form	Reproductive mode	Symbiont transfer mode	Predicted timing of spawning or larval release			Source
				Month(s)	Day(s)	Time	
<i>Acropora palmata</i>	Bra	Spa/Her	Hor	July to September	0 to 14 AFM	140 to 190 AS	Vermeij et al. 2018
<i>Orbicella faveolata</i>	Mas	Spa/Her	Hor	July to October	5 to 7 AFM	185 to 250 AS	Vermeij et al. 2018
<i>Diploria labyrinthiformis</i>	Mas	Spa/Her	Hor	April to November	10 to 13 AFM	70 BS to 10 AS	Vermeij et al. 2018
<i>Colpophyllia natans</i>	Mas	Spa/Her	Hor	July to October	6 to 8 AFM	35 to 110 AS	Vermeij et al. 2018
<i>Pseudodiploria strigosa</i>	Mas	Spa/Her	Hor	July to October	6 to 8 AFM	220-270 AS	Vermeij et al. 2018
<i>Montastraea cavernosa</i>	Mas	Spa/Gon	Hor	July to October	5 to 7 AFM	15-165 AS	Vermeij et al. 2018
<i>Dichocoenia stokesii</i>	Mas	Spa/Gon	Hor	August to November	month-round	100 to 160 AS	Vermeij et al. 2018
<i>Siderastrea siderea</i>	Mas	Spa/Gon	Hor	September to October	4 to 6 AFM	210-280 AS	Vermeij et al. 2018
<i>Favia fragum</i>	Mas	Bro/Her	Ver	Year-round	6 to 15 ANM	night time	Szmant-Froelich et al. 1985
<i>Porites porites</i>	Bra	Bro/Gon*	Ver	September to June	month-round	day and night time	Tomascik and Sander 1987 Chamberland VF, unpub. data

* Hermaphroditism observed in 3% of colonies in Bermuda

Table S2. Details on experimental designs for Experiment 1 and 2 including the number of experimental units (Petri dishes or PVC tiles), the total number of settlers monitored as well as the specific days after metamorphosis (d AM) on which prey capture abilities were monitored

Experiment 1: onset of prey capture

Species	Number of experimental dishes	Number of settlers per dish (range)	Total number of settlers monitored	Duration of observation (min)	Monitoring days (d AM)
<i>Acropora palmata</i>	4	4-15	65	60	0 to 10, 12, 14, 16, 18, 20
<i>Orbicella faveolata</i>	4	2-3	10	60	0 to 10
<i>Diploria labyrinthiformis</i>	12	8-15	132	60	0 to 10, 12, 14, 16, 18, 20
<i>Colpophyllia natans</i>	4	7-14	47	60	0 to 10, 12, 14, 16, 18, 20
<i>Pseudodiploria strigosa</i>	1	9	9	20	0 to 5, 7
<i>Montastraea cavernosa</i>	4	2-3	11	60	0 to 10
<i>Dichocoenia stokesii</i>	4	1-3	6	60	0 to 10
<i>Siderastrea siderea</i>	1	7	7	20	0 to 3
<i>Favia fragum</i>	34	1	34	60	0 to 10
<i>Porites porites</i>	1	4	4	20	0 to 5

Experiment 2: influence of water flow rate on prey capture

Species	Monitoring days (d AM)	Total number of PVC-tiles	Number of experimental tiles/settlers per water flow rate							
			0	5	10	15	20	25	30	35
<i>Orbicella faveolata</i>	9-12	25	5	5	5	5	5	-	-	-
<i>Diploria labyrinthiformis</i>	8-11	35	4	4	4	7	4	4	4	4
<i>Colpophyllia natans</i>	7-10	50	10	10	10	10	10	-	-	-
<i>Montastraea cavernosa</i>	8-11	20	3	4	4	3	3	3	-	-
<i>Favia fragum</i>	5-6	29	6	6	5	7	5	-	-	-

Table S3. Statistical output for Experiment 2 in which the influence of water flow on prey capture rates of newly settled coral polyps was assessed for five different species. Kruskal-Wallis H-tests were first performed to assess if water flow rate influenced the settlers' prey capture rates. If so, Mann-Whitney U-tests were run to determine optimal water flow rates for prey capture. Bonferroni corrections for multiple hypothesis testing were applied. Significant values are in bold

Orbicella faveolata

Kruskal-Wallis H-test: H=17.8, p=0.001

Mann-Whitney U-tests:

	0	5	10	15	20
0		1.000	1.000	0.311	0.049
5			1.000	0.037	0.004
10				0.990	0.209
15					1.000
20					

Montastraea cavernosa

Kruskal-Wallis H-test: H=16.2, p=0.006

Mann-Whitney U-tests:

	0	5	10	15	20	25
0		1.000	0.426	1.000	1.000	1.000
5			1.000	1.000	0.387	0.098
10				1.000	0.109	0.023
15					1.000	1.000
20						1.000
25						

Diploria labyrinthiformis

Kruskal-Wallis H-test: H=29.3, p=0.001,

Mann-Whitney U-tests:

	0	5	10	15	20	25	30	35
0		1.000	0.896	0.034	1.000	1.000	1.000	1.000
5			1.000	0.247	1.000	1.000	1.000	1.000
10				1.000	1.000	0.896	0.367	0.135
15					1.000	0.034	0.008	0.002
20						1.000	1.000	0.541
25							1.000	1.000
30								1.000
35								

Favia fragum

Kruskal-Wallis H-test: H=14.7, p=0.005

Mann-Whitney U-tests:

	0	5	10	15	19
0		1.000	1.000	0.077	0.004
5			1.000	1.000	0.212
10				1.000	0.443
15					1.000
19					

Colpophyllia natans

Kruskal-Wallis H-test: H=29.4, p=0.001

Mann-Whitney U-tests:

	0	5	10	15	20
0		0.434	0.008	1.000	1.000
5			1.000	0.115	0.009
10				0.001	<0.001
15					1.000
20					

Table S4. Statistical output for Experiment 2 in which the maximum prey capture rates of the five study species were compared when exposed to each of their optimal water flow rate for feeding. A Kruskal-Wallis H-tests was performed to assess if species displayed variable maximum prey capture rates overall, followed by Mann-Whitney U-tests to identify differences among pairs of species. A Bonferroni correction for multiple hypothesis testing was applied. Significant values are in bold

Kruskal-Wallis H-test: H=16.0, p=0.003

Mann-Whitney U-tests:

	<i>O. faveolata</i>	<i>D. labyrinthiformis</i>	<i>C. natans</i>	<i>M. cavernosa</i>	<i>F. fragum</i>
<i>O. faveolata</i>		0.864	0.93	0.751	0.003
<i>D. labyrinthiformis</i>			0.781	0.873	0.003
<i>C. natans</i>				0.666	0.001
<i>M. cavernosa</i>					0.007
<i>F. fragum</i>					