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Calcification rate and the stable carbon, oxygen, and nitrogen isotopes in the skeleton, host tissue, and zooxanthellae of bleached and recovering Hawaiian corals

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Abstract

We tested the effectiveness of stable isotopes as recorders of physiological changes that occur during coral bleaching and recovery. *Montipora capitata* and *Porites compressa* fragments were bleached in outdoor tanks with seawater temperature raised to 30 °C (treatment corals) for one month. Additional fragments were maintained at 27 °C in separate tanks (control corals). After one month, (0 months recovery), buoyant weight was measured and a subset of fragments was frozen. Remaining fragments were returned to the reef for recovery. After 1.5, 4, and 8 months, fragments were collected, measured for buoyant weight, and frozen. Fragments were analyzed for stable carbon and oxygen isotopic compositions of the skeleton ($\delta^{13}C_s$; $\delta^{18}O_s$) and nitrogen and carbon isotopic compositions of the host tissue ($\delta^{15}N_h$; $\delta^{13}C_h$) and zooxanthellae ($\delta^{15}N_z$; $\delta^{13}C_z$). $\delta^{13}C_s$ decreased immediately after bleaching in *M. capitata*, but not in *P. compressa*. $\delta^{18}O_s$ of both species failed to record the warming event. During the remaining months of recovery, $\delta^{13}C_s$ and $\delta^{18}O_s$ were more enriched in treatment than control corals due to decreases in calcification and metabolic fractionation during that time. Increased $\delta^{15}N_h$ of treatment *P. compressa* may be due to expelled zooxanthellae during bleaching and recovery. Increased $\delta^{15}N_z$ at 1.5 months in treatment fragments of both species reflects the increased incorporation of dissolved inorganic nitrogen to facilitate mitotic cell division and/or chl *a*/cell recovery. Changes in $\delta^{13}C_h$ and $\delta^{13}C_z$ at 1.5 months in treatment *M. capitata* indicated a large increase in heterotrophically acquired carbon relative to photosynthetically fixed carbon. We experimentally show that isotopes in coral skeleton, host tissue and zooxanthellae can be used to verify physiological changes during bleaching and recovery, but their use as a proxy for past bleaching events in the skeletal record is limited.

1. Introduction

Coral bleaching is primarily caused by elevated seawater temperatures and/or ultraviolet radiation, resulting in the loss of endosymbiotic zooxanthellae and a pale or white coral colony (Gleason and Wellington, 1993; Glynn, 1996; Brown, 1997; Wilkinson, 2000). For any given event, the extent of bleaching severity and mortality is variable (Fisk and Done, 1985; Oliver, 1985; Ghiold and Smith, 1990; Edmunds, 1994; Marshall and Baird, 2000; Loya et al., 2001). However, reasons for the variability are not fully understood. Stable isotopic analyses may be useful in further understanding the physiological changes that underlie variability in bleaching events.

Skeletal stable carbon isotopic composition of fast growing corals ($\delta^{13}C_s$) is primarily influenced by metabolic fractionation (e.g., McConnaughey, 1989; Grottoli, 1999; Grottoli and Wellington, 1999; Grottoli, 2002). Changes in $\delta^{13}C_s$ result from changes in photosynthesis and respiration (Swart, 1983; McConnaughey, 1989; Muscatine et al., 1989; Swart et al., 1996; McConnaughey et al., 1997; Grottoli and Wellington, 1999; Grottoli, 2002). In healthy corals, as solar irradiance decreases, $\delta^{13}C_s$ decreases (Cole and Fairbanks, 1990; Klein et al., 1992; Carriquiry et al., 1994; Grottoli, 1999; Grottoli and Wellington, 1999; Reynaud-Vaganay et al., 2001; Grottoli, 2002; Heikoop

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et al., 2002; Ferrier-Pages et al., 2003) due to decreases in photosynthesis. Analogously, in bleached corals, decreased chlorophyll *a* correlated with decreased $\delta^{13}C_s$ (Porter et al., 1989; Grottoli et al., 2004) also due to decreased photosynthesis. Heterotrophy also influences $\delta^{13}C_s$ through respiration. Naturally increased zooplankton concentrations result in decreased $\delta^{13}C_s$ (Felis et al., 1998; Grottoli and Wellington, 1999) since zooplankton $\delta^{13}C$ is low [−19.6‰ v-PDB ± 0.4 (1 standard error, 1SE)] relative to seawater. Nevertheless, for shallow fast growing non-bleached corals, the overall impact of heterotrophy on $\delta^{13}C_s$ is usually small relative to photosynthesis (Grottoli and Wellington, 1999). Changes in $\delta^{13}C_s$ during recovery from bleaching have not been fully investigated and may help identify past events in the coral record.

Skeletal stable oxygen isotopic composition in fast-growing corals ($\delta^{18}O_s$) is a well-established proxy for sea surface temperature (SST) and sea surface salinity (SSS) (e.g., Weil et al., 1981; Wellington et al., 1996; Fairbanks et al., 1997; Gagan et al., 2002) exhibiting species-specific offsets from equilibrium with seawater (Wellington et al., 1996). During bleaching events, $\delta^{18}O_s$ often, but not always, decreases as SST increases (Porter et al., 1989; Leder et al., 1991; Suzuki et al., 2000; Suzuki et al., 2003). Part of the difficulty in assessing $\delta^{18}O_s$ as a bleaching proxy from previous studies is although the occurrence of a bleaching event might be known, the bleaching status of the analyzed coral colonies was often unknown. Therefore, a controlled experiment is necessary to quantitatively evaluate the reliability of $\delta^{18}O_s$ to record bleaching events.

Maximum calcification occurs when seawater temperatures are close to mean maximum summer temperatures (Marshall and Clode, 2004). Temperatures above mean maximum summer temperatures result in decreased calcification rates (Abramovitch-Gottlib et al., 2002; Clausen and Roth, 1975). Skeletal extension rates are variably affected by bleaching: some species show a decrease during bleaching (Leder et al., 1991; Allison et al., 1996; Suzuki et al., 2000), others decrease immediately afterwards (Suzuki et al., 2003), while others experience complete cessation in skeletal extension (Goreau and Macfarlane, 1990). In one species, skeletal growth resumed to pre-bleaching rates once pigmentation and tissue depth recovered (Mendes and Woodley, 2002). This variability necessitates the systematic measurement of calcification during bleaching and recovery to accurately interpret changes in skeletal stable isotopes.

Host tissue ($\delta^{13}C_h$) and zooxanthellae ($\delta^{13}C_z$) stable carbon isotopic values are usually within 2‰ of each other (Muscatine et al., 1989; Risk et al., 1994; Reynaud et al., 2002; Grottoli et al., 2004) and depleted relative to the skeleton (Reynaud et al., 2002; Grottoli et al., 2004). The difference in $\delta^{13}C$ values between host tissue and zooxanthellae is considered diagnostic of the relative contribution of heterotrophy and photosynthesis to fixed carbon (Muscatine et al., 1989). For example, in Caribbean corals, $\delta^{13}C_h$ tends to decrease more with depth than does $\delta^{13}C_z$ (Muscatine et al., 1989), indicating that the ratio of heterotrophy to photosynthesis increases with depth as more isotopically depleted zooplankton are incorporated into the host tissue. No difference was found in the $\delta^{13}C_h$ and $\delta^{13}C_z$ of bleached versus non-bleached *Montipora capitata* or *Porites compressa* after three months of recovery, indicating that the ratio of heterotrophy to photosynthesis was the same under both conditions at that time (Grottoli et al., 2004). Any changes in the contribution of photosynthetically and heterotrophically acquired fixed carbon in earlier or later periods of recovery are unknown, but should also be detectable in $\delta^{13}C_h$ and $\delta^{13}C_z$.

Stable nitrogen isotopic composition of the host tissue ($\delta^{15}N_h$) is influenced by the presence or absence of symbiotic bacteria (Lesser et al., 2004) and zooxanthellae (Muscatine et al., 2005). Bleached corals are expected to have $\delta^{15}N_h$ similar to asymbiotic species and to approach values for symbiotic species through recovery. Isolated and in hospite zooxanthellae uptake dissolved inorganic nitrogen from solution, including ammonium, nitrate, and/or nitrite ions (Burris, 1983; D'Elia et al., 1983; Wilkerson and Trench, 1986). Zooxanthellae stable nitrogen isotopic composition ($\delta^{15}N_z$) likely reflects changes in the uptake of dissolved inorganic nitrogen from the seawater (Hoegh-Guldberg et al., 2004).

Together, isotopes in the skeleton, host tissue, and zooxanthellae should be useful indicators of physiological changes that occur during bleaching and recovery. In addition, skeletal isotopes may be useful as proxy indicators of past bleaching events. We hypothesize that for bleached versus non-bleached corals: (1) $\delta^{13}C_s$ decreases during bleaching due to decreased photosynthesis rates, and increases through recovery as photosynthesis recovers, (2) $\delta^{18}O_s$ decreases during bleaching due to increased seawater temperatures, and is not different from non-bleached corals through recovery, (3) $\delta^{15}N$ and $\delta^{13}C$ of host tissue and zooxanthellae record changes in the relative contribution of heterotrophy and photosynthesis, (4) calcification decreases during bleaching and increases throughout recovery, and (5) species-specific offsets occur for each variable. To address these hypotheses, we bleached *P. compressa* and *M. capitata* with elevated seawater temperatures for one month in outdoor flow-through tanks followed by 8 months of recovery on the reef. Calcification rate, $\delta^{13}C$ and $\delta^{18}O$ of the skeleton, and the $\delta^{15}N$ and $\delta^{13}C$ of the host tissue and zooxanthellae of temperature treated fragments were compared to untreated control fragments.

2. Materials and methods

2.1. Study site

Kaneohe Bay is on the windward side of Oahu, Hawaii and is 12.7 km long × 4.3 km wide (Bathen, 1968).

Seawater temperatures average 27 ± 1 °C [1 standard deviation (SD)] in summer/fall (June–October) and 24.5 ± 1.5 °C (1 SD) in winter/spring temperatures (November–May) (data from Hawaii Institute of Marine Biology weather station). Corals from this study were collected 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, consisting mainly of *P. compressa* Dana 1848 and *M. capitata* (Dana 1846) (=*Montipora verrucosa*). In addition, there are colonies of *Pocillopora damicornis* and the solitary coral *Fungia scutaria* found at shallower depths. *P. compressa* is a finger-like coral, ranging in color from yellow-brown to dark brown. *M. capitata* is a dark to medium brown coral, often observed to have beige to white tips. Its form ranges from plating (typically found at deeper depths) to branching (typically found at shallower depths). All fragments of *M. capitata* collected for this study were of the branching form.

2.2. Experimental design

In late August 2003, twelve large, healthy colonies of *P. compressa* and *M. capitata* were identified at 2 m depth. Eight fragments were collected from each colony for a total of 96 fragments and allowed to acclimate in the tanks for two weeks. All fragments were weighed using the buoyant weight technique (described below) to determine initial values. On 4 September 2003, four fragments from each colony were randomly placed in one of four 30 °C treatment tanks, and four were randomly placed in one of four ambient control tanks for one month. Within each treatment, one fragment from each colony was randomly assigned to 0, 1.5, 4, or 8 months recovery. Treatment tanks were fitted with three Aquarium Pharmaceuticals Rena Cal 200 W heaters, while ambient tanks had weighted PVC pipes of the same size to control for the presence of heaters. An Onset Corporation temperature logger monitored the seawater temperature of each tank every hour throughout the month. Hourly seawater temperatures averaged 26.8 °C ± 0.04 (1 SE) for the ambient tanks and 30.1 °C ± 0.05 (1 SE) for the treatment tanks. Daily seawater salinity in each tank was recorded with a refractometer. Daily salinity averaged 36.2‰ ± 0.3 (1 SE) in ambient tanks, and did not differ from treatment tanks (36.4‰ ± 0.3, 1 SE). Tanks were covered with neutral density mesh to mimic light levels of photosynthetically active radiation at 2 m depth. Inflow pipes were fitted with a 50 μm-filter. To minimize tank effects, coral were rotated between tanks of the same treatment weekly. To minimize positional effects, corals were rotated within each tank daily. The experiment was designed to mimic the timing, duration, and temperature of a natural bleaching event that occurred in Kaneohe Bay in late August to September 1996 (Jokiel and Brown, 2004).

On 4 October 2003, after one month in the tanks, buoyant weight was measured (described below) and 0 month recovery fragments were frozen at −80 °C to avoid degradation of tissue and zooxanthellae. Remaining fragments were returned to the reef at 2 m depth for in situ recovery. At 1.5 months (16 November 2003), 4 months (2 February 2004), and 8 months (4 June 2004) recovery, the respective coral fragments were collected, their buoyant weight measured, and frozen.

2.3. Laboratory analyses

2.3.1. Calcification

Calcification was determined by the buoyant weight technique (Jokiel et al., 1978), whereby fragments are weighed while suspended in seawater. This method is preferable to wet weight measurements in air, as it is not affected by amount of water in the skeleton, mucus on the colony, or commensal organisms within the skeleton as they are neutrally buoyant (Jokiel et al., 1978). Daily calcification rate was calculated as the difference between initial and final weights, divided by number of days elapsed. Beginning buoyant weights did not significantly differ between control and treatment fragments of *P. compressa* (control: 17.04 g ± 0.97, 1 SE; treatment: 16.04 g ± 0.80, 1 SE) and *M. capitata* (control: 17.74 g ± 1.33, 1 SE; treatment: 16.42 g ± 1.26, 1 SE) as determined with Student's *t*-tests. Therefore, any differences between control and treatment fragments are independent of beginning sizes.

2.3.2. Stable isotopic analyses

Coral tissue and zooxanthellae were removed from the skeleton of between four and six coral fragments (depending on the surviving number of fragments at each time period) from each treatment and recovery interval with a Water-pik and deionized water (Johannes and Wiebe, 1970), homogenized, and separated by centrifugation as described in Grottoli et al. (2004). Host tissue and zooxanthellae fractions were isolated onto pre-burned glass fiber filters under vacuum. Filters were combusted in a Carlo Erba NA 1500 Elemental Analyzer. Resulting N_2 and CO_2 gases were analyzed with a Finnigan Delta Plus isotope ratio mass spectrometer via a Finnigan ConFlow open split interface. Host tissue and zooxanthellae $\delta^{15}N$ values are reported relative to air ($\delta^{15}N$ = per mil deviation of the ratio of stable nitrogen isotopes ^{15}N:^{14}N relative to air). Host tissue and zooxanthellae $\delta^{13}C$ values are reported relative to Vienna Peedee Belemnite Limestone Standard (v-PDB) ($\delta^{13}C$ = per mil deviation of the ratio of stable carbon isotopes ^{13}C:^{12}C relative to v-PDB). Repeated measurements of internal standards ($n = 140$) had a standard deviation of ±0.23‰ for organic $\delta^{15}N$ and ±0.16‰ for organic $\delta^{13}C$.

Differences between carbon isotopic values of host tissue ($\delta^{13}C_h$) and zooxanthellae ($\delta^{13}C_z$) are indicative of the relative contribution of photosynthesis and heterotrophy in corals (Muscatine et al., 1989). No difference between

$\delta^{13}C_h$ and $\delta^{13}C_z$ or when $\delta^{13}C_h$ is greater than $\delta^{13}C_z$, heterotrophy has a very small or negligible contribution to the fixed carbon pool relative to photosynthesis. When $\delta^{13}C_h$ is less than $\delta^{13}C_z$, heterotrophy has a greater contribution to the fixed carbon pool than photosynthesis.

The skeleton dried for at least 24 h. Approximately, 0.25–0.5 mm of skeletal material was gently scraped from the tip of the coral branch with a diamond-tipped Dremmel tool (Grottoli et al., 2004) and acidified with 100% orthophosphoric acid in an automated carbonate Kiel device at 70 °C. $\delta^{13}C$ and $\delta^{18}O$ values of the resulting CO_2 were measured in a Finnigan MAT 252 triple collecting mass spectrometer. $\delta^{13}C_s$ and $\delta^{18}O_s$ values are reported relative to v-PDB standard ($\delta^{18}O$ = per mil deviation of the ratio of stable oxygen isotopes ^{18}O:^{16}O relative to v-PDB). Repeated measurement of internal standards ($n = 96$) had a standard deviation of $\pm 0.05‰$ or less for inorganic $\delta^{13}C$ and $\pm 0.08‰$ or less for $\delta^{18}O$. At least 10% of all measurements were made in duplicate.

2.4. Statistical analyses

Analysis of variance (ANOVA) was used to analyze the effects of species, genotype, temperature, and recovery interval on $\delta^{13}C_s$, $\delta^{18}O_s$, and rate of calcification (Electronic Annex 1). The additional effect of organic fraction type (host tissue or zooxanthellae) was tested on the organic $\delta^{15}N$ and $\delta^{13}C$ values. Species, temperature, recovery interval, and fraction type effects were considered fixed and were fully crossed. Genotype was a random effect and nested within species. Due to low sample size as a result of mortality during recovery, interaction terms involving genotype were combined with the residual. Synthetic mean square errors were generated. A posteriori slice tests (e.g., tests of simple effects, Winer, 1971) determined if bleached and control treatment averages significantly differed from each other within each species, recovery interval, and fraction type. Bonferroni corrections were not used (Quinn and Keough, 2002). The use of replicate genotypes across temperature treatments and recovery times reduced the overall variation between treatments. Since all fragments were reared under the exact same conditions except temperature during the first month and were similar sizes at the start, any differences between bleached and control corals for the measured variables were due to the temperature treatment alone, independent of natural seasonal variation.

Data were normally distributed as determined from plots of the residuals versus the predicted values for each variable. Statistical analyses were generated using SAS software, Version 8.02 of the SAS System for Windows. [Copyright © 1999–2001 SAS Institute Inc. SAS and all other SAS Institute Inc. product or service names are registered trademarks or trademarks of SAS Institute Inc., Cary, NC, USA.] P-levels $\leqslant 0.05$ were considered significant.

3. Results

Control fragments of *P. compressa* and *M. capitata* were non-bleached (dark brown in color) throughout the experiment, with average zooxanthellae densities of 2.2×10^6 cells/gdw $\pm 0.4 \times 10^6$ (1 SE) and 4.0×10^6 cells/gdw $\pm 0.6 \times 10^6$ (1 SE), respectively. After one month in tanks, treatment corals were completely bleached (white in color). After 1.5 months of recovery, fragments regained some color and were pale to light brown. By 4 months, all living treatment fragments of both species were visibly non-bleached and remained this way to the end of the experiment (8 months recovery). There was no mortality in either species during the one-month bleaching period. Once on the reef, 56% and 63% of remaining treatment *P. compressa* and *M. capitata*, respectively, died sometime during the 8 months. Coral mortality is comprehensively discussed in Rodrigues (2005) and does not affect the interpretation of data presented here.

At 0 months, treatment $\delta^{13}C_s$ was not significantly different from control fragments in *P. compressa* (Fig. 1a), while treatment $\delta^{13}C_s$ decreased by 0.9‰ in *M. capitata* (Fig. 1b). Both species had more enriched $\delta^{13}C_s$ in treatment than control fragments throughout the remainder of recovery. Differences were statistically significant for

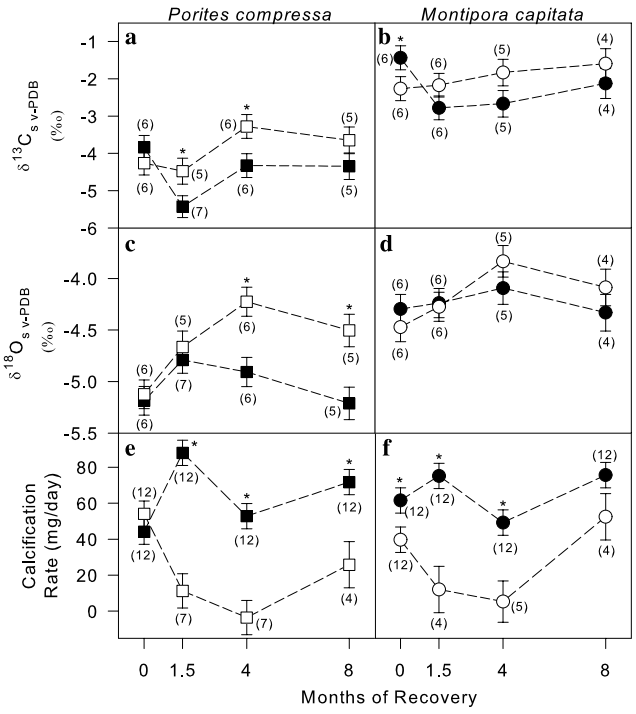

Fig. 1. Average (a and b) skeletal stable carbon isotopic ($\delta^{13}C$) values, (c and d) skeletal stable oxygen isotopic ($\delta^{18}O$) values, and (e and f) calcification rate in *P. compressa* (squares) and *M. capitata* (circles) at 0, 1.5, 4, and 8 months of recovery. All averages are ± 1 standard error. Symbols (*) indicate significant differences at $p \leqslant 0.05$ between control (filled) and treatment (open) averages at a given time interval by a posteriori slice tests. Sample size for each average provided in parentheses. Statistical analyses provided in Electronic Annex 1-1.

P. compressa at 1.5 and 4 months with increases of 0.9‰ and 1‰, respectively.

Treatment $\delta^{18}O_s$ at 0 and 1.5 months was not significantly different from control fragments in *P. compressa* (Fig. 1c). Treatment $\delta^{18}O_s$ was significantly enriched by 0.7‰ compared to control fragments at 4 and 8 months. Treatment $\delta^{18}O_s$ was not significantly different from controls throughout recovery in *M. capitata*, although the pattern was similar to that of *P. compressa*, with increases occurring at 4 and 8 months recovery (Fig. 1d).

Calcification rate at 0 months for treatment *P. compressa* was not significantly different from control fragments (Fig. 1e). Calcification significantly decreased during 1.5, 4, and 8 months by 13%, 2%, and 36%, respectively. For treatment *M. capitata*, calcification significantly decreased at 0, 1.5, and 4 months recovery by 65%, 16%, and 11%, respectively, and was not different at 8 months (Fig. 1f).

Treatment *P. compressa* $\delta^{15}N_h$ and $\delta^{15}N_z$ values were generally enriched compared to control fragments at all time intervals (Figs. 2a and b). These differences were only significant at 4 months when treatment $\delta^{15}N_h$ was 1‰ heavier, and at 1.5 months when treatment $\delta^{15}N_z$ was 1.6‰ heavier. Treatment *M. capitata* $\delta^{15}N_h$ and $\delta^{15}N_z$ did not differ from controls at any time, except at 1.5 months when $\delta^{15}N_z$ was heavier by 1.4‰ (Figs. 2c and d).

Treatment and control *P. compressa* did not differ in $\delta^{13}C_h$ and $\delta^{13}C_z$ throughout recovery (Figs. 2e and f). At 0 months, treatment $\delta^{13}C_h$ and $\delta^{13}C_z$ trended strongly towards lower, more depleted values. Treatment *M. capitata* $\delta^{13}C_z$ at 0 months and $\delta^{13}C_h$ at 1.5 months were significantly lower than controls by 1.7‰ and 0.9‰, respectively (Figs. 2g and h). The difference between $\delta^{13}C_h$ and $\delta^{13}C_z$ was close to zero for treatment and control *P. compressa*

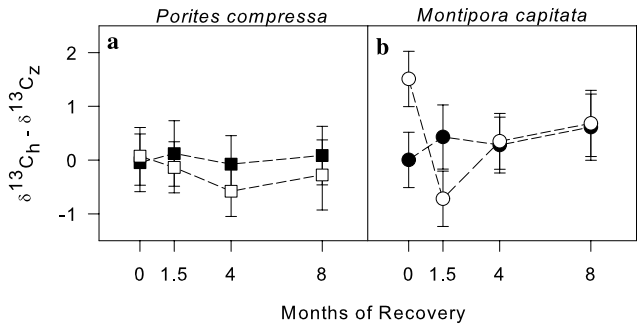

Fig. 3. The difference between $\delta^{13}C_h$ and $\delta^{13}C_z$ in (a) *P. compressa* (squares) and (b) *M. capitata* (circles) at 0, 1.5, 4, and 8 months of recovery for control (filled) and treatment (open) fragments. Heterotrophy contributes more to the fixed carbon pool when the difference is <0, while photosynthesis contributes more when the difference is ⩾0. All averages are shown ±1 SE. Statistical analyses provided in Electronic Annex 1-3.

(Fig. 3a), strongly positive at 0 months and negative at 1.5 months recovery for treatment *M. capitata* (Fig. 3b).

4. Discussion

4.1. Calcification

Although decreases in calcification and/or skeletal extension rates have been observed during bleaching (Abramovitch-Gottlib et al., 2002; Mendes and Woodley, 2002; Suzuki et al., 2003), only some studies have observed a decrease in growth for an extensive period of time after a bleaching event (Jokiel and Coles, 1977; Goreau and Macfarlane, 1990; Leder et al., 1991). These periods of slow growth or cessations in growth are often referred to as stress

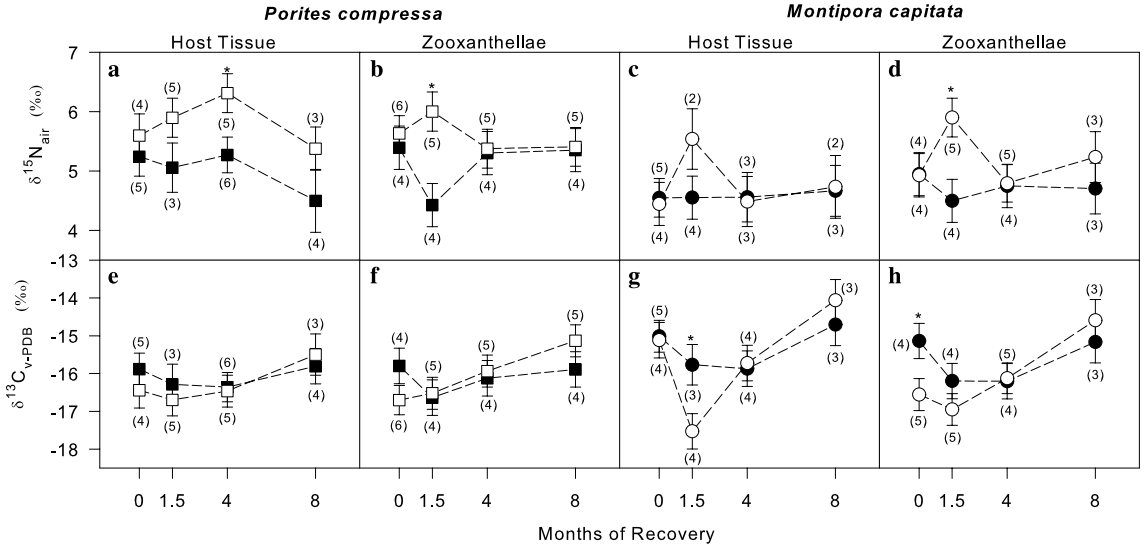

Fig. 2. Average (a and c) stable nitrogen isotopic ($\delta^{15}N$) values of the host tissue and (b and d) the zooxanthellae fractions, (e and g) stable carbon isotopic ($\delta^{13}C$) values of the host tissue and (f and h) zooxanthellae fractions in *P. compressa* (squares) and *M. capitata* (circles) at 0, 1.5, 4 and 8 months of recovery. All averages are ±1 standard error. Symbols (*) indicate significant differences at $p \leqslant 0.05$ between control (filled) and treatment (open) averages at a given interval by a posteriori least squares mean slice tests. Sample size for each average provided in parentheses. Statistical analyses provided in Electronic Annex 1-2.

bands in a coral record. Here, the most reduced period of growth lags the high SST component of the bleaching period, and lasts for 4 months in *M. capitata* and longer than 8 months in *P. compressa*. Therefore, the exact timing and duration of the stress band within a coral core varies by species exposed to the same event. Reconstructing bleaching event chronologies from the appearance of stress bands would require knowledge of species-specific variability to accurately detect the timing and duration of past events.

4.2. Skeletal $\delta^{13}C$

Decreases observed in $\delta^{13}C_s$ of both species immediately after bleaching is consistent with other studies (Porter et al., 1989; Carriquiry et al., 1994; Allison et al., 1996; Suzuki et al., 2000; Suzuki et al., 2003; Grottoli et al., 2004). In only one study (Leder et al., 1991), no consistent decrease was observed in bleached and non-bleached colonies from different depths. Since $\delta^{13}C_s$ changes with depth (Land et al., 1975; Weber et al., 1976; Grottoli, 1999; Rosenfeld et al., 2003), the Leder et al. (1991) results may have been confounded. Decreases in photosynthesis due to reduced light conditions also cause decreased $\delta^{13}C_s$ of healthy corals (Grottoli and Wellington, 1999; Reynaud-Vaganay et al., 2001; Grottoli, 2002). Previous studies have had to infer changes in photosynthesis to interpret their $\delta^{13}C_s$ results. Direct measurements indicated that bleached *P. compressa* and *M. capitata* had decreased rates of photosynthesis (Rodrigues, 2005) associated with decreased $\delta^{13}C_s$, shown here.

In both species, treatment $\delta^{13}C_s$ suddenly surpassed control $\delta^{13}C_s$ during recovery (Figs. 1a and b), rather than the gradual enrichment in treatment values that was expected. This is likely due to the substantial decrease in calcification that occurred (Figs. 1e and f). When calcification slows, the relative contribution of metabolic fractionation diminishes and $\delta^{13}C_s$ begins to approach isotopic equilibrium with seawater (McConnaughey, 1989). In contrast, after a relatively moderate natural bleaching event involving the same species and location, bleached colonies were depleted in $\delta^{13}C_s$ compared to non-bleached colonies (Grottoli et al., 2004). Under moderate bleaching conditions, corals maintain calcification, and $\delta^{13}C_s$ accurately records decreased photosynthesis. Furthermore, *Porites* spp. cores from the Great Barrier Reef were dramatically enriched in $\delta^{13}C_s$, while *Porites* spp. cores from Okinawa were depleted in $\delta^{13}C_s$ when bleached (Suzuki et al., 2000; Suzuki et al., 2003). Together, these results following natural events and the present experimental study indicate that the effect of bleaching on $\delta^{13}C_s$ is variable. Its value depends on bleaching severity, growth, and the relative contribution of kinetic and metabolic fractionation effects.

4.3. Skeletal $\delta^{18}O$

Coral $\delta^{18}O_s$ decreases by an average of 0.22‰ per 1 °C seawater warming (Weber and Woodhead, 1972). Therefore, we expected a 0.66‰ decrease in treatment $\delta^{18}O_s$ since they experienced a 3 °C increase in seawater temperature. However, no decrease occurred despite calcification rates being near normal. The salinity effect on $\delta^{18}O_s$ can be eliminated, since daily seawater salinity in the treatment (36.4‰ ± 0.3, 1SE) and control (36.2‰ ± 0.3, 1SE) tanks did not differ. Thus, the treatment corals of neither species actually recorded the 3 °C increase in SST.

While recovering on the reef, temperature and salinity, were the same for treatment and control corals. Therefore, enriched treatment $\delta^{18}O_s$ values (Figs. 1c and d) were likely associated with calcification rate (Figs. 1e and f). As calcification slows, the effect of kinetic fractionation decreases and $\delta^{18}O_s$ begins to reach isotopic equilibrium with seawater (McConnaughey, 1989). Suzuki et al. (2003) reported rapid ^{18}O enrichment in bleached *Porites* spp. cores from the Great Barrier Reef and Okinawa following known bleaching events in 1998. Interestingly, if isotopic measurements were taken from a coral core of unknown history, increases in $\delta^{18}O_s$ as observed in the present study would be interpreted as a cooling period rather than a month of intense warming followed by decreased calcification.

4.4. Detecting past bleaching events

Together, $\delta^{18}O_s$ and $\delta^{13}C_s$ have been suggested, though not successfully proven, to be a multi-proxy signal for past bleaching events (Porter et al., 1989; Leder et al., 1991; Carriquiry et al., 1994; Allison et al., 1996; Suzuki et al., 2000; Suzuki et al., 2003). Under controlled experimental conditions, $\delta^{18}O$ and $\delta^{13}C$ did not change with bleaching as expected. Slow growth masked the photosynthetic and temperature signals in $\delta^{13}C_s$ and $\delta^{18}O_s$, respectively. Stress bands resulting from slow growth or some other skeletal proxy (e.g., trace metals) may more reliably identify severe bleaching events in coral records.

4.5. Host and zooxanthellae $\delta^{15}N$

Control $\delta^{15}N_h$ and $\delta^{15}N_z$ values of both species are consistent with measurements from the Caribbean Sea, Indian and Pacific Oceans (Yamamuro et al., 1995; Heikoop et al., 2000a; M.P. Lesser, private communication). Particulate organic matter has been shown to be a source of $\delta^{15}N_h$ enrichment (Heikoop et al., 2000b). Here, all fragments were exposed to the same conditions in tanks and on the reef regardless of treatment or species, it is unlikely that differences in $\delta^{15}N_h$ are due to particulate organic input. However, azooxanthellate coral species are $\delta^{15}N_h$ enriched compared to zooxanthellate species (Muscatine et al., 2005). Gradual enrichment in treatment $\delta^{15}N_h$ of *P. compressa* (Fig. 2a) is consistent with low zooxanthellae concentrations during the first 4 months of recovery (Rodrigues, 2005). *M. capitata* is unusual in that it bleaches by reducing chlorophyll *a* per zooxanthellae and maintain-

ing total zooxanthellae densities (Grottoli-Everett and Kuffner, 1995; Rodrigues, 2005). This is consistent with no change in $\delta^{15}N_h$ (Fig. 2c). Therefore, $\delta^{15}N_h$ in both species reflects zooxanthellae concentration.

Increases in dissolved inorganic nitrogen (DIN) resulted in increased zooxanthellae densities and/or increased chl *a*/cell (reviewed in Fabricius, 2005). During recovery, corals might require a large influx of DIN to support zooxanthellae and chl *a* recovery. Increases in the rate of DIN incorporation would result in decreased isotopic fractionation, increased incorporation of ^{15}N from the nitrogen source, and more enriched $\delta^{15}N_z$ values. The elevated levels of $\delta^{15}N_z$ in treatment fragments of both species at 1.5 months (Figs. 2b and d) suggest that zooxanthellae up-regulate their incorporation of DIN to facilitate mitotic cell division and/or chl *a*/cell during the early phases of recovery.

4.6. Host and zooxanthellae $\delta^{13}C$

Depleted $\delta^{13}C_h$ is associated with increased feeding of isotopically depleted zooplankton at depth in symbiotic (Muscatine et al., 1989) and heterotrophic asymbiotic corals (Muscatine et al., 2005). The decrease in $\delta^{13}C_h$ of treatment relative to control *M. capitata* at 1.5 months (Fig. 2g) is consistent with dramatic increases in feeding at that time (Grottoli et al., in press). No difference between *P. compressa* control and treatment $\delta^{13}C_h$ (Fig. 2e) is consistent with no change in feeding rates when this species is bleached (Grottoli et al., in press). Therefore, depleted $\delta^{13}C_h$ at 1.5 months in *M. capitata* is associated with increased feeding of isotopically depleted zooplankton (average $\delta^{13}C$ of $-20‰$), while stability in $\delta^{13}C_h$ of *P. compressa* further emphasizes that this species does not adjust its feeding rate when bleached.

When the rate of photosynthesis decreases, zooxanthellae incorporate relatively more ^{12}C compared to ^{13}C, resulting in decreased $\delta^{13}C_z$ (Muscatine et al., 1989). Though not always statistically significant, the trend in treatment $\delta^{13}C_z$ (Figs. 2f and h) mirrors that of photosynthesis in both species (Rodrigues, 2005). Grottoli et al. (2004) observed no difference in bleached and non-bleached $\delta^{13}C_z$ of *P. compressa* and *M. capitata*, three months after a natural event, further corroborating that photosynthesis fully recovers by then (Rodrigues, 2005). Therefore, $\delta^{13}C_z$ tracks photosynthesis in bleached and recovering corals.

The difference between $\delta^{13}C_h$ and $\delta^{13}C_z$ is considered diagnostic of heterotrophically- relative to photosynthetically-acquired fixed carbon (Muscatine et al., 1989). Since differences remained near zero throughout recovery (Fig. 3a), heterotrophy was insignificant to the fixed carbon pool in *P. compressa* and control *M. capitata* (Fig. 3b). However, the *relative* contribution of photosynthesis was high for treatment *M. capitata* at 0 months because the contribution of heterotrophy was zero (zooplankton feeding was completely absent due to 50 μm tank filters). At 1.5 months, the strong negative difference between $\delta^{13}C_h$ and $\delta^{13}C_z$ for treatment *M. capitata* is consistent with observations of elevated feeding rates during recovery (Grottoli et al., in press). After 4 and 8 months, $\delta^{13}C_h$ was equal to or slightly greater than $\delta^{13}C_z$ indicating that heterotrophy had decreased to non-bleached levels and photosynthesis had a greater relative contribution to the carbon pool. These results are consistent with the results of Grottoli et al. (2004) and provide further evidence that *P. compressa* is mainly photoautotrophic, regardless of bleaching status, while the trophic status of *M. capitata* is plastic: photoautotrophic when non-bleached, heterotrophic during the early stages of recovery, and photoautotrophic again in the later stages of recovery.

4.7. Summary

Overall, stable isotopes in the skeleton, host tissue and zooxanthellae can be used to detect and further understand changes in calcification, photosynthesis and heterotrophy. $\delta^{18}O_s$ and $\delta^{13}C_s$ can not be reliably used as proxies for past bleaching events when calcification significantly slows. Alternatively, stress bands resulting from severely reduced calcification may be more reliable for identifying severe bleaching events. $\delta^{15}N_h$ reflect the loss of zooxanthellae during and after the bleaching event, while increases in $\delta^{15}N_z$ indicate increased incorporation of DIN early in recovery to facilitate mitotic cell division and/or chl *a*/cell. Together, $\delta^{13}C_s$, $\delta^{13}C_h$, and $\delta^{13}C_z$ indicate that *M. capitata* switches trophic sources between photoautotrophy and heterotrophy during bleaching and recovery, while *P. compressa* does not.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.gca.2006.02.014.

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