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Zooxanthellar symbiosis in planula larvae of the coral *Pocillopora damicornis*

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ABSTRACT

We characterized the planular-zooxanthellae symbiosis of the coral *Pocillopora damicornis* using criteria that are familiar in studies on corals. Similar to adult corals, planulae exhibited photoacclimation, as changes in symbiont chlorophyll *a* (chl *a*); changes in the light-saturation constant for photosynthesis (I_k); and, at insufficient light, fewer zooxanthellae, decreased respiration, increased weight loss, and increased sensitivity to photoinhibition. Numbers of zooxanthellae in newly-released planulae varied by at least three-fold within broods. Planulae with low *versus* high numbers of zooxanthellae (termed pale *versus* dark planulae, respectively) did not differ in symbiont chl *a* content, I_k, or biomass-specific rate of dark respiration. Pale planulae had lower rates of photosynthesis, but this difference vanished after three weeks, when zooxanthellar numbers increased by 225% in pale planulae and by 31% in dark planulae. Numbers of zooxanthellae also increased significantly in planulae cultured in ammonium-enriched seawater; ammonium also apparently prevented weight loss and induced settlement. Approximately 70% of photosynthetically-fixed carbon (labeled using ^{14}C) apparently was translocated from the zooxanthellae to their host. A comparison of planulae cultured at 0.3% *versus* 11% sunlight suggested that photosynthesis provided ~31% of the energy utilized by the latter. Overall, we conclude that the physiology of symbiosis in planulae of *P. damicornis* is broadly similar to symbiosis physiology in adult corals.

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1. Introduction

Due to their ecological importance and their value as model systems for understanding mutualism, coral-zooxanthellar symbioses have been studied intensively for decades. These symbioses integrate algal phototrophy with animal heterotrophy and thereby thrive in shallow, often nutrient-poor tropical seas. Many species of coral do not transmit zooxanthellae to their offspring, whereas others do (Harrison and Wallace, 1990; Trench, 1993). In the former, coral planula larvae lacking zooxanthellae depend on stored reserves for their nutrition; in the latter, zooxanthellate planulae might benefit energetically from hosting symbionts. Also, the initially aposymbiotic planulae of some species can establish symbiosis with zooxanthellae before they settle and metamorphose into juvenile corals (Schwarz et al., 1999), an ability that could be more common than is generally appreciated (van Oppen, 2001). Compared to adult corals, zooxanthellar symbiosis in planulae has not been well-studied; it could be especially significant given that larval energy budgets can be an important factor in larval dispersal potential (Bohonak, 1999) and thus adult biogeography (Mileikovsky, 1971; Strathmann, 1980).

Richmond (1982, 1987) showed that planulae of *Pocillopora damicornis* obtain photosynthetically-fixed carbon from their zooxanthellae and concluded that phototrophy can extend larval lifetime and hence dispersal. Consistent with this, Isomura and Nishihira (2001) found that planulae of *P. damicornis*, and also zooxanthellate planulae of the related species *Stylophora pistillata* and *Seriatopora hystrix*, survived better in the light than when kept in darkness. From histological examinations of *S. pistillata*, Titlyanov et al. (1998) concluded that zooxanthellar numbers are regulated by similar mechanisms in planulae and in adult corals. Edmunds et al. (2001) found that photosynthesis by zooxanthellate planulae of *Porites astreoides* was diminished at elevated temperature, which is a common feature of adult corals. Taken together these studies suggest the planular-zooxanthellar symbiosis is similar to the coral-zooxanthellar symbiosis and that vertical transmission of zooxanthellae, in addition to perpetuating successful combinations of host and symbiont genotypes across generations (Trench, 1993), creates functional symbioses in coral planulae.

The purpose of this study was to further investigate the potential significance of zooxanthellae in coral larvae. We analyzed planulae of *P. damicornis* using several criteria that are familiar in studies on adult corals including acclimation to different irradiance regimes, regulation of zooxanthellar proliferation, responses to ammonium as a potential source of nitrogen, the fate of photosynthetically-fixed carbon, and energy budgets constructed from photosynthesis–irradiance relationships and changes in biomass. By these criteria, planulae of *P. damicornis* appeared similar to published descriptions of adult corals.

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2. Materials and methods

2.1. Collection and maintenance of planulae

Colonies of *P. damicornis* were collected from the reef flat in East Agana Bay, Guam at depths of 0.5–1.5 m, held in a flow-through seawater tank, and exposed to ~25% sunlight. Planulae of *P. damicornis* measure approximately 0.35 mm^3 in size (Isomura and Nishihira, 2001) and were collected as described by Richmond and Jokiel (1984).

Following collection, planulae were placed in filtered seawater in glass Fernbach flasks and set in flowing seawater under shade. This seawater, and all seawater used to maintain planulae hereafter, was pumped from the fore reef of Pago Bay, Guam, at approximately 4 m depth. This seawater is clear and nutrient-poor, *e.g.*, 0.1–0.2 μM nitrate, ~0.2 μM phosphate, and no detectible ammonium (Marsh, 1977; Matson, 1991; E. A. Matson, University of Guam, personal communication). To obtain replicate samples, planulae were first combined in a 2 l beaker, swirled, and a sample of planulae was collected haphazardly in a 10-ml pipette. Those planulae were counted, and then that sample was adjusted, by the same method, to contain the number of planulae required. By repeating this process, the population was divided into replicate samples in 400-ml glass beakers (≤40 planulae) or in 800-ml glass beakers (>40 planulae) at densities of one planula per 8–10 ml of seawater.

Pale-colored and dark-colored planulae were collected as follows. Planulae were randomly divided into samples of 100, as above. From each of these sub-samples, the palest 10 and the darkest 10 planulae were sorted out by subjective visual inspection. These collections of ten were then pooled, to obtain larger populations of pale and of dark planulae.

2.2. Physical characterization of planulae

Numbers of zooxanthellae $planula^{-1}$ were determined by homogenizing 9–25 live planulae in filtered seawater using a dounce-type homogenizer, and counting zooxanthellae using a hemacytometer. In one experiment (Table 3), zooxanthellar counts were instead obtained from planulae that had been lyophilized to measure dry weights (see below). These planulae were first rehydrated overnight in 10% formalin in filtered seawater, and then homogenized. Zooxanthellar counts from these samples were ~twice what we expected. To investigate this discrepancy, we analyzed additional samples ($n = 14$) of lyophilized planulae (archived at −80 °C), from experiments in which zooxanthellar numbers previously had been obtained from live, replicate samples. This provided 14 sample-pairs of planulae, drawn randomly from each of 14 different populations, in which paired samples differed only in the method of analysis (live *versus* lyophilized). Results confirmed that lyophilized planulae yielded (on average) 2.2 times as many zooxanthellae as did live planulae (Appendix A). Thus most zooxanthellar numbers (except in Table 3) are underestimates by a factor of at least 2.2 (or more, if counts from lyophilized planulae were also underestimates). Except as indicated (see Results and Discussion), we did not correct for this error.

Chlorophyll was measured spectrophotometrically or fluorometrically. Homogenates (above) were centrifuged at 10,000 rpm (9200 *g*) for 4 min, and the zooxanthellar pellets were resuspended in 1 ml of 90% acetone (on ice), sonicated briefly, and extracted for ~24 h in the dark at 0–4 °C. Extracts were measured spectrophotometrically to quantify chlorophylls *a* (chl *a*) and c_2 (chl c_2) as described (Jeffery and Humphrey, 1975). Fluorometry was used to quantify chl *a* in dilute samples (excitation filter, 340–500 nm; emission filter, >665 nm). To measure chl *a* in individual planulae, one planula was sonicated (as above) in 7 ml of 90% acetone and extracted overnight in the dark at 0–4 °C.

Planular dry weights were determined from 20 to 26 planulae placed on a small piece (~12 mg) of pre-weighed aluminum foil. Seawater was removed using a small pipette and an absorbent tissue, and samples were stored at −80 °C. Later, all samples from an experiment were lyophilized for ~24 h and then weighed on a microbalance.

2.3. Measurement of respiration and net photosynthesis

Twenty-three to 25 planulae were placed in 1.6 ml of filtered and autoclaved seawater in a clear glass respirometry chamber that was mounted on an optical bench. Water at 28.0 °C was pumped through the chamber's jacket to regulate temperature. The chamber was illuminated from one side with a fiber optic illuminator (150 W tungsten-halogen lamp), and different levels of illumination were produced by placing neutral-density optical filters between the light source and the chamber. Light (400–700 nm = photosynthetically active radiation, PAR) passing through the chamber was measured with a cosine-corrected 2π sensor that faced the light source; these measured values of PAR are reported here, as means ± one standard deviation. Contents of the chamber were mixed continuously using a magnetic stirring bar at ~150 rpm, under which conditions the planulae swirled with the water.

Oxygen within the chamber was measured using a microcathode oxygen electrode. Oxygen and illumination were logged continuously using a chart recorder. Nine levels of illumination (and darkness) were used to determine overall photosynthesis–irradiance (*P–I*) relationships, which were summarized using the hyperbolic tangent function (Jassby and Platt, 1976). In replicated experiments (Tables 1 and 3) it was impractical to obtain complete *P–I* data for every sample. Instead, complete data were first obtained from one replicate of each experimental group. Other replicates were then tested in the dark (= dark respiration), at one or two levels of saturating illumination to measure maximum net photosynthesis (P^{net}_{max}), and a level of sub-saturating illumination below or near the compensation point (O_2 flux = 0). These data were used to estimate light-saturation constants (I_k) for net photosynthesis, for each sample.

Data were collected between 1030 and 1500 h, to lessen the potential for diurnal effects (Chalker et al., 1983). Within this window, it took three days to analyze all replicates in experiments (see below), which therefore ended over three days. All planulae remained under experimental conditions until they were analyzed. Planulae spent up to 1.5 h within the respirometry chamber, after which they were homogenized and characterized physically as described above.

2.4. Experimental conditions

Unless noted otherwise, beakers of planulae were placed in outdoor water tables that received unobstructed natural irradiance. Tents of clear acrylic sheet (~4 mm thick and blocking UV radiation) protected planulae from rain, and one layer of black fiberglass window screen shaded the tables by 43% of PAR (measured using a cosine-corrected, 2π sensor directed vertically). Water-table temperatures were recorded continuously and were similar to the corals' natural environment (Appendix A). Experimental irradiances were created by leaving beakers fully exposed (57 ± 1% ambient downward PAR; mean of eight different positions in the water tables ± one standard deviation), or by caging beakers individually with two (18 ± 2% PAR), three (11 ± 1% PAR) or eight (0.3 ± 0.2% PAR) layers of window screen. To estimate actual irradiance during the experiments reported in Table 1, total ambient downward PAR was recorded daily using an integrating radiometer (see Appendix A).

Planulae treated with ammonium or labeled with ^{14}C-bicarbonate (below) were kept outdoors under a roof of translucent white polyvinyl chloride. Downward irradiance varied during the day between ~7 and 15% of unobstructed PAR, and planulae were not further shaded.