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Effect of short-term exposure to elevated temperatures and light levels on photosynthesis of different host–symbiont combinations in the *Aiptasia pallida*/ *Symbiodinium* symbiosis

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Abstract

The physiology exhibited by symbioses between dinoflagellates (zooxanthellae) and hosts such as reef corals may be dictated by the host, the symbiont, or the synergistic effect of both partners. We compared the oxygen fluxes of two laboratory populations of the symbiotic sea anemone *Aiptasia pallida*, originally collected from Bermuda and Florida. *A. pallida* from Bermuda contained clade B zooxanthellae and *A. pallida* from Florida hosted clade A zooxanthellae. Both freshly isolated zooxanthellae and intact anemones were compared as a function of light intensity at culture (25°C) and elevated (32°C, 34°C) temperatures. Zooxanthellae isolated from the Florida anemones had higher net photosynthetic rates at 32°C (P_{max}: 6.2 ± 0.9 μmol O_2 h^{-1} μg chlorophyll *a* [Chl *a*]$^{-1}$ vs. 0.7 ± 3.6 for Bermuda algae), with the Bermuda algae exhibiting pronounced photoinhibition at higher irradiances. At 34°C, the clade A (Florida) symbionts had lower net photosynthesis than at 32°C (P_{max}: 3.6 ± 2.2 μmol O_2 h^{-1} μg Chl a^{-1}), but the Bermuda symbionts had high rates of net O_2 consumption at all irradiances. The intact Bermuda anemones, however, exhibited much less pronounced effects at elevated temperatures than did the isolated Bermuda zooxanthellae. Bermuda anemones containing the Florida symbionts had higher net oxygen fluxes than those with the Bermuda symbionts at elevated temperatures and irradiances. This study demonstrates that the physiology of the intact symbiosis is dictated by both partners and that studies of the intact symbiosis may not fully reveal the thermal liability of the algae.

Symbioses between cnidarians (e.g., corals, octocorals, sea anemones) and dinoflagellates (zooxanthellae) are crucial for the operation of coral reef ecosystems. Metabolic exchange between the partners is pivotal, with the zooxanthellae providing photosynthetic energy to the host while the host provides protection and nitrogenous wastes (Muscatine and Porter 1977). Much recent attention has been given to the diversity of these symbionts and their distribution (Baker 2003; Goulet and Coffroth 2004; Lajeunesse et al. 2004). There has been some consideration of how this diversity affects photosynthetic characteristics (reviewed in Lesser 2004). There is, however, little information about whether symbionts associated with the same host species display wide or narrow physiological ranges. For example, particular zooxanthella and host genotypes may have wide tolerances, allowing the association to exist over a wide latitudinal range or a large depth range on a reef. On the other hand, a host species might harbor different symbionts over a depth or latitudinal range, with the symbionts having narrower tolerances that are appropriate for a particular environment (Rowan and Knowlton 1995; Rowan 2004). How host differences might affect depth-related or latitudinal differences has received less attention.

The use of molecular techniques has facilitated the identification of zooxanthellae, and it is clear that there is a wide diversity. DNA sequences for small and large subunit ribosomal RNA have resulted in seven major clades (reviewed in Baker 2003), with each clade having multiple species (Rowan 1998). This diversity may have correlates in physiological differences. Increased seawater temperature can cause coral bleaching, i.e., the loss of zooxanthellae and/or their pigments (Glynn 1996). Numerous studies have shown that zooxanthellae can differ in their responses to elevated temperatures (Iglesias-Prieto and Trench 1997; Perez et al. 2001; Bhagooli and Hidaka 2003). The genetic identity of the zooxanthellae was not determined in these studies.

Physiological differences have been proposed to explain the distribution of zooxanthella genotypes. For example, corals in the *Montastraea* species complex harbor a variety of symbionts, the distribution of which may be related to irradiance levels and tolerance of elevated temperatures (Rowan 1998). Colonies of these corals in very shallow water (high irradiance) harbor clade A and/or B zooxanthellae, while

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those in deeper water (low irradiance) harbor clade C algae (Rowan and Knowlton 1995). Symbionts of the temperate sea anemone *Anthopleura elegantissima* exhibit a latitudinal gradient in distribution that may be related to temperature tolerance (Lajeunesse and Trench 2000). These genetic studies, however, did not include physiological experiments. Relatively few studies have considered both the physiology and genetic identity of zooxanthellae. These include synthesis of mycosporine-like amino acids (MAAs) (Banaszak et al. 2000), growth rates of cultures (Kinzie et al. 2001), photosynthesis irradiance curves at ambient temperatures (Savage et al. 2002), chlorophyll fluorescence (Iglesias-Prieto et al. 2004; Rowan 2004), and lipid composition (Tchernov et al. 2004).

The interaction of light and temperature is critical in coral bleaching events, yet few studies have examined how these interactions affect different host–symbiont genotypic combinations. In this study, we examine the effects of light and elevated temperature on oxygen flux in two laboratory clones of the sea anemones *Aiptasia pallida* from Bermuda and Florida. The Florida source population naturally experienced higher temperatures than that from Bermuda. Our objective was to determine how different isolated zooxanthellae and host–symbiont genotypic combinations varied in their photosynthesis–irradiance curves both at ambient and elevated temperatures. We determined the effect of elevated temperatures on isolated algae and naturally occurring intact anemones: Bermuda anemones with Bermuda zooxanthellae and Florida anemones with Florida zooxanthellae. In addition, we manipulated the association by infecting Bermuda anemones with Florida symbionts. We then determined how this new genotypic combination performed physiologically at ambient and elevated temperatures.

Methods

Experimental organisms—We used two clones of *Aiptasia pallida*. One clone, described in Cook et al. (1988), was originally collected in 1985 from Walsingham Pond (32.348°N, 64.710°W), an anchialine pond (landlocked marine pond with access to seawater via fissures or caves) in Bermuda (Thomas et al. 1991). Mean temperature of this pond from 1980 to 1989 was 23.2° ± 3.3°C (range = 19.0–27.7°C) (Thomas et al. 1991). The second clone was collected from a piling at the Key Largo Marine Research Laboratory (25.098°N, 80.441°W) in Key Largo, Florida, in March 1999. We used data from the National Oceanic and Atmospheric Agency moored buoy in Florida Bay near Long Key (LONF1; www.ndbc.noaa.gov; 24.84°N, 80.86°W) to provide comparable temperature data. From 1996 to 1999, the mean temperature at LONF1 was 26.5°C ± 4.0°C (range = 14.2–34.4°C). Symbiotic anemones and aposymbiotic individuals of the Bermuda clone (Muller-Parker et al. 1990) were routinely maintained in 1.2-μm-filtered sea water (FSW). Symbiotic anemones were kept in an incubator at 25°C and 55 μmol photons m^{-2} s^{-1} (12 h light : 12 h dark), and the aposymbiotic anemones were kept in darkness at room temperature. All anemones were fed biweekly with brine shrimp nauplii.

In addition to the native Bermuda and Florida combinations, we created a heterologous combination of Bermuda anemones with Florida zooxanthellae by infecting aposymbiotic Bermuda anemones with zooxanthellae freshly isolated from Florida anemones (cf. Perez et al. 2001). These anemones were maintained in culture for more than a year before use.

Genetic identification of zooxanthellae—The cladal identity of the zooxanthellae obtained from Florida and Bermuda anemones and the Florida symbionts that had been maintained in Bermuda anemones was confirmed using a polymerase chain reaction amplification of DNA encoding for the small subunit ribosomal RNA (ssRNA) as described in Goulet and Coffroth (2003*a*).

Respirometry—Dark respiration and net photosynthesis (P_{net}) were measured in glass incubation chambers. The chambers were fitted with magnetic stir bars and placed in a jacketed constant-temperature water bath. A Clark-type polarographic electrode (Yellow Springs Instruments #5331) was inserted into each chamber. The electrodes were connected to a YSI model #5300 dissolved-oxygen meter. Output from the meter was directed to an analog to digital converter (Sable Systems) and stored on a computer. The meter was calibrated with air-saturated seawater at each temperature and the data corrected for probe consumption at each temperature. A run at a given temperature began with a period of dark respiration until O_2 saturation values were ~80%. This was followed by measurements in the light at increasing light intensities. A 150-watt quartz halogen lamp fitted with an ultraviolet filter was used as a light source. By moving the light source and/or adding neutral density screening, we obtained eight light levels (19, 52, 78, 135, 198, 264, 335, and 531 μmol photon m^{-2} s^{-1}), determined with a cosine-corrected quantum sensor. All runs were performed in 0.2-μm-filtered FSW.

Oxygen flux of freshly isolated zooxanthellae (FIZ)—Zooxanthellae were obtained from *A. pallida* on the day of the experiment. Several anemones were ground in FSW using a Teflon and glass homogenizer. The pooled algal suspension was washed three times by centrifugation (550 × g) and then resuspended in 10 ml of 0.2 μm FSW. The final suspension was adjusted to between 200,000 and 500,00 cells ml^{-1} and placed in a darkened beaker with aeration and allowed to acclimate to the experimental water temperature (25°C, 32°C, or 34°C) for an hour. An aliquot of the suspension was then placed in a glass incubation chamber for oxygen flux measurements. After measurements at different irradiances, the suspension in the chamber was removed and a subset was taken for cell counts and chlorophyll measurements. The water temperature was then raised and the procedure was repeated. The complete series of measurements for a given cell suspension took about 4 h. We conducted runs on zooxanthellae from four samples of Florida anemones and from six samples of Bermuda anemones.

Cell counts involved a minimum of 10 hemocytometer fields. The remainder of the aliquot was filtered through a Whatman GF/A filter, which was homogenized on ice with

1–2 ml of 100% acetone. The resulting slurry was extracted with acetone rinses in the dark at 4°C for 24 h. Each extract was then centrifuged at 550 × g, and the absorbance of the supernatant was measured at 663, 630, and 750 nm. Chlorophyll *a* (Chl *a*) content was calculated using the equations of Jeffrey and Humphrey (1975). The cell sizes of zooxanthellae isolated from Florida and Bermuda *A. pallida* (Bermuda algae) were determined by measuring the long axis (Schoenberg and Trench 1980) of 50 dividing cells in three anemones from each location.

Oxygen flux of intact anemones—Each run consisted of a single intact anemone that was placed in a glass incubation chamber in 0.2 μm FSW and allowed to attach and acclimate to temperature for at least an hour prior to measurements. A perforated plexiglass plate separated the anemone from the stir bar. Following measurement of dark respiration, oxygen flux in the chamber was measured at increasing irradiances. The temperature was increased to the next level, the anemone was acclimated in the dark for at least an hour, and the oxygen flux measurements were repeated at increasing irradiances. When all runs for a given anemone were completed, the animal was ground up in FSW using a Teflon homogenizer. Aliquots of the homogenates were frozen for total protein determination. Following centrifugation, the algal pellet was cleaned by three cycles of washes and centrifugations. A subset of the final suspension was then taken for zooxanthella counts while the remainder was used for chlorophyll analysis (see previous). Total protein content of homogenates was determined by the Bradford technique (Bio-Rad; Bradford 1976), using bovine serum albumen standards. All reagents were made up in FSW. We used eight Florida anemones with Florida zooxanthellae, six Bermuda anemones with Bermuda zooxanthellae, and eight Bermuda anemones with Florida zooxanthellae.

Calculation of oxygen fluxes—Percent saturation was converted to oxygen concentration using nomagraphs for each temperature, and rates were calculated from the slopes of linear regression equations fitted to the oxygen content versus time plots. All rates were corrected for probe consumption. Photosynthesis–irradiance (P-I) curves were constructed for each anemone or cell suspension by fitting the raw data to the photoinhibition equation of Platt et al. (1980), using the nonlinear curve fitter of SigmaPlot (SPSS). Net fluxes were converted to gross photosynthetic rates (correcting for dark respiration) for the fitting routine, and then the fitted curves were converted to net photosynthetic rates. In some cases, notably the isolated Bermuda symbionts at 32°C and 34°C, oxygen fluxes in the light were more negative than dark respiration rates. Because the curve fitter required positive values, we used the most negative value as respiration in calculating gross P. Coefficients of the fitted P-I curves (P_{max}, the maximum photosynthetic rate; α, the initial slope of the curve; and β, the photoinhibition coefficient) were calculated by the curve fitter. In cases where β had high values, the curve fitter overestimated P_{max}. P_{max} in these instances was calculated by an iterative method, using a spreadsheet model of the photoinhibition equation and the values of α and β to interpolate P_{max}. One sample of Bermuda algae at 34°C yielded a mirror image (negative) P-I curve. To estimate P_{max} for this sample, we made all values positive, ran the curve fitter, and changed the sign of the resulting values for α and P_{max}. Compensation irradiances (I_c) were determined from the fitted curves, where net photosynthesis = 0; I_k, the irradiance at which photosynthesis becomes light saturated, was calculated from the initial linear portion of the curve (Muller-Parker 1984). Statistical comparisons of these coefficients were done using analysis of variance (ANOVA) of untransformed data, followed by the Tukey post hoc procedure.

For each group of anemones (cell suspensions) at a given temperature, we calculated the mean oxygen flux between replicates and 95% confidence intervals for each irradiance level. Overall, P-I curves were constructed using these mean values and proceeding as above. Ninety-five percent confidence limits were generated from these curves by the SigmaPlot curve fitter.

Results

Genetic identification of zooxanthellae—Zooxanthellae isolated from *Aiptasia pallida* from Florida displayed a characteristic clade A restriction fragment length polymorphism (RFLP) pattern (Rowan and Powers 1991) using either *Taq*I or *Dpn*II restriction enzymes. Zooxanthellae isolated from *A. pallida* from Bermuda exhibited a clade B RFLP pattern (Rowan and Powers 1991). Zooxanthellae originally from *A. pallida* from Florida that were introduced into *A. pallida* from Bermuda exhibited a clade A RFLP pattern.

Oxygen fluxes of freshly isolated zooxanthellae (FIZ)—Algae isolated from the Florida anemones exhibited similar net photosynthesis at 25°C and 32°C, as shown by both the comparisons of fitted curves (Fig. 1A,B) and of calculated net P_{max} values (Table 1; $p < 0.05$, ANOVA). The ninety-five percent confidence limits of the fitted curves were similar over the entire range of irradiances used. In contrast, net photosynthesis by these algae at 34°C was reduced by at least 50% at all irradiances (Fig. 1C), as was the calculated net P_{max} (Table 1; $p < 0.001$ for both 25°C and 32°C). This difference appears to be largely due to increased dark respiration shown in Fig. 1, as gross photosynthetic rates (P_{max} gross) were similar to those at the lower temperatures (Table 1; $p < 0.05$). The values of α (the initial slope of the P-I–curves), I_k, and β were similar at the three temperatures (Table 1; Tukey post hoc tests, $p > 0.05$), although values of α tended to increase with temperature. Values for I_c, the compensation irradiance, were higher at 34°C than at the two lower temperatures ($p < 0.05$). The values for β were less than 0.02 for all temperatures (Table 1), indicating little photoinhibition of the isolated Florida symbionts.

The Bermuda symbionts at 25°C were similar to the Florida algae in that they exhibited little photoinhibition (Fig. 1A). They had lower photosynthetic rates than the Florida symbionts at irradiances of 100 μmol photon m^{-2} s^{-1} (Fig. 1A; Table 1). These algae had markedly different responses at elevated temperatures (Fig. 1B,C). There was a dramatic decrease in net photosynthesis at 32°C; two of the six samples did not exhibit net photosynthesis at any irradiance.

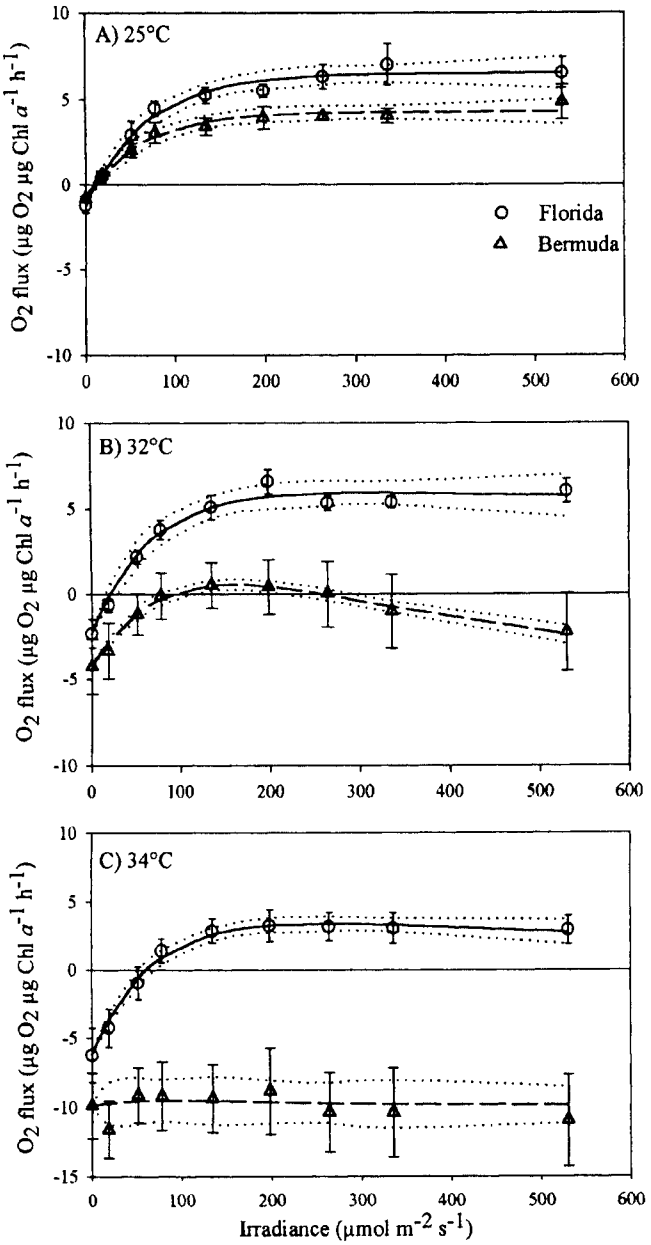

Fig. 1. Comparison of net photosynthesis-irradiance curves of freshly isolated zooxanthellae (FIZ) from Florida and Bermuda *Aiptasia pallida* compared at 25°C, 32°C, and 34°C (A–C). The points are the means of replicate runs ± 1 SE. The curves were fitted to the mean values from the photoinhibition equation of Platt et al. (1980). Dotted lines indicate 95% confidence limits of the fitted curves. Number of replicates is given in Table 1.

Dark respiration was approximately twice that of the Florida algae at this temperature (Fig. 1B; Table 1). Photoinhibition was clearly evident in the isolated Bermuda symbionts (Fig. 1B), with a mean β value of 1.3 (Table 1). At 34°C, these effects were more pronounced, with oxygen consumption at all irradiances exceeding that at 32°C (Fig. 1C). Dark respiration rates exceeded those of the Florida algae at this temperature (Fig. 1C). Although it is not obvious from Fig. 1C, photoinhibition was substantial in individual runs, yielding β values comparable with those at 32°C (Table 1). In general, α, I_c, and I_k showed the same trends with temperature as did those of the Florida algae (Table 1).

The average doublet size of Florida zooxanthellae (8.6 ± 0.5 μm) was not significantly different from that of Bermuda zooxanthellae isolated from Bermuda hosts (8.5 ± 0.6 μm; $p > 0.05$; $n = 150$ for each). Symbionts from Florida anemones, however, had significantly more Chl *a* per cell (2.8 ± 0.7 pg cell^{-1}; $n = 14$) than the Bermuda zooxanthellae (1.9 ± 0.6 pg cell^{-1}; $n = 10$; $p < 0.001$). At all experimental temperatures, productivity per cell (P_{max} net and P_{max} gross) was significantly higher in Florida FIZ compared with Bermuda FIZ ($p < 0.05$). All comparisons were done by *t*-tests.

Oxygen fluxes of intact anemones—P-I curves for the three anemone–symbiont combinations are presented in Fig. 2. In the intact native combination of Florida symbionts and *A. pallida* from the Florida Keys, Florida, anemones at 25°C had higher oxygen fluxes at all light levels than at 32°C and 34°C (Fig. 2). The P-I curves at 32°C and 34°C overlapped (Fig. 2). The calculated values for net P_{max} (Table 2) showed the same relationship. P_{max} net for 25°C was greater than at either of the higher temperatures (Tukey, $p < 0.001$ for both), while the values at the higher temperatures were not different ($p > 0.05$). Net oxygen production was observed at all saturating light levels.

Values for α, I_c, and I_k were not different at the three temperatures (Tukey procedure, $p > 0.05$), although I_c tended to increase with temperature (Table 2). The values for β are difficult to interpret, as several anemones showed exceptionally high values at 32°C but not at 34°C.

Bermuda anemones containing Bermuda symbionts exhibited the same general trends as the Florida anemones (Fig. 2). Net oxygen fluxes at 25°C were greater at all irradiances than at 32°C and 34°C (Fig. 2); however, in these anemones, fluxes at 32°C were greater than at 34°C. Calculated net P_{max} values showed the same pattern, with P_{max} net for 32°C being greater than that at 34°C (Table 2; $p < 0.001$). In contrast with the Florida anemones, there was no net photosynthesis at any irradiance at 34°C (Fig. 2C). As with the Florida anemones, several of the Bermuda anemones with Bermuda algae displayed significant photoinhibition at 32°C compared with 25°C, but this was not observed at 34°C (Table 2). These anemones, however, showed little net photosynthesis at this temperature. Other parameters of the P-I curves (α, I_c, and I_k) all increased with temperature.

Bermuda anemones with Florida symbionts displayed a third pattern of oxygen flux with temperature (Fig. 2). Net photosynthetic rates at 32°C were again intermediate between 25°C and 34°C, but did not differ from the rates at the other temperatures (95% confidence interval, Fig. 2B; P_{max} net, $p > 0.05$; Table 2). Unlike the Bermuda–Bermuda combination, the Bermuda anemones with Florida symbionts exhibited net photosynthesis at all saturating irradiances (Fig. 2).

At 25°C, Florida–Florida combinations had a higher P_{max} net than the other two ($p < 0.05$); the latter were not different (Fig. 2A). At 32°C and 34°C, however, the Bermuda anemones with Florida symbionts had higher net P_{max} values

Table 1. Parameters of photosynthesis-irradiance curves for freshly isolated zooxanthellae (FIZ) from the Florida and Bermuda samples of *Aiptasia pallida*. P_{max}, α, β, and I_c were determined from computer fits to the photoinhibition equation of Platt et al. (1980). Dark respiration values are from the respirometry data. Units for P_{max} net, P_{max} gross, and respiration are $\mu g\ O_2\ \mu g\ Chl\ a^{-1}\ h^{-1}$. Units of α and β are O_2 flux irradiance^{-1}, as in Fig. 1. Units of I_c and I_k are $\mu mol\ photon\ m^{-2}\ s^{-1}$. All values are means (±SD). Florida FIZ, $n = 4$ for all; Bermuda FIZ, $n = 6$ except for 34°C, where $n = 5$.

Temperature (°C)	P_{max} net	P_{max} gross	Respiration	α	β	I_c	I_k
Florida FIZ							
25	6.63 (1.50)	7.87 (1.12)	1.24 (0.87)	0.11 (0.01)	0.003 (0.003)	13.2 (11.2)	73.8 (14.8)
32	6.17 (0.89)	8.50 (1.89)	2.33 (1.67)	0.13 (0.06)	0.003 (0.005)	20.6 (9.1)	71.6 (19.6)
34	3.61 (2.15)	9.86 (2.10)	6.26 (4.00)	0.16 (0.07)	0.005 (0.004)	65.1 (40.5)	65.4 (18.9)
Bermuda FIZ							
25	4.55 (1.52)	5.39 (1.72)	0.86 (0.38)	0.08 (0.03)	0.002 (0.003)	14.1 (11.3)	74.9 (26.1)
32	0.68 (3.62)	4.94 (1.58)	4.26 (4.00)	0.09 (0.03)	1.267 (1.960)	32.9*(9.7)	57.7 (17.1)
34	−8.12 (5.34)	5.95 (1.40)	6.26 (3.93)	0.12 (0.06)	1.784 (2.143)	61.9†	54.6 (17.6)

* Two samples never reached compensation.
† Four samples never reached compensation.

than either the Florida–Florida or Bermuda–Bermuda combinations (Fig. 2B, C; Table 2). There was little photoinhibition of the Florida symbionts in Bermuda anemones at 25°C and 32°C, with β values ~0.01 (Table 2). At 34°C, the mean value for β was 0.18, indicating slight photoinhibition at the highest temperature ($0.06 > p > 0.05$ for comparisons at both temperatures; Tukey procedure). These anemones had similar values for α, I_c, and I_k at the three temperatures (Table 2; $p > 0.05$), again in contrast with the Bermuda anemones with Bermuda symbionts. Both groups of anemones with the Florida algae had lower compensation irradiances (I_c) than those with Bermuda algae at 34°C, consistent with the low rates of net photosynthesis by the Bermuda–Bermuda anemones.

The oxygen fluxes are normalized to total protein in Table 2. There were no differences in either net or gross productivity at 25°C or 32°C between any of the anemone groups when expressed this way. At 34°C, the net productivity of the Bermuda–Florida combination was greater than either the Bermuda–Bermuda or Florida–Florida combination, but gross photosynthetic rates did not differ (ANOVA, post hoc Bonferroni procedure).

Symbiont densities varied between the three groups. Bermuda anemones with Florida symbionts and Florida anemones with Florida symbionts did not differ in the number of symbionts they contained per unit protein ($p > 0.05$; $n = 8$ for each). Bermuda anemones with Bermuda symbionts contained two to three times the number of symbionts per mg protein of the other two groups ($p < 0.001$ for each comparison; ANOVA, post hoc Bonferroni procedure).

Oxygen fluxes of FIZ versus the intact symbiosis—In Figs. 3 and 4, we compare the O_2 fluxes of FIZ and intact native symbioses from Florida and Bermuda. At 25°C, zooxanthellae in intact *Aiptasia pallida* from Florida had higher net photosynthesis at saturating irradiances than freshly isolated algae from these anemones (Fig. 3A). This is reflected in the values for both P_{max}net ($p < 0.05$, *t*-test) and P_{max} gross ($p < 0.001$; Tables 1, 2). We note that the values for the intact Florida anemones were the highest net and gross photosynthetic rates (Table 2) in any of our experiments. At 32°C and 34°C, the P-I curves show that FIZ from these anemones had higher net photosynthesis than did the intact Florida anemones (Fig. 3B), although the calculated values for P_{max} (net and gross) were not significantly different at either temperature ($p > 0.05$; Tables 1, 2). In contrast, intact Bermuda anemones displayed higher net photosynthesis than Bermuda FIZ at all three experimental temperatures (Fig. 4A–C). Significant differences in P_{max} values were seen only at 25°C (intact > FIZ; $p < 0.001$).

Discussion

As sea water temperatures rise, there is a growing concern that cnidarians, such as corals, are approaching their maximum thermal limits (reviewed in Lesser 2004). The survival of corals and other symbiotic cnidarians in the wake of environmental changes will depend on the symbiosis' ability to either adapt, acclimate, or both (Coles and Brown 2003). Because the environment will affect the zooxanthellae, the host, and the intact symbiosis, all must withstand the changing environmental conditions. Given the genetic diversity of the symbionts, the combination of cnidarian host and symbiont genotypes will likely determine how the symbiotic unit survives as temperatures increase.

The sea anemone *Aiptasia pallida* provides a suitable test of the potential physiological response of different zooxanthella genotypes, within the same host species, both at ambient and elevated temperatures. This anemone has a wide geographic range in the western north Atlantic and Caribbean and naturally hosts zooxanthellae that belong to two distinct genetic clades. In Bermuda, *A. pallida* appears to contain only clade B zooxanthellae (Rowan and Powers 1991; Savage et al. 2002; this article). Our sample of *A. pallida* from the Florida Keys contained only clade A, although other individuals of this species from the Florida Keys may contain clade B or both (Santos et al. 2001). Latitudinal differences in symbiont clades have also been reported for the scleractinian corals *Plesiastrea versipora, Ac-*

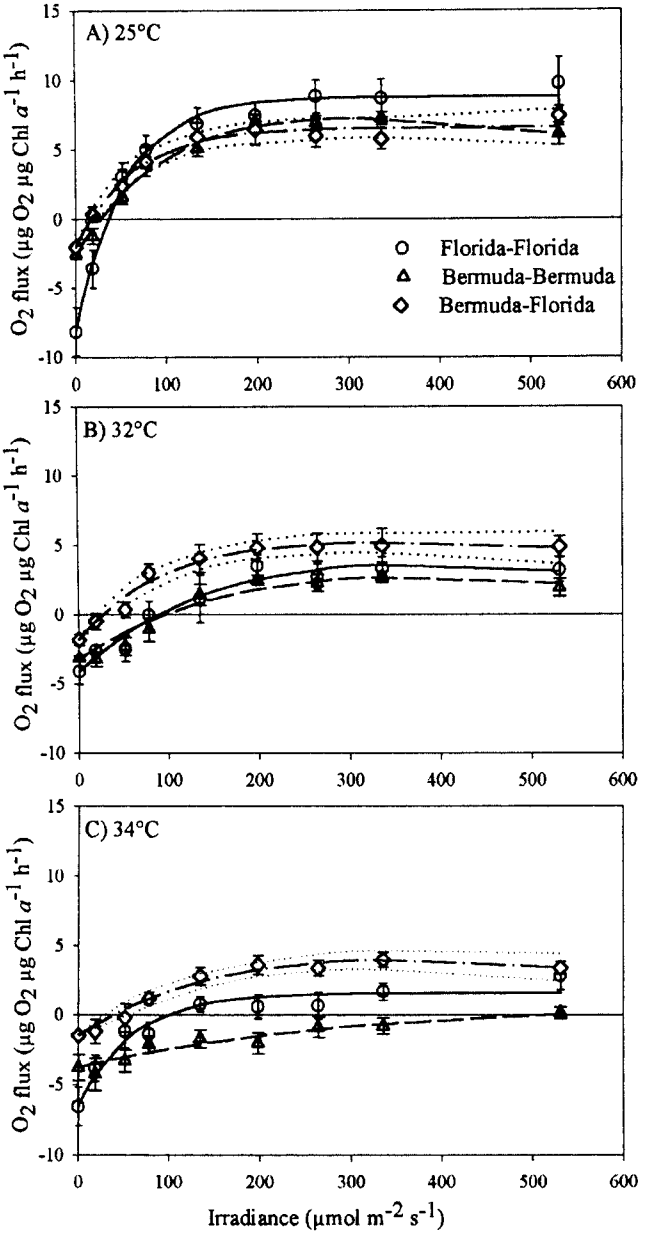

Fig. 2. Net photosynthesis–irradiance curves of intact *Aiptasia pallida* compared at 25°C, 32°C, and 34°C (A–C). Statistical parameters are as in Fig. 1. Number of replicates is given in Table 2. Florida–Florida = Florida anemones and symbionts, Bermuda–Bermuda = Bermuda anemones and symbionts, Bermuda–Florida = Bermuda anemones with Florida symbionts. Dotted lines indicate 95% confidence limits of the fitted curves for Bermuda anemones with Florida symbionts.

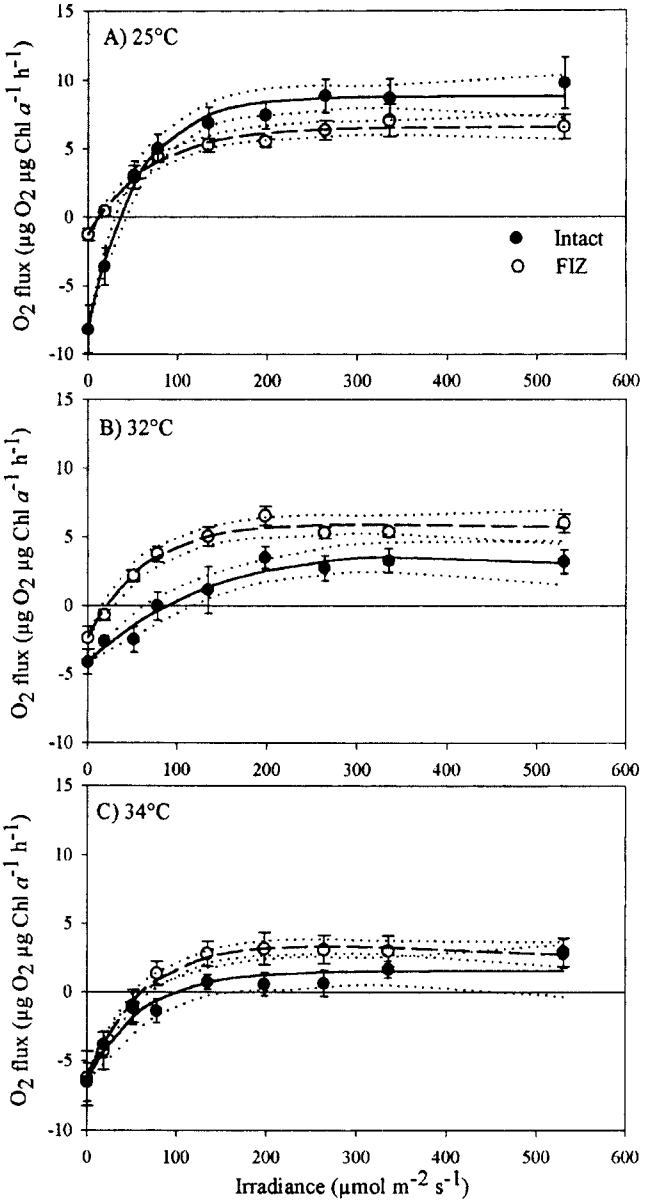

Fig. 3. Comparison of net photosynthesis–irradiance curves of Florida intact anemones (*A. pallida*) and Florida FIZ (symbionts) at 25°C, 32°C, and 34°C (A–C). Statistical parameters are as in Fig. 1.

ropora longicyathus, and *Seriatopora hystrix* (Loh et al. 2001; Rodriguez-Lanetty et al. 2001), the sea anemone *Anthopleura elegantissima* (Lajeunesse and Trench 2000), and the zoanthid *Palythoa caesia* (Burnett 2002).

Our article is the first to show that different zooxanthella genotypes that naturally occur within the same host species display temperature-related sensitivities of P–I curves despite long-term culture at one temperature (25°C). Bermuda anemones were collected from Walsingham Pond (mean temperature 23.2°C ± 3.3°C). Florida anemones were collected from the Florida Bay side of Key Largo (mean temperature 26.5°C ± 4.0°C). Responses of the algal symbionts from both locations were consistent with these temperature differences. Clade B symbionts from Bermuda were much more sensitive to elevated temperatures than the clade A symbionts from the Florida anemones.

Freshly isolated symbionts from Florida *A. pallida* (clade A) consistently had higher photosynthetic rates at ambient and elevated temperatures, while those from the Bermuda anemones exhibited marked photoinhibition at elevated tem-

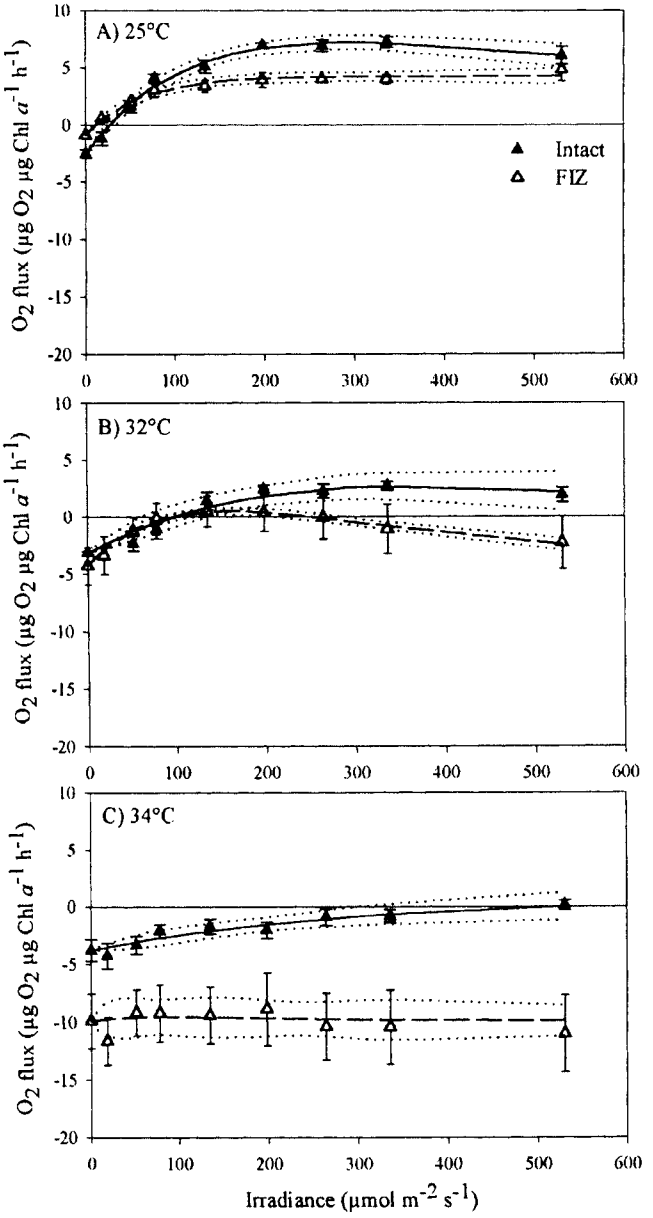

Fig. 4. Comparison of net photosynthesis–irradiance curves of Bermuda intact anemones (*A. pallida*) and Bermuda FIZ (symbionts) at 25°C, 32°C, and 34°C (A–C). Statistical parameters are as in Fig. 1.

peratures. These results are in agreement with the observation of Cook et al. (1990) that Bermuda corals and/or their zooxanthellae bleach at temperatures that normally do not affect corals at lower latitudes. Our value for P_{max} net for symbionts isolated from Florida anemones at 25°C was 6.6 µg O_2 µg Chl a^{-1} h^{-1}, which is very similar to that reported by Muller-Parker (1984) for FIZ from *Aiptasia pulchella* from Hawaii (6.4 µg O_2 µg Chl a^{-1} h^{-1}, using her value of 1.5 pg Chl *a* per cell for the conversion). They are, however, significantly higher than those reported by Savage et al. (2002) for FIZ from Bermuda *A. pallida* (1.8 µg O_2 µg Chl a^{-1} h^{-1}, converted to our units, versus 4.6; Table 1). These differences are unlikely to have resulted from differences in cell density (Muller-Parker 1984), as comparable densities were used in both studies. In addition, Florida and Bermuda zooxanthellae exhibited similar cell sizes.

In the intact anemone comparisons, Florida anemones had significantly higher net photosynthetic rates than did Bermuda anemones with their native clade B symbionts at 25°C and 34°C (Fig. 2; Table 2), although these rates declined with temperature for both groups. Clark and Jensen (1982) found that rates of carbon fixation by *A. pallida* from Key Largo increased with temperature to 32°C, supporting the idea that these symbionts are relatively warm adapted. Their experiments did not include 34°C and were conducted at a single irradiance of 175 µmol photon m^{-2} s^{-1}. When we infected Bermuda anemones with our Florida symbionts, this host–symbiont combination had significantly higher net photosynthetic rates at above ambient temperatures compared with the symbiosis between Bermuda *A. pallida* and its naturally occurring clade B zooxanthellae (Fig. 2; Table 2). Furthermore, the new association at 32°C and 34°C even performed better than the Florida anemone–Florida zooxanthella combination (Fig. 2; Table 2).

Comparisons between photosynthesis by the algal suspensions and intact associations lead to several interesting points. Within a host, algal self shading or CO_2 availability may limit zooxanthella photosynthesis (Lesser and Shick 1989), leading to higher photosynthetic rates by isolated zooxanthellae. This occurred in the Florida clade A zooxanthellae at elevated temperatures. Similarly, symbionts isolated from the marine hydroid *Myrionema amboinense* had higher photosynthetic rates than the intact symbiosis (Fitt and Cook 2001). Ralph et al. (2001) found that zooxanthellae isolated from *Cyphastrea serailia* had a higher thermal tolerance than within the host. In that association, it appears that the host was intolerant of elevated temperatures and therefore negatively affected the zooxanthellae within it, with the host being the more thermally sensitive partner. Perhaps this is the case for the Florida anemones because the isolated symbionts at 32°C and 34°C had higher photosynthetic rates than did the intact anemones.

On the other hand, reduced self shading in algal suspensions may exacerbate the effects of high irradiance levels and elevated temperature. Muller-Parker (1984) observed photoinhibition in isolated symbionts at high irradiances that was not seen in intact sea anemones. In our experiments, clade B zooxanthellae isolated from Bermuda anemones exhibited more severe effects of elevated temperature and irradiance than they did in the intact symbiosis. This was especially pronounced at 34°C, which resulted in dramatic oxygen uptake by the isolated algae at all irradiances. Bhagooli and Hidaka (2003) obtained similar results with the effects of elevated temperature and irradiance on maximum quantum yields in five species of scleractinian corals: isolated symbionts were generally more sensitive to elevated temperature and high irradiance than were the intact corals. Various reasons have been suggested for the advantages of being within host tissue such as host production of ultra violet–absorbing compounds and antioxidant protection (Lesser and Shick 1989) and host fluorescent pigments (Sa-

Table 2. Parameters of individual photosynthesis-irradiance curves for intact anemones from the Florida and Bermuda populations of *Aiptasia pallida*, and from Bermuda anemones with Florida symbionts. Data calculated as in Table 1. Units for P_{max} net, P_{max} gross, and respiration are μg O_2 μg mg protein^{-1} h^{-1}. Units for α, β, I_c and I_k as in Table 1. All values are means (±SD).

Temperature (°C)	P_{max} net	P_{max} gross	Respiration	α	β	I_c	I_k
Florida *A. pallida*–Florida symbionts (n = 8)							
25	257.2 (89.4)	460.0 (153.2)	202.8 (3.03)	0.31 (0.18)	0.01 (0.02)	36.5 (13.6)	405.7 (172.6)
32	95.9 (66.2)	217.8 (76.5)	121.9 (89.06)	0.08*(0.03)	0.60 (0.86)	95.8 (69.1)	399.5 (69.1)
34	62.3 (64.0)	237.0 (130.5)	174.7 (139.0)	0.29 (0.48)	0.08 (0.23)	102.6†(54.1)	416.3*(180.1)
Bermuda *A. pallida*–Bermuda symbionts (n = 6)							
25	287.8 (75.6)	386.0 (98.6)	98.2 (41.0)	0.11 (0.04)	0.01 (0.01)	25.5 (8.9)	95.4 (30.6)
32	121.9 (61.0)	253.8 (82.3)	131.9 (27.6)	0.05 (0.02)	0.50 (0.48)	99.8 (36.5)	150.2 (86.9)
34	−7.4 (49.5)	165.4 (67.6)	172.7 (103.4)	0.04 (0.04)	0.02 (0.05)	314.9‡(149.4)	222.2 (165.3)
Bermuda *A. pallida*–Florida symbionts (n = 8)							
25	258.8 (76.3)	331.2 (106.7)	72.4 (36.9)	0.11*(0.08)	0.01*(0.01)	26.4 (29.3)	90.8 (69.3)
32	185.5 (94.2)	246.9 (108.0)	61.4 (38.1)	0.08*(0.04)	0.01*(0.01)	29.6 (17.7)	101.7 (31.1)
34	138.7 (52.0)	206.4 (82.5)	67.7 (60.4)	0.06 (0.04)	0.18 (0.23)	45.7 (31.8)	125.0 (46.1)

* n = 7.
† Two samples never reached compensation.
‡ Three samples never reached compensation.

lih et al. 1997). The presence of a host factor may increase photosynthetic rates (Gates et al. 1999).

These studies indicate that following the effects of elevated temperature on intact corals by either respirometry or the use of pulse amplitude modulation (PAM)-fluorometry may not fully demonstrate the thermal sensitivity of the symbionts. Some symbionts may persist at temperatures in which the host cannot survive. Other symbionts may be living close to their upper thermal limits. Our findings demonstrate the importance of characterizing the physiology of both the intact association and the isolated zooxanthellae to determine which one of the partners is more susceptible to elevated temperatures.

The phenomenon of coral bleaching (i.e., the loss of zooxanthellae and/or their pigments) is often perceived as a stress response (Glynn 1996). Conversely, bleaching may be adaptive if it results in fitter host–symbiont combinations (Buddemeier and Fautin 1993). If a cnidarian host can host a diversity of zooxanthellae, environmental stress may result in the acquisition of zooxanthellae that best fit the host–symbiont genotypic combination for that environment. In *A. pallida,* the host–symbiont genotypic combination does affect zooxanthella photosynthetic rate. Conceivably, partner switching would occur to optimize physiological performance. In contrast, if a cnidarian can only host one zooxanthella type, as occurs in the majority of adult cnidarians (168 of 199 species) (references in Goulet and Coffroth 2003*b*), then the physiological flexibility of the association may determine if the symbiosis will survive environmental change.

Our results demonstrate that different host–symbiont genotypic combinations produce different photosynthetic rates during short-term exposure to elevated temperatures. Numerous researchers have tried to determine what is damaged in the zooxanthellae at elevated temperatures, proposing several mechanisms for the pathological effects of elevated temperature on zooxanthellae. These include the disorganization of thylakoid membranes (Iglesias-Prieto et al. 1992; Tchernov et al. 2004), disrupted electron flow to the dark reactions of photosystem II (Jones et al. 1998), elevated concentrations of damaging oxygen and hydroxide radicals (Lesser et al. 1990), and the loss of the D1 repair protein (Warner et al. 1999). Our findings indicate that, at least in some symbioses, the host may offset such damage, although how this might occur remains to be determined. Furthermore, these findings reiterate that, in a cnidarian–algal association, the physiological entity is the genotypic combination of host and symbiont (holosymbiont, Iglesias-Prieto and Trench 1997). Inserted into another host, the zooxanthellae may display a different photosynthetic ability and a different response to elevated temperatures. The unit of selection is therefore the symbiotic unit and not each partner separately.

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