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Changes in pigmentation associated with the bleaching of stony corals

Abstract—Bleaching (loss of pigmentation) is a common response by corals to environmental stress. Changes in pigmentation and the processes that caused them were studied in *Montastrea annularis* during a natural bleaching event off southeast Florida. Chlorophyll *c,* peridinin, and diadinoxanthin levels were 35, 17, and 20 times higher in normal corals than in bleached ones. Two processes, loss of zooxanthellae from the coral and loss of pigments from the remaining zooxanthellae, contributed to bleaching. Loss of zooxanthellar pigment accounted for 72% of the decrease in Chl *c.* The two processes contributed about equally to reduced carotenoid levels.

Environmental stress may be created by a change in ambient conditions (Stebbing 1981; Rosen 1982; Brown and Howard 1985). Loss of pigmentation, or bleaching, is thought to be a typical response to environmental stress by scleractinian corals and other invertebrates that contain endosymbiotic algae (i.e. zooxanthellae, Glynn 1984; Lessios et al. 1984). Alterations in light, temperature, or other environmental conditions (Yonge 1930; Lang 1973; Coles

and Jokiel 1978; Dustan 1979) have, under laboratory conditions or during field manipulations, been reported to initiate the departure of zooxanthellae from the host coral, the loss of pigment by the zooxanthellae, or both. Bleached corals seem more susceptible to invasion by parasites and competitors; ultimately the corals may die (Glynn 1984, 1988).

Many studies have documented the adaptation of coral pigments and zooxanthellae to light and other environmental variables (e.g. Prezelin 1976; Falkowski and Dubinsky 1981; Chang et al. 1983; Sandeman 1988). Few studies, however, have focused on bleaching as a response to environmental stress. The changes that occur in coral pigmentation during natural bleaching events are largely undescribed, even though the scales of these events may be basinwide to global (Ogden and Wicklund 1988). In the few cases where pigments were measured during natural coral bleaching events, only chlorophylls were considered (cf. Gladfelter 1988) Corals are rich in carotenoids (Chalker and Dunlap 1981) and changes in carotenoid concentrations may have important physiological implications for the coral and the coral-algal symbiosis.

Finally, bleaching is typically described through qualitative, visual observation. Little or no quantitative data have been gathered by which bleached and normal corals can be distinguished. Although such casual observations are useful when severe bleaching has occurred (i.e. most of the corals are white), a quantitative approach would be useful in identifying changes associated with
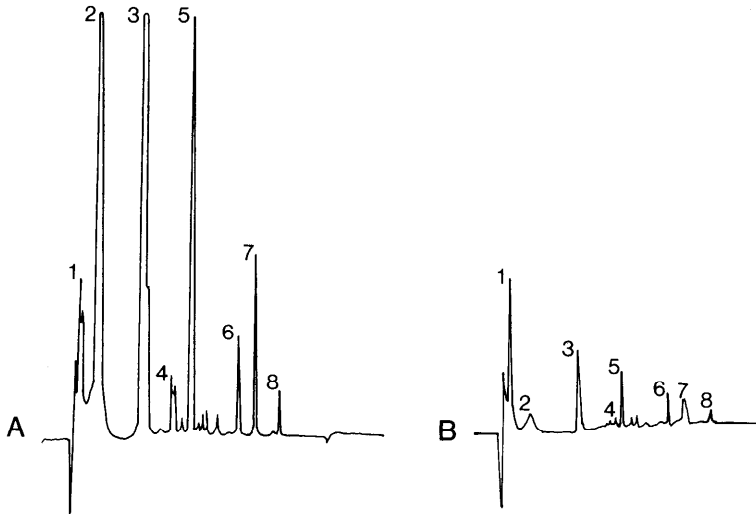
Fig. 1.   Typical chromatograms of (A) normally pigmented and (B) bleached corals collected from the waters off southeast Florida, December 1987. The same pigments, but at different concentrations, were found in each sample. Peaks monitored at 460 nm with a UV/vis detector represent: 1—unretained volume; 2—chlorophyll *c*; 3—peridinin; 4—astaxanthin (from the coral polyp tissue); 5—diadinoxanthin; 6—chlorophyll *b* (from a green alga associated with the coral skeleton); 7—chlorophyll *a*; 8—carotene.

the early stages of bleaching and with recovery. Few efforts have been made to quantitatively discriminate between normal and bleached corals, and none of them have measured carotenoids.

In 1987 and 1988, bleaching of scleractinian corals was reported worldwide; severe episodes occurred in the Caribbean Basin and off the southeast coast of Florida (Roberts 1987; Williams et al. 1987; Glynn 1988). In this note we describe the changes that occurred in the chlorophyll and carotenoid concentrations of *Montastrea annularis,* an important coral species, off southeast Florida. We ascertain whether bleached and normal corals can be distinguished on the basis of measured pigmentation and we determine the contribution of each of two mechanisms of bleaching—loss of zooxanthellae from the coral and loss of pigment from the zooxanthellae—during this particular event.

Colonies of *M. annularis,* a dominant southeast Florida coral species, were collected on 2 December 1987, from a reef 8 m deep at 26°05′N, 80°05′W. When a coral was encountered, it was classified as bleached or normal and collected. No attempt was made to collect remarkable ex-

amples of bleached or normal corals; a gradation in bleaching was visually apparent among the specimens. All colonies were hemispherical in shape and all were about 30 cm in diameter except one, normally pigmented, colony (~10-cm diam).

The samples were returned to the laboratory in a darkened cooler containing Dry-Ice. Pigments were extracted from coral tissue (2 cm², frozen, surface cores) with cold, 90% aqueous acetone. Carotenoids and chlorophylls were isolated quantitatively by high performance liquid chromatography (HPLC) with a Waters gradient elution system and a Beckman C8 octyl-ultrasphere reverse phase column. Procedural details are given by Kleppel et al. (1988). Pigments were identified by cochromatography of freshly prepared standards and by spectrophotometry (IBM Instr. Inc., model 9410 spectrophotometer). Published extinction coefficients (Mantoura and Llewellyn 1983) were used for quantitation.

Coral tissue was removed with an airbrush (Gil-Turnes and Corredor 1981; Knap and Sleeter 1984) for microscopic determination of zooxanthellar abundance. HPLC does not conserve the sample, and tissue removed for microscopy cannot be

Table 1. Statistics describing the mean levels and variability (SE) in normally (N) pigmented corals (two corals, five and four replicates) and bleached (B) corals (two corals, three and two replicates) collected off southeast Florida in December 1987.

	No. corals	No. reps.	x (μg cm^{-2})	SE	N:B
Bleached					
Chl c	2	9	2.43	0.66	
Perid.	2	9	2.15	0.30	
Diad.	2	9	0.99	0.17	
Chl a	2	9	6.35	1.05	
Normal					
Chl c	2	5	0.07	0.02	34.7
Perid.	2	5	0.13	0.02	16.5
Diad.	2	5	0.05	0.02	19.8
Chl a	2	5	0.71	0.10	8.9

reused for chromatography. Thus, zooxanthellae and pigment samples were taken from different, randomly chosen, locations on the same corals.

The same pigments were present in both kinds of corals (Fig. 1). Concentrations of the major zooxanthellar pigments, chlorophylls a and c, peridinin, and diadinoxanthin, however, were 9–34 times higher in normal corals than in bleached ones (Table 1). A two-level, nested, mixed-model ANOVA (Sokal and Rohlf 1981) confirms that differences between pigment concentrations in normal and bleached corals were significant (Table 2).

Chlorophyll b, a green algal pigment, was present in both normal and bleached corals (Fig. 1). The likely source of this pigment was endolithic green algae (Halldal 1968; Jeffrey 1968). Green algae do not contain Chl c, peridinin, or diadinoxanthin (pigments of dinoflagellate zooxanthellae), but they do contain Chl a. Because the Chl a contributed by the endolithic algae is indistinguishable from that contributed by the zooxanthellae, it is not feasible to use Chl

a as a zooxanthellar indicator pigment in this study. Therefore, we do not discuss Chl a further. Interestingly, Chl b concentrations were lower in bleached than in normal corals. Although extensive analysis of this phenomenon is beyond the scope of this note, one possible explanation is that the reduction in zooxanthellar pigmentation permitted greater light transmission to the endolithic green algae. This, in turn, may have mediated a decrease in green algal pigmentation.

Mean abundances of zooxanthellae were significantly higher (ANOVA $F = 154.5$; $P < 0.001$) in normally pigmented corals ($\bar{x} = 1.53 \times 10^6$ cells cm^{-2}; SE $= 8.0 \times 10^4$; $n = 10$) than in bleached specimens ($\bar{x} = 4.20 \times 10^5$ cells cm^{-2}; SE $= 3.0 \times 10^4$; $n = 10$). By dividing mean pigment concentrations (Table 1) by mean zooxanthellar abundances, estimates of the pigment content of an average, individual zooxanthella are obtained for normal and bleached corals (Table 3A). The pigment content per zooxanthella in normal corals was 4.5–9.4 times higher than in bleached corals. Thus, not only did bleached corals contain fewer zooxanthellae, but the zooxanthellae of bleached corals contained less pigment.

To determine the relative contribution of the two loss factors (loss of zooxanthellae from coral, loss of pigment from zooxanthellae), we compared the ratios of pigment content per zooxanthella in normal : bleached corals and of zooxanthellae cm^{-2} in normal : bleached corals (Table 3A). These ratios reflect the magnitudes of each loss factor. Dividing one ratio by the other (= W) gives an indication of the relative contribution of one pigment loss term (e.g. pigment zooxanthella^{-1}) to the other (zooxanthellae cm^{-2}).

The proportional contributions of loss of zooxanthellae, c, and loss of pigment zoo-

Table 2. Results of a two-level, nested ANOVA (mixed model) to identify the magnitudes of variance within and between coral types. Asterisks: *—$P < 0.05$; **—$P < 0.01$.

Source of variance	df	F-value Chl c cm^{-2}	Period. cm^{-2}	Diad. cm^{-2}	Chl a cm^{-2}
Between coral types	1.0	41.21*	269.30**	131.90**	58.05*
Within coral types	2.0	0.13	0.07	0.09	0.21
Within subgroups	10.0				

Table 3. A. Mean pigment content zooxanthella^{-1} (pg cell^{-1}) in normal (N) and bleached (B) corals, N:B ratios, and weighting factor, W. B. Analysis of pigment loss including total loss and losses associated with contributing processes.

	Chl c	Perid.	Diad.
A. Ratios			
Pigment zooxanthella^{-1}			
Normal	1.59	1.41	0.65
Bleached	0.17	0.31	0.12
N:B ratio	9.3	4.5	5.4
W*	2.6	1.3	1.5
B. Analysis of pigment loss			
Total loss (μg cm^{-2})	2.36	2.02	0.94
Contribution to pigment loss			
1. Loss of zooxanthellae (c)			
% c	28	43	40
μg cm^{-2}	0.66	0.87	0.38
2. Loss of pigment zooxanthella (p)			
% p	72	57	60
μg cm^{-2}	1.70	1.15	0.56

* W = N:B ratio of pigment zooxanthella^{-1}/N:B ratio of zooxanthellae cm^{-2}. Ratio of zooxanthellae cm^{-2} in normal:bleached corals = 3.6.

xanthella^{-1}, p, are related by $p = Wc$. The quantity of pigment lost, ΔQ, during bleaching is

$$\Delta Q = p\Delta Q + c\Delta Q. \qquad (1)$$

Substituting Wc for p,

$$\Delta Q = Wc\Delta Q + c\Delta Q, \qquad (2)$$

and

$$c = 1/(W + 1). \qquad (3)$$

It would appear that zooxanthellar pigment loss caused 72% of the reduction in Chl c (Table 3B). Among the carotenoids, the two factors contributed about equally to the loss of pigment.

Our observation that both loss factors contribute to bleaching corroborates the data of Gladfelter (1988), who noted that both zooxanthellae cm^{-2} and chlorophyll (a and c) content per zooxanthella were reduced in bleached, relative to normal, corals off St. Croix during 1987. However, she presented neither data for carotenoids nor estimates of the loss factors. Hoegh-Guldberg and Smith (1988) reported that bleached corals on reefs off Australia exhibited reduced numbers of zooxanthellae but that chlorophyll fluorescence per zooxanthella was

either the same or higher than in normal corals. Frequently, bleaching has been ascribed solely to the departure of zooxanthellae from the coral (Goreau 1964; Glynn 1988; Jaap 1988). In fact, decreased zooxanthellar pigment content may be at least as important as zooxanthellar loss in some bleaching events (Coles 1975).

At present, the environmental changes that initiate bleaching in corals are not well understood. Quantitative distinctions between bleached and normal corals and documentation of bleaching mechanisms (i.e. pigment loss, zooxanthellar loss) may permit coral bleaching events to be detected during their early stages. With such information, identification of the environmental forcing functions seems more likely.

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