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REPORT

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The tissue composition of *Montastraea franksi* during a natural bleaching event in the Florida Keys

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Abstract In this study, we used a correlative approach to (1) test for an association between bleaching and host tissue composition during a natural bleaching event, and (2) assess whether bleaching susceptibility varies between years. In August 1997, *Montastraea franksi* at 15-m depth on Conch Reef, Florida, bleached and the severity of the response varied among individuals. Seventy-five randomly selected colonies were quantified for bleaching using both an ordinal scale, assigned by eye, and a continuous scale, assessed using red, green, and blue (RGB) spectral analysis of photographs. Zooxanthella density and chlorophyll *a* content were evaluated as measures of bleaching, and coral tissue was analyzed for glycerol, free amino acids (FAA), protein, and mycosporine-like amino acids (MAAs); collectively, these are described as the "tissue composition." In 1998, most of the same coral colonies were analyzed for color and zooxanthella density to determine whether colony color in 1997 was correlated with color in 1998. In 1997, colonies of *M. franksi* that were ranked by color differed significantly in RGB brightness, zooxanthella density, and chlorophyll *a* content, but not in tissue composition. Similarly, a multivariate test for a linear relationship between color and tissue composition did not reveal a significant association. Analyses of corals in 1997 and 1998 revealed a significant positive relationship between color in both years (i.e., the same colonies were similarly colored in each year). These results are discussed in the context of the temporal scale of the sampling regime, the nature of the measured traits, and the adaptive bleaching hypothesis.

Keywords Coral bleaching · *Montastraea* · Tissue composition

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Introduction

Coral bleaching threatens the health and persistence of some of the most diverse and economically important marine ecosystems on earth (Berkelmans and Oliver 1999; Hoegh-Guldberg 1999). The serious consequences of these events, and the predicted increases in their frequency and severity associated with global warming (Berkelmans and Oliver 1999; Hoegh-Guldberg 1999), have provided ample incentive to better understand the mechanisms that drive the bleaching response.

Initial studies focused on the ecology and organismic consequences of bleaching (Ogden and Wicklund 1988; Porter et al. 1989) and provided significant insight into the causes of bleaching (Hoegh-Guldberg and Smith 1989) and the mechanisms by which the corals lose their algal symbionts (e.g., Gates et al. 1992). In the early 1990s, the application of molecular techniques resolved the genetic identity of the algal symbionts (Rowan and Powers 1991) and created the opportunity to test the role of algal diversity in determining patterns of coral bleaching (Rowan et al. 1997; Baker 2001). For example, within-colony patterns of bleaching in *Montastraea annularis* and *M. faveolata* in Panama can be explained by exquisite concordance with distribution of three clades of zooxanthellae that differ in their tolerance to temperature and light (Rowan et al. 1997). More recently, sophisticated techniques for measuring chlorophyll fluorescence have been applied to corals to identify the exact site and consequences of thermal damage to algal

photosynthesis (Warner et al. 1996, 1999; Jones et al. 1998; Gorbunov et al. 2000). When combined, the genetic and photophysiological data have dramatically improved our understanding of how coral symbioses respond to temperature anomalies. However, these data do not fully account for the documented differences in bleaching susceptibility of conspecifics that harbor the same algal genotypes (Warner et al. 1999). Such observations indicate that the coral host also plays an important and parallel role to the algal symbionts in mediating the bleaching response. In this context, we hypothesized that an investigation into the relationships between bleaching severity and the host tissue characteristics of affected colonies might provide a valuable first step in identifying host traits associated with bleaching susceptibility. Here we describe the results of such an analysis using the ecologically important Caribbean coral *Montastraea franksi*.

We initiated this work with two goals in mind: first, to identify measurable traits associated with bleaching that might be useful in developing testable hypotheses concerning the mechanism of bleaching of the holosymbiont [i.e., the animal host and the algal symbiont combined (sensu Iglesias-Prieto and Trench 1997)]; and second, to examine coral coloration in two consecutive years and test the adaptive bleaching hypothesis. According to this hypothesis, corals shuffle their symbionts during a bleaching event to acquire genotypes that are better adapted to environmental stress. As such, a coral exposed to a second disturbance should not bleach as severely as it did when exposed to the first.

As a means to achieve these goals, we exploited a natural bleaching event that occurred across the Florida Keys in the summer of 1997. In August 1997, corals in this area were exposed to seawater temperatures of >30 °C to 20-m depth (Warner et al. 1999), and we observed colonies of *Montastraea franksi* on Conch Reef (N24°56.807, W080°27.354) that ranged in color from normal, through pale and patchy, to completely white. This event provided us with a unique and unplanned opportunity to examine the role of host tissue traits in determining intraspecific variation in the propensity of *M. franksi* to bleach. We quantified bleaching in colonies representing a range of colors by ranking them on an ordinal color scale, assigning them a position along a continuous color scale, and measuring zooxanthella densities and chlorophyll *a* content. The tissue composition of the same corals was assessed by measuring the concentrations of glycerol, free amino acids (FAAs), soluble protein and mycosporine-like amino acids (MAAs), four constituents of the host that are all related to the functional integrity of the algal–cnidarian symbiosis. Glycerol was selected since it is the initial product of zooxanthella photosynthesis in the intact coral–algal symbiosis (Muscatine and Cernichiari 1969). FAAs have been demonstrated to function as a ''host factor'' and regulate algal metabolism (Gates et al. 1995), soluble protein is representative of host biomass and is reduced during some bleaching events (Szmant and Gassman 1990; Gates et al. 1992; but see Gleason and Wellington 1993), and MAAs provide protection against UV-bleaching (Gleason and Wellington 1993). These data were explored using single-factor and multivariate statistics to determine whether or not a relationship exists between one or more of the components of the host and the extent of coral bleaching.

The majority of the same corals (i.e., those that could be relocated) were re-sampled in July 1998 to determine whether bleaching in 1997 was associated with colony color and zooxanthella densities approximately 11 months later. It is important to note that between sampling, sea surface temperatures were below the local coral bleaching threshold for more than 8 months, and dipped below 25 °C during December 1997, as well as January, February, and April 1998 (National Oceanic and Atmospheric Administration 2001). Thus, the sampled colonies of *M. franksi* had the opportunity to recover from the thermal stress of 1997 before being sampled in 1998. We used the data collected for the same corals in 1997 and 1998 to examine the prediction implicit in the adaptive bleaching hypothesis (Buddemeier and Fautin 1993). Our results must be interpreted in the context of the serendipitous coincidence of a previously planned research trip, and an unanticipated natural event. Thus, it is not known when the sampled corals first were affected by thermal stress, or from where on the temporal scale of bleaching events the samples were taken.

Methods

Experimental design

At the height of the El Niño-related bleaching event in August 1997, 75 colonies of *M. franksi* were selected at 15-m depth on Conch Reef. The colonies were chosen to span a range of bleaching severity, and varied in color from dark brown/green (normal) to totally white (severely bleached). Each colony was marked with an aluminum tag, photographed and sampled for tissue composition between 18 and 22 August. Colony color was quantified using slides (Kodachrome 64) taken with a Nikonos V camera mounted on a quadrapod and fitted with a 28-mm lens and two strobes (Nikonos SB105). A 0.5×0.5-m quadrat was photographed, and each picture included a color standard of red, green, and blue bands. The standard was used to correct color differences caused by varying exposures and ambient light. Tissue composition was analyzed using pieces of coral (4 to 14 cm^2 area) removed with a hammer and chisel. Since most colonies were not uniformly colored (i.e., bleaching was patchy), samples were selected to represent the overall colony color. On shore, each sample was broken into three pieces that were processed independently for: (1) zooxanthella density, chlorophyll *a*, and protein; (2) FAA and glycerol; and (3) MAA.

To explore the role of recent (<11 months) history in determining the susceptibility to bleaching the following year, most of the corals were sampled again on 1 July 1998 for color and zooxanthella density (i.e., bleaching). Mean seawater temperature was lower in the 2 weeks preceding the July 1998 sampling (˜29 °C at 21-m depth) compared with August 1997. Nonetheless, bleached colonies of *M. franksi* were observed in the study population in 1998.

Coral color

Coral color was quantified by scanning slides (with a Microtek 1850S scanner) and analyzing the images with Adobe Photoshop 2.5. Brightness histograms of each entire colony (selected with the lasso software tool) were used to determine the mean red (R), green (G), and blue (B) brightness. With this technique, each color has a maximum brightness of 255, and a minimum of zero; a coral that is white over its entire surface has a brightness of 255 for each color. Brightness was adjusted for differences in exposures using the color standards, and the adjusted RGB values were collapsed to a single measure for each coral using principal component analysis [PCA; Jolliffe (1986)]. PCA was completed on the correlation matrix of the RGB brightness values using Systat 5.2 statistical software, and the principal component ($PC1_{Color}$) that explained the majority of the variance in the RGB brightness values was used as a measure of color (i.e., "whiteness"). Thus, $PC1_{Color}$ provided a continuous measure of color, and was determined for 1997 (1997-$PC1_{Color}$) and 1998 (1998-$PC1_{Color}$).

In addition to the quantitative analysis of color, the slides were used to assign the corals a subjective rank on an ordinal scale where 1 = normal (uniform brown/green); 2 = partial bleaching and mottling; 3 = severe bleaching with some normal brown/green coloration; and 4 = completely bleached. All slides were scored independently by the three authors and the values averaged to describe each colony.

Zooxanthellae, chlorophyll *a* and protein

The composition of coral tissue was determined using the slurry obtained by blasting a portion of the corals with filtered seawater (FSW, 0.45 μm) from a Water Pik (Johannes and Wiebe 1970). The slurry was analyzed with standard techniques for zooxanthella content [10 replicate counts on a hemocytometer (Edmunds 1994)], chlorophyll *a* (Gardella and Edmunds 1999), and soluble protein [Coomassie Brilliant Blue assay (Bradford 1976), as in Bruno and Edmunds (1998)]. The areas of the coral skeletons from which tissue was removed were measured with aluminum foil (Marsh 1970). Zooxanthella density was expressed as cells x GAP $10^6/cm^2$, chlorophyll *a* content as $\mu g/10^6$ cells, and protein as mg/cm^2.

Tissue composition

Glycerol and FAAs were measured in duplicate using concentrated tissue obtained by abrading a portion of the corals with a nylon toothbrush. The soluble protein content of this slurry was measured with the Coomassie Brilliant Blue assay (as in Bruno and Edmunds 1998), and used for normalizing the glycerol and FAA content. Glycerol was measured using an enzymatic procedure according to the manufacturer's protocol (Sigma, 337), with FSW blanks and a calibration line prepared using the stabilized glycerol supplied by the manufacturer. Glycerol concentrations were standardized to the area of the coral (to obtain $\mu g/cm^2$).

FAA concentrations were assessed using a spectrophotometric ninhydrin-protocol modified from Moore and Stein (1948). FSW blanks and a standard curve were prepared with pure FAAs that were combined to approximate the FAA pool of *Pocillopora damicornis* (Linnaeus) (Gates et al. 1995). The FAA content was calculated according to the standard curve, and normalized to the area of the coral (to obtain nM/cm^2).

MAAs were extracted from the remaining pieces of coral (skeleton plus tissue, ~1 cm^2 tissue surface area), using three 5-ml volumes of 80% methanol in water. Each extraction was carried out for 24 h at 4 °C. After each treatment with solvent, the sample was centrifuged for 5 min at 5,000 rpm and the supernatant decanted. To detect MAAs, the combined supernatant was scanned between 280 and 400 nm on a Shimadzu UV-2101PC spectrophotometer, using quartz cuvettes (1-cm pathlength). MAA content was assessed from the peak height at the wavelength of greatest absorbance (determined to be 310 nm), and normalized to surface area of the MAA sample (to obtain absorbance/cm^2); surface areas were measured as described above. This technique provides a relative rather than an absolute measure of MAA content, and assumes that the absorption spectra are similar in shape for all sampled colonies.

The overall tissue composition (glycerol, FAA, protein, and MAA) for each coral was collapsed to a single tissue metric using PCA (Jolliffe 1986). PCA was completed on the correlation matrix of the four tissue composition variables (each was log-transformed prior to PCA) using Systat 5.2, and the principal component ($PC1_{Composition}$) that explained the majority of the variance was used as a metric of tissue composition. $PC1_{Composition}$ provided an integrated and continuous measure of tissue composition.

Analyses

Two sets of analyses were applied to the 1997 data to test for an association between bleaching and tissue traits. First, single-factor analyses were used to determine whether subjective bleaching ranks corresponded to differences in continuous (instead of ordinal) measures of bleaching and tissue composition. A one-way MANOVA (dependent variables = 1997-$PC1_{Color}$, zooxanthella density, and chlorophyll *a*) was used to determine whether bleaching ranks corresponded to differences in color and zooxanthella characteristics. Zooxanthella density and chlorophyll *a* content were log-transformed to meet the statistical assumptions of normality and homoscedasticity. One-way MANOVA was also used to compare tissue composition (dependent variables = concentration of glycerol, FAA, protein, and MAA) among bleaching ranks. Where significant multivariate effects were detected, separate univariate ANOVAs were used to compare ranks, and Bonferonni post-hoc tests were used to distinguish effects. Second, correlation analyses were used to determine whether color was associated linearly with tissue composition. This analysis was completed using the principal components obtained from the analysis of RGB color and tissue composition (1997-$PC1_{Color}$ and $PC1_{Composition}$, respectively). The advantage of multivariate analyses is that they allow multiple dependent variables to be used in a single test of the hypothesis—for example, testing for an association between bleaching and tissue composition—that is protected from risks of type I error inherent in univariate approaches for each dependent variable. Additionally, multivariate analyses can provide an unambiguous inferential tool for accepting or rejecting the null hypotheses. An important caveat to these advantages is that there is no a priori rationale for choosing one dependent variable and a univariate approach. In the case of our study, we did not believe that the contemporary literature could be used to justify testing the effects of bleaching on coral tissue using only one dependent variable such as glycerol alone.

To determine whether the color (i.e., the degree of bleaching) of corals in 1997 was associated with their color in 1998, Pearson correlation analyses were used to test associations between years for color and zooxanthella density. These analyses were completed with the multivariate measures of color in 1997 (1997-$PC1_{Color}$) and 1998 (1998-$PC1_{Color}$), as well as the zooxanthella content in both years.

All statistical analyses were completed using Systat 5.2 statistical software, and data were tested for the assumptions of the analyses. Tests for normality were carried out with Kolmogorov Smirnov tests, or through graphical analyses of residuals. Residuals were also used to test for homoscedasticity.

Results

1997 sampling

The majority of *M. franksi* colonies were in various stages of bleaching, and only 5 of the selected 75 were healthy in color and subjectively ranked 1 (Fig. 1). Most of the remainder were severely or completely bleached (Fig. 1). Although colonies were typically mottled and not uniform in color, with a few exceptions all were assigned the same ranks by the three investigators. PCA of the red, green, and blue color brightness generated one principal component (1997-$PC1_{Color}$), which explained 98% of the variance and was correlated positively with the brightness of each color (component loadings ≥0.959 which indicates a strong relationship between each original variable and the new principal component). 1997-$PC1_{Color}$ ranged from −3.04 to 2.46, with large negative values corresponding to dark green/brown colonies (i.e., normal color), and large positive values corresponding to white colonies (i.e., bleached).

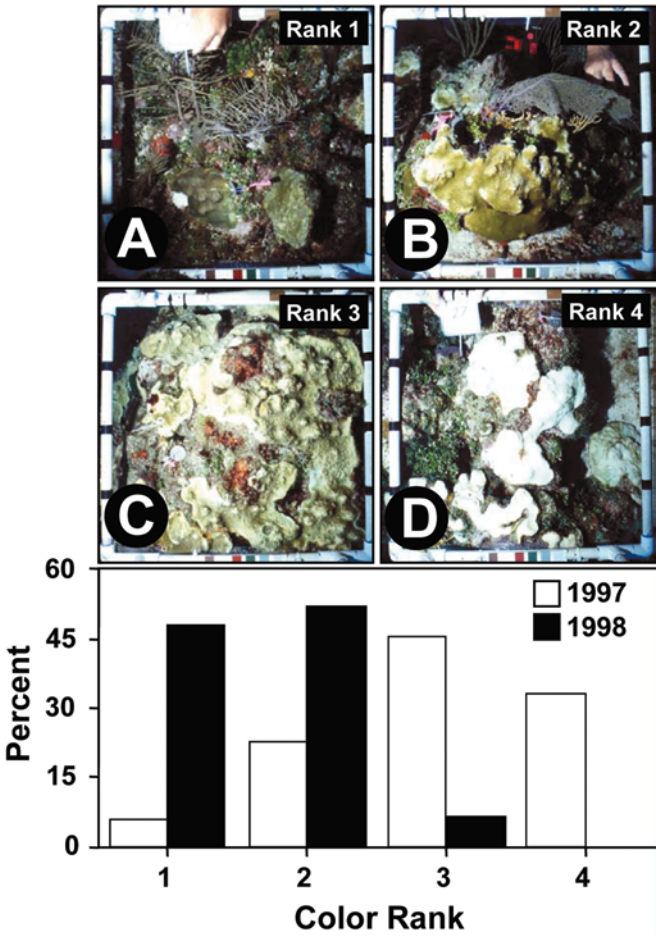

Fig. 1 Extent of bleaching in *Montastraea franksi* at 15-m depth. Pictures display representative colonies in 1997, and each frame (**a**–**d**) shows a colony that was assigned to a different bleaching rank: a healthy colony (rank 1); **b** partially bleached colony (rank 2); **c** severely bleached colony with some normal coloration (rank 3); and **d** completely bleached colony (rank 4). The quadrat is 0.5×0.5 m in size and has a standard color bar taped to one side (*bottom*). The *bar graph* displays the allocation of *M. franksi* colonies among the four bleaching ranks in 1997 ($n=75$) and 1998 ($n=51$)

Corals assigned to each of the four bleaching ranks differed significantly in color (1997-$PC1_{Color}$), zooxanthella density, and chlorophyll *a* content (MANOVA: Wilk's $\lambda=0.575$, $F=4.629$, $df=9$, 163, $P<0.001$). Separate univariate ANOVAs showed that the bleaching ranks differed significantly in multivariate color ($F=7.075$, $df=3$, 69, $P<0.001$), zooxanthella density ($F=7.949$, $df=3$, 69, $P<0.001$), and chlorophyll *a* content ($F=5.043$, $df=3$, 69, $P<0.001$) (Fig. 2). Post-hoc analyses showed that some, but not all, the ranks differed from one another for each trait (Fig. 2). Despite differing in color, zooxanthella density and chlorophyll *a* content, the corals assigned to the various bleaching ranks did not differ significantly in terms of FAA, glycerol, protein, and MAA content combined (MANOVA; Wilk's $\lambda=0.838$, $F=0.962$, $df=12$, 166, $P=0.487$). Based on the lack of significant variation in tissue composition among color ranks, the ranks were pooled to provide a general description of the tissue of *M. franksi*. Overall, the tissue contained 28.8 ± 1.1 nM/cm^2 FAA ($n=71$), 1.9 ± 0.2 μg/cm^2 glycerol ($n=70$), and 0.33 ± 0.01 mg/cm^2 protein ($n=73$), and had MAA levels corresponding to 0.25 ± 0.01 absorbance/cm^2 ($n=73$; all mean ±SE). Thus, the color rankings of *M. franksi* corresponded to significant differences in traits that are diagnostic of bleaching (Gates et al. 1992; Fitt et al. 1993), but not in traits that are characteristic of tissue composition.

As color is a continuous variable, an ordinal ranking scheme imposes artificial divisions among bleaching ranks. To overcome this limitation, PCA was used to generate multivariate measures of color (1997-$PC1_{Color}$, described above) and tissue composition. The PCA of tissue composition was carried out on the correlation matrix of log-transformed values of FAA, glycerol, protein, and MAA content, and generated one PC ($PC1_{Composition}$) that described much (44%) of the variance in tissue composition (Table 1). The second PC ($PC2_{Composition}$) described 26% of the variance. $PC1_{Composition}$ was characterized by high positive component loadings for FAA, MAA, and glycerol content, and a negative component loading for protein (Table 1). In other words, large positive values of $PC1_{Composition}$ correspond to high concentrations of FAA, glycerol, and MAA, but to low concentrations of protein.

The multivariate measure of color (1997-$PC1_{Color}$) was correlated significantly with zooxanthella density ($r=-0.447$, $n=73$, $P<0.001$) (Fig. 3) and chlorophyll *a* content ($r=0.281$, $n=73$, $P=0.016$; data not shown), although both relationships were weak ($r^2=0.199$ and 0.079, respectively). Nevertheless, the significant correlations demonstrate that paleness (identified with large, positive values for 1997-$PC1_{Color}$) was associated with a reduction of algal density and increased chlorophyll *a* in

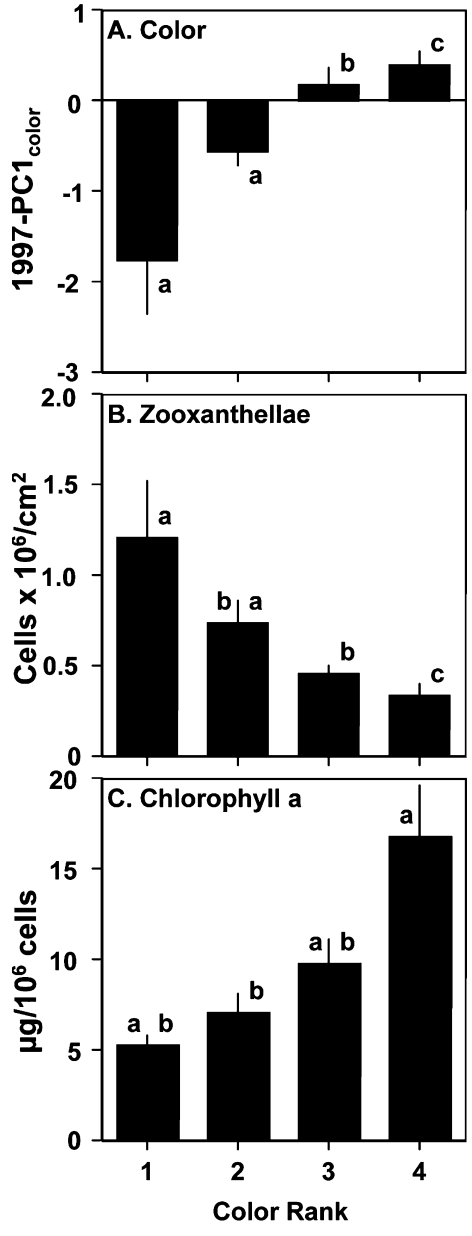

Fig. 2 Characteristics of colonies of *Montastraea franksi* in 1997 that were ranked 1 ($n=4$), 2 ($n=16$), 3 ($n=32$), or 4 ($n=23$) by color (means ±SE shown for untransformed data). **a** Color as determined by PCA of the RGB pixel brightness in digital images: large negative values correspond to green/brown colonies (i.e., normal) and large positive values correspond to pale/white colonies (i.e., bleached); **b** zooxanthella densities; **c** chlorophyll *a* content of the zooxanthella. Bars with the same letters are not significantly different as determined by ANOVA and Bonferonni post-hoc analysis; zooxanthellae and chlorophyll *a* were log-tranformed for statistical analysis. Refer to text for further details

Table 1 Principal component analysis (PCA) of four tissue traits in *Montastraea franksi* from 15-m depth in 1997 ($n=70$ colonies). The analysis was completed using log-transformed values for protein, free amino acids (*FAA*), glycerol, and mycosporine-like aminoacids (*MAA*). PCA generated two principal components: $PC1_{Composition}$ explained 71% more variance than $PC2_{Composition}$, but, together, they explained 70% of the variation in tissue composition

	$PC1_{Composition}$	$PC2_{Composition}$
Eigenvalue	1.766	1.035
Explained variance (%)	44.159	25.877
Component loadings		
Protein	–0.542	0.659
FAA	0.777	0.303
Glycerol	0.658	0.589
MAA	0.660	–0.402

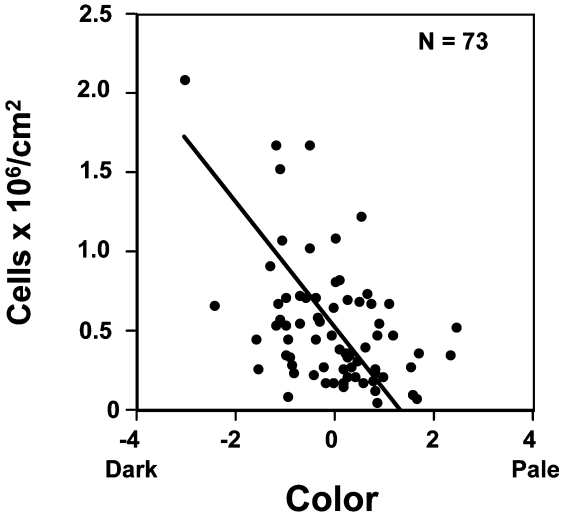

Fig. 3 Scatterplot showing relationship between color and zooxanthella density of *Montastraea franksi* in 1997 ($n=73$). Color was determined by PCA of the RGB pixel brightness. There is a weak negative correlation between color ($1997\text{-}PC1_{Color}$) and zooxanthella densities ($r=-0.447$, $n=73$, $P<0.001$), with pale colonies containing few zooxanthellae. Model II techniques were used to fit the geometric mean regression line to the data

the zooxanthellae remaining in the corals. In contrast, bleaching, as assessed by color ($1997\text{-}PC1_{Color}$), was not correlated significantly with multivariate tissue composition ($PC1_{Composition}$; $r=-0.124$, $n=70$, $P>0.232$) (Fig. 4), nor were zooxanthella densities (another measure of bleaching) and multivariate tissue composition ($r=0.114$, $n=70$, $P=0.348$). Thus, in 1997 the pale/white coloration of *M. franksi* was not associated with distinctive levels of glycerol, FAA, protein, and MAA.

Second sampling (1998)

Fifty-nine of the original 75 colonies of *M. franksi* were located in 1998 and sampled on 1 July. In contrast to 1997, in 1998 most colonies were relatively dark in color (i.e., were ranked 1 or 2). Three colonies (5%) were severely bleached with some normal coloration (rank 3), and none was completely white (rank 4) (Fig. 1). The PCA of the RGB brightness of the coral colonies generated one principal component ($1998\text{-}PC1_{Color}$), which explained 82% of the variance and was correlated

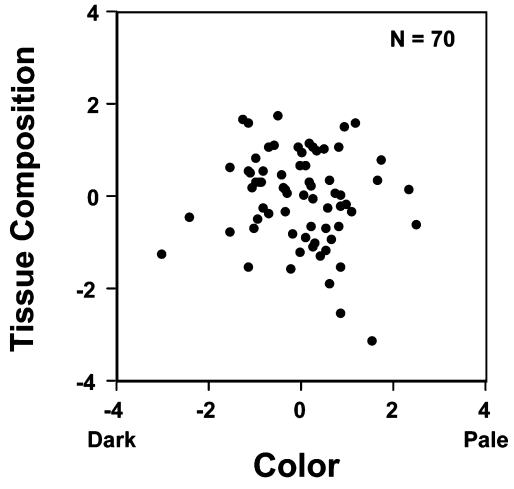

Fig. 4 Scatterplot showing relationship between color and tissue composition of *Montastraea franksi* in 1997 ($n = 70$). Color was determined by PCA of the RGB pixel brightness, and tissue composition was characterized by PCA of the free amino acid, glycerol, mycosporine-like amino acid, and protein content. Tissue composition displays the values for $PC1_{Composition}$ that describes 44% of the variation in dependent variables. There is no significant correlation between color (1997-$PC1_{Color}$) and tissue composition ($PC1_{Composition}$) ($r = -0.124$, $n = 70$, $P > 0.232$)

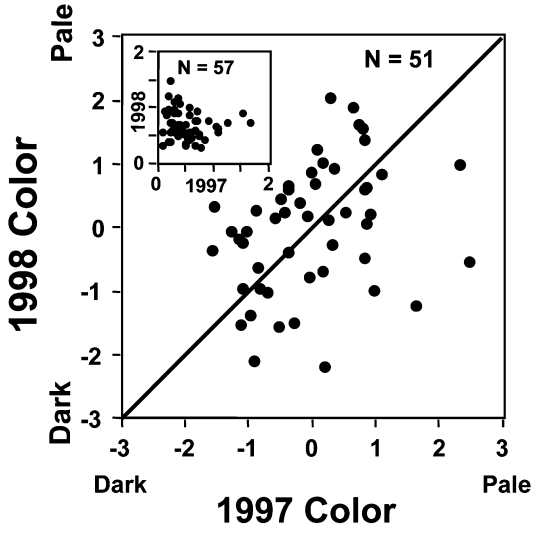

Fig. 5 Scatterplot displaying relationship between color in 1997 and 1998 for *Montastraea franksi*. *Color axes* display the values for 1997-$PC1_{Color}$ and 1998-$PC1_{Color}$ obtained from PCA of the RGB pixel brightness. There is a significant correlation between color in 1997 and 1998 ($r = 0.311$, $n = 51$, $P = 0.026$). Thus, colonies that were pale in 1997 also were pale in 1998, and colonies that were dark in 1997 also were dark in 1998; *solid line* indicates the expected outcome if the colors were identical in both years. *Inset* shows the zooxanthella density (axes scaled 0–2 $\times 10^6$ cells/cm^2) in 1997 and 1998: there is no significant correlation between the two ($r = -0.008$, $n = 57$, $P = 0.513$)

positively with the brightness of each color (component loadings $= 0.880$). Zooxanthella densities ranged from 0.27×10^6 to 1.51×10^6 cells/cm^2, and the quantitative color (1998-$PC1_{Color}$) ranged from −2.178 to 2.050. In contrast to the results from 1997, in 1998 there were no significant differences among bleaching ranks 1–3 for zooxanthella densities ($F = 0.526$, $df = 2, 48$, $P = 0.594$) or quantitative color (1998-$PC1_{Color}$) ($F = 2.980$, $df = 2, 48$, $P = 0.060$), although the statistical power was low (< 0.35) in both cases.

To assess whether bleaching severity is related to the short-term (< 11 months) history of previous bleaching events, coral color in 1997 (1997-$PC1_{Color}$) was compared with color in 1998 (1998-$PC1_{Color}$). Correlation analysis demonstrated that there was a significant relationship between color in both years ($r = 0.311$, $n = 51$, $P = 0.025$) (Fig. 5). However, there was no significant correlation between color in 1997 and zooxanthella density in 1998 ($r = -0.141$, $n = 59$, $P = 0.288$) (Fig. 5), or between zooxanthella densities in each year ($r = -0.088$, $n = 57$, $P = 0.513$). Thus, although coral color in 1997 and 1998 was related positively, zooxanthella densities in both years were unrelated, as were zooxanthella density in 1997 and color in 1998.

Discussion

This study is unique in sampling a large number of corals during a major bleaching event in Florida that coincided with the severe El Niño of 1997/1998. Our most significant finding is the discovery of differential bleaching yet congruent tissue composition among adjacent colonies of *M. franksi* in 1997. In other words, conspecifics that were strikingly different in color to the human eye were statistically indistinguishable in terms of tissue composition. It is tempting to suggest that this result is consistent with the view that zooxanthellae are more thermally sensitive than their animal host (Sharp et al. 1997; Fitt et al. 2001), because elevated temperatures result in changes in zooxanthella populations but do not affect the measured constituents of coral tissue. However, in the context of a temporal cascade of events beginning with the proximal effects of thermal stress and culminating in zooxanthella loss, there are at least three additional explanations for this finding. First, assuming that only clade "C" zooxanthellae are found in *M. franksi* (Rowan and Knowlton 1995; Warner et al. 1999), and that adjacent colonies were exposed to similar environmental conditions, then the among-colony differences in bleaching recorded in the present study could reflect the effects of host-mediated modulation of the consequences of thermal stress on zooxanthellae photophysiology. The mechanisms of such putative modulation are a matter of speculation but, for example, could include regulation of host-cell detachment, apoptosis, or exocytosis (Gates et al. 1992). Second, it is possible that we measured components of coral tissue that are sensitive to thermal stress, but sampled on a time scale uncoupled from the biologically significant events determining bleaching severity. For example, by sampling at a single time at an unknown point in the progression of a natural bleaching event, we have no indication of the changes that might have occurred

during the initial hours or days of the first effects of thermal stress. Coral tissue is known to respond to thermal stress within hours of exposure (Sharp et al. 1997; Gates and Edmunds 1999), and such changes may be critical in determining whether visible signs of bleaching subsequently occur. Third, it is possible that we selected components of coral tissue that are not sensitive to the effects of thermal stress. Currently, there are no data available that distinguish among these three explanations, and thus it remains unclear what role the coral host plays in determining susceptibility or how the host's biology is impacted by any thermal damage sustained by the photosynthetic apparatus of its symbiotic algae. Clearly, there is a critical need to study the coral host on time scales commensurate with the temporal resolution currently available for studies of zooxanthellae photosynthesis (i.e., seconds to hours).

Our results reveal that the tissue compositions of visibly pale and normally colored corals were essentially the same. Assuming that the bleaching threshold for *M. franksi* was reached on about 1 August 1997 (Warner et al. 1999; National Oceanic and Atmospheric Administration 2001), then zooxanthella densities may have been depressed for 3 weeks by the time the corals were sampled, yet there were no measurable effects on coral-host tissue composition. This conclusion does not contradict the inevitable negative consequences of zooxanthella loss for the supply of carbon to the coral (Porter et al. 1984; Edmunds and Davies 1986), or the effects on the carbon, nitrogen, and protein content of coral tissue (Glynn and D'Croz 1990; Szmant and Gassman 1990). Instead, it emphasizes that the nutritional consequences of bleaching (e.g., Glynn and D'Croz 1990; Szmant and Gassman 1990) develop slowly as the condition persists, and therefore may not be coincident with the timing of tissue discoloration. Just as bleaching (i.e., loss of color) is likely a delayed response to the effects of thermal stress on zooxanthella photosynthesis (Fitt et al. 2001), profound and measurable changes in the coral-host tissue composition are a delayed response to the effects of bleaching. For instance, changes in coral tissue composition occur at the end of a natural bleaching event (Porter et al. 1989), both in corals that have remained bleached for several months (Szmant and Gassman 1990) and in corals recovering from severe bleaching (Fitt et al. 1993). The time-lag between the onset of visible bleaching and measurable changes in tissue composition will likely vary depending on the species under consideration and its nutritional strategy [i.e., relative dependency on autotrophy; Glynn and D'Croz (1990)].

The zooxanthella densities in *M. franksi* are similar to those recorded in conspecifics and congenerics (Warner et al. 1999; Fitt et al. 2000). Moreover, the mean loss of 72% of the zooxanthellae from severely bleached *M. franksi* is similar to the 75% lost from bleached *M. annularis* (Porter et al. 1989). In addition to reducing zooxanthella density, bleaching can reduce chlorophyll *a* content by 48–65% (Porter et al. 1989; Gleason and Wellington 1993), although sometimes it is unaffected (Hoegh-Guldberg and Smith 1989). We recorded a different trend in *M. franksi*, where severely bleached colonies showed a 3.2-fold increase in chlorophyll *a* per cell. Fitt et al. (1993) reported a similar result for *M. annularis* recovering from bleaching (3 months after high temperatures ended), where chlorophyll *a* and zooxanthella densities were negatively correlated, and algal cells contained as much as 27 pg chlorophyll. This is ~5–10 times more than the normal summer levels for congeners at 13-m depth (Fitt et al. 2000), and 1.6-fold higher than the mean chlorophyll *a* content of completely bleached corals in the present study. Fitt et al. (1993) hypothesized that high chlorophyll *a* content of zooxanthellae in corals recovering from bleaching was a result of nutrient-stimulated zooxanthellae growth, where the nutrients were made available by the reduction in density of zooxanthella that normally would exploit this resource. This hypothesis offers one explanation for the high chlorophyll content of bleached *M. franksi* in the present study, although it seems unlikely that widespread zooxanthalla recovery would have occurred while temperatures remained high and conspecifics continued to bleach throughout August 1997 (Warner et al. 1999). Alternatively, high chlorophyll levels might have resulted from the selective loss of algae damaged by bleaching in the upper tissue layers (Jones and Hoegh-Guldberg 1999), thereby leaving the coral populated by a small number of cells that had previously been deep in the tissues. If deeper zooxanthellae in *M. franksi* are shade adapted, they would contain more chlorophyll than those in well-illuminated areas (Porter et al. 1984), and probably would be less affected by thermal stress because of the reduced potential for synergistic light effects (Jones et al. 1998). Thus, analysis of a recently bleached coral might reveal chlorophyll-rich zooxanthellae, at least until they too became negatively affected by the elevated temperatures and high light levels characteristic of their newly bleached hosts. The recent discovery that bleached corals can repopulate with novel and opportunistic zooxanthellae (Toller et al. 2001) raises the additional possibility that the high chlorophyll *a* levels in our bleached colonies might have been caused by the appearance of a chlorophyll-rich taxon of zooxanthellae. This possibility warrants further investigation, first to determine whether bleaching results in changes in the zooxanthellae genotypes inhabiting *M. franksi*, and, second, to determine whether chlorophyll content differs between opportunistic and specialist zooxanthellae (sensu Toller et al. 2001).

To our knowledge, the present analysis is among the first to quantify the most conspicuous feature of bleaching—unusual coloration—on a continuous scale (but see Thieberger et al. 1995; Holden and LeDrew 1998; Maguire et al. 2003). The results demonstrate that the RGB brightness of affected colonies can be collapsed to a single metric ($PC1_{Color}$) that describes most (98%) of the RGB variation. $PC1_{Color}$ is, in turn, correlated with zooxanthella and chlorophyll *a* content, and differs

among ordinal bleaching ranks, even though there is considerable mottling of individual colonies (e.g., Fig. 1b and c). Mottling of bleached corals is well known (e.g., Fig. 1 in Fitt et al. 1993), and has been attributed to small-scale spatial variation in zooxanthella taxa (Rowan et al. 1997), irradiance (Lesser et al. 1990), boundary layer characteristics (Patterson and Price 1992), acclimation (Brown et al. 2000), and regrowth of algal symbionts during recovery (Fitt et al. 1993). Because many colonies of *M. franksi* were mottled in the present study, the samples taken for zooxanthellae analysis, as well as the analysis of colony color using photographs, encompassed portions of the colony that differed in bleaching severity. This weakens tests of the putative relationships between pairs of colony-wide traits (for example, tissue composition versus color), and affects the mean and variance of traits associated with a specific bleaching severity (i.e., color). Within-colony variability in bleaching, together with the possibility of zooxanthella loss independent of colony color (Fitt et al. 2000), probably contributed to the lack of a correlation between colony color and other traits in the present study.

According to the adaptive bleaching hypothesis (ABH; Buddemeier and Fautin 1993), an initial bleaching should reduce the future damage by similar stressors, because bleaching facilitates the acquisition of a more suitable taxon of algae. Thus, bleaching could represent an acclimation strategy of the holosymbiont (sensu Iglesias-Prieto and Trench 1997) to elevated temperature. The present results provide insight into this possibility by demonstrating that coral color (i.e., degree of bleaching) was similar among colonies in 1997 and 1998, even though zooxanthellae densities in both years were not significantly related. In other words, colonies that were pale in 1997 were also pale in 1998, and their zooxanthella densities in 1997 were unrelated to those recorded in 1998. Within the short time frame of the two analyses (11 months), this result is not consistent with the ABH or acquired resistance to bleaching (i.e., acclimation), because bleaching in 1997 did not prevent bleaching in 1998. This conclusion must, however, be interpreted with caution and in the context of the recent history of the sampled corals, the severity of bleaching in 1997 and 1998, and the potential for recovery to occur in the time interval and thermal regime between sampling periods. Thus it is unknown whether earlier events influenced the degree of bleaching in 1997 and 1998 (as predicted by the ABH), or whether the temperature in July 1998 was sufficiently high to detect putative differences in bleaching due to events of the previous year. Finally, regardless of the potential relevance of the ABH to the present results, the most parsimonious hypothesis remains that corals had not recovered fully from their 1997 bleaching when they were again sampled in 1998 (Fitt et al. 1993). Clearly, there is a critical need for longer-term experiments to determine whether (if at all) one bleaching event can affect succeeding episodes.

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