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On the functional significance of molecular variation in *Symbiodinium*, the symbiotic algae of Cnidaria: photosynthetic response to irradiance

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ABSTRACT: The photosynthetic response to irradiance (*PI* response) of dinoflagellate algae of the genus *Symbiodinium* was quantified immediately after isolation from symbiosis with 9 species of corals and allied taxa on Bermuda. Significant variation in *PI* parameters was identified among the algal isolates, but no consistent differences between representatives of different *Symbiodinium* phylotypes were obtained. In a parallel analysis of *Montastraea franksi*, a dominant Bermudian coral species, colonies from 4 to 16 m (all bearing *Symbiodinium* of Phylotype B) were acclimated to high light conditions. The photoacclimatory response was slight. It included an increase in the dark respiration rate by algae from all collection depths, increased light use efficiency and decreased saturating irradiance of algae from 4 m, and increased maximal photosynthetic rate per unit chlorophyll (chl), but not per cell, for algae from 8 and 16 m. We conclude that generalisations about differences between the photosynthetic traits between *Symbiodinium* phylotypes are not valid, and that the wide depth distribution of *M. franksi* cannot be attributed to either depth-dependent association with multiple *Symbiodinium* phylotypes with different photosynthetic properties or strong photoacclimatory capabilities of its complement of *Symbiodinium*. These data suggest that *Symbiodinium* phylotype is not generally an important determinant of the abundance and distribution of symbioses on coral reefs.

KEY WORDS: Symbiosis · *Symbiodinium* · Zooxanthellae · *PI* response · Molecular diversity

INTRODUCTION

The symbiotic algae in scleractinian corals and other benthic Cnidaria (e.g. sea anemones, gorgonians) on coral reefs photosynthesise at appreciable rates, and much of the photosynthetic carbon fixed by the algae is transferred to the cnidarian tissues, where it is used as a respiratory substrate and for the synthesis of storage lipids and mucus (Trench 1993). This nutritional interaction is widely accepted to be crucial to the persistence and growth of the animals and general 'health' of coral reef ecosystems (Muscatine & Porter 1977, Muller-Parker & D'Elia 1997, Knowlton 2001).

The symbiotic algae in these benthic cnidarians are, almost without exception, dinoflagellates of the genus *Symbiodinium*. Several species of *Symbiodinium* have been described based on morphological and biochemical criteria (Blank & Trench 1986, Trench 1997), and the total molecular diversity among *Symbiodinium* has been expressed as comparable to the divergence 'between free-living dinoflagellates that are placed in different genera or even orders' (Rowan 1998). Virtually all molecular analyses of this genus have been conducted on rRNA genes and they have revealed that *Symbiodinium* comprises 2 broad groups; one containing Phylotype A and the other containing Phylotypes B, C and E (also known as D) (Rowan & Powers 1991a, Rowan & Knowlton 1995, Wilcox 1998, LaJeunesse 2001).

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The focus of this study is the functional significance of the molecular variation in *Symbiodinium*. The sequence variation in the rRNA genes of *Symbiodinium* is widely accepted to be selectively neutral and therefore does not translate directly into phenotypic variation among the *Symbiodinim* phylotypes. There is, however, persuasive evidence from field observations and experiments that *Symbiodinium* of different phylotypes exhibit distinctive traits, particularly in their response to temperature and irradiance (Rowan et al. 1997, Baker 2001, Toller et al. 2001a,b): Phylotype A has been described as 'invasive/opportunistic', B as 'sun specialists', C as 'shade specialists', and E as 'stress-tolerant' (Rowan 1998, Toller et al. 2001a). Implicit within these descriptions are 2 key predictions relating to the underlying physiological processes: that: (1) *Symbiodinium* of different phylotype display consistent differences; and (2) members of each phylotype have very limited acclimatory capabilities.

Relevant data are sparse and not entirely consistent. Variation among *Symbiodinium* isolates in long-term culture has been identified for photoacclimatory capabilities (Iglesias-Prieto et al. 1994), temperature tolerance (Warner et al. 1999) and production of mycosporine-like amino acids (Banaszak et al. 2000), but only the last appears to differ consistently between phylotypes. The traditional perspective founded on classical terrestrial plant ecophysiology (e.g. Bjorkman 1981), that the photoacclimatory capabilities of the algae contribute to a large depth range of some corals, has been confounded by the realisation that the *Symbiodinium* cells from different depths may be genetically distinct (Rowan & Knowlton 1995, Knowlton et al. 1997). Recent investigations include the demonstration that some *Symbiodinium* of Phylotype A show no detectable photoacclimatory response to depth (Bythell et al. 1997), but that *Symbiodinium* of Phylotype E acclimated to elevated irradiance display increased tolerance of high temperature (Brown et al. 2001).

The purpose of this study was to explore how *Symbiodinium* cells of known phylotype vary in their photosynthetic response to irradiance (PI response). This issue is of immediate relevance to both the core symbiotic function of *Symbiodinium*, photosynthate release to the animal, and to algal tolerance of the key abiotic stressors of high temperature and irradiance. The *PI* response was quantified from oxygen flux data using *Symbiodinium* cells immediately after isolation from the symbiosis as the experimental material. This experimental approach has been used successfully in a number of published studies (e.g. Muller-Parker 1984, Masuda et al. 1993, Goiran et al. 1996, Fitt & Cook 2001) and it ensures that the observed photosynthetic rates are not affected by factors independent of algal genotype, such as animal pigments or self-shading among algal cells in the intact association.

Two experimental designs were used with the objectives: (1) to test whether the *PI* response of *Symbiodinium* varied consistently between algae of different phylotypes; and (2) to identify the extent of the photoacclimatory capability of *Symbiodinium* cells in 1 coral species, namely, *Montastraea franksi*.

MATERIALS AND METHODS

Experimental design. The experiments were conducted at the Bermuda Biological Station for Research in summer 1999. All experimental material was allowed to acclimate for at least 2 wk on the laboratory wet bench in running seawater in large fibreglass tanks under natural light conditions (ca. 14:10 h light:dark cycle, maximal mid-day irradiance at the tank surface 1500 µmol quanta m^{-2} s^{-1} PAR). The acclimation period was well in excess of the time required for algae, including *Symbiodinium*, to acclimate to new irradiance conditions (Richardson et al. 1983, Harland & Davies 1994), and comparison with published data (e.g. Kühl et al. 1995) suggested that the maximum irradiance and total radiation dose were higher than are usually experienced by most symbioses in the field (but see 'Discussion').

The first experiments explored the *PI* response of *Symbiodinium* of different phylotypes. Samples of 9 species of animal hosts were collected from shallow reefs and mangrove ponds of depths of up to 6 m: the scleractinian corals *Agaricia* sp., *Montastraea cavernosa*, *M. franksi*, *Oculina diffusa* and *Porites astreoides*; the sea anemones *Aiptasia pallida*, *Bartholomea annulata* and *Condylactis gigantea*; and the jellyfish *Cassiopeia xamachana*. All animals appeared healthy during the acclimation period and appreciable bleaching was not observed.

The second experiments used colonies of *Montastraea franksi* collected from depths of 4, 8 and 16 m and brought to the laboratory in black bags to minimise light exposure. The exact collection sites were not monitored for incident radiation; however, long-term field studies of comparable sites on Bermuda have 90% of surface irradiance penetrating to 5 m, 40% to 10 m and 20% to 15 m (R. Smith pers. comm.). Half of the colonies from each depth were kept in running seawater in the dark prior to analysis within 24 h of collection, and the rest were allowed to acclimate for at least 2 wk, as above.

Isolation of *Symbiodinium* cells. Tissue homogenates were obtained by air-brushing (corals) or maceration (anemones and jellyfish) in the defined medium JTB, comprising 420 mM NaCl, 26 mM $MgSO_4$, 23 mM

$MgCl_2$, 9 mM KCl, 9 mM $CaCl_2$, 2 mM $NaHCO_3$, 10 mM HEPES, pH 8.2 (Wang & Douglas 1997). Microscopical analysis (phase contrast 1000×) confirmed that this procedure disrupted the animal cells but not the algal cells, and that more than 98% of the *Symbiodinium* cells were viable, as assessed with the fluorescein diacetate viability stain for membrane integrity (Sigma). To obtain preparations of isolated *Symbiodinium* cells, a sample of each homogenate was washed 3 times by centrifugation (1000 × *g* for 2 min) followed by gentle resuspension in JTB. The suspensions were free of animal debris and nematocysts detectable by light microscopy (1000×), and are referred to as 'freshly-isolated algal preparations'. The algal density in each isolated algal preparation was estimated using a haemocytometer and modified to give a standard final density of 5×10^5 cells ml^{-1} JTB. The preparations were used immediately for analysis of *PI* response and chlorophyll (chl) extraction.

Photosynthetic response of freshly isolated algal preparations to irradiance. Each algal sample was added to the glass cuvette of a Clarke-type oxygen electrode with an integral polariser and magnetic stirrer (Digital Model 10), for quantification of oxygen flux. The cuvette (1 to 2.5 ml variable volume) was maintained at 25 ± 0.5°C by a recirculating water bath, and the electrode was calibrated using sodium hydrosulphite in JTB (0 oxygen) and air-bubbled JTB (saturated oxygen, approximately 203 nmol O_2 ml^{-1}; Goiran et al. 1996). White light of different intensities was provided by a variable-irradiance halogen lamp (HB50) calibrated with a 2π quantum light sensor/datalogger (Li-Cor LI-1400). The experiments were conducted in a light-proof room, allowing the algal samples to be exposed to a wide range of accurately measured irradiances between 0 and 1050 µmol quanta m^{-2} s^{-1}. After a steady dark respiration rate had been achieved, each algal sample was exposed to increasing light intensities, with 6 min at each level. For each value of light intensity, the rate of oxygen production/consumption was quantified for the latter 3 min. At least 6 replicate algal samples (each from a different host individual/colony) were assayed for each animal species or experimental group.

Oxygen flux was expressed per unit algal number, chl *a* content and total protein. Using a non-linear regression subroutine in the Excel 97 spreadsheet program, *PI* curves were fitted iteratively to the data to give the best R^2 value using the following equation (e.g. Evans et al. 2000):

$$P = \left(\frac{\alpha I + P_{max} - \sqrt{(\alpha I + P_{max})^2 - 4\alpha\theta I P_{max}}}{2\theta} \right) - R$$

where P is photosynthetic rate, α is light use efficiency, I is photon flux density, P_{max} is maximum rate of photosynthesis, θ is curvature and R is dark respiration rate. This equation does not take into account the photoinhibitory decrease in photosynthesis often observed in algae exposed to high irradiances (Platt et al 1980) and therefore, points on the *PI* curves which showed inhibition were not included in the curve fitting analysis. Values for R, α, P_{max}, I_c (compensation light intensity) and I_k (saturation light intensity) were derived from the fitted equation. Values of each parameter for each animal species or experimental group were tested for homogeneity of variance, and the statistical significance of between-group differences was tested using 1-way ANOVA.

Molecular typing of *Symbiodinium*. DNA was isolated from a sample of each homogenate by the method of Rowan & Powers (1991b). Briefly, the isolated algae were washed with centrifugation in ice-cold zooxanthellae isolation buffer (ZIB) (0.4 M NaCl, 10 mM Na_2 EDTA, 20 mM Tris, 8 mM dithiothreitol, pH 8.2) and then in ice-cold DNA extraction buffer (DNAB) (0.4 M NaCl, 50 mM Na_2 EDTA, pH 8) before an overnight incubation at 50°C with 0.5 mg Proteinase K ml^{-1} DNAB. The digests were incubated with 1.5% cetyltrimethylammonium bromide, 1 M NaCl, 1.5 µg glycogen ml^{-1} (final concentrations), and DNA was extracted in chloroform and precipitated in ice-cold ethanol with 0.3 M sodium acetate, pH 6.5. The phylotype of the *Symbiodinium* was determined by PCR-RFLP analysis of the small subunit rRNA gene. The rDNA fragment was amplified with the 'host-excluding' primers ss3z and ss5z (Rowan & Powers 1991b), and digested with the diagnostic restriction enzymes *Taq*I and *Dpn*II (Bythell et al. 1997).

Chlorophyll analysis. A sample of each freshly isolated algal preparation was vacuum-filtered onto a Whatman GF/A glass microfibre filter (25 mm in diameter), rinsed with 2 ml ice-cold JTB and brought to dryness with the vacuum. The filter was pounded with 1 ml acetone/DMSO (9:1 v/v) in an ice-cold pestle and mortar. Preliminary tests confirmed that the chl pigments were completely extracted by a 2-step protocol: (1) incubation for 5 h in the dark at 4°C and collection of the supernatant after centrifugation at 10 000 × *g* for 10 min at 4°C; (2) resuspension of the pellet in 1 ml fresh acetone/DMSO by vortexing, followed by overnight incubation and centrifugation (10 000 × *g* for 10 min, 4°C). The total volume of the combined supernatants was measured to the nearest 10 µl using accurate micropipettes (Gilson). This solution was centrifuged (10 000 × *g* for 10 min, 4°C) and the absorbance of 1 ml of the supernatant was quantified at 630 and 663 nm against an acetone/DMSO blank. The concentrations of chls *a* and c_2 were calculated using the equations of Jeffrey & Humphrey (1975).

Table 1. *PI* parameters for *Symbiodinium* preparations from 9 symbioses. Values are mean ± SE with number of replicates given in parentheses in the first column. The results of 1-way ANOVA tests for variation among host species are shown for each parameter and the superscripted letters indicate homogenous subsets from Tukey's HSD post hoc analyses for each column. Data for I_k and α required square-root transformation before statistical analysis to obtain homogenous variances. *Porites astreoides*-I and *P. astreoides*-II refer to the algae from 2 groups of *P. astreoides* colonies with distinct *PI* parameters

(a) P_{max} (nmol oxygen min^{-1})

Animal species bearing *Symbiodinium*	per 10^6 *Symbiodinium* cells	per µg chl *a*	per µg protein
Phylotype A			
Bartholomea annulata (6)	7.53 ± 0.19^{c,d}	1.63 ± 0.19^{b,c,d}	11.29 ± 1.90^{b}
Cassiopeia xamachana (6)	10.43 ± 0.90^{b,c}	1.50 ± 0.06^{b,c,d}	4.92 ± 0.37^{c,d}
Porites astreoides-I (3)	20.97 ± 2.35^{a}	10.40 ± 6.53^{a}	34.92 ± 8.12^{a}
Porites astreoides-II (3)	7.40 ± 0.87^{c,d}	1.26 ± 0.10^{c,d,e}	9.21 ± 1.14^{b,c}
Condylactis gigantea (5)	5.34 ± 0.95^{d}	1.79 ± 0.31^{b,c}	6.15 ± 2.44^{b,c,d}
Phylotype B			
Condylactis gigantea (2)	3.83 ± 0.75^{d}	2.24 ± 0.36^{b}	4.35 ± 0.51^{c,d}
Aiptasia pallida (6)	4.39 ± 0.26^{d}	0.93 ± 0.11^{c,d,e}	4.31 ± 0.50^{c,d}
Montastraea franksi (6)	5.97 ± 0.37^{d}	1.43 ± 0.07^{b,c,d}	1.68 ± 0.06^{d}
Oculina diffusa (6)	4.76 ± 0.21^{d}	1.12 ± 0.14^{c,d,e}	1.96 ± 0.24^{d}
Phylotype C			
Agaricia sp. (6)	12.42 ± 1.28^{b}	0.43 ± 0.05^{e}	2.05 ± 0.39^{d}
Montastraea cavernosa (6)	7.21 ± 0.58^{c,d}	0.73 ± 0.08^{d,e}	0.73 ± 0.09^{d}
ANOVA	$F_{10,46}$ = 41.61, p < 0.001	$F_{10,46}$ = 17.56, p < 0.001	$F_{10,46}$ = 69.16, p < 0.001

(b) *R* (nmol oxygen min^{-1})

Animal species bearing *Symbiodinium*	per 10^6 *Symbiodinium* cells	per µg chl *a*
Phylotype A		
Bartholomea annulata (6)	0.59 ± 0.23^{c}	0.13 ± 0.08^{b}
Cassiopeia xamachana (6)	1.45 ± 0.23^{b,c}	0.21 ± 0.03^{b}
Porites astreoides-I (3)	6.55 ± 2.55^{a}	2.14 ± 0.79^{a}
Porites astreoides-II (3)	0.82 ± 0.22^{c}	0.14 ± 0.04^{b}
Condylactis gigantea (5)	0.72 ± 0.25^{c}	0.23 ± 0.07^{b}
Phylotype B		
Condylactis gigantea (2)	0.61 ± 0.61^{c}	0.35 ± 0.35^{b}
Aiptasia pallida (6)	0.74 ± 0.23^{c}	0.15 ± 0.04^{b}
Montastraea franksi (6)	1.22 ± 0.18^{b,c}	0.29 ± 0.04^{b}
Oculina diffusa (6)	0.14 ± 0.07^{c}	0.03 ± 0.02^{b}
Phylotype C		
Agaricia sp. (6)	3.44 ± 0.63^{b}	0.12 ± 0.02^{b}
Montastraea cavernosa (6)	1.91 ± 0.25^{b,c}	0.19 ± 0.02^{b}
ANOVA	$F_{10,46}$ = 14.31, p < 0.001	$F_{10,46}$ = 9.05, p < 0.001

(c) α (nmol oxygen min^{-1} µmol quanta m^{-2} s^{-1})

Animal species bearing *Symbiodinium*	per 10^6 *Symbiodinium* cells	per µg chl *a*
Phylotype A		
Bartholomea annulata (6)	0.071 ± 0.013^{b}	0.015 ± 0.004^{b}
Cassiopeia xamachana (6)	0.075 ± 0.004^{b}	0.011 ± 0.001^{b}
Porites astreoides-I (3)	0.255 ± 0.124^{a}	0.077 ± 0.030^{a}
Porites astreoides-II (3)	0.076 ± 0.010^{b}	0.013 ± 0.001^{b}
Condylactis gigantea (5)	0.094 ± 0.030^{b}	0.031 ± 0.009^{b}
Phylotype B		
Condylactis gigantea (2)	0.208 ± 0.103^{a}	0.121 ± 0.057^{a}
Aiptasia pallida (6)	0.270 ± 0.130^{a}	0.087 ± 0.060^{a,b}
Montastraea franksi (6)	0.039 ± 0.005^{b,c}	0.009 ± 0.001^{b}
Oculina diffusa (6)	0.030 ± 0.003^{b,c}	0.007 ± 0.0011^{b}
Phylotype C		
Agaricia sp. (6)	0.211 ± 0.020^{a}	0.007 ± 0.001^{b}
Montastraea cavernosa (6)	0.093 ± 0.022^{b}	0.010 ± 0.003^{b}
ANOVA	$F_{10,46}$ = 2.97, 0.001 < p < 0.01	$F_{10,46}$ = 3.06, 0.001 < p < 0.01

Table 1 (continued)

(d) Compensatory irradiance (I_c) and saturating irradiance (I_k) (µmol quanta m^{-2} s^{-1}) Animal species bearing *Symbiodinium*	I_c	I_k
Phylotype A		
Bartholomea annulata (6)	$10.2 \pm 5^{b,c}$	$134.6 \pm 2.5^{a,b}$
Cassiopeia xamachana (6)	$19.3 \pm 2.5^{a,b,c}$	161.4 ± 14.3^{a}
Porites astreoides-I (3)	35.3 ± 7.3^{a}	170.4 ± 40.4^{a}
Porites astreoides-II (3)	$11.2 \pm 2.7^{b,c}$	$190.6 \pm 4.1^{a,b}$
Condylactis gigantea (5)	$16.3 \pm 8.1^{b,c}$	$105.4 \pm 38.1^{a,b}$
Phylotype B		
Condylactis gigantea (2)	2.4 ± 2.4^{c}	24.4 ± 4.8^{b}
Aiptasia pallida (6)	$7.5 \pm 3.0^{b,c}$	54.4 ± 15.7^{b}
Montastraea franksi (6)	33.6 ± 3.4^{a}	199.7 ± 25.5^{a}
Oculina diffusa (6)	$5.0 \pm 2.4^{b,c}$	170.2 ± 10.5^{a}
Phylotype C		
Agaricia sp. (6)	$22.0 \pm 4.8^{a,b}$	$88.5 \pm 19.6^{a,b}$
Montastraea cavernosa (6)	$29.2 \pm 6.1^{a,b}$	136.4 ± 26.3^{a}
ANOVA	$F_{10,46} = 41.61$, $p < 0.001$	$F_{10,46} = 2.70$, $0.01 < p < 0.05$

Quantification of protein. The protein content of a known volume of each homogenate was quantified using the Bradford reagent (Sigma), following the manufacturer's instructions, with bovine serum albumin dissolved in JTB as protein standard. Algal density in the symbioses was expressed as the number of cells per unit total protein, which approximates to an index of alga:host ratio because the algal protein contributes less than 5% of the total (unpubl. results). Host surface area was not used as an index of host biomass because it cannot be applied to non-coral hosts and does not take into account variation in coral tissue thickness either between coral species or with depth.

RESULTS

PI response of *Symbiodinium* of different phylotypes

The *Symbiodinium* cells in the 9 animal species used to test the *PI* response of *Symbiodinium* of different phylotypes were assigned by PCR-RFLP analysis to the previously described Phylotypes A, B and C. All samples of 8 species bore a single phylotype: A in *Bartholomea annulata*, *Cassiopeia xamachana* and *Porites astreoides*; B in *Aiptasia pallida*, *Montastraea franksi* and *Oculina diffusa*; and C in *Agaricia* sp. and *Montastraea cavernosa*. For the last species, *Condylactis gigantea*, Phylotype A was detected in 5 individuals, B in 2 and both in 4. On a per algal cell basis, the chl content varied significantly among animal species for total chl ($F_{9,59} = 18.00$, $p < 0.001$), chl *a* content ($F_{9,59} = 15.75$, $p < 0.001$) and chl c_2 content ($F_{9,59} = 13.57$, $p < 0.001$), but no consistent differences between *Symbiodinium* of different phylotype were evident. Similarly, the variation in number of *Symbiodinium* per unit total protein was significant ($F_{10,64} = 14.71$, $p < 0.001$), but not correlated with algal phylotype.

The mean *PI* parameters obtained for the freshly isolated *Symbiodinium* preparations from each animal species are shown in Table 1 (data for the 4 individuals of *Condylactis gigantea* bearing mixed infections of *Symbiodinium* Phylotypes A and B are excluded from the *PI* analysis). Significant variation in *PI* parameters was evident (see statistical analysis in Table 1), but inspection of the groupings of the treatments identified by the post hoc tests reveals that *PI* parameters are: (1) not uniform for all members of 1 phylotype and; (2) not consistently different between phylotypes. Notably, the algae (of Phylotype A) from 3 colonies of *Porites astreoides* (displayed in Table 1 as *Porites astreoides*-I) had a significantly higher P_{max} and R than all other preparations (of all phylotypes) including the other 2 *P. astreoides* colonies tested (*P. astreoides*-II in Table 1). Furthermore, among algae of Phylotype B, the preparations from the corals *Oculina diffusa* and *Montastraea franksi* had significantly lower light use efficiency (α) per unit algal cell and a significantly higher saturating irradiance (I_k) than those from the anemones *Aiptasia pallida* and *Condylactis gigantea*. Furthermore, the algal preparations of Phylotypes A and B from *Condylactis gigantea* differed significantly in α but no other *PI* parameters (P_{max}, R, I_c or I_k) calculated.

PI response of *Symbiodinium* from *Montastraea franksi* collected from different depths

Symbiodinium of Phylotype B was the sole alga detected in all samples of *Montastraea franksi* used for

PI analysis. The algal density in the coral colonies varied significantly with collection depth, with a significantly higher density in the colonies from 8 m than those from 4 m (Fig. 1A). Over the acclimation period, no significant change in algal density was observed. The total chl content per algal cell was significantly lower for coral colonies collected from 4 m than from 8 and 16 m and, on acclimation, the corals at 8 and 16 m (but not 4 m) exhibited significant reduction in chl content per algal cell (Fig. 1B). The effect was greater for colonies from 16 than 8 m, and it was paralleled by a slight but unmistakable paling in colour of the coral colonies from 16 m over the acclimation period.

Analysis of the *PI* response of the freshly isolated algal preparations (Table 2) revealed that collection depth had no significant impact on P_{max} and R; however, algae from colonies at 4 m had a significantly lower α on a per cell and per unit chl *a* basis than algae from colonies at 8 and 16 m. The algae from colonies at 4 m also had a significantly higher value of I_c than algae from colonies at 16 m and of I_k than at both 16 and 8 m. Following acclimation, R was elevated for algae from all collection depths, whether expressed per cell or per unit chl *a*. The algae from colonies at 4 m displayed an increase in α and decrease in I_k, in both cases, to values that did not differ significantly from values for algae from colonies at 8 and 16 m. Moreover, the algae from colonies at 8 and 16 m displayed an increase in P_{max} on a per unit chl, but not cell number, basis.

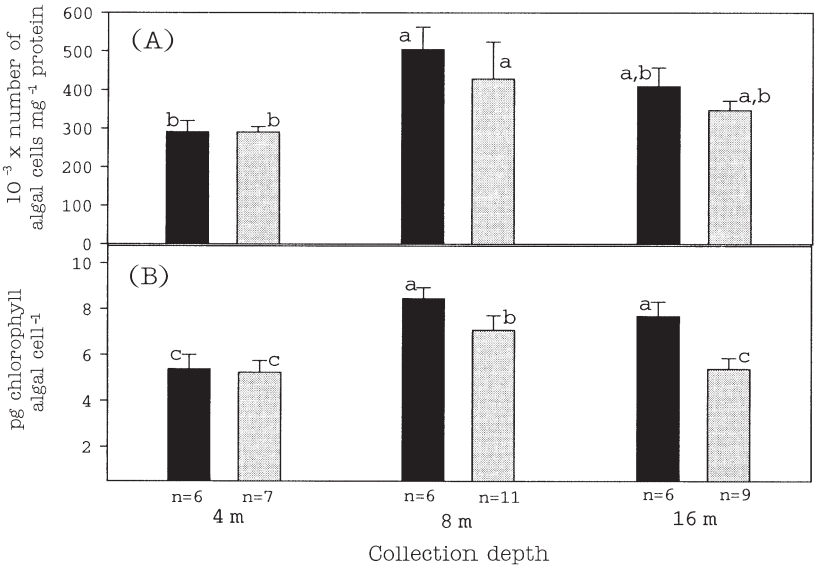

Fig. 1. Response of the algal cells in the coral *Montastraea franksi* collected from 4, 8 and 16 m, and acclimated for at least 2 wk on the laboratory wet bench. Data are mean ± SE with number of replicates (n) indicated; closed bars, before acclimation; open bars, after acclimation. (A) Algal density, 2-way ANOVA: depth, $F_{2,39} = 8.07$, $p < 0.001$; acclimation, $F_{1,39} = 1.51$, $p > 0.05$; interaction, $F_{1,39} = 1.51$, $p > 0.05$. (B) Chlorophyll content per algal cell, 2-way ANOVA: depth, $F_{2,39} = 26.08$, $p < 0.001$; acclimation, $F_{1,39} = 17.70$, $p < 0.001$; interaction, $F_{1,39} = 5.41$, $0.001 < p < 0.01$. For both (A) and (B), values with the same letters (above each bar) are not significantly different by Tukey's post hoc analysis

DISCUSSION

Variation in *PI* response among freshly isolated preparations of *Symbiodinium*

Data obtained in this study suggest that the *PI* response of *Symbiodinium* does not map directly onto phylotype. Statistically significant variation in 1 or more *PI* parameters was obtained within each of the 3 phylotypes, and representatives of no phylotype differed significantly in all *PI* parameters from any other phylotype (Table 1). Since these data were obtained for material acclimated to uniform conditions, any phylotype-specific patterns should, in principle, have been revealed.

There is, however, a caveat to this interpretation: that the subject of acclimation was the intact symbiosis (coral, sea anemone, jellyfish) and the subject of the *PI* experiments was freshly isolated algal preparations. As a consequence of variation in animal architecture, animal pigments, carbon dioxide concentrating mechanisms in the intact symbiosis and algal density between host species (e.g. Porter 1976, Fitt et al. 2000, Salih et al. 2001, Leggat et al. 2002), algal cells in the different host species are unlikely to be acclimated to exactly the same light conditions. Detailed inspection of the data provides the basis to assess whether this issue may influence the overall conclusion that the *PI* response of *Symbiodinium* does not map onto phylotype. The *PI* parameters of Phylotype B vary with host architecture (sea anemone vs coral), even though rDNA sequence data suggest that the *Symbiodinium* cells in the coral *Montastraea franksi* are more closely related to the algae in the sea anemone *Aiptasia pallida* than in the coral *Oculina diffusa* (Savage 2001). Comparison of the *PI* parameters in Table 1 suggests that the *Symbiodinium* in the sea anemones *A. pallida* and *Condylactis gigantea* may have been acclimated to a lower irradiance than those in the corals. The high photosynthetic efficiency (α) and low saturating irradiance (I_k) of the algae from the sea anemones shows that they

allocated more resources into light harvesting (Björkman 1981, Falkowski & Raven 1997). Two factors may have contributed directly to this difference: the significantly higher densities of *Symbiodinium* in *A. pallida* and *C. gigantea* (0.9 to 1.2×10^6 cells μg^{-1} protein) than in *M. franksi* and *O. diffusa* (0.3 to 0.5×10^6 cells μg^{-1} protein), potentially resulting in self-shading (Goiran et al. 1996) in the former; and increased light availability in the corals arising from internal reflection from the skeleton (Kühl et al. 1995). However, the link between *PI* response and host architecture/algal density is not consistent across the full data set. In particular, combining the data for all species, *PI* parameters and algal density are not correlated, even though both vary significantly with host species. Furthermore, the greatest difference in *PI* response was obtained for

Table 2. *PI* parameters for *Symbiodinium* preparations from *Montastraea franksi* within 24 h of collection (pre-acclimation) and after incubation on the laboratory wet-bench for at least 2 wk (post-acclimation). All values are mean ± SE (n = 6). The results of 2-way ANOVA for each parameter are shown and superscripted letters indicate homogenous subsets from Tukey's HSD post hoc analyses

(a) P_{max} (nmol oxygen min^{-1})

Collection depth (m)	per 10^6 *Symbiodinium* cells		per µg chl *a*	
	Before acclimation	After acclimation	Before acclimation	After acclimation
4	5.32 ± 0.39[a]	5.97 ± 0.37[a]	1.32 ± 0.08[a]	1.43 ± 0.07[a]
8	5.89 ± 0.61[a]	6.70 ± 1.08[a]	0.93 ± 0.10[b]	1.40 ± 0.33[a]
16	5.53 ± 0.41[a]	5.80 ± 0.45[a]	0.88 ± 0.05[b]	1.49 ± 0.16[a]
ANOVA				
Depth	$F_{2,30} = 0.76$, $p > 0.05$		$F_{2,30} = 1.01$, $p > 0.05$	
Acclimation	$F_{1,30} = 1.38$, $p > 0.05$		$F_{1,30} = 8.87$, $0.001 < p < 0.01$	
Interaction	$F_{2,30} = 0.11$, $p > 0.05$		$F_{2,30} = 1.31$, $p > 0.05$	

(b) R (nmol oxygen min^{-1})

Collection depth (m)	per 10^6 *Symbiodinium* cells		per µg chl *a*	
	Before acclimation	After acclimation	Before acclimation	After acclimation
4	0.86 ± 0.18[b]	1.22 ± 0.18[a]	0.21 ± 0.05[b]	0.29 ± 0.04[a]
8	0.64 ± 0.21[b]	1.06 ± 0.26[a]	0.10 ± 0.03	0.29 ± 0.04[a]
16	0.57 ± 0.14[b]	0.99 ± 0.33[a]	0.09 ± 0.02[b]	0.25 ± 0.09[a]
ANOVA				
Depth	$F_{2,30} = 0.69$, $p > 0.05$		$F_{2,30} = 1.62$, $p > 0.05$	
Acclimation	$F_{1,30} = 4.64$, $0.01 < p < 0.05$		$F_{1,30} = 7.05$, $0.01 < p < 0.05$	
Interaction	$F_{2,30} = 0.01$, $p > 0.05$		$F_{2,30} = 0.30$, $p > 0.05$	

(c) α (nmol oxygen min^{-1} µmol quanta m^{-2} s^{-1})

Collection depth (m)	per 10^6 *Symbiodinium* cells		per µg chl *a*	
	Before acclimation	After acclimation	Before acclimation	After acclimation
4	0.019 ± 0.003[b]	0.039 ± 0.005[a]	0.005 ± 0.001[b]	0.009 ± 0.001[a]
8	0.039 ± 0.008[a]	0.035 ± 0.006[a]	0.006 ± 0.001[a]	0.007 ± 0.002[a]
16	0.048 ± 0.006[a]	0.059 ± 0.028[a]	0.008 ± 0.001[a]	0.016 ± 0.008[a]
ANOVA				
Depth	$F_{2,30} = 3.06$, $p > 0.05$		$F_{2,30} = 2.77$, $p > 0.05$	
Acclimation	$F_{1,30} = 1.90$, $0.01 < p < 0.05$		$F_{1,30} = 4.47$, $0.01 < p < 0.05$	
Interaction	$F_{2,30} = 0.97$, $p > 0.05$		$F_{2,30} = 1.67$, $p > 0.05$	

(d) Compensatory irradiance (I_c) and saturating irradiance (I_k) (µmol quanta m^{-2} s^{-1})

Collection depth (m)	I_c		I_k	
	Before acclimation	After acclimation	Before acclimation	After acclimation
4	64.9 ± 22.3[a]	33.6 ± 3.4[a]	381.9 ± 61.2[a]	199.7 ± 25.5[b]
8	21.1 ± 6.8[a]	31.2 ± 5.0[a]	203.8 ± 38.0[b]	232.6 ± 13.9[b]
16	13.5 ± 3.9[b]	14.9 ± 3.4[b]	136.1 ± 18.3[b]	149.5 ± 34.5[b]
ANOVA				
Depth	$F_{2,30} = 6.29$, $0.001 < p < 0.01$		$F_{2,30} = 8.70$, $p = 0.001$	
Acclimation	$F_{1,30} = 0.65$, $p > 0.05$		$F_{1,30} = 2.60$, $p > 0.05$	
Interaction	$F_{2,30} = 2.35$, $p > 0.05$		$F_{2,30} = 5.49$, $0.001 < p < 0.01$	

members of Phylotype A from a single coral species, *Porites astreoides* (Table 1). The basis of this intraspecific variation is unknown; all the colonies of *P. astreoides* were collected from the same reef area and depth, bore algal populations at comparable density and chl content (see 'Results'), and did not differ by eye in the intensity of yellow-brown animal pigmentation. The genetic relatedness between the different *P. astreoides* colonies was, however, unknown.

Taken together, these considerations suggest that *Symbiodinium* vary in their photosynthetic response to irradiance and that the results cannot be attributed exclusively to inter-host differences. Relating to our original objectives (see 'Introduction'), these results indicate that there is no general justification for the description of certain *Symbiodinium* phylotypes (usually A and B) as 'sun-loving' and others (notably C) as 'shade-loving' or suited to low light environments.

Of the various patterns observed, the most striking is the combination of high α and P_{max} displayed by the algae in certain *Porites astreoides* colonies. A comparable pattern has been obtained for *Symbiodinium* in some colonies of the shallow-water coral *Goniastrea aspera* (J. Bythell pers. comm.). This pattern is contrary to the generalisation that high light use efficiency occurs at the expense of investment in carbon fixation, including P_{max} (Richardson et al. 1983, Evans 1996). However, certain algae, including the dinoflagellates *Amphidinium carterae* and *Peridinium cinctum*, acclimated to low light levels have a higher α and P_{max} than conspecifics acclimated to high light levels (Richardson et al. 1983). It has been suggested that this pattern arises when P_{max} is limited by photoinhibition at high, but not low, light levels (Richardson et al. 1983).

Acclimatory capability of *Symbiodinium* from *Montastraea franksi*

The *Montastraea franksi-Symbiodinium* symbioses at depths of 4 to 16 m differed with respect to both the density and the photosynthetic apparatus of *Symbiodinium* cells. The significantly lower *Symbiodinium* density in corals from 4 compared to 8 m can be interpreted in terms of the costs associated with maintaining a high algal density in high light (shallow) environments where light is not limiting; the costs may include damage from reactive oxygen species produced during photosynthesis (Asada 1996) and nutritional demands on the coral host (Steen 1986). However, not all *Symbiodinium* symbioses respond to differences in depth in this fashion. There are reports of symbioses in shallow waters with higher (e.g. Titlyanov et al. 1996), lower (e.g. Berner et al. 1987) or comparable (e.g. Falkowski & Dubinsky 1981, Fitt & Cook 2001) *Symbiodinium* densities to those in deeper waters. Turning to the depth-related differences in the photosynthetic apparatus, algae in shallow-water corals are predicted to have a higher P_{max}, I_c and I_k and lower α than those in deep-water corals. The data obtained were in broad agreement with these expectations, indicating that, prior to the experimental acclimation, the algae in corals at 8 and 16 m were acclimated to lower light environments than those at 4 m.

A key concern in any experiment involving transplantation is the possibility that the symbiosis exhibits a non-specific stress response, for example to mechanical damage or change in conditions. Such a response is generally evident within 10 d and involves loss of algae (Steen & Muscatine 1987, Hannack et al. 1997, A. M. Savage pers. obs.). Assurance that the responses of the *Montastraea franksi* colonies transferred from the field to the high irradiance conditions of the laboratory wet bench were not confounded by a generalised stress response comes from the finding that the algal densities in colonies from different depths were maintained during acclimation for at least 14 d. Constant algal densities have also been observed in other studies of photoacclimation (Falkowski & Dubinsky 1981, Muller-Parker 1985, Harland & Davies 1994). Significant reduction in the chl content per algal cell was, however, observed for *Symbiodinium* in colonies from 8 and 16 m, consistent with many published studies of algal responses to increased irradiance (Falkowski & Dubinsky 1981, Chang et al. 1983, Richardson et al. 1983, Helmuth et al. 1997).

Although alteration in the content of photosynthetic pigments indicated that changes occurred in the photosynthetic machinery of the algae during acclimation to laboratory conditions, there was remarkably little variation in the *PI* parameters. The principal statistically significant changes comprised respiration (*R*) in colonies from all depths and increased P_{max} per µg chl *a* in colonies from 8 and 16 m (correlated with a significant decrease in chl *a* content of these colonies). Increases in respiration have previously been observed in *Symbiodinium* acclimated to high light conditions (Muscatine et al. 1984, Porter et al. 1984). In addition, acclimation of the shallow-water (4 m) colonies resulted in increased α and consequent decrease in I_k, a response very similar to that described for *Symbiodinium* from the corals *Pocillopora verucosa* and *Pavona praetorta* (Richardson et al. 1983). The change in α may have resulted from rearrangement of the photosynthetic components on the thylakoid membranes, which enhances the efficiency of electron transport, or changes in the light harvesting activity of accessory pigments (which were not measured here). These results were contrary to expectation because they are indicative of a photoacclimatory response to a

'lower' light environment, even though the midday irradiance during acclimation regularly exceeded 1000 µmol quanta m^{-2} s^{-1}, considerably higher than the averaged irradiance at 4 m. One possible explanation for the apparently paradoxical result is that the field irradiance may be enhanced by a process roughly analogous to sunflecks penetrating a forest canopy: ripples on the surface water may concentrate light such that irradiance of up to 4000 µmol quanta m^{-2} s^{-1} are reached at points on the shallow reefs (Iglesias-Prieto & Trench 1994, Lesser et al. 2000).

The most important aspect of these results, however, is the small scale of the variation in *PI* parameters between *Symbiodinium* from corals at 4 and 16 m and the minimal responses to transplantation to the high light environment of the laboratory wet bench. Two factors that may have contributed to these results are a very limited photoacclimatory capability of *Symbiodinium* and less variation of the light environment within the corals than of the external light environment. The latter can be achieved by coral-mediated photoacclimation, for example through modification of the concentration and composition of pigments that either screen the algae from excessive light or enhance photon capture at low light intensities (e.g. Muller-Parker 1984, Lesser & Shick 1989, Masuda et al. 1993, Salih et al. 2001), through extension/retraction of the polyps (e.g. Muller-Parker 1984, Day 1994) and, in the long term, by modified growth patterns resulting in changing polyp density or overall colony morphology (Porter et al. 1984, Helmuth et al. 1997). Consistent with an important contribution of the host to photoacclimation, *Symbiodinium* cells in long-term culture exhibit a greater photoacclimatory capability than *Symbiodinium* freshly isolated from the symbiosis (Muller-Parker 1984). This observation is consistent with an important impact of the host on photoacclimation.

The dominant *Symbiodinium* in *Montastraea franksi* on Bermuda is Phylotype B, but Phylotypes A, B, C and E have all been detected in this coral species in the Caribbean (Savage 2001, Toller et al. 2001a). Our demonstration in this study of no consistent inter-phylotype differences in the *PI* responses of *Symbiodinium* cells indicates that it would be premature to interpret the low specificity of the symbiosis in the Caribbean as an indication of greater functional variation in the algae in this coral in the Caribbean than on Bermuda.

CONCLUDING COMMENTS

Strands in the literature on the photosynthetic response of *Symbiodinium* in corals and allied animals offer the perspective that these symbioses colonise a wide range of photic environments either through the photoacclimatory responses of *Symbiodinium* (Iglesias-Prieto et al. 1994) or by hosting multiple alternative *Symbiodinium* genotypes with different photosynthetic properties (e.g. Rowan et al. 1997, Baker 2001). This study illustrates that such a perspective may not encompass the full complexity or diversity of symbioses. First, the photosynthetic characteristics of *Symbiodinium* cannot be deduced reliably from the usual current method, molecular typing of the rRNA genes, and consequently, it is not valid to make generalisations about the photosynthetic traits of different phylotypes. Second, the success of at least 1 coral species, *Montastraea franksi*, a dominant species over a wide range of depths around Bermuda, is not dependent on photoacclimation by its complement of *Symbiodinium*.

It is widely recognised that the benefit a host gains from its complement of symbionts may vary among symbiont genotypes and with environmental circumstance (Douglas 1998). However, the distribution and abundance of a host species on a reef is likely to be influenced by many more factors than simply the genotype of *Symbiodinium* that it contains.

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