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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 ¹⁴C) 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.

Keywords

symbiodinium; photosynthesis; photoacclimation; energy budgets; ammonium; photosynthetically fixed carbon

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

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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; Strathman, 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.

Materials and methods

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 mm³ 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.

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 1). 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 (9,200 g) for 4 min, and the zooxanthellar pellets were resuspended in 1 ml of 90% acetone (on ice), sonicated briefly, and extracted for ~ 24 hr 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 20-26 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 hr and then weighed on a microbalance.

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 (150W 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 $\pm$ 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 ($P_{\max}^{\text{net}}$), 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 hr, 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 hr within the respirometry chamber, after which they were homogenized and characterized physically as described above.

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 2a). Experimental irradiances were created by leaving beakers fully exposed ($57 \pm 1\%$ ambient downward PAR; mean of eight different positions in the water tables $\pm$ one standard deviation), or by caging beakers individually with two ($18 \pm 2\%$ PAR), three ($11 \pm 1\%$ PAR) or eight ($0.3 \pm 0.2\%$ 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 2b).

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 ~ 7-15% of unobstructed PAR, and planulae were not further shaded.

High-Light Experiment

To test the ability of planulae to photoacclimate, seven replicate samples of 23-25 newly-released planulae (age, 10-100 hr) were analyzed for respiration and net photosynthesis, numbers of zooxanthellae, and chl *a* and *c*₂. Seven other replicates were dried and weighed. Data from these 14 samples defined the initial condition ($n = 7$) of their population, which was further subdivided (35 planulae per sample) and then placed under "high light" (57% ambient PAR; 14 samples), and under "medium light" (18% ambient PAR; 14 samples). After 17-19 days, seven replicate samples from each group were analyzed for respiration and net photosynthesis, number of zooxanthellae, and chl *a* and *c*₂; after 19 days, the other seven samples of each group were analyzed for dry weight.

Low-Light Experiment

This experiment was identical to the High-Light Experiment (above) except that samples ($n = 8$) were maintained under low light (11% ambient PAR) and under very low light (0.3% ambient PAR).

Experiments on pale and dark planulae

To test if pale and dark planulae maintain their different chl-*a* phenotypes over time, we first measured chl *a* in 32 individual, newly-released planulae of each type. Other samples of 40-42 planulae were then maintained at high light (57% ambient PAR, $n = 1$) and at medium light (18% ambient PAR, $n = 1$) for 18 days, after which we measured chl *a* those individual planulae that survived.

To measure weight loss by pale *versus* dark planulae over time, seven samples of newly-released planulae of each type were analyzed for respiration and net photosynthesis and then

lyophilized and weighed; zooxanthellar numbers were obtained from the lyophilized planulae. Concurrently, seven other samples of 29 planulae of each type were placed under high light (57% ambient PAR) for 20-22 days, after which they were analyzed the same way. During this 22-day experiment, we measured $P_{\max}^{\text{net}}$ of one sample of each type at 4- or 5-day intervals. These planulae spent < 1 hr in the laboratory and were then returned to the experiment; samples were chosen for measurement randomly, but without replacement.

Ammonium-Enrichment Experiment

To test responses to ammonium, ten samples of 4-8-day-old planulae (12-14 planulae per sample) were each analyzed for numbers of zooxanthellae and for chl *a*. Ten other samples of 24-26 planulae were dried and weighed. Data from these 20 samples defined the initial condition of their population, which was further subdivided (44 planulae per sample) and then placed in filtered seawater (control planulae, $n = 10$), in filtered seawater plus 4 μM NH_4Cl (low-ammonium planulae, $n = 10$), or in filtered seawater plus 18 μM NH_4Cl (high-ammonium planulae, $n = 10$). Ammonium chloride was added (or not) every two days, when cultures were cleaned (above). Ammonium remaining after two days was compared to that in freshly-made additions using an ammonia-selective electrode filled with the manufacturer's electrolyte and connected to a millivolt meter, as described (Garside et al., 1978). By this method (limit of detection $\sim 1 \mu\text{M}$) ammonium could not be detected in plain seawater, and measurements indicated that $\geq 60\%$ of added ammonium persisted for two days. The experiment ended after 21-23 days, when the samples of planulae were randomly divided into two unequal parts: nine to 15 planulae were analyzed for numbers of zooxanthellae and for chl *a*, and 20-25 planulae were dried and weighed.

Analysis of photosynthetically-fixed carbon

Photosynthetically-fixed carbon was labeled with $\text{NaH}^{14}\text{CO}_3$ ($0.1 \mu\text{Ci } \mu\text{g}^{-1}$). The labeled planulae were 22-27 days old, and were used to examine the partitioning of photosynthate between zooxanthellae and host tissue (Battey, 1992). Specific methods are given in Appendix 2c.

Statistical analyses

One-way ANOVAs followed by post hoc analyses (Tukey's Honestly Significant Difference) were used to compare planulae from different experimental treatments ($\alpha = 0.05$; Sokal and Rohlf, 1995). Two-group comparisons were completed with Student's *t*-tests. The homogeneity of variances was examined using Bartlett's test. When the statistical assumption of equal variances was violated ($\alpha = 0.05$) the more conservative Games and Howell test for multiple comparisons with heterogeneous sample variances was used ($\alpha = 0.05$, Games and Howell, 1976). Error intervals are standard deviations.

Results

High-Light Experiment

After 19 days, planular dry weight decreased by 25% at high light (hereafter, high-light planulae) and by 23% at medium light (hereafter, medium-light planulae); the slight difference between groups was not significant (Table 1, High-Light Experiment). Rates of dark respiration after 17-19 days were similar to the initial condition, suggesting that weight loss had little effect on overall metabolism. Zooxanthellar number in both treatment groups was higher than the initial 11,000 zooxanthellae planula⁻¹, but no difference was significant. Newly-released planulae and high-light planulae had similar amounts of chl *a*, and medium-light planulae had significantly more (by $\sim 50\%$). Compared to the initial condition, zooxanthellae in high-light planulae tended to have less chl *a*, whereas zooxanthellae in medium-light planulae tended

toward more. Consequently, zooxanthellae in high-light planulae had significantly less chl *a* cell⁻¹ (by 28%) than did zooxanthellae in medium-light planulae. There was no significant difference among groups in either chl *c*₂ content (not presented) or chl *a*:*c*₂ ratio (Table 1).

Photosynthesis *versus* irradiance relationships (*P-I* curves) of one sample of planulae from each group are shown in Appendix 3a. Other samples were tested to determine rates of dark respiration, and of net photosynthesis at an irradiance below the onset of light saturation ($76 \pm 9 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ PAR) and at light saturation ($P_{\text{max}}^{\text{net}}$; at $1690 \pm 120 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ PAR). Medium-light planulae resembled the initial condition (Table I; $P_{\text{max}}^{\text{net}}$ through I_k); high-light planulae differed from the initial condition in having lower $P_{\text{max}}^{\text{net}}$; they differed from medium-light planulae in having higher I_k , which indicates a lower photosynthetic efficiency at sub-saturating irradiance.

Low-Light Experiment

Dry weight decreased by 24% at low light (hereafter, low-light planulae) and by 32% at very low light (hereafter, very-low-light planulae); this difference was significant (Table 1, Low-Light Experiment). Dark respiration of low-light planulae was similar to the initial condition, but decreased by 52% in very-low-light planulae. Numbers of zooxanthellae also decreased (by 36%) in very-low-light planulae, whereas they increased (by 64%) in low-light planulae. Based on dry weights of zooxanthellae from adult *P. damicornis* ($2.7 \pm 0.23 \times 10^{-1} \mu\text{g}$ per 10^3 cells; $n = 3$ coral colonies; methods in Appendix 2d), 54% of the dry-weight difference between low-*versus* very-low-light planulae could be attributed to zooxanthellar biomass. (For this calculation, zooxanthellar numbers reported in Table 1 were corrected by multiplying by 2.2, as explained in Materials and methods and in Appendix 1) Planular chl *a* mirrored zooxanthellar numbers, decreasing by 46% in very-low-light planulae, and increasing by 83% in low-light planulae. Although changes in zooxanthellar chl *a* (from the initial condition) were not significant in either treatment, very-low-light planulae had significantly less chl *a* cell⁻¹ (by 19%) than did low-light planulae. There was no significant difference among the groups in either chl *c*₂ content (not presented) or chl *a*:*c*₂ ratio (Table 1).

Photosynthesis-irradiance curves from one sample of planulae from each group are shown in Appendix 3b. Low-light planulae resembled the initial condition, but very-low-light planulae had lower photosynthetic rates. The other low-light and very-low-light samples were tested at four levels of illumination: darkness, and one below light-saturation ($70 \pm 6 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ PAR) and two above saturation (549 ± 41 and $1610 \pm 150 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ PAR). Low-light planulae were indistinguishable from the initial condition in these tests (Table I, $P_{\text{max}}^{\text{net}}$ through I_k ; $P_{\text{max}}^{\text{net}}$ is the value obtained at $1610 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ PAR).

Very-low-light planulae were different. They had lower $P_{\text{max}}^{\text{net}}$ (Table 1), and oxygen production decreased by 38% at 1610 compared to $550 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ PAR (paired *t*-test, $P = 0.001$). (Net photosynthesis at $550 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ PAR represents $P_{\text{max}}^{\text{net}}$ of very-low-light planulae in Table 1.) The same comparison showed minimal photoinhibition of photosynthesis in low-light planulae (3% decrease, paired *t*-test, $P = 0.001$). In contrast, photosynthesis by very-low-light planulae at sub-saturating irradiance, represented by I_k , was unexceptional (Table 1).

Pale and dark planulae

Newly-released planulae ranged from off-white (= pale) to dark brown (= dark) in color. We collected populations of the two extremes (see Materials and methods), and found that their chl-*a*-planula⁻¹ distributions did not overlap, nor did they include the mean chl *a* content of their unsorted population (Fig. 1a). After 18 days at medium light (18% ambient PAR, as above), average chl *a* planula⁻¹ increased in both pale and in dark planulae (Fig. 1b). Many of

these 18-day-old planulae (59% and 46%, respectively) had more chl *a* than any planula in their groups tested initially (Fig. 1a); given 18-day survivorships > 80%, these observations indicate that increased chl *a* after 18 days was not due to selective mortality of low-chl-*a* planulae. In contrast, pale and dark planulae maintained at high light (57% ambient PAR) for 18 days did not change (Fig. 1c).

Because the planulae analyzed above were pooled from several corals, it was possible that pale *versus* dark planulae came from different parents. To test this, we examined individual broods and found pale and dark planulae in each one. Four of these broods were large enough for comparative analyses of pale and dark siblings (Table 2, Appendix 4). On average, pale planulae had 65% fewer zooxanthellae and 69% less chl *a* than dark planulae had, and thus both types of planulae had the same amount of chl *a* zooxanthella⁻¹. Pale planulae weighed 17% less, and only 14% of this weight difference was accounted for by their lower number of zooxanthellae (calculated as above, using corrected data in Table 2). Pale planulae had lower rates of dark respiration (Table 2), but not when normalized to dry weight (data in Table 2; paired *t*-test, *P* > 0.5). Pale planulae had much lower $P_{\max}^{\text{net}}$. However, similar I_k in pale and in dark planulae, like similar chl *a* zooxanthella⁻¹ (above), implied that zooxanthellae in both types of planulae were physiologically similar.

The dichotomy of pale *versus* dark planulae might provide insight into the energetic benefit of photosynthesis. Specifically, we suspected that pale planulae, having lower photosynthetic capacity, would rely more on stored energy and therefore lose more weight as they aged. We tested this hypothesis using pale and dark planulae pooled from several parents.

Again, pale planulae initially weighed less than dark planulae, this time by 7% (Table 3); one-half of this difference could be attributed to fewer zooxanthellae (calculated as above, using uncorrected data in Table 3). Pale and dark planulae lost similar amounts of weight during three weeks (25% and 24%, respectively), contrary to our expectation. Initially, pale planulae had 70% fewer zooxanthellae than dark planulae had, but this difference was only 24% after 20-22 days (Table 3). Zooxanthellar number increased in pale planulae by 225% and in dark planulae by 31%. (Note that zooxanthellar numbers reported in Table 3 are ~ twice as great as those reported in Tables 1 and 2, as a result of a difference in methods; see Materials and methods and Appendix 1.) These significant increases contrast with previous observations on planulae kept at 57% ambient PAR (Table 1, High Light; and by inference from measures of chl *a* in Fig. 1c). Actual irradiance might have been different this time (ambient PAR was not recorded during this experiment), as was temperature, which averaged 1.2 C higher than previously.

Again, newly-released pale and dark planulae differed substantially in $P_{\max}^{\text{net}}$, but that difference disappeared after 20-22 days owing to a significant increase in $P_{\max}^{\text{net}}$ of pale planulae (Table 3, Appendix 5). Measurements of $P_{\max}^{\text{net}}$ taken during the experiment revealed steady increases, more so in pale planulae (Fig. 2). Dark respiration and I_k were similar in pale and in dark planulae both initially and after three weeks; however, values of both parameters increased substantially after three weeks (Table 3).

Ammonium-Enrichment Experiment

After 21-23 days, dry weight decreased by 14% in planulae held in filtered seawater (control planulae), which otherwise resembled the initial condition (Table 4). Dry weights were obtained from only four samples of planulae held in seawater plus 4 μM NH_4Cl (low-ammonium planulae), because many of these planulae settled and metamorphosed during the experiment. Their dry weight decreased by 9%, but the *n* of 4 makes statistical analysis of this parameter impractical. Planulae held in seawater plus 18 μM NH_4Cl (high-ammonium planulae), in contrast to other planulae in this study (Tables 1 and 3), did not lose significant

dry weight (Table 4). Both low- and high-ammonium planulae had more zooxanthellae than control planulae had (by 75% and 68%, respectively), and they had more chl *a* (by 77%); the amount of chl *a* zooxanthella⁻¹ did not change significantly after three weeks, in any group. Higher zooxanthellar number in high-ammonium planulae *versus* control planulae could account for 46% of the dry-weight difference between these groups (calculated as above, using corrected data in Table 4).

The lower final recovery of low-ammonium planulae in this experiment was significant (mean number of planulae recovered per sample $\pm$ one standard deviation: control, 36 ± 4 ; low-ammonium, 20 ± 11 ; high-ammonium, 36 ± 5 ; Tukey's HSD, $n = 10$, $P < 0.01$).

Photosynthetically-fixed carbon

Planulae fixed 169 ± 19 cpm (mean $\pm$ standard error, $n = 3$) of ¹⁴C planula⁻¹ in the light; fixation in the dark was $3.8 \pm 0.4\%$ of that in the light (mean $\pm$ standard error, $n = 3$). Radiolabeled planulae placed in the dark lost fixed ¹⁴C slowly: $100 \pm 4\%$ of the initial radioactivity remained after 3 hr; $91 \pm 4\%$ remained after 6 hr; and $74 \pm 2\%$ remained after 20 hr (means $\pm$ standard errors, $n = 2$).

The amount of photosynthetically-fixed ¹⁴C translocated from zooxanthellae to host tissue was estimated indirectly, by measuring the chl *a*-specific radioactivity of whole planulae *versus* zooxanthellae. One determination implied that 74% of the total photosynthetically-fixed carbon was in host tissue. A second determination, using a longer processing time, gave a value of 69%. The higher value in the first determination suggests that these estimates were not seriously inflated by "leakage" of ¹⁴C from zooxanthellae during their isolation (see Appendix 2c).

Discussion

Numbers of zooxanthellae in planulae of *P. damicornis*

We do not know why zooxanthellar numbers determined from live *versus* lyophilized planulae differed by 2.2-fold (Appendix 1). We noticed that live planulae were "stickier," suggesting that zooxanthellae might have been lost from live planulae by adhering to the homogenizer. Or, live zooxanthellae may be more fragile than lyophilized and then formalin-fixed zooxanthellae, and thus more susceptible to rupture during homogenization. Because losses are more readily explained than excesses, the larger numbers – from the lyophilized planulae – are the better estimates of true zooxanthellar numbers. While these observations call into question how many zooxanthellae planulae really had (counts from lyophilized planulae could be underestimates, too), they do not affect testing null hypotheses – hypotheses of *no difference* between groups (Tables 1, 2, and 4) – because one estimate is simply a linear transformation of the other (Appendix 1). Thus where our goal was hypothesis-testing (Tables 1-4) we analyzed and presented whichever data we had collected. On the other hand, in the Discussion below we are more interested in accurate zooxanthellar numbers for specific groups of planulae. Here, and given data from live planulae (Tables 1, 2, and 4), a much better estimate of true zooxanthellar number is obtained by multiplying those data by 2.2. We make that correction throughout the Discussion, below.

On a dry-weight basis, numbers of zooxanthellae in our unsorted planulae were similar to those reported for adult corals: $1.6 - 3.4 \times 10^5$ zooxanthellae mg⁻¹ planula (data in Table 1, except very-low-light planulae) *versus*, e.g., 2.8×10^5 zooxanthellae mg⁻¹ tissue reported for adult *Pocillopora edouxi* from Guam (Davies, 1984). In contrast, newly-released planulae from one colony of *P. damicornis* in Okinawa (Isomura and Nishihira, 2001) averaged only ~ one-seventh as many zooxanthellae planula⁻¹ as we observed. Although we used dry weight to

quantify planular size, whereas Isomura and Nishihira (2001) used linear measurements, planulae in the latter study were not obviously much smaller than ours. Thus, this difference in zooxanthellar numbers could be a real physiological difference between two geographically separated populations. Perhaps it relates to different kinds of zooxanthellae: Around Guam (Rowan, 2004 and unpublished) and elsewhere (Magalon et al., 2007), *P. damicornis* associates with at least four distinct types of *Symbiodinium*. All of the material in the present study had the higher-temperature adapted zooxanthellae referred to as *Symbiodinium* genotype *D* (*sensu* Rowan, 2004); identities of zooxanthellae in the planulae from Okinawa (Isomura and Nishihira, 2001) were not reported.

We further observed that zooxanthellar numbers varied substantially among newly-released planulae (Tables 2 and 3). The decimal that we termed pale planulae were comparable – in appearance, zooxanthellar number, and chl *a* content – to moderately-bleached corals (personal observations). The biological basis of this variation is unknown. No data suggest that pale planulae, or the zooxanthellae in pale planulae, were “unhealthy.” The observation that pale planulae weighed slightly less than dark planulae suggests the possibility that pale planulae were released when slightly less mature. This could explain their lower numbers of zooxanthellae if zooxanthellae proliferate disproportionately during the final phase of brooding (Edmunds et al., 2001).

Zooxanthellae often did proliferate in planulae after their release from corals (Tables 1, 3, and 4). Two observations imply that this proliferation was regulated. First, the greater proliferation of zooxanthellae in pale compared to dark planulae (Table 3) implies that proliferation was inversely proportional to population density. Similar observations have been made on adult corals, and attributed to greater availability of nutrients (Cook et al., 1994; Falkowski et al., 1993; Rees, 1991) or of space (Jones and Yellowlees, 1997) when fewer zooxanthellae are present. Assuming similar nutrient content and given only slightly different sizes for pale and dark planulae, both conditions predict the greater proliferation of zooxanthellae in pale planulae, which presumably was reflected in rapidly increasing $P_{\max}^{\text{net}}$ (Fig. 2).

The second observation implying regulated zooxanthellar proliferation in planulae came from an ammonium-enrichment experiment, discussed below.

Photophysiology of planulae of *P. damicornis*

Maximum net photosynthetic rates of newly-released, unsorted planulae (Table 1) were similar to those reported by Richmond (1987) for planulae of *P. damicornis* in Hawaii (average $P_{\max}^{\text{net}}$ of 5.4 nmol O₂ planula⁻¹ hr⁻¹). Richmond (1987) reported saturation-constant (I_k) values of 50 and 70 μmol photons m⁻² s⁻¹ PAR indicate greater photosynthetic efficiencies at low irradiance than we observed (I_k = 157–199 μmol photons m⁻² s⁻¹ PAR; Tables 1 and 3, Initial Condition; Table 2). Our higher values are more similar to those of shallow-water corals in general, *e.g.*, Chalker et al. (1983).

The overall phototrophic capacity of planulae, summarized as $P_{\max}^{\text{net}}$ normalized to oxygen uptake in the dark ($P_{\max}^{\text{net}}:R$), was generally lower than that of adult *P. damicornis*. Newly-released, unsorted planulae had $P_{\max}^{\text{net}}:R$ of 1.1 or 1.2 and had similar values after 17–19 days at medium or low light (data in Table 1). In contrast, $P_{\max}^{\text{net}}:R$ decreased to 0.73 at high light due to significantly decreased $P_{\max}^{\text{net}}$; a non-significant increase in R perhaps contributed (data in Table 1). A higher $P_{\max}^{\text{net}}:R$ of 1.7 was observed in one batch of newly-released dark planulae, but that declined to 1.2 after 20–22 days due to increased dark respiration (data in Table 3). Such age-related changes in physiology might reflect, in part, acclimation by planulae to a free-living state. In comparison, the average $P_{\max}^{\text{net}}:R$ of adult *P. damicornis* from Guam, measured at a similar temperature (28.3 C), was 1.5 (Rowan, 2004). Lower $P_{\max}^{\text{net}}:R$ of planulae *versus* adult corals might reflect different respiratory requirements (*e.g.*, motile

planulae *versus* sessile adults) or different constraints on O₂ and CO₂ diffusion to/from seawater (Patterson, 1992; Shick, 1990). Richmond (1987) reported lower values of $P_{\max}^{\text{net}}$: R for newly-released, unsorted planulae of *P. damicornis* (0.83 and 0.53) in Hawaii; perhaps those planulae had fewer zooxanthellae than ours had.

We examined photoacclimation in planulae because this phenomenon is well-studied in corals. Zooxanthellae in corals acclimated to lower irradiance typically contain more chl *a* and they capture photons more efficiently, as evident in a lower I_k (Chalker et al., 1983; Porter et al., 1984). We observed these phenotypes in planulae kept at 18% *versus* 57% ambient PAR (Table 1, Medium Light *versus* High Light), implying that planulae of *P. damicornis* photoacclimated like corals. Planulae kept at 11% ambient PAR (Table 1, Low Light) exhibited no ill effects, lost no more weight than planulae in the High-Light Experiment, and increased their numbers of zooxanthellae. In contrast, their siblings kept at 0.3% ambient PAR (Table 1, Very Low Light) had phenotypes attributable to chronic light insufficiency. These were the only planulae, in all experiments, that lost zooxanthellae and chl *a*. Similar results have been reported for light-deprived corals, *e.g.*, Hoegh-Guldberg and Smith, 1989; Stambler, 1998. We further observed that light-limited photosynthesis was not seriously impaired in very-low-light planulae, but they experienced photoinhibition at super-saturating irradiance; this probably reflects the loss of photoprotective mechanisms (Long et al., 1994; Walters, 2005) that very-low-light planulae would not have needed. Also, substantially decreased respiration by very-low-light planulae should have off-set the loss of metabolic energy resulting from limited photosynthesis, as proposed for corals (Anthony and Hoegh-Guldberg, 2003; Davies, 1980; McCloskey and Muscatine, 1984).

In summary, planulae of *P. damicornis* appeared to acclimate to different irradiance similarly to adult corals. Corals have an obvious need for long-term photoacclimation, as they occupy essentially permanent irradiance environments. Planulae, as plankton carried by mixing seas, might not. Or, as phototactic motile organisms (Kawaguti, 1941), planulae might maximize daily photosynthetic production by photoacclimating to lower irradiance and staying deeper when surface irradiance is high and moving upward when surface irradiance is lower, *e.g.*, Ault, 2000. It is also possible that photoacclimation by planulae is not an ecological strategy, but rather a consequence of a close functional similarity to corals.

Effects of ammonium on planulae of *P. damicornis*

We examined responses of planulae to ammonium because this phenomenon is also well-studied in corals and related symbioses. For corals and other cnidarians, the ability to assimilate ammonium efficiently is dependent on hosting zooxanthellae (Miller and Yellowlees, 1989; Roberts et al., 1999b). In corals, μM quantities of ammonium result in more zooxanthellae, presumably because zooxanthellar proliferation is normally suppressed by nitrogen limitation (Falkowski et al., 1993; Hoegh-Guldberg and Smith, 1989). For example, adult *P. damicornis* treated with 20 μM ammonium (for four weeks) or with 17 μM ammonium (for 12 weeks) had 114% or 200% (respectively) more zooxanthellae than non-treated corals had (Muller-Parker et al., 1994; Stimson, 1991). Our observation that planulae treated with 4 or 18 μM ammonium (for three weeks) had an average of 72% more zooxanthellae than controls (Table 4) indicates that the planulae assimilated ammonium, and implies that their zooxanthellae were regulated by nitrogen availability.

Planulae treated with 18 μM ammonium were exceptional in not losing a significant amount of dry-weight during three weeks (Table 4). This implies that ammonium either decreased catabolism or it promoted biosynthesis, or both. Ammonium did promote zooxanthellar growth (above), and based on the dry-weight of zooxanthellae from adult *P. damicornis*, zooxanthellae can account for ~ one-half of the greater dry-weight of high-ammonium *versus* control planulae. The remaining dry-weight difference may represent host-tissue growth. For example,

Muller-Parker et al. (1994) found that host protein of adult *P. damicornis* doubled when corals were maintained in 20 μM ammonium versus seawater for eight weeks; Roberts et al. (1999a) found that starved zooxanthellate anemones (*Anemonia viridis*) gained buoyant weight when provided with 20 μM ammonium whereas starved anemones in plain seawater lost weight. Ammonium-enhancement of host growth can be attributed to zooxanthellae, but the mechanisms are not certain (Wang and Douglas, 1998; Roberts et al., 1999b; Tanaka et al., 2006).

A possible third effect of ammonium on planulae was that 4 μM ammonium induced settlement and metamorphosis. This statement is provisional because we did not record settlement events but rather only numbers of planulae remaining at experiment's end. However, it was obvious that the low recovery of low-ammonium planulae (45%) compared to control or high-ammonium planulae (both 82%) was largely due to increased settlement. Low-ammonium planulae were the only planulae, in all experiments, that demonstrated substantial settlement behavior. A settlement-promoting effect of ammonium could be indirect, e.g., by promoting biofilms (Webster et al., 2004), although biofilms should not have been extensive in beakers cleaned every two days.

Or, ammonium might have stimulated settlement directly (Meadows and Campbell, 1972; Pawlik, 1992). Nitrogen is a scarce nutrient and colonies of *P. damicornis* might obtain considerable nitrogen from dissolved ammonium when available at $\geq 0.1 \mu\text{M}$ (Hoegh-Guldberg and Williamson, 1999). Seawater from the fore reef of Pago Bay, Guam (our control treatment) contains little ammonium, e.g., 0.22 μM total dissolved inorganic nitrogen (nitrate, nitrite, and ammonium combined; Marsh 1977), with ammonium undetectable (E. A. Matson, University of Guam Marine Laboratory, personal communication). Given that planulae of *P. damicornis* assimilated ammonium (above), perhaps they also evaluated its concentration and responded to an amount favorable to coral-colony growth – 4 μM – by settling. By this interpretation, 18 μM ammonium (the high-ammonium treatment) might not induce settlement if it implies eutrophic conditions to which corals are not well-adapted.

Bassim and Sammarco (2003) found that 20 μM ammonium harmed planulae of the Caribbean coral *Diploria strigosa*, which lack zooxanthellae. These authors suggested that zooxanthellae, by assimilating ammonium, might protect other species of planulae from ammonium toxicity. We did not test that hypothesis, but our observations support it, as we saw no ill effects of 18 μM ammonium on planulae of *P. damicornis*.

The energetics of planulae of *P. damicornis*

For photosynthesis to support overall energy needs of a symbiosis, photosynthetically-fixed carbon must be translocated to host tissue (Muscatine, 1990). Our estimate that ~ 70% of photosynthate was translocated is much larger than Richmond's (1982) estimate of 18% for planulae of *P. damicornis* in Hawaii. The discrepancy may result from methodological differences, from real differences in planulae from Guam versus Hawaii, or from the fact that we analyzed planulae aged 23-27 days whereas Richmond used newly-released planulae (< 5 days old; R. Richmond, University of Hawaii, personal communication). Any estimate of photosynthate translocation is approximate, and results from different studies are difficult to compare (Battey, 1992); our estimate of ~ 70% for planulae of *P. damicornis* is comparable to most values for adult corals (Battey, 1992; Davies, 1984; Muscatine, 1990).

We assume that planulae in our experiments had two sources of energy: catabolism of their own biomass and photosynthesis. Planulae of *P. damicornis* can metamorphose into floating, potentially feeding polyps (Richmond, 1985), but we saw none; any polyps would have found little food in their filtered seawater. We measured biomass catabolism as decrease in dry weight. Lost weight probably was lipid (Harii et al., 2007), which comprises ~ 70% by dry weight of

newly-released planulae and therefore represents a large energy reserve that non-feeding planulae utilize as they age (Richmond, 1987; Harii et al., 2007). Carbohydrate comprises only ~ 13% of dry-weight (Richmond, 1987) and thus should be comparatively insignificant. Under these assumptions, and further assuming that structural lipid is ~ 9% of dry weight (an estimate for adult *Pocillopora capitata*; Patton et al., 1977), unsorted planulae kept at high, medium, or low light (data in Table 1) utilized 37-41% of their storage lipid during 19 days; pale and dark planulae utilized 42% and 39% (respectively; data in Table 3) during 20-22 days.

These values do not support Richmond's assumption that planulae of *P. damicornis* catabolize biomass only to support net oxygen consumption (Richmond, 1987). For example, using a theoretical 19-day PAR treatment record for medium-light planulae (Appendix 2b), and a *P-I* relationship defined by the averages of initial and final values of dark respiration, of $P_{\max}^{\text{net}}$, and of I_k for these planulae (data in Table 1), we estimate a 19-day consumption of $0.81 \mu\text{mol O}_2 \text{ planula}^{-1}$. At an equivalence of 2 l of oxygen to 1 g of lipid respired (Schmidt-Nielsen, 1997), this equates with 9 μg of lipid, which accounts for only 35% of the weight these planulae lost. The same estimate for high-light planulae (data in Table 1) equates with 7 μg of lipid respired, or only 24% of their lost weight. Thus, energy budgets based on oxygen fluxes do not balance with observed dry-weight losses.

The unaccounted-for lost weight in planulae is analogous to the unaccounted-for photosynthetically-fixed carbon in energy budgets of adult corals (Davies, 1984; Muscatine, 1990). Davies (1984) attributed the missing carbon to mucus secretion. We noticed that planulae of *P. damicornis* secreted mucus, which, as an energy sink, could account for some of their lost weight. Another possible energy sink for planulae is motility. If planulae were relatively inactive during respirometry, measured oxygen fluxes would have underestimated their routine energy consumption, and thus the lipid needed to support it. Qualitative observations argue against this idea, however. Planulae of *P. damicornis* swim actively against currents (Kawaguti, 1941; our observations), and when stirring in the respirometry chamber was stopped, it was obvious that planulae were active. Typically, planulae appeared much less active under culture conditions. Thus it seems unlikely that respirometry underestimated routine energy consumption; it may have overestimated it.

The discrepancy between energy utilization implied by oxygen fluxes *versus* dry-weight losses means that contributions of photosynthesis to planular energetics can not be determined from these data alone. Another, albeit indirect, approach is to compare planulae that had different capacities for photosynthesis, while assuming that other components of their energy budgets did not differ. For example, planulae kept at 0.3% ambient PAR would have fixed negligible carbon compared to siblings kept at 11% PAR (Table 1; Very Low Light *versus* Low Light, respectively). Our calculations (Appendix 2e) imply that the benefit of photosynthesis to low-light planulae was equivalent to 11 μg of lipid respired. Adding this to the 24 μg of lipid these planulae probably did utilize (i.e., their dry-weight loss, not corrected for a probable increase in zooxanthellar mass; see Table 1) implies a total energy expenditure equivalent to ~ 35 μg of respired lipid, ~ 31% of which came from photosynthesis.

Less dramatic comparisons of planulae with different capacities for photosynthesis revealed no clear energetic benefit of photosynthesis. Lower realized photosynthetic rates at lower treatment irradiances imply 19-day oxygen consumption values of 0.62, 0.81, and $0.97 \mu\text{mol O}_2 \text{ planula}^{-1}$ for high-light, medium-light, and low-light planulae, respectively (data in Table 1; calculated as above). However, dry-weight losses were, if anything, inversely related to these estimates (29, 26, and 24 μg , respectively; Table 1). Similarly, pale and dark planulae lost the same dry-weight (26 μg ; Table 3) despite the estimated 40% lower net photosynthetic capacity of pale planulae (averaged over the experiment; data in Table 3). These observations suggest that planulae utilized stored energy at a rate that was largely independent of quantitative

differences in energy obtained *via* photosynthesis. This suggests that much of the budget for photosynthetically-obtained energy was “discretionary,” supporting activities that we did not measure, such as motility and mucus production, that varied depending on the amount of energy available.

Our observations do not suggest that zooxanthellar symbiosis in planulae of *P. damicornis* is primarily part of a life-history strategy to maximize larval duration *via* phototrophy. Were that so, planulae should have at least halved their dry-weight losses by restricting energy expenditures to those implied by measured oxygen fluxes. Other traits of these planulae imply a life-history that minimizes larval duration: They are large, brooded to maturity, and they do settle and metamorphose soon after release if substrate is available (Harrigan, 1972). In this context, zooxanthellae in planulae of *P. damicornis* should be most beneficial after planulae settle, when as juvenile corals they would immediately benefit from symbiosis with zooxanthellae that are well-adapted to their environment (Rowan, 2004).

For planulae that do not settle and instead end up in the plankton, photosynthesis might become more important as stored energy is depleted. Support for this idea comes from observations of photoacclimation (Table 1) and changes in zooxanthellar numbers (Tables 3 and 4) and in photosynthetic capacities (Table 3, Fig. 2), which show that physiology can change. Indeed, Richmond (1987) observed a 5% survival rate of planulae of *P. damicornis* after 103 days of culture. Moreover, zooxanthellae should allow planulae of *P. damicornis* to conserve nitrogen (Wang and Douglas, 1998) and to obtain new nitrogen by assimilating ammonium (above) and perhaps nitrate (Marubini and Davies, 1996; Grover et al., 2003), if they encounter it. Being motile, planulae might migrate diurnally between shallower and deeper habitats to take advantage of both light and dissolved inorganic nitrogen, respectively, as some phytoplankton do (Eppley et al., 1968; Lieberman and Shilo, 1994).

Using planulae of *P. damicornis* to study symbiosis

Our observations indicate that zooxanthellar symbiosis in planulae is broadly similar to that in adult *P. damicornis*. While this is not surprising, it also could not have been assumed *a priori*, given that larval and adult stages of marine invertebrates tend to differ. For some research on symbiosis, planulae of *P. damicornis* might offer advantages compared to working with corals: Planulae do well under static conditions, which facilitates treatments (*e.g.*, addition of nutrients, inhibitors, or potential toxins; changed salinity; changed temperature). Because planulae are small, morphologically simple, and unattached to substrate, they can be exposed to relatively well-defined irradiance. Lacking skeletons, planulae can be processed for biochemical studies quickly and quantitatively. Pale *versus* dark planulae provide quantitative variation in host:symbiont biomass, among siblings, without stressful pre-treatment.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

- Anthony KRN, Hoegh-Guldberg O. Variation in coral photosynthesis, respiration and growth characteristics in contrasting light microhabitats: an analogue to plants in forest gaps and understoreys? *Funct. Ecol* 2003;17:246–259.
- Ault TR. Vertical migration by the marine dinoflagellate *Prorocentrum triestinum* maximises photosynthetic yield. *Oecologia* 2000;125:466–475.
- Bassim KM, Sammarco PW. Effects of temperature and ammonium on larval development and survivorship in a scleractinian coral (*Diploria strigosa*). *Mar. Biol* 2003;142:241–252.
- Batley, JF. Carbon metabolism in zooxanthellae-coelenterate symbioses. In: Reisser, W., editor. *Algae and symbioses: plants, animals, fungi, viruses, interactions explored*. Biopress Limited; Bristol, UK: 1992. p. 153–187.
- Bohonak AJ. Dispersal, gene flow, and population structure. *Q. Rev. Biol* 1999;75:21–45. [PubMed: 10081813]
- Chalker BE, Dunlap WC, Oliver JK. Bathymetric adaptations of reef-building corals at Davies Reef, Great Barrier Reef, Australia. II. Light saturation curves for photosynthesis and respiration. *J. Exp. Mar. Biol. Ecol* 1983;73:37–56.
- Cook CB, Muller-Parker G, Orlandini CD. Ammonium enhancement of dark carbon fixation and nitrogen limitation in zooxanthellae symbiotic with the reef corals *Madracis mirabilis* and *Montastrea annularis*. *Mar. Biol* 1994;118:157–165.
- Davies PS. Respiration in some Atlantic reef corals in relation to vertical distribution and growth form. *Biol. Bull* 1980;158:187–194.
- Davies PS. The role of zooxanthellae in the nutritional energy requirements of *Pocillopora eydouxi*. *Coral Reefs* 1984;2:181–186.
- Edmunds PJ, Gates RD, Gleason DF. The biology of larvae from the reef coral *Porites astreoides*, and their response to temperature disturbances. *Mar. Biol* 2001;139:981–989.
- Eppley RW, Holm-Hansen O, Strickland JDH. Some observations on the vertical migration of dinoflagellates. *J. Phycol* 1968;4:333–340.
- Falkowski PG, Dubinsky Z, Muscatine L, McCloskey L. Population control in symbiotic corals: ammonium ions and organic materials maintain the density of zooxanthellae. *Bioscience* 1993;43:606–611.
- Games PA, Howell JF. Pairwise multiple comparison procedures with unequal N's and/or variances: A Monte Carlo study. *J. Educ. Stat* 1976;1:113–125.
- Garside C, Hull G, Murry S. Determination of submicromolar concentrations of ammonia in natural waters by a standard addition method using a gas-sensing electrode. *Limnol. Oceanogr* 1978;23:1073–1076.
- Grover R, Maguer JF, Allemand D, Ferrier-Pages C. Nitrate uptake in the scleractinian coral *Stylophora pistillata*. *Limnol. Oceanogr* 2003;48:2266–2274.
- Harrigan, JF. Ph.D. thesis. University of Hawaii; Honolulu: 1972. The planula larva of *Pocillopora damicornis*: lunar periodicity of swarming and substratum selection behavior.
- Harii S, Nadaoka K, Yamamoto M, Iwao K. Temporal changes in settlement, lipid content and lipid composition of larvae of the spawning hermatypic coral *Acropora tenuis*. *Mar. Ecol. Prog. Ser* 2007;346:89–96.
- Harrison, P.; Wallace, CC. Reproduction, dispersal, and recruitment of scleractinian corals. In: Dubinsky, Z., editor. *Ecosystems of the world, volume 25: coral reefs*. Elsevier; New York: 1990. p. 133–207.
- Hoegh-Guldberg O, Smith GJ. Influence of the population density of zooxanthellae and supply of ammonium on the biomass and metabolic characteristics of the reef corals *Seriatopora hystrix* and *Stylophora pistillata*. *Mar. Ecol. Prog. Ser* 1989;57:173–186.
- Hoegh-Guldberg O, Williamson J. Availability of two forms of dissolved nitrogen to the coral *Pocillopora damicornis* and its symbiotic algae. *Mar. Biol* 1999;133:561–570.
- Isomura N, Nishihira M. Size variation of planulae and its effect on the lifetime of planulae in three pocilloporid corals. *Coral Reefs* 2001;20:309–315.
- Jassby AD, Platt T. Mathematical formulation of the relationship between photosynthesis and light for phytoplankton. *Limnol. Oceanogr* 1976;21:540–547.

- Jeffery SW, Humphrey GF. New spectrophotometric equations for determining chlorophylls *a*, *b*, *c*₁ and *c*₂ in higher plants, algae and natural phytoplankton. *Biochem. Physiol. Pflanz* 1975;167:191–194.
- Jones RJ, Yellowlees D. Regulation and control of intracellular algae (= zooxanthellae) in hard corals. *Philos. Trans. R. Soc. Lond., B* 1997;352:457–468.
- Kawaguti S. On the physiology of reef corals V. Tropisms of coral planulae, considered as a factor of distribution of the reefs. *Palao. Trop. Biol. Stn. Stud* 1941;2:319–328.
- Lieberman OS, Shilo M. The physiological ecology of a freshwater dinoflagellate bloom population: vertical migration, nitrogen limitation, and nutrient uptake kinetics. *J. Phycol.* 1994;30:964–971.
- Long SP, Humphries S, Falkowski PG. Photoinhibition of photosynthesis in nature. *Annu. Rev. Plant. Physiol. Plant. Mol. Biol* 1994;45:633–662.
- Magalon H, Flot J-F, Baudry E. Molecular identification of symbiotic dinoflagellates in Pacific corals in the genus *Pocillopora*. *Coral Reefs* 2007;26:551–558.
- Marsh JA. Terrestrial inputs of nitrogen and phosphorus on fringing reefs of Guam. *Proc. 3rd Intl. Coral. Reef. Symp* 1977;1:331–336.
- Marubini F, Davies PS. Nitrate increases zooxanthellae population density and reduces skeletogenesis in corals. *Mar. Biol* 1996;127:319–328.
- Matson EA. Nutrient chemistry of the coastal waters of Guam. *Micronesica* 1991;24:109–135.
- McCloskey LR, Muscatine L. Production and respiration in the Red Sea coral *Stylophora pistillata* as a function of depth. *Proc. R. Soc. Lond. B Biol. Sci* 1984;222:215–230.
- Meadows PS, Campbell JJ. Habitat selection by aquatic invertebrates. *Adv. Mar. Biol* 1972;10:271–382.
- Mileikovsky SA. Types of larval development in marine bottom invertebrates, their distribution and ecological significance: a re-evaluation. *Mar. Biol* 1971;10:193–213.
- Miller DJ, Yellowlees D. Inorganic nitrogen uptake by symbiotic marine cnidarians: a critical review. *Proc. R. Soc. Lond. B Biol. Sci* 1989;237:109–125.
- Muller-Parker G, McCloskey LR, Hoegh-Guldberg O, McAuley PJ. Effect of ammonium enrichment on animal and algal biomass of the coral *Pocillopora damicornis*. *Pac. Sci* 1994;48:273–283.
- Muscatine, L. The role of symbiotic algae in carbon and energy flux in reef corals. In: Dubinsky, Z., editor. *Ecosystems of the world, volume 25: coral reefs*. Elsevier; New York: 1990. p. 75–87.
- Patterson MR. A chemical-engineering view of cnidarian symbioses. *American Zoologist* 1992;32:566–582.
- Patton JS, Abraham S, Benson AA. Lipogenesis in the intact coral *Pocillopora capitata* and its isolated zooxanthellae: Evidence for a light-driven carbon cycle between symbiont and host. *Mar. Biol* 1977;44:235–247.
- Pawlik, JR. Induction of marine invertebrate larval settlement-evidence for chemical cues. In: Paul, VJ., editor. *Explorations in chemical ecology: ecological roles of marine natural products*. Cornell University Press; Ithaca: 1992. p. 189–236.
- Porter JW, Muscatine L, Dubinsky Z, Falkowski PG. Primary production and photoadaptation in light- and shade-adapted colonies of the symbiotic coral *Stylophora pistillata*. *Proc. R. Soc. Lond. B Biol. Sci* 1984;222:161–180.
- Rees TAV. Are symbiotic algae nutrient-deficient? *Proc. R. Soc. Lond. B Biol. Sci* 1991;243:227–233.
- Richmond RH. Energetic considerations in the dispersal of *Pocillopora damicornis* (Linnaeus) planulae. *Proc. 4th Intl. Coral Reef Symp* 1982;2:153–156.
- Richmond RH. Reversible metamorphosis in coral planula larvae. *Mar. Ecol. Prog. Ser* 1985;22:181–185.
- Richmond RH. Energetics, competency, and long-distance dispersal of planula larvae of the coral *Pocillopora damicornis*. *Mar. Biol* 1987;93:527–533.
- Richmond RH, Jokiel PL. Lunar periodicity in larva release in the reef coral *Pocillopora damicornis* at Enewetak and Hawaii. *Bull. Mar. Sci* 1984;34:280–287.
- Roberts JM, Davies PS, Fixter LM. Symbiotic anemones can grow when starved: nitrogen budget for *Anemonia viridis* in ammonium-supplemented seawater. *Mar. Biol* 1999a;133:29–35.
- Roberts JM, Davies PS, Fixter LM, Preston T. Primary site and initial products of ammonium assimilation in the symbiotic sea anemone *Anemonia viridis*. *Mar. Biol* 1999b;135:223–236.
- Rowan R. Thermal adaptation in reef coral symbionts. *Nature* 2004;430:742. [PubMed: 15306800]

- Schmidt-Nielsen, K. Animal physiology. 5th edn.. Cambridge University Press; Cambridge, UK: 1997.
- Schwarz JA, Krupp DA, Weis VM. Late larval development and onset of symbiosis in the scleractinian coral *Fungia scutaria*. Biol. Bull 1999;196:70–79.
- Shick JM. Diffusion limitation and hyperoxic enhancement of oxygen-consumption in zooxanthellate sea-anemones, zoanthids, and corals. Biol. Bull 1990;179:148–158.
- Sokal, RR.; Rohlf, JF. Biometry. 3rd ed.. WH Freeman; San Francisco: 1995.
- Stambler N. Effects of light intensity and ammonium enrichment on the hermatypic coral *Stylophora pistillata* and its zooxanthellae. Symbiosis 1998;24:127–146.
- Stimson JS. The temporal pattern and rate of release of zooxanthellae from the reef coral *Pocillopora damicornis* (Linnaeus) under nitrogen-enrichment and control conditions. J. Exp. Mar. Biol. Ecol 1991;153:63–74.
- Strathmann RR. Why does a larva swim so long? Paleobiology 1980;6:373–376.
- Tanaka Y, Miyajima T, Koike I, Hayashibara T, Ogawa H. Translocation and conservation of organic nitrogen within the coral-zooxanthella symbiotic system of *Acropora pulchra*, as demonstrated by dual isotope-labeling techniques. J. Exp. Mar. Biol. Ecol 2006;336:110–119.
- Titlyanov EA, Titlyanova TV, Loya Y, Yamazato K. Degradation and proliferation of zooxanthellae in planulae of the hermatypic coral *Stylophora pistillata*. Mar. Biol 1998;130:471–477.
- Trench RK. Microalgal-invertebrate symbioses: a review. Endocytobiosis Cell Res 1993;9:135–175.
- van Oppen MJH. *In vitro* establishment of symbiosis in *Acropora millepora* planulae. Coral Reefs 2001;20:200.
- Walters RG. Towards and understanding of photosynthetic acclimation. J. Exp. Bot 2005;56:435–447. [PubMed: 15642715]
- Wang J, Douglas AE. Nitrogen recycling or nitrogen conservation in an alga-invertebrate symbiosis? J. Exp. Biol 1998;201:2445–2453. [PubMed: 9679106]
- Webster NS, Smith LD, Heyward AJ, Watts JEM, Webb RI, Blackall LL, Negri AP. Metamorphosis of a Scleractinian coral in response to microbial biofilms. Appl. Environ. Microbiol 2004;70:1213–1221. [PubMed: 14766608]

Fig. 1.

Chlorophyll *a* contents of individual pale (hatched bars) versus dark (solid bars) planulae of *Pocillopora damicornis*. a. Newly-released ($\leq$ four days old) planulae; the vertical arrow indicates the average chlorophyll *a* planula⁻¹ of the unsorted planulae from which these pale and dark planulae were obtained. b. As in a, after 18 days at 18% ambient irradiance. c. As in a, after 18 days at 57% ambient irradiance.

Fig. 2. Changes in rates of maximum net photosynthesis ($P_{\max}^{\text{net}}$) in pale (open circles) and in dark (filled circles) planulae of *Pocillopora damicornis* during 22 days under 57% sunlight. Each datum represents a different replicate sample. Slopes of the two linear regressions are not zero (ancova, $P < 0.05$) and are different (ancova, $P = 0.021$).

Characteristics of planulae of *Pocillopora damicornis* when newly-released and after culture at different light regimes

Table 1

Parameter	High-Light Experiment (n = 7)			Low-Light Experiment (n = 8)		
	Initial Condition	High Light	Medium Light	Initial Condition	Low Light	Very Low Light
Dry weight (µg)	115 ± 9 ^{a,b}	86 ± 7 ^a	89 ± 7 ^b	101 ± 7 ^a	77 ± 4 ^a	69 ± 4 ^a
Zoox (10 ³ cells)	11.0 ± 1.3	13.0 ± 1.5	15.0 ± 4.1	7.3 ± 1.0 ^a	12.0 ± 2.2 ^a	4.7 ± 0.9 ^a
Chl <i>a</i> (ng/planula)	62 ± 5 ^a	60 ± 7 ^b	91 ± 17 ^{a,b}	41 ± 9 ^a	75 ± 13 ^a	22 ± 4 ^a
Chl <i>a</i> (pg/cell)	5.7 ± 0.6	4.8 ± 0.9 ^a	6.7 ± 1.7 ^a	5.6 ± 0.9	6.3 ± 1.0 ^a	5.1 ± 0.97 ^a
Chl <i>a:c2</i>	3.0 ± 0.9	3.8 ± 1.6	3.3 ± 0.9	3.9 ± 0.5	5.2 ± 1.2	4.9 ± 2.0
Dark respiration	-6.1 ± 0.9	-6.6 ± 0.6	-5.8 ± 1.1	-4.8 ± 0.3 ^a	-5.0 ± 1.6 ^b	-2.3 ± 0.5 ^{a,b}
<i>P</i> _{net}	6.8 ± 0.9 ^a	4.8 ± 1.1 ^a	6.0 ± 1.3	5.8 ± 0.6 ^a	5.5 ± 1.2 ^b	1.2 ± 0.9 ^{a,b}
<i>I</i> _k	182 ± 18	211 ± 30 ^a	160 ± 17 ^a	199 ± 29	177 ± 25	171 ± 45

Planulae were analyzed in two separate experiments (High-Light and Low-Light), 10-100 hr after release from parent corals (Initial Condition) and after an additional 17-19 days at 57% (High Light), 18% (Medium Light), 11% (Low Light), or 0.3% (Very Low Light) of ambient sunlight. Data are means ± one standard deviation. Dry weight is per planula; Zoox, number of zooxanthellae planula⁻¹; Chl *a*, chlorophyll *a* planula⁻¹ or zooxanthella⁻¹ (cell); Chl *a:c2*, mass ratio of chlorophylls *a* and *c2*; Dark respiration, oxygen consumption (indicated as negative oxygen production) in darkness in nmol O₂ planula⁻¹ hr⁻¹; *P*_{net}, light-saturated net photosynthetic rate in nmol O₂ planula⁻¹ hr⁻¹; *I*_k, saturation constant of net photosynthesis in µmol photons m⁻² s⁻¹ PAR

^aWithin an experiment, means that share a superscript are significantly different (Tukey's HSD test or Games and Howell method, *P* < 0.05)

^bWithin an experiment, means that share a superscript are significantly different (Tukey's HSD test or Games and Howell method, *P* < 0.05)

Table 2Characteristics of newly-released pale *versus* dark planulae from four colonies of *Pocillopora damicornis*

<i>Parameter</i>	Type of Planula		<i>P-value</i>
	<i>Pale</i>	<i>Dark</i>	
Dry weight (μg)	115 $\pm$ 16	138 $\pm$ 11	0.04
Zoox (10^3 cells)	2.8 $\pm$ 1.0	8.1 $\pm$ 2.4	0.03
Chl <i>a</i>	17 $\pm$ 7	55 $\pm$ 18	0.008
Chl <i>a</i> (pg/cell)	5.3 $\pm$ 1.7	5.8 $\pm$ 1.3	0.6
Dark respiration	-5.2 $\pm$	-6.5 $\pm$	0.01
$P_{\text{max}}^{\text{net}}$	-0.4 $\pm$	4.3 $\pm$ 2.4	0.0001
<i>I</i> _k	181 $\pm$ 10	189 $\pm$ 14	0.4

Planulae were 10-100 hr old; pale and dark planulae represent the least-pigmented decimal and the most-pigmented decimal (respectively) of one brood, as determined subjectively. Data are means ($n = 4$) $\pm$ one standard deviation. Dry weight is per planula; Zoox, number of zooxanthellae planula⁻¹; Chl *a*, chlorophyll *a* planula⁻¹ or zooxanthella⁻¹ (cell); Dark respiration, oxygen consumption in darkness (indicated as negative oxygen production) in nmol O₂ planula⁻¹ hr⁻¹; $P_{\text{max}}^{\text{net}}$, light-saturated net photosynthetic rate in nmol O₂ planula⁻¹ hr⁻¹; *I*_k, saturation constant of net photosynthesis in $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ PAR. *P-value* is the probability that pale and dark planulae were not different (paired *t*-test, two-tailed, $n = 4$)

Table 3

Characteristics of “pale” and of “dark” planulae of *Pocillopora damicornis* when newly-released and after three weeks of culture

Parameter	Initial Condition		Three Weeks	
	Pale	Dark	Pale	Dark
Dry weight (μg)	102 ± 6^a	110 ± 5^a	76 ± 3^a	84 ± 4^a
Zoox (10^3 cells)	$7.1 \pm 1.5^{a,b}$	23.2 ± 1.9^a	23.1 ± 3.7^b	$30.4 \pm 4.9^{a,b}$
Dark respiration	$-4.0 \pm 0.5^{a,c}$	$-4.4 \pm 0.6^{d,d}$	$-6.7 \pm 0.8^{a,d}$	$-7.4 \pm 0.5^{b,c}$
$P_{\max}^{\text{net}}$	$1.5 \pm 0.8^{a,b,c}$	7.4 ± 1.8^a	8.2 ± 0.6^b	8.7 ± 0.8^c
I_k	$157 \pm 27^{a,c}$	$164 \pm 11^{b,d}$	$282 \pm 31^{a,d}$	$284 \pm 18^{b,c}$

Newly-released planulae (Initial Condition) were 10-100 hr old; pale and dark planulae represent the least-pigmented decimal and the most-pigmented decimal (respectively) of an unsorted newly-released population, as determined subjectively. Planulae were cultured for 20-22 days (Three Weeks) at 57% ambient PAR. Dry weight, per planula; Zoox, number of zooxanthellae per planula; Dark respiration, oxygen consumption in darkness in $\text{nmol O}_2 \text{ planula}^{-1} \text{ hr}^{-1}$; $P_{\max}^{\text{net}}$, light-saturated net photosynthetic rate in $\text{nmol O}_2 \text{ planula}^{-1} \text{ hr}^{-1}$; I_k , saturation constant of net photosynthesis in $\mu\text{mol photons m}^{-2} \text{ s}^{-1} \text{ PAR}$

^a Means that share a superscript are significantly different (Tukey's HSD test or Games and Howell method, $P < 0.05$)

^b Means that share a superscript are significantly different (Tukey's HSD test or Games and Howell method, $P < 0.05$)

^c Means that share a superscript are significantly different (Tukey's HSD test or Games and Howell method, $P < 0.05$)

^d Means that share a superscript are significantly different (Tukey's HSD test or Games and Howell method, $P < 0.05$)

Table 4Characteristics of planulae of *Pocillopora damicornis* cultured with or without added ammonium

<i>Parameter</i>	<i>Initial Condition</i>	<i>Control</i>	<i>Low NH₄⁺</i>	<i>High NH₄⁺</i>
Dry weight (μg)	88 ± 5 ^a	76 ± 7 ^{a,b}	(80 ± 8)	85 ± 7 ^b
Zoox (10 ³ cells)	9.9 ± 1.7 ^{a,b}	10.1 ± 1.4 ^{c,d}	17.7 ± 3.0 ^{a,b}	17.0 ± 4.2 ^{b,d}
Chl <i>a</i> (10 ⁻² μg/planula)	6.9 ± 0.9 ^{a,b}	7.0 ± 1.4 ^{c,d}	12.4 ± 1.2 ^{a,c}	12.4 ± 1.6 ^{b,d}
Chl <i>a</i> (10 ⁻⁶ μg/cell)	7.0 ± 0.8	7.0 ± 1.0	7.1 ± 1.0	7.6 ± 1.7

Planulae were analyzed when 4-6 days old (*Initial Condition*) and after culture for another 21-23 days in seawater only (*Control*), seawater plus 4 μM NH₄Cl (*Low NH₄⁺*), and seawater plus 18 μM NH₄Cl (*High NH₄⁺*); *n* = 10 in each group except datum in parentheses is *n* = 4 (these data not included in statistical analysis). Dry weight, per planula; Zoox, number of zooxanthellae per planula; Chl *a*, chlorophyll *a* per planula or per zooxanthella (cell).

^a Means that share a superscript are significantly different (Tukey's HSD test, *P* < 0.05)

^b Means that share a superscript are significantly different (Tukey's HSD test, *P* < 0.05)

^c Means that share a superscript are significantly different (Tukey's HSD test, *P* < 0.05)

^d Means that share a superscript are significantly different (Tukey's HSD test, *P* < 0.05)