

Bleaching and lipids in the Pacific coral *Montipora verrucosa*

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ABSTRACT: Endosymbiotically-derived fixed carbon is drastically reduced in bleached corals as a result of decreased chlorophyll *a* per zooxanthella or the number of zooxanthellae per cm² of coral tissue. Under such conditions, corals may have to rely on other sources of energy including stored lipids. In this study, I investigated the relationship between coral bleaching and lipid concentrations when bleaching was induced by increased total solar irradiance.

I hypothesized that bleached corals would have a lower lipid level than non-bleached corals and that progressively longer bleaching periods would result in successively lower lipid concentrations. Bleaching was induced via increased solar irradiance by transplanting fragments of *Montipora verrucosa* from 10 m to 1 m depth for 4, 8 or 14 days. Corals appeared paler in color after 3 days of exposure and chlorophyll *a* concentrations were significantly lower in all bleached corals after 4, 8 and 14 days of exposure. Chlorophyll *a* levels in bleached corals did not recover to normal levels after 10 or 6 days following exposure. Lipid levels in bleached coral fragments did not differ significantly from control fragments at any time during the experiment. These data indicate that *M. verrucosa* does not depend on lipid energy reserves during short bleaching periods. Decreased metabolism, increased heterotrophy, gamete resorption or some combination of these factors during the early stages of bleaching may compensate for the immediate decrease in photosynthetically-derived fixed carbon in this species. Future studies on the changes in lipids over a longer period of time is needed in order to fully assess the importance of lipid reserves in bleached corals.

INTRODUCTION

Over the past 15 years the incidence of widespread bleaching events has increased on coral reefs throughout the world. Coral bleaching is characterized by the loss of photosynthetic pigments and has been correlated to factors such as elevated seawater temperatures, increased ultraviolet radiation or combinations of the two (Coles & Jokiel, 1978; Jokiel, 1980; Hoegh-Gulberg & Smith, 1989; Cook *et al.*, 1990; Glynn, 1993; Gleason & Wellington, 1993). Bleaching can result in interruption of coral growth, reduction of reproductive output and eventually death (Jokiel & Coles, 1977; Glynn & D'Cruz, 1990; Szmant & Gassman, 1990). The magnitude of bleaching events and the rate of coral recovery varies within and between both sites and species (Brown & Suharsono, 1990; Fitt *et al.*, 1993). The reasons for these variations in bleaching responses however are poorly understood. One physiological component that may account for inter- and intra-specific differences in bleaching susceptibility is lipid concentrations. Under standard physiological conditions, fatty acids and glycerol are synthesized by zooxanthellae from photosynthetically-fixed carbon and are translocated to the host where they are either metabolized or transformed and stored primarily in the form of wax esters and triglycerides (Batey & Patton, 1984). Lipids in corals are stored predominantly in the animal host with concentrations ranging from 29% of dry biomass in *Morastrea annularis*, to 30-40% of dry biomass in various Hawaiian species (Harland *et al.*, 1992; Stimson, 1987). However, under bleached conditions corals may rely heavily on lipid stores due to decreases in the number of zooxanthellae and/or chlorophyll per zooxanthella which leads to lower photosynthetic rates, and thus reduced quantities of translocated lipid. In the Caribbean, temperature-stressed corals showed 39-73% lower lipid concentrations than non-bleached conspecifics 6 months after the onset of the event (Porter *et al.*, 1989). This lower lipid level was presumed to be the result of an estimated 50% decrease in translocated carbon. However, it is not known whether or not lipid levels decrease in corals bleached by increased solar irradiance nor how rapidly lipid levels change in response to bleaching. In this study I examined the initial effects bleaching by solar irradiation had on the total lipid content of the Pacific coral, *Montipora verrucosa*. I hypothesized that bleached corals would have a lower lipid level than non-bleached corals, and that progressively longer bleaching periods would result in successively lower lipid concentrations. Also, given that increased solar irradiance causes chlorophyll levels to decrease, I expected lipid and chlorophyll *a* levels in corals to be correlated (Hoegh-Gulberg & Smith, 1989; Gleason & Wellington, 1993). Finally, I briefly monitored lipid and chlorophyll *a* levels in the corals following exposure to increased irradiance, since little is known about coral recovery following short bleaching periods.

MATERIALS AND METHODS

The experiment was conducted between July 12 and July 26, 1994, at the Lighthouse Point (LP) and Bridge to Nowhere (BTN) sites on Coconut Island, Hawai'i. Fifteen coral colonies of the plating morph of *Montipora verrucosa* were tagged at a depth of 10 m at the LP site. Two fragments with minimum dimensions of 15 cm² were broken off from each of these parent colonies. One fragment was transplanted to a depth of 1 m at the BTN site (treatment fragment) and the other fragment remained directly beside the parent colony as a control for transplantation (control fragment)(Fig. 1). Transplanted and control fragments were affixed to a uniform substrate plastic mesh platform. Treatment fragments were induced to bleach by exposing them to increased solar irradiance via transplantation from a low light (LP site at 10 m) to a high light site (BTN at 1 m) for 4, 8 or 14 days. Transplanted coral fragments were exposed to a 70% increase in average total irradiance over that observed at 10m (based on integrated light measurements made every 2 nm using a LiCor Li-1800UW underwater spectroradiometer between 300 - 700 nm). Integrated UVB (300 - 320 nm), UVA (320 - 400 nm) and photosynthetically active radiation (PAR 400 - 700 nm) levels were approximately 99%, 93% and 66% greater at 1 m than at 10 m respectively. Treatment fragments were transplanted to the BTN site to better replicate the low wave action conditions present at 10 m at the LP site (LP site is beside a dock with high boat traffic). These sites are separated by approximately 100 m, occur in the same small lagoon and have similar sedimentation and temperature regimes. Hourly temperature values were recorded both at the 1 m BTN and at the 10 m LP site using two HOBO brand miniature data loggers. The two sites differed by less than 1.0°C on average and neither temperature regime was high enough to induce bleaching (LP average temp. = 26.5 ± 2.0 °C and BTN average temp. = 27.0 ± 1.0°C)(Jokiel & Coles, 1977).

SAMPLING METHOD

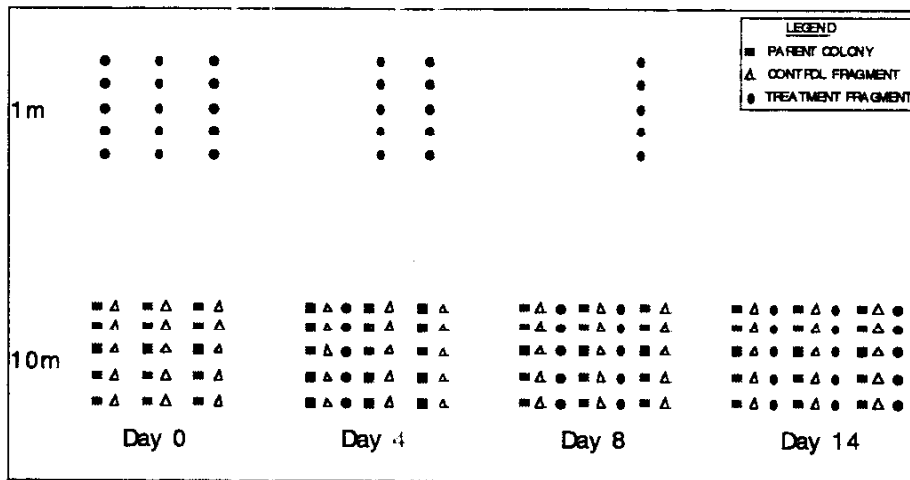


Figure 1. Sampling schedule. Initially (day 0), two fragments each were broken off of each of 15 parent colonies at 10 m (squares = parent colonies). One fragment was transplanted to 1 m (circle = treatment fragment) and the other fragment was placed directly beside the parent colony as a control for transplantation (triangles = control fragments). After 4 days, 5 randomly chosen treatment fragments were returned to 10 m. The same process was repeated on day 8 and day 14. At each time interval, both fragments and the parent colonies were sampled and chlorophyll a and total lipid levels were determined.

Two subsamples from each fragment and parent colony were taken by drilling with a 1.25 cm cork borer through the coral plate. One subsample was analyzed for chlorophyll a concentrations and the other for total lipid levels in order to determine initial levels of both of these parameters. Since lipid and chlorophyll a levels did not vary significantly within a coral plate (determined prior to conducting the experiment), a single sample from each fragment and parent colony adequately

represented the concentrations of these parameters ($F = 2.167$, $n = 6$, $p < 0.08$; $F = 1.596$, $n = 6$, $p < 0.194$ respectively).

After four days (July 16), all of the treatment fragments, control fragments and the parent colonies were again sampled and analyzed for total lipid and chlorophyll *a* levels. Five of the fifteen treatment fragments were randomly selected, returned to their original sites at 10 m and subsequently monitored for signs of recovery. This procedure was repeated again on days 8 and 14 (July 20 and July 26) of the experiment thus returning all treatment fragments to their original depth (Fig. 1).

Chlorophyll *a* and lipid analyses were performed as follows: Chlorophyll *a* was extracted from fresh, finely-ground samples according to the method described by Jeffrey and Humphrey (1975) and reported in $\mu\text{g}/\text{cm}^2$. Lipids were extracted from finely-ground samples (samples had been frozen at -50°C for 2 weeks prior to extraction) in a chloroform:methanol (2:1,v:v) solution. Extracts were then washed once with 0.88% potassium chloride solution, three-times with a methanol:water solution (1:1,v:v) and dried at 50°C for 24 hours before weighing. Animal tissue biomass was determined following lipid extraction by burning the skeleton and remaining tissue residue in a muffle furnace at 450°C for 6 hours. Lipid content in corals was reported as % lipid per gram dry tissue weight. This method differs slightly from Harland *et al.* (1992) where samples were decalcified prior to lipid extraction which can result in lipid loss during the decalcification process (triglycerides can hydrolyze in acid solutions and the glycerol component of the molecule is then soluble).

The data were analyzed by pairwise comparisons between parent colonies and control fragments as well as between control fragments and treatment fragments to determine if lipid and/or chlorophyll *a* levels had changed in treated fragments. The null hypothesis was that the difference between the above mentioned pairs was less than or equal to zero and was rejected at an alpha level of 0.05 by means of a paired ANOVA on each sampled date. Comparisons of the lipid and chlorophyll *a* levels in the treatment fragments that had recovered for 10, 6 and 0 days were examined by means of an ANOVA. The relationship between lipid and chlorophyll *a* levels was assessed by means of a correlation analysis.

RESULTS

Both the parent colonies and the control fragments were observed to have normal coloration throughout the experiment. Their chlorophyll *a* levels were not significantly different from each other at any time (Table 1)(Fig. 2). Treatment fragments were initially observed to have similar pigmentation as the controls but began to pale on the third day of exposure to high light (Table 1). They became progressively paler with increasing exposure time. Chlorophyll *a* levels were significantly lower in treatment fragments than in control fragments on days 4, 8 and 14 (Table 1)(Fig. 2).

During this interval, lipid levels in the parent colony, control fragment and treatment fragment samples did not differ (Table 1)(Fig. 2). Further indication that bleaching, as indicated by decreased chlorophyll *a*, was not accompanied by a decrease in lipid levels was revealed by a lack of a significant correlation between chlorophyll *a* and the percent lipid per gram dry weight ($F=1.734$, $n=171$, $p<0.190$) (Fig. 3).

Recovery in bleached fragments was assessed by directly comparing lipid and chlorophyll *a* levels in fragments that had bleached and recovered for 4 and 10 days, 8 and 6 days and 14 and 0 days, respectively. Lipid levels and chlorophyll *a* levels were not significantly different between treatment fragments that had recovered for 10, 6 and 0 days ($F=0.124$, $p<0.884$, $n=15$; $F=0.243$, $p<0.788$, $n=15$ respectively).

Table 1: Results of pairwise comparisons. CHL a = chlorophyll a, P-CT = pairwise comparison between parent colonies and control fragments, CT-T = pairwise comparison between control fragments and treatment fragments, n = number of samples.

	DAY 0		DAY 4		DAY 8		DAY 14	
	P<	n	P<	n	P<	n	P<	n
CHL a								
P-CT	0.78	14	0.28	15	0.91	10	0.44	4
CT-T	0.38	14	0.00	15	0.00	10	0.04	4
%LIPID								
P-CT	0.98	14	0.63	15	0.26	10	0.55	4
CT-T	0.53	14	0.33	15	0.30	10	0.19	4

DISCUSSION

The upper surface of all treatment fragments appeared paler in color after 3 days of bleaching and remained pale throughout the experiment. This overall paler appearance was reflected in lower chlorophyll *a* levels. The decrease in pigment levels did not recover to pre-bleach levels during the course of the experiment irrespective of the length of exposure to high light. Lipids did not decrease in bleached fragments of *M. verrucosa* over the course of two weeks. Rather, a general trend towards increased lipid levels in the parent colony, control fragment and the treatment fragment was observed. *M. verrucosa* has a natural lunar cycle to its lipid levels that corresponds to spawning (Stimson, 1987). Since the experiment was initiated immediately following the July spawning, the observed trend in increased lipids seems to be a reflection of this natural cycle of reproduction. The lack of a significant correlation between chlorophyll *a* and the percent lipid per gram dry weight is consistent with the observation that decreases in chlorophyll *a* were not accompanied by decreases in lipid levels (Fig. 3). Studies by Fitt *et al.* (1993) showed that lipids in 3 bleached *Montipora annularis* colonies were lower than in 3 unbleached colonies 6 months following bleaching. There are several possible reasons for the discrepancy between this study and that of Fitt *et al.* (1993). Lipids may be metabolized very slowly in bleached corals making decreases in lipid levels apparent only long after initial bleaching. However, shading experiments by Harriott (1993) indicated that lipid levels in the Hawaiian coral *Pocillopora damicornis* decreased in just one week. When corals were shaded, zooxanthellae were initially unable to maintain photosynthesis at the same level as when in full sunlight. Under these conditions, lipid stores were metabolized in order to fulfill the corals' daily energetic demands. This situation is analogous to bleaching in that bleached corals suffer from decreased photosynthetically-derived carbon as well. Based on this evidence, one might expect to observe a change in lipid levels in the first week of bleaching. But *M. verrucosa* is a much slower growing species than *P. damicornis* and predictions of a rapid response in *Montipora* based on the latter coral may be unrealistic. *Montipora verrucosa* in Hawai'i lives in a more extreme habitat than Caribbean corals. Therefore it may be naturally more capable of coping with stresses such as bleaching making it difficult to detect any lipid responses after a short bleaching period. *M. verrucosa* spawns every month during the summer. Eggs released during spawning can be up to 70% lipid by dry weight (Arai *et al.*, 1993). If stressed corals can resorb lipids from unreleased eggs, or can delay ripening eggs for spawning, then decreases in total lipid levels could possibly only be detectable over a longer period of time.

Corals may increase heterotrophy in order to supplement their nutritional demands. However, it is suspected that heterotrophy may only account for 10% of the corals diet in some species (Wellington, 1982). Lipid levels simply do not change when corals are bleached. In this study treated fragments were compared with genetically identical control fragments in a pairwise fashion. This is a very robust experimental method because it controls for genetic variation between corals. Paired comparisons such as these have not been used in any previous experiments which examined lipids in corals. I believe that my results are convincing evidence that lipids do not change within the first two weeks of bleaching. The hypothesis that lipid levels in

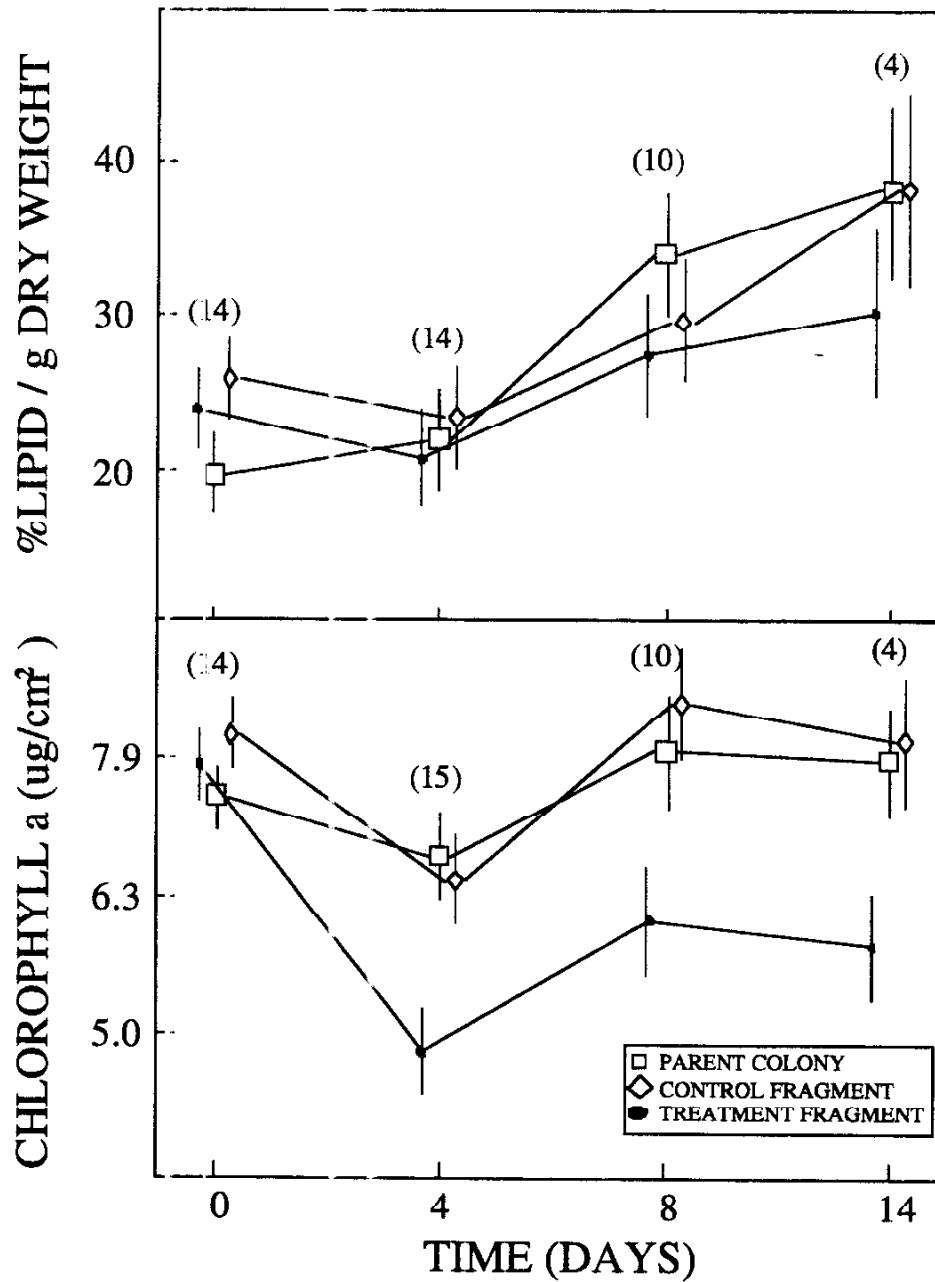


Figure 2. Average %lipid per gram dry weight (\pm one standard error) and average chlorophyll a ($\mu\text{g}/\text{cm}^2$) (\pm one standard error) on day 0, 4, 8 and 14. Open squares, open diamonds and solid circles represent parent colonies, control fragments and treatment fragments, respectively, and are offset slightly from one another so that error bars do not overlap. The number of parent colonies, control fragments and treatment fragments are indicated in parentheses.

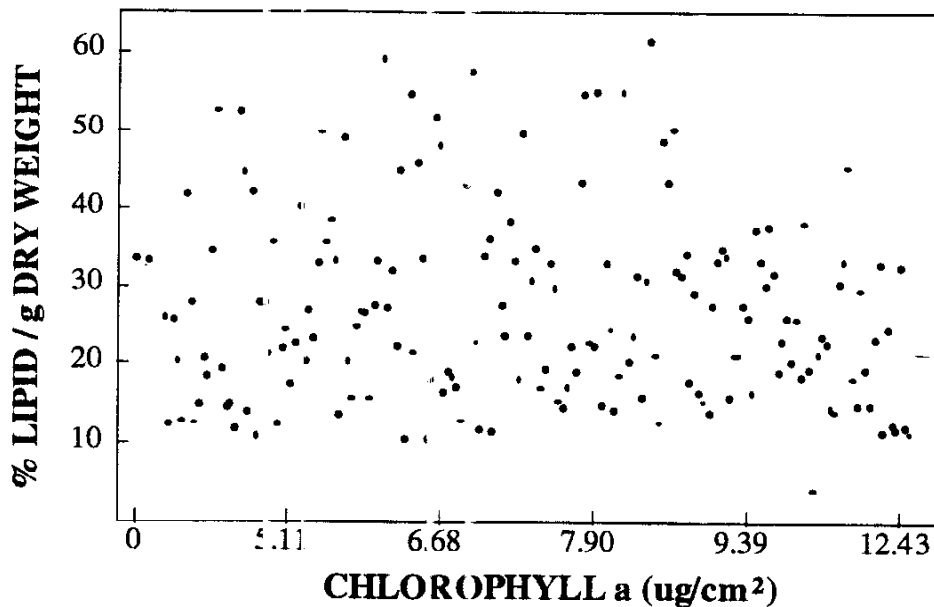


Fig. 3. Correlation between % lipid per gram dry weight and log chlorophyll a ($\mu\text{g}/\text{cm}^2$).

recently bleached corals would be lower, and that lipid content in corals would decrease as the length of the bleaching period increased, was rejected. No lipid response was detectable in bleached fragments over the course of the initial 2 weeks. Running the experiment for 1, 2 and 3 months would determine whether or not lipid levels change in bleached corals over a longer period of time. As well, monitoring gamete production and release over the same period in bleached and unbleached corals would reveal whether or not gametes are being resorbed or being prevented from developing during bleaching.

After natural bleaching events such as those observed in the Caribbean in 1983 and 1987, some coral species recovered more rapidly and more frequently than others. The ability to withstand and recover from prolonged bleaching events (i.e., several months) may yet be related to the amount of lipid stores, and may yield some insight into why some coral species recover more quickly from bleaching events than others. However, lipids do not appear to play a role in short-term coral bleaching and recovery.

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