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A comparison of the thermal bleaching responses of the zoanthid *Palythoa caribaeorum* from three geographically different regions in south Florida

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Abstract

Coral bleaching involves the loss of symbiotic algae (zooxanthellae) from reef corals and other cnidarians during periods of environmental stress, particularly elevated temperature. In this study we compared the thermal bleaching responses of the zoanthid *Palythoa caribaeorum* from three populations along the southeast coast of Florida. Winter (2002–2003) and summer (2003) samples from three geographically separate sites were experimentally exposed to increased temperatures and the loss of zooxanthellae was measured. Population densities of zooxanthellae were analyzed and their genetic identity determined using PCR-DGGE analysis of the internal transcribed spacer region 2. The results showed that samples of *P. caribaeorum* from reefs that experienced the smallest range in annual seawater temperature released the most zooxanthellae. Seasonal comparisons revealed that winter samples lost more zooxanthellae than summer samples. *P. caribaeorum* harbored two genetic types of zooxanthellae, C1 and D1a. Individual colonies contained populations of only C1 or D1a, or combinations of C1 and D1a. However, these genotypic patterns did not relate latitudinal distribution nor to differences in experimental thermal tolerance.

Keywords: Coral bleaching; DGGE; *Palythoa caribaeorum*; *Symbiodinium*; Symbiosis; Zoanthid

1. Introduction

The zoanthid *Palythoa caribaeorum* is an abundant cnidarian that occurs throughout shallow coral reef environments of the Caribbean, Bermuda and south Florida, including the Florida Keys. All individuals of *P. caribaeorum* throughout this range harbor dinoflagellate endosymbionts, commonly called zooxanthellae. Dino-

flagellates in the genus *Symbiodinium* represent the most common of these photosynthetic endosymbionts among coral reef-dwelling cnidarians (Trench, 1997; Rowan, 1998; Baker, 2003). The presence of *Symbiodinium* contributes substantially to the productivity, survival, and success of their animal hosts (Muscatine and Porter, 1977). Zooxanthellae increase the fitness of their scleractinian hosts by enhancing calcification (Barnes and Chalker, 1990), mediating elemental nutrient flux (D'Elia and Weibe, 1990), and providing photosynthetically-fixed carbon (Muscatine, 1990). The algae benefit from a supply of CO₂ and nutrients (Cook et al., 1988), protection from grazers, and protection from UV damage (e.g.

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Lesser and Shick, 1989). The symbiosis between cnidarians and zooxanthellae is also ecologically important, as it contributes to primary productivity of coral reefs (McLaughlin and Zahl, 1959), aids in recycling and conservation of nutrients (Taylor, 1982; Cook, 1985), and promotes construction of reef frameworks and habitats by reef-building corals.

Recently, the stability of tropical and subtropical environments has been disrupted by an overall increase in baseline seawater temperatures and ocean-warming anomalies such as the El Niño Southern Oscillation. This increase in global temperature has had significant, negative impacts on coral reefs. Goreau (1990), Glynn (1991), and Hayes and Goreau (1991) were among the first to suggest that increasing seawater temperatures could push reef corals beyond their thermal limits. Subsequent studies have linked worldwide coral bleaching to thermal stress (Goreau et al., 1993; Hoegh-Guldberg and Salvat, 1995; Brown, 1997; Winter et al., 1998; Hoegh-Guldberg, 1999).

Different cnidarians have different sensitivities during bleaching events, but the reasons for these differing responses are not clear (Cook et al., 1990; Fitt and Warner, 1995, see Loya et al., 2001). These differences have been attributed to tissue tolerances of host cnidarians, and to thermal and irradiance tolerances exhibited by zooxanthellae (Fitt, 1985; Iglesias-Prieto et al., 1992; Trench, 1993; Fitt and Warner, 1995; Perez et al., 2001; Goulet et al., 2005). Temperature and irradiance are factors that have been found to influence *Symbiodinium* populations in some tropical and subtropical symbioses (Iglesias-Prieto et al., 1992, 2004; Savage et al., 2002; Tchernov et al., 2004; Goulet et al., 2005). Irradiance and temperature have also been found to be the primary environmental parameters that contribute to bleaching. Rowan et al. (1997) suggested that some corals are protected from bleaching by hosting genetically distinct symbionts that are more tolerant of high irradiance and temperature.

Cnidarians also exhibit geographical variation in the bleaching responses to temperature. Coles et al. (1976) showed that subtropical corals and zooxanthellae from the Hawaiian Islands were more thermally sensitive to elevated seawater temperatures than tropical corals of the same species at lower latitudes from the Marshall Islands. Similarly, Cook et al. (1990) found that corals in Bermuda bleached at temperatures that would be normal for corals at lower latitudes in the Caribbean. LaJeunesse and Trench (2000) showed that 2 species of *Symbiodinium* inhabit the temperate anemone *Anthopleura elegantissima* over a wide range of the Pacific coast of the U. S., and suggested that the biogeographical distribution pattern of dinoflagellates associated with these anemones might be related to the temperature cline created by latitude.

This possible link between geographic location and host/algal composition is the basis for the present study. Specifically, we compared the thermal bleaching responses of *P. caribaeorum* at three latitudes along the east coast of Florida: Palm Beach, Ft. Lauderdale, and American Shoals (Lower Florida Keys). We assayed the thermal bleaching responses of these populations to increased experimental temperatures. The following questions were addressed: 1) Does the experimentally induced high temperature bleaching responses of *P. caribaeorum* correlate with geographic distribution? 2) How does seasonal acclimatization affect bleaching responses to initiated high temperature? 3) Are different *Symbiodinium* spp. associated with geographically separated populations of *P. caribaeorum* along the east coast of Florida?

2. Materials and methods

2.1. Zoanthid collections

Colonies of the zoanthid *P. caribaeorum* were collected using SCUBA from three reefs along the east coast of southern Florida. The northernmost site was off the coast of Palm Beach (PB) in Palm Beach County (26°43.130'N; 80°01.840'W); the middle latitude was off Ft. Lauderdale (FT) in Broward County (26°11.060'N; 80°05.585'W); the third site was American Shoals (AK) in the Lower Florida Keys (24°33.44'N; 81°31.10'W) (Fig. 1). These sites have differences in annual maximum and minimum seawater temperatures and monthly mean seawater temperatures (Table 1) that correlate with latitude.

Samples of unshaded colonies were collected from each site, at approximately the same depth (3–5 m) during winter and summer months in 2002–2003 (Table 1). Single samples of 6–8 separate colonies were collected at each site using a hammer and chisel. Each sample was approximately 10 × 10 cm. The samples were transported to the laboratory within 12 h after collection in coolers filled with natural seawater and allowed to acclimate to room light (100 μmol photons m⁻² s⁻¹) and a temperature of 25 ± 1 °C for a period of 12 h. After acclimation each sample was divided into four smaller pieces consisting of 3–5 live polyps.

2.2. Experimentally induced release of symbionts

The release of algal symbionts at various temperatures (“bleaching response”) was studied using the protocols of Hoegh-Guldberg and Smith (1989) and Perez et al. (2001). The divided pieces were placed in individual 20 ml glass scintillation vials filled with 0.45 μm filtered seawater (FSW), and the vials were sealed tightly. One vial remained

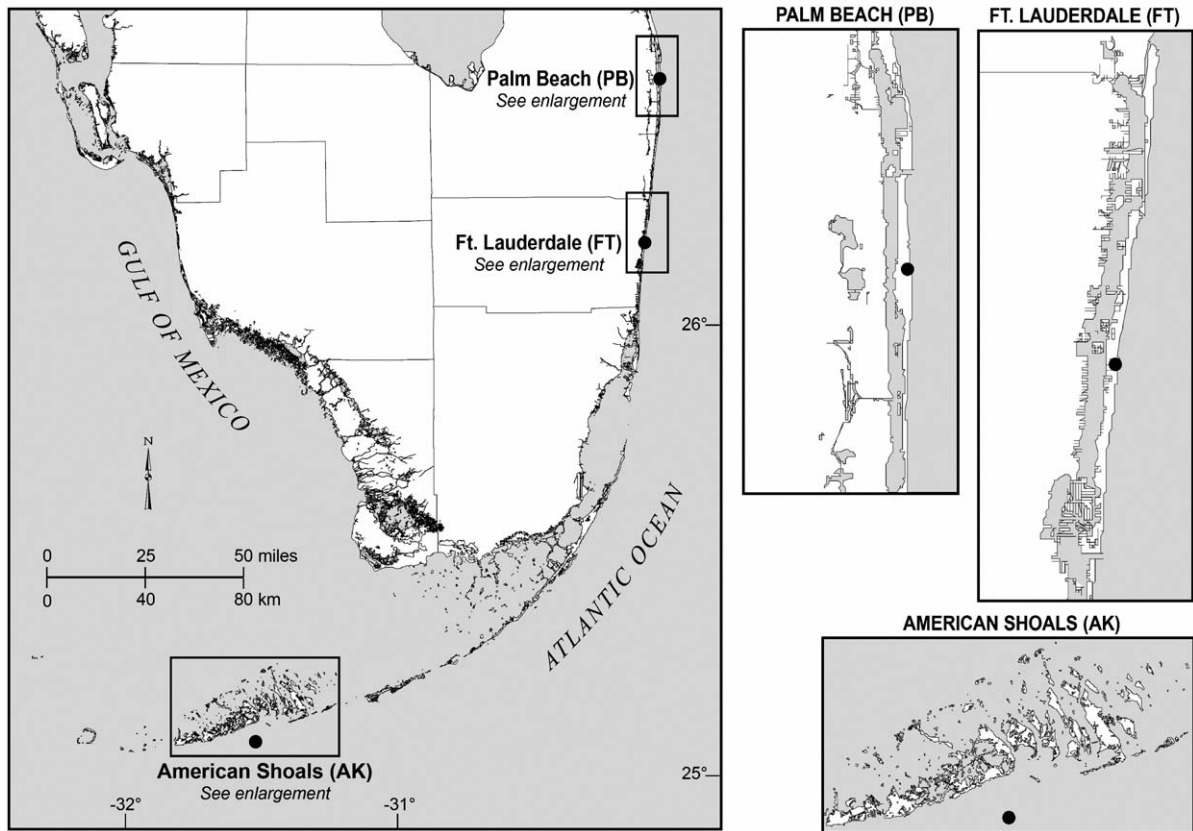


Fig. 1. Map of Florida showing the locations of the three study sites: Palm Beach ($26^{\circ}43.130'N$; $80^{\circ}01.840'W$), Ft. Lauderdale ($26^{\circ}11.060'N$; $80^{\circ}05.585'W$), and American Shoals ($24^{\circ}33.44'N$; $81^{\circ}31.10'W$) where *Palythoa caribaeorum* collections were made in the winter of 2002–2003 and summer of 2003.

at $26^{\circ}C$ while the other three were directly transferred to preheated water baths with experimental temperatures of 30, 32, and $34^{\circ}C$. This was done for each sample. Irradiances were increased on all four water baths from 100 to $300 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ using 100 W halogen lamps. The vials were kept at these temperatures under 12 h light/dark photoperiods for 48 h, and then removed for determination of percentage of zooxanthellae released by the zoanthids.

2.3. Determination of zooxanthellae release

The release of zooxanthellae was calculated by determining the number of cells that were released in the vials and the number remaining in host tissue. After removal of the polyps from the scintillation vials, each piece of zoanthid was rinsed with FSW, and these rinses were added to the seawater remaining in the vial for subsequent determination of released algae. We found initial complications with the quantitative extraction of symbiotic algae from the *P. caribaeorum* tissue, due mainly to the large

amount of carbonate sediment that these zoanthids assimilate in their tissues. To minimize this problem, zoanthid polyps were transferred to a Petri dish where they were cut into smaller pieces using a scalpel, and then the pieces were sonicated using a Fisher Model 60 Sonic Dismembrator at a power setting of $<5 \text{ W (RMS)}$ for 3–5 min. This power setting was the highest setting that allowed quantitative analyses of zooxanthellae left in the host. Higher settings tended to break the cell walls of the zooxanthellae (Kemp, personal observation). The sonicated tissue was then homogenized in FSW using a Teflon and glass homogenizer, centrifuged, and the suspended pellet was washed two more times by centrifugation. The final pellet was re-suspended in 5 ml FSW. Numbers of zooxanthellae were determined from 10 replicate haemocytometer counts, which were converted to total numbers.

After the 48 h incubation period, the seawater in the scintillation vials was transferred to 50 ml centrifuge tubes. The polyps and interior of the scintillation vials were rinsed with FSW, and the rinses were added to the water in the centrifuge tubes. The samples were homogenized in

Table 1
Site location, temperature parameters, and date of *Palythoa caribaeorum* collections

Site	Latitude and longitude	Date of collection	Temperature at time of collection (°C)	Maximum and minimum surface seawater temperatures
Palm Beach (PB)	26°43.130'N;	Winter: Feb. 2003	Winter: 21.7 °C	Lake Worth ^a Max. 33.1 °C
	80°01.840'W	Summer: Sept. 2003	Summer: 30.6 °C	Min. 14.7 °C
Fort Lauderdale (FT)	26°11.060'N;	Winter: Dec. 2002	Winter: 25.0 °C	Fort Lauderdale ^b Max. 31.0 °C
	80°05.585'W	Summer: Sept. 2003	Summer: 29.4 °C	Min. 19.0 °C
American Shoals (AK)	24°33.440'N;	Winter: March 2003	Winter: 26.1 °C	Sand Key ^c Max. 32.4 °C
	81°31.100'W	Summer: Sept. 2003	Summer: 30.6 °C	Min. 17.6 °C

Maximum and minimum surface seawater temperatures for Palm Beach and American Shoals were taken from the nearest available data stations deployed by the National Data Buoy Center (NDBC) (<http://www.ndbc.noaa.gov/Maps/Florida.shtml>) and Fort Lauderdale seawater temperatures were supplied by Broward County Department of Planning and Environmental Protection. ^a, ^cTemperature data recorded from 1991–2003.

^bTemperature data recorded from 2000–2003.

FSW using a Teflon and glass homogenizer, washed three times by centrifugation and the final pellets were re-suspended in 1 ml of FSW. The released cell numbers were determined from 10 replicate haemocytometer counts. The release of zooxanthellae was expressed as percentage of cells released from the original total cell number:

$$\% \text{ released cells} = \frac{\text{Expelled cells}}{\text{Expelled cells} + \text{cells in zoanthid}} \times 100$$

2.4. Symbiont DNA extraction, PCR amplification, and DGGE

To identify the symbionts in each experiment, DNA was isolated from zooxanthellae obtained from the colonies used in the release experiments. 3 to 5 additional polyps from each colony were transected to expose the coelenteron, and samples of the gastrodermal tissue were collected by scraping with a scalpel. This gastrodermal material, containing algae but free of mesogleal collagen and sediment, was suspended in DMSO buffer (20% dimethylsulfoxide, 0.25 M EDTA in saturated aqueous sodium chloride; *Seutin et al., 1991*) and stored in 1.5 ml microcentrifuge tubes at 10 °C.

All subsequent DNA extractions, PCR amplifications, and denaturing-gradient gel electrophoresis (DGGE) were done using the protocols of *LaJeunesse et al. (2003)*. DNA was extracted using the Wizard DNA preparation protocol (Promega). Between 20 and 40 mg of material was placed into 1.5 ml microcentrifuge tubes with 250 µg of 0.5 mm

glass beads and 600 µl nuclei lysis buffer (Promega) and beaten for 140 s at 2400 rpm in a Biospec Mini-Beadbeater. The lysate was then incubated with 0.1 mg ml⁻¹ proteinase K for 1 h at 65 °C, followed by incubation with 6 µg ml⁻¹ RNase at 37 °C for 10 min. 250 µl of protein precipitation buffer (Promega) was then added and the extract incubated on ice for 10–15 min. After centrifugation for 5 min at 15,000 g, 600 µl of supernatant was transferred to a second 1.5 ml tube with 700 µl 100% isopropanol and 50 µl 3 M sodium acetate, pH 5.6. After incubation on ice for 10 min, the precipitated DNA was centrifuged and the pellet washed with 70% ethanol. The DNA was centrifuged again for 5 min, dried, and re-suspended in 95 µl H₂O and 5 µl of 10× Tris-EDTA (10 mM Tris Base, 1 mM EDTA Solution, 0.05% Tween 20, pH 9).

Denaturing-gradient gel electrophoresis (DGGE) was used to analyze the ITS2 region of nuclear ribosomal RNA genes (*LaJeunesse, 2001, 2002*). PCR-DGGE analyses of the ITS2 region were conducted using the forward primer, “ITSintfor2” (5'-GAATTGCAGA ACTCCGTG-3') (*LaJeunesse and Trench, 2000*), which anneals to a “*Symbiodinium*-conserved” region in the middle of the 5.8S ribosomal gene, and the highly conserved reverse primer that anneals to the LSU “ITS2CLAMP” (5'-CGCCCGCCGC GCCCGCGCC CGTCCC GCCG CCCC GCCC GGGATCCATA TGCTTAAGTT CAGCGGGT-3'), an ITS-reverse universal primer modified with a 39-bp GC clamp (italicized) (*LaJeunesse and Trench, 2000*). A “touchdown” amplification protocol with annealing conditions 10 °C above the final annealing

temperature of 52 °C was used to ensure PCR specificity. The annealing temperature was decreased by 0.5 °C after each of 20 cycles. Once the annealing temperature reached 52 °C, amplified samples were loaded onto an 8% polyacrylamide denaturing gradient gel (45–80% urea-formamide gradient; 100% consists of 7 M urea and 40% deionized formamide) and separated by electrophoresis for 9.5 h at 150 V at a constant temperature of 60 °C (LaJeunesse, 2002). Samples were run with previously published standards (LaJeunesse, 2002). The gel was stained with Sybr Green (Molecular Probes) according to the manufacturer's specifications and photographed.

2.5. Statistical analysis

All statistical analyses were performed using Sigma-Stat® (Version 3.2). The arcsine transformation was applied to all percentage data. Data sets that satisfied assumptions of normality and equality of variance were analyzed by ANOVA or a *t*-test using a significance level (*P*-value) of no greater than 0.05 to identify significant differences. Tukey post hoc multiple comparison tests were performed whenever overall treatment effects were found.

3. Results

3.1. Winter samples: effect of temperature on algal release from *P. caribaeorum*

The dates of collection of the winter samples and surface seawater temperatures were as follows: Palm Beach, February 7, 2003, water temperature=21.7 °C; Ft. Lauderdale, December 12, 2002, water temperature=25.0 °C; American Shoals, March 23, 2003, water temperature=26.1 °C (Table 1). Two-way ANOVA of the arcsine-transformed data from the laboratory release experiments revealed significant algal loss from *P. caribaeorum* at each of the three elevated temperatures, with the greatest expulsion at 32 and 34 °C (Fig. 2). Two-way ANOVA of the arcsine-transformed data revealed significant effects of site and temperature, but there was no interaction between site and temperature. Two-way ANOVA of the arcsine-transformed data revealed that overall Fort Lauderdale zoanthids had lower release of zooxanthellae than either American Shoals or Palm Beach, but no significant differences were found between Palm Beach and American Shoals (Tukey post hoc multiple comparison, $P > 0.05$). One-way ANOVA of the data for each temperature between sites showed an overall effect on algal expulsion at 26, 30, and 34 °C ($P < 0.01$ for each temperature). At these temperatures, samples from American Shoals lost significantly higher proportions of

symbionts than samples of *P. caribaeorum* from Fort Lauderdale; these were the only significant between-site differences (Fig. 2).

3.2. Summer samples: effect of temperature on the release of algae from *P. caribaeorum*

The collection dates and surface seawater temperatures were as follows: Palm Beach, September 4, 2003, water temperature=30.6 °C; Ft. Lauderdale, September 10, 2003, water temperature=29.4 °C; American Shoals, September 14, 2003, water temperature=30.6 °C (Table 1). As with the winter samples, algal expulsion for the summer samples increased significantly with temperature at all sites (Fig. 3; two-way ANOVA $P < 0.001$). Overall, samples of *P. caribaeorum* from American Shoals had higher rates of release than those from Fort Lauderdale or Palm Beach; zoanthids from the latter sites did not differ (Fig. 3; Tukey post hoc multiple comparison, $P > 0.05$). However, one-way ANOVA of arcsine-transformed data for each temperature between sites showed no overall effect on algal expulsion at any temperature ($P > 0.05$ for each temperature).

3.3. Seasonal effects on the thermal bleaching response

Comparisons of the thermal bleaching responses of *P. caribaeorum* in winter and summer are shown at each site in Fig. 4. Overall, winter zoanthids collected from Palm Beach and American Shoals experienced greater amounts of algal loss than did summer samples ($P < 0.01$). However, significant seasonal differences in bleaching responses were only found at 26 and 34 °C for Palm

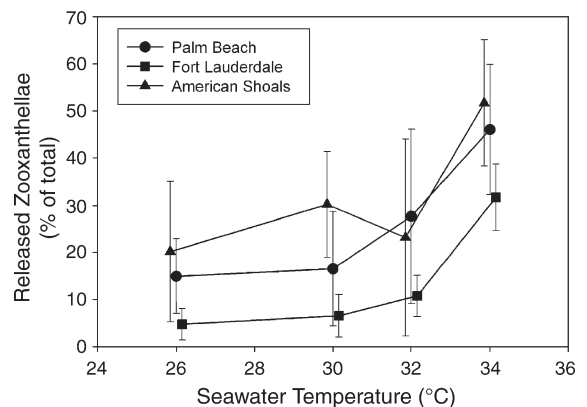


Fig. 2. Release of symbiotic algae from winter collections of *Palythoa caribaeorum*. Colonies were collected during the winter months of 2002–2003 and exposed to elevated temperatures in the laboratory for 48 h. Means + S.E.M. Sample sizes: PB ($n = 6$); FT ($n = 8$); AK ($n = 8$). (one-way ANOVA of arcsine-transformed data: $P < 0.05$).

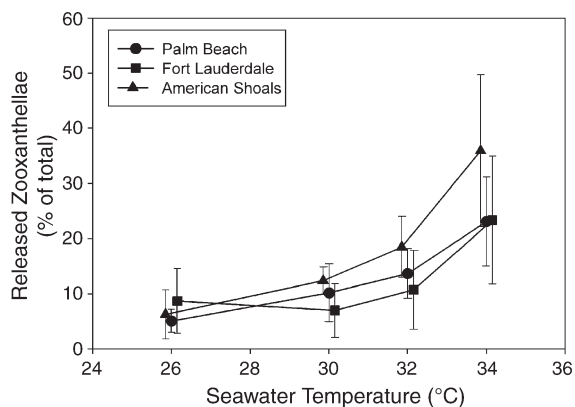


Fig. 3. Release of symbiotic algae from summer collections of *Palythoa caribaeorum*. Colonies were collected during the summer month of September 2003, and exposed to elevated temperatures in the laboratory for 48 h. Means + S.E.M. Sample sizes: PB ($n=8$); FT ($n=8$); AK ($n=8$). No significant differences between sites at a single given temperature were found. (one-way ANOVA of arcsine-transformed data: $P<0.05$).

Beach and at 26, 30, and 34 °C for American Shoals (Fig. 4a,b); one-way ANOVA of arcsine-transformed data; $P<0.05$; Tukey post hoc multiple comparison, $P<0.05$). Zoanthid samples collected from American Shoals showed seasonal differences in experimental bleaching responses (two-way ANOVA, $P<0.01$). Again, winter samples of *P. caribaeorum* experienced the greatest amount of algal loss at all tested temperatures and significant differences were revealed at 26, 30 and 34 °C (Fig. 4c) (one-way ANOVA of arcsine-transformed percent released data $P=0.035$) (Tukey post hoc multiple comparison, $P<0.05$). In contrast there were no seasonal differences observed for Fort Lauderdale at any temperature (Fig. 4c) (Tukey multiple comparison, $P=0.851$).

3.4. Identification of *Symbiodinium* samples using PCR-DGGE

Analyses by PCR-DGGE of ITS2 variants revealed two distinctive *Symbiodinium* spp. One belonged to clade C, characterized previously as type C1 (LaJeunesse, 2001), and the second was type D1a in clade D (LaJeunesse, 2002). Each was found in *P. caribaeorum* from Palm Beach, Fort Lauderdale, and American Shoals (Fig. 5a,b). Colonies were either found to have only C1 populations, only D1a populations or some combination of both (Fig. 5b).

Five of the six Palm Beach colonies sampled in winter contained only C1, while the other contained both C1 and D1a populations of zooxanthellae. Two of the eight summer samples from Palm Beach contained only C1, four contained only D1a, and the remaining two had mixtures of C1 and D1a. Four of the eight colonies sampled at the

Fort Lauderdale site in winter had C1 populations, while the other four had mixtures of C1 and D1a. Five of the eight summer samples from the Fort Lauderdale site contained only C1, one D1a, and two possessed mixed populations of C1 and D1a.

Four of the eight colonies sampled at American Shoals in winter contained only C1 populations, two contained only D1a, and two had mixtures of C1 and D1a. In the summer samples four of eight colonies had only C1 populations, and three colonies had a mixture of C1 and D1a;

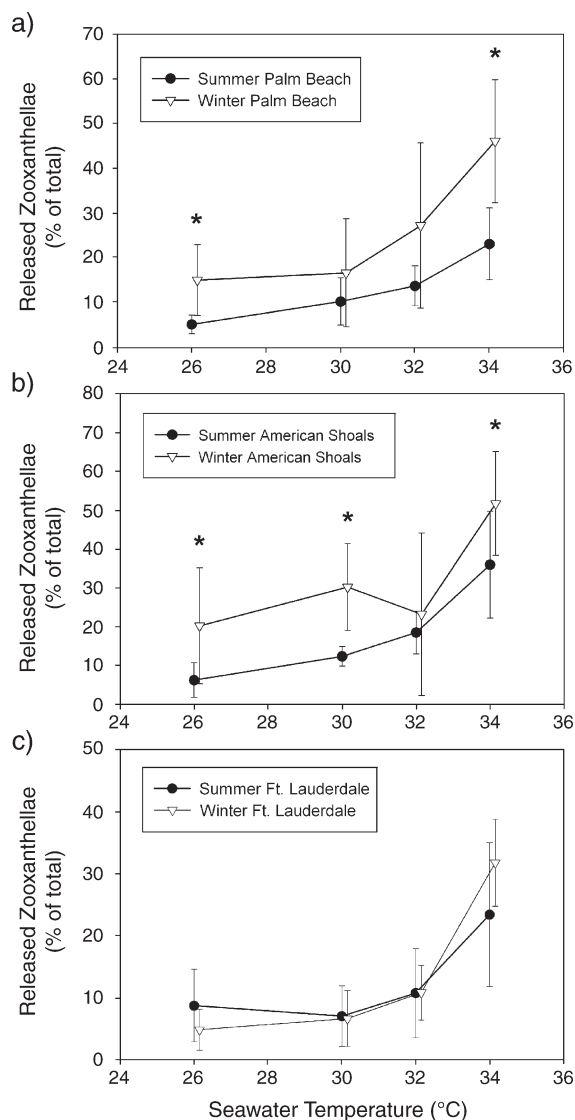


Fig. 4. Seasonal variation of released algae from *Palythoa caribaeorum* over 48 h as a result of increased water temperature. a) PB winter samples ($n=6$) and summer samples ($n=8$); b) AK winter samples ($n=8$) and summer samples ($n=8$); c) FT winter samples ($n=8$) and summer samples ($n=8$); Means + S.E.M.; *Significantly different between seasons (Tukey post hoc multiple comparison $P<0.05$).

none of these samples were only D1a. The other colony sampled in the summer had a strain of *Symbiodinium* sp. that differed from all of the others that were found in this study. This unidentified strain apparently belongs to clade C, but no effort was made to identify these algae beyond the cladal level (Fig. 5b).

Given the recent interest in clade D zooxanthellae as temperature-tolerant algae (Baker et al., 2004; Rowan, 2004; Chen et al., 2005), we examined our release data to

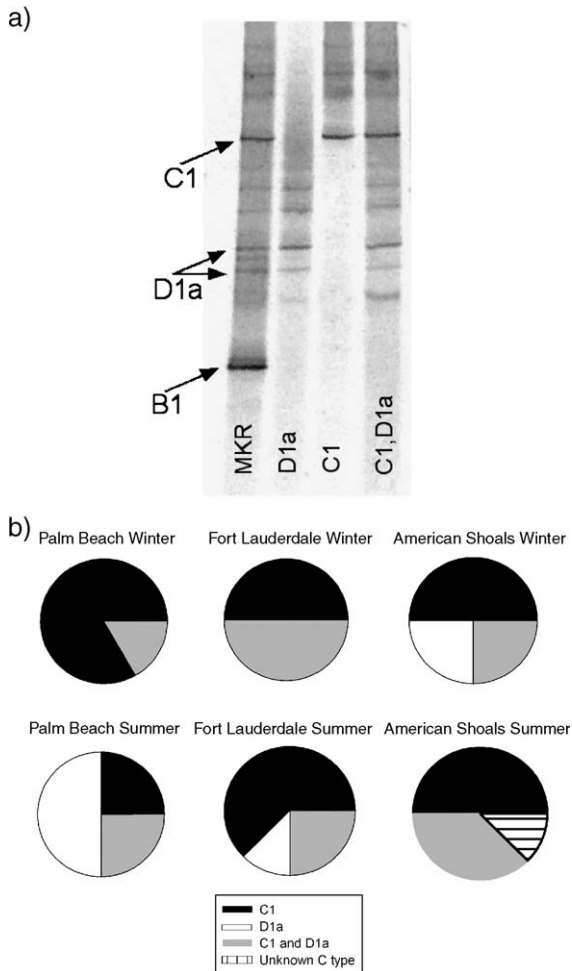


Fig. 5. The distribution of *Symbiodinium* genotypes (ITS2 region) in the summer and winter samples of *Palythoa caribaeorum*. a) PCR-DGGE fingerprint profiles of the ITS2 region showing the genotypes involved. The gel profile is presented as a reverse image. The first gel lane is a marker lane with some common PCR-DGGE profiles of *Symbiodinium* found in the Caribbean. Designated symbiont types are indicated at the bottom of the gel; capital letters indicate clades, and numbers represent ITS2 types (LaJeunesse, 2002). b) Pie charts showing the symbiont composition of colonies of *P. caribaeorum* that were experimentally bleached. Each chart shows the percentage distribution of genotypes among the colonies sampled. $n=8$ for each except for Palm Beach (winter; $n=6$).

Table 2
Effect of symbiont type on the release of symbionts by *Palythoa caribaeorum*

A. Release from zoanthids containing either C1 or D1a symbionts			
<i>32°: (summer only)</i>			
Clade	<i>N</i>	% release \pm SD	<i>P</i> ^a
C1	11	15.7 \pm 7.3	>0.05
D1a	5	13.0 \pm 5.8	
<i>34°: (summer only)</i>			
C1	11	31.4 \pm 15.4	>0.05
D1a	6	25.1 \pm 12.8	
B. Release based on the presence of D1a symbionts (including mixed populations)			
<i>32°: (winter samples)</i>			
Clade	<i>N</i>	% release \pm SD	<i>P</i> ^a
D1a present	9	13.5 \pm 10.1	>0.05
D1a absent	13	24.3 \pm 19.5	
<i>34°: (winter samples)</i>			
D1a present	9	42.9 \pm 17.7	>0.05
D1a absent	13	42.9 \pm 12.0	
<i>32°: (summer samples)</i>			
D1a present	11	13.9 \pm 5.8	>0.05
D1a absent	13	14.6 \pm 7.2	
<i>34°: (summer samples)</i>			
D1a present	11	24.5 \pm 9.4	>0.05
D1a absent	13	30.0 \pm 14.5	

^a*t*-test of transformed data (angular transformation).

For these analyses, data from all sites were pooled, and the release by winter and summer samples at 32° and 34° were used.

see if there was any relationship with cladal identity. We pooled the data from all sites at each season, and compared the percent release at 32° and 34°. The data were compared in two ways. First, we looked at the release from zoanthids harboring either C1 or D1a symbionts. Since only two zoanthids from the winter samples contained only D1a, this analysis was restricted to the summer samples. Our second approach was to compare all zoanthids with D1a symbionts (including mixed populations) with those without them. Both analyses yielded similar results: there was no relationship between symbiont complement and release (Table 2).

4. Discussion

We compared the thermal bleaching responses of *P. caribaeorum* from three latitudinally separated localities along the east coast of south Florida. Colonies from each region responded to experimental heating by expelling their symbionts, a reaction displayed by many zooxanthellate cnidarians (e.g., Hoegh-Guldberg and Smith, 1989). The

magnitude of each response, however, differed among each site. Colonies from Fort Lauderdale exhibited the least amount of algal loss during winter and summer experiments (Figs. 2 and 3), while contradictory to our expectations, colonies from American Shoals in the Florida Keys exhibited the greatest amount of algal loss during both winter and summer experiments (Figs. 2 and 3). Colonies from Palm Beach, the site with the lowest annual mean temperature, were intermediate. The results of Coles et al. (1976) suggested that difference in coral thermal tolerances should correspond with differences in the ambient temperature patterns between geographic areas.

Data collected from the National Data Buoy Center (NDBC) and Broward County Department of Planning and Environmental Protection indicate that the monthly mean seawater temperatures of the Lower Keys (i.e. American Shoals) were almost always greater than those at the other sites. This site experienced the least fluctuation in monthly mean seawater temperatures. In addition, the Lower Keys are the only site with a monthly mean seawater temperature greater than 30 °C during the summer. Yet, *P. caribaeorum* from American Shoals showed the highest levels of zooxanthellae loss when exposed to experimentally increased seawater temperatures. With respect to monthly mean seawater temperature differences, Fort Lauderdale was the intermediate site of the three, but zoanths from Fort Lauderdale showed the greatest thermal tolerance to an increase in seawater temperature, and in all but one instance (summer at 26 °C) exhibited the lowest percent algae released for both the winter and summer bleaching experiments.

One explanation for this pattern is that hosts that experience the widest range of temperatures harbor symbionts with similarly wide tolerances. An example of this is the 1988 bleaching event reported from Bermuda by Cook et al. (1990). These authors found that corals from patch reefs in shallow lagoons, which experience greater ranges of seawater temperatures than those on rim reefs with more oceanic exposure, did not bleach as much as did corals on the outer rim reefs. Cook et al. (1990) suggested corals that normally experience wide temperature ranges should be less sensitive to thermal stress. *P. caribaeorum* from Palm Beach and Fort Lauderdale, might be more tolerant of thermal stress because they must acclimate annually to broader ranges in temperature.

LaJeunesse and Trench (2000) showed that two species of zooxanthellae inhabit the temperate anemone *A. elegantissima* over a large geographic range along the west coast of the United States. In northern populations they found *A. elegantissima* associated with *Symbiodinium muscatinei* while the southern populations also contained *S. californium*. Additionally, they often found

mixed populations of *S. muscatinei* and *S. californium* associating with *A. elegantissima* at southern collection sites. LaJeunesse and Trench (2000) suggest that the biogeographical distribution of dinoflagellates associated with *A. elegantissima* might be related to the temperature cline created by latitude. Our PCR-DGGE analysis of the symbiotic algae associated with *P. caribaeorum* colonies revealed a dynamic assemblage of two different clades of *Symbiodinium* sp. Colonies with only clade C1 and/or D1a were found in all samples during both winter and summer collections. Additionally, colonies containing both C1 and D1a were found at all sites in both winter and summer. This suggests that any differences that we observed in bleaching responses were not related to genetic differences between the symbionts, at least at the taxonomic level we examined. We do not imply by this that genetic differences between the symbionts – or the hosts – are not involved in between-site differences. Unfortunately, we did not examine the identity of symbionts released from polymorphic colonies, so we do not know if selective release had occurred.

LaJeunesse (2002) and LaJeunesse et al. (2003) stated that *Symbiodinium* types C1 and D1a are common “host-generalists” occurring in the Caribbean. Collections of *P. caribaeorum* from Caribbean coasts of Mexico and Belize contain C1 (LaJeunesse, 2002). It has been suggested that members of the D clade of *Symbiodinium* are opportunistic symbionts that occupy open niches of recently bleached corals that are in the process of recovering. (Baker, 2001, 2003; Baker et al., 2004; Van Oppen, 2001). This may explain why *Symbiodinium* D occurs unpredictably in various scleractinians and other hosts, including the Pacific zoanthid *Palythoa caesia* (Burnett, 2002). However, it is now clear that corals in the Caribbean and Florida can recover from bleaching events without the involvement of members of this clade (Thornhill et al., 2006).

Baker (2003) suggests that *Symbiodinium* D may be a useful tool in monitoring the health of the coral communities by signaling recent and/or recurrent stress events. The abundance of *Symbiodinium* D1a in summer samples of *P. caribaeorum* may indicate that the colonies sampled may have recently or are currently under stress and/or in the process of recovering to their steady-state symbiont communities. To date, few studies have examined the physiological associations of *Symbiodinium* D within hosts (i.e. the “holobiont”), but the work of Baker et al. (2004) and Rowan (2004) suggests that members of this clade may have greater thermal tolerances than *Symbiodinium* C. In our samples of *P. caribaeorum*, we did not find any indication that colonies with only *Symbiodinium* D1a were more heat tolerant

than those containing *Symbiodinium* C1 or having mixtures of C1 and D1a.

There are other explanations for the bleaching responses that we observed. One is that there has been little temperature-related selection of symbiont genotypes at the sites in our study, at least for the ITS markers that we used. A second possibility is that environmental or physiological factors other than temperature maintain these host–symbiont populations. For example, niche partitioning between genetic strains of *Symbiodinium* may occur within *P. caribaeorum* tissue in response to different light fields (e.g. Rowan and Knowlton, 1995; Baker, 2001). *P. caribaeorum* is notable for the amount of particulate inorganic material it contains. Haywick and Mueller (1997) found that *P. caribaeorum* can assimilate a variety of minerals from the reef, including aragonite, calcite, magnesium calcite, and minor quantities of siliciclastic components. This inorganic material can account for up to 45% of the total biomass, and likely affects the optical properties of the tissue. Enriquez et al. (2005) found symbiotic scleractinian corals to be among the most efficient light collectors in nature, because the massive CaCO₃ skeleton acts as a Lambertian reflector, aiding zooxanthellae in the absorption of solar energy. Although no similar studies have been done with zoanthids, we suggest that the crystalline material in the mesoglea may increase the light field of the zooxanthellae at the colonies' surface while potentially shading symbiont populations within the colonies' interior. Such differences in light field could influence the partitioning of *Symbiodinium* types in "optimal" microhabitats within the host. A third explanation of the bleaching patterns that we observed could be genetic differences between the host populations, rather than of the symbionts.

To our knowledge, there have been no studies comparing the thermal sensitivities of host–symbiont combinations collected during winter versus summer months. In our study, the winter samples showed an increase in released algae at every temperature (Fig. 4). One explanation for seasonal differences is that the zoanthids harbored different symbiont populations, but our findings do not support this (Fig. 5). Rather, it appears that seasonal acclimation of the host or the symbiont is involved. Downs et al. (2002) found elevated levels of antioxidants and heat shock proteins in summer corals, which would reduce the propensity for bleaching. Several studies have shown that corals have greater densities of zooxanthellae in winter months (Stimson, 1997; Fagoonee et al., 1999; Fitt et al., 2001). The combination of increased symbiont density and decreased "host defenses" would make winter corals (or zoanthids) more susceptible to elevated temperature.

However, we did not determine symbiont densities or antioxidant levels in our samples.

The spatial and seasonal variations in thermal susceptibilities experienced by *P. caribaeorum* and its zooxanthellae during this study may indicate the importance of acclimatory processes experienced by the animal, the zooxanthellae, or both (Dunne and Brown, 2001). It is important to note that this study revealed there is an increase/decrease in thermal susceptibilities that correlate to both seasonal changes and local environmental conditions. It is proposed that the dynamic relationship between *P. caribaeorum* and its symbionts may be partially explained by the possible abundance of microhabitats within *P. caribaeorum* tissue layers due to the large amounts of sediment found within the mesoglea.

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