Photoacclimation and the effect of the symbiotic environment on the photosynthetic response of symbiotic dinoflagellates in the tropical marine hydroid *Myrionema amboinense*

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Abstract

Symbiotic dinoflagellates of the genus *Symbiodinium* and residing in the tropical hydroid *Myrionema amboinense* acclimate to low photon flux associated with low light 'shade' environments by increasing the amount of photosynthetic pigments per algal cell. The photosynthetic light intensity (PI) curves suggested that the low-light pigment response involved an increase in the number of photosynthetic units (PSU) in the chloroplast in addition to any increases in PSU size. Comparisons of light-dependent portion of the *P–I* curves of freshly isolated zooxanthellae (FIZ) with those from symbionts within the intact animal suggest that the host cell environment reduced average light levels reaching the symbiotic algae by more than half. This phenomenon may protect the algae from photobleaching of pigments and/or photoinhibition of photosynthesis at high light intensities present in shallow water habitats. In addition, maximum photosynthesis (P_{max}) of symbionts removed from the host cell was higher than that recorded from dinoflagellates in the intact association, suggesting that the availability of carbon dioxide for photosynthesis may be limited in the intact hydroid. Shaded polyps contained fewer zooxanthellae and had less tissue biomass (measured as protein) than unshaded polyps. However symbionts from shaded polyps acclimated to the low light intensities by increasing chlorophyll levels and photosynthetic rates. The higher photosynthetic rates may have resulted from increased availability of carbon dioxide associated with lower symbiont density. Calculations of the contribution of zooxanthellae carbon to the host animal's respiratory demand (CZAR) showed that zooxanthellae from shaded polyps living in the field potentially provide about the same amount of carbon to their host as zooxanthellae from polyps living in the field in unshaded high light intensities.

Keywords: Photoacclimation; Symbiotic dinoflagellates; Zooxanthellae

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1. Introduction

Reef corals and other invertebrates containing symbiotic dinoflagellates of the genus *Symbiodinium* (hereafter also referred to as zooxanthallae) often inhabit a range of light habitats. Some of these hosts occur over a wide depth gradient, experiencing changes in both light quantity and light quality, while others may be found at one depth in either shade or exposed to full sunlight where only quantity of light changes (Trench, 1993; Iglesias-Prieto and Trench, 1997a). The ability of zooxanthellae to adapt to differences in light quality and quantity appears to be of selective advantage to the intact association in terms of maintaining optimal levels of photosynthesis and photosynthetically fixed carbon translocated from alga to animal, the failure of which might ultimately limit distribution of the coral (i.e., Muscatine, 1980; Fabricius and Klumpp, 1995; Iglesias-Prieto and Trench, 1997b).

Acclimation of algal symbioses to low light conditions may involve changes in algal density within a host. Although an increase in algal density may result in increased photon capture, most symbiotic cnidarians respond to reduced photon flux at the same depth (i.e., shading) by maintaining relatively constant densities of zooxanthellae while increasing pigment content (Houck, 1978; Svoboda and Porrmann, 1980; Falkowski and Dubinsky, 1981; fed anemones *Aiptasia pulchella* in Porter et al., 1984; Muller-Parker, 1985; Harland and Davies, 1994; Helmuth et al., 1997). Only in the coral *Montipora verrucosa* (Houck, 1978) and laboratory-starved and field collected *Aiptasia pulchella* and possibly *Anthopleura elegantissima* (Muller-Parker, 1985, 1987; Saunders and Muller-Parker, 1997) were symbiont densities higher in reduced light. In contrast, most corals exhibit a decrease in the density of zooxanthellae with depth (four out of six species in Drew, 1972; Dustan, 1979; McCloskey and Muscatine, 1984; Battey and Porter, 1988; Kaiser et al., 1993; Masuda et al., 1993; one of two species in Leletkin et al., 1996, Fitt et al., 2000). McCloskey and Muscatine (1984) have suggested that reduced densities of symbionts at depth may minimize algal self-shading in low light, thereby increasing efficiency of light capture. However, coral hosts also decrease their respiratory biomass with depth (i.e., Davies, 1977; McCloskey and Muscatine, 1984; Porter et al., 1984; Battey and Porter, 1988; Masuda et al., 1993; Fitt et al., 2000), such that algal densities expressed as a unit of tissue biomass often stay constant over their depth range in a fashion consistent with the concept of density regulation (Fitt and Cook, 2000).

Acclimation to low light conditions may also involve changes in pigment composition of symbiotic dinoflagellates, leading to increased light-capturing ability. Zooxanthellae experiencing reduced photon flux density (PFD) associated with shade, rather than shifts in light quality accompanying increased depth, typically have more chlorophyll *a* and accessory pigments (chlorophyll c_2 and peridinin) per cell than those in higher light (Redjalde, 1976; Titlyanov et al., 1980; Falkowski and Dubinsky, 1981; Chang et al., 1983; Muller-Parker, 1984; Harland and Davies, 1994; Iglesias-Prieto and Trench, 1994; Helmuth et al., 1997). However, pigments in zooxanthellae from *Aiptasia diaphana* (Svoboda and Porrmann, 1980) and *Montipora verrucosa* (Houck, 1978; Chang et al., 1983) showed similar levels of chlorophyll *a* when moved from a high to a low light

intensity, indicating little photoadaptive ability of some genotypes of symbiotic dinoflagellates.

Studies of changes in pigment composition as a function of depth have yielded more variable results. Most have documented increases in chlorophylls *a* and c_2 per zooxanthella in corals with increasing depth (Titlyanov et al., 1980; McCloskey and Muscatine, 1984; Porter et al., 1984; Warnock, 1985; Battey and Porter, 1988; Kaiser et al., 1993; Masuda et al., 1993; Leletkin et al., 1996), while zooxanthellae pigment composition in other corals appears not to change with depth (*Agaricia agaricites* and *Acropora palmata* in Warnock, 1985; *Pocillopora verrucosa* in Titlyanov et al., 1980). Finally, photosynthetic pigments in *Montipora foliosa* decreased between the depths of 45 and 55 m (Titlyanov et al., 1980). Hosts harboring more than one type of symbiontic dinoflagellate (i.e., Rowan and Knowlton, 1995) have been hypothesized to 'switch' to more tolerant symbionts in different habitats (i.e., Buddemeier and Fautin, 1993).

Zooxanthellae may increase photon capture via change in pigmentation by increasing numbers of photosynthetic units (PSU) in their chloroplasts as well as by increasing the size of the PSU (see Iglesias-Prieto and Trench, 1994). Early interpretation of photoacclimation in phytoplankton (Falkowski and Owens, 1980; Prezelin, 1981) and zooxanthellate corals (Falkowski and Dubinsky, 1981) held that either the number of PSU or size of PSU changed. It is now known that 'both strategies are found within algal classes' (Falkowski and LaRoche, 1991). An example of both mechanisms from the cnidarian literature is the zooxanthellate anemone *Aiptasia pulchella* (Chang et al., 1983; Muller-Parker, 1985). Other studies on zooxanthellae symbioses have documented evidence of at least one of these mechanisms of photoacclimation (i.e., Dustan, 1979; Titlyanov et al., 1980, Zvalinskii et al., 1980; Masuda et al., 1993; Harland and Davies, 1994; Iglesias-Prieto and Trench, 1994).

Photoacclimation of zooxanthellae in their host results in higher carbon fixation rates at subsaturating light conditions, with acclimation capabilities of the specific zooxanthellae correlated with the ecological distribution of their respective hosts (Chang et al., 1983; Trench, 1993; Iglesias-Prieto and Trench, 1997b). A number of studies have now demonstrated that daily carbon fixation in deeper corals is often comparable to that of shallow water morphs when expressed per unit surface area or tissue biomass (Barnes and Taylor, 1973; Wethey and Porter, 1976a,b; McCloskey and Muscatine, 1984; Battey and Porter, 1988; Masuda et al., 1993; Fabricius and Klumpp, 1995; Leletkin et al., 1996).

The present study addresses mechanisms by which symbiotic dinoflagellates residing in the shallow-water hydroid *Myrionema amboinense* acclimate to low light intensities associated with 'shade' habitats. This particular algal symbiosis has become a useful model in illustrating interactions between symbiotic dinoflagellates and marine cnidarian hosts at the cellular level (Cook and Fitt, 1990; Trench, 1993; McAuley and Cook, 1994; Fitt and Cook, 2000; Fitt, 2000). Calculations of the contribution of photosynthetically fixed carbon from zooxanthellae in polyps from shaded and full natural light conditions to the host hydroid's respiratory demand are made, and most importantly, the effect of the symbiotic habitat on the photosynthetic performance of the zooxanthellae is addressed.

2. Materials and methods

2.1. Maintenance of experimental organisms

Colonies of *Myrionema amboinense* were collected during the winter from the backreef lagoon adjacent to the Discovery Bay Marine Laboratory on the north coast of Jamaica. Specimens were either used the same day for experiments or maintained in the laboratory as described below. Colonies collected fresh from the field experienced natural day:night light cycles of approximately 13:11 h light:dark. Exposed or 'high-light' colonies were collected from the middle of a lagoon (1.3–1.5 m deep) or shallow rocks (0.2 m), where they were unshaded from surrounding mangroves during the middle of the day. Maximum light intensities, measured with a Li-Cor flat cosine-corrected sensor at noon on a cloudless January day at 1 m depth, reached 1500–1900 μmol m^{-2} s^{-1}. While there were several hydroid colonies around the edge of the lagoon that were at least partially shaded during the day, it it was not possible to determine if they were continuously shaded. Therefore 'shaded' colonies from high light habitats in the lagoon (1.5 m deep) were covered for 4 weeks with a 0.25-m^2 piece of black plastic placed 0.3 m above the colonies such that they never received direct sunlight. There was no obvious effect of the black plastic shading on water circulation or temperature in the otherwise calm lagoon. Maximum light intensities measured horizontally from under the shade on a cloudless day at noon were approximately 300 μmol m^{-2} s^{-1}. Ambient seawater temperatures in January in Jamaica were 26–27°C.

Some colonies of *M. amboinense* were collected from the lagoon (1.5 m) and maintained in a laboratory wet table under 14:10-h light:dark cycle (daylight fluorescent, 35 μmol m^{-2} s^{-1}) at 27±1°C for 4 weeks before experiments were conducted. These animals were fed *Artemia* nauplii once each day by placing the hydroid colonies in a petri dish containing the live food for 1 h, then placing the colonies back in the wet table.

2.2. Oxygen flux measurements

Oxygen flux in filtered (0.45 μm) seawater (FSW) was measured either from intact polyps or from suspensions of algae. Freshly isolated symbionts (FIZ) were obtained by macerating 50–100 polyps in a glass tissue grinder with FSW. The resulting slurry was filtered through three layers of cheesecloth to remove any large pieces of animal tissue, then repeatedly centrifuged ($1500\times g$) and washed with FSW until little animal tissue was seen by microscopic observation. The remaining algal pellet was resuspended in FSW and density adjusted to $0.5–1.0\times10^6$ cells ml^{-1}.

Dissolved oxygen concentration was determined for seawater surrounding intact polyps and freshly isolated algal symbionts using a YSI Model 53 oxygen electrode. The electrode was connected to a chart recorder. A slide projector halogen lamp was used as the light source. Approximately eight to 10 polyps, each with approximately 8 mm of stalk, were held in place in glass chambers containing 5 ml of FSW with an O-ring. The rubber ring pinned the end of the stalks against the side of the chamber but did not interfere with water circulation around the electrode, generated by a magnetic stir bar.

For FIZ measurements the chambers contained 3 ml of algal suspension. The chambers were kept in a water jacketed (26±1°C) glass electrode chamber. Rates of oxygen consumption or evolution were recorded continuously on the chart recorder over 5–30-min intervals. Light intensity was varied between 0 (dark) and 2500 μmol m^{-2} s^{-1} by using neutral density screening and adjusting the distance from the slide projector. Dark respiration was measured by covering the water-jacketed chambers with aluminum foil. Oxygen levels under experimental conditions were never below 80% of saturation. The hyperbolic tangent function was used to analyze the *P* versus *I* curves (i.e., Chalker et al., 1983).

2.3. Algal and animal biomass parameters

After measurements of oxygen flux, the samples were removed from the glass chambers. Intact animals were homogenized in FSW in a glass tissue grinder before analysis. Numbers of zooxanthellae were determined from six to eight replicate haemocytometer counts of homogenates and were expressed as symbionts per polyp. Size of zooxanthellae was determined from measurements of longest diameters of dividing cells using a micrometer. Chlorophylls were extracted in 90% acetone either from whole animal (0.2 and 1.3 m) or algal suspensions after being pelleted and frozen in 0.2 ml of distilled water. Absorbances at 663 and 630 nm were read on a spectrophotometer on duplicate samples and amounts of chlorophylls *a* and c_2 calculated using the equations of Jeffrey and Humphrey (1975).

Protein content of total homogenates and isolated zooxanthellae were analyzed by the methods of Lowry et al. (1951). Slurrys were freeze-thawed 3 times and thoroughly homogenized in a glass tissue grinder before analysis. Animal protein content was calculated as the difference of these values.

2.4. Translocation

Two methods of estimating the amount of photosynthate released to the host were used in this study. The older method, which is widely recognized as underestimating translocation, involved measuring the amount of photosynthetically fixed ^{14}C that was released by the symbiotic algae in *M. amboinense* by methods similar to those of Trench (1971). Algae were isolated as previously described and adjusted to concentrations of $0.5–1.0\times10^6$ cells ml^{-1} in 1 ml of FSW in replicate test tubes. One ml of host homogenate (supernatant of the first centrifugation during isolation) was added back to the cell suspension, and 30 μl of a stock solution of $NaH^{14}CO_3$ (1 mCi ml^{-1}) was added. The test tubes were sealed with Parafilm™ and placed in indirect sunlight (500–800 μE m^{-2} s^{-1}) in a water bath (27±1°C) for 1 h. Following incubation, the test tubes were centrifuged ($2000\times g$) for 5 min to separate the supernatant from the algal pellet. Aliquots of the supernatant were acidified to liberate unincorporated $NaH^{14}CO_3$. The samples were neutralized with NaOH and preserved with 80% ethyl alcohol. Concentrations of ^{14}C in the pellets and supernatant fractions were determined from replicate 0.1-ml aliquots counted on a Beckman model LS 6800 scintillation counter.

The ‘growth rate method’ of calculating rate of translocation is based on the

assumption that all photosynthetically fixed carbon is translocated to the host, except for that used for zooxanthellae assimilation and growth (Muscatine et al., 1984). In the case of *Myrionema amboinense*, where about 15% of the zooxanthellae divide daily (doubling time, 8 days) and each zooxanthella contains about 60 pg C cell^{-1} (Fitt, unpublished), an alga producing 15 and 25 pg C h^{-1} for 13 h of saturating light would theoretically translocate 88 and 93%, respectively, of their fixed-C to the host. Therefore an average 'growth method' translocation rate of 90% was used in Table 3. The peak mitotic index for both high-light and shade zooxanthellae was assumed to be about 15% for this method of calculating translocation (Fitt, 2000).

2.5. Contribution of algae to animal respiration (CZAR)

The contribution of translocated carbon to the host's respiratory demand (CZAR) was calculated according to Muscatine and Porter (1977). For discussions of assumptions and limitations of this method see Muscatine et al. (1981) and McCloskey and Muscatine (1984). Briefly:

$$\mathrm{CZAR} = \frac{(P_g)\,(10\text{ h of maximum photosynthesis day}^{-}1)}{(R_a)\,(24\text{ h of respiration day}^{-1})} \times (\%T)$$

where P_g is gross photosynthesis h^{-1}; R_z is respiration of the algae calculated from biomass ratios h^{-1}; R_a is respiration of the animal h^{-1}; and $\%T$ is percent of the photosynthetically fixed carbon translocated to the host. Oxygen data were transformed to carbon equivalents assuming the respiratory quotient = 1.0 and photosynthetic quotient = 1.0. The ratio of algal to animal protein biomass was used to calculate R_a from whole animal respiration ($R_z + R_a$).

2.6. Statistical analyses

Untransformed data were used in all analyses. Two-sample *t*-tests were used to compare samples with two groups, while one-way ANOVA followed by the post-hoc Tukey HSD procedure was used for multiple group comparisons.

3. Results

3.1. Algal and animal biomass parameters

Fig. 1 summarizes the chlorophyll content of zooxanthellae from *Myrionema amboinense* maintained for 4 weeks under various light conditions in the field and in the laboratory. Those colonies kept under low irradiances (laboratory, shaded in the field) had higher levels of both chlorophyll *a* (Fig. 1A) and c_2 (Fig. 1B) than any of the hydroids in the high light conditions in the field. The shallowest (0.2 m) high-light colonies had the lowest chlorophyll content of any algae in this study. The resulting ratios of chlorophyll *a*:chlorophyll c_2 were slightly over 1.0 for the high light

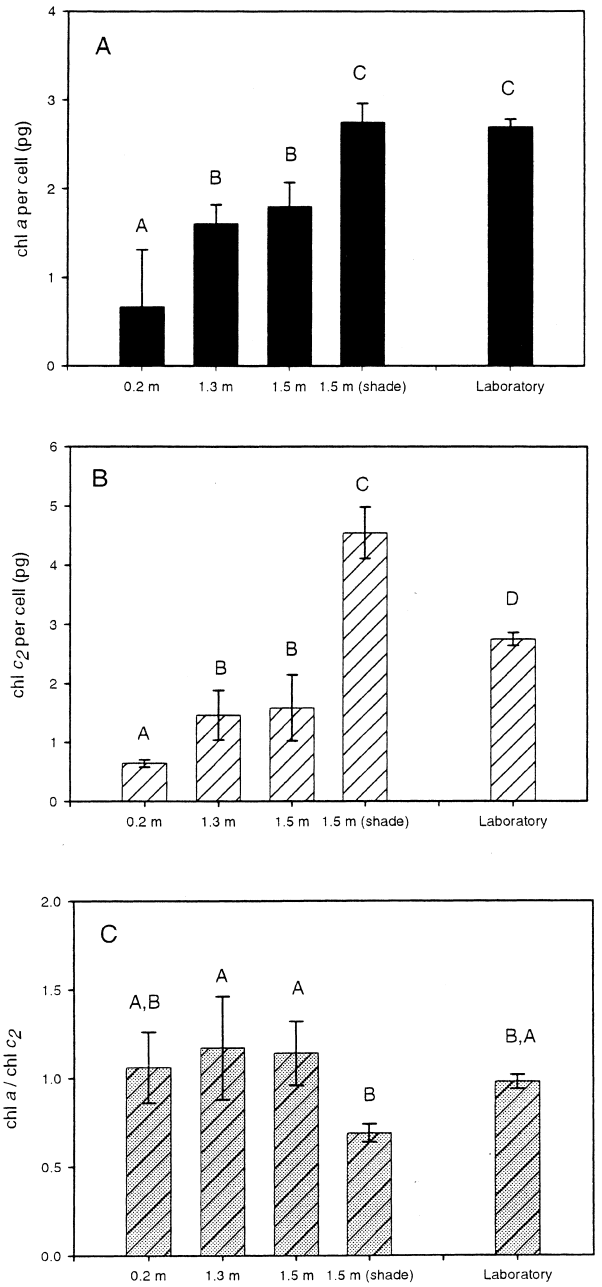

Fig. 1. Chlorophyll content of zooxanthellae from *Myrionema amboinense* maintained under various irradiance conditions in the field and in the laboratory. (A) Chlorophyll *a* content per cell. Letter designations indicate statistically similar groups ($p<0.001$ for all comparisons; Tukey HSD post-hoc comparisons), $n=6$ for all field samples, $n=4$ for laboratory samples. (B) Chlorophyll c_2 content per cell. Statistical conventions as in (A), except that $p<0.01$ for all comparisons. (C) Chlorophyll *a*:chlorophyll c_2 ratios. Statistical conventions as in (B).

zooxanthellae, less than 1.0 for the shaded zooxanthellae in the field, and essentially 1.0 for those kept in the laboratory (Fig. 1C).

Polyps of the shaded colonies in the field contained only about half of the zooxanthellae complement of high light polyps maintained at the same depths, as did colonies kept in the laboratory (Table 1). However, symbiont densities were equivalent in polyps in low and high light conditions when expressed on a per animal protein basis. There were no significant differences in the maximum diameters of dividing zooxanthellae under any of the experimental conditions (Table 1; ANOVA, $p > 0.05$ for all comparisons).

3.2. Photosynthesis–light intensity curves

P_{max} (maximum rate of gross photosynthesis) and α (light-dependent portion of the P–I curve) were virtually identical for both high-light and shade-adapted field colonies from the same depths when expressed on a per chlorophyll *a* basis, as was α (initial slope of P–I curve) and I_k (the irradiance at which photosynthesis is saturated) (Table 2). However, when compared on a per symbiont basis, intact shade-adapted colonies showed both increased P_{max} and α (Table 2), as did intact hydroids kept in low light laboratory conditions (data not shown). P–I curves exhibited different patterns when freshly isolated zooxanthellae were examined. FIZ from high light colonies had higher rates of P_{max} when expressed either on a per cell or per chl *a* basis than shaded hydroids, and had higher values of α when expressed on a chlorophyll basis; α was similar on a per cell basis. There was little evidence of photoinhibition in intact hydroids, with only two experiments out of 30 showing reduced oxygen production at high light intensities (>2000 μmol m^{-2} s^{-1}).

The P–I parameters of intact colonies compared to FIZ from hydroids maintained under similar conditions of depth and irradiance showed significant differences (Table 2). For colonies maintained under high light conditions, P_{max} values (per chl and per cell) were 2–3 times as high for FIZ than intact polyps as were values for α; I_k was about a third as high. Differences were less apparent in the colonies maintained under shaded conditions. In contrast to the high light samples, P_{max} for algae from colonies in

Table 1
Zooxanthellae and protein content of *Myrionema amboinense* under various natural light conditions in the field and in laboratory[a]

Condition	Zooxanthellae per polyp ($\times 10^{-5}$)	Total protein per polyp	Algal protein/ total protein	Zooxanthellae per mg animal protein ($\times 10^{-6}$)
High light:				
Field (1.3 m)	2.41±0.35 (6)	0.17±0.03 (6)	0.37±0.04 (6)	2.34±0.41 (6)
Field (1.5 m)	2.40±0.35 (15)	nd	nd	nd
Shade:				
Field (1.3 m)	1.34±0.12 (6)	0.13±0.03 (6)	0.60±0.13 (6)	2.69±0.89 (6)
Field (1.5 m)	1.15±0.21 (6)	nd	nd	nd
Laboratory	1.16±0.05 (6)	nd	nd	nd

[a] Data expressed as means±S.D. (*n*).

Table 2
Comparisons of photosynthesis–light intensity (P–I) curves for symbiotic dinoflagellates (=zooxanthellae) residing in intact *Myrionema amboinense* (IA) or freshly isolated zooxanthellae (FIZ), when maintained in high light or shade conditions[a]

Zooxanthellae	Intact animals ($n=6$)	Freshly isolated ($n=4$)
(A) Gross photosynthesis		
pg O_2 h^{-1} (μg chl a)$^{-1}$:		
Shade	8.7±0.9	9.7±0.8[a]
High light	8.6±0.3[b]	17.6±2.3[a,b]
pg O_2 h^{-1} zooxanthella^{-1}:		
Shade	24.5±2.7[c]	26.5±2.3[e]
High light	14.4±4.7[c,d]	34.2±4.7[d,e]
(B) I_k(μE m^{-2} s^{-1})		
Shade	181±46[f]	65±23[f]
High light	181±20[g]	71±30[g]
(C) α		
pg O_2 h^{-1} (μg chl a)$^{-1}$:		
Shade	0.05±0.01[h]	0.15±0.05[h,j]
High light	0.05±0.01[i]	0.25±0.11[i,j]
pg O_2 h^{-1} zooxanthella^{-1}:		
Shade	0.13±0.04[k,l]	0.43±0.15[k]
High light	0.08±0.09[l,m]	0.45±0.19[m]

[a] P_{max}, maximum photosynthesis at saturating light intensities; I_k, light intensity at which P_{max} first achieved; α, initial slope of light-dependent portion of P–I curve. Mean±S.D.

Within each set of comparisons, lettered pairs of means are significantly different from one another ($p<0.05$, ANOVA).

shaded conditions was similar for both FIZ and intact animals ($p>0.05$), but α (per cell) was significantly greater, and I_k significantly less, for FIZ than for whole polyps ($p<0.05$).

3.3. Contribution of zooxanthellae to animal respiration (CZAR)

Values of P_{max} (per zooxanthella) for shaded polyps were nearly twice those of high light polyps from the same depth (Table 3). Since shaded polyps had 50% fewer symbionts than those in high light, the maximum photosynthetic rates per polyp were similar for shaded and high light colonies. Laboratory maintained animals exhibited photosynthetic rates closer to those of high light adapted animals. To estimate the contribution of zooxanthellae to animal respiration (CZAR) it was necessary to estimate relative respiration rates of algal and animal components. This was done by using the ratio of algal protein to total (animal plus algal) protein (Table 1, 0.37±0.07 $n=6$ for high-light colonies and 0.60±0.13 $n=6$ for shade-adapted colonies). CZAR was calculated using two estimates of carbon translocation from alga to host. The ^{14}C method yielded a mean translocation rate of 52.2±9.8% ($n=4$), determined from experiments on high light polyps only, while the ‘growth method’ yielded an estimate of

Table 3
Photosynthesis, respiration and CZAR (contribution of zooxanthellar photosynthesis to host respiratory demand over 24 h) in intact polyps of *M. amboinense* maintained under various natural light conditions in the field and in laboratory[a]

Condition	Photosynthesis (pg O_2 zooxanthella^{-1} h^{-1})	Respiration (pg O_2 zooxanthella^{-1} h^{-1})	Photosynthesis (μg O_2 polyp^{-1})	P_g:R	CZAR (%)[b]	CZAR (%)[c]
High light:						
Field (1.3 m)	11.9	5.0	2.87	2.4	83	135
Field (1.5 m)	14.4±4.7	4.1±1.3	3.44±1.12	3.5±1.1	121±39	203±66
Shade:						
Field (1.3 m)	nd	nd	nd	nd	nd	nd
Field (1.5 m)	24.5±2.7	5.7±0.5	2.81±0.31	4.3±0.5	232±26	412±46
Laboratory	15.0	4.1	1.73	3.6	194	327

[a] CZAR was calculated using estimates of percent translocation by both the ^{14}C and growth rate methods (see Section 2). Mean±S.D. ($n=6$).
[b] CZAR calculated using translocation estimates from ^{14}C (=55% of total C).
[c] CZAR calculated using translocation estimates from growth rate method (=90% of total C).

90% (see Section 2). Table 3 shows that regardless of the method used to calculate CZAR, zooxanthellae released enough carbon to cover >80% of their host's respiratory requirements. High light animals exhibited CZAR's from 83 to 200%, while zooxanthellae from shaded field animals and those maintained in the laboratory contributed 2–4 times the carbon required to meet their host's respiratory demand.

4. Discussion

4.1. Energetic contribution of zooxanthellae to Myrionema amboinense

Acclimation of the dinoflagellate symbionts in *Myrionema amboinense* to low photon flux in shallow backreef habitats involved an increase in photosynthetic pigments per alga and a decrease in symbiont density, with the net result that the amount of carbon fixed per polyp was essentially the same as for polyps found in natural high light habitats. The data show that the amount of photosynthetically fixed C translocated from symbionts in shaded polyps can potentially contribute at least as much carbon to their hosts as zooxanthellae from polyps in high natural light intensities. Even hydroids maintained at sub-saturating intensities in the laboratory could receive the same relative level of carbon input from their symbionts as hydroids in the field, though it is clear that zooplankton availability in the laboratory was probably much less than in the field (Fitt, 2000).

Some of our assumptions used in the calculations of CZAR may not be accurate (equal symbiont growth rate in hydroids living in shade versus high-light, and that host/symbiont respiration rates are proportional to protein ratios). Nonetheless, it is clear that CZAR values calculated in this study were as high or higher in hydroids in shady field conditions than in hydroids in high light conditions. Those hydroids maintained in

the laboratory were especially interesting, since irradiance levels (35 μmol m^{-2} s^{-1}) were far below saturation; photoacclimation occurred in these hydroids and they appeared to do quite well in lower light conditions. Shade-adapted zooxanthellae in the coral *Stylophora pistillata* showed similar changes as those seen in *M. amboinense*, with less total protein, increased chlorophyll *a* per symbiont, about the same photosynthesis per unit surface area (corals) or polyp (hydroid), and calculated CZAR values at P_{max} as high or higher as those in high light at P_{max} light intensities (Porter et al., 1984).

There was little difference in our translocation rates in shade (92%) and high light (88%) adapted zooxanthellae when calculated by the 'growth method'. Similarly, Muscatine et al. (1984) presented translocation rates in high-light and shade-adapted colonies of *Stylophora pistillata* by three different methods using ^{14}C, yielding translocation rates ranging from 36 to 84%, all generally lower than the 'growth method' which estimated 97% translocation from both shade- and high light-adapted zooxanthellate corals. This same species of coral translocated about 60% (^{14}C method) of photosynthate at both 5 and 30 m on the Great Barrier Reef (Gattuso et al., 1993). However, at least two studies have determined that translocation decreases with depth. Battey and Porter (1988) found that translocation rates declined from over 40 to 50% in the Caribbean reef coral *Montastrea annularis* at 0.5–10 m to less than 30% at 40 m. Similarly McCloskey and Muscatine (1984) showed that zooxanthellae in the Red Sea coral *Stylophora pistillata* translocate only 11% of their photosynthetically fixed carbon at 35 m compared to 38.2% at 3 m as determined by ^{14}C methods, but 98.11% (3 m) vs. 95.2% (35 m) by the 'growth method'.

4.2. Photoacclimation of zooxanthellae of M. amboinenese

The observed reduction of zooxanthellae density in shaded polyps of *M. amboinense* differs from all other studies of shade acclimation in symbiotic dinflagellates. Typically, zooxanthellae densities change little when hosts grow under reduced light environments that are otherwise similar (Houck, 1978; Svoboda and Porrmann, 1980; Falkowski and Dubinsky, 1981; Porter et al., 1984; Muller-Parker, 1985; Harland and Davies, 1994; Helmuth et al., 1997). Our data are more similar to depth-related patterns of some reef corals, in which numbers of zooxanthellae per unit coral surface decreased with reduced light at depth (Drew, 1972; Dustan, 1979; McCloskey and Muscatine, 1984; Battey and Porter, 1988; Kaiser et al., 1993; Masuda et al., 1993; Fitt et al., 2000). The reduced symbiont population in deeper corals has been interpreted in two ways: (1) fewer algae may reduce self-shading, thereby increasing the light-capturing efficiency of the algae, or (2) some hosts in low light conditions may have lower tissue biomass per unit surface area (i.e., fewer gastrodermal cells?) and therefore may be maintaining constant algal:host biomass ratios (see Porter et al., 1984). In the case of *M. amboinense* the ratio of algal biomass to animal biomass remains relatively constant under high and low light conditions, supporting the latter hypothesis (Table 1; Fitt and Cook, 2000).

The increase in the photosynthetic pigment content in zooxanthellae of *M. amboiense* in response to lower irradiance is similar to the photoadaptive responses of zooxanthellae found in a number of symbiotic cnidarians experiencing changes in light quantity with shading and light quality/quantity at deeper depths (Titlyanov et al., 1980;

Falkowski and Dubinsky, 1981; Chang et al., 1983; McCloskey and Muscatine, 1984; Parker and Muscatine, 1984; Porter et al., 1984; Battey and Porter, 1988; Kaiser et al., 1993; Masuda et al., 1993; Leletkin et al., 1996), as well as those of many phytoplankton species (Falkowski and Owens, 1980; Prezelin, 1981). However, zooxanthellae in some hosts do not increase pigment levels in response to reduced photon flux (Houck, 1978; Svoboda and Porrmann, 1980; Titlyanov et al., 1980; Warnock, 1985), and those in the coral *Montipora foliosa* were found to have lower levels of pigment at depth (Titlyanov et al., 1980). Such variable patterns of acclimation appear to support the interpretations of Chang et al. (1983) and Iglesias-Prieto and Trench (1997a) that different types (clades) or species of *Symbiodinium* adapt to low light by different mechanisms.

The values for α, P_{max} and I_k of the $P–I$ curves in this study are consistent with the interpretation that the mechanism of adaptation of pigments to lower light in this zooxanthella is by increase of the number of photosynthetic units, in addition to any increase in size of the PSU (see Prezelin, 1981; Falkowski and LaRoche, 1991; Iglesias-Prieto and Trench, 1994; cf. Titlyanov et al., 1980, Zvalinskii et al., 1980; Dustan, 1979; Masuda et al., 1993; Harland and Davies, 1994). In addition, our comparison of I_k values for FIZ with I_k values of intact hydroids suggests that the host cell habitat reduces the amount of light reaching the average symbiont by 50–70%, similar to phenomena seen in fungoid corals (Masuda et al., 1993). Such light reduction likely involves both attenuation by host cell tissues and self-shading by the algal cells, with resulting non-saturating light intensities thought to be at least partially responsible for significant decreases in the photosynthetic rate per zooxanthella with increasing size of the giant clam *Tridacna gigas* (Fisher et al., 1985). Functional responses to attenuation of light reaching intracellular algae, as documented in the current study, would also act to protect the photosynthetic apparatus from photobleaching and photoinhibition. This would be particularly important for symbiotic dinoflagellates growing in very shallow depth where *M. ambionense* occurs. It is interesting to note that evidence of photoinhibition (reduced oxygen production at high light intensities) in intact *M. amboinense* was noted in only two out of over 30 experiments.

4.3. P–I responses of intact symbioses versus FIZ: limitation by light or CO_2 in intact polyps?

Symbiotic algae often occur in high densities in host tissue, on the order of 10^6 cells per mg host protein (e.g., Table 1). It has been suggested by several authors that photosynthesis at these densities would be limited either by shading by host tissue or other zooxanthellae (i.e., Crossland and Barnes, 1977; Fisher et al., 1985), or by CO_2 availability (i.e., Phipps and Pardy, 1982; Muscatine et al., 1989a,b), or both. Comparisons of the photosynthetic characteristics of isolated zooxanthellae versus intact associations with the same history of photoacclimation lend insight into which of these factors might be operating. If shading were occurring in the intact symbiosis, the initial light-dependent portion of the $P–I$ curve would exhibit a shift to the right relative to those of FIZ (lowered α, higher I_k). P_{max} should not be affected, assuming that the shading still allows enough light to reach saturation. However, if CO_2 limitation is

occurring in intact animals, then effects will largely be seen on the level of P_{max}. For instance, α and I_c might not differ in intact symbioses if no shading is occurring, but P_{max} will be less and I_k will likely be lower than FIZ, since limited CO_2 would reduce saturation levels. Of course, both shading and CO_2 limitation could occur simultaneously. The basis of differences in CO_2 availability to zooxanthellae inside of host cells compared to freshly isolated in our experiments and those listed below obviously lies in the presence or absence of host tissue and concomitant differences in pH, various ions, and carbon concentrating mechanisms. For instance, mechanisms of uptake and availability of inorganic carbon in FIZ in Na^+-rich seawater at pH 8.3 are thought to differ markedly from those found in Na^+-poor intracellular habitats with widely fluctuating pH (Goiran et al., 1996, 1997).

Only a few studies have examined zooxanthellar photosynthesis in this fashion. Crossland and Barnes (1977) compared *P–I* curves for FIZ and intact pieces of the coral *Acropora acuminata*. Both zooxanthellae in intact corals and the higher concentration of FIZ (9.4 μg chl *a* ml^{-1}) had higher values for I_k and lower P_{max} compared to dilute suspensions of FIZ (2.1 μg chl *a* ml^{-1}). These affects were attributed to increased zooxanthellar self shading. Fisher et al. (1985) interpreted similar reductions in I_k, α and P_{max} of increasing sizes of the giant clam *T. gigas* as the result of shading from both host and algal tissues. In addition, Masuda et al. (1993) and Goiran et al. (1996) present similar evidence of reduced light in the symbiotic habitat for zooxanthellae living in fungoid corals and microcolonies of the coral *Galaxea fascicularis*, respectively, as well as evidence of photoinhibition at the highest intensities for FIZ, with little to no change in P_{max}. In contrast, there were no differences in α, P_{max} or I_k between FIZ and symbionts in the intact sea anemone *Aiptasia pulchella* (Muller-Parker, 1984), and neither shading nor CO_2 limitation appeared to occur in this anemone; photoinhibition was reported in FIZ but not observed in the intact anemone.

We found evidence in intact *M. amboinense* for limitation of photosynthesis by both light and CO_2 relative to photosynthesis of their FIZ. Symbionts in hydroids maintained in high-light conditions had reduced α (both per cell and per chl *a*) and higher I_k relative to FIZ, indicating that shading affected photosynthesis in the intact animal. However, P_{max} was reduced even at 2000 μE m^{-2} s^{-2}, suggesting that maximum photosynthesis was probably limited by CO_2 availability. We postulate that lack of CO_2 also explains the reduced P_{max} previously observed in the *A. acuminata* study (Crossland and Barnes, 1977). In a related study, Dennison and Barnes (1988) found evidence that CO_2 was more available for photosynthesis and calcification in experiments involving water motion, in contrast to unstirred or calm water conditions. Other algal symbioses also appear to lack adequate carbon dioxide in the immediate vicinity of algal cells residing in the host. For instance, Phipps and Pardy (1982) found that additions of up to 1 mM bicarbonate to the medium containing symbiotic *Hydra viridis* led to increased photosynthetic rates of the intact animal, suggesting that carbon dioxide may be a limiting factor for photosynthesis of symbiotic *Chlorella* sp. inside of *H. viridis* digestive cells. Similar observations were made by Reisser (1980) on *Chlorella* sp. living symbiotically with *Paramecium bursaria* and Burris et al. (1983) for zooxanthellae living in and recently isolated from the Pacific coral *Seriatopora hystrix*. In addition, Muscatine et al. (1989a) found that P_{max} per zooxanthellae decreased with

increasing density of symbionts, indicating that carbon dioxide may also be in in limited supply to zooxanthellae living at normal densities of about 10^6 cm^{-2} in the Red Sea coral *Stylophora pistillata* (see also Muscatine et al., 1989b).

Symbiotic dinoflagellates isolated from hydroids from the shaded habitat, in contrast to those from hydroid colonies living in high light, did not have higher P_{max} than those in the intact host. Maximum photosynthesis by these low-light adapted algae appeared not to be limited by CO_2, an observation perhaps related to reduced algal densities in shaded polyps. While the densities of zooxanthellae in host tissue expressed in terms of mg protein were virtually identical in shaded and high light *M. amboinense*, as well as in the anemone *A. pulchella* (ca. $2.3–2.6\times10^6$ cells (mg host protein)$^{-1}$), symbionts in the hydroid are roughly 15% larger (diameter) than those in the anemone, or about 50% greater in volume with photosynthesis rates up to 3 times higher (Muller-Parker, 1984). Such physiological differences may explain why photosynthesis of symbiotic dinoflagellates living in the anemone *A. pulchella* are apparently not limited by availability of CO_2, whereas photosynthesis in *M. amboinense* is. Alternatively, differences in carbonic anhydrase availability (Weis et al., 1989; Weis, 1993) or other CO_2-concentrating mechanisms (i.e., Al-Moghrabi et al., 1997; Allemand et al., 1998) may exist in the two hosts. In this way, and in many other respects (McAuley and Cook, 1994; Fitt and Cook, 2000; Fitt, 1999), symbiotic dinoflagellates in *M. amboinense* appear to act similarly to symbionts in reef corals, and therefore be a useful model system in investigating cellular events in these ecologically important symbioses.

Acknowledgements

Editorial comments and suggestions from Drs. S. Chang, G. Muller-Parker, L. McCloskey, and R.K. Trench improved earlier drafts of this paper. We especially thank L. McCloskey for help with *P–I* curve analyses. Contribution ≠699, Discovery Bay Marine Laboratory, University of The West Indies in Jamaica, ≠019 from the Key Largo Marine Research Laboratory and ≠1383 of the Harbor Branch Oceanographic Institution. [SS]

References

Allemand, D., Furla, P., Benazet-Tambutte, S., 1998. Mechanisms of carbon acquisition for endosymbiont photosynthesis in Anthozoa. Can. J. Bot. 76, 925–941.

Al-Moghrabi, S., Goiran, C., Allemand, D., Speziale, N., Jaubert, J., 1997. Inorganic carbon uptake for photosynthesis by the symbiotic coral/dinoflagellate association. II. Mechanisms for bicarbonate uptake. J. Exp. Mar. Biol. Ecol. 199, 227–248.

Barnes, D.J., Taylor, D.L., 1973. In situ studies of calcification and photosynthetic carbon fixation in the coral monastrea annularis. Helgolander wiss Meeresunters 24, 284–291.

Battey, J., Porter, J.W., 1988. Photoadaptation as a whole organism response in *Montastrea annularis*. In: 4th International Coral Reef Symposium, Townsville, Australia, Vol. 3, pp. 79–88.

Buddemeier, R.W., Fautin, D.G., 1993. Coral bleaching as an adaptive mechanism: a testable hypothesis. Bioscience 43, 320–326.

Burris, J.E., Porter, J.W., Lang, W.A., 1983. Effect of carbon dioxide concentration on coral photosynthesis. Mar. Biol. 75, 113–116.

Chang, S.S., Prezelin, B.B., Trench, R.K., 1983. Mechanisms of photoadaptation in three strains of the symbiotic dinoflagellate *Symbiodinium microadriaticum*. Mar. Biol. 76, 219–229.

Chalker, B.E., Dunlap, W.C., Oliver, J.K., 1983. 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. 73, 37–56.

Cook, C.B., Fitt, W.K., 1990. Some effects of dissolved inorganic nutrients on the growth of zooxanthellae in the hydroid *Myrionema amboinense*. In: Nardon, P. et al. (Ed.), Endocytobiology IV. INRA, Paris, pp. 285–288.

Crossland, C.J., Barnes, D.J., 1977. Gas exchange studies with the staghorn coral *Acropora acuminata* and its zooxanthellae. Mar. Biol. 40, 185–194.

Davies, P.S., 1977. Carbon budgets and vertical zonation of Atlantic reef corals. In: Proc. 3rd Int. Coral Reef Symp, pp. 391–396.

Dennison, W.C., Barnes, D.J., 1988. Effect of water motion on coral photosynthesis and calcification. J. Exp. Mar. Biol. Ecol. 115, 67–77.

Drew, E.A., 1972. The biology and physiology of alga-invertebrate symbioses. I. The density of symbiotic algal cells in a number of hermatypic hard corals and alcyonarians from various depths. J. Exp. Mar. Biol. Ecol. 9, 71–75.

Dustan, P., 1979. Distribution of zooxanthellae and photosynthetic chloroplast pigment of the reef-building coral *Montastrae annularis* Ellis and Solander in relation to depth on a West Indian coral reef. Bull. Mar. Sci. 29, 79–95.

Fabricius, K.E., Klumpp, D.W., 1995. Widespread mixotrophy in reef-inhabiting soft corals: the influence of depth, and colony expansion and contraction on photosynthesis. Mar. Ecol. Prog. Ser. 125, 195–204.

Falkowski, P.G., Dubinsky, Z., 1981. Light-shade adaptation of *Stylophora pistillata*, a hermatypic coral from the Gulf of Eilat. Nature 289, 172–174.

Falkowski, P.G., Owens, T.G., 1980. Light-shade adaptation: two strategies in marine algae. Plant Physiol. 66, 592–595.

Falkowski, P.G., LaRoche, R., 1991. Acclimation to spectral irradiation in algae. J. Phycol. 27, 8–14.

Fisher, C.R., Fitt, W.K., Trench, R.K., 1985. Photosynthesis and respiration in *Tridacna gigas* (Mollusca, Bivalvia) as a function of irradiance and size. Biol. Bull. 169, 230–245.

Fitt, W.K., 2000. Cellular growth of host and symbiont in a cnidarian-zooxanthellae symbiosis. Biol. Bull. 198, 110–120.

Fitt, W.K., Cook, C.B., 2000. The effects of feeding and addition of dissolved inorganic nutrients in maintaining the symbiosis between dinoflagellates and a tropical marine cnidarian (submitted).

Fitt, W.K., McFarland, F.K., Warner, M.E., Chilcoat, G.C., 2000. Seasonal patterns of tissue biomass and densities of symbiotic dinoflagellates in reef corals and relation to coral bleaching. Limnol. Oceanogr. 45, 677–685.

Gattuso, J.P., Yellowlees, D., Lesser, M., 1993. Depth- and light-dependent variation of carbon partitioning and utilization in the zooxanthellate scleractinian coral *Stylophora pistillata*. Mar. Ecol. Prog. Ser. 92, 267–276.

Goiran, C., Al-Moghrabi, S., Allemand, D., Jaubert, J., 1996. Inorganic carbon uptake for photosynthesis by the symbiotic coral/dinoflagellate association. I. Photosynthetic performances of symbionts and dependence on sea water bicarbonate. J. Exp. Mar. Biol. Ecol. 199, 207–225.

Goiran, C., Allemand, D., Galgani, I., 1997. Transient Na stress in symbiotic dinoflagellates after isolation from coral-host cells and subsequent immersion in seawater. Mar. Biol. 129, 581–589.

Harland, A.D., Davies, P.S., 1994. Time-course of photoadaptation in the symbiotic sea anemone *Anemonia viridis*. Mar. Biol. 119, 45–51.

Helmuth, B.S.T., Timmerman, B.E.H., Sebens, K.P., 1997. Interplay of host morphology and symbiont microhabitat in coral aggregations. Mar. Biol. 130, 1–10.

Houck, J.E., 1978. The potential utilization of scleractinian corals in the study of marine environments. Unpublished Ph.D. dissertation, University of Hawaii, 178 pp.

Iglesias-Prieto, R., Trench, R.K., 1994. Acclimation and adaptation to irradiance in symbiotic dinoflagellates. I. Responses of the photosynthetic unit to changes in photon flux density. Mar. Ecol. Prog. Ser. 113, 163–175.

Iglesias-Prieto, R., Trench, R.K., 1997a. Acclimation and adaptation to irradiance in symbiotic dinoflagellates. II. Response of chlorophyll-protein complexes to different photon-flux densities. Mar. Biol. 130, 23–33.

Iglesias-Prieto, R., Trench, R.K., 1997b. Photoadaptation, photoacclimation and niche diversification in invertebrate-dinoflagellate symbioses. In: Proc. 8th Int coral Reef Symp., Vol. 2, pp. 1319–1324.

Jeffrey, S.W., Humphrey, G.F., 1975. New spectrophotometric equations for determining chlorophylls a, b, c, and c_2 in higher plants, algae and natural phytoplankton. Biochem. Physiol. Pflanzen 167, 191–194.

Kaiser, P., Schlichter, D., Fricke, H.W., 1993. Influence of light on algal symbionts of the deep water coral *Leptoseris fragilis*. Mar. Biol. 117, 45–52.

Leletkin, V.A., Titlyanov, E.A., Dubinsky, Z., 1996. Photosynthesis and respiration of the zooxanthellae in hermatypic corals habitated on different depths of the Gulf of Eilat. Photosynthetica 32, 481–490.

Lowry, O.H., Rosenborough, H.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with Folin phenol reagent. J. Biol. Chem. 193, 265–275.

Masuda, K., Goto, M., Maruyama, T., Miyacki, S., 1993. Adaptation of solitary corals and their zooxanthellae to low light and UV radiation. Mar. Biol. 117, 685–691.

McAuley, P.J., Cook, C.B., 1994. Effects of host feeding and dissolved ammonium on cell division and nitrogen status of zooxanthellae in the hydroid *Myrionema amboinense*. Mar. Biol. 121, 343–348.

McCloskey, L.R., Muscatine, L., 1984. Production and respiration in the Red Sea coral *Stylophora pistillata* as a function of depth. Proc. R. Soc. London 222, 215–230.

Muller-Parker, G., 1984. Photosynthesis-irradiance responses and photosynthetic periodicity in the sea anemone *Aiptasia pulchella* and its zooxanthellae. Mar. Biol. 82, 225–232.

Muller-Parker, G., 1985. Effect of feeding regime and irradiance on the photophysiology of the symbiotic sea anemone *Aiptasia pulchella*. Mar. Biol. 90, 65–74.

Muller-Parker, G., 1987. Seasonal variation in light-shade adaptation of natural populations of the symbiotic sea anemone *Aiptasia pulchella* (Calgren, 1943) in Hawaii. J. Exp. Mar. Biol. Ecol. 112, 165–183.

Muscatine, L., 1980. Productivity of zooxanthellae. In: Falkowski, P.G. (Ed.), Primary Productivity in the Sea. Plenum Press, New York, London, pp. 381–402.

Muscatine, L., Porter, J.W., 1977. Reef corals: mutualistic symbioses adapted to nutrient-poor environments. Bio. Sci. 27, 454–460.

Muscatine, L., McCloskey, L.R., Marian, R.E., 1981. Estimating the daily contribution of carbon from zooxanthellae to coral animal respiration. Limnol. Oceanogr. 26, 601–611.

Muscatine, L., Falkowski, P., Porter, J., Dubinskky, Z., 1984. Fate of photosynthetically-fixed carbon in light and shade-adapted colonies of the symbiotic coral *Stylophora pistillata*. Proc. R. Soc. London B 222, 181–202.

Muscatine, L., Falkowski, P.G., Dubinsky, Z., Cook, P.A., McCloskey, L.R., 1989a. The effect of external nutrient resources on the population dynamics of zooxanthellae in a reef coral. Proc. R. Soc. London B 236, 311–324.

Muscatine, L., Porter, J.W., Kaplan, I.R., 1989b. Resource partitioning by reef corals as determined from stable isotope composition. I. $\delta_{13}C$ of zooxanthellae and animal tissue vs depth. Mar. Biol. 100, 185–193.

Parker, G.M., Muscatine, L., 1984. Photosynthesis-irradiance responses and photosynthetic periodicity in the sea anemone *Aiptasia pulchella* and its zooxanthellae. Mar. Biol. 82, 225–232.

Phipps, Jr. D.W., Pardy, R.L., 1982. Host enhancement of symbiont phototsynthesis in the *Hydra*-algae symbiosis. Biol. Bull. 162, 83–94.

Porter, J.W., Muscatine, L., Dubinsky, Z., Falkowski, P.G., 1984. Primary production and photoadaptation in light- and shade-adapted colonies of the symbiotic coral, *Stylophora pistillata*. Proc. Rot. Soc. Lond. B. 222, 161–180.

Prezelin, B.B., 1981. Light reactions in photosynthesis. In: Platt, T. (Ed.), Canadian Bulletin of Fisheries and Aquatic Sciences (No. 201). Blackwell, Oxford, pp. 101–106.

Redjalde, R., 1976. Light adaptation strategies of hermatypic corals. Pacific Sci. 30, 212.

Reisser, W., 1980. The metabolic interactions between *Paramecium bursaria* Ehrbg. and *Chlorella* spec. in the *Paramecium bursaria*-symbiosis. III. The influence of different CO_2 concentrations and of glucose in the photosynthetic and respiratory capacity of the symbiotic unit. Arch. Microbiol. 125, 291–293.

Rowan, R., Knowlton, N., 1995. Intraspecific diversity and ecological zonation in coral-algal symbiosis. Proc. Natl. Acad. Sci. USA 92, 2850–2853.

Saunders, B.K., Muller-Parker, G., 1997. The effects of temperature and light on two algal populations in the temperature sea anemone *Anthopleura elegantissium* (Brandt, 1835). J. Exp. Mar. Biol. Ecol. 211, 213–224.

Svoboda, A., Porrmann, T., 1980. Oxygen production and uptake by symbiotic *Aiptasia diaphana* (Rapp), (Anthozoa, Coelenterata) adapted to different light intensities. In: Smith, D.C., Tiffon, Y. (Eds.), Nutrition in the Lower Metazoa. Pergamon, Oxford, New York, pp. 87–99.

Titlyanov, E.A., Shaposhmikovy, M.G., Zvalinskii, V.I., 1980. Photosynthesis and adaptation of corals to irradiance. I. Contents and native state of photosynthetic pigments in symbiotic microalga. Photosynthetica 14, 413–421.

Trench, R.K., 1971. The physiology and biochemistry of zooxanthellae symbiotic with marine coelenterates. II. Liberation of fixed ^{14}C by zooxanthellae in vitro. Proc. R. Soc. London (B) 4177, 237–250.

Trench, R.K., 1993. The cell biology of plant-animal symbiosis. Annu. Rev. Plant Physiol. 30, 485–531.

Warnock, T.E., 1985. The photosynthetic pigment composition of *Symbiodinium* (=*Gymnodinium*) *microadriaticum* in relation to cnidarian host and depth or origin. Ph.D. thesis, U. West Indies, Jamaica.

Weis, V.M., 1993. Effect of dissolved inorganic carbon concentration on the photosynthesis of the symbiotic sea anemone *Aiptasia pulchella* Carlgren: role of carbonic anhydrase. J. Exp. Mar. Biol. Ecol. 174, 209–225.

Weis, V.M., Smith, G.J., Muscatine, L., 1989. A ‘CO2 supply’ mechanism in zooxanthellate cnidaria of carbonic anhydrase. Mar. Biol. 100, 195–202.

Wethey, D.S., Porter, J.W., 1976a. Sun and shade differences in productivity of reef corals. Nature 262, 281–282.

Wethey, D.S., Porter, J.W., 1976b. Habitat-related patterns of productivity of the foliaceous reef coral, *Pavona praetorta* Dana. In: Mackie, G.O. (Ed.), Coelenterate Ecology and Behavior. Plenum, New York, pp. 59–66.

Zvalinski, V.I., Letekin, V.A., Titlyanov, E.A., Shaposhnikova, M.G., 1980. Photosynthesis and adaption of corals to irradiance. II. Oxygen exchange. Photosynthetica 14, 422–430.