The following supplement accompanies the article

Host growth and survivorship varies with endosymbiotic algal partner in developing cnidarians

Daniel M. Poland[1], Mary Alice Coffroth[2,*]

*Corresponding author: coffroth@buffalo.edu

Marine Ecology Progress Series 612: 87–100 (2019)

SUPPLEMENT

Larval rearing and settlement

Aposymbiotic planula larvae were collected from maternal colonies of the surface-brooding octocoral *Briareum asbestinum* during the 2007 spawning event on the Florida Bay coast of Long Key, Florida Keys (24^{o} 49.942' N, 80^{o} 48.622' W). Larvae were maintained in 0.2 µm-filtered seawater (FSW) at Keys Marine Lab, Florida, USA. To monitor *in situ* acquisition of symbionts, approximately 500-600 larvae were added to five mesh-covered jugs with gorgonian skeletal branches as settlement substrate. Branches were scrubbed clean in fresh water and dried in the sun to eliminate contaminant symbionts before use. These jugs were transported to the natal site where planulae settled and metamorphosed. Branches were removed and suspended approx. 0.5 m off the seafloor as *in situ* controls once settlement was complete (~5 days). The remaining larvae were used in the laboratory experiment conducted at the Aquarium of Niagara (AON) in Niagara Falls, NY. Initially the larvae were maintained at the Keys Marine Lab and after they began to elongate (3-4 days), they were supplied cleaned gorgonian skeletal branches as settlement substrate. After settlement at the Keys Marine Lab (approximately 3 weeks), these experimental branches with partially or fully metamorphosed juveniles were shipped to the State University of New York at Buffalo, where they were maintained in filtered artificial seawater (ASW, Instant Ocean, Spectrum Brands) for a four-day acclimation period. Before starting the experiment, juveniles were treated for an additional 24 hours with antibiotics (9.0 mg chloramphenicol, 45.0 mg ampicillin and 90.0 mg streptomycin per liter ASW) and transported to the AON.

Experimental treatment conditions

Following the initial acclimation period, polyps were counted on all branches. Based on these counts, branches were divided into 11 treatment tanks at the AON. A total of 11-12 branches per aquarium (380-430 single-polyp colonies) were suspended in approximately 25-30L ASW (Instant Ocean, Spectrum Brands) using monofilament arrays. Each aquarium was supplied with an air stone and a 200-Watt submersible aquarium heater and loosely covered in plastic wrap to avoid water transfer between treatments. Salinity of ASW ranged from 31-32 ppt and all tanks were illuminated by overhead light banks with 40-Watt 5,000K color scale lighting (full spectrum color rendering: 92), on a 14:10 hour light/dark cycle, delivering a light intensity of approximately 15-55% of light recorded at the natal site of Long Key, FL, and 25-95% of light at a 4 m *B. asbestinum* population in lower Keys depending on the time of day (mid-day or morning/evening, respectively). Temperature and light intensity were recorded every hour using underwater HOBO data loggers (Onset Computer Corp.). To account for variability in light intensity and other environmental variables, treatment re-

plicates were alternated so that replicates were not adjacent and the position of each replicate was switched approximately monthly.

The juveniles were kept in ASW with weekly/biweekly feedings of macerated frozen *Artemia* nauplii to provide a heterotrophic source of nutrients to maintain productivity in the treatments (Fitt 2000). The feedings were performed for approximately 30 minutes with gentle stirring before partial water changes. Bacterial and non-*Symbiodinium* algal growth was manually cleaned from branches every week and 50% of the ASW was changed each week. A complete ASW change was made every 2-3 weeks.

Quantitative real time PCR (qPCR) reactions

Each assay included cloned *Symbiodinium* A (formerly Clade A) ITS rDNA standards at 10^{o} to 10^{-7} dilutions for standard curves. The reaction volume of 20 µl included 5-10 ng genomic DNA (host+symbiont), 1X PCR Buffer (1.5 mM $MgCl_2$, 10 mM Tris-HCl, 50 mM KCl), 200 mM dNTPs, 0.1 µM of each primer, 0.5 µl each SYBR Green and ROX fluorescent dyes, and 1 U *Taq* (New England Biolabs). The amplification conditions were: 94 ^{o}C for 5 min. for initial denaturing, followed by 40 cycles of 94^{o}C for 30 s, 63^{o}C for 33 s and 72^{o}C for 45 s, ending with a melt-curve analysis from 55^{o}C to 95^{o}C for 30 s. The presence of *Symbiodinium* A194 was apparent by a peak in the melt curve at approximately 85^{o}C, which was used as a signal for the presence of the target DNA. Non-target DNA amplifications showed melt-curve peaks below 80^{o}C and this was consistent between different *Breviolum* cultures (B-types) non-target samples included as controls.

Table S1. Number of juveniles sampled (and percent of samples that did not amplify) for symbiont identificaton in each treatment over the 44 week experimental period. ET= experiment terminated; NS=not sampled. For actual numbers that amplified for each treatment/time period, see Fig. 2

week/treatment	**2**	**4**	**12**	**20**	**28**	**44**
B184 (1)	20 (0)	10 (0)	60 (3.3)	59 (3.4)	60 (6.7)	73 (4.1)
B224 (2)	20 (0)	10 (0)	60 (35)	60 (43.3)	60 (26.7)	87 (59.8)
A194 (3)	10 (0)	10 (0)	60 (1.7)	38 (7.9)	101 (18.8)	ET
Mix (4)	40 (0)	35 (11.4)	60 (13.7)	60 (11.7)	60 (15)	78 (21.8)
“control” (9)	20 (20)	20 (30)	60 (1.7)	60 (40)	142 (64.8)	ET
Mix+Heat (12)	30 (0)	15 (0)	30 (26.7)	30 (30)	30 (16.7)	30 (43.3)
in situ	30 (56.7)	NS	NS	30 (23.3)	NS	30 (40.0)

Fig. S1.

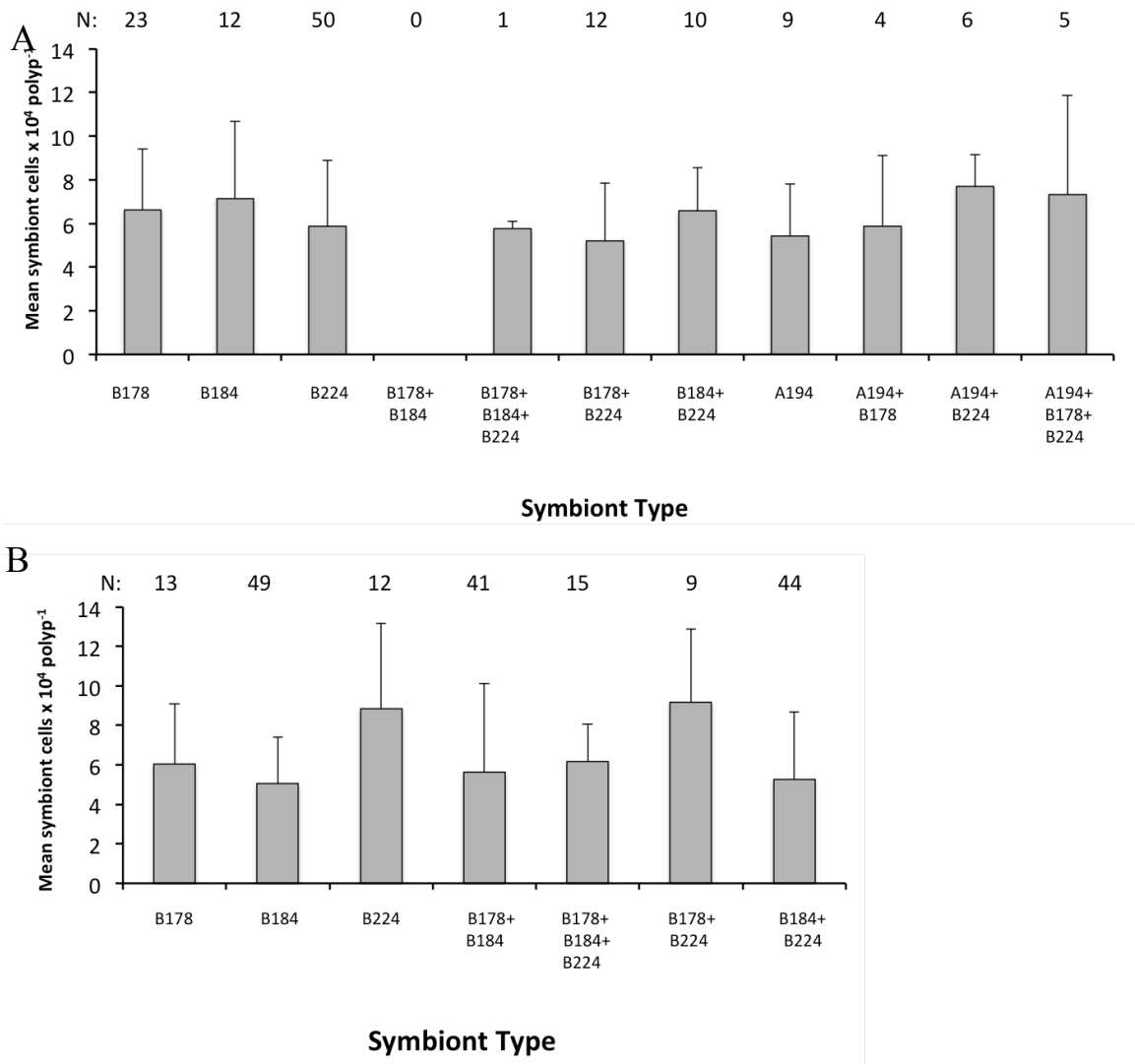

Fig. S1. Mean symbiont densities (+ one standard deviation) in juveniles with different symbiont populations from non-B treatments (A194 and "control") at week 28 (A), and B treatments at week 44 (B). Here juveniles are categorized according to the symbionts they harbor, not treatment origin. Symbiont types B178+B184 were not encountered at week 28. At both week 28 and 44, cell counts and symbiont type were determined for the same polyps. Sample sizes (numbers of samples that amplified) are given above the columns. Cell densities are based on four replicate counts per polyp.

LITERATURE CITED

Fitt WK (2000) Cellular growth of host and symbiont in a cnidarian-zooxanthellar symbiosis. Biol Bull 198:110–120