

Acclimation and adaptation to irradiance in symbiotic dinoflagellates. I. Responses of the photosynthetic unit to changes in photon flux density

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ABSTRACT: The photosynthetic responses to variations in photon flux density were determined for *Symbiodinium microadriaticum*, the symbiont of the Caribbean jellyfish *Cassiopeia xamachana*, *S. kawagutii*, the symbiont of the Indo-Pacific stony coral *Montipora verrucosa*, and *S. pilosum*, the symbiont of the Caribbean zoanthid *Zoanthus sociatus*. Photosynthetic responses were characterized in terms of cellular pigment content, photosynthesis versus irradiance (*P-I*) relationships, and number and size of the photosynthetic unit (PSU). Analyses of the responses under 2 light regimes of 40 and 250 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$ indicate that: (1) the 3 different species cultured under identical conditions possess different photosynthetic characteristics; (2) the 3 species acclimate to low photon flux density by simultaneously increasing the number and size of their PSU; (3) PSU characteristics were not correlated with the parameters of their respective *P-I* curves; (4) the 3 species analyzed have different photo-acclimatory capabilities which can be correlated with their respective ecological distribution as endosymbionts. As different species demonstrate characteristic responses, photo-acclimatory adjustment in symbiotic dinoflagellates may be under genetic constraints, and thus may represent photo-adaptation (sensu O. Björkman). As a whole, the results suggest that symbiont photo-adaptation may constitute an important axis of niche diversification for the intact associations.

KEY WORDS: Photo-acclimation · Photosynthesis · Symbiotic dinoflagellates · Photosynthetic unit · Niche diversification

INTRODUCTION

Symbiotic dinoflagellates form a heterogeneous assemblage which includes members of at least 7 genera in 4 orders (Banaszak et al. 1993). These algae establish specific mutualistic associations with invertebrate hosts ranging from protozoans to mollusks (Taylor 1974, Trench 1987, 1992, 1993). Symbiotic dinoflagellates are commonly referred to in the literature by the trivial term 'zooxanthellae', although this term lacks any taxonomic significance as it has been used to describe any brown symbiont, including diatoms

(Trench 1992, 1993). There is a large body of evidence demonstrating that symbiotic dinoflagellates are more species diverse than previously recognized. Although it is very difficult to apply the Biological Species Concept to asexual microalgae (Trench & Blank 1987), dinoflagellate symbionts isolated from different host species and maintained in culture under constant growth conditions consistently show significant morphological, biochemical, physiological, behavioral and genetic differences (Rowan & Powers 1992, Trench 1992, 1993, Banaszak et al. 1993, McNally et al. 1994).

Symbiotic associations between dinoflagellates and invertebrates are among the most important primary producers in oligotrophic tropical oceans. In coral reefs, symbiotic invertebrates are responsible not only for the high gross production characteristic of these

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ecosystems (Muscatine 1980), but for the construction and maintenance of the reef framework (Goreau & Goreau 1959, Stanley 1981). Carbon fixation and photosynthate translocation by the algal component of these symbioses represent in some instances more than 100% of the basal metabolic requirements of the intact association (Muscatine 1980, Muscatine et al. 1981, Falkowski et al. 1984, Porter et al. 1984). Photosynthetic activity by the symbiotic algae has been implicated in the high CaCO_3 deposition rates of hermatypic corals (Goreau & Goreau 1959, Goreau 1977, Chalker 1981).

The vertical distribution of symbiotic invertebrates includes the entire euphotic zone. Reef corals live in a strong depth-mediated photon flux and spectral gradient that spans over 2 orders of magnitude (Porter 1980, Porter et al. 1984, Wyman et al. 1987). In addition, the 3-dimensional complexity of the coral reef produces shaded habitats at all depths (Sheppard 1981, Chang et al. 1983). The amount of light received by endozoic algae is modified by the host tissues, and in some cases it is attenuated by self-shading due to close packing of symbionts (Trench & Fisher 1983). The vertical distribution patterns of individual symbioses are very predictable; dominance of one species of coral in parts of the depth gradient produces obvious zonation patterns in Caribbean reefs (Goreau 1959, Porter 1972, Kinzie 1973, Jackson 1991). Some symbiotic species have very limited vertical distribution. For example, the chondrophore *Vellela vellela* is restricted to the air-sea interface, exposed to full sunlight; the zoanthid *Zoanthus sociatus* inhabits very shallow reef crest environments (Goreau 1959), while the scleractinian *Leptoseris fragilis* is found exclusively at depths between 95 and 145 m, exposed to very dim light (Schlichter & Fricke 1991). By contrast, others have wide vertical distributions that include the majority of the euphotic zone. This stratification may be the result of the evolution of adaptations of the symbionts to particular light regimes. In this context, it can be hypothesized that the vertical distribution ranges of individual symbiotic associations are, at least in part, the result of the relative capabilities of their specific symbionts to exploit distinct photic environments.

Given the importance of algal photosynthesis to the nutrition of the intact association, it is not surprising that the responses of symbiotic invertebrates to differences in their photic environments have been extensively studied (Drew 1972, Wetthey & Porter 1976, Dustan 1979, 1982, Titlyanov et al. 1980, Zvalinskii et al. 1980, Falkowski & Dubinsky 1981, Trench et al. 1981, Porter et al. 1984, Wyman et al. 1987). In the studies cited, photosynthetic responses of the symbionts were deduced from the oxygen production of the intact association. Unfortunately, the variability in host res-

piration can mask the photosynthetic responses of the algae (Spencer-Davies 1980, Edmunds & Spencer-Davies 1988). To isolate the photo-acclimatory responses of the algal component from animal interferences, Chang et al. (1983) characterized the photosynthetic responses of 3 species of symbiotic dinoflagellates in culture, using ^{14}C assimilation methods. Although their results clearly show that different species of *Symbiodinium* respond differently to changes in light regime, their interpretations of the responses of the photosynthetic unit (PSU) were made based on theoretical models in vogue at the time, and not by direct assessment of PSU features. By direct assessment of changes in the PSU, we analyzed the photosynthetic responses of 3 species of *Symbiodinium* to reduced photon flux density in culture and found that the 3 species photo-acclimate by differentially increasing the number and size of the PSU. Analyses of their photosynthetic responses in terms of daily production indicate that different species of *Symbiodinium* possess different photo-acclimatory capabilities.

MATERIALS AND METHODS

Algal cultures. Cultures of *Symbiodinium microadriaticum* Freudenthal, *S. kawagutii* and *S. pilosum* Trench & Blank were grown axenically in 500 ml flasks containing 250 ml of ASP-8A (Blank 1987) at 26°C, with a 14:10 h light:dark photoperiod. Light was provided by cool white fluorescent lamps delivering either 40 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$ [low light (LL) hereafter], or 250 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$ [high light (HL)] of photosynthetically active radiation (PAR). Photon flux densities were measured inside the culture vessels with a QSL-100 4 π sensor (Biospherical Instruments, Inc., San Diego, CA, USA). Cells were acclimated to both light regimes for at least 3 mo prior to the experiments. Cultures were maintained at densities below 1.0×10^5 cells ml^{-1} to minimize self-shading. All the determinations described below were performed on cells in early exponential growth to ensure homogeneous nutrient status.

Photosynthesis vs irradiance measurements. Photosynthesis vs irradiance determinations (*P-I* curves) (5 replicates per species per light treatment) were made with a Clark-type O_2 electrode (Rank Bros., Bottisham, UK) at 26°C. Temperature within the jacketed chamber was controlled by an external recirculating water bath (VWR Scientific, Inc., San Francisco, CA, USA). Cells were concentrated by gentle centrifugation (1500 rpm, 5 min) in a clinical centrifuge and resuspended in ASP-8A. Three ml of cell suspension containing approximately 1.0×10^6 cells ml^{-1} was placed in the electrode chamber. O_2 tension was reduced to

20% saturation by bubbling with N_2 gas (Kurz & Alberte 1983), and $NaHCO_3$ was added to a final concentration of 5 mM to prevent CO_2 limitation. Cells were exposed sequentially to 16 different irradiances (ranging from 6 to 2490 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$). Dark respiration was obtained by averaging the O_2 consumption values obtained at the beginning and at the end of each experiment. Illumination was provided by a high power fiber optics illuminator (Cuda Products, Jacksonville, FL, USA). The maximum output of the illuminator (2490 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$) was attenuated by different combinations of neutral density filters. Experimental irradiances were measured with a QSL-100 4 π sensor. The electrode was calibrated with air-saturated medium. Data were captured directly by means of a personal computer equipped with an analog/digital converter (Datacan IV, Sable Systems, Salt Lake City, UT, USA). Maximum rates of net photosynthesis (P_{max}) were calculated from the average photosynthetic rates obtained for the 4 higher irradiances (375, 714, 1360, and 2490 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$). The photosynthetic efficiencies (α) were calculated from the linear regression of the light-limited photosynthetic rates in the 0 to 60 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$ range (9 points including dark respiration). The light saturation intensity for maximal photosynthesis (I_k) was obtained from the intersection of the regression of the light-limited photosynthesis and P_{max} . $P-I$ parameters were also obtained by non-linear fitting of the data to the Jassby & Platt (1976) equation. For simplicity, only the parameters obtained by the linear fitting are presented. At the end of each experiment, cell density was estimated in triplicate with a hemacytometer from a 0.1 ml aliquot; the remainder of the sample was concentrated by centrifugation and stored at -20°C for pigment analysis. Daily production (P_d) was calculated for 14 h of net photosynthesis at the experimental light fields, subtracting 10 h of dark respiration.

Pigment analyses. Samples of algal cells (2.9 ml) were extracted in acetone:dimethyl sulfoxide (DMSO) (90:10, v:v) (Iglesias-Prieto et al. 1992). Cell pellets were resuspended in 0.9 ml of acetone and centrifuged for 10 s at high speed in a microcentrifuge; the supernatant was saved. The pellet was resuspended in 0.025 ml of DMSO and mixed vigorously for 30 s with a Vortex mixer. The acetone supernatant was added to the DMSO-extracted sample. Finally the extract was clarified by centrifugation. This operation was repeated 2 or 3 times until a white pellet was obtained. Chlorophyll *a* (chl *a*) and chl *c*₂ concentrations were determined spectrophotometrically using the equations of Jeffrey & Humphrey (1975) on a Hewlett Packard 8452A diode array spectrophotometer. Aliquots (50 μl) from the original cultures were concentrated by centrifugation at $8000 \times g$ for 10 min and extracted in acetone:DMSO as

described above. Pigments were transferred to dimethyl-ether, concentrated under N_2 gas and separated by thin-layer chromatography (TLC) as described by Jeffrey (1974). Peridinin and chl *a* were eluted from the TLC plate and their concentrations determined spectrophotometrically as described by Pr zelin (1976). Chl *a* concentrations in native chl-protein complexes were determined using an extinction coefficient of $60 \text{ mM}^{-1} \text{ cm}^{-1}$ (Shiozawa et al. 1974).

Determinations of P_{700} content. Cells were harvested by centrifugation at $8000 \times g$ for 10 min, resuspended in ice-cold TB buffer [100 mM tris-borate (TB), pH 8.0, 2 mM $MgCl_2$, 2 mM Na-EDTA, 1 mM phenylmethylsulphonyl fluoride], and broken by 3 passes through a French pressure cell at 8.3×10^7 Pa (Iglesias-Prieto et al. 1991, 1993). The lysate was clarified by centrifugation at $500 \times g$ for 10 min at 4°C to remove large debris and unbroken cells. The samples were centrifuged at $225\,000 \times g$ for 2 h at 4°C , and the pellets resuspended in 3 ml of ice-cold TB. Thylakoid membranes were made 1% (v:v) with *n*-dodecyl β -D-maltoside (DDM), and solubilized with a Potter-Elvehjem homogenizer. The solubilized material was clarified by centrifugation at high speed (12 000 rpm, $13\,300 \times g$) in a microcentrifuge for 5 min at 4°C , diluted to approximately 16.6 mM chl *a* (absorbance ≈ 1.00) with ice-cold TB, and rendered 10 mM and 100 mM with methyl viologen and Na ascorbate, respectively. P_{700} concentrations were determined spectrophotometrically by light-induced bleaching at 698 nm, using 726 nm as the isobestic point, and a molar extinction coefficient of $64 \text{ mM}^{-1} \text{ cm}^{-1}$ (Shiozawa et al. 1974). P_{700} determinations were made with a Hewlett Packard 8452a diode array spectrophotometer as described by Smith & Alberte (1991). Actinic illumination was provided by a high power fiber optics illuminator, filtered with a broad-band blue filter delivering 40 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$. The actinic beam was triggered manually with a mechanical shutter with a full width at half maximum (FWHM) < 0.1 s. The diode array was protected by a broad-band red filter. Under the solubilization conditions described above, approximately 85% of the chl *a* present in the purified thylakoids was recovered from the supernatant from which the P_{700} determinations were made. Preliminary experiments with repetitive extraction of the pellet showed no detectable P_{700} activity. Similar results were obtained for the water-soluble peridinin-chl *a*-protein (sPCP) and chl *a*-chl *c*₂-peridinin-protein (acpPC) complexes (Iglesias-Prieto et al. 1993). The solubility efficiencies were evaluated from spectroscopic determinations of chl *a* content in the DDM-insoluble pellet after 90% acetone extraction.

Chl *a*: P_{700} ratios reported here were corrected for solubilization efficiency as well as for the amount of chl *a*

present as sPCP and acpPC in the supernatant. Cellular concentrations of P_{700} were calculated from the molar ratios of chl *a*: P_{700} and the cellular chl *a* content.

P_{680} determinations. The concentration of P_{680} was determined by the method of Emerson & Arnold (1932), as described by Mishkind & Mauzerall (1980) and Kursar & Alberte (1983). Rates of O_2 evolution were determined under single-turnover saturating light flashes with a Clark-type O_2 electrode at 26°C. Repetitive light flashes were provided by 2 synchronously triggered flash lamps (Strovolave 1539A, General Radio, MA, USA). Cells were concentrated by gentle centrifugation at 1500 rpm in a clinical centrifuge, and resuspended in ASP-8A, at densities of approximately 1×10^6 cells ml^{-1} . The algal suspension was bubbled with N_2 gas to reduce the O_2 tension to 20% saturation and the sample was made 5 mM (final concentration) $NaHCO_3$ to prevent CO_2 limitation. Optimal O_2 :flash yields (about 20% P_{max}) were obtained with flashes delivered between 10 and 20 Hz. Cells were exposed to flashing light for 5 min before the measurements were performed. Oxygen flux was recorded for 5 to 10 min. The flash lamps were assumed to produce a single photosystem II (PS II) turnover since they have nominal FWHM between 1.6 and 7.5 μs (Falkowski et al. 1981), which are 2 orders of magnitude smaller than the reported PS II turnover rates for several algal species (Mishkind & Mauzerall 1980). P_{680} determinations were performed with saturating flash intensities. Analyses of the O_2 yield as a function of the relative flash intensity demonstrated that even at half of the maximum output, flashes were saturating. At the end of each experiment cells were harvested by centrifugation, extracted in acetone:DMSO, and chl concentrations determined

spectrophotometrically as previously described. Dark respiration was measured at the beginning and at the end of each experiment. O_2 yields were calculated by adding the rates of dark respiration to the rates of O_2 evolution and dividing by the number of flashes. The Emerson & Arnold photosynthetic unit number was obtained as the molar ratio of chl *a*: O_2 evolved per flash. Assuming that 4 photons are required to evolve a molecule of O_2 , the Emerson & Arnold number was divided by 4 to obtain the molar ratio of chl *a*: P_{680} . The ratio of total pigment: P_{680} was calculated from the chl *a*:chl *c*₂ and chl *a*:peridinin molar ratios. Cellular content of P_{680} was calculated as the product of cellular chl *a* concentrations and the molar ratio of chl *a*: P_{680} .

RESULTS

Consistent with previous observations (Chang et al. 1983), the 3 species of *Symbiodinium* analyzed had significantly higher cellular concentrations of photosynthetic pigments when grown at LL than when grown at HL ($p < 0.01$) (Table 1). Comparisons of the molar ratios of chl *a*:chl *c*₂ and chl *a*:peridinin indicate that in LL cells the cellular content of accessory pigments were disproportionately larger than chl *a* content relative to HL cells (Table 2). The only deviation from this pattern was observed in the chl *a*:chl *c*₂ ratio in *S. microadriaticum*.

The photosynthetic responses of *Symbiodinium microadriaticum* grown at HL and LL are shown in Fig. 1. P_{max} normalized to cell number was 24% higher in LL cells ($p < 0.01$), and LL cells showed an 82% higher α and a 30% lower I_k than HL cells (Table 3). Dark respiration was 56% higher in LL cells ($p < 0.05$), but no significant differences were observed in the $P_{max}:R$ (respiration) ratio. When the results were normalized to chl *a*, P_{max} of LL cells was 36% ($p < 0.01$) lower than HL

Table 1. *Symbiodinium* spp. Pigment composition of cells grown under 2 experimental photon flux densities, 250 μmol quanta $m^{-2} s^{-1}$ (HL) and 40 μmol quanta $m^{-2} s^{-1}$ (LL). Pigment concentrations are expressed as μmol cell⁻¹; values are means of 5 replicates. Values in parentheses represent 1 SEM. The data were analyzed by 1-way ANOVA

	LL	HL	p
<i>S. microadriaticum</i>			
Chl <i>a</i>	3.70 (0.14)	1.92 (0.04)	<0.01
Chl <i>c</i> ₂	1.75 (0.05)	0.95 (0.02)	<0.01
Peridinin	5.51 (0.18)	2.11 (0.05)	<0.01
<i>S. kawagutii</i>			
Chl <i>a</i>	2.92 (0.14)	2.20 (0.10)	<0.01
Chl <i>c</i> ₂	1.42 (0.06)	0.95 (0.04)	<0.01
Peridinin	4.61 (0.22)	2.89 (0.14)	<0.01
<i>S. pilosum</i>			
Chl <i>a</i>	8.52 (0.23)	4.53 (0.21)	<0.01
Chl <i>c</i> ₂	4.16 (0.11)	2.03 (0.09)	<0.01
Peridinin	11.60 (0.32)	5.49 (0.26)	<0.01

Table 2. *Symbiodinium* spp. Ratios of photosynthetic pigments in cells grown under LL and HL conditions

	LL	HL
<i>S. microadriaticum</i>		
Chl <i>a</i> :chl <i>c</i> ₂	2.10	2.02
Chl <i>a</i> :peridinin	0.67	0.91
Chl <i>a</i> :total pigment	0.34	0.39
<i>S. kawagutii</i>		
Chl <i>a</i> :chl <i>c</i> ₂	2.06	2.31
Chl <i>a</i> :peridinin	0.63	0.76
Chl <i>a</i> :total pigment	0.33	0.36
<i>S. pilosum</i>		
Chl <i>a</i> :chl <i>c</i> ₂	2.06	2.23
Chl <i>a</i> :peridinin	0.73	0.83
Chl <i>a</i> :total pigment	0.35	0.36

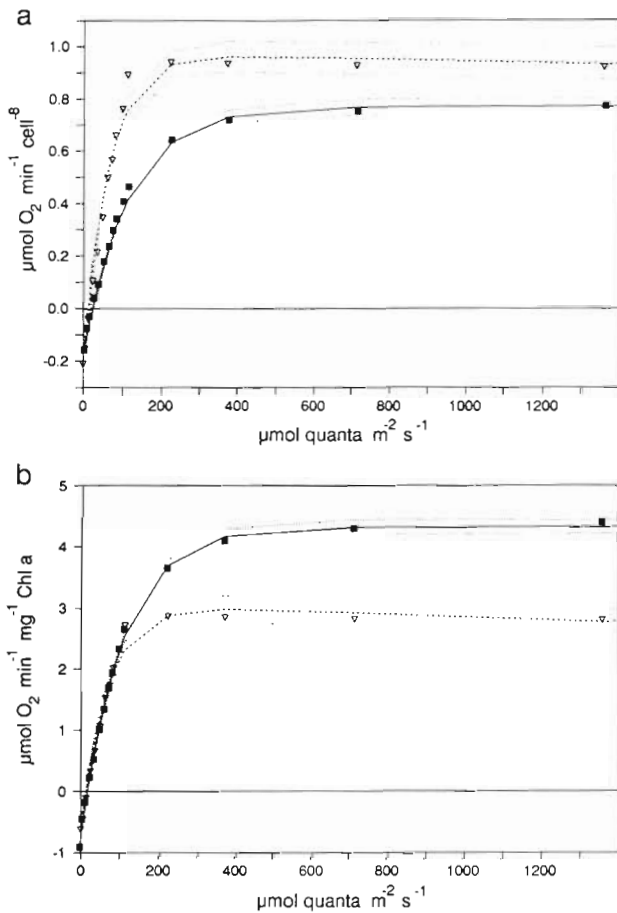


Fig. 1. *Symbiodinium microadriaticum*. Photosynthesis vs irradiance curves normalized to (a) cell number and (b) chl *a* content, for cells grown at high (—■—) and low (---△---) photon flux densities. For clarity, the values of photosynthesis at the highest irradiance ($2490 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$) were not included. Each symbol represents the average of 5 individual determinations. For comparison, pooled data were fitted with a non-linear procedure to the Jassby & Platt (1976) equation. Dotted lines indicate the 95% limits of confidence of the fits

cells (Table 4). Photosynthetic efficiencies (α) of cells grown under the 2 light regimes in terms of chl *a* were identical (Table 4). Normalization of P_{max} to cell volume was not performed since cultured symbiotic dinoflagellates are polymorphic; tetrads, and cells in coccoid and motile stages, have different dimensions, and they occur simultaneously in growing cultures (Schoenberg & Trench 1980, Trench & Blank 1987). Synchronized cell cultures have never been achieved.

The P - I curves normalized to cell number and chl *a* content from HL and LL cultures of *Symbiodinium kawagutii* are shown in Fig. 2. Statistical analyses of the P - I parameters normalized to cell number show that P_{max} values of LL cells were 32% lower than those of HL cells ($p < 0.01$). Photosynthetic efficiencies of LL cells were 35% higher ($p < 0.01$) than those measured

Table 3. *Symbiodinium* spp. Photosynthesis-irradiance parameters normalized to cell number. P_{max} and respiration (R) values are expressed as $\mu\text{mol O}_2 \text{min}^{-1} \text{cell}^{-1}$. I_k values are expressed as $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$. Values are means of 5 replicates. Values in parentheses are 1 SEM. The data were analyzed by 1-way ANOVA. ns: not significant

	LL	HL	p
<i>S. microadriaticum</i>			
P_{max}	9.268 (0.432)	7.499 (0.231)	<0.01
$\alpha (\times 10^{-10})$	1.038 (0.006)	0.570 (0.021)	<0.01
I_k	109.1 (7.3)	154.0 (10.0)	<0.01
R	1.834 (0.291)	1.178 (0.049)	<0.05
$P_{\text{max}}:R$	6.08 (0.86)	6.41 (0.40)	ns
<i>S. kawagutii</i>			
P_{max}	5.302 (0.116)	7.908 (0.304)	<0.01
$\alpha (\times 10^{-10})$	0.871 (0.022)	0.643 (0.022)	<0.01
I_k	67.4 (1.0)	122.7 (1.7)	<0.01
R	0.438 (0.058)	1.135 (0.116)	<0.01
$P_{\text{max}}:R$	13.71 (1.95)	7.37 (3.11)	<0.01
<i>S. pilosum</i>			
P_{max}	10.910 (0.818)	15.580 (0.336)	<0.01
$\alpha (\times 10^{-10})$	0.787 (0.022)	0.643 (0.022)	ns
I_k	85.5 (2.8)	124.9 (5.2)	<0.01
R	2.029 (0.357)	2.070 (0.468)	ns
$P_{\text{max}}:R$	6.28 (1.32)	8.99 (1.58)	ns

for HL cells. The I_k of LL cells was 45% less than HL cells ($p < 0.01$). Dark respiration of LL cells decreased by 61%, and the $P_{\text{max}}:R$ ratio was 86% higher relative to HL cells ($p < 0.01$) (Table 3). When the P - I parameters were normalized to chl *a*, P_{max} of LL cells was 48% lower than HL cells ($p < 0.01$). There was no significant difference in α (Table 4).

The photosynthetic responses of *Symbiodinium pilosum* grown under LL and HL conditions, normalized to cell number and chl *a* content, are shown in Fig. 3. The

Table 4. *Symbiodinium* spp. Photosynthesis-irradiance parameters normalized to chl *a*. Values are means of 5 replicates. P_{max} values are expressed as $\mu\text{mol O}_2 \text{mg}^{-1} \text{chl a min}^{-1}$. I_k values are $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$. Values in parentheses are 1 SEM. The data were analyzed by 1-way ANOVA. ns: not significant

	LL	HL	p
<i>S. microadriaticum</i>			
P_{max}	2.728 (0.099)	4.283 (0.130)	<0.01
α	0.032 (2.23×10^{-5})	0.032 (5.56×10^{-7})	ns
I_k	106.1 (7.3)	154.0 (10.0)	<0.01
<i>S. kawagutii</i>			
P_{max}	2.044 (0.019)	4.046 (0.071)	<0.01
α	0.030 (2.43×10^{-7})	0.033 (3.94×10^{-6})	ns
I_k	67.4 (1.0)	122.5 (1.7)	<0.01
<i>S. pilosum</i>			
P_{max}	1.432 (0.044)	3.883 (0.112)	<0.01
α	0.020 (3.36×10^{-6})	0.035 (1.39×10^{-6})	<0.01
I_k	85.5 (2.8)	125.9 (5.2)	<0.01

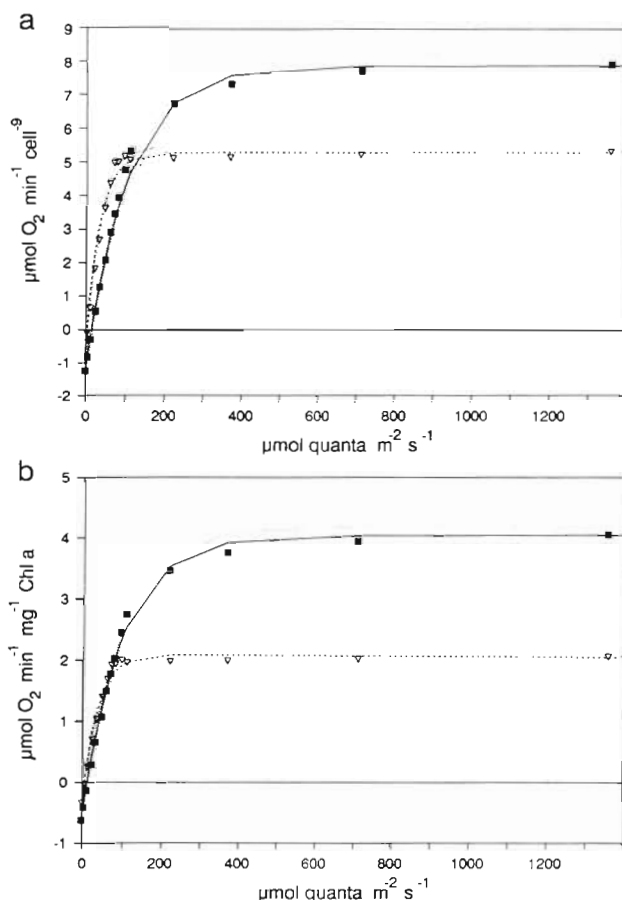


Fig. 2. *Symbiodinium kawagutii*. Photosynthesis vs irradiance curves normalized to (a) cell number and (b) chl *a* content, for cells grown at high (—■—) and low (---△---) photon flux densities. For clarity, the values of photosynthesis at the highest irradiance ($2490 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$) were not included. Each symbol represents the average of 5 individual determinations. For comparison, pooled data were fitted with a non-linear procedure to the Jassby & Platt (1976) equation. Dotted lines indicate the 95 % limits of confidence of the fits

P_{max} of LL cells, normalized to cell number, is 30 % less than HL cells ($p < 0.01$). The α values were identical. I_k values for LL cells were 32 % lower than HL cells ($p < 0.01$). No differences were detected in the dark respiration and $P:R$ ratios (Table 3). When the parameters of the $P-I$ curves were normalized to chl *a*, P_{max} and α were 63 % ($p < 0.01$) and 42 % ($p < 0.01$) lower, respectively, in LL cells (Table 4).

Characteristics of the PSU of *Symbiodinium microadriaticum* cultured at high and low photon flux densities are summarized in Table 5. The size of PS II (PSU- O_2) in terms of chl *a* (chl *a*: P_{680}) of LL cells was 37 % higher than in HL cells ($p < 0.01$). When PSU- O_2 was calculated based on total pigments (chl *a* + chl *c*₂ + peridinin), the value obtained for LL cells was 61 % higher than HL cells ($p < 0.01$). On a cellular basis, P_{680}

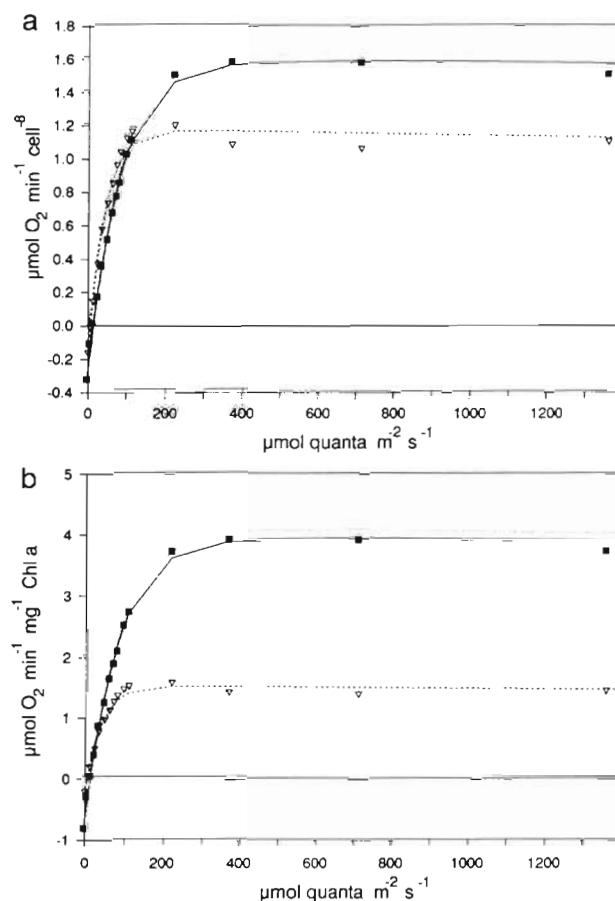


Fig. 3. *Symbiodinium pilosum*. Photosynthesis vs irradiance curves normalized to (a) cell number and (b) chl *a* content, for cells grown at high (—■—) and low (---△---) photon flux densities. For clarity, the values of photosynthesis at the highest irradiance ($2490 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$) were not included. Each symbol represents the average of 5 individual determinations. For comparison, pooled data were fitted with a non-linear procedure to the Jassby & Platt (1976) equation. Dotted lines indicate the 95 % limits of confidence of the fits

was 35 % higher in LL cells. The size of PS I (PSU- P_{700}) of LL cells was significantly smaller than in HL cells (11 %) ($p < 0.01$) when the values were calculated in terms of chl *a*. However, when PSU- P_{700} values were calculated in terms of total pigment, no difference was detected. The most dramatic differences observed in terms of the organization of the PSU in this species were (1) a doubling of the cellular concentration of P_{700} in LL cells, and (2) a higher molar ratio of PS I:PS II in LL cells than in HL cells.

A summary of PSU characteristics obtained for *Symbiodinium kawagutii* exposed to both light treatments is presented in Table 5. Chl *a*-based PSU- O_2 was smaller in LL cells (12 %) ($p < 0.01$). When PSU- O_2 was normalized to total pigment, the results were not significantly different. LL cells had a 50 % higher ($p <$

Table 5. *Symbiodinium* spp. Photosynthetic unit characteristics of cells grown at high (250 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$) and low (40 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$) photon flux densities. Values in parentheses are 1 SEM ($n = 15$ for P_{680} determinations and $n = 9$ for P_{700}). Data were analyzed by 1-way ANOVA

	LL	HL	p
<i>S. microadriaticum</i>			
Chl <i>a</i> :O ₂ (molar)	2527 (85)	1851 (105)	<0.01
Chl <i>a</i> :P ₆₈₀ (molar)	632 (21)	463 (26)	<0.01
Total pigment:P ₆₈₀ (molar)	1934 (65)	1200 (68)	<0.01
P ₆₈₀ cell ⁻¹ (moles cell ⁻¹⁸)	5.899 (0.198)	4.355 (0.267)	<0.01
Chl <i>a</i> :P ₇₀₀ (molar)	677 (19)	767 (37)	<0.01
Total pigment:P ₇₀₀ (molar)	2073 (57)	1990 (97)	ns
P ₇₀₀ cell ⁻¹ (moles cell ⁻¹⁸)	5.497 (0.147)	2.599 (0.126)	<0.01
PS I:PS II	0.931	0.596	
<i>S. kawagutii</i>			
Chl <i>a</i> :O ₂ (molar)	1912 (60)	2081 (61)	<0.01
Chl <i>a</i> :P ₆₈₀ (molar)	460 (15)	520 (15)	<0.01
Total pigment:P ₆₈₀ (molar)	1462 (46)	1429 (42)	ns
P ₆₈₀ cell ⁻¹ (moles cell ⁻¹⁸)	6.380 (0.196)	4.250 (0.133)	<0.01
Chl <i>a</i> :P ₇₀₀ (molar)	913 (19)	817 (37)	<0.01
Total pigment:P ₇₀₀ (molar)	2793 (60)	2243 (83)	<0.01
P ₇₀₀ cell ⁻¹ (moles cell ⁻¹⁸)	3.217 (0.062)	2.721 (0.096)	<0.01
PS I:PS II	0.504	0.640	
<i>S. pilosum</i>			
Chl <i>a</i> :O ₂ (molar)	1750 (62)	1714 (89)	ns
Chl <i>a</i> :P ₆₈₀ (molar)	432 (15)	428 (22)	ns
Total pigment:P ₆₈₀ (molar)	1247 (65)	1140 (68)	<0.01
P ₆₈₀ cell ⁻¹ (moles cell ⁻¹⁸)	19.850 (0.632)	10.080 (0.553)	<0.01
Chl <i>a</i> :P ₇₀₀ (molar)	1025 (32)	808 (14)	<0.01
Total pigment:P ₇₀₀ (molar)	2921 (92)	2150 (38)	<0.01
P ₇₀₀ cell ⁻¹ (moles cell ⁻¹⁸)	8.387 (0.251)	5.620 (0.100)	<0.01
PS I:PS II	0.423	0.558	

0.01) cellular concentration of PS II than HL cells. PSU- P_{700} values of LL cells were higher (12 and 25 % for chl *a* and total pigment, respectively) than those of HL cells ($p < 0.01$). Correlated with the higher PSU- P_{700} values observed in LL cells, the cellular content of P_{700} was 18 % higher than in HL cells ($p < 0.01$). The PS I:PS II molar ratio of LL cells was 0.50, whereas that obtained for HL cultures was 0.64.

The PSU characteristics obtained for *Symbiodinium pilosum* are summarized in Table 5. No differences were detected in PSU-O₂ based on chl *a*. A small (9 %) but significantly higher PSU-O₂, based on total pigment, was observed in LL cells ($p < 0.01$). The cellular content of P_{680} for LL cells was 97 % higher than for HL cells ($p < 0.01$). PSU- P_{700} based on chl *a* and total pigment, as well as the cellular P_{700} content of LL cells, were higher than in HL cells (27, 36, and 49 % respectively) ($p < 0.01$). The PS I:PS II molar ratio of HL cells was higher (0.56) than that of LL cells (0.42).

Comparisons of daily production (P_d) estimated for their respective light fields indicate that in all 3 species, LL cells are less productive than HL cells (Table 6) ($p < 0.01$). To assess the efficacy of photo-acclimation to low photon flux density, the estimated P_d of LL cells was compared with theoretical values calculated,

assuming no acclimation, for 14 h of photosynthesis at 40 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$ using the P - I curves obtained for HL cells (HL*). The results indicate that in *Symbiodinium microadriaticum*, P_d in LL cells was 97 % higher

Table 6. *Symbiodinium* spp. Comparisons of daily production of cells acclimated to high and low photon flux densities. Values are means of 5 replicates. Values in parentheses represent 1 SEM. Production is expressed as $\mu\text{mol O}_2 \text{ cell}^{-6} \text{ d}^{-1}$. HL* values were calculated, assuming that no photoacclimation had occurred, by the integration of cellular photosynthesis and respiration at 40 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$ on the P - I curves obtained from cells grown at high photon flux density. Probabilities resulted from 1-way ANOVAs between the LL and HL* data. When the same test was used to compare HL and LL treatments it resulted in $p < 0.01$ in the 3 species. ns: not significant

Species	LL	HL	HL*	p
<i>S. microadriaticum</i>	2.386 (0.200)	5.592 (0.189)	1.209 (0.091)	<0.01
<i>S. kawagutii</i>	2.382 (0.087)	5.962 (0.307)	1.480 (0.119)	<0.01
<i>S. pilosum</i>	3.826 (0.423)	11.850 (0.327)	3.531 (0.429)	ns

than in HL cells ($p < 0.01$). In *S. kawagutii* the values of P_d for LL cultures were 61 % higher than HL cells ($p < 0.01$). In *S. pilosum* no significant difference was detected.

DISCUSSION

The success of mutualistic associations between invertebrates and dinoflagellates in environments with very different photic regimes may be attributed, at least in part, to the photo-acclimatory capacities of their specific algal symbiont. The evidence presented here indicates that the 3 species of *Symbiodinium* analyzed effectively modify their photosynthetic machinery in response to changes in photon flux density. Furthermore, the results indicate that when different species of symbiotic dinoflagellates are cultured under identical conditions, their photosynthetic responses are different. Comparative analyses of the *P-I* parameters indicate significant differences in the photosynthetic performance of the 3 species when cultured under identical conditions (Table 7). Similar responses were reported previously for cultured symbiotic dinoflagellates (Chang et al. 1983), but the present effort represents the first attempt to document such responses by direct measurements of PSU features. Each individual species demonstrated a characteristic pattern of photo-acclimation.

Analyses of chromophore ratios and spectroscopic characteristics indicate that concomitant with the increase in total pigment observed in LL cells, there is a relative enrichment of accessory pigments in relation to chl *a*. Details of the distribution of pigments among chlorophyll-protein complexes of the cells employed in this study will be published separately.

The ubiquitous increase in cellular concentrations of photosynthetic pigments demonstrated by primary producers exposed to low irradiances can be the re-

sult of 2 non-exclusive photo-acclimatory mechanisms: an increase in the optical cross section of the PSU (increase in PSU size), and/or increases in the number of PSU (Prézelin 1981, 1987, Larkum & Barrett 1983, Richardson et al. 1983, Falkowski & LaRoche 1991). The results presented here indicate that the 3 symbiotic dinoflagellates analyzed adjust their photosynthetic apparatus to low photon flux density by a combination of the 2 mechanisms (Table 5), but the changes were differential, independent and species specific.

There are only a few reports in which dinoflagellate PSU characteristics have been determined. Prézelin & Alberte (1978) reported chl *a*-based PSU- P_{700} sizes of 600 for the free-living dinoflagellates *Glenodinium* sp. (= *Heterocapsa pygmaea*) and *Gonyaulax polyedra* (total pigment PSU- P_{700} were 1702 and 1715, respectively). Perry et al. (1981) reported a PSU- P_{700} (total chl) for *G. polyedra* grown at 300 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$ of 445. Dubinsky et al. (1986), working with *Prorocentrum micans*, showed that cells grown at 70 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$ possessed a PSU- P_{700} of 1107, while cells cultured at 600 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$ had a PSU- P_{700} of 695. To the best of our knowledge there is only 1 previous report of PSU- P_{700} for symbiotic dinoflagellates (Falkowski & Dubinsky 1981). Symbionts isolated from the coral *Stylophora pistillata* from habitats with high photon flux density (50 to 90 % of the surface irradiance) had a chl *a*: P_{700} molar ratio of 425 ± 50 , whereas those collected from shade environments (0.3 to 1.0 % of the surface irradiance) had a chl *a*: P_{700} ratio of 1650 ± 175 . P_{700} content of cells collected at both light environments was the same (approximately $5.8 \times 10^{-18} \text{ mol cell}^{-1}$) (Falkowski & Dubinsky 1981). The PSU- P_{700} values reported in the present communication are very similar to those reported previously for several dinoflagellates and diatoms (Falkowski & Dubinsky 1981, Falkowski et al. 1981, Dubinsky et al. 1986, Owens & Wold 1986), the only exception being the results reported by Perry et al. (1981), which are consistently smaller.

There are 2 important factors that can potentially interfere with P_{700} determinations in dinoflagellates:

(1) A considerable fraction of chl *a* is associated with chl-protein complexes which cannot be sedimented by low speed centrifugation [even after centrifugation for 2 h at $225\,000 \times g$, a large portion of the light-harvesting complexes (Iglesias-Prieto et al. 1991, 1993) remains in the supernatant]. This unsedimented chl *a* must be taken into account during calculations of chl *a*: P_{700} . Experiments in which thylakoids were centrifuged at $20\,000 \times g$ for 2 h prior to solubilization produced a chl *a*: P_{700} ratio of 470 ± 49 ($n = 5$), whereas the ratio obtained for preparations processed as described in the 'Materials and methods' was 913 ± 20 ($n = 9$).

Table 7. Comparison of the photosynthetic performance of the 3 species of *Symbiodinium* cultured under identical conditions. Data were compared with a 1-way ANOVA

	F-test	p
250 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$		
P_{max}	148.72	0.0001
α	132.09	0.0001
I_k	5.05	0.0278
Respiration	3.55	0.0721
40 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$		
P_{max}	22.83	0.0001
α	29.06	0.0001
I_k	16.74	0.0003
Respiration	8.42	0.0052

(2) The surfactant Triton X-100, which is commonly employed for P_{700} determinations, appears to have a deleterious effect on PS I in dinoflagellates (Iglesias-Prieto et al. 1993). Determinations of chl *a*: P_{700} molar ratios using different concentrations of Triton X-100 as a surfactant (0.01 to 1.0%, v:v) failed to produce ratios smaller than 1756.

Analyses of dinoflagellate PSU in terms of P_{680} are even more scarce in the literature than those for P_{700} . Zvalinskii et al. (1980) determined that for freshly isolated symbionts from *Pocillopora verrucosa*, the chl *a*-based PSU- P_{680} values ranged from 325 at 20 m to 467 at 45 m. The P_{680} content was identical in both instances (4.48×10^{-18} mol cell⁻¹). Similar results were obtained by Dubinsky et al. (1986) from *Prorocentrum micans* cultured at 70 and 600 μ mol quanta m⁻² s⁻¹ (chl *a*: P_{680} ratios of 725 and 525, respectively). Chl *a*-based PSU- O_2 obtained for the 3 symbiotic dinoflagellate species examined here are similar to those reported for diatoms and free-living and symbiotic dinoflagellates (Zvalinskii et al. 1980, Falkowski et al. 1981, Gallagher et al. 1984, Dubinsky et al. 1986).

Zvalinskii et al. (1980) and Falkowski & Dubinsky (1981) concluded that symbiotic dinoflagellates acclimate to low irradiances by increasing PSU size. In contrast, our results indicate that the 3 species of symbiotic dinoflagellates analyzed photo-acclimate by simultaneously enlarging the antenna and the number of PSU. The observed differences may be the result of ambiguities associated with the definition of PSU in terms of only 1 photosystem (Falkowski & LaRoche 1991). In both previous PSU analyses, conclusions were made by measuring PSU in terms of either PS I or PS II, but not both (Zvalinskii et al. 1980, Falkowski & Dubinsky 1981). The notion that PS I:PS II stoichiometries are variable, and that the variations are dependent on irradiance, has been firmly established for most primary producers (Falkowski et al. 1981, Myers & Graham 1983, Barlow & Alberte 1985, Chow et al. 1988, 1990, Guenther et al. 1988).

A possible explanation for the observed differences in the photo-acclimatory mechanisms used by freshly isolated and cultured algae may be related to the nutrient status of the algae. Comparisons of the photosynthetic responses of *Symbiodinium pulchrum* (the symbiont of the sea anemone *Aiptasia pulchella*) in culture and *in hospite* show significant differences (Chang et al. 1983, Muller-Parker 1984). Cultured cells (in log-phase growth) are nitrogen sufficient, whereas algal cells *in hospite* may be deficient in nitrogen (Cook et al. 1988, Muscatine et al. 1989; cf. Trench 1993). It is possible that under nutrient-limiting conditions, increasing PSU size could be less demanding in terms of nitrogen than synthesizing a complete PSU. Alternatively, it is also possible that species of sym-

bionts isolated from different host species possess different mechanisms of photo-acclimation (Chang et al. 1983). Symbiotic dinoflagellates form a heterogeneous assemblage of at least 7 different genera in 4 orders (Banaszak et al. 1993). There are numerous reports documenting biochemical, physiological, behavioral and genetic differences among members of the genus *Symbiodinium* (Trench 1987, Trench & Blank 1987, Rowan & Powers 1992, McNally et al. 1994). Differences in photosynthetic responses of isolated symbiotic dinoflagellates cultured at identical light regimes have been documented, and presumably reflect different photo-acclimatory mechanisms (Chang et al. 1983). These authors deduced the mechanisms of photo-acclimation from conceptual models and not from direct assessment of PSU (see below). Variations in photo-acclimatory mechanisms, comparable with those observed among different species, have been documented for different clones of the diatom *Skeletonema costatum* (Gallagher et al. 1984, Gallagher & Alberte 1985). Analyses of the photoacclimatory mechanisms employed by symbiotic dinoflagellates are too scarce to make any secure generalizations about their distribution within the genus *Symbiodinium*.

The evidence presented here indicates that the parameters of the *P-I* curves cannot be directly correlated with PSU characteristics. Using conceptual models of PSU organization, several authors have proposed that changes in PSU organization can be detected by simultaneous analyses of the *P-I* curves and the photosynthetic pigment content alone (Prézelin 1981, 1987). In the particular case of dinoflagellates, most of the photo-acclimation studies have been performed using this approach without any direct PSU assessment (Prézelin 1976, Prézelin & Sweeney 1978, Chang et al. 1983, Prézelin & Matlick 1983). Our results indicate that interpretations based exclusively on pigment and *P-I* curve analyses could result in conclusions that clearly contradict the empirical observations. For example, the conceptual models (Prézelin 1981, 1987) predict that cell-specific values of P_{max} must be proportional to the number of PSU per cell. Analyses of PSU in the present communication indicate that this is not necessarily the case. Increases in PSU number resulted in higher P_{max} per cell only in *Symbiodinium microadriaticum*, whereas P_{max} for LL cells in the other 2 species was significantly lower than in HL cells. These observations indicate that reliable physiological PSU characterizations required the direct measurement of PSU features. Similar discrepancies between the measured PSU features and those predicted by the theoretical model have been reported in several algae and cyanobacteria (Falkowski et al. 1981, Barlow & Alberte 1985, Gallagher & Alberte 1985, Dubinsky et al. 1986). Based on the P_{max}

values obtained for *S. kawagutii* in this study using O_2 evolution, and those obtained for the same species (same clonal population) by Chang et al. (1983) using ^{14}C incorporation, the calculated photosynthetic quotients (PQ) of the LL and HL cells were 2.13 and 1.27, respectively. The PQ value for HL cells conforms to the expected for cells using NO_3^- as a source of nitrogen; the deviation from the expected PQ in LL cells requires further investigation.

As a result of their photo-acclimatory capabilities, free-living microalgae can maintain high cell division rates in a variety of light regimes (Prézelin 1981, 1987, Richardson et al. 1983). As symbiotic dinoflagellates *in hospite* are believed to exhibit very low cell division rates (Muscatine et al. 1989), the function of photo-acclimation in these organisms may be the maintenance of high photosynthate translocation rates under different light regimes. Given the importance of translocation to the overall nutrition of holosymbionts (Muscatine 1980, Porter et al. 1984), the different photo-acclimatory responses demonstrated by the 3 species of symbiotic dinoflagellates analyzed suggest that in the absence of any other variable, intact associations specifically harboring the 3 dinoflagellate species would be differentially effective in exploiting any particular light environment. The observed differences in photo-acclimatory capabilities of the algae may result in an effective niche diversification of the intact associations. Differences in the effectiveness of photo-acclimation to low photon flux density in different dinoflagellate species may represent true adaptive (sensu Björkman 1981) responses to both the predictability and amplitude of the light fields present in their natural environments. *Symbiodinium microadriaticum* is the symbiont of the Caribbean jellyfish *Cassiopeia xamachana*. The motile nature of *C. xamachana* allows them to actively 'select' their photic regime (Zahl & McLaughlin 1959). The range of irradiances encountered by *C. xamachana* in its natural habitat is very large, and unpredictable. *C. xamachana* can be found at various depths in coral reefs and coastal lagoons, including narrow channels below mangrove canopies. Although this environment possesses low average irradiances, it may be subject to short-duration episodes of high photon flux density (sun-flecks), similar to those observed below the canopies of tropical rain forests (Pearcy et al. 1985). The photosynthetic responses of *S. microadriaticum* grown at low photon flux density, in particular the high cell-specific P_{max} values, indicate that this organism can effectively utilize short pulses of high photon flux density. Under these conditions a large portion of P_d could be the result of the photosynthesis performed under short periods of saturating light.

In contrast, *Symbiodinium kawagutii* and *S. pilosum* are the symbionts of colonial sessile organisms (the scleractinian *Montipora verrucosa* from the Indo-Pacific, and the Caribbean zoanthid *Zoanthus sociatus*, respectively). Given their sessile nature, these 2 species occupy photic environments that can be characterized by high predictability. The growth pattern of these organisms is indeterminate (Potts 1984, Jackson & Coates 1986, Jackson 1991). Individual colonies may be exposed to relatively constant photic regimes for periods ranging from tens to hundreds of years. Our results indicate that both algal species acclimated to low irradiances by increasing the number of reaction centers per cell, but this increase did not result in higher cell-specific P_{max} values. This observation indicates that their photosynthetic capacities are probably limited by the concentration of carbon fixing enzymes, or electron transfer chains (Falkowski et al. 1981, Prézelin 1981, 1987, Chang et al. 1983). Given the high predictability of their photic environments, it would be 'superfluous' for these 2 species to synthesize more carbon fixing enzymes or electron transfer components than those required at one particular depth.

The low efficiency of photo-acclimation to low light exhibited by *Symbiodinium pilosum* appears to be consistent with the vertical pattern of distribution of the intact association, since the *Zoanthus sociatus* morph, from which the cells employed in the present study were isolated, occupy exclusively the shallow parts of the reef (Goreau 1959). *Montipora verrucosa* demonstrates a wide vertical distribution, although growth rates of this species appear to have a negative correlation with irradiance (Coles & Jokiel 1978). The evidence presented here indicates that the low light photo-acclimation abilities of *S. kawagutii* are higher than those of *S. pilosum*, but cannot account for the growth patterns observed in the field (Coles & Jokiel 1978). Maximal surface irradiances in coral reefs can be as high as $2500 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$. Due to the concentration of light by waves (glitter), flashes of high photon flux densities ($>4000 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$) have been recorded in shallow parts of the reef (Jokiel 1988). In this context the 'high light' ($250 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$) experimental light field employed in the present study represents approximately only 10% of the maximal intensities that organisms would normally encounter in their natural environment.

Assuming that coral symbionts were members of a single species, Connell (1978) suggested that niche diversification among species plays only a minor role in maintaining the high diversity observed in coral reefs. Data presented in this communication clearly demonstrate that different species of *Symbiodinium* respond differently to equivalent variations in light. Given the high degree of specificity exhibited by sym-

biotic associations involving dinoflagellates and invertebrates (Trench 1987, 1992), and the importance of algal metabolism for both calcification and nutrition of the intact associations (Goreau 1977, Muscatine 1980, Porter et al. 1984), the results presented here suggest that the role of photo-adaptation as an important axis of niche diversification in reef corals should be reassessed.

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