

The ecological distribution and comparative photobiology of symbiotic dinoflagellates from reef corals in Belize: Potential implications for coral bleaching

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Abstract

The photobiology and distribution of dinoflagellates in the genus *Symbiodinium* was investigated for eight common reef coral species over a depth range of 1–25 m on a coral reef in Belize. The genetic identification of symbionts using polymerase chain reaction–denaturing gradient electrophoresis of the internal transcribed spacer 2 region revealed marked differences in host specificity and depth zonation for certain symbiont types. Each host taxon was found to associate with a limited subset of symbionts that exist in the region. Intraspecific variation was greatest at the shallower sites (1–8 m), where as many as five distinctive symbionts were distributed among a single host population (e.g., *Montastraea faveolata*). At depth (15–25 m), variation from colony to colony was minimal, where one algal type associated with most or all the colonies of a species. The maximal photochemical efficiency and light-acclimated efficiency of photosystem II (PSII) were determined by active chlorophyll fluorescence and used to assess potential differences in photosynthetic potential. Under normal ambient conditions, little or no physiological differences were noted among different symbionts occurring in the same species of coral at a particular depth, yet interspecific differences in PSII efficiency were noted between coral species at the same depth. Short-term bleaching experiments showed that symbionts B1 and C7 within *M. faveolata* experienced a higher degree of thermally induced photoinhibition relative to A4a symbionts in *Porites astreoides*. The differential patterns of PSII inactivation observed within *M. faveolata* could be explained by the presence of different symbiont populations within this coral. Differences in in situ maximum excitation pressure on PSII between symbionts within some corals may provide a predictive measure of how different species of coral or individual colonies with different symbionts would respond to natural thermal stress events.

The ecological success of reef-building corals is reliant on the symbiotic relationship that these organisms have with a diverse group of dinoflagellates within the genus *Symbiodinium*, commonly referred to as zooxanthellae. In the past decade, there has been a significant increase in our understanding of the genetic diversity of these algae. Reconstructed phylogenies from molecular markers such as nuclear rDNA and chloroplast ribosomal DNA divide the genus into at least eight highly divergent clades or lineages, designated “A” through “H” (Rowan and Powers 1991; LaJeunesse 2001; Coffroth and Santos 2005). Molecular markers such as the internal transcribed spacer regions 1 and 2 (ITS1, ITS2) (LaJeunesse 2001; Van Oppen

et al. 2001; LaJeunesse 2002), have provided greater taxonomic resolution within each clade. This has led to the characterization of numerous genetically distinct *Symbiodinium* “types” within each clade. Ecologically, they are distributed differently across the available diversity of coral and other reef-dwelling zooxanthellate cnidarian species. Many of these types also show distinctive bathymetric and biogeographic distributions at various spatial scales. The host community on a coral reef appears to be dominated by a few commonly distributed types, or host generalists, that are found in a wide range of host taxa. In contrast, >90% of symbiont diversity is represented by host-specific and/or more ecologically rare algal types (LaJeunesse 2002; LaJeunesse et al. 2003).

Although our understanding of the genetic diversity of *Symbiodinium* has improved, we are just beginning to understand the extent of physiological differences among these algae. Previous work has clearly shown that there is a high degree of morphological and physiological diversity within this genus, and in general there is no clear correlation between ecological or physiological performance and the generic clades commonly used to identify these algae (Iglesias-Prieto and Trench 1997; Savage et al. 2002; Tchernov et al. 2004). In particular, it is eminently

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clear that algal types between and within each major clade display different susceptibilities to environmental perturbation. Differential photoinactivation and damage induced by thermal stress under natural and experimental conditions highlight these physiological differences. Such disparities factor into the phenomena of coral bleaching, when reef-building corals expel significant numbers of zooxanthellae when exposed to elevated seawater temperature (Hoegh-Guldberg 1999; Warner et al. 1996). Further, for corals that have the capacity to harbor multiple symbiont types, algal distribution can be strongly influenced by light or temperature within some Caribbean and Pacific reef-building corals and, in some instances, can explain patterns of differential algal loss across a coral colony (Rowan et al. 1997; Ulstrup and Van Oppen 2003).

Active chlorophyll fluorescence has become a common tool for investigating the photobiology of *Symbiodinium*, and in particular, for investigating the physiological impact that elevated seawater temperature and light has on photosynthetic processes within these algae. Previous work has shown that some zooxanthellae in culture and within several species of reef-building corals display a high susceptibility to thermally induced loss of photosystem II (PSII) activity (Warner et al. 1996; Jones et al. 1998; Tchernov et al. 2004), which can lead to irreversible damage to the PSII reaction center and expulsion from the coral host (Warner et al. 1999; Lesser and Farrell 2004). Likewise, chlorophyll fluorescence methods have been used to document typical diel fluctuations in PSII function in the field. For example, studies using either pulse amplitude modulation (PAM) fluorometry or fast repetition rate (FRR) fluorometry have shown that *Symbiodinium* possess several photoprotective pathways, which are used during the course of the day to dissipate excess excitation energy. These include down-regulation of PSII reaction centers as well as energy dissipation via nonphotochemical pathways originating in the light-harvesting antennae, such as the reduction in the functional absorption cross section (σ) of PSII through an active xanthophyll cycle (Brown et al. 1999; Gorbunov et al. 2001; Lesser and Gorbunov 2001).

As of yet, few studies have approached in situ physiological comparisons that have also accounted for the identity of the *Symbiodinium* sp. within multiple species of corals. Although unaware of the symbiont's taxonomy, significant differences in in situ diel patterns of effective quantum yield of PSII ($\Delta F/F_m'$), electron transport, and nonphotochemical quenching have been noted between several species of Pacific and Caribbean corals (Hoegh-Guldberg and Jones 1999; Levy et al. 2004). However, in some cases, there is significant variability in these parameters between replicate colonies of the same coral species, thereby making it difficult to discern if shifts in photosynthetic activity recorded by chlorophyll fluorescence are also attributable to microscale colony variation in light or other uncontrolled external factors that may influence the fluorescence signal (Levy et al. 2004).

By measuring in situ differences in fluorescence yield in the dark and at peak sunlight and accounting for symbiont type, Iglesias-Prieto et al. (2004) noted similar patterns in the maximum excitation pressure over PSII (Q_m) between

the two eastern Pacific corals *Pocillopora verrucosa* and *Pavona gigantea*, which harbored clade D and C zooxanthellae, respectively. Both species displayed the same degree of Q_m even though they are found at different depths. This result indicated that symbionts within the deeper coral, *P. gigantea*, would experience >30% higher light pressure as compared to *P. verrucosa* and suffer from chronic photoinactivation if moved to shallow waters. Thus, differential use of light by specific symbionts can serve as an axis for niche diversification and explain, in part, the vertical distribution of the corals in which they reside. This present study used rapid in situ fluorescence analysis to investigate the potential for significant physiological differences in different populations of *Symbiodinium* spp. residing within different corals at the same depth and across a bathymetric gradient. Differences in the maximal photochemical efficiency and light acclimated efficiency of PSII were used to assess the potential disparity, or similarity, in how symbiotic combinations may react to thermal stress similar to that seen during coral bleaching.

Materials and methods

Work was conducted at three reef locations in front of Carrie Bow Cay, Belize. In September 2002, three sites were established to the northeast seaward side of the island: one site in the back reef lagoon directly behind the reef crest (2-m depth), one site at 8-m depth on the fore reef, and one site at 25-m depth at the outer ridge of the fore reef slope. In addition to these locations, some corals were also sampled for symbiont identification at approximately 15 m at the top of the outer ridge site in 2002.

Algal identifications—In 2002 the following coral species were selected from the following depths: 2 m: *Montastraea faveolata*, *Porites astreoides*, *Siderastrea siderea*, and *Siderastrea radians*; 8 m: *M. faveolata*, *Montastraea annularis*, *Montastraea cavernosa*, *P. astreoides*, *S. siderea*, and *Stephanocoenia intersepta*; 15 m: *M. faveolata*, *M. annularis*, *M. cavernosa*, *P. astreoides*, and *S. intersepta*; and 25 m: *Agaricia lamarki*, *M. faveolata*, *M. cavernosa*, *P. astreoides*, *S. siderea*, and *S. intersepta*. In 2003, samples were taken once more from colonies of *M. faveolata*, *P. astreoides*, and *S. siderea* at the 2-m, 8-m, and 25-m locations, whereas *M. annularis* was sampled at the 8-m site also. Because of a limited number of *M. faveolata* colonies at 2 m, the same colonies were sampled in both years. New corals were selected in 2003 at the 8-m and 25-m sites, with the exception of six colonies of *M. faveolata* and *M. annularis*, which were sampled in both years at 8 m. In each year, 4–13 coral colonies were chosen, and the chlorophyll fluorescence was measured (described later) on the uppermost portion of the colony facing direct downwelling irradiance, after which a 2-cm piece of coral was removed for algal isolation from the same area previously sampled for chlorophyll fluorescence.

Algae were removed from collected coral fragments with an airbrush and isolated from the coral tissue by centrifugation. Algal pellets were stored in a DNA preservation buffer (20% dimethyl sulfoxide, 0.25 mol L⁻¹

ethylenediaminetetraacetic acid in sodium chloride-saturated water) (Seutin et al. 1991) until processed further. DNA was extracted using a Wizard isolation kit (Promega) following the protocols listed in LaJeunesse et al. (2003). The ITS2 was amplified using previously developed primers ITS2 clamp and ITSintfor2 with a touch-down polymerase chain reaction (PCR) protocol, and amplified PCR fragments were run on denaturing gradient gels (45–80%) as previously described (LaJeunesse 2002). Band patterns (fingerprints) from denaturing gradient gels were compared to a database from the same coral species sampled at other locations in the Caribbean. If any band pattern appeared unique, the prominent band was excised from the gel, eluted overnight, re-amplified, and subsequently cycle-sequenced in both directions using an ABI big dye terminator cycle sequencing ready reaction kit (PE Applied Biosystems) and analyzed on an Applied Biosystems 310 sequencer. Resulting sequences were then aligned against previously collected ITS2 data from the Caribbean to confirm their ITS2 designation following established terminology for these methods (LaJeunesse 2001; LaJeunesse 2002).

In situ algal photobiology—Photochemical capacity of PSII was measured two times each day with a pair of submersible pulse amplitude modulated fluorometers (Diving PAM, Walz). Dark-acclimated maximum PSII quantum yield (F_v/F_m) was recorded in the early morning (between 05:00–05:30 h) near sunrise and the effective quantum yield ($\Delta F/F'_m$), also referred to as the PSII activity in the light-acclimated state, was recorded at mid-day (between 12:00–13:30 h) during peak sunlight exposure. Measurements were performed at each site on different days with only occasional light cloud cover during sampling times in 2002 and clear days in 2003. Maximal light intensity was $1,664 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$, $762 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$, and $397 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$ at 2 m, 8 m, and 25 m in 2003 and similar values were recorded in 2002. In addition to the colonies sampled for algal DNA typing, four to eight additional random colonies of each species listed above were sampled for chlorophyll fluorescence in 2002 at the 8-m site. Preliminary work showed that there was no significant difference between morning F_v/F_m values and those recorded approximately one hour after sunset (data not shown). For each coral, three independent readings of F_v/F_m and $\Delta F/F'_m$ were taken by moving the fiber optic of the fluorometer to a new location within approximately 1–2 cm of the starting measuring point. An opaque polyvinyl chloride cover was attached to the end of each fiber optic bundle to standardize the distance between the coral surface and the optics and to provide 30 s of dark acclimation before each $\Delta F/F'_m$ measurement. The maximum excitation pressure over PSII (Q_m) was calculated as in Iglesias-Prieto et al. (2004) as $Q_m = 1 - [\Delta F/F'_m / F_v/F_m]$. Nonphotochemical quenching (NPQ) of chlorophyll fluorescence was calculated as $\text{NPQ} = [F_m - F'_m] / F'_m$.

Short-term experimental thermal stress—Two short-term thermal stress experiments were conducted in 2003 to test

for differences in thermally induced photoinhibition between *M. faveolata* and *P. astreoides*. These two species were selected based on preliminary in situ data that indicated the two coral species displayed different levels of maximum excitation pressure over PSII, and *M. faveolata* can show greater signs of bleaching in the field (M. Warner, unpubl. data). For each experiment, two pieces from replicate colonies ($n = 3$ colonies species⁻¹, fragment size approximately 15 cm^2) of *M. faveolata* and *P. astreoides* were sampled from 8 m, and one piece from each colony was placed into one of two shallow opaque plastic bins receiving a continuous supply of seawater (1.3 L min^{-1}) and aeration provided by air stones. One bin was heated by two aquarium heaters (Visitherm Aquarium Systems, Mentor, Ohio), and water temperature was monitored by submersible temperature loggers (Water TempPro, Onset Instruments). The water temperature in each bin fluctuated because of daily solar heating such that the control bin had a daily temperature range of 29.5–31.3°C, and the heated bin had a temperature range of 31.6–33.1°C. Window screening was placed over both bins to replicate the near maximal irradiance ($750 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$) measured at the 8-m site with a submersible light sensor (Li-Cor 192SA, LiCor Biosciences, Lincoln, Nebraska).

For each sample, replicate measurements of F_v/F_m ($n = 3$) were recorded at 05:30 h each day for three days, and $\Delta F/F'_m$ was recorded at 20:00 h and then 24 h and 48 h thereafter in the experimental run. For the second thermal stress experiment, samples were collected from new colonies of *M. faveolata* and *P. astreoides* at 8 m, and F_v/F_m was recorded at the start of the experiment (21:00 h), 24 h, and 48 h following thermal exposure. At the end of the second experiment, dark-acclimated corals were also sampled for rapid induction of chlorophyll fluorescence using a Handy PEA fluorometer (Hansatech instruments). The polyphasic fluorescence transient (Strasser et al. 1995) was recorded after applying a 1-s pulse of saturating red light (peak at 650 nm, $3,000 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$). Relative variable fluorescence was calculated as $V_t = (F_t - F_o) / (F_m - F_o)$, where F_t is the fluorescence at time t , and F_o and F_m are the minimal and maximal fluorescence values, respectively. The initial rates of photochemistry (dV/dt_o) were calculated by measuring the initial slope of relative variable fluorescence between 20 μs and 300 μs (Strasser et al. 2000). At the end of each experiment, zooxanthellae were isolated from all corals for algal typing as described previously.

Statistical analyses—Data were tested for assumptions of normality and homogeneity of variance, and if necessary, were transformed before further analyses. Possible differences in in situ F_v/F_m , $\Delta F/F'_m$, Q_m , and NPQ within and between different corals were tested by a one-way analysis of variance (ANOVA) for each site. Data collected from coral species studied in both years were compared by a two-factor ANOVA to investigate potential differences between sampling years. When appropriate, intra- and interspecific differences were

compared by Tukey/Kramer multiple comparisons testing.

Results

Algal distributions—Across their reef distributions, each coral species associated with either one or, more commonly, a particular subset of symbiont types. Genetic characterizations identified a total of 15 distinguishable ITS types. *Symbiodinium* clades A (A3, A3b, A4, and A4a), B (B1, B5, B17), and C (C1a, C3, C3e, C3g, C7, C16, C54) were well represented. One colony of *M. faveolata* at 2 m hosted a clade D population (type D1a) (Table 1). In some cases, two fingerprints could be distinguished in the same sample, yet there was always a more prominently stained band, which was scored as the dominant alga. There was significant variation in the dominant symbiont when comparing different colonies at a particular depth in six out of the eight coral species surveyed. This trend was most prominent between colonies of *S. intersepta*, *M. faveolata*, and, to a lesser extent, *M. annularis* that displayed a high level of variation in algal type at the cladal and intra-cladal levels, especially at the shallow and intermediate depths (Table 1). Conversely, all colonies of *Siderastrea radians* and *S. siderea* contained a single dominant alga at all sampling locations (types B5 and C,3 respectively, Table 1).

As expected, corals capable of harboring multiple symbionts across a bathymetric range displayed a transition from the dominant symbiont type(s) when comparing the same species at several depths, however this transition was not always consistent between different species. For example, equivalent amounts of type A or B zooxanthellae were found in *M. faveolata* at 2 m, whereas from 8–25 m the host population transitioned to exclusively hosting the C7 symbiont. *P. astreoides* colonies at 2 m contained type A4, at 8 m, however, a closely related type, A4a, was found in 80% of the colonies as well as three colonies sampled at 15 m. Meanwhile all *P. astreoides* sampled at 25 m contained C1a, a symbiont type unique to this coral species (Table 1).

In a majority of cases, the pattern of symbiont distribution within a particular coral species was consistent between the two sampling years. Some variability was

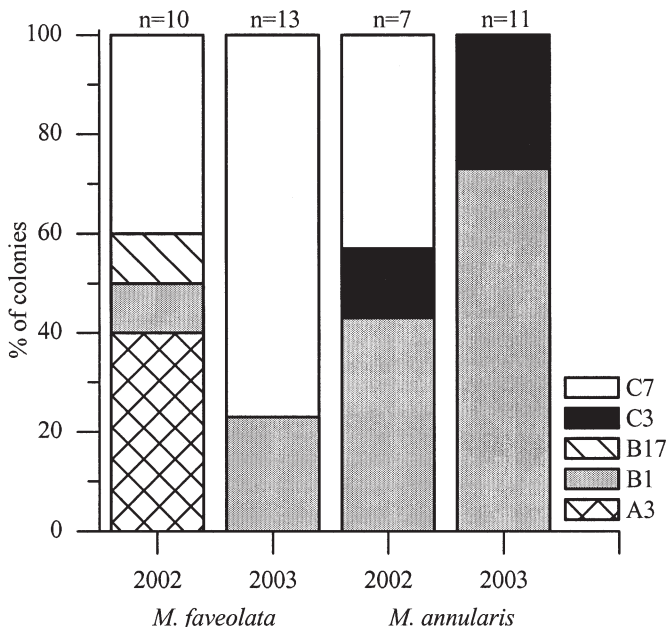


Fig. 1. Distribution of zooxanthellae types identified by ITS2 within *M. faveolata* and *M. annularis* at 8-m depth in 2002 and 2003.

noted for *M. faveolata* at the intermediate 8-m site wherein an equal number of colonies sampled in 2002 contained A3 or C7 symbionts, whereas in 2003, the dominant symbiont in the majority of colony samples was the C7 alga while the A3 alga was not detected (Fig. 1). Notably, of the six fixed colonies of *M. faveolata* that were sampled in both years, three contained A3 in 2002. Slightly less between-year variability in symbiont type was found for *M. annularis* at the same depth (Fig. 1), and these two coral species showed marked differences in symbiont composition in 2003, as the C7 symbiont dominated colonies of *M. faveolata* while the B1 symbiont was more common on the top portions of *M. annularis* at 8-m depth.

In situ PSII performance—In some cases, the high variability in algal type (at the level of ITS designation) between different colonies of the same coral species

Table 1. Host species and symbiont types identified by PCR–denaturing gradient electrophoresis at four depths sampled in 2002 and 2003 during in situ fluorescence measurements. Superscripted numbers represent the number of sampled colonies found to harbor the particular ITS2 type.

Coral	Depth			
	2 m	8 m	15 m*	25 m
<i>A. lamarki</i> *	na	na	na	C3b ⁴ , C3a ¹
<i>M. cavernosa</i> *	na	C3 ⁶ , C3e ¹ , C3g ¹	C3 ¹ , C3e ¹ , C3g ³	C3e ⁵
<i>M. annularis</i>	na	B1 ¹¹ , C7 ⁶ , C3 ¹	C7 ⁶	na
<i>M. faveolata</i>	A3 ³ , B1 ² , B17 ¹ , D1a ¹	A3 ⁴ , C7 ¹⁴ , B1 ⁴ , B17 ¹	C7 ⁸	C7 ¹⁰
<i>P. astreoides</i>	A4 ⁴	A4a ⁸ , A4 ²	A4a ³	C1a ⁵
<i>S. siderea</i>	C3 ⁶	C3 ¹²	na	C3 ⁶
<i>S. radians</i> *	B5 ⁶	na	na	na
<i>S. intersepta</i> *	na	A3b ² , A3 ¹ , C16 ² , C3 ¹ , C54 ¹	A3b ² , A3 ² , C3 ¹ , C16 ²	C16 ⁴ , C3 ²

na, species was not present at study site at that depth.
* Samples collected only in 2002.

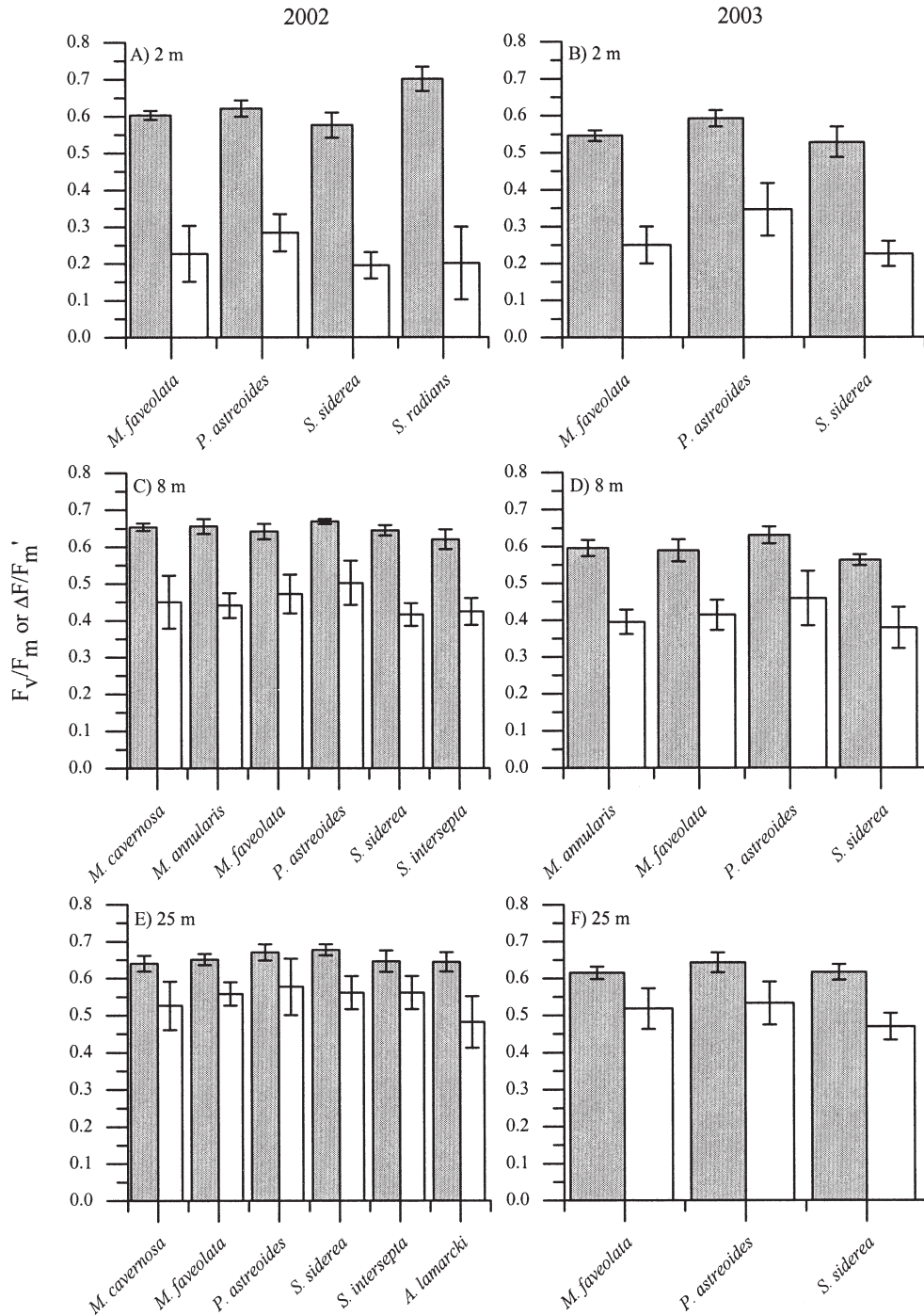


Fig. 2. Dark-acclimated quantum yield, F_v/F_m (shaded bars), and mid-day effective quantum yield, $\Delta F/F_m'$ (open bars), in different coral species residing at (A, B) shallow, (C, D) intermediate, and (E, F) deep reef locations recorded in 2002 (A, C, and E) and 2003 (B, D, and F). For each bar, $n = 4-15 \pm$ SD.

precluded adequate replication to test for intraspecific differences in fluorescence parameters (e.g., *S. intersepta* at all depths and *M. faveolata* at 2 m). When sample sizes were adequate, no significant relationships were detected at the intraspecific level in any measured parameters of chlorophyll fluorescence in either year, thus fluorescence data for each coral species were combined in each year before further statistical analysis. Slight yet significant

differences were detected in dark-acclimated quantum yield (F_v/F_m) between some corals at each site in each year (Fig. 2A–F). At the 2-m site in 2002, F_v/F_m within *S. radians* was significantly higher ($p < 0.001$) than all other corals, and there was no significant difference in F_v/F_m between other corals sampled, whereas in 2003, F_v/F_m in *P. astreoides* was significantly higher as compared to *M. faveolata* and *S. siderea* (Fig. 2 A,B). Likewise, *P.*

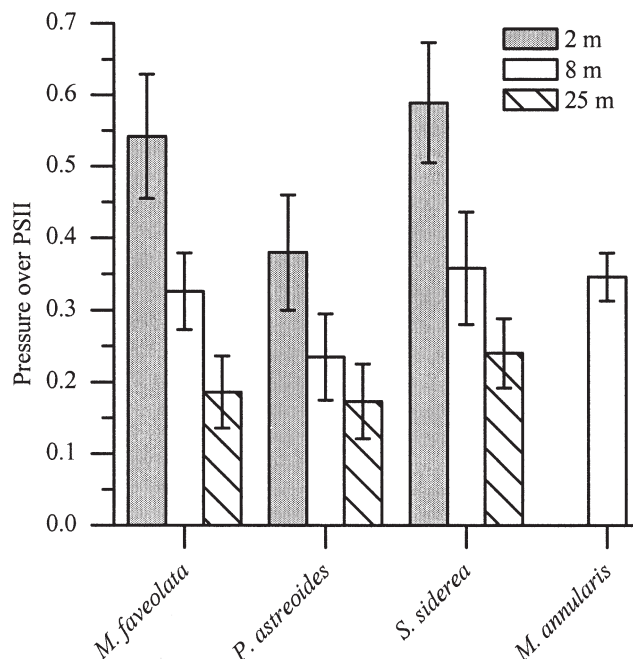


Fig. 3. Maximal excitation pressure over PSII (Q_m) in zooxanthellae within three coral species sampled in 2003 at 2-m, 8-m, and 25-m depth. For each bar, $n = 6-15 \pm$ SD.

astreoides displayed the highest light-acclimated PSII activity with a significantly higher $\Delta F/F'_m$ at 2 m compared to all other corals at this site in both years. At 8 m, symbionts within *P. astreoides* consistently had the highest F_v/F_m values of all corals sampled in both years. Meanwhile, $\Delta F/F'_m$ in *P. astreoides* at 8 m was significantly greater than all corals except *M. faveolata* in 2002, whereas mid-day effective quantum yield in *S. siderea* (0.416 ± 0.031) was significantly lower ($p < 0.001$) than these two corals (Fig. 2C).

In 2003, $\Delta F/F'_m$ in *P. astreoides* was significantly higher than all other corals at the intermediate site (Fig. 2D). At 25 m, dark-acclimated F_v/F_m in *P. astreoides* and *S. siderea* was significantly greater than all other corals in 2002, and this parameter was significantly higher in *P. astreoides* relative to other corals sampled in 2003 ($p = 0.015$) (Fig. 2E,D). In general, there was less difference in mid-day PSII activity between coral species at the deepest site, with the exception of $\Delta F/F'_m$ for *A. lamarki*, which was significantly lower ($p < 0.0001$) than all other corals sampled in 2002 (Fig. 2E). In 2003, the effective quantum yield in *P. astreoides* at 25 m was significantly greater ($p = 0.024$) than *S. siderea*, but not *M. faveolata* (Fig. 2F). Nonphotochemical quenching (not shown) and maximum excitation pressure over PSII (Q_m) covaried with depth, as both variables decreased in all corals when comparing samples from shallow to deep sites (Fig. 3). Maximum excitation pressure over PSII and nonphotochemical quenching were significantly lower in symbionts within *P. astreoides* as compared to algae within *M. faveolata* and *S. siderea* at 2 m and *M. annularis*, *M. faveolata*, and *S. siderea* at 8 m ($p < 0.006$ for 2-m sites and $p < 0.005$ for 8-m sites) (Fig. 3). At 25 m, maximum excitation pressure

over PSII was significantly higher in *S. siderea* compared to *M. faveolata* and *P. astreoides* (Fig. 3).

Experimental thermal stress—Brief exposure to elevated temperature (33.1°C) resulted in a significant loss in dark- and light-acclimated PSII activity within symbionts in both *P. astreoides* and *M. faveolata* relative to control samples held at a maximal temperature of 31.3°C (Fig. 4A). By 36 h, there was a greater loss in effective quantum yield in the heat-treated *M. faveolata* as compared to *P. astreoides*, yet some recovery was noted when F_v/F_m was recorded at sunrise the next day. However, this loss in PSII activity within *M. faveolata* continued to occur to a greater extent relative to *P. astreoides* throughout the rest of the experiment. When the algal type within each replicate colony of *M. faveolata* was accounted for and compared to the fluorescence response, two replicates contained the B1 symbiont and showed a higher degree of partial daily recovery of dark-acclimated quantum yield during the thermal stress relative to the third replicate, which harbored the C7 symbiont and showed a complete loss of night-time recovery in F_v/F_m by 48 h (Fig. 4B). All samples of *P. astreoides* contained A4a zooxanthellae.

When the thermal stress experiment was repeated with three more colonies of *M. faveolata* from 8 m, all three colonies were found to contain the C7 symbiont and showed a fluorescence pattern that closely matched the one C7 replicate from the first experiment (Fig. 4B, compare closed to open squares). The fragments of *P. astreoides* used in the second thermal experiment contained A4a zooxanthellae and showed a similar photosynthetic response as seen in the first experiment shown in Fig. 4A (data not shown). Figure 5 shows the rapid chlorophyll-fluorescence induction curves at the end of the second thermal stress experiment, which displayed a typical polyphasic kinetic pattern with O-J-I-P transients that represent the successive reduction of the electron acceptor pool of PSII (Strasser et al. 1995). Exposing *M. faveolata* to 33.1°C for 48 h resulted in a significant rise ($p < 0.001$) in the initial portion of the fluorescence transient at approximately 1 ms, which is seen as the “J” step in the fluorescence transient, representing the accumulation of Q_A to Q_{A^-} in PSII. Meanwhile, there were no significant differences between the rapid fluorescence transients recorded in *P. astreoides* held at the two temperatures by this time (Fig. 5A,B). The significant rise in the fluorescence transient in *M. faveolata* was clearly seen when comparing the slope at the origin of the relative variable fluorescence curves (dV/dt_0) measured between 10 μs and 300 μs (inset of Fig. 5B).

Discussion

Algal distributions and in situ photobiology—The genetic diversity of *Symbiodinium* coupled with the capacity for some coral species to harbor multiple symbionts has led to the suggestion that flexibility in the degree of specificity between both partners may serve as a mechanism whereby some reef-building corals could acclimatize to climate change (Baker 2003). Although it is important to empiri-

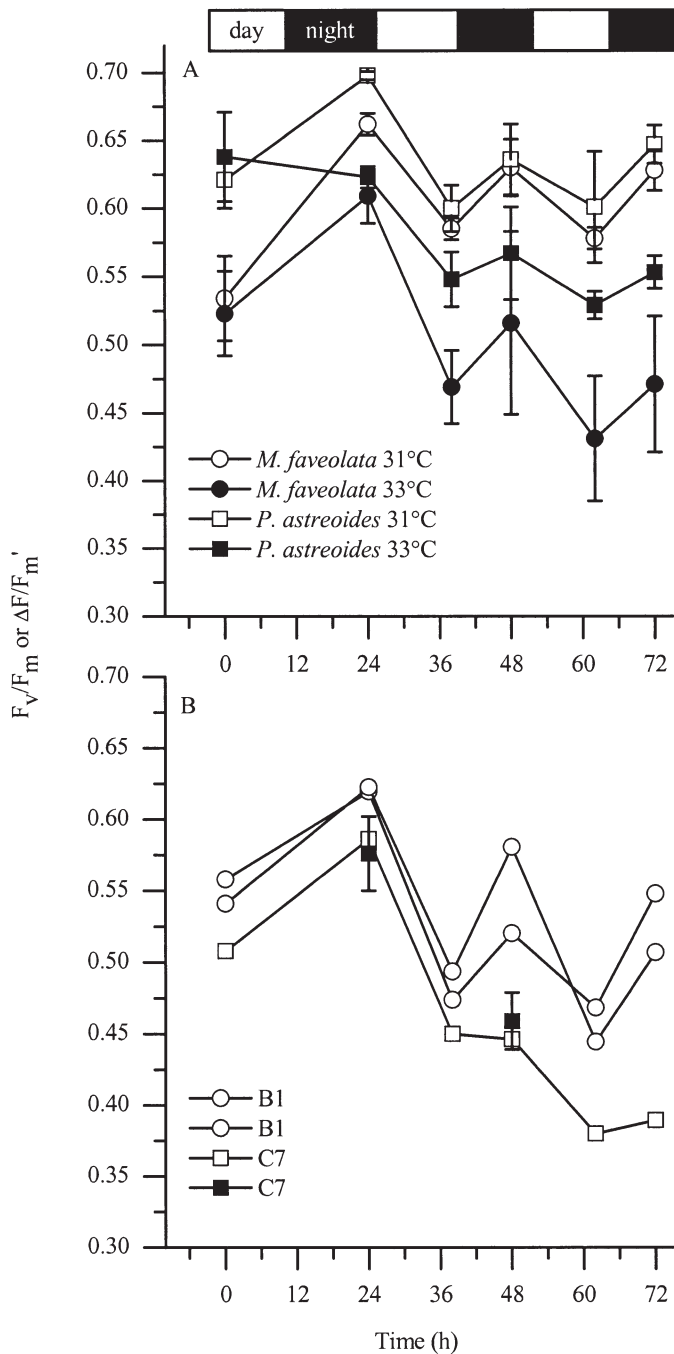


Fig. 4. (A) Diel fluctuation in dark acclimated PSII quantum yield, F_v/F_m , and light acclimated effective quantum yield, $\Delta F/F_m'$, in zooxanthellae within *M. faveolata* and *Porites astreoides* exposed to a maximal daily temperature of 31.6°C or 33.1°C and (B) the ITS type of zooxanthellae within each replicate of *M. faveolata* shown in panel A and three additional replicates of *M. faveolata* (filled squares) in a second independent thermal experiment.

cally test for potential symbiont change and acclimatization, there is also a need to better understand potential physiological differences between these symbionts within different reef corals in relation to their bathymetric distribution across a reef.

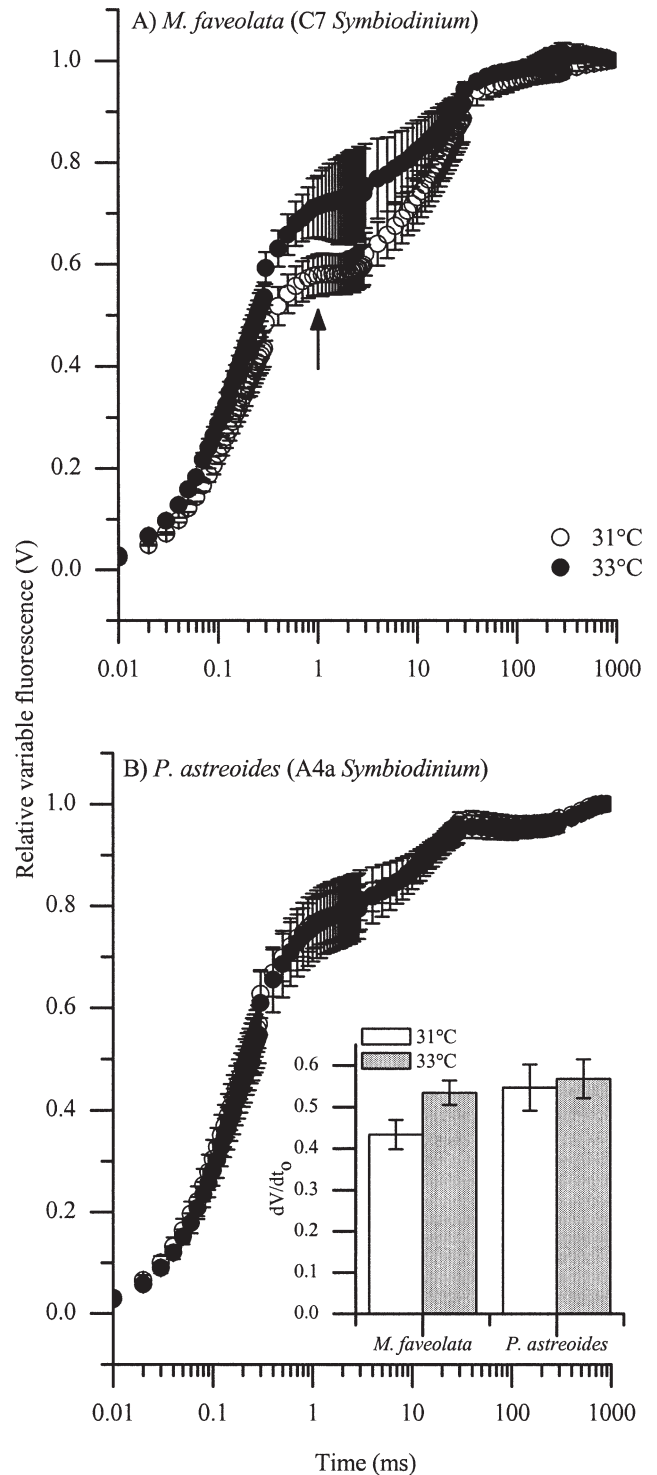


Fig. 5. Rapid induction of chlorophyll fluorescence in zooxanthellae within (A) *M. faveolata* and (B) *P. astreoides* after exposure to a maximal temperature of 31.6°C or 33°C for 48 h. Arrow designates the "J" step of the induction curve. Inset in panel B represents the slope of the initial fluorescence rise from the induction curves in both panes. For each point, $n = 3 \pm SD$.

Algal surveys from this work revealed a range of symbiont diversity between different corals with several representative algal ITS types that have been noted to

occur in northern areas of the Yucatan (Mexico) as well as other areas of the Caribbean (LaJeunesse 2002). Each of the host taxa we studied associated with a narrow subset of the diversity of *Symbiodinium* spp. that occur in this region (LaJeunesse et al. 2004 unpub. data). Host identity appears to be the most important factor determining the distribution of a particular symbiont. Beyond this, external conditions, notably irradiance, influence the distribution of these symbionts on a bathymetric scale. Intraspecific variability in algal type was observed at the shallow and intermediate sites in some hosts (e.g., *M. faveolata* and *S. intersepta*), and similar symbiont variability was previously noted at a similar depth range (5 m) in *M. annularis* and *M. faveolata* in Panama (Rowan et al. 1997). While a clade B alga was dominant in the uppermost portions of colonies of *M. faveolata* between 1–5 m in Panama, this current study shows that the C7 alga is quite common in sun-exposed portions of *M. faveolata* at intermediate depths (8 m) in this region of the western Caribbean. The presence of different symbionts in two adjacent colonies of the same coral under the same light field is explainable. First, different algal types exposed to a light field intermediate between their respective adaptive ranges would function similarly. Without a clear competitive advantage, the dominance of one algal type over another may result from subtle dynamic events under internal and/or external control, such as cellular mechanisms involved in initiation of the symbiosis or physical forcing from light, temperature, or nutrients. Second, while two or more symbionts from different clades are evolutionarily divergent, we may presume that convergence in physiological adaptations to high or low light has occurred among symbionts in different clades (Tchernov et al. 2004). Thus, while genetically unique, several *Symbiodinium* may share the same ecotype designation based on similar distribution and physiology.

At a particular depth, no significant differences in in situ chlorophyll fluorescence were measured between different algae from different colonies of the same coral species. If slight differences do exist, a greater number of colonies may require monitoring, because several of our comparisons were skewed by highly unequal sample sizes of different symbiont types. Denaturing gradient electrophoresis (DGGE) analysis consistently revealed one prominent band that was scored as the dominant alga, which was assumed to contribute the most to the fluorescence signal. Some DGGE samples did show faint secondary bands that matched other algal types known to occur in the host at other depths. To what degree the presence of additional, albeit secondary, algal populations influence chlorophyll fluorescence signals remains open for further investigation (Bhagooli and Hidaka 2004). Investigating the photophysiology of certain coral species (e.g., the *Montastraea annularis* complex) is further complicated since dominance of a particular symbiont may shift over time in response to the prevailing environment (Thornhill et al. 2006). The apparent loss of A3 zooxanthellae in *M. faveolata* colony samples at 8 m in 2003 (Fig. 1) may have represented a short-term shift from the dominance of this alga or further microscale variation in symbiont type. Excessive

spatial and temporal sampling across an individual colony would be needed to confirm this finding. Likewise, one must also consider the potential influence the host coral may have in modulating photosynthetic response such as by skeletal reflectance (Enriquez et al. 2005), tissue thickness and polyp contraction (Brown et al. 2002), or different host pigments (e.g., pociloporins or green fluorescent protein homologues) which may lessen available light (Dove 2004).

As expected, there was a significant effect of depth for both F_v/F_m and $\Delta F/F'_m$ in all corals (Fig. 2), as fluorescence yields increased when moving from shallow to deep reef habitats. Previous studies have documented a similar trend in corals in other areas of the Caribbean (Lesser and Gorbunov 2001; Warner et al. 2002) as well as in cultured zooxanthellae grown under different light intensities (Robison and Warner in press). Low variable fluorescence measured in shallow waters largely reflects down-regulation of PSII activity due to photoacclimation. Type A4 or A4a zooxanthellae within *P. astreoides* consistently displayed higher values of dark- and light-acclimated PSII activity relative to most other zooxanthellae in other corals at the same depths in both years. This higher level of PSII capacity could mean that these symbionts are more resistant to photodamage on a daily basis as well as during further physical perturbation such as high temperature exposure.

Maximum excitation pressure over PSII was significantly lower within *P. astreoides* at 2 m and 8 m as compared to *M. faveolata*, *M. annularis*, and *S. siderea*. The maximum excitation pressure represents the interaction of photochemical and nonphotochemical processes that are occurring simultaneously in the algae and serves as a rapid proxy of their physiological state. As Q_m approaches 1.0, a higher proportion of PSII reaction centers are closed, thereby increasing the potential for photoinhibition (Iglesias-Prieto et al. 2004; Maxwell et al. 1995). While the average maximal PSII excitation pressure found for *M. faveolata* and *S. siderea* at 2 m (0.57 ± 0.025) was approximately the same level recorded for *Pavona gigantea* undergoing chronic photostress when transplanted from 10 m to 2.7 m (Iglesias et al. 2004), it is clear that this level of Q_m does not inhibit photochemical capacity to such a degree to impede the coral species in this current study from successfully inhabiting this shallow depth. Conversely, the higher Q_m noted in *S. siderea* at 25 m in 2003 may indicate that the symbionts within this coral are operating at higher photosynthetic rates compared to those in *P. astreoides* and *M. faveolata* at this depth. Whereas Iglesias et al. (2004) demonstrated how differences in Q_m between corals residing at different depths provide evidence for niche diversification via symbiont performance, this current work shows that Q_m differences between coral species residing at the same depth is much smaller and cannot be interpreted in the same manner. Nevertheless, this work does show that, in some cases, significant differences in maximum excitation pressure do correlate with the potential for disruption in PSII function when a physical stress such as elevated temperature is applied (described following).

Experimental thermal stress—Under thermal stress, the symbionts within *P. astreoides* and *M. faveolata* displayed heat-induced photoinhibition via a drop in F_v/F_m and $\Delta F/F'_m$, corresponding to similar findings of previous studies (Warner et al. 1996; Jones et al. 1998; Warner et al. 1999). However, PSII activity declined at a slower rate in *P. astreoides* (Fig. 5A). *M. faveolata* colonies bearing two different symbiont types also reacted differently to this thermal stress. The C7 symbiont had a greater loss of photosynthetic potential than the B1 alga (Fig. 4). In particular, this alga had lost the capacity for recovery from dynamic diel photoinactivation as $\Delta F/F'_m$ remained low at the end of each day, while B1 symbionts in other colonies achieved some degree of recovery. This loss in diel recovery of $\Delta F/F'_m$ represents a chronic loss in photosynthetic function, similar to that seen in zooxanthellae under acute thermal and light stress (Franklin et al. 2004). The reduction or loss in daily synthesis of new PSII reaction centers most likely contributes to this decline in daily recovery (Warner et al. 1999; Takahashi et al. 2004).

Further analysis of rapid chlorophyll *a* fluorescence induction revealed impairment in the rate of primary photochemistry in PSII in the C7 alga, as the slope at the origin of the relative variable fluorescence curve and the J-step of fluorescence induction increased significantly after thermal exposure, whereas this effect was not seen in the A4a alga within *P. astreoides* (Fig. 5). Similar results for thermal disruption in electron transport in PSII have been noted in terrestrial plants (Srivastava et al. 1997), cultured *Symbiodinium kawagutii* (Iglesias-Prieto 1997), and recently in zooxanthellae within Pacific corals following shorter periods of thermal stress than used in this study (Hill et al. 2004). The rise in the initial portion of the fluorescence induction curve is most likely caused, in part, by a loss of re-oxidation capacity of the primary electron acceptor quinone (Q_A) as has been noted in some cultured zooxanthellae (Iglesias-Prieto 1997; Robison and Warner in press), which can lead to further irreversible damage to the acceptor side of the PSII reaction center (Warner et al. 1999). Interestingly, the V(J) of *P. astreoides* was higher than that for *M. faveolata* at the start of the experiment, thereby indicating that symbionts within *P. astreoides* had some reduced electron flow prior to thermal exposure. This difference may represent a photoacclimatory response in *P. astreoides* that may further confer some protection to the alga in this coral once thermal stress is applied. However, further work is needed to fully understand how other factors such as shifts in absorption cross section coupled with pigment and/or algal cell loss may influence the fluorescence induction signal during thermal stress.

Previous work has shown that in some cases corals that display a greater reduction in in situ $\Delta F/F'_m$ are more susceptible to natural bleaching (Lombardi et al. 2000). An important result of this current work is the link between in situ maximum excitation pressure over PSII and the potential for thermally enhanced photoinhibition related to coral bleaching. The rise in Q_m correlates to an elevated level of nonphotochemical quenching in these algae (Iglesias-Prieto 2004; this study, data not shown) and may explain one pathway by which some zooxanthellae

become susceptible to PSII damage during thermal stress. The capacity to increase energy dissipation via nonphotochemical pathways to alleviate potential over-reduction of PSII and subsequent oxidative stress is an important defense mechanism in many photoautotrophs (Macintyre et al. 2000; Holt et al. 2004) and in some zooxanthellae during thermal stress (Warner et al. 1996). As normal diel energy dissipation is already high in symbionts within corals such as *M. faveolata*, *M. annularis*, and *S. siderea*, additional stress that negatively impacts photosynthetic electron flow can overwhelm the remaining sources of nonphotochemical energy dissipation in these algae.

It is important to note that the relationship between elevated Q_m and bleaching potential is not necessarily ubiquitous for all *Symbiodinium*-host coral combinations. For example, *Siderastrea radians* displayed a significant reduction in effective quantum yield and high nonphotochemical fluorescence quenching at the 2-m site, yet this coral has shown to be quite tolerant to bleaching conditions (Warner et al. 1996). Likewise, *S. radians* still maintained high F_v/F_m , which is in contrast to *M. faveolata* and *S. siderea*, that both displayed lower F_v/F_m and Q_m . Resistance to thermal stress may be caused by several things, including additional capacity to elevate NPQ, sustained PSII repair, compensatory electron flow through remaining functional reaction centers (Behrenfeld et al. 1998), or structural differences in the thylakoid membrane (Iglesias-Prieto et al. 1992; Tchernov et al. 2004). While rapid in situ chlorophyll fluorescence signatures can provide insight into the daily homeostasis and stress response of these algae, additional work is needed in this area to better understand the affect that intraspecific and interspecific variation in symbiont type may have on these symbioses.

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