

# Effect of bleaching on lipid content and composition of Okinawan corals

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**ABSTRACT:** Lipid contents and compositions of the bleached Okinawan corals were analyzed for the first time. Bleached corals collected at Sesoko Okinawa, Japan (26°38'N, 127°52'E), after the 1998 bleaching event showed decreased lipid content with a concomitant decline of wax in the lipid composition. The reduction in the lipid content of corals showed diversity depending on their colony morphology. On the whole, corals of massive morphology were relatively rich in lipid even after the bleaching event, and hence lesser reduction in the lipid content compared with the cases for those of branching morphology. Furthermore, there was a positive correlation between lipid content and zooxanthellae density in the bleached corals, suggesting again the importance of symbiont for the supply of lipid to the host cells, and hence for the survival of the bleaching event.

**KEY WORDS:** bleaching, lipid composition, lipid content, reef-building coral.

## INTRODUCTION

Reef-building corals are rich in lipids. Our previous work found that the zooxanthellate scleractinian corals found in the Okinawan sea (26°38'N, 127°52'E) contained large amounts of storage lipid, ranging from 14 to 37% of dry tissue weight.<sup>1</sup> These storage lipids are the result of translocation of photosynthate from symbiont zooxanthellae to host coral tissues.<sup>2,3</sup> There are good evidences that the lipid production is light-dependent and driven by photosynthesis of dinoflagellates harbored in coral tissue.<sup>4–7</sup> Thus, symbiosis plays a pivotal role in the energy budget of reef-building corals.

Bleaching in stony corals is the result of disruption of symbiosis between the coral hosts and photosynthetic microalgal endosymbionts (zooxanthellae). Given the fundamental role of the algal symbiosis in meeting the energy requirement, bleaching may significantly affect the lipid dynamics of stony corals. Coral bleaching events of unprecedented frequency and global extent have been reported during the last two decades.<sup>8–10</sup>

However, despite the physiological significance of bleaching in the coral biology, little is known about its effect on lipid content and profiles. Thus, the aim of this study is to describe the effect of bleaching on lipid content and compositions of reef-building scleractinian corals.

## MATERIALS AND METHODS

### Corals and lipid extraction

Samples of bleached and normal corals were collected from the reef flat in front of Sesoko Station, Tropical Biosphere Research Center, University of the Ryukyus (26°38'N, 127°52'E), Okinawa, Japan, in October 1998 (after the mass bleaching event) at a depth of 1–3 m. Because of extensive mortality, normal samples were not available for corals *Stylophora pistillata*, *Fungia fungites*, *Galaxea fascicularis* and *Porites cylindrica* at Sesoko. For this reason, the normal specimens collected in June 1996 were analyzed as references for these corals. Samples (about 10 cm<sup>2</sup> in size) decalcified as described previously<sup>1</sup> were washed thoroughly with tap water, and the lipids were extracted with the method of Folch.<sup>11</sup> In this study, no separation of symbiotic algae from the hosts was done. The solvent was flashed under a nitrogen stream at

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50°C. The crude lipid fraction was weighed, re-dissolved in chloroform-methanol (2:1 by vol.) to a concentration of 1 mg lipid/mL, and then stored at -20°C until use.

### Analysis of lipid composition

For analysis of total lipid composition, aliquots of lipid extract (50 µg) were applied to high performance thin layer chromatography (HPTLC) plates (10 cm × 10 cm, Merck, Darmstadt, Germany). The plates were first developed to top off the plates with hexane and successively benzene, and finally to half-length with hexane/ether/acetic acid (70:30:1, by vol.) in the same direction. After drying in a stream of air, the plates were immersed in phosphoric acid/33% acetic acid/sulfuric acid/0.5% copper sulfate (5:5:0.5:90, by vol.) for 40 s and heated at 130°C for 12 min.<sup>12</sup> The chromatograms were scanned by an image scanner (GT-9000, Epson, Tokyo, Japan) with a gray scale mode. The software used for scanning was Adobe Photoshop (Adobe Systems Inc., San Jose, USA). Percent compositions of the lipids were determined on the basis of band intensity by an image analysis program of the National Institute of Health.

### Density of zooxanthellae cells

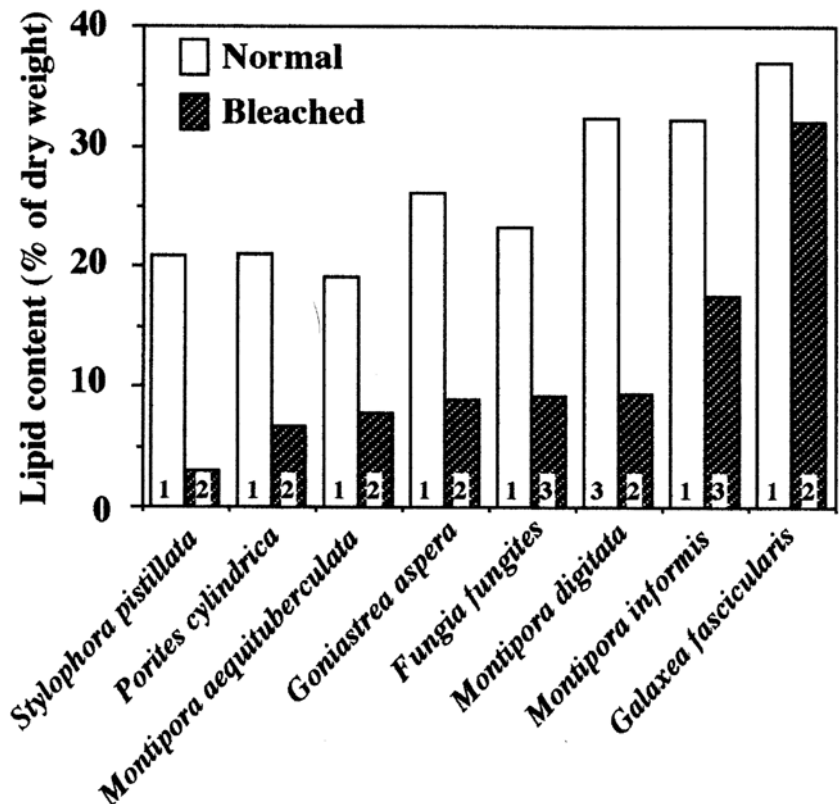
Coral tissue samples were fixed and preserved in 10% formaldehyde solution, then decalcified in 10% acetic acid solution containing 10% formaldehyde. A part of decalcified soft tissues were homogenized in a Teflon-piston grinder, and cell counts were made using a hemocytometer. For those severely bleached samples, a known volume (5 µL) of ground solution was examined using usual slide glass.

### Protein determination

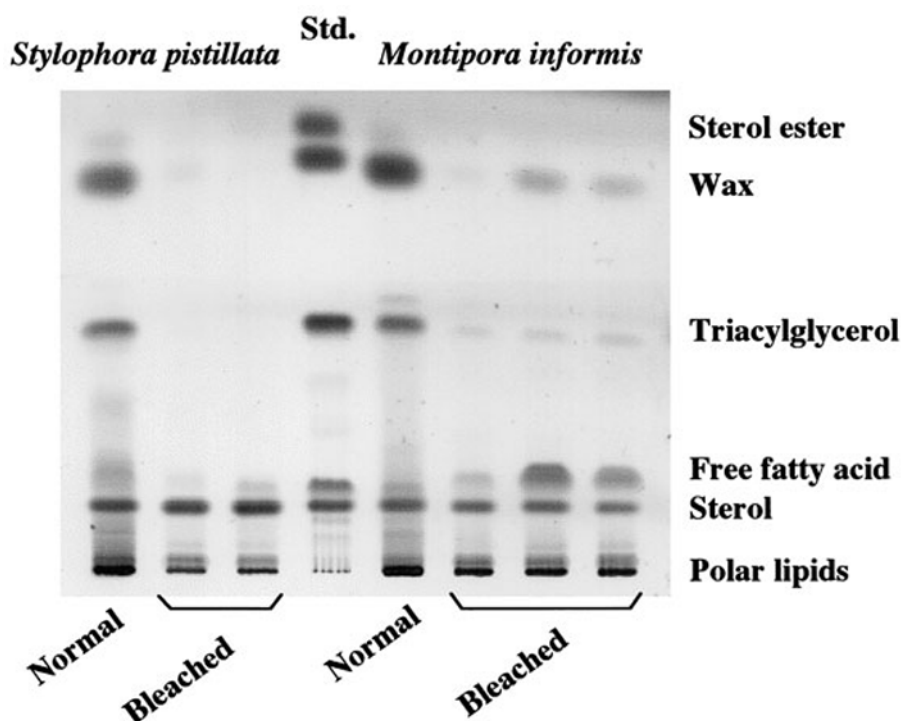
The biomass unit used for zooxanthellae density was tissue protein in this study. The coral homogenates were dissolved in boiling solution of 0.5 N NaOH (30 min, twice). Protein concentration was determined by the Lowry method using bovine serum albumin as standards.<sup>13</sup>

### RESULTS

Figure 1 shows the lipid content of bleached and non-bleached reef building corals collected at



**Fig. 1** Lipid content of normal (blank column) and bleached (hatch line column) corals. Values in the columns are the number of samples. Samples were analyzed in triplicate, and data are the mean values of two or three specimens except for single samples.



**Fig. 2** High performance thin layer chromatography chromatograms of lipid from normal and bleached corals. Chromatograms for *Stylophora pistillata* and *Montipora informis* are representatively shown. Standards used were cholesterol (sterols); oleic acid (free fatty acid); triolein (triacylglycerol); stearyl oleate (wax); and cholesteryl oleate (sterol ester). Polar lipid essentially denotes phospholipids.

Sesoko Island, Okinawa, Japan, before and after the 1998 bleaching event. As stated in the method section, normal samples for corals *Stylophora pistillata*, *Fungia fungites*, *Galaxea fascicularis* and *Porites cylindrica* were not available at Sesoko after the 1998 bleaching event. Thus, the samples collected in June 1996 at the same location served as normal references for these corals. The bleaching significantly decreased the lipid content of the reef building corals. Averaged lipid content was  $26.5 \pm 2.3\%$  for normal corals, and was  $9.0 \pm 1.5\%$  for bleached samples ( $P < 0.01$  by Student's *t*-test). The extent of decreases in the lipid contents appeared to vary with the colony morphology. As a whole, corals of massive or plate-like morphology showed lesser reduction in the lipid content compared with the branching corals.

Analysis of lipid composition by HPTLC plates revealed decreased intensities of wax and triacylglycerol bands in the bleached samples than in the normal corals (Fig. 2). The largest constituent in the lipid from normal corals was wax, and was decreased by bleaching in most cases (Fig. 3). A similar decreasing trend by bleaching was also noted with the storage lipid triacylglycerol. Thus, the bleaching decreased the storage lipid content, and consequently elevated the relative proportion of membrane constituent polar lipids and sterols (Fig. 3).

Our previous study demonstrated a strong correlation between the lipid content and the ratio of

wax to sterol proportion in the coral *Goniastrea aspera*.<sup>14</sup> Thus, the present study examined the correlation throughout 8 species of reef-building corals (Fig. 4). Again, a strong positive correlation was noted between the lipid content and the ratio of wax to sterol proportion. Thus, the correlation first established in *Goniastrea aspera* can now be expanded across the species of stony corals. This result also reinforced that the wax was the major determinant for the lipid content of reef building corals.

As the zooxanthellae plays a pivotal role in the energy budget of symbiotic corals, correlation between lipid content and the numbers of zooxanthellae in the host tissues was studied with bleached corals. A positive and statistically significant correlation was obtained between these two parameters (Fig. 5). However, a rather scattered data distribution was also notable.

## DISCUSSION

This study is the first to show the lipid composition of bleached Okinawan corals. Coral bleaching is caused by the disruption of the symbiotic system of zooxanthellate corals. Although a variety of stresses have been implicated in the induction of coral bleaching, the thermal stress above the coral's acclimatization capacity has been suggested as the primary causative agent of large-scale

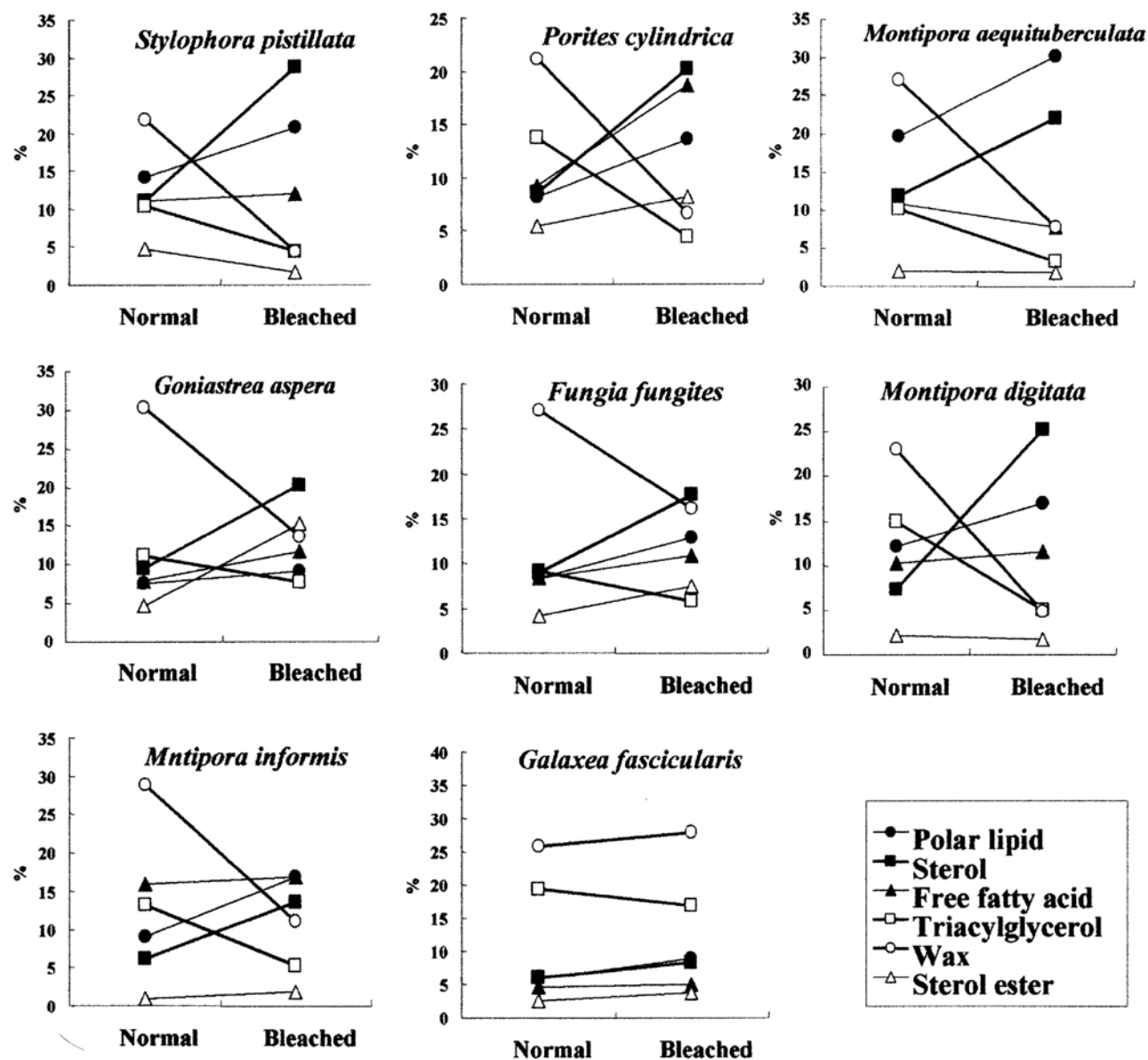


Fig. 3 Lipid composition of normal and bleached corals. Percent compositions of major known lipids are shown. Data are means of triplicate analyses.

coral bleaching.<sup>15–19</sup> The year 1998 experienced the strongest El Nino on record. Consequently, sea surface temperatures (SST) at Sesoko, Okinawa were higher throughout 1998 than in 10 preceding years, peaking at 2.8°C above average during August, and the average SST from July to September was 30.4°C.<sup>20</sup> These higher SST above the critical level caused extensive coral bleaching and mortality at Sesoko, as was the case in other parts of the world.

Zooxanthellate corals contain large amounts of lipids derived mostly from algal photosynthetic CO<sub>2</sub> fixation. Coral bleaching is evoked by the impairment of CO<sub>2</sub> fixation under high SST and

high light intensities.<sup>18</sup> Thus, it can consequently be expected that bleaching may result in decreased lipid content compared with the normal samples. However, a previous experiment failed to observe the decreased lipid level in light-induced bleached coral.<sup>21</sup> The corals irradiated with elevated light intensity for 14 days displayed only a decreased chlorophyll level but not lipid level. These researchers concluded that the irradiance period was insufficient to induce the changes in lipid content. In 1998 at Sesoko, Okinawa, high SST lasted about 3 months from July to September, and resulted in large-scale coral bleaching and mortal-



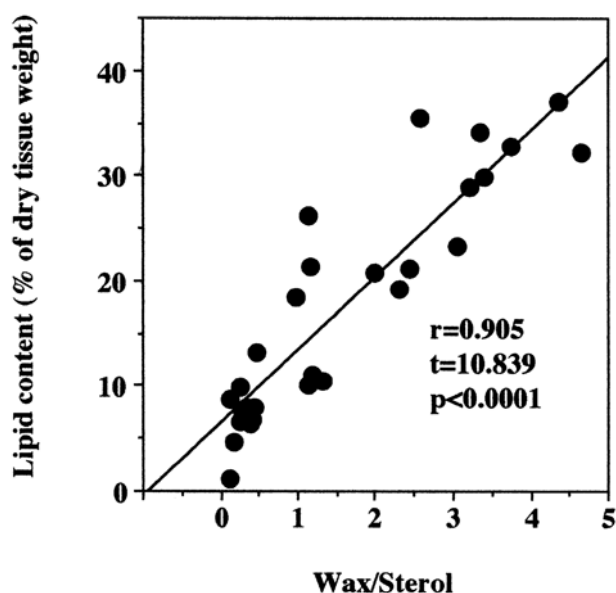


Fig. 4 Correlation between lipid content and the ratio of wax to sterol proportion.

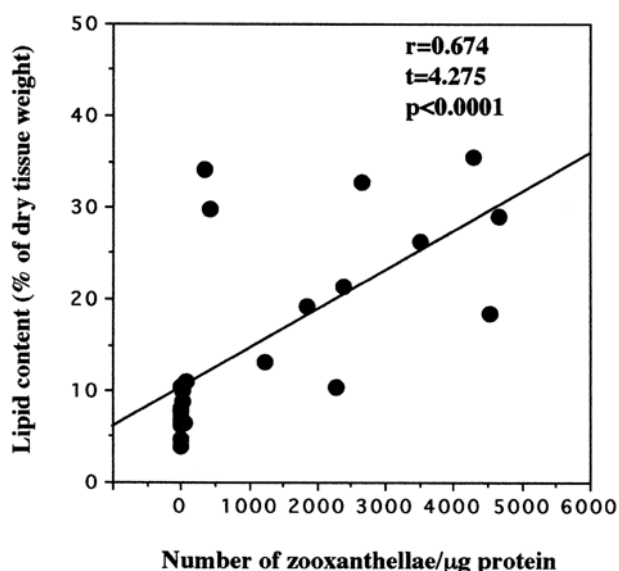


Fig. 5 Correlation between lipid content and zooxanthellae density.

ity.<sup>20</sup> Bleaching significantly lowered the lipid content in all stony corals studied (Fig. 1). This decline of lipid content in bleached coral was mainly explained by the reduced levels of wax and triacylglycerol (Figs 2,3). Our recent study showed that the biosynthesis of storage lipid wax was directly coupled with the photosynthesis.<sup>22</sup> Thus, the decreased lipid deposit in the bleached corals

should be reasonably ascribed to lowered algal lipid supply to the host tissues. This proposition may be supported by the positive correlation between lipid content and the zooxanthellae number in the host tissue (Fig. 5).

It is also noteworthy that the extent to which the lipid level decreased by bleaching varied with the colony morphology. Massive corals retained relatively high lipid levels than the branching corals even after the bleaching event, suggesting that the massive types had some mechanisms to protect the symbionts from heat and light stress which may allow photosynthesis to continue. Several lines of studies showed that the differential susceptibility of corals to bleaching was linked to colony-morphology.<sup>10,20,23,24</sup> Corals of massive morphs were rather resistant to bleaching than those of branching.<sup>19,20,24</sup> Thus, the susceptibility of stony coral to bleaching appeared to coincide with the extent in the reduction of the storage lipid level by bleaching. The tolerant species may be able to house the symbiotic algae even under elevated SST, resulting in continuous algal energy production (Fig. 5). Moreover, the genetic constitution of symbiotic algae in the massive corals may tolerate the higher SST compared with those housed in more susceptible species.<sup>25</sup> Therefore, the stresses imposed on corals may cause a change not only in the numbers but also the types of algae in the host cells.

The alternate explanation for relatively high lipid levels in massive corals may be the heterotrophic mode of nutrition. The massive corals may take up planktons to compensate the reduced algal lipid supply.

Our previous work demonstrated the correlation between the lipid content and the ratio of wax to sterol proportion in the coral *Goniastrea aspera*.<sup>14</sup> This correlation was further extended across the species of reef building corals. As shown in Fig. 4, a positive correlation between these two parameters was reproduced. The correlation may be useful for the studies employing a small sample size. It is often difficult to have sufficient amounts of samples in field studies. Measurement of lipid content by the gravimetric method usually requires a relatively large mg order of samples. However, only a  $\mu\text{g}$  order of specimen is needed for the estimation of the ratio of wax to sterol by HPTLC plates.

In conclusion, this study showed a decreased lipid content in the bleached coral for the first time. The decrease of lipid content was most reasonably explained by the reduction in wax content, probably due to decreased lipid supply from symbiont algae. Therefore, the lipid of storage fuel, as well as membrane component, may be a critical factor for the survival of a bleaching event.

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