

# Stability of symbiotic dinoflagellate type in the octocoral *Briareum asbestinum*

Andrew R. Hannes<sup>1</sup>, Marcos Barbeitos<sup>1</sup>, Mary Alice Coffroth<sup>2,\*</sup>

<sup>1</sup>Department of Biological Sciences, State University of New York at Buffalo, Buffalo, New York 14260, USA

<sup>2</sup>Graduate Program in Evolution Ecology and Behavior and Department of Geology, State University of New York at Buffalo, Buffalo, New York 14260, USA

**ABSTRACT:** Coral bleaching—the loss of photosynthetic algal symbionts from their cnidarian hosts—can lead to coral mortality and subsequent reef degradation. To understand the phenomenon of coral bleaching it is imperative to understand natural fluctuations in symbiotic dinoflagellate diversity and density. In this study, *Symbiodinium* type, based on length variation in domain V of the chloroplast large subunit of rDNA (cp23S rDNA), and cell densities were followed in 2 populations of the octocoral *Briareum asbestinum* over 1 yr. *Symbiodinium* type varied little over the course of the study despite anomalously cold sea surface temperatures during January 2003, when *B. asbestinum* populations experienced a significant loss of symbionts. This provides some of the first evidence that Caribbean octocorals are susceptible to cold-water bleaching events. Furthermore, the symbiont stability observed within *B. asbestinum* contributes to an increasing number of studies suggesting that many zooxanthellate coral species may not respond to fluctuating environments by changing symbionts.

**KEY WORDS:** Bleaching · Coral · *Symbiodinium* · Symbiosis · Zooxanthellae · Diversity

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## INTRODUCTION

Over the past 2 decades the condition of coral reefs worldwide has attracted universal attention and the increased incidence of coral bleaching, defined as the loss of symbiotic algae (dinoflagellates within the genus *Symbiodinium*) or their pigmentation, has been especially alarming. Bleaching has been linked to thermal stress, high ultraviolet light exposure, sedimentation, disease and low salinity (Glynn & D'Croz 1990, Gleason & Wellington 1993, Douglas 2003). Symbiotic dinoflagellates provide vital contributions to coral nutrition and physiology (Muscatine 1990) and loss of these algal symbionts (i.e. bleaching) often results in host death. Several studies have tracked symbiont cell densities in scleractinians and octocorals during natural seasonal changes in temperature and irradiance levels (Coles & Jokiel 1978, Glynn & D'Croz 1990, Fitt & Warner 1995, Fitt et al. 2000, Lasker 2003) and a few studies report on temporal variation in symbiont diversity within their cnidarian host (Goulet &

Coffroth 2003a, Chen et al. 2005, Thornhill et al. 2006a,b). However, there are no studies that examine the fluctuation of these characteristics together in octocorals, which are often the dominant cnidarians on reefs (Lasker & Coffroth 1983, Sanchez et al. 2003).

The genus *Symbiodinium* is a heterogeneous group of many species and strains that display different infectivity, behavior, ultrastructure, molecular characteristics and physiologies (reviewed in Trench 1997, Rowan 1998, Baker 2003, Coffroth & Santos 2005, Stat et al. 2006). This diversity is found not only among host taxa, but also, in some instances, within a single host species (reviewed in Goulet 2006, Baker & Romanski 2007). For example, in some host taxa the complement of *Symbiodinium* varies with fluctuating thermal and irradiance levels (Rowan et al. 1997, Baker 2001, Toller et al. 2001, van Oppen et al. 2001, Berkelmans & van Oppen 2006, Oliver & Palumbi 2009). This raises the possibility of seasonal change in symbiont composition. Temporal variation has been examined among scleractinian corals where symbiont assemblages varied seasonally

\*Corresponding author. Email: coffroth@buffalo.edu

in some species (Chen et al. 2005), while in many cases no seasonal pattern was discerned in studies over 3 and 5 yr periods (Thornhill et al. 2006a,b) or during extreme temperature fluctuations over the course of a year (Rodriguez-Lanetty et al. 2003).

Decreases in the density of symbiotic dinoflagellates are generally proportional to increases in temperature (Coles & Jokiel 1978, Glynn & D'Croz 1990, Fitt & Warner 1995). In a 2 to 5 yr study of 5 scleractinian species, Fitt et al. (2000) found the lowest densities of symbiotic dinoflagellates at the end of the summer season when seawater temperatures were high (termed 'seasonal bleaching' by Fitt et al. 2000). Aside from seasonal bleaching, there are numerous studies documenting, forecasting and modeling widespread bleaching events caused by anomalously warm sea-surface temperatures (SSTs) (reviewed in Hughes et al. 2003, McWilliams et al. 2005, Donner et al. 2007, Carpenter et al. 2008); however, there are few reports detailing the effects of anomalously cold SST's (Shinn 1966, Porter et al. 1982, Hoegh-Guldberg et al. 2005).

Abnormally warm and cold temperatures affect photosynthetic organisms by increasing photoinhibition, eventually leading to cellular damage (Lyons 1973, Krause 1992). Saxby et al. (2003) provided evidence that corals exposed to reduced temperatures ( $\leq 14^{\circ}\text{C}$ ) experience similar physiological symptoms as those exposed to elevated temperatures, which, depending on the duration of thermal exposure and light regime, can cause partial to total bleaching and mortality.

Few studies have followed temporal variation in *Symbiodinium*–octocoral symbioses even though octocorals are among the most abundant cnidarians of Caribbean reef communities, with up to 40 species co-existing in a single coral community (Lasker & Coffroth 1983, Sanchez et al. 2003). In the octocoral *Plexaura kuna*, *Symbiodinium* densities were lowest during the summer months (Lasker 2003), yet the predominant *Symbiodinium* genotype did not vary over 10 yr of sampling, demonstrating tremendous temporal stability (Goulet & Coffroth 2003a).

In the present study we monitored temporal patterns in *Symbiodinium* diversity and cell density in the octocoral *Briareum asbestinum* (encrusting form). *B. asbestinum* is a gonochoric brooding gorgonian that is widely distributed throughout the Caribbean Sea (Brazeau & Lasker 1992). Like most Caribbean octocorals, *B. asbestinum* harbors mainly Clade B *Symbiodinium*, which is acquired via horizontal transmission during the juvenile stage (LaJeunesse 2002, Goulet & Coffroth 2004). However, finer level characterization of the symbionts reveals the presence of variation within the Clade B *Symbiodinium* harbored by *B. asbestinum* (Santos et al. 2003) and studies of the establishment of the symbiosis indicate that *B. asbestinum* polyps can

host other symbiont types shortly after metamorphosis of the planula larva into a polyp (M. A. Coffroth unpubl. data). Moreover, *B. asbestinum* can shift symbionts after an artificial bleaching event (Lewis & Coffroth 2004). This raises the question of whether *B. asbestinum* also shows variation after exposure to natural seasonal events and smaller scale stresses. The goal of the present study was to determine whether temporal variation in *Symbiodinium* taxa and density occur within the octocoral *B. asbestinum*.

## MATERIALS AND METHODS

**Study site and collection of corals.** Two populations of 30 *Briareum asbestinum* colonies were repeatedly sampled from the middle Florida Keys during 2002 and 2003. The Point population was located approximately 30 m offshore from Long Key in Florida Bay ( $24^{\circ}49.949' \text{N}$ ,  $80^{\circ}48.660' \text{W}$ ) at a depth of 1.5 m; the Craig Key population was located approximately 150 m offshore from Craig Key in the Atlantic Ocean ( $24^{\circ}49.791' \text{N}$ ,  $80^{\circ}46.743' \text{W}$ ) at a depth of 2.0 m. These 2 sites were chosen because they contain high densities of *B. asbestinum* and potentially experience different environmental conditions that may differentiate the coral–symbiont dynamics of the 2 populations. Tissue was collected from the lower third of each colony in June and October 2002 and January, March and June 2003.

**Temperature measurements.** We monitored *in situ* seawater temperatures at each site from October 2002 to June 2003 using HOBO H-01-001-01 temperature loggers (Onset Computer Corporation; accuracy within  $\pm 0.1^{\circ}\text{C}$ ). Additional temperature measurements from January 1993 to January 2009 were obtained from Florida Bay at the Long Key Buoy (LONF1), which is operated and maintained by the National Oceanic and Atmospheric Administration's (NOAA's) National Data Buoy Center, approximately 2.0 km from our Point site. These temperatures were used as a proxy for seawater temperature at the study sites when the temperature loggers were not deployed.

We compiled data from the LONF1 buoy corresponding to 12 yr of hourly SST measurements, from January 1994 to December 1995 and from July 1998 to December 2008. Temperature readings from December 1995 to July 1998 were fragmented and therefore were not used. These data were good predictors of the hourly temperatures obtained with the HOBO loggers at Craig and Point from October 20th, 2002 to June 6th 2003 (linear regression: Craig  $r^2 = 0.84$ ,  $F_{1,1543} = 7,996.14$   $p < 0.001$ ; Point  $r^2 = 0.98$ ,  $F_{1,1544} = 32,702.49$   $p < 0.001$ ).

Daily minimum and maximum temperatures were analyzed using classical seasonal decomposition (CSD) to remove the seasonal and trend-cycle components.

The trend-cycle component was estimated using 5 mo weighted centered moving average (Makridakis & Wheelwright 1989). Graphical inspection of temperature series revealed that the months of January were consistently the coldest during the survey period. We used CSD residuals obtained for the months of January 1995, 1999 and for all Januaries from 2001 to 2008 to test if the winter of 2003 was significantly colder than the other winters within the 12 yr period. To test if the summer of 2002 was significantly warmer, we used residuals obtained for the hottest part of that season (from June until August) for the years of 1994, 1995 and from 1999 to 2007. Because the time series was frequently interrupted during the sampling period, we could not use all available seasons. Residuals were subjected to Kruskal-Wallis ANOVA followed by Bonferroni-corrected pair-wise Mann-Whitney tests.

**Characterization of *Symbiodinium* diversity.** The *Symbiodinium* populations harbored by *Briareum asbestinum* were distinguished by length variation in domain V of the chloroplast large subunit of rDNA (cp23S-rDNA) following standard protocols and comparing the resultant fragments to known standards (cp23S-rDNA type, Santos et al. 2003). This technique rapidly identifies *Symbiodinium* isolates within a phylogenetic framework and allows detection of multiple *Symbiodinium* cp23S-rDNA types of a single clade in a single sample at very low concentrations (estimated at 10 to 1000 cells, Santos et al. 2003). cp23S-rDNA type is designated by clade and bp size of the cp23S-rDNA fragment. *B. asbestinum* typically contains *Symbiodinium* cp23S-rDNA types B178 and/or B184 (i.e. Clade B and a cp23S-rDNA fragment of 178 or 184 bp), which are analogous to ITS2 type B19 and B1, respectively, based on sequence of the internal transcribed

spacer region 2 (ITS2) of nuclear ribosomal DNA (Cofroth & Santos 2005).

To determine whether cp23S-rDNA type varied with sampling position on the colony, tissue samples were taken from the top, middle and bottom of 10 *Briareum asbestinum* colonies adjacent to the marked colonies at the Craig Key and Point sites in August 2002. To determine whether *Symbiodinium* type varied with height above the substrate, colonies were sampled at <10 cm, between 10 and 20 cm and at >20 cm above the substrate. These samples were collected from marked survey colonies at the Craig Key and Point sites in January 2003. Eight additional colonies that were not included in the annual monitoring were sampled from the Point site to attain a sufficient sample size for the >20 cm height.

**Determination of *Symbiodinium* density.** To determine symbiont density, an approximately 4 mm<sup>3</sup> sample of frozen tissue was macerated in a tissue homogenizer with 5 ml of 0.2 µm filtered seawater and passed through a 125 µm mesh to remove tissue. A 1 ml aliquot was preserved in 10% formalin to calculate cell counts and *Symbiodinium* densities were averaged across 4 hemocytometer counts. Cell densities were computed using the product of length, width and thickness of the tissue samples as an approximation of tissue volume.

## RESULTS

### Temperature measurements

The study sites experienced very similar temperatures over the course of a year and from year to year (Fig. 1). Sampling logistics did not permit sampling during late summer months (Aug and Sep) when water temperature

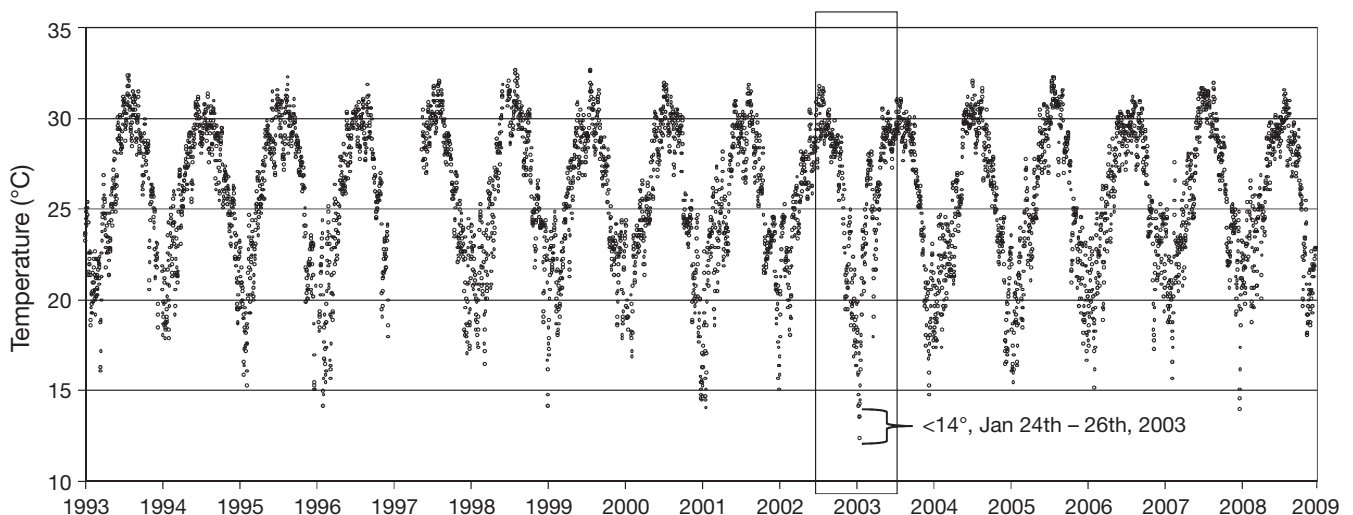


Fig. 1. Minimum daily seawater temperature recorded in Florida Bay at the Long Key Buoy (LONF1) between January 1993 and January 2009. Open rectangle: time period in which the 2 populations of *Briareum asbestinum* were sampled. The 3 d in January 2003 (24th to 26th) when seawater temperatures were <14 °C are indicated

is typically greatest. However, the October 2002 sampling occurred before water temperatures began to decrease, which captured the peak of elevated water temperatures (Fig. 1).

Kruskal-Wallis ANOVA employing errors computed for the months of January was highly significant (Pearson's  $\chi^2 = 36.65$ ,  $df = 8$ ,  $p < 0.001$ ) and Bonferroni corrected ( $\alpha = 0.05/8 = 0.00625$ ) pair-wise Mann-Whitney tests found significant differences between January 2003 and all other months with the exception of January 2001 (Table 1). We also computed the numbers of hourly readings below  $14^\circ\text{C}$  at the buoy for all months of January from 1993 to 2008. January 2003 was the only month when temperatures actually dropped below  $14^\circ\text{C}$  (37 h) and hence this month had a significantly larger number of extreme readings (Pearson's  $\chi^2 = 484.02$ ,  $df = 14$ ,  $p < 0.001$ ) than the other Januaries within this 15 yr window (Fig. 1). A SST reading of  $14^\circ\text{C}$  at buoy LONF1 is equivalent to  $14.2$  to  $14.4^\circ\text{C}$  at Point and  $14.8$  to  $15.3^\circ\text{C}$  at Craig (95% CIs) hence these sites were slightly warmer. HOBO readings at the sites show that only 8 h were spent below  $14^\circ\text{C}$  at Craig and 11 h at Point for January 2003. However, readings at these sites were taken every 3 h as opposed to the hourly readings at the buoy.

Kruskal-Wallis ANOVA was also significant for the summer SST data (Pearson's  $\chi^2 = 40.16$ ,  $df = 10$ ,  $p < 0.001$ ); however, results of the pairwise comparison revealed that the maximum temperatures during the summer of 2002 were not significantly higher than any other year but 2004 (Mann-Whitney  $U = 3169.50$ , Z-score = 2.94,  $p < 0.005$ ).

### Characterization of *Symbiodinium* diversity

The *Symbiodinium* assemblages in the 2 *Briareum asbestinum* populations were dominated by *Symbiodinium* B178 and were stable over a year period, with only 7 of the 60 monitored colonies experiencing a change in cp23S-rDNA type (Table 2). The remaining colonies harbored exclusively B178 for the duration of study. When symbiont change was observed no clear pattern emerged. Log-linear analysis on a 3-way contingency table revealed that genotype frequencies did not change significantly between populations or over time ( $\chi^2 = 2.65$ ,  $df = 12$ ,  $p = 0.99$ ).

Tissue samples taken from different locations within a colony generally contained the same symbiont type.

Table 1. Temperature ranges and results for pair-wise Mann-Whitney tests for January from 1993 to 2008. Ranges for the sites are 95% CIs obtained via linear regression (see 'Materials and methods' for details). Analyses were conducted with the temperature residuals obtained from classical seasonal decomposition. Because of discontinuities in the time series, we only tested months for which the  $U$  statistic was reported. Because multiple comparisons were employed,  $p$ -values were Bonferroni corrected ( $\alpha = 0.00625$ ). **Bold:** significant. LONF 1: Long Key Buoy

Month	Range ( $^\circ\text{C}$ ) LONF 1	Range ( $^\circ\text{C}$ ) Craig Key	Range ( $^\circ\text{C}$ ) Point	Mann-Whitney $U$	Z-score	$p$
Jan 93	18.6–26.3	18.8–26.7	18.8–27.1			
Jan 94	17.9–25.3	18.1–25.6	18.4–26.0			
Jan 95	15.9–24.5	15.9–25.0	16.1–24.9	220.0	-3.66	<b>0.000250</b>
Jan 96	18.3–24.4	18.5–24.8	18.8–25.2			
Jan 98	17.3–25.5	17.5–25.9	17.5–26.3			
Jan 99	14.2–24.4	15.0–24.9	14.4–24.8	270.0	-2.96	<b>0.003110</b>
Jan 00	18.6–26.3	18.8–26.7	19.0–27.1			
Jan 01	14.1–23.6	14.9–24.2	14.2–24.8	467.5	0.18	0.860308
Jan 02	15.1–26.3	15.9–26.8	15.3–26.7	276.0	-2.87	<b>0.004079</b>
Jan 04	17.4–24.2	18.1–24.8	17.6–24.6	198.5	-3.96	<b>0.000074</b>
Jan 05	15.5–24.2	16.3–24.8	15.7–24.6	220.0	-3.66	<b>0.000252</b>
Jan 06	16.7–24.5	17.4–25.0	16.9–24.9	192.0	-4.05	<b>0.000050</b>
Jan 07	18.8–26.0	19.4–26.5	19.0–26.9	109.0	-5.22	<b>0.000000</b>
Jan 08	14.0–26.0	14.8–26.5	14.2–26.9			
Jan 03	12.4–22.6	12.5–23.2 <sup>a</sup>	11.3–24.4 <sup>a</sup>			

<sup>a</sup>Actual ranges obtained from HOBO readings

Table 2. *Symbiodinium* spp. in *Briareum asbestinum*. *Symbiodinium* cp23S-rDNA type within 7 coral colonies that exhibited symbiont variation during the monitoring period from June 2002 to June 2003. Letter before colony number (C: Craig Key; P: Point): source population for each colony. 178/184 indicates periods when both B178 and B184 cp23S-rDNA types were present. The other 54 colonies harbored only cp23S-rDNA type B178 throughout the study.

**Bold: 184 was present**

	Colony designation						
	C4	C14	P1	P8	P9	P14	P15
<b>2002</b>							
June	178	178	178	178	<b>178/184</b>	<b>184</b>	<b>184</b>
October	178	<b>178/184</b>	<b>184</b>	<b>184</b>	178	<b>184</b>	178
<b>2003</b>							
January	<b>178/184</b>	178	178	178	178	<b>184</b>	178
March	178	178	178	178	178	<b>184</b>	178
June	178	178	178	178	178	<b>178/184</b>	178

Additionally, no relationship between height above the substrate and symbiont composition was detected with 31 of the 33 colonies sampled at different heights above the substrate, harboring only *Symbiodinium* B178.

### *Symbiodinium* density

Average *Symbiodinium* sp. concentrations for each sampling period ranged from  $3.19 \times 10^5$  cells  $\text{cm}^{-3}$  (March 2003) to  $3.94 \times 10^5$  cells  $\text{cm}^{-3}$  (June 2002) at the

Craig Key population and from  $2.69 \times 10^5$  cells  $\text{cm}^{-3}$  (June 2003) to  $3.48 \times 10^5$  cells  $\text{cm}^{-3}$  (October 2002) at the Point population. It should be noted that during all sampling events there was no visible difference in the polyp pigmentation of the sampled colonies. However, cell count results revealed that the cell densities in the Craig Key population were consistently higher than in the Point population (repeated measures ANOVA,  $F_{1,217} = 14.79$ ,  $p < 0.001$ ). Furthermore, cell densities decreased significantly over the course of the study ( $F_{4,868} = 7.62$ ,  $p < 0.001$ ) and this decrease was consistent between populations since the interaction term was non-significant ( $F_{4,868} = 0.28$ ,  $df = 4$ ,  $p = 0.892$ ) (Fig. 2). Bonferroni corrected pairwise comparison using the dependent samples Student's t-test revealed that the significant decrease at the Point population occurred between October 19th, 2002 and January 26th, 2003 (Student's  $t = 6.30$ ,  $df = 57$ ,  $p < 0.001$ ); and between October 19th, 2002 and March 27th, 2003 at the Craig Key population (Student's  $t = 4.13$ ,  $df = 56$ ,  $p < 0.001$ ). The Point and Craig populations lost 22.5 and 18.9%, respectively, of their resident symbionts during these significant decreases. It should be noted that during the January 2003 sampling event, the Craig Key population was sampled on the 23rd, 1 d before the SST's dropped below  $14^\circ\text{C}$ , whereas, the Point population was sampled on January 26th, the 3rd consecutive day of SSTs below  $14^\circ\text{C}$  (Fig.2). As of June 12th, approximately 6 mo after the cold SST event, the colonies at both populations had not recovered to the cell density levels observed before the cold SST event.

Kruskal-Wallis ANOVA was used to test for significant differences in cell counts among genotypes in the months when a change in genotypes occurred (Fig. 3).

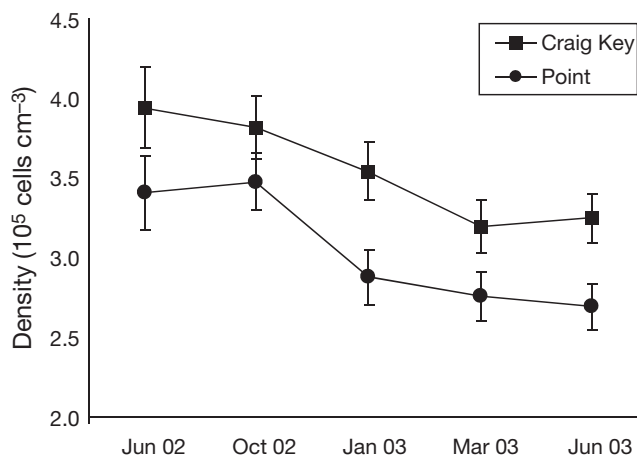


Fig. 2. *Symbiodinium* spp. Mean cell density over the 5 sampling periods from June 2002 to June 2003. Error bars:  $\pm 95\%$  CI

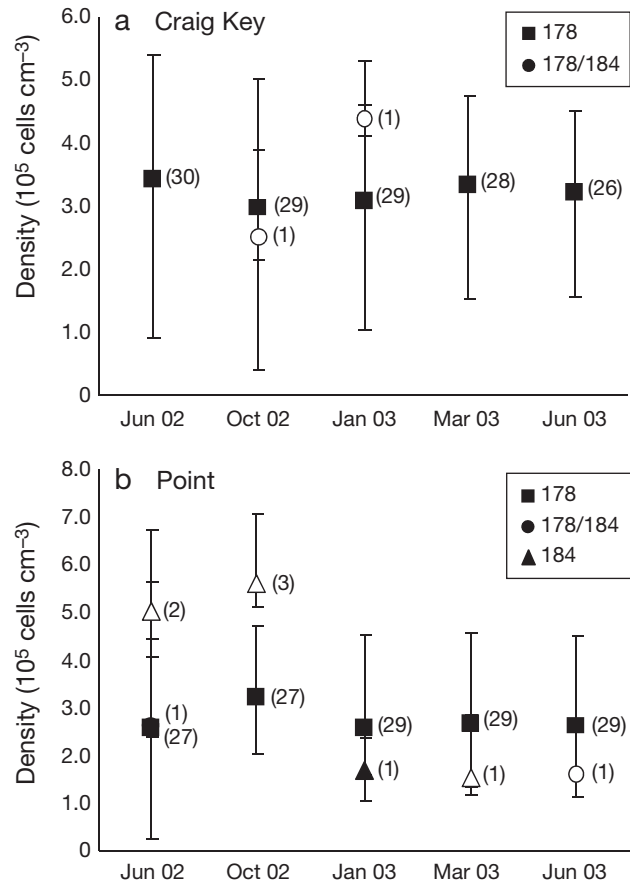


Fig. 3. *Symbiodinium* spp. Temporal variation in cell density grouped by genotype at the (a) Craig Key and (b) Point populations. Symbols: median; error bars: minimum–maximum ranges. Open symbols: symbols corresponding to genotypes that differed significantly with respect to cell density. Parentheses: sample sizes (n) used in the Kruskal-Wallis ANOVA

Although it appears that colonies with B184 have significantly greater cell densities during many of the sampling events, these results could be an artifact of smaller sample sizes. Greater sampling effort is needed to further test this pattern.

## DISCUSSION

The high correlation between the Long Key Buoy (LONF1), Craig Key and Point temperatures indicate that the buoy data were good predictors of SSTs at our study sites. Thus, corals at the study sites experienced anomalously cold SSTs during January 2003, which corresponded to a significant loss of symbionts in both populations, and repeatedly experienced daily averages that exceeded  $31^\circ\text{C}$  during the summer of 2002. Despite the extreme temperature fluctuations that

occurred throughout the year, these octocorals did not change symbiont composition.

*Briareum asbestinum* associates with at least 2 types of Clade B *Symbiodinium* (B178 and B184) with the abundance of each type varying between sites (D. Poland & M. A. Coffroth unpubl. data). *Symbiodinium* B178 was the dominant symbiont within *B. asbestinum* at the study sites, but in some samples (3.3 to 6.7%) only B184 was detected and in a few others (3.3%) mixtures of B178 and B184 were found. Thus, within sites (and within colonies) the *Symbiodinium* types form a mosaic with no evident patterns of distribution. Furthermore, the small variation in symbiont genotype that was observed, did not correlate with any of the environmental variables investigated. These data corroborate other studies that have found similar long-term stability in symbiont diversity in several octocoral and scleractinian species (Goulet & Coffroth 2003b, Kirk et al. 2005, Thornhill et al. 2006a,b, Stat et al. 2009). Despite the fact that many of these studies encompassed bleaching events (Goulet & Coffroth 2003b, Thornhill et al. 2006a, Stat et al. 2009) or diseased corals (Kirk et al. 2005), the overriding trend is one of stability in symbiont composition.

Stability of symbiosis appears to be a common trait among Caribbean octocorals and is supported by the general low level of bleaching experienced by most Caribbean octocoral taxa (*Erythropodium* and, in some cases, *Briareum* and *Plexaurella*, which are notable exceptions). Caribbean octocorals almost exclusively harbor *Symbiodinium* type B184 (Santos et al. 2003, Goulet & Coffroth 2004; analogous to ITS2 type B1 of LaJeunesse 2001, 2002) while scleractinians host a variety of *Symbiodinium* 'types' (LaJeunesse 2002). Those octocorals that do bleach (i.e. *Erythropodium* and, in some cases, *Briareum* and *Plexaurella*) tend to harbor Clade C *Symbiodinium* (Goulet & Coffroth 2004, van Oppen et al. 2005). *Symbiodinium* B184/B1 is characterized as a generalist (LaJeunesse, 2002), but the variability in bleaching seen between octocorals and scleractinians that harbor B184/B1 suggests either a variation in the response of the holobiont to bleaching stressors or variation within *Symbiodinium* B184. Santos et al. (2004) confirmed fine scale diversity and specificity within the type B184. Future studies should focus on this diversity as a basis for the differences in bleaching in response to a given environmental stressor between octocorals and other cnidarians.

Although *Symbiodinium* type B184 was relatively rare at our study sites, this type dominates some *Briareum asbestinum* populations in the Florida Keys (D. Poland pers. comm.) and can often exist with *Symbiodinium* B178 at background levels (Lewis & Coffroth 2004), which would account for the few instances of

symbiont change observed over the year. For example, Lewis & Coffroth (2004) found that within *B. asbestinum* the proportion of *Symbiodinium* B184 to B178 increased after dark-induced bleaching, demonstrating the potential for change in symbiont composition within a host as the environmental conditions change (as seen in the *Montastraea* species complex, Rowan et al. 1997, Toller et al. 2001, and in *Acropora millepora*, Jones et al. 2008). Lewis & Coffroth (2004) suggested that in this species B184 may be a low-level symbiont type that may be more tolerant of bleaching stressors (Lewis & Coffroth 2004). In the present study, the colonies harboring B184 often exhibited higher cell densities, but since B184 was often found in combination with B178, it is unclear what cell types contributed to the higher densities.

Coral bleaching in general and the seasonal fluctuations reported in previous studies are often associated with increasing SST (Fitt et al. 2000, Stimson 1997, Brown et al. 1999, Fagoonee et al. 1999, Warner et al. 2002, Lasker 2003, Costa et al. 2005). As noted, the SSTs at the study sites fluctuated from 12.5 to 32.7°C. There was no temperature related variation in symbiont density in response to the elevated summer SSTs. The elevated summer temperatures of 2002 are comparable with that in 1998 when bleaching in the upright morph of *Briareum asbestinum* was recorded at sites in the northern and southern Florida Keys, but this was accompanied by a pathogenic infection that led to high levels of mortality (Harvell et al. 2001). Site differences, *B. asbestinum* morphology, marginally lower temperatures in 2002 compared with 1998 and possibly pathogen abundance may account for the different response. The lack of fluctuation in cell density and symbiont type during the summer months may signify that these holobionts (collected from shallow waters that routinely experience this range of temperatures, Fig. 1) maintain the association over this range of environmental fluctuations. Furthermore, the contrasting results of these 2 studies (Harvell et al. vs. the present study) emphasize that small differences in temperature may be important and suggest that other environmental factors may also exacerbate the effects.

In January 2003 both *Briareum asbestinum* populations experienced a low-level of bleaching (~20% cell loss) in response to anomalously cold SSTs of <14°C for 8 to 11 h. The significant decrease in cell density at the Point population was observed during the January 2003 sampling event, most likely because colonies were sampled on January 26th, the third day of temperatures <14°C. A significant decrease in cell density was not detected at the Craig population until March 2003, most likely due to the fact that these colonies were sampled on January 23rd, 1 d before the temperatures dropped below 14°C. Although not planned, this sam-

pling strategy provided insight into the level and duration of cold water stress that causes bleaching in *B. asbestinum*. The effects of cold water stress on *B. asbestinum* observed in the present study are concordant with others studies documenting cold water stress on Atlantic and Pacific scleractinian species, which indicate SSTs <14°C lead to the breakdown of the coral-algal symbioses (Shinn 1966, Porter et al 1982, Saxby et al 2003, Hoegh-Guldberg 2005). The present study also provides information concerning the time needed for an octocoral to recover from a low-level bleaching event; as of June 12th, 2003, neither population had recovered to pre-bleaching symbiont densities.

The dramatically different response demonstrated by *Briareum asbestinum* to the elevated temperatures in 1998 and 2002 indicates how close many corals live to their thermal limits. Considering the magnitude and rate of change forecasted for the tropical marine environment in the near future (IPCC 2007), it is clear that bleaching events will continue and intensify. The present study contributes to an increasing number of investigations that suggest many coral species may not respond to fluctuating environments by changing symbionts and points to the need for more studies like this one (and those of Thornhill et al. 2006a,b, Jones et al. 2008, Sampayo et al. 2008 and Stat et al. 2009) that track the longer term trends of coral-algal symbiosis. The higher *Symbiodinium* densities retained by the Craig populations (Fig. 2), indicate that there may be significant spatial variation in algal densities in coral populations. We ruled out the possibility that this spatial variation is due to differences in the genotypic composition of the symbionts, but distinguishing between causation due to environmental factors and/or host genotype is beyond the scope of the present study. However, if further experiments demonstrate that higher *Symbiodinium* densities mean greater resistance to bleaching, and spatial variation in symbiont densities is detected in other coral populations, this will have implications for management and policy strategies in terms of prioritizing areas where synergistic impacts should be mitigated in order to minimize incidence of bleaching.

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