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Coral Reefs

The effects of prolonged "bleaching" on the tissue biomass and reproduction of the reef coral *Montastrea annularis*

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Abstract. Colonies of *Montastrea annularis* from Carysfort Reef, Florida, that remained bleached seven months after the 1987 Caribbean bleaching event were studied to determine the long term effects of bleaching on coral physiology. Two types of bleached colonies were found: colonies with low numbers of zooxanthellae with normal pigment content, and a colony with high densities of low-pigment zooxanthellae. In both types, the zooxanthellae had an abnormal distribution within polyp tissues: highest densities were observed in basal endoderm and in mesenteries where zooxanthellae are not normally found. Bleached corals had 30% less tissue carbon and 44% less tissue nitrogen biomass per skeletal surface area, but the same tissue C:N ratio as other colonies that either did not bleach (normal) or that bleached and regained their zooxanthellae (recovered). Bleached corals were not able to complete gametogenesis during the reproductive season following the bleaching, while recovered corals were able to follow a normal gametogenic cycle. It appears that bleached corals were able to survive the prolonged period without nutritional contribution from their zooxanthellae by consuming their own structural materials for maintenance, but then, did not have the resources necessary for reproduction. The recovered corals, on the other hand, must have regained their zooxanthellae soon after the bleaching event since neither their tissue biomass nor their ability to reproduce were impaired.

Introduction

The ability of some scleractinian corals to grow fast and to form reef structures is generally attributed to their symbiosis with dinoflagellates. These algal endosymbionts provide the coral host with organic compounds that constitute the major portion of the animal's diet (Muscatine 1967; Muscatine and Porter 1977; Muscatine et al. 1981; 1984). The algal translocate is believed to provide most of the energy for maintenance, tissue and skeletal growth and possibly reproduction for many reef corals.

Recent episodes of massive expulsion or loss of zooxanthellae by reef corals have attracted the attention of reef ecologists around the world. Most notable of these wide-spread "bleaching" events are the 1982–1983 bleachings throughout the eastern Pacific and the western Indo-Pacific regions (Glynn 1983, 1984) and the more recent bleaching that occurred during the late summer and fall of 1987 throughout the Caribbean, Bahamas and Florida (Williams et al. 1987; Ogden and Wicklund 1988). The former event is attributed to high seawater temperatures or altered sealevels during the 1982–1983 El Niño event (Glynn 1983, 1984, 1988); the latter bleaching event also is being attributed to high seawater temperatures (Atwood et al. 1988; Causey 1988; Jaap 1988) but the supporting data is less conclusive. Numerous other reports of bleachings, most related to local events such as salinity drops following storms, or pools of warm waters during hot summers, are common in the literature (e.g. Goodbody 1961; Goreau 1964; Jaap 1979, 1985; Oliver 1985; Acevedo and Goenaga 1986). In some cases mass mortality of corals has followed the bleachings (Glynn 1983), but in most cases many of the corals survive the bleaching and gradually regain their zooxanthellae. The long-term effect of bleaching on the physiology of surviving corals has not been previously reported.

Coral bleaching can be functionally defined as the loss of the normal brownish color characteristic of live healthy corals. The loss of coloration, while generally attributed to the loss of zooxanthellae can also result from a loss of pigmentation by the zooxanthellae without a loss of the zooxanthellae themselves (Hoegh-Guldberg and Smith 1989). In most of the studies cited above, bleaching was assumed to be due to the loss of zooxanthellae, but zooxanthellae density was not always measured.

Since some species of reef coral are believed to receive most of their nutrition from photosynthetic materials translocated by their zooxanthellae (above references), processes such as growth and reproduction might be af-

fected by bleaching. Coral tissue composition and biomass could also be affected by the loss of zooxanthellae if the corals had to utilize storage products, and eventually structural materials, to support maintenance metabolism.

The present study reports the effects of bleaching on the physiology of *Montastrea annularis,* the major framework-forming coral on Caribbean and western Atlantic reefs. Nine coral colonies were sampled in a study to determine whether the loss of zooxanthellae has an effect on coral biomass characteristics and their ability to reproduce sexually. A second paper will report on the growth rates and skeletal stable isotope compositions of these same corals.

Materials and Methods

Sample collection

During the summer of 1987, at Carysfort Reef, Florida (Fig. 1), many (but not all) large heads of *M. annularis* were observed to bleach, and this bleaching was recorded with an underwater video camera by J. Halas and Lt. M. White, Key Largo National Marine Sanctuary. In May of 1988 the coloration of the colonies in these tapes was used to identify and select three large colonies that were bleached in October, 1987 and still remained bleached seven months later (bleached colonies), three colonies that had bleached but had recovered their zooxanthellae during the six month interval (recovered colonies), and three colonies that had normal coloration at the time of the filming (normal colonies). Sample size was limited because the study was conducted within the National Marine Sanctuary.

Carysfort Reef is located approximately 20 km offshore and is part of the series of barrier reefs known as the Florida Reef Tract. The colonies of *Montastrea annularis* used in this study are located on the seaward (eastern) side of the reef. The bleached colonies were all at 13 m depth while the normal and recovered ones were at depths from 9 to 13 m. The shallower colonies were high relief massive "heads" of 1 to 2 m height, while the deeper colonies were more encrusting or platey and only 0.5 to 1 m high. *M. annularis* is a hermaphroditic species with an annual reproductive cycle (Szmant 1986 and in preparation). Oogenesis in Puerto Rican specimens begins in May and continues through July and August. Spermatogenesis begins later by mid-July, and is completed by mid-August. Spawning occurs sometime during late August through September depending on the lunar cycle of the particular year. Samples were collected from each colony on four occasions at approximately monthly intervals from May through August, 1988, so as to examine the various stages of the reproductive cycle.

A pneumatic drill powered by a SCUBA tank and equipped with a hole saw was used to remove small cores of tissue and skeleton. Cores 5 cm in diameter were used for tissue biomass studies and cores 2.5 cm in diameter for histological studies. A single core of each size was collected from each colony during each sampling date. The three colonies selected for the "bleached" classification were much lighter in color than the normal and recovered colonies, and had a mottled appearance which did not change during the study period; samples were taken from the lighter colored portions of the colony. (These colonies had regained normal coloration when examined on June 3rd 1989). Cores for biomass studies were returned to the laboratory and kept in running seawater until they could be processed (always less than 24 h); cores for histological examination were preserved in the field with Zenker's fixative (Barszcz and Yevich 1975).

Biomass samples

Tissues were removed from the coral skeleton with a jet of high-pressure air and seawater from an artist's airbrush. This method generates a coral tissue/zooxanthellae slurry that is much more concentrated than that generated by the more commonly used Water-Pik method (Johannes and Wiebe 1970). The slurry was homogenized for 30 s with an Ultra-Turrax homogenizer, the volume of homogenate recorded and sub-samples taken for zooxanthellae counts, chlorophyll analysis and tissue elemental composition. Samples for zooxanthellae counts were preserved with Lugol's iodine solution and zooxanthellae densities determined with a hemocytometer. Samples for chlorophyll analysis were filtered onto Whatman GF/A glass fiber filters and kept frozen for later extraction. Chlorophyll was extracted from the filters by immersion in 100% acetone in the freezer for 24 h. Chlorophyll absorbances were read at 750, 663 and 630 nm and chlorophyll *a* concentrations calculated with the equations in Jeffrey and Humphrey (1975). Samples for elemental analysis were lyophilized in a Savant Speed-Vac, pulverized with an agate mortar and pestle, and sub-samples analyzed for carbon and nitrogen content with a Carlo Erba Model 1106 Elemental Analyzer. Surface areas of the upper (living) surface of the cores were determined by the aluminum foil method of Marsh (1970).

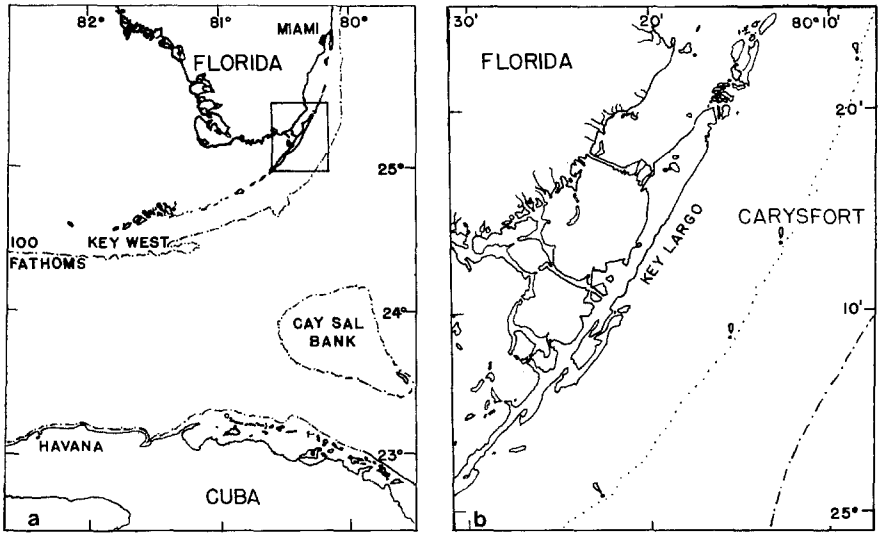

Fig. 1. a Map of the south Florida region showing the Florida Reef Tract and the location of the study site (box).
b Expansion of the area within the box showing the location of Carysfort Reef on the outer edge of the Reef Tract

Histology

The preserved small core samples were rinsed in tap-water, decalcified in 10% hydrochloric acid, dehydrated and cleared with Technicon's S-29 and UC-670 histological reagents, respectively, and embedded in Paraplast. Coral polyps were embedded such that both vertical and transverse sections were obtained. Sections 7 μm thick were stained by Heidenhain's azocarmine-aniline blue method (Luna 1968) and examined with an Olympus BH-T microscope at magnifications up to 600 ×. Gametocytes when present were classified as to developmental stage according to the criteria described in Szmant-Froelich et al. (1985). A mean of 17 (range: 7 to 50) polyps were examined per sample.

Statistical analyses

Tests for homogeneity of variances showed that for most biomass parameters measured the variances were heterogenous, which was not surprising given the small number of samples. Therefore, non-parametric analysis of variance (Kruskal-Wallis test) was used to determine whether significant differences existed in biomass characteristics between the three groups of corals. For data sets where the Kruskal-Wallis indicated significant variation, the Kolmogorov-Smirnov two-sample test was used to determine which groups differed significantly from each other. The statistical calculations were done with SPSS (Hull and Nie 1981) on a Vax computer.

Results

Zooxanthellae density and chlorophyll content

The mean zooxanthellae densities expressed as cells per cm^2 of surface area for each of the three groups of colonies for each date are summarized in Fig. 2. One of the colonies in the "bleached" category (No. 67) was plotted separately from the other two bleached colonies because it had significantly higher zooxanthellae densities than the other two bleached corals and also higher than those of the normal and recovered corals. The two remaining bleached corals had lower zooxanthellae densities than the recovered and unbleached corals on three of the four sampling dates, but these differences were only statistically significant ($P=0.02$) when the data from all four dates were pooled. The mean zooxanthellae density of the bleached corals was not significantly different from those of the normal and recovered ones when the data for coral No. 67 was included in the analysis. There was no statistical difference between zooxanthellae densities of the recovered and unbleached corals.

Chlorophyll data is only available for the June and July sample dates. The mean chlorophyll concentrations for each group for each date, expressed both per algal cell and per surface area, is presented in Fig. 3. Colony No. 67 was again plotted separately because it differed significantly from the other two bleached corals. With the exception of colony No. 67, there was no significant difference between groups in the amount of chlorophyll *a* per algal cell (Fig. 3a). Zooxanthellae from colony No. 67 had an order of magnitude less chlorophyll *a* per cell than those of all other colonies sampled. All three bleached corals did have, however, significantly less chlorophyll *a* per live surface area than did the recovered and normal

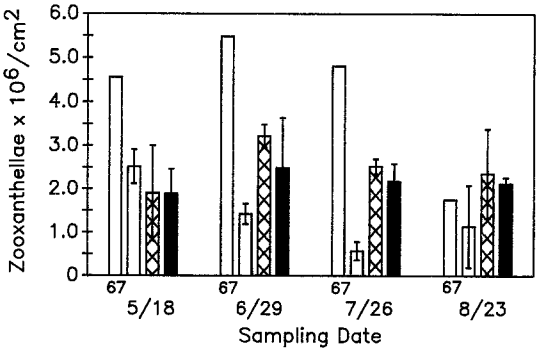

Fig. 2. Zooxanthellae densities in samples of tissues collected from colonies of *Montastrea annularis* from Carysfort Reef 7 to 10 months after the 1987 bleaching event. Values are means of samples from three colonies, except for the bleached corals: bleached colony No. 67 had unusually high zooxanthellae densities and was plotted separately. Error bars = 1 SD; □ = bleached; ⊠ = recovered; ■ = normal

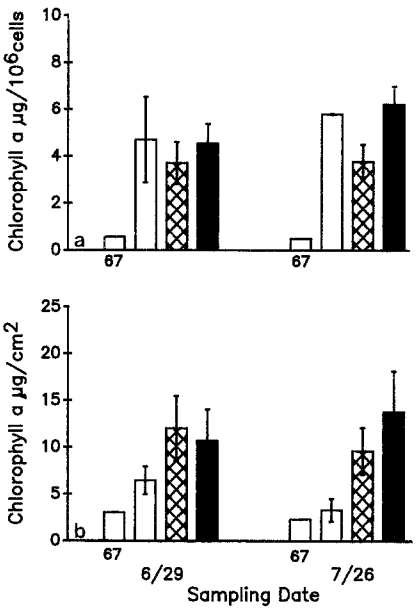

Fig. 3a, b. Chlorophyll concentrations in tissues of colonies of *Montastrea annularis* from Carysfort Reef. **a** Chlorophyll content per zooxanthellae cell. **b** Chlorophyll density per tissue surface area. Means (±1 SD) of samples from three colonies of each type except for bleached ones as explained in Fig. 2. □ = bleached; ⊠ = recovered; ■ = normal

colonies ($P<0.001$; Fig. 3b). Again, there was no significant difference between the recovered and normal colonies in chlorophyll *a* per surface area.

Distribution of zooxanthellae within coral tissue

Zooxanthellae in scleractinian corals are normally found in highest densities in the tentacles, oral disc area and in the coenenchyme between polyps and few if any zooxanthellae are normally found in the lower parts of a polyp. This was the distribution of zooxanthellae observed in tissue samples from all of the normal and recovered colonies (Fig. 4A–C), but not in the three bleached ones

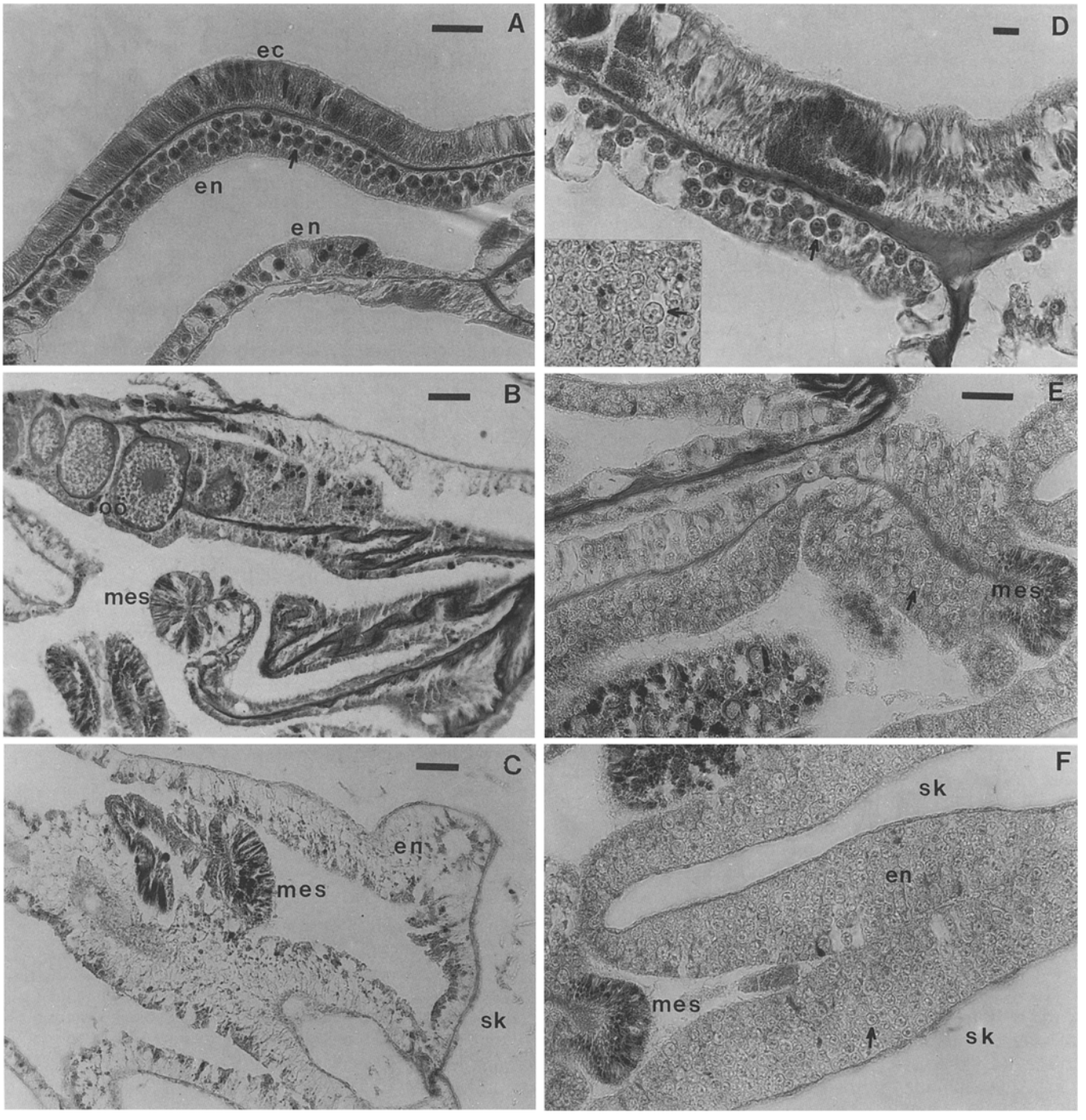

Fig. 4 A–F. Photomicrographs of histological preparations of tissue from normal and bleached colonies of *Montastrea annularis* showing the distribution of zooxanthellae within polyps. **A** Vertical section through the coenenchyme of a normal specimen showing a high density of zooxanthellae in the endodermal layer. **B** Section through a normal polyp showing a mesentery with oocytes and a mesenterial filament. Note absence of zooxanthellae in these tissues. **C** Vertical section through the lower (basal) portion of a normal polyp demonstrating absence of zooxanthellae in this area. **D** Section through the coenenchyme of a bleached specimen (colony No. 67) containing a low density of normal appearing zooxanthellae in the endoderm. The insert in the lower left is of zooxanthellae from the basal endoderm of the same polyp. **E** Section of a mesentery and mesenterial filament of bleached colony No. 67 containing a high density of empty-looking zooxanthellae. **F** Vertical section through the basal area of a polyp from colony No. 67 showing the basal endoderm fully packed with abnormal zooxanthellae. ec = ectoderm; en = endoderm; mes = mesenterial filament; oo = oocyte; sk = areas where skeleton had been. **A**, **E** and **F** = 30 μm; **B** and **C** = 50 μm; **D** = 10 μm

(Fig. 4D–F). In the latter there were abnormally dense populations of algal cells that completely filled endodermal tissues at the base of the polyp (Fig. 4F). These tissues in normal polyps seldom have any zooxanthellae (Fig. 4C). Mesenterial filaments of the bleached colonies also had much higher densities of zooxanthellae (Fig. 4E) than did normal or recovered corals (Fig. 4B). Furthermore, the zooxanthellae in the basal endoderm and mesenteries of the bleached corals had an abnormal empty appearance (Fig. 4D insert). This contrasts with the zooxanthellae found in the distal endodermal layers of bleached polyps, which had normal appearance and densities (Fig. 4A and D).

Tissue biomass

Two aspects of coral tissue (animal plus zooxanthellae) biomass were measured: the carbon to nitrogen ratio (C:N) of the tissues and the amount of tissue C and N per skeletal surface area (Fig. 5). Bleached colony No. 67 did not differ from the other two bleached colonies in either of these two measures, and thus was included in the statistical comparisons.

The tissue C:N ratios of the three groups of colonies were very similar to each other (Fig. 5a). No statistically significant differences were found between the C and N biomass per surface area of recovered and normal colonies, but the bleached colonies had 30 to 50% less tissue C and N per skeletal surface area than the recovered and normal colonies (Fig. 5b and c). These differences were highly significant ($P < 0.001$).

Gametogenesis

Oogenesis had already begun in the normal and recovered colonies at the time of the first sampling (May

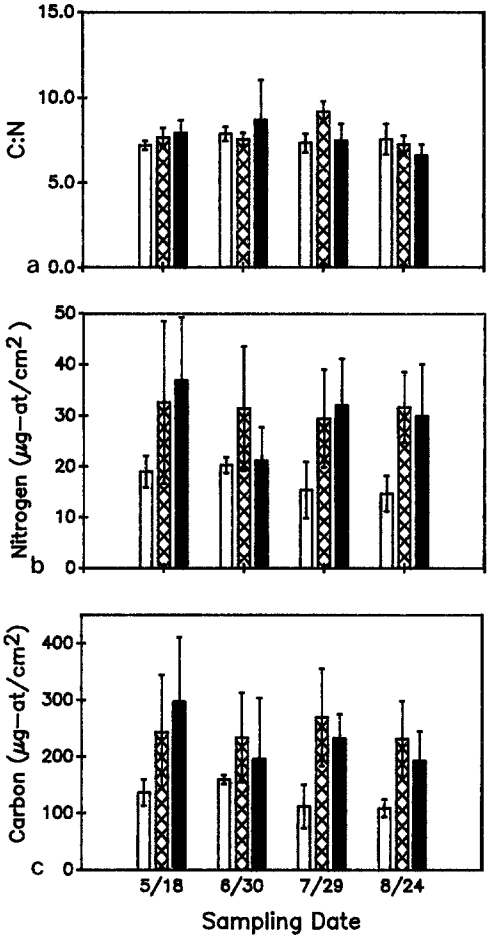

Fig. 5a–c. Carbon and nitrogen content of whole tissues samples (coral plus zooxanthellae) from colonies of *Montastrea annularis* from Carysfort Reef. Means (±1 SD) of three colonies of each type. **a** C to N atomic ratios of the tissues. **b** Tissue N per skeletal surface area. **c** Tissue C per skeletal surface area. □ = bleached; ⊠ = recovered; ■ normal

Table 1. Gametogenic stages observed in histological preparations of tissues of the coral *Montastrea annularis* collected from Carysfort Reef during the reproductive season following the 1987 bleaching event. Oocyte stages (O): I = primary oogonia; II = early oocyte before vitellogenesis; III = vitellogenic period; IV = mature oocyte. Spermatocyte stages (S): I = primary spermatogonia; II = early spermatocytes; III = late spermatocytes before nuclear condensation. – = no gametocytes observed; * = samples lost

Colony type and number	Sample dates							
	May 18		June 29		July 25		August 24	
	O	S	O	S	O	S	O	S
Normal								
80	III	–	III	–	III	I, II	*	
81	II, III	–	III	–	III	I	*	
82	III	–	III, IV	–	III	–	III	III
Recovered								
70	III	–	III	–	III	II	*	
71	III	–	III	–	III	II	*	
72	II, III	–	III	–	III	–	III	III
Bleached								
65	–	–	II, III	–	–	–	*	
66	–	–	III	–	–	–	*	
67	–	–	–	–	–	–	*	

18, 1988; Table 1 and Fig. 4B). Most of the oocytes observed were classified as being in Stage III, characterized by active vitellogenesis. A few Stage II oocytes (early vitellogenesis) were also observed. None of the bleached colonies had any evidence of oogenesis. In samples collected on June 29, 1988, one of the bleached colonies had a few early Stage III oocytes, one had numerous mid-Stage III oocytes and colony No. 67 had none; all of the normal and recovered colonies had large numbers of late Stage III oocytes but no spermatocytes. On July 25, 1988, all of the normal and recovered colonies had mature oocytes, and early spermatocytes (Stages I and II) were observed in over half of these colonies. No gametocytes were observed in samples from any of the three bleached colonies. Unfortunately, most of the samples from August 24, 1988 were ruined during processing. The two useable samples, one from each a normal and a recovered colony, had mature oocytes and spermaries. These two colonies (Nos. 82 and 72, respectively) were ones which did not have spermaries during the July sampling.

Discussion

High temperatures are claimed to be associated with the 1987 Caribbean-wide bleaching (Atwood et al. 1988; Jaap 1988). Hoegh-Guldberg and Smith (1989) reported that experimental bleaching of two species of Pacific coral after exposure to high light intensity was due to pigment loss by the zooxanthellae, but that exposure to high temperatures caused bleaching due to loss of zooxanthellae. They did not find loss of pigment from high temperatures, nor loss of zooxanthellae from high light intensities. If these results can be generalized, the high temperatures reported above could have been responsible for bleaching due to loss of zooxanthellae but not to loss of pigment. However, both types of bleaching were found in the present study. The white or creamy coloration of the colonies of *Montastrea annularis* used in this study we assumed to be due to loss of zooxanthellae, but this was the case for only two of the three colonies. Our third bleached colony (No. 67) had the highest density of zooxanthellae (almost twice those of the normal colonies), but these zooxanthellae had only one-tenth the chlorophyll *a* content per cell of those from the other eight colonies. Furthermore, we observed two distinct populations of zooxanthellae in No. 67: a normal-appearing (but low density) one located in the endoderm of the oral disk (Fig. 4D) and tentacles, and an empty-appearing, high density one in the mesenterial filaments and basal endoderm (Fig. 4D–F). High densities of abnormal appearing zooxanthellae were also observed in the same areas of the basal endoderm of the other two bleached corals. Kleppel et al. (1989) found that samples from two bleached colonies of *M. annularis* sampled in December, 1987; only three to four months after the bleaching, had both lower densities of zooxanthellae and lower concentrations of pigment per cell, but did not report on the physical appearance of the algae. It appears that most of the zooxanthellae in two of our bleached colonies had recovered their normal pigment content, but for some reason had not been able to achieve normal cell densities. Possible explanations for the latter include continued high expulsion rates, low cell division rates or a combination of the two. There is no way to determine *a posteriori* whether any of the recovered colonies had bleached due to pigment loss rather than due to loss of symbionts. Since, in either case, the photosynthetic capacity of the coral will be reduced, it is likely that the physiological effects of bleaching will be similar whether colonies lose their symbionts or whether the symbionts are retained without chlorophyll. It is also not possible to determine from this study which type of bleaching was most common, given the small sample size to which we were limited by Sanctuary authorities.

The unusual distribution of the abnormal zooxanthellae remaining in the bleached colonies is difficult to explain. The basal endoderm of *Montastrea annularis* generally has few zooxanthellae, but, in bleached polyps the basal endoderm was fully packed with algal cells. Host cells are unlikely to continue normal function under such conditions. Higher than normal densities were also found in mesenterial endoderm, where low numbers of atrophied cells are frequently found in normal polyps possibly in the process of being digested. The gross morpholoy of the unusually-located zooxanthellae was also abnormal: cells appeared empty, as if major organelles were missing, although the pyrenoids and assimilation bodies remained visible (Fig. 4D insert). The nuclei were not distinguishable with light microscopy. As bleached corals may have catabolized structural material for maintenance, zooxanthellae may have been concentrated in the polyps' remaining tissues. The vacant appearance of the mesenterial zooxanthellae may also indicate that those algal cells had lost their photosynthetic pigments and were in the process of being digested by the host.

Zooxanthellae have been shown to provide their coral hosts with large quantities of organic materials, especially high-caloric value lipids and carbohydrates (e.g. Muscatine 1967; Muscatine and Cernichiari 1969; von Holt and von Holt 1968; Battey and Patton 1984). Several investigators have estimated that algal translocate provides up to 100% of the host's daily energy requirement, and may also provide materials for growth and reproduction (e.g. Wethey and Porter 1976; Muscatine and Porter 1977; Muscatine et al. 1981, 1984). In general, the materials translocated are characterized by high C:N ratios. Corals apparently utilize much of the translocate to support respiration, and thus have very high oxygen consumption to ammonium excretion (O:N) ratios (>100; Szmant et al. 1989). Based on this knowledge, we expected to find that corals which had remained bleached for a prolonged period would have lower tissue biomass and C:N ratios than normal or recovered corals. These expectations were further supported by the results of Glynn et al. (1985) which showed bleached colonies of *Pocillopora* to have lower lipid content than unbleached animals, and Szmant-Froelich and Pilson (1980) which showed that starved colonies of *Astrangia danae* had lower lipid: protein ratios than fed animals. Our results do show the expected decrease in tissue biomass for the bleached colonies (Fig. 5), but not the decrease in C:N

ratio. The average C:N ratio of the Carysfort corals of about 7.5 was considerably lower than that of colonies of *M. annularis* from the Bahamas (C:N of about 11; Szmant et al. 1989). Our present results suggest that the Florida corals had less storage material to sustain them during periods of reduced nutritional input, so that the bleached corals may have had to depend on catabolism of structural material as soon as bleaching began.

We also expected to find that the bleached corals and perhaps the recovered ones, with their reduced nutritional supplies, would not have the resources necessary to initiate or complete sexual reproduction. The production of gametes requires large amounts of energy and new biomass, and many animals are known to accumulate and store materials for much of the year for this purpose (Townsend and Calow 1981). *Montastrea annularis* has a relatively brief reproductive period, with oogenesis taking only three months to complete, which contrasts with the prolonged periods of gametogenesis found for most other Caribbean reef corals studied to date (Szmant 1986). This suggests that in *M. annularis*, the materials for vitellogenesis are derived from storage products accumulated prior to the initiation of gametogenesis. Our results confirmed that the bleached corals were not able to complete gametogenesis. The few early oocytes found in the June samples were no longer evident in the July samples, suggesting that the oocytes may have been resorbed. The recovered corals apparently had regained their zooxanthellae sufficiently early so that they were able to maintain their tissues and recover their reserves for reproduction.

In conclusion, many colonies of the important reef-building coral *Montastrea annularis* became bleached during the fall-1987 Caribbean-wide bleaching event. Many of these bleached colonies had recovered their pigment and/or zooxanthellae by the next spring, but others were still bleached a year later. The recovered corals had normal tissue biomass and C:N ratio, and were able to complete the energy-intensive process of gametogenesis. We do not know how long these animals remained bleached before they recovered, but the evidence suggests that they recovered quickly since they had normal growth rates subsequent to the bleaching (Leder et al. unpublished). The bleached corals all had some zooxanthellae a year later, but those of one of the three colonies sampled had lowered chlorophyll *a* content. All three bleached colonies had lower tissue biomass and C:N ratios, and were not able to produce gametes during the normal reproductive season. These bleached animals also had slower growth rates subsequent to the bleaching than the normal or recovered colonies (Leder et al. unpublished). While we recognize that a sample size of three is less than optimal, and that caution should be exercised in the interpretation of our data, we believe that the differences in coral tissue biomass and reproduction between the bleached corals and the normal and recovered corals are significant and real; they were reproducible over a four month period. Our results illustrate once again the importance of zooxanthellae to the host's well-being and success, but they also show that this species can persist for prolonged periods of time with minimal contributions from its algal symbionts. The degree to which the algal contribution can be replaced by phagotrophy will be reported elsewhere.

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