Quicklook Report:

Coral spawning and larval observations, upper Florida Keys, 2016

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Introduction and Methods:

The SEFSC benthic team has led efforts, over many years, to document coral spawning and various aspects of larval ecology and early life history of upper Keys *Acropora palmata* and *Orbicella faveolata* (Miller et al. 2016). The 2016 coral spawning season has followed two consecutive years of severe late summer thermal stress in the Florida Keys (http://coralreefwatch.noaa.gov/vs/gauges/florida_keys.php) including mass coral bleaching of both these species. Based on published literature and observations by our team last year ((Levitan et al. 2014), less spawning was observed in 2015 by *O. faveolata* colonies that had bleached in 2014 compared to those that did not, Fisch et al. Pers. Comm.), there was expectation that spawning and/or larval performance might be compromised as a consequence of this severe and repeated stress on the parental population.

Along with collaborators, we performed spawning observations and attempted gamete collection at Horseshoe Reef, Elbow Reef, Sand Island Reef, Grecian Rocks and Molasses Reef. Based on past experience, observations at each site were targeted to the nights within the expected lunar window when spawning by the target species was most likely. This was a somewhat different strategy than past years, where we invested more effort documenting lack of spawning on nights prior and after. Partly as a result, we were able to dedicate one boat-night observation to a site recently reported by the Florida Aquarium dive team (slightly northwest of the Benwood wreck) with an aggregation of a third ESA-listed coral species, *Dendrogyra cylindrus*.

As in the past 2-3 years, a major goal of SEFSC larval investigations for 2016 involved basic characterization of early life history, specifically the longevity of the pelagic larval phase and the timing and dynamics of settlement competency for both species, as well as experiments testing settlement habitat quality (in collaboration with FIU). Hence, much of our attention was on the larval phase. Other significant collaborating groups (Vaughn & Page of Mote Tropical Marine Lab and Coffroth of SUNY-Buffalo) were also engaged in larval culture for the purposes of settling them for experiments and culture of benthic corals. These groups coordinated closely in exchanging both larvae and observations of their condition.

The practice of coral larval culture is largely an art. Current methods used by SEFSC include culturing batches of larvae in ~ 10l chambers which have mesh floor and constant drip input around the circumference of the chamber to encourage gentle but continuous movement/mixing of the larvae. These chambers are housed in bins in a re-circulating seawater system with UV sterilization, filtration to 1 or 5 um, and temperature control. These chambers have been very successful in culturing and settling cohorts of coral larvae over extended periods of time (e.g. swimming for over a month in most years) with minimal labor, generally requiring only a cleaning out of the mesh floor on the chambers every few days. Settlement has been particularly facilitated in these flow-through chambers as a large number of larvae can be exposed to settlement substrates sequentially over a long period of time. SEFSC has also utilized table-top square, smooth-sided static bins with a small bubbler and water changes 2-3 times per day (with 1um filtered, temperature controlled seawater) for larval culture over shorter periods of time. Our collection and treatment of reef water for larval culture was consistent with recent years (bulk storage of seawater hauled from fore-reef areas in Teflon-lined trash cans with filtration [i.e., pumped through canister filters]).

Results and Discussion:

Spawning observations (and fate of different collections) are summarized in Table 1. We did observe and collect a small number of *D. cylindrus* eggs, a first for us, on 20 Aug. A large proportion of the ~ 30 colonies at the site spawned, but all as females (Fig 1). No male spawning was observed so no fertilization was possible.

Overall, spawning by *A. palmata* was considered reasonably good, and better than had been expected based on prior thermal/bleaching stress. However, no spawning or virtually no spawning was observed at Sand Island nor Molasses on the most likely nights (based on past observational record). Also, the timing and combination of spawning by individual genets did not conform well to past observations. For example, most genets at Elbow spawned on night 3 AFM (After Full Moon), whereas we have only observed this many genets spawning together on night 5 AFM in the past (Miller et al. 2016). Two separate cohorts of *A. palmata* were fertilized and cultured (one from each night), and showed very high rates of fertilization (up to 90%) seemingly due to the relatively high number of parents (i.e. 5) that were available on the same night (many years we have only 2 or 3). The 8/21 batch was split between SEFSC, the SUNY Buffalo, and the Mote teams. The smaller 8/22 batch was cultured by SUNY Buffalo and Mote. Sperm samples were also allocated to collaborating groups for genome sequencing (Penn State Univ) and cryo-archiving (Smithsonian).

Spawning by *O. faveolata* at both Grecian Rocks (a highly clonal site) and Horseshoe (few clones) was good on both expected nights (6 and 7 AFM). Diver attention was focused on collecting spawn so complete genet-specific observations of spawning (i.e. which genets spawned which night) were not possible. However, two large genets (up to 10 colonies each) at Grecian Rocks spawned profusely on both nights. At Horseshoe, 8 of 18 observed genets spawned on the 24th (night 6 AFM) and 12 of 28 genets spawned on the 25th (night 7 AFM). Four separate cohorts of *O. faveolata* larvae were cultured (one from each of 2 nights from each of 2 sites), though overall fertilization appeared to be rather low, ranging from ~20-60% among the four cohorts.

Development and behavior of the *A. palmata* larvae was more or less as expected through day 8 or so AS (after spawn). Due to the high fertilization, these cultures were easy to care for. However, at about

day 8, the batch culture, which had been kept in a static table-top bin, more or less spontaneously converted from normal planulae larvae to 'cauliflowers'. This phenomenon of metamorphosis in the water column is often observed at low levels in larval cohorts, but has not been observed to occur so suddenly to this extent. Consequently, many of the larval and settlement experiments were curtailed. We can speculate that this phenomenon may have been accelerated by keeping the larvae at too high density in a static bin (most culture in years past has been in the drip-through chambers). This metamorphosis without attachment has been described in the literature to result from a specific chemical cue produced by crustose coralline algae-associated bacteria (Siboni et al. 2012; Tebben et al. 2011), though no CCA was in contact with the larval culture bin. The *A. palmata* larvae that had been sequestered in aliquots in glass dishes to assess larval longevity showed morphology and behavior consistent with previous years, with high proportion of planulae still swimming nicely and displaying high settlement capacity at over five weeks of age.

Development and behavior of the *O. faveolata* cohorts was much more problematic. Again, fertilization was observed to be relatively low so this always presents some challenge in culture. However, each cohort cultured by SEFSC sequentially suffered catastrophic declines. We had placed a portion of the best-looking cohort (Grecian Rocks 8/24) in one of the drip-through chambers on day 2 AS, but it had essentially completely died and fouled the chamber within 2 days, whereas the remainder of this cohort in a countertop bin crashed a day later. A portion of the Horseshoe 8/24 cohort was subsequently placed in the drip-through chamber (on day 4 AS), and had also crashed by the following day. The larvae appeared to simply disappear or melt away. Additionally, aliquots of 200 swimming, healthy-looking larvae (GR 8/24 cohort) were sequestered in individual temperature-controlled glass dishes (n=10) on day 2 AS for the larval longevity study. These larvae also showed very high rates of abnormal morphology (e.g. lumpy and mis-shapen) and mortality over the course of 4 days when the experiment was aborted (i.e. day 6 AS, total survival ~ 30 % whereas the expectation would be more like 70% based on previous years).

After subsequent crash of all *O. faveolata* cohorts cultured by SEFSC, we received additional larvae back from Mote (Horseshoe 8/25 cohort) on 30 Aug and 1 Sept. The larvae transferred on 30 Aug appeared normal, swimming planulae, but by the morning of 31 Aug, were observed with large numbers of mis-shapen larvae and broken up cell masses (which were still swimming). The larvae transferred on 1 Sept (i.e. day 7 AS) mostly still appeared as swimming planulae, though with a substantial number of round spinning larvae which might be ' cauliflowers'. By the morning of 2 Sept, a large proportion of this batch of *O. faveolata* appeared as very obvious cauliflowers or partial cauliflowers (i.e. with swollen oral ends with obvious mouth and tentacle ridges forming but aboral end remaining narrow and 'planula-looking'), a phenomenon not observed in previous years.

Lastly, we observed abnormal results in fixed samples of fertilization assays for *O. faveolata*, though predominantly in specific parental crosses. For these experiments, we fix an aliquot of eggs/embryos in Z-fix (a buffered formalin fixative) at 6-8 hr after fertilization in order to score them at a later time. We have successfully used this method for several years. However, this year, fixed samples from several of the parental crosses contained unfertilized eggs (solid, smooth spheres, seemingly discounting a fixation problem) but no intact cleaving embryos. Rather, substantial amounts of cellular debris, seemingly broken up cleaving embryos, were evident (Fig 2) making these assays impossible to accurately score for fertilization success.

There is no way to know for sure the cause or causes of the anomalous development patterns and poor survivorship/performance of *O. faveolata* larval cohorts observed by SEFSC. Too-high density cultures in table-top bins may have accelerated declines in some larval batches, but this does not explain the poor performance (high rates of deformity and mortality) in the drip-through chambers and small glass dishes nor the abnormal appearance of the fixed fertilization samples. Mote and Coffroth at KML both succeeded in settling moderate numbers of *O. faveolata* for long-term *ex situ* culture. It is not clear how their rate of settlement success compared with previous years.

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References

Levitan DR, Boudreau W, Jara J, and Knowlton N. 2014. Long-term reduced spawning in *Orbicella* coral species due to temperature stress. *Marine Ecology Progress Series* 515:1-10.

Miller M, Williams D, and Fisch J. 2016. Genet-specific spawning patterns in *Acropora palmata*. *Coral Reefs*:1-6.

Siboni N, Abrego D, Seneca F, Motti CA, Andreakis N, Tebben J, Blackall LL, and Harder T. 2012. Using Bacterial Extract along with Differential Gene Expression in *Acropora millepora* Larvae to Decouple the Processes of Attachment and Metamorphosis. *PLoS ONE* 7:e37774.

Tebben J, Tapiolas DM, Motti CA, Abrego D, Negri AP, Blackall LL, Steinberg PD, and Harder T. 2011. Induction of Larval Metamorphosis of the Coral *Acropora millepora* by Tetrabromopyrrole Isolated from a <italic>Pseudoalteromonas</italic> Bacterium. *PLoS ONE* 6:e19082.

Table 1. 2016 Spawning observations on upper Florida Keys reefs by species and site. ARB = R/V Research Diver of Aquarius Reef Base (FIU).

Date (AFM*)	Site	Observing Team/Boat	Species	Action
20 Aug (2)	Horseshoe	ARB	*A. palmata*	Substantial spawning by main, monotypic thicket. No fertilization possible. Sperm samples preserved for genome sequencing (Penn State Univ) and cryo-archiving (Smithsonian)
20 Aug (2)	'FlAq Forest'^	SEFSC	*Dendrogyra cylindrus*	~ 75% of 30 colonies observed spawned eggs, no male spawning
21 Aug (3)	Elbow	SEFSC	*A. palmata*	Substantial spawning by 3 genets, minor spawning by 2 additional. Two genets had sperm cryo-archived (Smithsonian). Main larval cohort for subsequent studies from this collection.
21 Aug (3)	Horseshoe	ARB	*A. palmata*	No spawning signs
21 Aug (3)	Molasses	FIU small boat	*A. palmata*	Tiny dribble from singe genet at this site (ML3)
22 Aug (4)	Elbow	SEFSC	*A. palmata*	Smaller amounts of spawn from same 5 genets as night before. Additional larval cohort fertilized from these.
22 Aug (4)	Sand Island	ARB	*A. palmata*	No spawning signs
22 Aug (4)	Molasses	FIU small boat	*A. palmata*	No spawning signs
23 Aug (5)	Elbow	SEFSC	*A. palmata*	No spawning signs
23 Aug (5)	Sand Island	ARB	*A. palmata*	No spawning signs
24 Aug (6)	Grecian Rocks	SEFSC/FIU	*O.faveolata*	Substantial spawning by most genets. Larval cohort created
24 Aug (6)	Horseshoe	ARB	*O.faveolata*	Substantial spawning; 8 of 18 genets observed; Larval cohort created
25 Aug (7)	Grecian Rocks	SEFSC/FIU	*O. faveolata*	Smaller spawn than night before, dominated by 2 abundant genets, Larval cohort created
25 Aug (7)	Horseshoe	ARB	*O. faveolata*	Substantial spawning; 12 of 28 genets observed

*After Full Moon ^ Coordinates provided by Florida Aquarium; site discovered during Dcyl rescue collections, July16

Fig 1: *Dendrogyra cylindrus* spawning eggs on 20 Aug 2016 at a site just northwest from the Benwood wreck. Left panel shows eggs departing from individual branch, right panel shows cloud of eggs in the water column

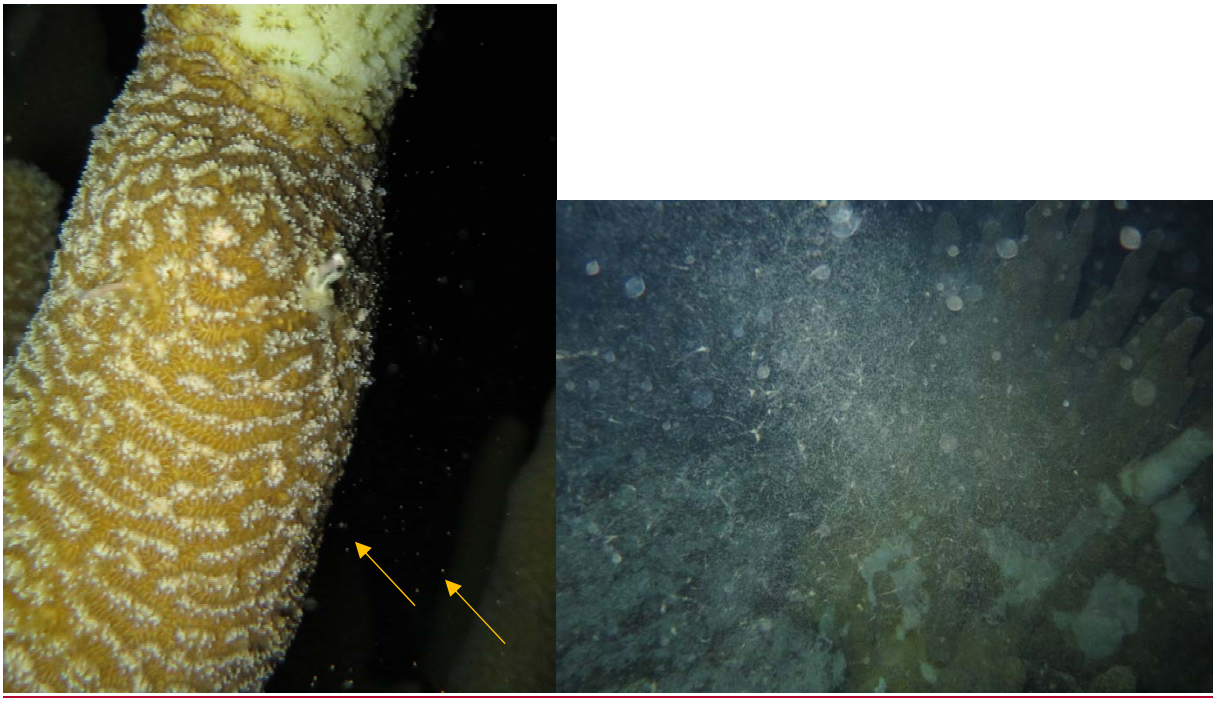

Fig 2: Example of a formalin-fixed sample (~7hr post-fertilization) showing only unfertilized eggs and cellular debris, seemingly from the breakup of cleaving embryos. These samples were impossible to accurately score fertilization success.