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RESEARCH ARTICLE

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Bleaching in *Amphistegina gibbosa* d'Orbigny (Class Foraminifera): observations from laboratory experiments using visible and ultraviolet light

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Abstract Bleaching (visible loss of symbiont color) in populations of the diatom-bearing foraminifer *Amphistegina* has been recorded from reefs worldwide since 1991. Field studies and previous laboratory experiments have strongly implicated solar radiation as a factor in bleaching stress. The influence of spectral quality and quantity of photosynthetically active radiation (PAR) and ultraviolet radiation (UV) on growth rates and bleaching in *Amphistegina gibbosa* was investigated in the laboratory using fluorescent sources of PAR ('blue' with a spectral peak at 450 nm and 'white' with a 600-nm spectral peak) and biologically effective ultraviolet radiation [UVB (280–320 nm)]. Growth rate, as indicated by increase in maximum shell diameter, saturated at a PAR of 6–8 μmol photon m^{-2} s^{-1}, increased in 'blue' light, and was not influenced by UVB ≤ 0.0162 W m^{-2}. Frequency of bleaching increased with increasing PAR photon flux density and with exposure to shorter wavelengths, with or without an increase in total energy. Growth was significantly inhibited by UVB at 0.105 W m^{-2}. Specimens in treatments exposed to UVB to PAR ratios >0.003 became dark in color, rather than bleaching, which previous cytological studies indicate is a photo-protective response. Implications of these experiments are that environmental factors that affect either the spectral quality or quantity of solar radiation can influence bleaching in *Amphistegina*.

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Introduction

Amphistegina spp. are foraminifers that bear diatom symbionts and are found abundantly on coral reefs and tropical carbonate shelves worldwide (Langer and Hottinger 2000). These protists live predominantly on hard and phytal substrates at depths determined largely by the penetration limits of visible radiation (Hallock 1999; Hohenegger et al. 1999). Representatives of this genus can exploit a wide depth range, from intertidal to 120 m (Hallock 1999) using phototaxic behavior (Zmiri et al. 1974) and modification of their tests. They secrete hyaline calcite tests, which are typically thicker at shallower depths, reducing penetration of solar radiation into the test (Hallock et al. 1986). Other adaptive mechanisms, such as symbiont diversity (Lee et al. 1995) or photoprotective compounds, particularly micosporine-like amino acids (MAA) (Dunlap and Shick 1998), may also contribute, though data are lacking.

Bleaching, the visible loss of symbiont color, was first documented in field populations of *A. gibbosa* d'Orbigny in the Florida Keys (USA) in 1991 (Hallock et al. 1993). Similar bleaching was originally observed in the laboratory by Hallock et al. (1986) during experiments to assess growth rates in response to photosynthetically active radiation (PAR) at 400–700 nm under fluorescent sources. Those experiments revealed that individuals grown for 90 days at PAR photon flux densities (PFD) of 14 and 40 μmol photon m^{-2} s^{-1} were not significantly different in size or shape but exhibited partial loss of symbiont color, while individuals grown at 6 μmol photon m^{-2} s^{-1} were not significantly different in maximum diameter, but produced thinner tests and maintained a healthy golden-brown color. Cytological studies of bleaching in these protists has revealed that visible loss of color results from the deterioration of the diatom endosymbionts, followed by deterioration of the host endoplasm (Hallock et al. 1993; Talge and Hallock 1995). Comparison of partly bleached specimens collected from field populations with specimens partly

bleached in laboratory experiments demonstrated that cytological responses are statistically identical (Talge and Hallock 2003). Field observations of symptoms accompanying bleaching, including reproductive failure (Hallock et al. 1995), increased susceptibility to predation (Hallock and Talge 1994) and shell breakage (Toler and Hallock 1998), clearly indicate that bleaching is a stress response. The 1991 onset of bleaching in Florida Keys populations of *A. gibbosa* coincided with global stratospheric ozone depletion following the eruption of Mount Pinatubo (Randel et al. 1995), an event that Shick et al. (1996) described as a 'natural experiment' on the effects of ozone depletion on reef organisms. Hallock et al. (1993) postulated that bleaching in field populations is a symptom of stress induced by exposure to ultraviolet B radiation [UVB (280–320 nm)], particularly, increases in UVB relative to PAR. To pursue this hypothesis, Hallock et al. (1995) exposed healthy individuals to three treatments of PAR (from a 'white' fluorescent source) and ultraviolet radiation [UV (300–400 nm)]: 16 μmol photon m^{-2} s^{-1} PAR plus 0.005 W m^{-2} UV, 16 μmol photon m^{-2} s^{-1} PAR with no UV, and 8.8 μmol photon m^{-2} s^{-1} PAR with no UV. Results indicated that growth rate, as indicated by increase in shell diameter, was not influenced by these PAR or UV dosages, but that bleaching increased with both PAR PFD and UV dosage.

Thus, the optimal PAR PFD for *A. gibbosa* in laboratory culture has been determined to be between 6 and 14 μmol photon m^{-2} s^{-1}. However, the spectral quality and quantity used in previous laboratory experiments (Hallock et al. 1986, 1995) were measured using broadband PAR and UV sensors. Moreover, the spectral quality of fluorescent PAR sources used in these experiments differed substantially from that of solar radiation penetrating clear tropical waters. Most notably, the longer wavelengths between 550 and 700 nm are rapidly attenuated by seawater (Kirk 1994), resulting in a relative increase in the blue and green wavelengths with depth. Fluorescent sources described as 'white', which are typically used in culture chambers, deliver longer wavelengths, peaking around 650 nm or are spectrally 'flat' between 500 and 650 nm. Because a photon of longer wavelength delivers less energy than a photon of shorter wavelength (e.g. Kirk 1994), foraminifers maintained under a white fluorescent source may respond differently than specimens maintained under a source emitting higher energy, shorter wavelengths. For example, Fitt and Warner (1995) found that a 'blue' source of PAR reduced photosynthetic potential more in *Montastraea annularis* (Ellis and Solander) than did a 'white' source.

Experiments presented here build upon previous research to refine understanding of *A. gibbosa*'s PAR requirements for growth, as well as to assess the role of spectral quality in bleaching. Specifically, the first experiment compares responses to blue and white fluorescent PAR sources to investigate the relationship between spectral quantity (PFD) and quality (wavelength) on growth and bleaching. Subsequent experiments compare responses to PAR PFD, with and without UVB exposure.

Materials and methods

The following methods are common to all experiments. For clarity and brevity, variations specific to individual experiments are presented with the results of each experiment. Abbreviations used to denote experimental treatments are presented in Table 1.

Reef rubble was collected from 30 m on Conch Reef in the Florida Keys (24° 59′ N, 80° 25′ W) and scrubbed according to methods detailed by Williams et al. (1997). Specimens from this site and depth were used for experiments because suitable specimens (described below) were consistently more abundant than at other sites routinely sampled (see Hallock et al. 1995 or Williams et al. 1997). Prior to the beginning (≤ 3 weeks) of an experiment, sediments and live *A. gibbosa* were placed in sealed containers in a culture chamber maintained at 25°C and 12 h light/dark cycle using a 'white' fluorescent source (Fig. 1) providing a PAR PFD of approximately 5 μmol photon m^{-2} s^{-1}. Talge and Hallock (2003) found that laboratory-maintained specimens were cytologically indistinguishable from newly collected field specimens after more than a month in experimental conditions.

Healthy individuals (i.e., those with golden-brown color and exhibiting pseudopodial activity) of approximately the same maximum diameter (ranging from 0.6 to 0.95 mm depending on the experiment) were isolated and randomly placed into 15×60 mm plastic petri dishes containing nutrient-enriched Erdschreiber culture medium (Hallock et al. 1986). Three replicates per treatment, each containing 12–15 specimens, were randomly assigned to a treatment level. The diameter of each specimen in a replicate was measured using a stereomicroscope equipped with an ocular micrometer, and each dish was sealed with plastic wrap.

During all experiments, PAR was delivered in a 12-h light/dark cycle using 15 W fluorescent sources (Fig. 1), either warm white (General Electric) or blue (Marine-Glo). Diffusers were placed over the PAR sources to distribute visible radiation more evenly and to absorb extraneous UV (250–400 nm) produced by the fluorescent sources. In experiments II and III, UVB-exposed treatments were exposed to a 15 W UVB bulb (NIS) (Fig. 2) for 4 h in the middle of the 12-h light (PAR) cycle. All treatments were shielded from UVC radiation (250–280 nm) by cellulose acetate film (0.11-mm thickness, with zero transmission in the range 250–290 nm). For UVB-shielded treatments ['No UVB' and 'Lo UVB' (Table 1)], Mylar-D [0.13-mm thickness (Dupont)] was used to filter the UVB (3% transmission at 280–320 nm and 93% transmission at 400–700 nm). Spectral irradiance measurements were made at 2-nm intervals using an Optronics OL754 portable high-

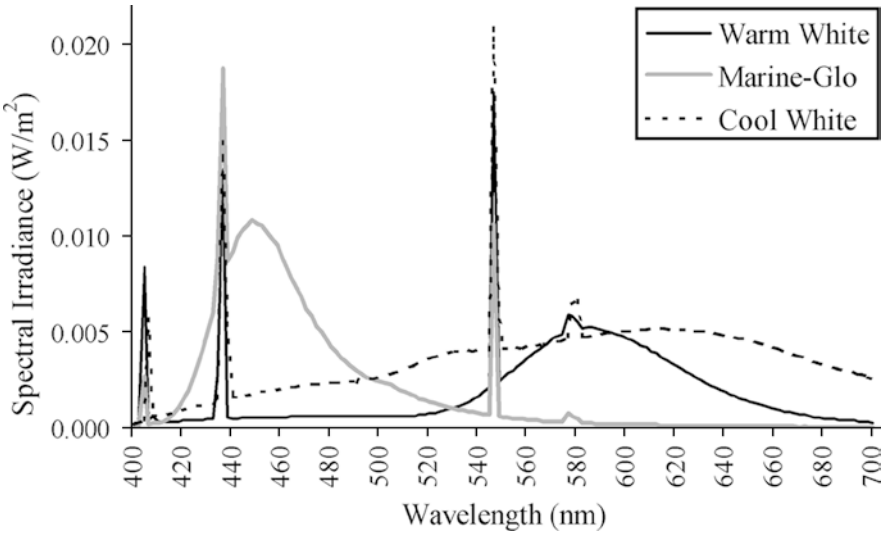

Fig. 1 Spectral irradiance (W m^{-2}) of each photosynthetically active radiation (PAR) source used in experiments I–III. All sources were 15-W bulbs and were assessed at 2-nm intervals using an Optronics spectroradiometer. The 'warm white' source was only used in experiment I; the 'Marine-Glo' was used in all experiments; the 'cool white' is the typical light source used in culture chambers and is shown only for comparison

accuracy UV–visible spectroradiometer (200–800 nm range). Measurements were made prior to each experiment (Table 1). The spectral quality and quantity of irradiance applied to a specific treatment were measured independently for each treatment by placing the Optronics sensor in the same place as the treated specimens in the environmental chamber before the experiment began. For each of these measurements the sensor was placed beneath the same diffusers (plastic film and/or Mylar-D filters) as in the specific treatment, to approximate most closely the spectral quality and quantity reaching the foraminifers.

During the experiments, cultures were maintained at approximately 25 ± 2°C, for 31–34 days. Culture medium was changed weekly. Maximum test diameter of each specimen was measured using a stereomicroscope equipped with an ocular micrometer and each specimen was assessed approximately weekly for the presence of bleaching. Mean growth rates in μm day^{-1} for each treatment were determined by dividing mean increase in shell diameter by the duration of the experiment in days.

For each experiment, general linear model factorial analysis of covariance (ANCOVA) was performed using STATISTICA (Statsoft 2001) on the measured diameter of each specimen over all experimental intervals to test for replicate and treatment effects. Data from each experiment were reciprocally transformed (Zar 1984) so that assumptions of normality and homoscedasticity would be met. In all cases replicates were not significantly different ($P > 0.29$) so the replicate effect was eliminated from the model. Multiple comparisons by Tukey's honestly significant difference (HSD) test (Statsoft 2001) were performed within all significant effects.

Bleaching was assessed as the number of affected individuals per replicate at the conclusion of the experiment. For each experiment, the number of bleached individuals in each replicate was compared for treatment effects using a Kruskal-Wallis (non-parametric) one-way analysis of variance (ANOVA) followed by a multiple-comparison procedure that uses rank sums to make pairwise comparisons (Conover 1980).

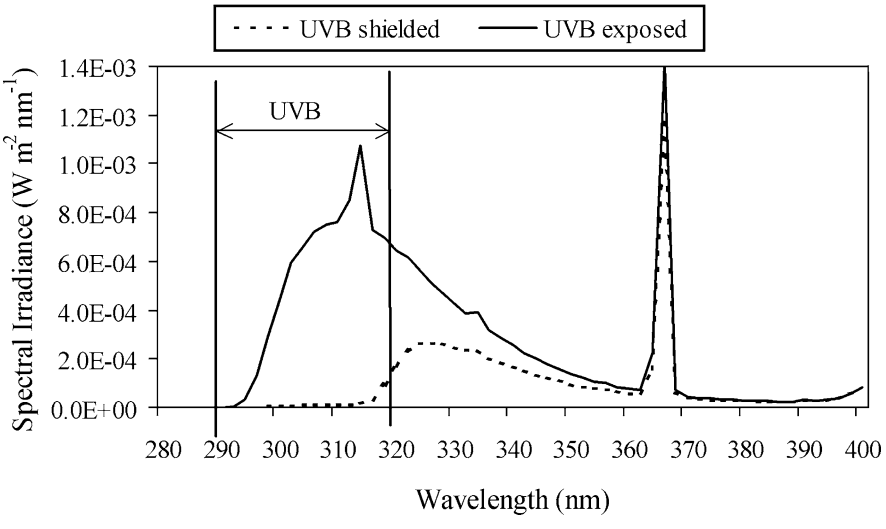

Fig. 2 Ultraviolet spectral irradiance (W m^{-2}) of the UVB source (15-W bulb) for experiments II and III demonstrating the UVB shielding by Mylar film; assessed at 2-nm intervals using an Optronics spectroradiometer

Table 1 Summary of experimental treatments, growth and bleaching results from all experiments. All treatments included three replicates, each containing 12 (experiment II) or 15 (experiments I and III) individual foraminifers. The quantity of UVB is shown as a maximum dose rate and a daily dose rate based on a 4-h exposure period (1 W = 1 J s^{-1}). Total energy is the sum of energy measured between 290 and 700 nm at 2-nm intervals using an Optronics spectroradiometer

Treatment	PAR PFD (μmol photon m^{-2} s^{-1})[a]	UVB (W m^{-2})[b]	Daily UVB (J m^{-2} day^{-1})[c]	Total energy (W m^{-2})	UVB/PAR	Growth rate (μm day^{-1})	Bleaching
Experiment I							
Blue Hi	7.7	[d]	[d]	1.97	[d]	10.8	27%
Blue Lo	2.5	[d]	[d]	0.64	[d]	6.7	7%
White Hi	7.8	[d]	[d]	1.64	[d]	9.3	0%
White Lo	2.8	[d]	[d]	0.58	[d]	5.1	0%
Experiment II							
Hi/UVB	12.0	0.0168	242	3.09	0.0055	9.6	89%
Hi/NoUVB	11.0	0.0008	11	2.80	0.0003	8.7	72%
Med/UVB	8.8	0.0166	239	2.26	0.0074	7.6	56%
Med/NoUVB	8.1	0.0007	10	2.07	0.0003	8.6	33%
Lo/NoUVB	4.0	0.0003	4	1.01	0.0003	2.5	3%
Experiment III							
Hi PAR/Hi UVB	6.2	0.1048	1,509	1.75	0.0668	2.5	[e]
Hi PAR/Lo UVB	5.6	0.0051	73	1.49	0.0036	7.7	[e]
Lo PAR/Hi UVB	2.0	0.1074	1,546	0.68	0.2168	0.8	[e]
Lo PAR/Lo UVB	1.8	0.0049	71	0.51	0.0110	1.4	[e]

[a]Photosynthetically active radiation (PAR) converted from W m^{-2} using $E = hc/\lambda$, where E is the energy of a photon in Joules, h is Planck's constant, c is the speed of light and λ represents wavelength
[b]Maximum dose rate
[c]Daily dose rate
[d]Values $< 10^{-5}$
[e]Specimens darkened rather than bleaching

Results

Experiment I

The first experiment examined the effect of PAR spectral quality (color) and quantity (PFD) on growth and bleaching. Four treatments were applied (Table 1): high and low PFD of both white and blue fluorescent sources (Fig. 1) addressed using a simple 2×2 factorial design.

Growth rates were significantly higher in the treatments exposed to 'Blue' light (ANOVA, $P = 0.0067$); and higher in the 'Hi' PFD treatments (ANOVA, $P < 0.0001$) (Fig. 3), but there was no significant interaction in spectral quality (color) and quantity (PFD) (ANOVA, $P = 0.8451$).

Frequency of bleaching was significantly different between treatments (Kruskal-Wallis, $P = 0.0165$) (Fig. 4). Multiple comparisons indicated that, in treatments exposed to blue light, significantly ($\alpha = 0.05$) more individuals exhibited bleaching than in treatments exposed to white light. In addition, significantly more individuals exhibited bleaching in the 'Blue Hi' treatment than in the 'Blue Lo' treatment.

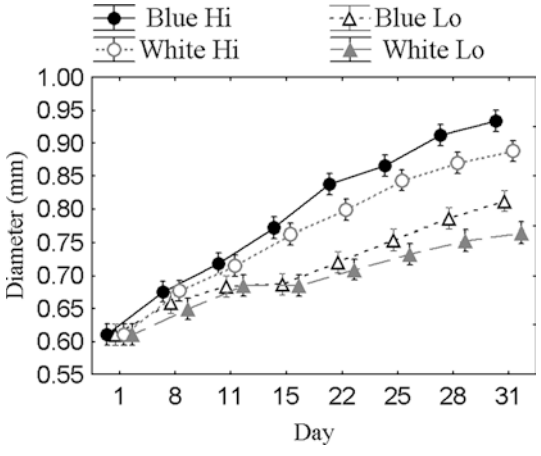

Fig. 3 Effects of PAR quality and quantity on growth (mean diameter ± SE) during experiment I. *'Blue Hi'* indicates 7.7 μmol m^{-2} s^{-1} of the blue PAR source; *'Blue Lo'* indicates 2.5 μmol m^{-2} s^{-1} of the blue PAR source; *'White Hi'* indicates 7.8 μmol m^{-2} s^{-1} of the white PAR source; *'White Lo'* indicates 2.8 μmol m^{-2} s^{-1} of the white PAR source (see Table 1)

Experiment II

The second experiment consisted of five treatments (Table 1) used to test the effect of PAR intensity and UVB presence on growth and bleaching in *A. gibbosa*. The blue (Marine-Glo) and UVB (NIS) sources were used and the 'Hi/NoUVB' and 'Med/NoUVB' treatments were shielded with Mylar-D to filter the UVB while 'Hi/UVB', 'Med/UVB' and 'Lo/UVB' treatments were exposed to UVB. The 'Lo/NoUVB' treatment was not balanced with a 'Lo/UVB' treatment due to space limitations.

Growth rates were significantly affected by PAR PFD (ANOVA, $P < 0.0001$), with growth in the 'Hi' and 'Med' PFD treatments greater than in the 'Lo' treatments (Tukey HSD, $P < 0.0001$), but not significantly different from each other (Tukey HSD, $P = 0.3878$) (Fig. 5). Doses of UVB used in these experiments had no significant effect on growth rate (ANOVA, $P = 0.2435$) (Fig. 6).

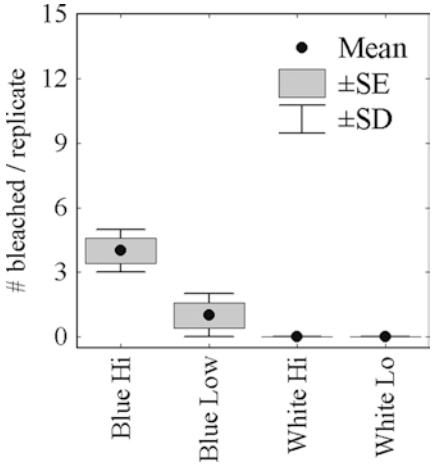

Fig. 4 Effects of PAR quality and quantity on bleaching (mean number per replicate of 15) in experiment I. *'Blue Hi'* indicates 7.7 μmol m^{-2} s^{-1} of the blue PAR source; *'Blue Lo'* indicates 2.5 μmol m^{-2} s^{-1} of the blue PAR source; *'White Hi'* indicates 7.8 μmol m^{-2} s^{-1} of the white PAR source; *'White Lo'* indicates 2.8 μmol m^{-2} s^{-1} of the white PAR source (see Table 1)

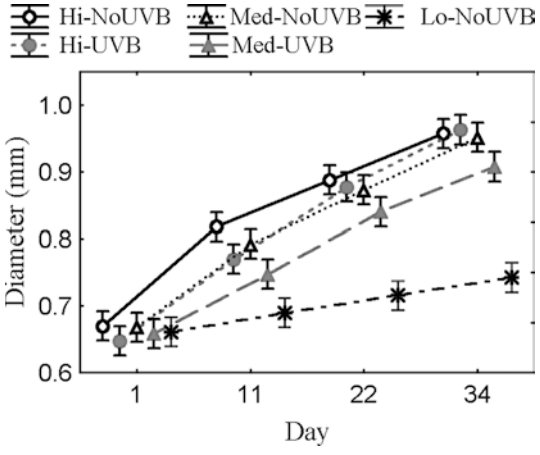

Fig. 5 Effects of PAR and UVB on growth (mean diameter ± SE) during experiment II. Treatments were exposed to PAR at *'Hi'* (11–12 μmol m^{-2} s^{-1}) and *'Med'* (approx. 8 μmol m^{-2} s^{-1}) intensities of blue PAR with and without UVB. Treatments with *'UVB'* were exposed to 0.016 W m^{-2} for 4 h a day, treatments with *'NoUVB'* were exposed to less than 0.0006 W m^{-2} during those 4 h. Additionally a *'Lo'* (approx. 4 μmol m^{-2} s^{-1}) PAR treatment with *'NoUVB'* was included (see Table 1)

Bleaching frequency was significantly different among treatments (Kruskal-Wallis, $P = 0.005$), being highest in the 'Hi' treatments and decreasing to the 'Lo' treatment (Figs. 6, 7). At the 'Hi' and 'Med' PAR PFD, more individuals exhibited bleaching with 'UVB' (56–89%, Table 1) than in comparable 'NoUVB' treatments (33–72%, Table 1) ($\alpha/2 = 0.025$, $df = 10$).

Experiment III

In the final experiment two PAR (Marine-Glo) PFD and two UVB irradiances were tested (Table 1). Due to an initial calculation error, the UVB irradiances were

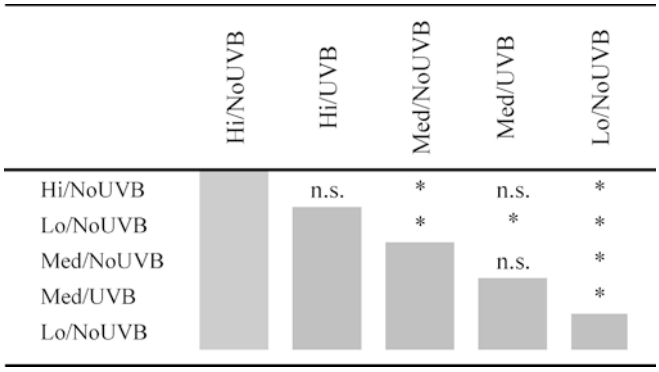

Fig. 6 Pair-wise comparisons of bleaching between treatments in experiment II. Significant differences ($\alpha/2 = 0.025$) are indicated with *asterisks*; differences that are not significant are noted as *n.s.*; bleaching was assessed at the end of the experiment

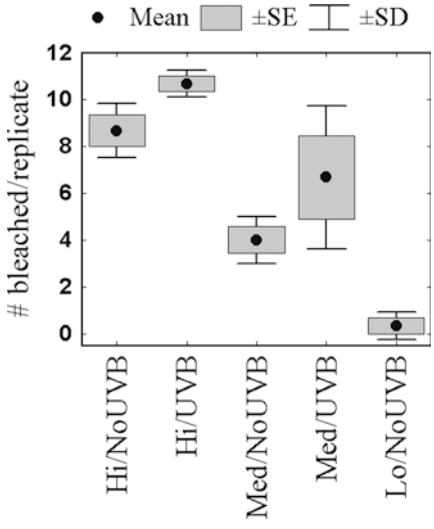

Fig. 7 Effects of PAR and UVB on bleaching (mean number per replicate of 12) in experiment II. Treatments were exposed to PAR at *'Hi'* (11–12 μmol m^{-2} s^{-1}) and *'Med'* (approx. 8 μmol m^{-2} s^{-1}) intensities of blue PAR with and without UVB. Treatments with *'UVB'* were exposed to 0.016 W m^{-2} for 4 h a day, treatments with *'NoUVB'* were exposed to less than 0.0006 W m^{-2} during those 4 h. Additionally a *'Lo'* (approx. 4 μmol m^{-2} s^{-1}) PAR treatment with *'NoUVB'* was included (see Table 1)

higher than originally planned. The treatments shielded with Mylar-D still received a substantial amount of UVB due to the high intensity emitted by the source so they are labeled as 'Lo UVB'. The same general hypotheses from experiment II, investigating the effect of PAR and UVB on growth rates and treatment effect on bleaching frequency, were addressed using a simple 2×2 factorial design.

Growth rate was significantly higher in the 'Hi PAR' treatments (ANOVA, $P < 0.0001$) and the 'Lo UVB' treatments (ANOVA, $P < 0.0001$) (Fig. 8). A significant interaction between PAR and UVB effects reflects the significantly higher growth rate in the 'Hi PAR/Lo UVB' treatment compared to the others (Tukey, $P < 0.0001$).

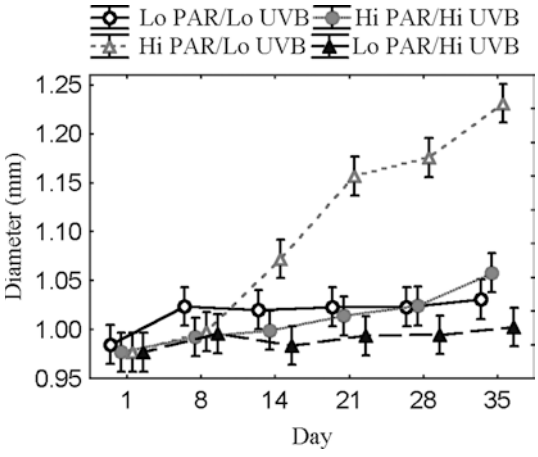

Fig. 8 Effects of PAR and UVB on growth (mean diameter ± SE) during experiment III. Experimental conditions are described in Table 1. Treatments exposed to *'Hi PAR'* were exposed to approximately 6 μmol m^{-2} s^{-1} while those exposed to *'Lo PAR'* were exposed to approximately 2 μmol m^{-2} s^{-1}. Treatments exposed to *'Hi UVB'* were exposed to 0.099–0.096 W m^{-2} of UVB while those exposed to *'Lo UVB'* were exposed to approximately 0.005 W m^{-2} (see Table 1)

There were no significant differences in bleaching frequency (Kruskal-Wallis, $P = 0.0761$) between treatments. Rather than typical bleaching, in this experiment 2–3 of the 15 individuals per replicate became unevenly dark brown in color across all treatments.

Discussion

Optimal PAR

The results of this series of experiments reinforce previous observations by Hallock et al. (1986,1995) that *A. gibbosa* have a remarkably limited optimum irradiance in the laboratory, given that this species of protist can live over an approximately 45-m euphotic depth range. Optimal radiant energy appears to be 1–2 W m^{-2} delivered by PAR PFD of 6–8 μmol m^{-2} s^{-1}, representing a daily dose rate of 6–8×10^4 J m^{-2} day^{-1}. Higher PAR PFD or spectral shifts to higher-energy wavelengths induces bleaching, while exposure to lower PAR PFD reduces growth rates.

Growth rates were highest (8.7–10.8 μm day^{-1}), and comparable to those reported by Hallock et al. (1986), at PAR PFD between 7.7 and 12.0 μmol photon m^{-2} s^{-1} (Table 1). At equal PAR PFD, higher energy blue radiation resulted in significantly faster growth than the longer 'white' wavelengths. Total available energy was about 20% higher under blue sources than white at the same PAR PFD. Furthermore, energy uptake by diatom photosynthetic pigments peaks at 450 and 675 nm with low uptake inbetween the peaks (550 nm) (Iturriaga et al. 1988), while the spectra of the PAR sources (Fig. 1) peaked at 450 nm (blue) and 580 nm (white). Thus, at equal PAR PFD, specimens growing under the blue source were exposed to more useable energy, explaining the higher growth rates.

The blue, Marine-Glo source was chosen for these experiments because its spectral characteristics are more similar to 'deep-reef' spectral characteristics than those of white fluorescent sources. Higher energy, shorter wavelengths produced bleaching in a higher percentage of individuals than did longer wavelengths at equivalent PAR PFD; some individuals experienced partial bleaching in each blue-source treatment (Table 1, Fig. 4). However, frequency of bleaching generally increased with increase in total energy, even within the range of irradiance that produced the highest growth rates (i.e., 8–12 μmol photon m^{-2} s^{-1}). An exception was seen in experiment I, where some bleaching occurred in the 'Blue Lo' treatments (0.64 W m^{-2}), while none occurred in the 'White Hi' treatments (1.64 W m^{-2}). The highest growth rate that was achieved with no bleaching symptoms (9.3 μm day^{-1}) occurred in the experiment I 'White Hi' treatment (7.8 μmol photon m^{-2} s^{-1}).

A plausible argument might be made that specimens used in these experiments were preconditioned to low PAR PFD by maintenance at 5 μmol m^{-2} s^{-1} in the laboratory prior to the experiments. However, the experiments ran longer than the pre-experimental holding time, so if photoacclimatization had occurred, the specimens could presumably re-acclimatize to the higher experimental PAR PFD. This was certainly the case for previously reported experiments (Hallock et al. 1986), which began with juveniles only 1 week old and continued for up to 4 months. Additionally, bleaching observed in the experiments described here appeared, usually in the 2nd or 3rd week, and progressed gradually, rather than as an immediate reaction to the experimental treatments. Talge and Hallock (1995) documented that bleaching in field-collected *A. gibbosa* is a cumulative, degenerative process, and Talge and Hallock (2003) further verified that the cytological characteristics of experimentally bleached specimens were consistent with those of partly bleached specimens taken directly from field samples. Moreover, the standard procedure of maintaining specimens at PAR PFD−2 s^{-1} emerged from recurring observations in the early 1980s that exposure to higher intensities in culture induced loss of symbiont color (Hallock et al. 1986).

Optimal PAR PFD for growth of *A. gibbosa* found in these and prior experiments (Hallock et al. 1986, 1995) indicate that supra-optimal intensities can occur to depths of 30 m or more in clear reef waters. Based on August 1994 hourly PAR measurements from several depths on Conch Reef reported by Lesser (2000) and assuming optimal PAR PFD of 8 μmol photon m^{-2} s^{-1}, excess available PAR (available/optimal) was calculated and averaged for measurements collected between the hours of 1000 and 1500. At 3 m on Conch Reef, 128 times optimal PAR PFD are available; at 18 m, 45 times; and at 30 m, 9 times.

UVB tolerance

The UVB total daily dose rates and maximum dose rates administered in experiments II and III (Table 1) are comparable to rates measured at Conch Reef between 30 June and 10 July 1993 by Gleason and Wellington (1995).[1] The UVB maximum dose rate used in experiment II (0.0168 W m^{-2}) is similar to the maximum dose rate that Gleason and Wellington measured at 24 m (0.02–0.03 W m^{-2}). However, because UVB exposure was 4 h per day in our experiments, the daily dose rate was approximately 240 J m^{-2} day^{-1} (1 W = 1 J s^{-1}), which is less than half the 500–600 J m^{-2} day^{-1} measured in the field at 24 m. In experiment III (Table 1), the maximum dose rate of 0.11 W m^{-2} is 4–5 times higher than measured at 24 m at Conch Reef, and roughly half the maximum dose measured at 10 m (0.22–0.28 W m^{-2}). The daily dose rate was about 1,500 J m^{-2} day^{-1}, which is double that measured at 24 m and one fourth of the daily dose rate measured at 10 m (Gleason and Wellington 1995). The 'Lo' maximum and daily dose rates of UVB used in the experiment III 'Hi PAR/LoUVB' treatment (0.0051 W m^{-2} and 72 J m^{-2} day^{-1}) are comparable to mid-summer doses that can reach 40 m at Conch Reef, based on an attenuation coefficient of 0.2, estimated from Gleason and Wellington's (1995) measurements at 10 and 24 m.

However, given that *A. gibbosa* can tolerate only a fraction of available PAR at depths less than about 40 m, it follows that they only tolerate a similar fraction of available subsurface UVB. Therefore, ecologically relevant UVB to PAR ratios are necessary to evaluate the possible role of UVB in bleaching stress. Using measurements made by Gleason and Wellington (1995) on Conch Reef, UVB to PAR ratios were 0.006 at 3 m, 0.002 at 10 m and 0.0004 at 24 m. Thus, the range of UVB ratios used in experiment II (0.0003 and 0.007, see Table 1) encompass those measured at Conch Reef. These treatments did not result in suppressed growth (Fig. 5) but did induce bleaching in the foraminifers (Fig. 7). However, whether the bleaching was accelerated specifically by UVB or simply by the increase in total energy cannot be discerned from these experiments.

In Experiment III, a calculation error resulted in unrealistically high UVB to PAR ratios for all treatments except the 'Hi PAR/Lo UVB' (Table 1) treatment, which was comparable to the UVB to PAR ratio at depths less than 10 m. Some specimens from all treatments in experiment III became dark brown and less uniform in color, rather than bleaching. Talge (2002) reported that *A. gibbosa* specimens exposed to 13–15 μmol photon m^{-2} s^{-1} PAR plus 0.0156 W m^{-2} s^{-1} UVB for only 4 days appeared anomalously dark. When examined cytologically, the symbionts were deteriorating as they do when a foraminifer bleaches. The difference was that a dense, dark-staining material was evident in the pore cups that line the underside of the chamber walls. This dense material appeared to be shielding the symbionts, which are housed in the pore cups, and may represent a short-term defense mechanism to minimize damage from solar radiation.

[1]Sensors used by Gleason and Wellington (1995) measured UVB between 300 and 320 nm while our experimental doses were calculated from 290 to 320 nm. However, note from Fig. 2 that our artificial UVB source emitted very little irradiance between 290 and 320 nm; on average only 4% of the maximum dose rate was emitted in this range making the laboratory magnitudes comparable to measurements in their field study.

Possible mechanism

Photoinhibition in algal cells was described by Neale (1987, p 41) as 'a reaction to supra-optimal PFDs that is exhibited even by healthy, exponentially growing cultures'. Photoinhibition can occur with PAR or UV exposure, though UV magnifies the effects seen with PAR alone (Richardson et al.1983; Neale 1987). Photoinhibition has been implicated as the cellular mechanism involved in coral bleaching (Warner et al. 1999) by leading to the production of oxygen free radicals resulting in oxidative stress to the cell (e.g. Lesser and Shick 1989; Lesser 1997). Fitt and Warner (1995) also found that blue wavelengths were more effective in inducing bleaching stress in *M. annularis*.

Cytological examination of partly bleached *A. gibbosa*, whether field-collected or laboratory-induced, revealed damage consistent with oxidative stress to cellular membranes and organelles (Talge 2002). Moreover, there are two major differences between laboratory and field conditions that may induce bleaching in the laboratory at light intensities that are optimal for growth (see also Hallock et al. 1986, 1995). In the laboratory, during light exposure periods, specimens were continuously exposed to the same irradiance. In the field, irradiance continuously changes. Future experiments could be designed to determine if equal PAR PFD, delivered from a randomized light source, would induce more or less bleaching than a continuous source. Furthermore, in the field there is usually some water motion, while in the laboratory there is little to none. We attempted an experiment on a slowly rotating table, but heat from the motor driving the turntable terminated the experiment. Either lack of water motion or constant irradiance may allow accumulation within the host endoplasm of damaging concentrations of oxygen free radicals, which are naturally produced during photosynthesis (e.g., Neale 1987). When isolated from the foraminiferal host, the diatom symbionts are routinely grown in substantially higher irradiance [6–10 W m^{-2} (see, e.g., Lee et al. 1983)]. Future experiments should be designed to test these and other possibilities.

Ecological Implications

A. gibbosa are found abundantly at depths where available visible and UV irradiance is as much as 100 times optimal. What adaptations enable these pro-

tists to avoid damage from excess PAR? What happened in 1991 that resulted in the failure of those adaptations to prevent widespread bleaching in these protists?

Phototaxic behavior appears to be the primary mechanism by which *A. gibbosa* individuals can modulate their exposure to solar radiation. Behavioral experiments by Zmiri et al. (1974) on specimens of *A. radiata* found that shorter wavelengths of visible radiation increased positive phototaxis at lower PFDs than the longer wavelengths, and that negative geotaxis increased under shorter wavelengths. Their results could be interpreted as the expression of an adaptive strategy ensuring that deeper-dwelling individuals, which are naturally exposed to shorter wavelengths of solar radiation (e.g. Kirk 1994), gain exposure to adequate PAR by being less cryptic (moving more and climbing higher) than shallower-dwelling individuals. Zmiri et al. (1974) did not report negative phototaxis at the PAR PFD used in their experiments. However, the 0.02–2 μmol photon m^{-2} s^{-1} PAR used would not be expected to induce negative phototaxis even in this deep-euphotic species (Hohenneger et al. 1999). Nevertheless, the results reported by Zmiri et al. (1974) demonstrated extreme behavioral sensitivity to both spectral quality and quantity. Both positive and negative phototaxis have been observed in *A. gibbosa* under culture conditions (authors' unpublished observations).

In addition to behavioral responses, *Amphistegina* spp. appear to have several other adaptations to deal with supra-optimal irradiance. Hallock et al. (1986) found that specimens grown under 14 and 40 μmol photon m^{-2} s^{-1} PAR produced significantly more heavily calcified tests than the specimens grown under lower PAR PFD. As observed in experiment III and documented cytologically by Talge (2002), specimens exposed to a sudden, severe increase in irradiance may darken, indicating movement of opaque material between the symbionts and the outer wall, presumably partly shading the symbionts. At least 10 species or varieties of diatom symbionts have been isolated from *A. gibbosa* (Lee et al. 1995). This diversity may allow members of the species to exploit a range of habitats by harboring symbionts that can tolerate higher irradiance at shallower depths and symbionts that require lower irradiance at the deeper depths, as has been demonstrated in zooxanthellate corals (e.g., Rowan 1998; Baker 2000). In addition, photoprotective compounds such as MAA occur in many organisms (e.g., Dunlap and Shick 1998), and preliminary data indicate their occurrence in *Amphistegina* (S.K. Toler, personal communication). Future research may demonstrate that all of these strategies are involved in the remarkable biogeographic and euphotic-depth range of this species.

One seemingly contradictory result from these experiments is that the highest growth rates were observed in the treatments that also suffered the highest frequency of bleaching. Given the previously-reported dependence of the growth and calcification of *Amphistegina* spp. on their symbionts (Muller 1978; ter Kuile et al. 1987; ter Kuile and Erez 1991), consistent observations from both field and laboratory studies that partly bleached individuals continue to grow seems paradoxical. The partitioning of the foraminiferal cytoplasm into ectoplasm and endoplasm may help explain this apparent paradox. The ectoplasm forms the granuloreticulopodia, whose functions include feeding, motility, and chamber addition (Travis and Bowser 1991). The endoplasmic functions include housing the symbionts, lipid storage, and reproduction. Thus, if bacteria and diatoms are available as food sources, the ectoplasm can continue to function, even when degradation of the symbionts and of the endoplasm occurs (Talge and Hallock 2003). This separation of function also provides insight into why reproduction is so profoundly impacted by bleaching (Hallock et al. 1995; Talge et al. 1997). Toler and Hallock (1998) further showed that the tests of bleached individuals had a reduced organic matrix suggesting that the foraminfers may have been malnourished during chamber construction.

The results of these experiments do not confirm that increased UVB to PAR ratios resulting from stratospheric ozone depletion triggered the onset of bleaching in *A. gibbosa* populations in the Florida Keys and elsewhere in summer 1991. However, the results strongly support the hypothesis that an increase in higher energy, shorter wavelength radiation reaching the seafloor can induce bleaching. At otherwise optimal PAR PFD, some bleaching occurred under a 'blue' PAR source that delivered 20% more energy than the 'white' source. Stratospheric ozone depletion at 20–30° N latitude, where the Florida reef tract is located, has been approximately 2–4% per decade, resulting in cumulative depletion on the order of 10% (Shick et al. 1996). That decrease in stratospheric ozone has been responsible for a roughly 20% increase in DNA-weighted UVB dose (Madronich 1992) reaching the Earth's surface. Given that a 20% increase in energy in the blue wavelengths can trigger bleaching in *A. gibbosa*, it is not unreasonable to suspect that the cumulative ozone depletion prior to 1991, compounded by the approx. 4% transient global decline following the Mount Pinatubo eruption in June 1991, may have played an important role in the abrupt onset of bleaching in *Amphistegina* populations in Florida and elsewhere (Hallock et al. 1993; Williams et al. 1997).

Conclusions

1. Optimal PAR PFD for growth in laboratory culture for *A. gibbosa* is 6–8 μmol photon m^{-2} s^{-1}; optimum total daily dose of PAR is approximately 6–8×10^4 J m^{-2} day^{-1}.
2. Higher-energy blue radiation (peaking at 450 nm) induced more bleaching, even at lower PAR PFD, than lower-energy white radiation (peaking at 580 nm).

3. UVB irradiance, known to occur in reef environments at depths in excess of 10 m (0.10 W m^{-2}), can inhibit growth in *A. gibbosa*.
4. At the irradiance optimum of these protists, small changes that increase exposure either to shorter, higher energy wavelengths or to total radiant energy can induce bleaching in *A. gibbosa*.
5. Given that the irradiance optimum of *A. gibbosa* is only a fraction of the PAR PFD that can penetrate to depths in excess of 40 m in reef waters, behavior probably plays a critical role in the modulation of exposure by these protists.

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References

Baker AC (2000) Reef corals bleach to survive change. Nature 411:765–766

Conover WJ (1980) Practical nonparametric statistics, 2nd edn. John Wiley, New York, pp 229–233

Dunlap WC, Shick JM (1998) Ultraviolet radiation-absorbing micosporine-like amino acids in coral reef organisms: a biochemical and environmental perspective. J Phycol 34:418–430

Fitt WK, Warner ME (1995) Bleaching patterns of four species of Caribbean reef corals. Biol Bull 189:298–307

Gleason DF, Wellington GM (1995) Variation in UVB sensitivity of planula larvae of the coral *Agaricia agaricites* along a depth gradient. Mar Biol 123:63–703

Hallock P (1999) Symbiont-bearing Foraminifera. In: Sen Gupta BK (ed) Modern Foraminifera. Kluwer, Great Britain, pp 123–139

Hallock P, Talge HK (1994) A predatory foraminifer, *Floresina amphiphaga* n. sp., from the Florida Keys. J Foramin Res 24:210–213

Hallock P, Forward LB, Hansen HJ (1986) Influence of environment on the test shape of *Amphistegina*. J Foramin Res 16:224–231

Hallock P, Talge HK, Smith K, Cockey EM (1993) Bleaching in a reef-dwelling foraminifer, *Amphistegina gibbosa*. Proceedings of the 7th International Coral Reef Symposium, Guam, vol 1, pp 44–49

Hallock P, Talge HK, Cockey EM, Muller RG (1995) A new disease in reef-dwelling foraminifera: implications for coastal sedimentation. J Foramin Res 25:280–286

Hohenegger J, Yordanova E, Nakano Y, Tatzreiter F (1999) Habitats of larger foraminifera on the upper reef slope of Sesoko Island, Okinawa, Japan. Mar Micropaleontol 36:109–16

Iturriaga R, Mitchell BG, Kiefer DA (1988) Microphotometric analysis of individual particle absorption spectra. Limnol Oceanogr 33:128–135

Kirk JTO (1994) Light and photosynthesis in aquatic ecosystems. Cambridge University Press, Cambridge

Kuile BH ter, Erez J (1991) Carbon budgets for two species of benthonic symbiont-bearing foraminifera. Biological Bulletin 180:489–495

Kuile BH ter, Erez J, Lee JJ (1987) The role of feeding in the metabolism of larger symbiont bearing foraminifera. Symbiosis 4:335–350

Langer MR, Hottinger L (2000) Biogeography of selected "larger" foraminifera. Micropaleontology 46[Suppl 1]:105–126

Lee JJ, McEnery ME, Koestler RJ, Lee MJ, Reidy J, Shilo M (1983) Experimental studies of symbiont persistence in *Amphistegina lessonii*, a diatom-bearing species of larger foraminifera from the Red Sea. Endocytobiology 2:487–514

Lee JJ, Morales J, Symons A, Hallock P (1995) Diatom symbionts in larger foraminifera from Caribbean hosts. Mar Micropaleontol 26:99–105

Lesser MP (1997) Oxidative stress causes coral bleaching during exposure to elevated temperatures. Coral Reefs 16:187–192

Lesser MP (2000) Depth-dependent photoacclimatization to solar ultraviolet radiation in the Caribbean coral *Montastraea faveolata*. Mar Ecol Prog Ser 192:137–151

Lesser MP, Shick JM (1989) Photoadaptation and defenses against oxygen toxicity in zooxanthellae from natural populations of symbiotic cnidarians. J Exp Mar Biol Ecol 134:129–141

Muller PH (1978) ^{14}Carbon fixation and symbiont loss in a foraminiferal-algal symbiont system. J Foramin Res 8:35–41

Madronich S (1992) Implications of recent total atmospheric ozone measurements for biologically active ultraviolet radiation reaching the Earth's surface. Geophys Res Lett 19:37–40

Neale PJ (1987) Algal photoinhibition and photosynthesis in the aquatic environment. In: Kyle DJ, Osmond CB, Arntzen CJ (eds) Photoinhibition. Elsevier, Amsterdam

Randel WJ, Fei W, Russell JM III, Waters JW, Froidevaux L (1995) Ozone and temperature changes in the stratosphere following the eruption of Mt. Pinatubo. J Geophys Res 100:16753–16764

Richardson K, Beardall J, Raven JA (1983) Adaptation of unicellular algae to irradiance: an analysis of strategies. New Phytol 93:157–191

Rowan R (1998) Diversity and ecology of zooxanthellae on coral reefs. J Phycol 34:407–417

Shick JM, Lesser MP, Jokiel PL (1996) Effects of ultraviolet radiation on corals and other coral reef organisms. Glob Change Biol 2:527–545

StatSoft Inc (2001) STATISTICA for Windows.Computer program manual. StatSoft, Tulsa, Okla., USA. http://www.statsoft.com. Cited 2 March 2003

Talge HK (2002) Cytology of symbiont loss (bleaching) in *Amphistegina gibbosa* (Class Foraminifera). PhD dissertation, University of South Florida, Tampa, Florida

Talge HK, Hallock P (1995) Cytological examination of symbiont loss in a benthic foraminifera, *Amphistegina gibbosa*. Mar Micropaleontol 26:107–113

Talge HK, Hallock P (2003) Ultrastructural responses to bleaching in *Amphistegina gibbosa* (Foraminifera). J Eucaryot Microbiol 50:324–333

Talge HK, Williams DE, Hallock P, Harney JN (1997) Symbiont loss in reef foraminifera: consequences for affected populations. Proceedings of the 8th International Coral Reef Symposium, Panama, vol 1, pp 589–594

Toler SK, Hallock P (1998) Shell malformation in stressed *Amphistegina* populations: relation to biomineralization and paleoenvironmental potential. Mar Micropaleontol 34:107–115

Travis JL, Bowser SS (1991) The motility of Foraminifera. In: Lee JJ, Anderson OR (eds) Biology of Foraminifera. Academic, New York, p 91–155

Warner ME, Fitt WK, Schmidt GW (1999) Damage to photosystem II in symbiotic dinoflagellates: a determinant of coral bleaching. P Natl Acad Sci USA 96:8007–8012

Williams DE, Hallock P, Talge HK, Harney JN, McRae G (1997) Responses of *Amphistegina gibbosa* populations in the Florida Keys (U.S.A.) to a multi-year stress event (1991–1996). J Foramin Res 27:264–269

Zar JH (1984) Biostatistical analysis. Prentice Hall, Englewood Cliffs, N.J.

Zmiri A, Kahan D, Hochstein S, Reiss Z (1974) Phototaxis and thermotaxis in some species of *Amphistegina* (Foraminifera). J Protozool 21:133–138