Marine Biology (2004) 145: 621–631
DOI 10.1007/s00227-004-1337-3

RESEARCH ARTICLE

A. G. Grottoli · L. J. Rodrigues · C. Juarez

# Lipids and stable carbon isotopes in two species of Hawaiian corals, *Porites compressa* and *Montipora verrucosa*, following a bleaching event

Received: 7 March 2003 / Accepted: 13 February 2004 / Published online: 20 March 2004


**Abstract** Mass coral bleaching events have occurred on a global scale throughout the world's tropical oceans and can result in large-scale coral mortality and degradation of coral reef communities. Coral bleaching has often been attributed to periods of above normal seawater temperatures and/or calm conditions with high levels of ultraviolet radiation. Unusually high shallow-water temperature ( > 29°C) in Kaneohe Bay, Hawaii, USA, in late summer (20 August–9 September) and fall (1–7 October) of 1996 produced visible bleaching of two dominant corals, *Porites compressa* Dana, 1864 and *Montipora verrucosa* Dana, 1864. The present study examined chlorophyll *a* (chl *a*), total lipid concentrations, and lipid class composition in corals of both species in which the entire colony was non-bleached, moderately bleached, or bleached. Skeletal, host tissue, and algal symbiont $\delta^{13}$C values were also measured in non-bleached and bleached colonies. In additional unevenly bleached colonies, paired samples were collected from bleached upper surfaces and non-bleached sides. Samples were collected on 20 November 1996 during the coral recovery phase, a time when seawater temperatures had been back to normal for over a month. Chl *a* levels were significantly lower in bleached colonies of both species compared with non-bleached specimens, and in bleached areas of unevenly bleached single colonies. Total lipid concentrations were significantly lower in bleached *P. compressa* compared with non-bleached colonies, whereas total lipid concentrations were the same in bleached and non-bleached *M. verrucosa* colonies. The proportion of triacylglycerols and wax esters was lower in bleached colonies of both species. Both bleached and non-bleached *M. verrucosa* had from ~17% to 35% of their lipids in the form of diacylglycerol, while this class was absent in *P. compressa*. $\delta^{13}$C was not significantly different in the host tissue and algal symbiont fractions in non-bleached and bleached samples of either species. This suggests that the ratio of carbon acquired heterotrophically versus photosynthetically was the same regardless of condition. Skeletal $\delta^{13}$C was significantly lower in bleached than in non-bleached corals. This is consistent with previous findings that lower rates of photosynthesis during bleaching results in lower skeletal $\delta^{13}$C values. The two species in this study displayed different lipid class compositions and total lipid depletions following bleaching, suggesting that there is a difference in their metabolism of lipid reserves and/or in their temporal responses to bleaching and recovery.

Communicated by J.P. Grassle, New Brunswick

A. G. Grottoli (✉) · L. J. Rodrigues · C. Juarez
Department of Earth and Environmental Science,
University of Pennsylvania, 240 South 33rd Street,
Philadelphia, PA 19104-6316, USA
E-mail: grottoli@sas.upenn.edu
Tel.: +1-215-8989269
Fax: +1-215-8980964

## Introduction

Under sustained physiologically stressful conditions, corals lose their algal symbionts and/or their photosynthetic pigments, causing the colony to appear pale or white. This is referred to as bleaching. The primary environmental factors that cause mass bleaching are sustained elevated seawater temperatures (Glynn 1996; Brown 1997; Wilkinson 2000) and/or increased ultraviolet radiation (Gleason and Wellington 1993; Glynn 1996; Brown 1997; Wilkinson 2000). For any given event, bleaching severity and mortality vary among individual corals, coral species, depths, and geographic locations (e.g. Fisk and Done 1985; Harriott 1985; Oliver 1985; Ghiold and Smith 1990; Edmunds 1994; Hoegh-Guldberg and Salvat 1995; Baird and Marshall 1998; Marshall and Baird 2000; Wilkinson 2000; Loya et al. 2001; Obura 2001; Stimson et al. 2002). However, the physiological mechanisms underlying this bleaching

variability are poorly understood. Research has concentrated on the variation in the algal symbiotic dinoflagellate *Symbiodinium* type (e.g. Rowan et al. 1997; Baker 2001; LaJeunesse 2001; Toller et al. 2001), density (Stimson et al. 2002), and physiology (e.g. Warner et al. 1996; Lesser 1997), to explore this variation in bleaching. However, less research has been oriented towards examining the effect of bleaching on the animal-host fraction. This paper examines the changes in total lipids, lipid classes, and stable carbon isotopic composition of the coral skeleton, host tissue, and algal symbionts in two species of Hawaiian corals 44 days after a bleaching event.

On an annual cycle, corals experience a natural variation in tissue biomass, lipid, and photosynthetic pigment concentrations. Tissue biomass in some Caribbean corals has been observed to be highest during winter and spring and lowest during the late summer and fall (Fitt et al. 2000), while the opposite pattern has been observed in the total lipids in several Hawaiian coral species (Stimson 1987) and in the total lipids and wax esters in Japanese *Goniastrea aspera* (Oku et al. 2003). For four Indo-Pacific coral species, tissue thickness is greatest during the cooler wet season and thinnest during the warmer dry season (Brown et al. 1999). In several Caribbean coral species (Fitt et al. 2000; Warner et al. 2002), the Hawaiian coral *Pocillopora damicornis* (Stimson 1997), *Acropora formosa* in Mauritius (Fagoonee et al. 1999), and four Indo-Pacific corals (Brown et al. 1999), the algal symbiont and chlorophyll *a* (chl *a*) concentrations tend to be highest during the cooler season (winter/spring or wet) and lowest during the warmest season (summer/fall or dry), when solar irradiance levels are also at their highest. In non-mass bleaching years, such natural variation in pigmentation typically is not apparent (Stimson 1997; Fagoonee et al. 1999; Fitt et al. 2000; Warner et al. 2002). However, if stressful conditions such as sustained elevated seawater temperatures occur, large decreases in pigmentation result in visible bleaching (Brown et al. 1999; Fitt et al. 2000; Warner et al. 2002).

In healthy corals, the bulk of photosynthetically fixed carbon is translocated from the endosymbiotic algae (*Symbiodinium* spp.) to the coral host, providing it with up to 100% of its daily metabolic energy requirements (e.g. Muscatine and Cernichiari 1969; Muscatine et al. 1981, 1984; Patton and Burris 1983; Falkowski et al. 1984, 1993; Spencer Davies 1984; Harland et al. 1991). Excess fixed carbon is stored in the host tissue as lipids (Patton et al. 1977; Patton and Burris 1983; Battey and Patton 1984), representing significant energy reserves (Edmunds and Spencer Davies 1986; Stimson 1987; Harland et al. 1993). Up to 90% of total lipids is in the host tissue (Patton et al. 1977), and total lipid concentrations of 10–40% of dry biomass have been reported for a number of Caribbean, Red Sea, Japanese, and Hawaiian corals (Stimson 1987; Porter et al. 1989; Harland et al. 1993; Grottoli-Everett 1995; Yamashiro et al. 1999).

In bleached corals, decreases in algal symbiont densities and/or chl *a* levels are accompanied by a net decrease in photosynthesis (Porter et al. 1989; Fitt and Warner 1995; Lesser 1997; Lombardi et al. 2000; Warner et al. 2002). Under these circumstances, corals may rely heavily on their energy stores to support their metabolic energy needs. Proteins can serve as an energy source under some circumstances, while carbohydrates represent a small portion of a coral's energy reserves (Porter et al. 1989; Anthony et al. 2002), which are generally best used short term. However, the primary role of lipids is to serve as long-term energy reserves. When bleaching stress and subsequent recovery are prolonged, corals may have to rely on energy reserves for several weeks or months while chl *a* levels and photosynthesis rates remain low. Representing up to 40% of the dry weight of corals, lipids should be the primary energy reserve. It is estimated that *Pocillopora damicornis*, *Montipora verrucosa*, and *Porites lobata* maintained under cloudy conditions contain enough lipid to sustain their normal caloric demand for 28, 114, and 71 days, respectively (Spencer Davies 1991). Several studies on bleached Caribbean corals showed that without the usual nutritional input from their algal symbionts, energy reserves and/or tissue biomass decreased (Porter et al. 1989; Fitt et al. 1993, 2000). However, this pattern is not observed in all coral species when bleached (Grottoli-Everett 1995; Fitt et al. 2000; Edmunds et al. 2003) (see "Discussion" for details). [Note that the Hawaiian *Montipora verrucosa* has more recently also been referred to as *Montipora capitata*. The species is referred to as *M. verrucosa* throughout this text for clarity.]

Of the total lipids, triacylglycerol and wax esters are the main storage lipids in corals, and can account for 40–73% of total lipids (Harland et al. 1993; Yamashiro et al. 1999; Oku et al. 2002). Examination of the changes in total lipids and in lipid class composition should provide insight into how corals are consuming their lipid reserves.

Changes in photosynthesis and lipid content in bleached corals should impact the stable carbon isotopic composition ($\delta^{13}C = {}^{13}C{:}^{12}C$ relative to Vienna Peedee Belemnite Limestone Standard) of the coral skeleton, host tissue, and algal symbionts. Coral skeletal $\delta^{13}C$ is primarily influenced by metabolic fractionation, due to changes in symbiont photosynthesis and host respiration (Swart 1983; McConnaughey 1989; McConnaughey et al. 1997; Grottoli 1999, 2002; Grottoli and Wellington 1999). As solar irradiance decreases, the rate of photosynthesis decreases and coral skeletal $\delta^{13}C$ values decrease (Cole and Fairbanks 1990; Klein et al. 1992; Carriquiry et al. 1994; Grottoli and Wellington 1999; Heikoop et al. 2000; Reynaud-Vaganay et al. 2001; Grottoli 2002). Heterotrophy also influences skeletal $\delta^{13}C$ through coral host respiration (Felis et al. 1998; Grottoli and Wellington 1999; Grottoli 2002; Reynaud et al. 2002), but the impact of heterotrophy on the skeletal $\delta^{13}C$ is usually small relative to the effect of light (Grottoli and Wellington 1999; Grottoli 2002). During

coral bleaching, decreases in photosynthesis appear to result in decreases in skeletal $\delta^{13}C$ (Porter et al. 1989; Carriquiry et al. 1994; Allison et al. 1996; Suzuki et al. 2000, 2004). In addition, coral tissue and algal symbiont $\delta^{13}C$ values are usually within 2‰ of each other (Muscatine et al. 1989; Risk et al. 1994; Reynaud et al. 2002) and very depleted relative to the skeleton (Reynaud et al. 2002). The difference in $\delta^{13}C$ values between the host tissue and algal symbiont fractions is diagnostic of the relative contribution of heterotrophically acquired fixed carbon and photosynthetically fixed carbon to the coral (Muscatine et al. 1989; Risk et al. 1994; Reynaud et al. 2002). For example, in Caribbean corals, host tissue $\delta^{13}C$ tends to decrease more with depth than does algal symbiont $\delta^{13}C$ (Muscatine et al. 1989), suggesting that the ratio of heterotrophy to photosynthesis increases with depth. Changes in host tissue and algal symbiont $\delta^{13}C$ values during bleaching or recovery could be diagnostic of the relative contribution of photosynthesis and heterotrophy to the coral during that time. For example, an increase in the difference between host tissue and algal symbiont $\delta^{13}C$ values during bleaching or recovery would indicate that the ratio of heterotrophy to photosynthesis has also increased, and vice versa. No change in the difference between coral host and algal symbiont $\delta^{13}C$ values during bleaching or recovery would indicate that the ratio of heterotrophic to photosynthetic sources of fixed carbon was unchanged during that time.

To address the relationship between lipids and bleaching in Hawaiian corals, we measured chl *a*, total lipid concentration, and lipid class composition in non-bleached, moderately bleached, and completely bleached *Porites compressa* and *Montipora verrucosa* as well as partially bleached colonies of both species. Samples were collected 3 months after the onset of a warm seawater temperature event (44 days after seawater temperatures had returned to normal) that caused bleaching in many coral colonies of both species. To detect changes in the sources and utilization of carbon due to coral bleaching, the $\delta^{13}C$ of the skeleton, host tissue, and algal symbiont fractions were measured in non-bleached and bleached corals. This snapshot of two species of Hawaiian corals exposed to the same environmental conditions allows for the comparison of lipids, chl *a*, and $\delta^{13}C$ in bleached and non-bleached corals of the same species and for a preliminary assessment of possible species-specific differences in their physiological and biogeochemical responses to bleaching.

## Materials and methods

### Study site

Kaneohe Bay is on the windward side of Oahu, Hawaii. It is a eutrophic bay, 12.7 km long×4.3 km wide, with diurnal tidal fluctuations of 0.5–2 m (Bathen 1968). Mean summer/fall temperatures (June–October) average 27 ± 1°C and winter/spring temperatures (November–May) average 24.5 ± 1.5°C (data from Hawaii Institute of Marine Biology weather station). Corals in this study were collected at 2 m depth from the Point Reef (Coconut Island), Kaneohe Bay, Hawaii (21°26.18′N; 157°47.56′W). Coral cover on the reef slope approaches 100%, extending from the surface to 8.5 m depth and consisting primarily of *Montipora verrucosa* Dana, 1864 and *Porites compressa* Dana, 1864, with some *Pocillopora damicornis*, and a few solitary *Fungia scutaria* corals at shallower depths. Both *P. compressa* and *M. verrucosa* contain *Symbiodinium* spp. algal symbionts from the C clade (LaJeunesse, personal communication). *P. compressa* contains type C15 and *M. verrucosa* contains C31 at 2 m depth (LaJeunesse, personal communication). *P. compressa* is a finger-like coral, ranging in color from yellow-brown to dark brown. *M. verrucosa* is a dark to medium brown coral, with beige to white tips. Its form ranges from plating (predominantly on deeper reefs) to branching (predominantly on shallower reefs), and more than one form can be expressed within a single colony. All fragments of *M. verrucosa* used in this study were collected from the branching form.

### Bleaching event and coral sampling

On average, seawater temperatures in Kaneohe Bay are lowest from November through March, and warmest from June through September, with sea surface temperature (SST) excursions above 28°C for a couple of weeks in late summer. The SSTs for 1995 represent a typical year (Fig. 1). However, summer and early fall SSTs for 1996 were above average. From May to October seawater temperature was >28°C for 80 days. During 27 of those days (20 August–9 September and 1–7 October) SSTs were 2–3°C above normal (>29°C) (Fig. 1). At the same time, winds were low and the water column was clearer than usual (Jokiel and Brown, submitted). *M. verrucosa* was visibly bleached by 31 August 1996, and *P. compressa*, by 5 October 1996. This was the first temperature-induced bleaching event to be observed in Kaneohe Bay (Jokiel, personal communication). By March 1997, there was no sign of bleaching on the reef, all of the corals were brown, and there was no significant mortality (Grottoli, personal observation).

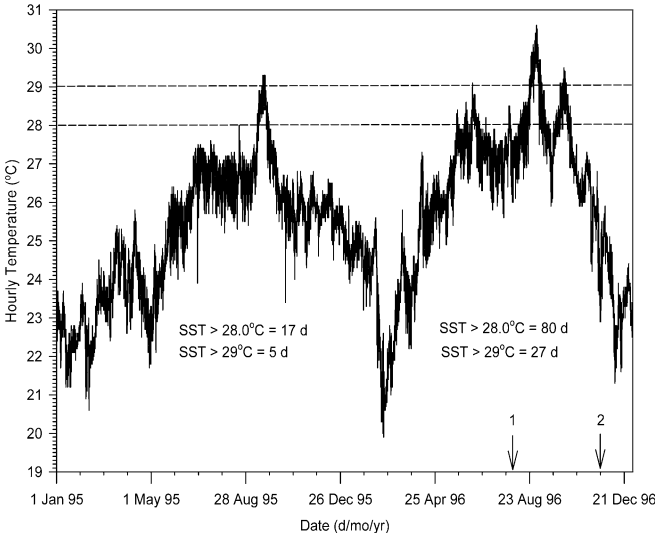


**Fig. 1** Hourly seawater temperature at 2 m depth on Point Reef, Hawaii Institute of Marine Biology (HIMB), Kaneohe Bay, Hawaii, during a normal year (1995) and a year with unusually warm sea surface temperatures (*SST*; 1996). *Dashed lines* indicate 28°C and 29°C (*arrow 1* onset of major warming event; *arrow 2* all coral fragments in this study were collected at 2 m depth on Point Reef). Data from HIMB weather station

On 20 November 1996, 3 months after the onset of elevated seawater temperatures, some corals were uniformly dark brown (non-bleached), some were medium to light brown (moderately bleached), while others were very pale or white (bleached) over the entire colony surface (top and sides). This was 45 and 80 days since *P. compressa* and *M. verrucosa* were first observed to bleach, respectively, and 44 days since seawater temperatures had returned to normal for that time of year. The non-bleached colonies may have already recovered their chl *a* or never bleached initially, while the completely bleached colonies may or may not have started to recover their chl *a*. All of the corals, including the completely bleached colonies, displayed normal tentacle extension and were alive. At least four branch tips from 32 *P. compressa* colonies and at least four branch tips from 32 *M. verrucosa* colonies were collected at 2 m depth and frozen. Based on color, approximately one-third of the colonies of each species were uniformly non-bleached, one-third were moderately bleached, and one-third were bleached. Coral color is correlated with chl *a* concentration (Edmunds et al. 2003) and is commonly used to identify non-bleached and bleached corals (e.g. Jokiel and Coles 1974; Lombardi et al. 2000; Edmunds et al. 2003). Some coral colonies were not uniformly affected, but were bleached white on the top, while their sides were a healthy, non-bleached dark brown. Pairs of samples were collected from 16 such *P. compressa* and 4 *M. verrucosa* colonies on the same date: one sample from the bleached top and one from the non-bleached side of each colony.

### Chl *a*, total lipids, lipid classes, and $\delta^{13}C$ analyses

Chl *a* and total lipid concentrations, lipid class composition, and $\delta^{13}C$ (of the skeleton, host tissue, and algal symbionts) were measured on four separate branch tips (~1 cm long) from each coral colony. Chl *a* was extracted from ground samples in 100% acetone (Jeffrey and Humphrey 1975) and was used as a measure of bleaching, irrespective of algal symbiont density. Endolithic algae were not visible in the samples and assumed not to contribute any significant amount of chl *a* to the samples. Total lipids were extracted from ground samples in a 2:1 chloroform/methanol solution, washed in 0.88% KCl followed by 1:1 methanol/water solution. The extract was dried to a constant weight (Harland et al. 1991; Grottoli-Everett and Kuffner 1995). Chl *a* and total lipids were measured on ground coral samples (skeleton + coral host tissue + algal symbionts) and normalized to total ash-free dry weight of the organic fraction of the coral (host tissue + algal symbionts). This normalization is important, because the polyp structure and the penetration thickness of the coral tissue into the skeleton of each species are different, and normalization to tissue biomass is more robust (Edmunds and Gates 2002). Grinding and extracting whole coral samples allowed for the lipid and chl *a* content to be compared between species and between colonies with different degrees of bleaching.

Total lipids were separated into lipid classes by applying the lipid extract to Chroma Rods, and separating them into phospholipids, diacylglycerol, cholesterol, free fatty acids, triacylglycerol, and wax esters (or hydrocarbons) by thin layer chromatography in 90:10:1 (v/v/v) hexane/ether/acetic acid (Wakeham et al. 1993; Yamashiro et al. 1999; Oku et al. 2002). The amount of each lipid class relative to its internal standard was determined by flame ionization detection with an Iatroscan Mark III analyzer by R. Harvey.

$\delta^{13}C$ of the skeleton, host tissue, and algal symbiont was measured on completely non-bleached and bleached colonies of *M. verrucosa* and non-bleached colonies of *P. compressa*. Due to sample loss, bleached *P. compressa* host tissue and algal symbiont $\delta^{13}C$ was measured from the bleached tops of unevenly bleached colonies. For $\delta^{13}C$ analyses, the host tissue and algal symbionts were removed with a Water-pik (Johannes and Wiebe 1970) and separated by centrifugation. The host tissue fraction was isolated onto pre-burned glass fiber filters under vacuum and rinsed with deionized water. The algal symbiont fraction was acidified to remove any skeletal fragments then isolated onto a glass fiber filter in the same way as the host tissue. Filters containing the organic fraction were combusted in a Carlo Erba NA 1500 Elemental Analyzer via a Finnigan ConFlow open split interface. The $\delta^{13}C$ values of the resulting $CO_2$ were measured in a Delta Plus mass spectrometer at Stanford University. The top 100–200 μm of the skeletal material was gently scraped from the very tip of the coral branch with a diamond-tipped Dremmel tool, yielding ~300 μg of skeletal sample, ground to a fine powder to homogenize it, and then 80 μg was subsampled for stable isotope analysis. The subsample was acidified with 100% ortho-phosphoric acid in an automated Kiel carbonate device, and the $\delta^{13}C$ values of the resulting $CO_2$ were measured in a Finnigan MAT 252 triple-collecting mass spectrometer at the University of Pennsylvania. All $\delta^{13}C$ values were reported as the per mil deviation relative to the Vienna Peedee Belemnite Limestone Standard (v-PDB). Host tissue and algal symbiont $\delta^{13}C$ values have a measurement error of $\pm 0.15‰$ or less, and skeletal $\delta^{13}C$ values have a measurement error of $\pm 0.05‰$ or less. At least 20% of all measurements were made in duplicate.

### Statistical analyses

A one-way non-parametric Kruskal–Wallis test was used to test for significant differences in mean chl *a* and total lipid concentrations between uniformly non-bleached, moderately bleached, and completely bleached *P. compressa* and *M. verrucosa*. If the model was significant, an a posteriori non-parametric multiple comparisons test (Zar 1984) was used to determine which means significantly differed from each other. The non-parametric tests were used because chl *a* and total lipid data were not normally distributed. A completely different set of corals was used to determine if there were significant differences in the total lipid and chl *a* concentrations in the tops versus sides of unevenly bleached corals. These data were normally distributed as determined by Shapiro–Wilk tests. Pairwise Student's *t*-tests were used to test for significant differences in mean chl *a* and total lipid concentrations between bleached tops and non-bleached sides of each species. A one-way ANOVA was performed on the mean skeletal, host tissue, and algal symbiont $\delta^{13}C$ values in non-bleached and bleached corals of both species. Normality for the $\delta^{13}C$ data groups was confirmed with Shapiro–Wilk tests. SAS statistical software was used for all statistical analyses, and $P < 0.05$ was considered significant. Since all of the coral fragments were collected on the same date, seasonal changes in chl *a*, algal symbiont concentration, tissue biomass, and total lipids (Stimson 1987; Fitt et al. 2000) do not confound the results of this study within each species. Differences in chl *a*, total lipids, lipid classes, and $\delta^{13}C$ between non-bleached, moderately bleached, and bleached colonies within *P. compressa* and within *M. verrucosa* are probably due to bleaching alone. However, the natural seasonal variation in the measured variables may differ between these two species in Hawaii and must be taken into consideration when comparing them.

## Results

Uniformly bleached coral colonies had significantly lower chl *a* concentrations than non-bleached corals for both *Porites compressa* (Table 1; Fig. 2A) and *Montipora verrucosa* (Table 1; Fig. 2B). Chl *a* levels were 85% and 80% lower in uniformly bleached *P. compressa* and *M. verrucosa* compared to non-bleached colonies, respectively. In moderately bleached colonies, chl *a* levels were 38% and 35% lower than in non-bleached *P. compressa* and *M. verrucosa*, respectively. In unevenly bleached colonies, chl *a* concentrations were significantly lower on the bleached top than on the non-bleached side of colonies in both species (Table 2; Fig. 3).

**Table 1** *Porites compressa*, *Montipora verrucosa*. Statistical evaluation of mean chlorophyll *a* and total lipid concentrations in non-bleached, moderately bleached, and completely bleached corals. Results of one-way non-parametric Kruskal–Wallis test (since data were not normally distributed) using SAS statistical software (*df* degrees of freedom)

| | *P. compressa* | | | | *M. verrucosa* | | | |
|---|---|---|---|---|---|---|---|---|
| | $X^2$ | *df* | *n* | *P* | $X^2$ | *df* | *n* | *P* |
| Chl *a* | 27.56 | 2 | 32 | < 0.0001 | 27.20 | 2 | 32 | < 0.0001 |
| Total lipid | 14.14 | 2 | 32 | < 0.0009 | 0.90 | 2 | 32 | < 0.640 |

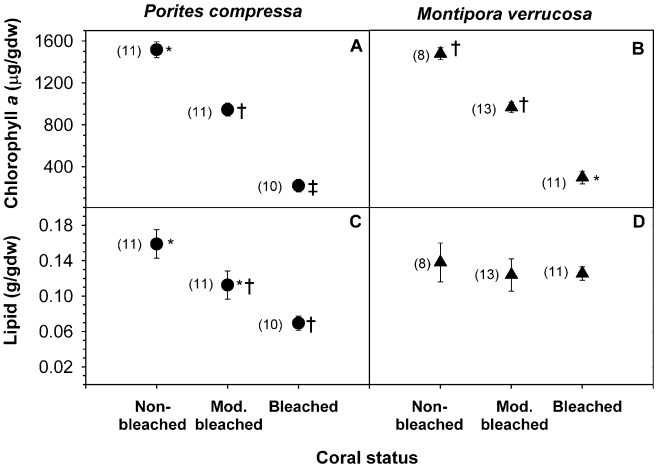


**Fig. 2A–D** *Porites compressa*, *Montipora verrucosa*. Mean concentration of chlorophyll *a* and total lipid (± 1 SE) in non-bleached, moderately bleached, and bleached coral colonies. Significant decreases in chlorophyll *a* in: bleached *P. compressa* (**A**) and *M. verrucosa* (**B**) corals parallel significant decreases in total lipids in *P. compressa* (**C**); there was no significant difference in total lipids in *M. verrucosa* (**D**). Symbols (*, †, ‡) indicate significant differences between means by a posteriori non-parametric multiple comparisons according to Zar (1984). Sample size for each mean in *parentheses* [*gdw* grams dry weight of whole coral tissue (host tissue + algal symbionts + lipid); *mod.* moderately]. Statistical analyses in Table 1

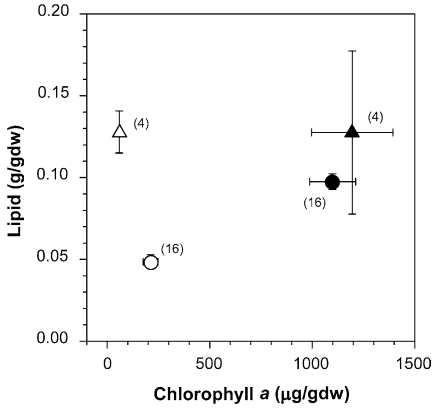


**Fig. 3** *Porites compressa*, *Montipora verrucosa*. Mean concentration of chlorophyll *a* and total lipids (± 1 SE) in unevenly bleached corals. *P. compressa* (*circles*), *M. verrucosa* (*triangles*); bleached tops (*open symbols*), non-bleached sides (*solid symbols*). Sample size for each mean is in *parentheses*. Statistical analyses in Table 2

**Table 2** *Porites compressa*, *Montipora verrucosa*. Statistical paired comparison of the chlorophyll *a* and total lipid concentrations in the bleached tops and non-bleached sides of unevenly bleached corals. Data were normally distributed according to a Shapiro–Wilk test for normality. *P. compressa*: chl *a* $W = 0.93$, $P < W$ 0.05; total lipids $W = 0.96$, $P < W$ 0.20; *M. verrucosa*: chl *a* $W = 0.90$, $P < W$ 0.31; total lipids $W = 0.89$, $P < W$ 0.22. The Wilk's statistic (*W*) ranges from 0 (non-normal distribution) to 1 (normal distribution) and a $P < W$ of ≥0.05 indicates data are normally distributed (*df* degrees of freedom)

| | *P. compressa* | | | *M. verrucosa* | | |
|---|---|---|---|---|---|---|
| | *t* | *df* | *P* | *t* | *df* | *P* |
| Chl *a* | 7.34 | 15 | < 0.0001 | 5.72 | 3 | < 0.01 |
| Total lipid | 6.39 | 15 | < 0.0001 | 0.01 | 3 | < 0.99 |

Mean total lipid concentrations were 56% lower in uniformly bleached than in non-bleached *P. compressa* colonies, while moderately bleached colonies had lipid concentrations that were 31% lower than those of non-bleached corals (Table 1; Fig. 2C). In unevenly bleached *P. compressa* colonies, total lipid concentrations were 50% lower on the bleached tops compared to on non-bleached sides (Table 2; Fig. 3). In *M. verrucosa*, mean lipid concentrations did not significantly differ between bleached, moderately bleached, and non-bleached colonies (Table 1; Fig. 2D), nor between the bleached tops and non-bleached sides of unevenly bleached colonies (Table 2; Fig. 3). Interestingly, total lipid concentrations in all corals in this study were lower than during non-bleaching years for both species. Non-bleached *P. compressa* and *M. verrucosa* had total lipid concentrations that were on average ~16% and ~24% lower, respectively, than summer/fall lipid levels during previous non-bleaching years, despite their appearance and high chl *a* content (Stimson 1987; Grottoli-Everett 1995; Grottoli-Everett and Kuffner 1995).

Within the total lipids, differences in lipid class composition between non-bleached and bleached corals were detected. For both species, visibly bleached corals had lower levels of the storage lipids triacylglycerol and wax esters, higher levels of phospholipids, and no detectable difference in free fatty acids and cholesterol compared to non-bleached corals (Table 3). However, most noticeable was the distinct lack of diacylglycerol in *P. compressa* regardless of bleaching status, whereas the percentage of diacylglycerol was higher in bleached than non-bleached *M. verrucosa*.

**Table 3** *Porites compressa*, *Montipora verrucosa*. Percent composition of lipid classes in the categories bleached and non-bleached corals. Two colonies (e.g. PC22 and PC44) of each category were analyzed (measurements made by R. Harvey, Chesapeake Biological Lab, UMCES) (*TG* triacylglycerol; *WE* wax esters; *HC* hydrocarbons; *DAG* diacylglycerol; *CS* cholesterol; *FFA* free fatty acids; *PL* phospholipids; *ND* below detection limit)

| Lipid class | *P. compressa* | | | | *M. verrucosa* | | | |
|---|---|---|---|---|---|---|---|---|
| | Non-bleached | | Bleached | | Non-bleached | | Bleached | |
| | PC22 | PC44 | PC07 | PC30 | MC68 | MC79 | MC74 | MC80 |
| TG | 21.92 | 23.76 | 10.95 | 9.07 | 14.75 | 8.93 | ND | ND |
| WE (or HC) | 11.63 | 21.94 | ND | ND | 8.99 | 4.48 | ND | ND |
| PL | 39.58 | 27.55 | 54.36 | 52.94 | 38.81 | 45.30 | 56.52 | 61.46 |
| FFA | 19.53 | 21.88 | 23.39 | 25.50 | 16.72 | 8.53 | 4.86 | 5.05 |
| CS | 7.33 | 4.87 | 11.03 | 9.07 | 3.32 | 2.25 | 3.91 | 4.01 |
| DAG | ND | ND | ND | ND | 17.41 | 30.52 | 34.72 | 29.48 |

**Table 4** *Porites compressa*, *Montipora verrucosa*. Results of one-way model III ANOVA of skeletal, host tissue, and algal symbiont $\delta^{13}$C levels in non-bleached and bleached corals. Data were normally distributed according to a Shapiro–Wilk test for normality. *P. compressa*: skeleton $W = 0.96$, $P < W$ 0.70; host tissue $W = 0.93$, $P < W$ 0.35; algal symbionts $W = 0.99$, $P < W$ 1.00; *M. verrucosa*: skeleton $W = 0.86$, $P < W$ 0.13; host tissue $W = 0.92$, $P < W$ 0.40; algal symbionts $W = 0.87$; $P < W$ 0.16. The Wilk's statistic ($W$) ranges from 0 (non-normal distribution) to 1 (normal distribution) and a $P < W$ of ≥0.05 indicates data are normally distributed (*df* degrees of freedom)

| | *P. compressa* | | | *M. verrucosa* | | |
|---|---|---|---|---|---|---|
| | *F* | *df* | *P* | *F* | *df* | *P* |
| $\delta^{13}$ Skeleton | 5.29 | 14 | < 0.039 | 4.46 | 7 | < 0.079 |
| $\delta^{13}$ Host tissue | 1.88 | 11 | < 0.202 | 0.09 | 7 | < 0.770 |
| $\delta^{13}$ Algal symbionts | 1.81 | 10 | < 0.211 | 0.49 | 7 | < 0.509 |

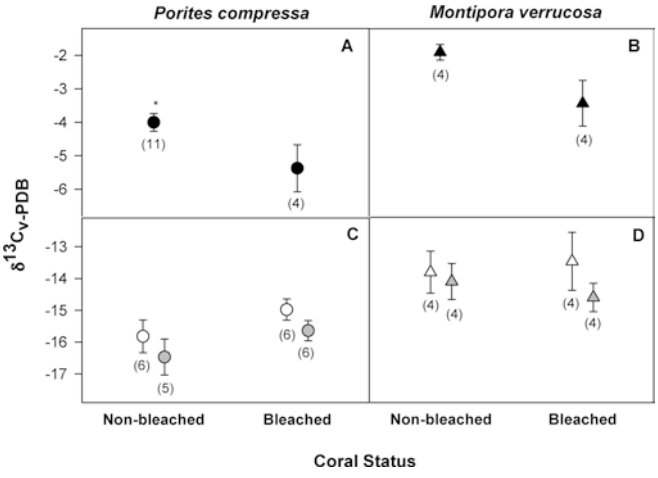


**Fig. 4A–D** *Porites compressa*, *Montipora verrucosa*. Mean skeletal $\delta^{13}$C (±1 SE) in non-bleached and bleached: **A** *P. compressa* (*circles*) and **B** *M. verrucosa* (*triangles*). Mean host tissue (*open symbols*) and algal symbiont (*gray symbols*) $\delta^{13}$C (±1 SE) in: **C** *P. compressa* and **D** *M. verrucosa*. Sample size for each mean in *parentheses*. Statistical analyses in Table 4. *Asterisk* indicates significant differences between means

Skeletal $\delta^{13}$C was significantly lower in bleached than in non-bleached *P. compressa* corals and lower (though the decrease was marginally significant) in *M. verrucosa* colonies (Table 4; Fig. 4A, B). This decrease in skeletal $\delta^{13}$C with bleaching was similar in both species, with a ~2‰ offset. Coral host tissue and algal symbiont $\delta^{13}$C values did not significantly differ between bleached and non-bleached corals of either species (Table 4; Fig. 4C, D). Overall, an a posteriori Tukey test revealed that skeletal $\delta^{13}$C was significantly heavier than either organic fraction, while the $\delta^{13}$C of the host and algal symbiont fractions did not significantly differ from each other (one-way ANOVA: $F = 453$, $df = 61$, $P < 0.0001$: data were normally distributed according to a Shapiro–Wilk test for normality: $W = 0.99$, $P < 0.84$).

## Discussion

To investigate the relationship between lipids and bleaching in Hawaiian corals, we measured the chl *a* concentration, total lipid concentration, and lipid class composition in non-bleached, moderately bleached, and completely bleached Hawaiian *Porites compressa* and *Montipora verrucosa*. Chl *a* concentrations decreased in moderately bleached and in bleached corals of both species, consistent with observations of lower chl *a* levels in many other studies (e.g. Coles and Jokiel 1978; Porter et al. 1989; Fitt et al. 1993, 2000; Warner et al. 1996; Ambarsari et al. 1997; D'Croz et al. 2001; Hueerkamp et al. 2001).

Completely bleached *P. compressa* colonies had significantly lower lipid concentrations than non-bleached colonies. A decrease in lipid biomass in *P. compressa* is consistent with findings for Caribbean *Montastraea annularis* and *Agaricia lamarcki* showing that tissue biomass, total carbon, total lipid, protein, carbohydrates, and total nitrogen decreased when these corals were bleached (Porter et al. 1989; Szmant and Gassman 1990; Fitt et al. 1993, 2000) and with decreases in tissue biomass in bleached *Montastraea faveolata* (Fitt et al. 2000). We found no significant difference in total lipid concentrations between non-bleached and bleached colonies of *M. verrucosa*. No change in lipid concentrations was also found by Grottoli-Everett (1995)

when Hawaiian *M. verrucosa* corals were experimentally bleached by elevating light and ultraviolet radiation levels. These *M. verrucosa* results are also consistent with findings for Caribbean *Montastraea franksi*, showing that protein, glycerol, and tissue biomass do not decrease when bleached (Fitt et al. 2000; Edmunds et al. 2003). Interestingly, in non-mass-bleaching years, unstressed *P. compressa* (Stimson 1987) and *M. verrucosa* (Stimson 1987; Grottoli-Everett 1995; Grottoli-Everett and Kuffner 1995) are ~32% and 37% lipid in the summer/fall, respectively, whereas the non-bleached corals of both species in this study ranged from 13% to 16% at the same time of year. Photosynthesis to respiration ($P/R$) ratios decrease as seawater temperature increases (Coles and Jokiel 1977; Jokiel and Coles 1990; Fitt and Warner 1995). Thus at above normal temperatures and lower $P/R$ values even the non-bleached corals in the present study had lower lipid reserves in 1996 compared to corals during non-bleaching years.

Our results from the unevenly bleached corals revealed that *P. compressa* had lower lipid levels locally in bleached tops compared to the non-bleached sides, suggesting that there was no re-allocation of lipid resources from the healthy sides to the bleached tops of the colony. Increases in susceptibility to disease or mortality in bleached areas of a coral colony (Meesters and Bak 1993; Mascarrelli and Bunkley-Williams 1999) may be due to the local depletion of energy reserves. However, in *M. verrucosa*, lipid concentrations did not significantly differ between the bleached tops and the healthy sides of colonies. Lipid levels within *M. verrucosa* colonies also did not change when coral colonies were unevenly bleached by exposing parts of the colonies to elevated light and ultraviolet radiation levels for 9 days (Grottoli-Everett and Kuffner 1995). This suggests that *M. verrucosa* may either re-allocate lipid resources from the non-bleached to the bleached portions of the colony and/or have a lower metabolic rate, which allows for the conservation of lipids irrespective of bleaching condition. For coral such as *P. compressa*, with lowered total lipid levels in bleached portions of the colony, decline of health or partial death of bleached portions of the colony may, in part, be due to the local depletion of energy reserves.

Possible mechanisms for the lack of lipid consumption in visibly bleached *M. verrucosa* compared to visibly bleached *P. compressa* in our study were explored. First, the $P/R$ ratio in *M. verrucosa* was 25–30% higher than that in *P. compressa*, both at normal (25°C) and elevated (30°C) seawater temperatures, because *M. verrucosa* has a proportionately lower respiration rate (Coles and Jokiel 1977). A lower respiration rate would have allowed *M. verrucosa* to conserve its energy reserves, including lipids, compared to *P. compressa*. Coles and Jokiel (1977) have shown that coral species that maintain higher $P/R$ ratios at elevated temperatures appear to be more resistant to bleaching and may be more likely to survive bleaching.

Second, perhaps bleached *M. verrucosa* colonies used their carbohydrate and protein stores preferentially to support metabolic demand while maintaining their lipid reserves. This is difficult to assess since specific energy reserve levels have only been measured in bleached and non-bleached *Montastraea annularis*, *Agaricia lamarcki* (Porter et al. 1989), and *Montastraea franksi* (Edmunds et al. 2003). For *M. annularis* and *A. lamarcki*, total lipids, protein, and carbohydrates were depleted by 39–73% after 5 months of recovery, suggesting that in bleached corals all three energy reserves are drawn upon during recovery. For *M. franski*, glycerol, free fatty acids, and protein were not depleted after 3 weeks of recovery from bleaching. Additional study of the lipid, protein, and carbohydrate reserves in bleached *M. verrucosa* and other species is needed to evaluate the proportion of each energy reserve used during bleaching and recovery.

Third, while the timing of the seasonal variation in chl *a*, algal symbiont, total lipid, and storage lipid concentration, as well as total biomass can vary among species (Stimson 1987, 1997; Brown et al. 1999; Fagoonee et al. 1999; Fitt et al. 2000; Warner et al. 2002; Oku et al. 2003), both *M. verrucosa* and *P. compressa* have similar annual total lipid cycles, with higher lipid levels in the summer than in the winter/spring (Stimson 1987). Thus, the species-specific differences in lipid content observed in our study are probably not due to differences in the timing of their natural cycle in total lipid concentration.

Finally, bleached *M. verrucosa* may have begun to recover at the time the samples were collected and had started rebuilding their lipid reserves. Indications are that chl *a* levels and algal symbiont concentrations recover within a couple of months to a year after bleaching (Jokiel and Coles 1990; Fitt et al. 1993, 2000) and that tissue biomass and energy reserve levels take longer, usually more than a year, to return to pre-bleaching levels even if chl *a* and algal symbiont concentrations are normal (Fitt et al. 1993, 2000). It seems unlikely that the completely bleached *M. verrucosa* colonies (or completely bleached *P. compressa* colonies for that matter) had begun to recover their lipid reserves, because 44 days after temperatures had returned to normal they were still visibly bleached. Their low chl *a* levels reflect a limited ability to photosynthetically fix large amounts of carbon for lipogenesis and storage. In addition, *M. verrucosa* bleached for more than a month longer than *P. compressa*, further limiting its ability to photosynthesize excess fixed carbon for lipogenesis and storage. Thus, the higher levels of total lipids in bleached *M. verrucosa* relative to bleached *P. compressa* are not likely due to enhanced recovery in *M. verrucosa*.

Other factors that could influence total lipid concentrations in corals are mucus secretions and gametogenesis or spawning. Lipid-based mucus is secreted by corals for protection (Crossland et al. 1980), and eggs and larvae contain measurable amounts of lipids (Arai 1993; Ward 1995). In the corals in this present study,

major spawning had already occurred prior to the onset of bleaching for both species (Grottoli, personal observations). Mucus secretion measurements were not made, and their contribution to changes in total lipids cannot be evaluated here.

Overall, a lower respiration rate in *M. verrucosa* is consistent with the conservation of lipid reserves in bleached relative to non-bleached colonies. However, the complexity of the effect of bleaching superimposed on the natural variation in photosynthetic pigmentation, energy reserves, and metabolic rates needs to be more closely studied in order to confirm these findings. Perhaps the mechanism for conserving total lipids in bleached *M. verrucosa* is similar to the mechanism for conserving protein, tissue biomass, and glycerol in bleached *Montastraea franksi* (Fitt et al. 2000; Edmunds et al. 2003).

For a small number of samples, the relative lipid class composition was analyzed. The proportion of triacylglycerol and wax esters in non-bleached *P. compressa* and *M. verrucosa* were similar to non-bleached Caribbean (*Porites porites*, *Montastraea annularis*), Japanese (*Pocillopora damicornis*, *Pocillopora verrucosa*, *Stylophora pistillata*, *Montipora aequituberculata*, *Acropora microphthalma*, *Porites lutea*, *Porites cylindrical*, *Fungia fungites*, *Galaxea fascicularis*, *Galaxea aspera*, *Oulastrea crispate*) and Red Sea (*Montipora digitata*, *P. verrucosa*, *S. pistillata*, *Goniastrea retiformis*) corals (Harland et al. 1993; Yamashiro et al. 1999; Oku et al. 2002). In bleached *P. compressa*, decreases in total lipids were due mostly to decreases in storage lipids such as triacylglycerol and wax esters. Although both bleached and non-bleached *M. verrucosa* had the same total lipid levels, the relative composition of the lipid classes was different: triacylglycerol and wax esters were lower, while diacylglycerol was higher. Caribbean *M. annularis* and *Montastraea faveolata* corals also consume their storage lipids following short, 24-h stress events (either 24 h with sediment stress or 35 h with heat stress) (Niebuhr 1999). Interestingly, *P. compressa* did not contain any diacylglycerol irrespective of its bleaching status. The complete lack of diacylglycerol in *P. compressa* at that time of year may be responsible for the overall decrease in total lipids following bleaching stress and may be diagnostic of corals that consume their lipid stores when bleached. Despite the small sample size, this preliminary assessment of changes in the proportion of lipid classes reveals two strategies for lipid use during bleaching: (1) to use easily available lipid stores, or (2) to shift the lipid class composition. The implications of both strategies for coral recovery bear further investigation.

To detect changes in the sources and utilization of carbon during bleaching, the $\delta^{13}C$ of the coral skeleton, host tissue, and algal symbiont fractions in non-bleached and bleached *M. verrucosa* and *P. compressa* was measured. Both species showed a consistent decrease in skeletal $\delta^{13}C$ of 1.4‰ in bleached compared to non-bleached colonies, with a species-specific offset. Our findings are consistent with several previous studies that also found a decrease in skeletal $\delta^{13}C$ with bleaching (Porter et al. 1989; Carriquiry et al. 1994; Allison et al. 1996; Suzuki et al. 2000, 2004). In only one study were there no systematic changes in skeletal $\delta^{13}C$ when two bleached *Montastraea annularis* colonies from 13.7 m depth were compared with two non-bleached *M. annularis* colonies from 8.5 and 13.7 m depth and two recovered colonies from 9.8 m depth (Leder et al. 1991). Skeletal $\delta^{13}C$ is known to change with depth (Land et al. 1975; Weber et al. 1976; Grottoli 1999) and may have confounded any patterns in isotope response to bleaching in the Leder et al. (1991) study. A decrease in skeletal $\delta^{13}C$ in coral paleoclimate proxy records could help identify past bleaching events. Any offset in skeletal $\delta^{13}C$, as seen between *P. compressa* and *M. verrucosa*, needs to be taken into account when comparing skeletal $\delta^{13}C$ paleo-records from different species.

Host tissue and algal symbiont $\delta^{13}C$ values of both species were not significantly different between non-bleached and bleached corals, suggesting that, overall, the ratio of heterotrophically acquired carbon to photosynthetically acquired carbon was the same under both conditions. However, a trend towards higher $\delta^{13}C$ in the host tissue of bleached *P. compressa* (0.84‰ higher) and *M. verrucosa* (0.34‰ higher) compared to non-bleached colonies is worth discussing. For any chemical reaction, the lighter isotope is preferentially incorporated into the reaction over the heavier isotope. In the case of carbon, $^{12}C$ is preferentially respired over $^{13}C$. Therefore, as lipid reserves are consumed, the $^{12}C$-rich lipid molecules would be respired first, leaving behind lipids and tissues more enriched in $^{13}C$. When bleached corals consume their energy reserves, we would expect the $\delta^{13}C$ of the remaining tissue to increase as the isotopically lighter lipids (those with higher $^{12}C$ content) are respired. This trend was observed in the data. Although the increase was not statistically significant, the results suggest that the hosts consume their isotopically lighter tissues/lipids, leaving isotopically heavier tissues/lipids behind. The measured decrease in skeletal $\delta^{13}C$ indicates that photosynthesis decreased, while the trend towards an increase in host tissue $\delta^{13}C$ values suggests that bleached corals respired their stored energy reserves.

Overall, the present study provides evidence that bleached *P. compressa* and *M. verrucosa* consumed their own lipid reserves following bleaching, when nutritional input from their algal symbionts was reduced or missing. Differences in their lipid class composition indicate that the two species may differ in their use of specific lipid reserves. Differences in their $P/R$ values appear to influence whether or not corals consume energy reserves, and may identify corals more likely to survive a bleaching event. $\delta^{13}C$ measurements of the host tissue and algal symbionts are consistent with findings that corals consume their lipid stores when bleached. However, additional study is needed to measure the changes in lipids and other physiological parameters during

bleaching and over a long period of recovery in order to follow species-specific differences in energy-reserve consumption and/or restoration due to bleaching. In addition, lower skeletal $\delta^{13}C$ in bleached corals may also be useful for identifying past bleaching events in coral paleoclimate proxy records.

**Acknowledgements** We thank the Hawaii Institute of Marine Biology (HIMB), P. Jokiel and all members of HIMB for logistical field support, E. Druffel for her encouragement, R. Harvey for the lipid class analyses, M. Lesser, and two anonymous reviewers for their insightful comments, as well as C. Grottoli, R. Dunbar, and D. Mucciarone. Funding for this study was provided to A.G. by the PADI Foundation, the Mellon Foundation, EPA STAR Fellowship, Dreyfus Postdoctoral Fellowship, and to L.R. by a William Penn Fellowship. This research complies with the current laws of the country in which the research was performed.

## References

Allison N, Tudhope AW, Fallick AE (1996) Factors influencing the stable carbon and oxygen isotopic composition of *Porites lutea* coral skeletons from Phuket, South Thailand. Coral Reefs 15:43–57

Ambarsari I, Brown BE, Barlow RG, Britton G, Cummings D (1997) Fluctuations in algal chlorophyll and carotenoid pigments during solar bleaching in the coral *Goniastrea aspera* at Phuket, Thailand. Mar Ecol Prog Ser 159:303–307

Anthony KRN, Connolly SR, Willis BL (2002) Comparative analysis of energy allocation to tissue and skeletal growth in corals. Limnol Oceanogr 47:1417–1429

Arai T (1993) Lipid composition of positively buoyant eggs of reef building corals. Coral Reefs 12:71–75

Baird AH, Marshall PA (1998) Mass bleaching of corals on the Great Barrier Reef. Coral Reefs 17:376

Baker AC (2001) Reef corals bleach to survive change. Nature 411:765

Bathen KH (1968) A descriptive study of the physical oceanography of Kaneohe Bay, Oahu, Hawaii. Hawaii Institute of Marine Biology, University of Hawaii, 14, Honolulu

Battey JF, Patton JS (1984) A reevaluation of the role of glycerol in carbon translocation in zooxanthellae–coelenterate symbiosis. Mar Biol 79:27–38

Brown BE (1997) Coral bleaching: causes and consequences. Coral Reefs 16[Suppl]:129–138

Brown BE, Dunne RP, Ambarsari I, Le Tissier MDA, Satapoomin U (1999) Seasonal fluctuations in environmental factors and variations in symbiotic algae and chlorophyll pigments in four Indo-Pacific coral species. Mar Ecol Prog Ser 191:53–69

Carriquiry JD, Risk MJ, Schwarcz HP (1994) Stable isotope geochemistry of corals from Costa Rica as proxy indicator of the El Niño/Southern Oscillation (ENSO). Geochim Cosmochim Acta 58:335–351

Cole JE, Fairbanks RG (1990) The southern oscillation recorded in the $\delta^{18}O$ of corals from Tarawa Atoll. Paleoceanography 5:669–683

Coles SL, Jokiel PL (1977) Effects of temperature on photosynthesis and respiration in hermatypic corals. Mar Biol 43:209–216

Coles SL, Jokiel PL (1978) Synergistic effects of temperature, salinity and light on the hermatypic coral *Montipora verrucosa*. Mar Biol 49:187–195

Crossland CJ, Barnes DJ, Borowitzka MA (1980) Diurnal lipid and mucus production in the staghorn coral *Acropora acuminata*. Mar Biol 60:81–90

D'Croz L, Mate JL, Oke JE (2001) Responses to elevated seawater temperature and UV radiation in the coral *Porites lobata* from upwelling and non-upwelling environments on the Pacific coast of Panama. Bull Mar Sci 69:203–214

Edmunds PJ (1994) Evidence that reef-wide patterns of coral bleaching may be the result of the distribution of bleaching-susceptible clones. Mar Biol 121:137–142

Edmunds PJ, Gates RD (2002) Normalizing physiological data for scleractinian corals. Coral Reefs 21:193–197

Edmunds PJ, Spencer Davies P (1986) An energy budget for *Porites porites* (Scleractinia). Mar Biol 92:339–347

Edmunds PJ, Gates RD, Gleason DF (2003) The tissue composition of *Montastraea franksi* during a natural bleaching event in the Florida Keys. Coral Reefs 22:54–62

Fagoonee I, Wilson HB, Hassell MP, Turner JR (1999) The dynamics of zooxanthellae populations: a long-term study in the field. Science 283:843–845

Falkowski PG, Dubinsky Z, Muscatine L, Porter JW (1984) Light and bioenergetics of a symbiotic coral. Bioscience 34:705–709

Falkowski PG, Dubinsky Z, Muscatine L, McCloskey L (1993) Population control in symbiotic corals. Bioscience 43:606–611

Felis T, Patzold J, Loya Y, Wefer G (1998) Vertical water mass mixing and plankton blooms recorded in skeletal stable carbon isotopes of a Red Sea coral. J Geophys Res 103:30,731–30,739

Fisk DA, Done TJ (1985) Taxonomic and bathymetric patterns of bleaching in corals, Myrmidion Reef (Queensland). In: Gabrié C, et al (eds) Proc 5th Int Coral Reef Congr. Antenne Museum—EPHE, Moorea, Tahiti, pp 149–154

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

Fitt WK, Spero HJ, Halas J, White MW, Porter JW (1993) Recovery of the coral *Montastrea annularis* in the Florida Keys after the 1987 Caribbean "bleaching event". Coral Reefs 12:57–64

Fitt WK, McFarland FK, Warner ME, Chilcoat GC (2000) Seasonal patterns of tissue biomass and densities of symbiotic dinoflagellates in reef corals and relation to coral bleaching. Limnol Oceanogr 45:677–685

Ghiold J, Smith SH (1990) Bleaching and recovery of deepwater, reef, and reef-dwelling invertebrates in the Cayman Islands. Caribb J Sci 26:52–61

Gleason DG, Wellington GM (1993) Ultraviolet radiation and coral bleaching. Nature 365:836–838

Glynn P (1996) Coral reef bleaching: facts, hypotheses and implications. Global Change Biol 2:495–509

Grottoli AG (1999) Variability in skeletal stable isotopes and maximum linear extension in reef corals at Kaneohe Bay, Hawaii. Mar Biol 135:437–449

Grottoli AG (2002) Effect of light and brine shrimp levels on skeletal $\delta^{13}C$ values in the Hawaiian coral *Porites compressa*: a tank experiment. Geochim Cosmochim Acta 66:1955–1967

Grottoli AG, Wellington GM (1999) Effect of light and zooplankton on skeletal $\delta^{13}C$ values in the eastern Pacific corals *Pavona clavus* and *Pavona gigantea*. Coral Reefs 18:29–41

Grottoli-Everett AG (1995) Bleaching and lipids in the Pacific coral *Montipora verrucosa*. In: Gulko D, Jokiel PL (eds) Ultraviolet radiation and coral reefs. UNIHI-Sea Grant, Honolulu, Hawaii, pp 107–114

Grottoli-Everett AG, Kuffner IB (1995) Uneven bleaching within colonies of the Hawaiian coral *Montipora verrucosa*. In: Gulko D, Jokiel PL (eds) Ultraviolet radiation and coral reefs. UNI-HI-Sea Grant, Honolulu, Hawaii, pp 115–120

Harland AD, Fixter LM, Spencer Davies P, Anderson RA (1991) Distribution of lipids between the zooxanthellae and animal compartment in the symbiotic sea anemone *Anemonia viridis*: wax esters, triglycerides and fatty acids. Mar Biol 110:13–19

Harland AD, Navarro JC, Spencer Davies P, Fixter LM (1993) Lipids of some Caribbean and Red Sea corals: total lipid, wax esters, triglycerides and fatty acids. Mar Biol 117:113–117

Harriott VJ (1985) Mortality rates of scleractinian corals before and during a mass bleaching event. Mar Ecol Prog Ser 21:81–88

Heikoop JM, Dunn JJ, Risk MJ, Schwarcz HP, McConnaughey TA, Sandeman IM (2000) Separation of kinetic and metabolic isotope effects in carbon-13 records preserved in reef coral skeletons. Geochim Cosmochim Acta 64:975–987

Hoegh-Guldberg O, Salvat B (1995) Periodic mass-bleaching and elevated sea temperatures: bleaching of outer reef slope communities in Moorea, French Polynesia. Mar Ecol Prog Ser 121:181–190

Hueerkamp C, Glynn PW, D'Croz L, Mate JL, Colley SB (2001) Bleaching and recovery of five eastern Pacific corals in an El Niño–related temperature experiment. Bull Mar Sci 69:215–236

Jeffrey SW, Humphrey GF (1975) New spectrophotometric equations for determining chlorophylls *a*, *b*, *c1* and *c2* in higher plants, algae and natural phytoplankton. Biochem Physiol Pflanz (BPP) 167:191–194

Johannes RE, Wiebe WJ (1970) Method for determination of coral tissue and biomass and composition. Limnol Oceanogr 15:822–824

Jokiel PL, Coles SL (1974) Effects of heated effluent on hermatypic corals at Kahe Point, Oahu. Pac Sci 28:1–18

Jokiel PL, Coles SL (1990) Response of Hawaiian and other Indo-Pacific reef corals to elevated temperature. Coral Reefs 8:155–162

Klein R, Patzold J, Wefer G, Loya Y (1992) Seasonal variations in the stable isotopic composition and the skeletal density pattern of the coral *Porites lobata* (Gulf of Eilat, Red Sea). Mar Biol 112:259–263

LaJeunesse TC (2001) Investigating biodiversity, ecology, and phylogeny of endosymbiotic dinoflagellates in the genus *Symbiodinium* using the ITS region: in search of a 'species' level marker. J Phycol 37:866–880

Land LS, Lang JC, Barnes DJ (1975) Extension rate: a primary control on the isotopic composition of West Indian (Jamaican) scleractinian coral skeletons. Mar Biol 33:221–233

Leder JL, Szmant AM, Swart PK (1991) The effect of prolonged "bleaching" on skeletal banding and stable isotopic composition in *Montastrea annularis*. Coral Reefs 10:19–27

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

Lombardi MR, Lesser MP, Gorbunov MY (2000) Fast repetition rate (FRR) fluorometry: variability of chlorophyll *a* fluorescence yields in colonies of the corals, *Montastraea faveolata* and *Diploria labyrinthiformes* recovering from bleaching. J Exp Mar Biol Ecol 252:7584

Loya Y, Sakai K, Yamazato K, Nakano Y, Sambali H, van Woesik R (2001) Coral bleaching: the winners and the losers. Ecol Lett 4:122–131

Marshall PA, Baird AH (2000) Bleaching of corals on the Great Barrier Reef: differential susceptibilities among taxa. Coral Reefs 19:155–163

Mascarrelli PE, Bunkley-Williams L (1999) An experimental field evaluation of healing in damaged, unbleached and artificially bleached star coral, *Montastraea annularis*. Bull Mar Sci 65:577–586

McConnaughey T (1989) $^{13}C$ and $^{18}O$ isotopic disequilibrium in biological carbonates. I. Patterns. Geochim Cosmochim Acta 53:151–162

McConnaughey TA, Burdett J, Whelan JF, Paull CK (1997) Carbon isotopes in biological carbonates: respiration and photosynthesis. Geochim Cosmochim Acta 61:611–622

Meesters EH, Bak RPM (1993) Effects of coral bleaching on tissue regeneration potential and colony survival. Mar Ecol Prog Ser 96:189–198

Muscatine L, Cernichiari E (1969) Assimilation of photosynthetic products of zooxanthellae by a reef coral. Biol Bull (Woods Hole) 137:506–523

Muscatine L, McCloskey LR, Marian RE (1981) Estimating the daily contribution of carbon from zooxanthellae to coral animal respiration. Limnol Oceanogr 26:601–611

Muscatine L, Falkowski PG, Proter JW, Dubinsky Z (1984) Fate of photosynthetic fixed carbon in light- and shade-adapted colonies of the symbiotic coral *Stylophora pistillata*. Proc R Soc Lond B Biol Sci 222:181–202

Muscatine L, Porter JW, Kaplan IR (1989) Resource partitioning by reef corals as determined from stable isotope composition. I. $\delta^{13}C$ of zooxanthellae and animal tissue versus depth. Mar Biol 100:185–193

Niebuhr DH (1999) Environmental stress in hard coral: evaluating lipid as an indicator of sub-lethal stress on short time scales. PhD thesis, College of William and Mary, Williamsburg, Va., USA

Obura DO (2001) Can differential bleaching and mortality among coral species offer useful indicators for assessment and management of reefs under stress? Bull Mar Sci 69:421–442

Oku H, Yamashiro H, Onaga K, Iwasaki H, Takara K (2002) Lipid distribution in branching coral *Montipora digitata*. Fish Sci (Tokyo) 68:517–522

Oku H, Yamashiro H, Onaga K, Sakai K, Iwasaki H (2003) Seasonal changes in the content and composition of lipids in the coral *Goniastrea aspera*. Coral Reefs 22:83–85

Oliver J (1985) Recurrent seasonal bleaching and mortality of corals on the Great Barrier Reef. In: Gabrié C, et al (eds) Proc 5th Int Coral Reef Congr. Antenne Museum—EPHE, Moorea, Tahiti, pp 210–206

Patton JS, Burris JE (1983) Lipid synthesis and extrusion by freshly isolated zooxanthellae (symbiotic algae). Mar Biol 75:131–136

Patton JS, Abraham S, Benson AA (1977) Lipogenesis in the intact coral *Pocillopora capitata* and its isolated zooxanthellae: evidence for a light-driven carbon cycle between symbiont and host. Mar Biol 44:235–247

Porter JW, Fitt WK, Spero HJ, Rogers CS, White MW (1989) Bleaching in reef corals: physiological and stable isotopic responses. Proc Natl Acad Sci USA 86:9342–9346

Reynaud S, Ferrier-Pages C, Sambrotto R, Juillet-Leclerc A, Jaubert J, Gattuso J-P (2002) Effect of feeding on the carbon and oxygen isotopic composition in the tissues and skeleton of the zooxanthellate coral *Stylophora pistillata*. Mar Ecol Prog Ser 238:81–89

Reynaud-Vaganay S, Juillet-Leclerc A, Jaubert J, Gattuso J-P (2001) Effect of light on skeletal $\delta^{13}C$ and $\delta^{18}O$, and interaction with photosynthesis, respiration and calcification in two zooxanthellate scleractinian corals. Palaeogeogr Palaeoclim Palaeoecol 175:393–404

Risk MJ, Sammarco PW, Schwarcz HP (1994) Cross-continental shelf trends in $\delta^{13}C$ in coral on the Great Barrier Reef. Mar Ecol Prog Ser 106:121–130

Rowan R, Knowlton N, Baker A, Jara J (1997) Landscape ecology of algal symbionts creates variation in episodes of coral bleaching. Nature 388:265–269

Spencer Davies P (1984) The role of zooxanthellae in the nutritional energy requirements of *Pocillopora eydouxi*. Coral Reefs 2:181–186

Spencer Davies P (1991) Effect of daylight variations on the energy budgets of shallow-water corals. Mar Biol 108:137–144

Stimson JS (1987) Location, quantity and rate of change in quantity of lipids in tissue of Hawaiian hermatypic corals. Bull Mar Sci 41:889–904

Stimson J (1997) The annual cycle of density of zooxanthellae in the tissues of field and laboratory-held *Pocillopora damicornis* (Linnaeus). J Exp Mar Biol Ecol 214:35–48

Stimson J, Sakai K, Sembali H (2002) Interspecific comparison of the symbiotic relationship in corals with high and low rates of bleaching-induced mortality. Coral Reefs 21:409–421

Suzuki A, Kawahata H, Tanimoto Y, Tsukamoto H, Gupta LP, Yukino I (2000) Skeletal isotopic record of a *Porites* coral during the 1998 mass bleaching event. Geochem J 34:321–329

Suzuki A, Gagan MK, Fabricius K, Isdale PJ, Yukino I, Kawahata H (2004) Skeletal isotope microprofiles of growth perturbations in *Porites* corals during the 1997–98 mass bleaching event. Coral Reefs (in press)

Swart PK (1983) Carbon and oxygen isotope fractionation in scleractinian corals: a review. Earth Sci Rev 19:51–80

Szmant AM, Gassman NJ (1990) The effects of prolonged "bleaching" on the tissue biomass and reproduction of the reef coral *Montastrea annularis*. Coral Reefs 8:217–224

Toller WW, Rowan R, Knowlton N (2001) Zooxanthellae of the *Montastreea annularis* species complex: patterns of distribution of four taxa of *Symbiodinium* on different reefs and across depths. Biol Bull (Woods Hole) 201:348–359

Wakeham SG, Hedges JI, Lee C, Pease TK (1993) Effects of poisons and preservatives on the composition of organic matter in a sediment trap experiment. J Mar Res 51:669–696

Ward S (1995) The effect of damage on the growth, reproduction and storage of lipids in the scleractinian coral *Pocillopora damicornis* (Linnaeus). J Exp Mar Biol Ecol 187:193–206

Warner ME, Fitt WK, Schmidt GW (1996) The effects of elevated temperature on the photosynthetic efficiency of zooxanthellae in hospite from four different species of reef coral: a novel approach. Plant Cell Environ 19:291–299

Warner ME, Chilcoat GC, McFarland FK, Fitt WK (2002) Seasonal fluctuations in the photosynthetic capacity of photosystem II in symbiotic dinoflagellates in the Caribbean reef-building coral *Montastrea*. Mar Biol 141:31–38

Weber JN, Deines P, Weber PH, Baker PA (1976) Depth related changes in the $^{13}C/^{12}C$ ratio of skeletal carbonate deposited by the Caribbean reef-frame building coral *Montastrea annularis*: further implications of a model for stable isotope fractionation by scleractinian corals. Geochim Cosmochim Acta 40:31–39

Wilkinson C (2000) Status of coral reefs of the world: 2000. Australian Institute for Marine Science, Townsville

Yamashiro H, Oku H, Higa H, Chinen I, Sakai K (1999) Composition of lipids, fatty acids and sterols in Okinawan corals. Comp Biochem Physiol B 122:397–407

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