The role of symbiotic dinoflagellates in the temperature-induced bleaching response of the subtropical sea anemone *Aiptasia pallida*

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Abstract

Coral bleaching involves the loss of symbiotic dinoflagellates (zooxanthellae) from reef corals and other cnidarians and may be a stress response of the host, algae or both. To determine the role of zooxanthellae in the bleaching process, aposymbiotic sea anemones from Bermuda (*Aiptasia pallida*) were infected with symbionts from other sea anemones (*Aiptasia pallida* from Florida, *Bartholomea annulata* and *Condylactis gigantea*). The expulsion of algae was measured during 24-h incubations at 25, 32 and 34°C. Photosynthetic rates of freshly isolated zooxanthellae were also measured at these temperatures. The *C. gigantea* (Cg) symbionts were expelled in higher numbers than the other algae at 32°C. Photosynthesis by the Cg algae was completely inhibited at this temperature, in contrast to the other symbionts. At 34° all of the symbionts had increased expulsion rates, and at this temperature only the symbionts from Florida *A. pallida* exhibited any photosynthesis. These results provide the first evidence that the differential release of symbionts from the same host species is related to decreased photosynthesis at elevated temperatures, and support other findings suggesting that zooxanthellae are directly affected by elevated temperatures during bleaching events.

Keywords: Aiptasia pallida; Coral bleaching; Photosynthesis; Sea anemone; Symbiosis; Zooxanthellae

1. Introduction

Under certain environmental conditions, particularly thermal stress (Porter et al.,

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1989; Cook et al., 1990; Gates, 1990; Glynn and D'Croz, 1990), corals with algal symbionts (zooxanthellae) lose their algae-derived coloration during bleaching episodes. Bleaching can result from the loss of algal pigments, loss of algae from animal tissues, or both (Hoegh-Guldberg and Smith, 1989; Kleppel et al., 1989). It is generally thought that coral bleaching involves the mass expulsion of zooxanthellae (Hoegh-Guldberg and Smith, 1989). Iglesias-Prieto et al. (1992) proposed that the algae were a sensitive component in high temperature-associated bleaching. In support of this proposal, during high temperature bleaching zooxanthellae are photosynthetically compromised (Fitt and Warner, 1995; Warner et al., 1999) and differential loss of symbiont genotypes from individual corals can occur (Rowan et al., 1997).

A high degree of quantitative and qualitative variability of coral bleaching events exists at both spatial and temporal scales (Glynn, 1996; Fagoonee et al., 1999). Variability in the extent, timing and severity of stress conditions, and differences in the degrees of tolerance to stress between hosts and symbiotic algae may explain this variation (Clark and Jensen, 1982; Gladfelter, 1988; Rowan et al., 1997). There is now overwhelming evidence that zooxanthellae form a genetically heterogeneous group, based on morphological, functional and molecular differences (Trench, 1997; Baker and Rowan, 1997; Rowan, 1998). This algal diversity is probably a major reason for the spatial variability of coral bleaching events (Rowan and Knowlton, 1995; Rowan et al., 1997).

Recent studies have clearly shown that temperature increases, similar to those measured during bleaching episodes, affect both the dark and light reactions of photosynthesis by zooxanthellae (Jones et al., 1998; Iglesias-Prieto et al., 1992; Fitt and Warner, 1995; Iglesias-Prieto, 1997; Lesser, 1996). Yet few studies have reported differences in temperature tolerance among zooxanthellae and how this variation may contribute to patterns of coral bleaching (Warner and Fitt, 1996; Warner et al., 1999).

The present study was conducted to investigate how thermal bleaching responses would vary in a symbiotic cnidarian host containing different algal symbionts. To address this question, we exposed sea anemones containing various zooxanthellae to elevated temperatures, and measured the expulsion of algae, and the photosynthetic rates of the symbionts at these temperatures. This is the first report to demonstrate that the differential release of genetically distinct zooxanthellae from the same host species during exposure to elevated temperatures is associated with decreased symbiont photosynthesis.

2. Materials and methods

2.1. Collection of source hosts and isolation of zooxanthellae

Symbiotic and aposymbiotic *Aiptasia pallida* (Verrill) were taken from a clone originally isolated in Bermuda (Cook et al., 1988). *Condylactis gigantea* (Weinland), *Bartholomea annulata* (LeSeur), and *Aiptasia pallida* (Verrill) were collected from sites in West Summerland Key, Florida, for use as sources of zooxanthellae. Table 1 summarizes the taxonomy of the symbionts associated with these hosts. *A. pallida* were

Table 1
The identity of symbiotic zooxanthellae used in this study: cladal designations are from analyses of ssRNA genes, following the original designations of Rowan and Powers (1991)

Host species/collection site	Symbiont species	Symbiont clade
Aiptasia pallida (Verrill)/Bermuda	*Symbiodinium bermudense* (Banaszak et al., 1993)	B (Rowan, 1998)
Aiptasia pallida (Verrill)/Florida Keys	'*S. bermudense*' (Stochaj and Grossman, 1997)	A (Goulet and Cook, unpublished)
Condylactis gigantea (Weinland)/Florida Keys	*S. cariborum* (Banaszak et al., 1993)	n.d.[a]
Bartholomea annulata (LeSeur)/Florida Keys	Not described	n.d.[b]

[a] Symbionts of *C. gigantea* from Puerto Morelos, Mexico have been found to be a mixture of A and C (T.C. LaJeunesse and R.K. Trench, pers. commun.).

[b] Symbionts of *B. annulata* from Barbados have been found to be clade A (T.C. Lajeunesse and R.K. Trench, pers. commun.).

taken from a rock in < 0.5 m of water in Florida Bay, while the other anemones were collected from seagrass beds in 2 m on the ocean side of the key. These specimens were taken to the laboratory within 24 h of collection. Zooxanthellae were obtained from a small (≈ 1 cm) piece of tentacle from each actinian species. The tentacle pieces were homogenized in 0.45-μm Millipore-filtered seawater (MFSW) using a Teflon and glass homogenizer. The resulting crude suspension was washed three times by centrifugation ($550 \times g$) and then resuspended in MFSW. The final isolates were used for the re-infection of aposymbiotic *A. pallida*.

2.2. Re-infection of aposymbiotic Aiptasia pallida

Aposymbiotic *A. pallida* were re-infected with zooxanthellae isolated from Florida *A. pallida* (Ap), *B. annulata* (Ba) and *C. gigantea* (Cg) by injecting the isolated algae into the coelenterons with a Pasteur pipette drawn out to a fine tip. For convenience, we refer to the algae by these abbreviations, and to the symbionts of the Bermuda strain of *A. pallida* as ApB. The re-infected anemones and the Bermuda strain of symbiotic and aposymbiotic *A. pallida* were kept in 50 ml Petri dishes (10–20 individuals each) with 0.45 μm MFSW. Anemones were given satiation feedings of *Artemia* nauplii hatched in MFSW. The dishes were kept at 25°C and 12-h light/dark photoperiod at an irradiance of 100 μmol m^{-2} s^{-1}. The reinfected anemones attained stable populations of symbionts within one month. Approximately 1 year after re-infection, these anemones and their asexually derived progeny were used in experiments. Uninjected aposymbionts kept under the same conditions of light, temperature and feeding remained free of zooxanthellae, indicating that the aposymbiotic anemones were initially algae-free and that maintenance conditions did not cause accidental cross-contamination.

2.3. Biomass determinations

Populations of zooxanthellae in anemones were determined from cell counts of tissue

homogenates. Each anemone was separately homogenized in approximately 1 ml of MFSW. The homogenate was centrifuged at $550 \times g$ and the pellet was washed three times with MSFW. The final suspension (1 ml) was preserved with a drop of 1% formalin and Lugol's solution. Cell numbers were determined with 14–20 replicate haemocytometer counts; dividing cells were counted as two (Guillard, 1973).

Algal suspensions were individually filtered through 0.45 μm glass fiber filters. A few drops of 1% $MgCO_3$ were added to the filters during filtration. The filters were placed in 5 ml plastic tubes with 1–2 ml of 100% acetone and homogenized on ice using a Teflon pestle under dim light. The final volume was brought to 10 ml with acetone and the centrifuge tubes were stored in the dark at 4°C for 24 h. Each extract was centrifuged at $550 \times g$ for 20 min, and the absorbance of the supernatant was measured at 663, 630, and 750 nm. Chlorophyll-*a* content was calculated using the equations of Jeffrey and Humphrey (1975).

Anemone size was estimated using oral disc diameter, a convenient non-destructive technique that has been shown to correlate with wet weight (Clayton and Lasker, 1985). We defined the oral disc diameter as the widest distance between opposite tentacle bases. Measurements were made 3 days after feeding and 1–2 h after the onset of the light period. The anemones were allowed to expand fully, and measurements were made by using calipers placed directly above and as close as possible to the oral disc without making contact with the anemones.

2.4. Measurement of algal release

Five anemones infected with each zooxanthella type and five symbiotic Bermuda *A. pallida* were randomly selected and placed in individual 50 ml capacity Petri dishes with 40 ml of MFSW at 25°C. After 24 h, the water in the dishes was replaced with MFSW preheated at the experimental temperature (25, 32, or 34°C). Incubations took place at this temperature in an environmental chamber fitted with cool white fluorescent lights on a 12-h light/dark photoperiod with an irradiance of 75 $\mu E\ m^{-2}\ s^{-1}$.

After the 24-h incubation period, the water from the dishes was transferred to 50 ml plastic centrifuge tubes. The walls and bottom of the dishes were rinsed with a stream of MFSW (dish walls were not wiped), and the rinse was added to the water in the centrifuge tubes. These samples contained the algae released after 24 h. Any algae still adhering to the walls after this procedure were not included, so that algal release was under-estimated. However, examinations of filter wipes indicated minimal numbers of algae remained. Each anemone was homogenized in MFSW as above. The Teflon pestle and glass homogenizer was rinsed with MFSW and the rinses were combined with the anemone homogenate. These samples contained the algae remaining within the host. The homogenates were centrifuged at $550 \times g$ for 20 min to collect the algae and the algal pellets were re-suspended in approximately 1 ml of MFSW. The suspension volume was determined by drawing the solution into a graduated 1 ml pipette. Cell numbers in these suspensions were determined by hemocytometer counts. Algal release was expressed as % cell release (Eq. (1)):

$$\%\ \text{released cells} = \frac{\text{Expelled cells}}{\text{Expelled cells} + \text{cells in anemone}} \times 100 \tag{1}$$

2.5. Measurement of oxygen fluxes

Dark respiration (R) and net photosynthesis (P_{net}) was measured using suspensions of zooxanthellae isolated from the reinfected anemones and symbiotic *A. pallida*. The anemones were maintained at 25°, and replicate ($n=2$) suspensions were prepared from each host–symbiont combination by homogenizing anemones in MFSW. The crude suspensions of freshly isolated zooxanthellae (FIZ) were washed by centrifugation ($550\times g$) followed by re-suspension with MFSW until microscopic inspection showed little remaining host tissue. The final suspensions used in the oxygen flux determinations were obtained by centrifugation followed by re-suspension of the resulting algal pellet with air-saturated, MFSW (approximately 10^6 cells ml^{-1}) pre-heated to each of the experimental incubation temperatures (25, 32, and 34°C).

The final algal suspensions (3–8 ml) were added to glass incubation chambers fitted with magnetic stir bars and Clark-type polarographic electrodes (Yellow Springs Instruments #5331). The electrodes were connected to a YSI model #5300 dissolved oxygen meter. The chambers were placed in a jacketed constant temperature water bath (YSI #5301B) at the appropriate temperature (25, 32, or 34°C). Illumination was provided by cool-white fluorescent lamps producing 100 μE m^{-2} s^{-1}. MFSW used in these experiments had initial O_2 saturation values in excess of 90%. Oxygen measurements in the dark were followed by measurements in the light and were made every 5 min. Incubation periods lasted 30 min, or until saturation levels changed 10% from initial values. Percent saturation was converted to oxygen concentration, and flux rates were calculated from the slopes of linear regression equations fitted to the oxygen content versus time plots. Blank controls were subtracted from R and P_{net}. Gross productivity (P_g) was calculated by adding the O_2 consumed in the dark (R) to P_{net}, and gross photosynthesis to respiration ratios (P:R) was calculated by dividing P_g by R. Aliquots of the algal suspensions were taken for cell counts and chl *a* analysis. Productivity was expressed as μg O_2 10^6 $cells^{-1}$ min^{-1} or μg O_2 μmol chl a^{-1} min^{-1}.

2.6. Statistical analysis

All statistical analyses were performed using SigmaStat® (Version 3.2). The arc sine transformation was applied to data on P:R ratios and per cent expulsion. Datasets that satisfied assumptions of normality and equality of variance were analyzed by ANOVA. The nonparametric Kruskal–Wallis procedure was used when these assumptions were not met. Multiple comparison tests (Student–Newman–Keuls, SNK) were performed whenever overall treatment effects were found.

3. Results

3.1. Biomass parameters of infected anemones

Biomass characteristics of the various host–zooxanthella combinations 1 year after

Table 2
Biomass characteristics of *Aiptasia pallida* symbiotized with different strains of zooxanthellae (mean±S.E.M.; sample size in parentheses)

Symbiont	Oral disc diameter (mm)	Algal density (cells mm^4 oral diameter)	Chlorophyll content (jig Chl-*a* $cell^{-1}$)
ApB	61.90±4.08 (15)[a]	$4.62\pm0.83\times10^4$ (15)[a]	1.92±0.3 (6)[b]
Ap	69.57±3.02 (15)[a]	$3.95\pm0.47\times10^4$ (15)[a]	0.68±0.19 (5)[a]
Ba	60.83±4.98 (15)[a]	$1.24\pm0.16\ 10^4$ (15)[b]	1.08±0.24 (5)[b]
Cg	50.17+3.00 (15)[b]	$0.74\pm0.08\times10^4$ (15)[c]	1.37±0.19 (5)[b]

[a,b] Means with similar superscripts are not significantly different from each other (SNK; $P<0.05$). Anemones with Ap, Ba and Cg471 symbionts were assayed 1 year after establishment of symbiosis. ApB anemones contained native symbionts (Bermuda strain).

infection are summarized in Table 2. Anemones harboring Cg zooxanthellae were significantly smaller (oral disc diameter) than anemones symbiotic with the other symbionts (one-way ANOVA; $P=0.009$, SNK: $P<0.05$). Algal cell densities were highest in ApB anemones and anemones with Ap algae. Algal cell densities were lowest in anemones containing Cg algae, while anemones with Ba algae were intermediate (One-way ANOVA on ranks $P\leq0.001$; SNK: $P<0.05$). Chlorophyll *a* (chl *a*) content ranged from 0.68 to 1.92 pg Chl-a^{-1} $cell^{-1}$, with Ap zooxanthellae having significantly lower chl *a* per cell than the other algae (one-way ANOVA $P=0.012$; Student–Newman–Keuls pair-wise multiple comparison (SNK): $P<0.05$).

3.2. Effect of temperature on expulsion of zooxanthellae

Algal expulsion increased significantly with temperature in all anemone–symbiont combinations (Fig. 1), although there was no effect of symbiont type on expulsion rate when the analysis was performed over all three temperatures (two-way ANOVA; $F=124.2$; for temperature, $P<0.001$; for type, $P=0.158$; for interaction, $P=0.201$). When the data for each temperature were analyzed separately, there was a significant effect of symbiont type on expulsion at 32°C (one-way-ANOVA of arc sine transformed percent expulsion at 32°C: $P<0.05$) but not at 25 or 34°C. At 32°C anemones with Cg algae had lost a significantly higher percentage of symbionts than the other anemone–zooxanthella combinations (SNK multiple comparison tests, $P<0.05$). At 34° all of the anemones exhibited increased algal expulsion, ranging from 30 to 50% of the total symbiont populations over the 24-h period (Fig. 1).

The only mortality observed during the study was of that of a single Ba anemone during the 34°C treatment.

3.3. Effect of temperature on productivity of zooxanthellae

The productivity of all suspensions of zooxanthellae decreased at elevated temperatures (Figs. 2–4). This decrease was largely due to decreased photosynthesis, rather than increases in dark respiration (Figs. 2,3). The negative effect of temperature on photosynthesis was most pronounced in Cg algae, which were the only symbionts that did not photosynthesize at 32°C. ANOVA of both the production rates (per algal cell and

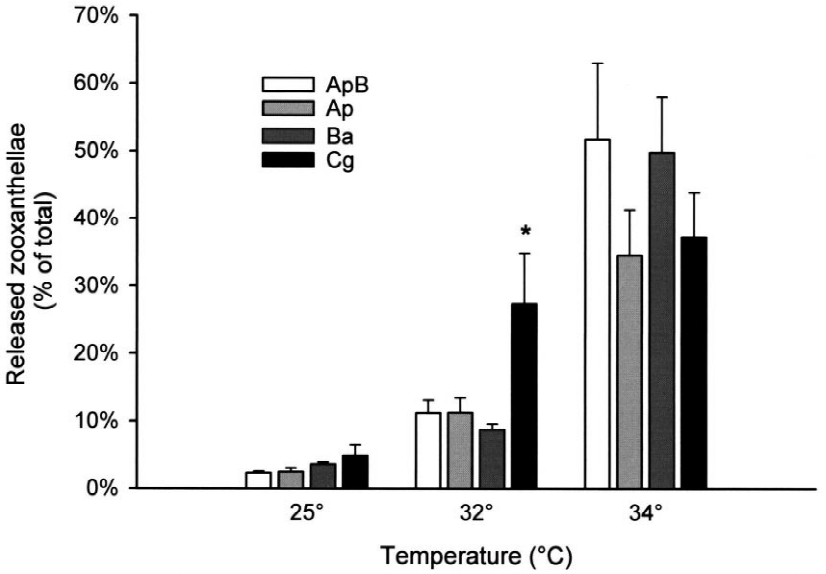

Fig. 1. Release of algae from *Aiptasia pallida* over 24 h as a function of zooxanthella type and temperature. ApB: zooxanthellae from *A. pallida* from Bermuda; Ap: zooxanthellae from *A. pallida* from the Florida Keys; Ba: zooxanthellae from *Bartholomea annulata*; Cg: zooxanthellae from *Condylactis gigantea*; Means + S.E.M., $n = 5$. * Significantly different from others at 32°C (SNK; $P < 0.05$).

per μg chl *a*) and Pg:R ratios at each temperature treatment showed no effect of symbiont type ($P > 0.05$) with the exception of the Pg:R ratios at 32°C (Fig. 4). The Pg:R ratio of Ap (algae from Florida *A. pallida*) at 32°C was significantly higher than that of the other symbionts at that same temperature (SNK; $P < 0.05$) Production rates per chl *a* (Fig. 2) and per cell (Fig. 3) were highest in Ap algae at 25 and 32°C. At 34°, O_2 consumption in the light exceeded that in the dark for the ApB, Ba and Cg algae (Figs. 2,3), and the Ap algae were the only ones to show positive values for Pg:R at this temperature (Fig. 4). Given that Ap zooxanthellae had the lowest chlorophyll-*a* content per cell (Table 2), oxygen produced per unit of chlorophyll-*a* was also the highest in Ap (Fig. 2).

We plotted P_{gross}:R for each type of zooxanthella at each temperature tested against the respective algal expulsion values (Fig. 5). There was a significant negative correlation between photosynthesis and expulsion ($r = -0.807$, $P < 0.01$; Pearson product–moment correlation), with expulsion increasing dramatically at P_{gross}:R < 1.

4. Discussion

4.1. Infectivity of zooxanthellae in Aiptasia pallida

Aposymbiotic *A. pallida* (Bermuda strain) established stable symbioses with symbionts from three species of sea anemone in our study. However, infections with

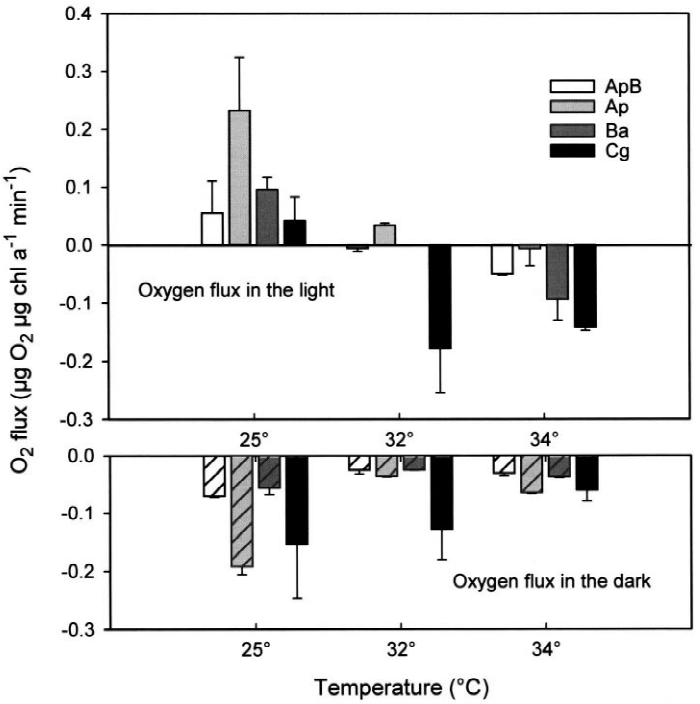

Fig. 2. Chlorophyll-specific O_2 fluxes of freshly isolated zooxanthellae in the light and dark as a function of temperature. Zooxanthella designations as in Fig. 1. Solid bars denote fluxes in the light, hatched bars denote fluxes in the dark; means + S.E.M.; $n = 2$.

symbionts from two zoanthids (*Palythoa caribbaeorum* and *Zoanthus sociatus)* and a scyphozoan (*Cassiopea xamachana)* did not persist (Perez, unpublished observations). Schoenberg and Trench (1980) were also unsuccessful in establishing symbioses between *Aiptasia tagetes* (= *A. pallida*; Sterrer, 1986) and symbionts from *Z. sociatus* and *Palythoa mammilosa*. These authors successfully infected *A. tagetes* with symbionts from *C. xamachana* but not from *Condylactis gigantea*, in contrast to our results.

Previous studies of infections with zooxanthellae have shown that host physiology is affected by the kind of symbiont. This includes growth and asexual reproduction in *Aiptasia pulchella* (Kinzie and Chee, 1979), strobilization of scyphozoan polyps (Fitt, 1985) and growth and survival of tridacnid clams (Fitt, 1985). After 1 year, anemones containing *C. gigantea* (Cg) symbionts were smaller than the others in our study, and had the lowest density of symbionts (16–60% of the other groups; Table 2). Symbionts from Florida *A. pallida* (Ap) had the highest densities, similar to those of the native Bermuda *A. pallida* (ApB algae), while densities of *Bartholomea annulata* (Ba) algae were intermediate between these and the Cg algae (Schoenberg and Trench, 1980). In contrast, zooxanthellae from a variety of hosts infecting the anemone *Cereus pedunculatus* all achieved densities comparable to those of the normal symbiotic host (Davy et al., 1997).

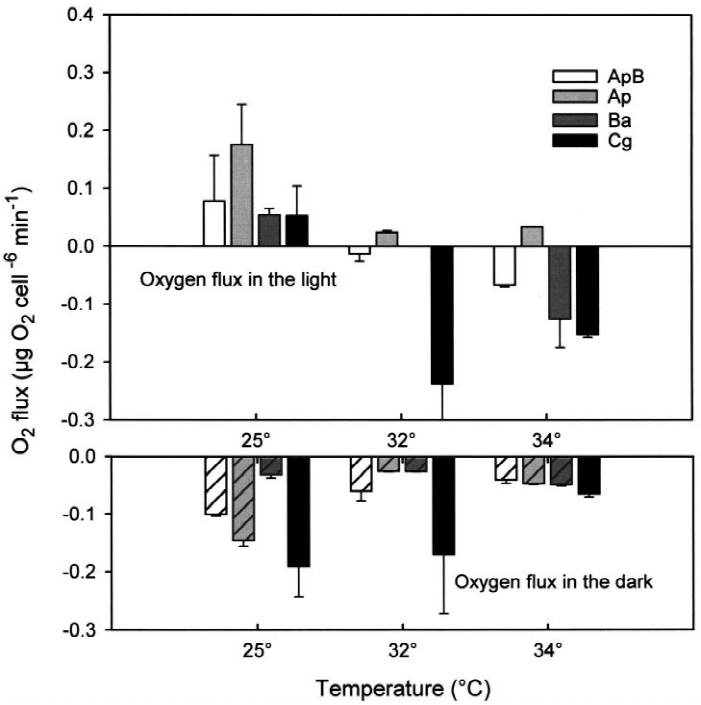

Fig. 3. Cell-specific O_2 fluxes of freshly isolated zooxanthellae in the light and dark as a function temperature. Zooxanthella designations as in Fig. 1. Solid bars denote fluxes in the light, hatched bars denote fluxes in the dark; means + S.E.M.; $n = 2$.

The symbionts in our study differed in chl *a* content, although they were maintained under similar conditions of irradiance and feeding. In particular, Ap algae had one-third of the chl *a* content of ApB algae. As these symbionts had similar densities in host tissue, differential photoadaptation due to self-shading does not seem likely. Self-shading would also not explain why Cg algae at the lowest density also had more chl *a* than did Ap algae. These differences in pigment content indicate a genetic basis (Chang and Trench, 1982), including differences in cell size between the symbiont groups (Schoenberg and Trench, 1980; Fitt, 1985).

4.2. Effect of temperature on expulsion of zooxanthellae from A.pallida

The expulsion of both heterologous and homologous zooxanthellae increased with temperature during the 24 h exposures (Fig. 1). At 34°C, all four groups of symbionts were released in large numbers, up to 50% of the total population. Presumably longer exposure times would have resulted in greater loss of all symbionts. The 32° treatment produced different expulsion rates, depending on the symbiont involved. In particular, the Cg zooxanthellae were released at twice the rate of other algae at this temperature.

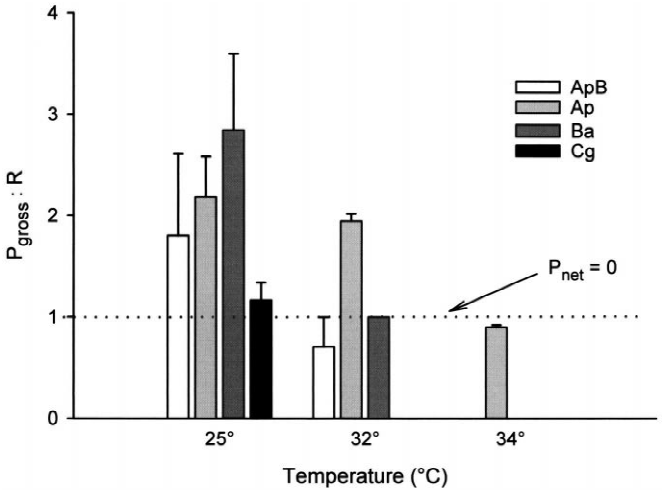

Fig. 4. Ratio of gross production to respiration of freshly isolated zooxanthellae as a function of temperature. Zooxanthella designations as in Fig. 1. The dotted line indicates where gross photosynthesis equals respiration (net photosynthesis = 0). Means + S.E.M., $n = 2$. Note: Cg algae at 32° and ApB, Ba and Cg algae at 34° did not photosynthesize; these bars with zero or negative values are not shown in the figure.

Since anemones containing Cg algae had the lowest density of symbionts, the net effect on algal loss was greatest in these.

4.3. Effects of temperature on photosynthesis

In our study, photosynthesis by freshly isolated heterologous and homologous

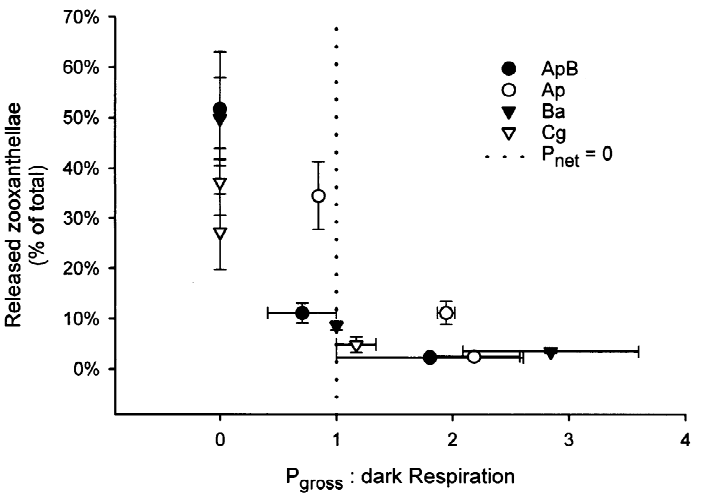

Fig. 5. Relationship between mean values of P_{gross}:R ($n = 2$) and the temperature-dependent expulsion ($n = 5$) of different zooxanthellae from *A. pallida*. Zooxanthella designations as in Fig. 1. Means ± S.E.M., $n = 12$.

zooxanthellae was impaired at 32 and 34°C (Fig. 2–4). As with expulsion, the most sensitive symbionts were the Cg algae, which exhibited no photosynthesis at either 32 or 34°C. Photosynthesis by the ApB and Ba zooxanthellae was inhibited at 34°C; only the Florida Ap algae were capable of photosynthesis at this temperature. Fitt and Warner (1995) reported that exposure to 32° had different effects on photosynthesis on zooxanthellae from the corals *Montastraea annularis* and *M. cavernosa,* and that corals with the more sensitive symbionts (*M. annularis*) were more susceptible to bleaching.

Iglesias-Prieto et al. (1992) and Trench (1993) have emphasized that understanding coral bleaching involves knowing whether the host or symbiont (or both) perceives and responds to environmental signals that cause expulsion. Bioenergetic studies of *A. pallida* have indicated that zooxanthellae are less temperature tolerant than the host (Clark and Jensen, 1982). Our finding that the most temperature-sensitive symbionts (Cg algae) were expelled at the highest rates from *A. pallida* at 32° implies that the alga is the more temperature-sensitive component. Symbiont expulsion from *Aiptasia pulchella* involves the release of intact host cells containing symbionts (Gates et al., 1992). These observations suggest that host cells detect affected symbionts and respond by detachment from the mesoglea and from neighboring cells. Temperature-induced symbiont expulsion in *A. pallida* occurs after the symbionts become photosynthetically compromised to the point that they are no longer autotrophic (Fig. 5; see also Nii and Muscatine, 1997). Thus, the loss of oxygen production and/or the cessation of symbiont translocation in the light could be signals that trigger the expulsion process.

The different responses of zooxanthellae from the two populations of *A. pallida* in this study suggest adaptation to local temperature regimes. Ap zooxanthellae (from Florida Keys *A. pallida*) were the only algae in this study to photosynthesize at 34°C (Fig. 4), and to exhibit net photosynthesis at 32°C (Figs. 2–4). The Ap anemones were taken from a small intertidal pool in Florida Bay that probably experienced higher temperatures than the seagrass bed on the ocean side where the other Florida anemones in this study were collected. As indicated in Table 1, the Ap algae from Florida and the native Bermuda *A. pallida* symbionts are genetically distinct, belonging to different clades (Goulet and Cook, unpublished data). The photosynthesis and expulsion data suggest that symbionts from the higher latitude Bermuda populations are less tolerant of elevated temperatures than the Ap algae from the Florida Keys. Clark and Jensen (1982) reported temperatures as high as 33°C at collection sites of *A. pallida* in Florida. This seawater temperature is rarely encountered in Bermuda, where corals bleach at temperatures that are within the normal range of lower latitude populations (Cook et al., 1990). LaJeunesse and Trench (2000) have recently found that the algal symbionts of the sea anemone *Anthopleura elegantissima* comprise two species that exhibit a latitudinal gradient along the Pacific coast of the US, and suggested that this distribution is due to temperature tolerance of the symbionts.

The differential effects of temperature on photosynthesis on genetically different zooxanthellae that we and others have observed could be due to differences in the sensitivity of mechanisms that prevent heat damage (Jones et al., 1998; Warner et al., 1999). The reduced chlorophyll fluorescence yield of zooxanthellae in bleaching corals indicates damage to components of the electron transport system of Photosystem II (PSII), possibly at the site of the D1 protein that serves to dissipate excess light and heat energy (Fitt and Warner, 1995; Warner and Fitt, 1996; Warner et al., 1999). Studies with

cultured zooxanthellae have led to similar conclusions (Iglesias-Prieto et al., 1992; Iglesias-Prieto, 1997). High temperature could also directly affect Calvin cycle reactions. Recent work has also indicated that the initial impairment of photosynthesis during temperature-associated bleaching involves damage to carboxylation mechanisms (Jones et al., 1998). Another possibility is that there are differences in Rubisco activase, an enzyme implicated in heat stressed CO_2 fixation in higher plants (Feller et al., 1998).

Thus, the results obtained in this study support the findings of previous studies indicating that elevated temperature stress elicits the bleaching of zooxanthellate cnidarians by affecting the photosynthetic machinery of the algae. Hence, bleaching patterns seen in field populations may be partly explained by the differential ability of different symbiotic algae in coping with temperature stress (Rowan et al., 1997). In addition, patterns of temperature sensitivity among zooxanthellae correlate well with temperature characteristics of different habitats (e.g. Iglesias-Prieto and Trench, 1997). That is, hosts residing in habitats experiencing wide temperature ranges may benefit from associating with more tolerant zooxanthellae.

Acknowledgements

We thank Marguerite Koch-Rose and Alex Marsh for their advice and suggestions, R.K. Trench and T. LaJeunesse for sharing unpublished data, and T. Goulet and two anonymous reviewers for constructive comments on the manuscript. This project was supported through the Master of Science program of the Department of Biological Sciences, Florida Atlantic University, and ONR grant N00014-96-1-0602 to CBC. This is Contribution # 1365 of Harbor Branch Oceanographic Institution. [SS]

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