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Acquisition and assimilation of carbon in non-bleached and bleached corals

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ABSTRACT: Reef-forming corals cycle carbon (C) between the coral host, their endosymbiotic algae, and their skeleton. At elevated sea-surface temperatures this relationship breaks down and the corals bleach by expelling their endosymbiotic algae or these algae lose their photosynthetic pigments. The effect of thermally induced bleaching on the C cycling of 2 ecologically important coral species was investigated. The acquisition and assimilation of photoautotrophically and heterotrophically acquired C was examined via pulse-chase labeling experiments in thermally bleached and non-bleached *Montipora capitata* and *Porites compressa* corals. In non-bleached corals photoautotrophic and heterotrophic C were acquired and assimilated very differently. Namely, photoautotrophically acquired C was used to meet short-term metabolic demands and calcification, whereas heterotrophically acquired C was retained in both the coral host and endosymbiotic algae. In bleached corals there was a dramatic reduction in the assimilation of photoautotrophically acquired C by the endosymbiotic algae, in the translocation of C from the algae to the coral host, and in the C assimilated in the skeleton. The level of heterotrophically acquired C assimilated into bleached corals was similar to that in non-bleached corals, and was a direct source of organic C to the endosymbiotic algae. This host-to-endosymbiotic algal supply of heterotrophic C may stimulate endosymbiotic algal recovery. These findings show the importance of both photoautotrophic and heterotrophic C to coral function and demonstrate that both play a crucial role in the recovery from bleaching.

KEY WORDS: *Porites compressa* · *Montipora capitata* · Coral bleaching · Resilience · Stable isotope · Heterotrophy · Photoautotrophy

INTRODUCTION

Mass coral bleaching events caused by elevated seawater temperatures are increasing in frequency (Hoegh-Guldberg 1999) and intensity and threaten to reduce reefs globally by 60% by the year 2030 (Hughes et al. 2003). During such events, some corals bleach and die, others bleach and recover, and some do not visibly bleach at all (Edmunds 1994, Marshall & Baird 2000, Stimson et al. 2002). The causes of this differential response appear to be due to one or more of the following factors: endosymbiotic algal type (Baker 2001, Rowan 2004), endosymbiotic algal density (Stimson et al. 2002), coral morphology (Loya et al. 2001), heterotrophic feeding capacity (Grottoli et al. 2006), and energy reserve concentrations (Rodrigues & Grottoli 2007, Anthony et al. 2009).

When healthy, zooxanthellate corals acquire carbon (C), i.e. food, by 2 means. (1) Corals are effective predators and acquire up to 60% of their fixed C by capturing zooplankton (heterotrophy) from the water column (Goreau et al. 1971, Grottoli et al. 2006, Palardy et al. 2008). (2) The endosymbiotic algae (i.e. zooxanthellae) photosynthetically fix C (photoautotrophy) far in excess of their daily metabolic needs. The vast majority of that C is translocated to the coral host, supplying it with up to 100% of its daily metabolic requirements (Muscatine et al. 1981, Falkowski et al. 1993, Grottoli et al. 2006,

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Palardy et al. 2008). Photosynthesis is also tightly coupled with calcification such that as photosynthesis increases, calcification rates increase (Goreau 1959, Gattuso et al. 1999, Furla et al. 2000).

At elevated seawater temperatures the relationship between coral host and endosymbiotic algae can break down, resulting in the loss of endosymbiotic algae and/or their photosynthetic pigments, and making live corals appear bleached or white. When bleached, coral photosynthesis decreases, the supply of photoautotrophically acquired C decreases, and the coral must meet its metabolic energy requirements from stored energy reserves and/or from heterotrophically acquired C (Grottoli et al. 2006, Rodrigues & Grottoli 2006, Rodrigues & Grottoli 2007).

Previous work has shown that the proportionate contribution of photoautotrophically and heterotrophically acquired C differs among coral species and between bleached and non-bleached corals (Grottoli et al. 2006). However, it is unknown if C from both trophic sources can equally be used for major functions like host tissue growth, endosymbiotic algal growth, and calcification in corals. In addition, it is unknown if C utilization patterns change with bleaching and if that could dramatically influence which coral species are better able to recover from bleaching events. To track the acquisition and allocation of both photoautotrophically and heterotrophically acquired C, bleached and non-bleached *Montipora capitata* and *Porites compressa* corals were exposed to ^{13}C-labeled seawater dissolved inorganic C (DI-^{13}C) and ^{13}C-labeled rotifers (^{13}C-rotifers). The acquisition and allocation of the photoautotrophic and heterotrophic stable isotope label was traced in the coral host tissue, endosymbiotic algae, and skeleton over the course of 7 d.

MATERIALS AND METHODS

Maintenance. Coral specimens were collected from a fringing reef (2–4 m depth) surrounding Moku O Lo'e Island at the Hawaii Institute of Marine Biology in Kaneohe Bay, Hawaii on 11 August 2006. Five large healthy colonies of *Montipora capitata* (branching type) and *Porites compressa* were identified from which 24 coral branch tip fragments (5 cm tall) were collected. The fragments were attached to 20 × 20 mm ceramic tiles, which were placed in 16 flow-through seawater tanks and allowed to acclimatize for 7 d. On 18 August 2006, 8 fragments from each colony (reef controls) were returned to the reef at a depth and location as close as possible to their original colonies. The remaining 16 fragments from each colony were equally divided among non-bleached and bleached treatment tanks. The temperature in the 8 bleached treatment tanks was gradually raised to an average of 30.2°C over the course of a week, using industrial aquarium heaters. Those in the non-bleached treatment tanks were maintained at ambient temperature, which averaged 27.5°C. Temperatures were recorded in each tank and on the reef every 15 min using Hobo UA-002-08 temperature loggers. Daily average temperatures for each tank and on the reef were calculated from all 96 measurements made over each 24 h period. The average (±1 SE) treatment and control tank temperatures were calculated from the daily average values for all treatment and control tanks, respectively. These conditions were maintained for 3.5 wk (see Fig. 1). The light intensities (average ±1 SE) in the tanks were reduced to those observed at collection depth (midday: 12 715 ± 1386 lx) by covering the tanks with 2 layers of neutral-density mesh (midday: 10 429 ± 1874 lx). Light intensity was measured using Hobo UA-002-64 light loggers. On 12 September 2006, the heaters were turned off and the bleached treatment coral tanks were allowed to return to ambient temperature conditions. The reef control fragments were collected from the reef and placed in the tanks, and all coral fragments were allowed to acclimatize to ambient temperature in flow-through tanks for 1 wk. Throughout this entire 5.5 wk period, corals were fed freshly caught zooplankton from the adjacent reef for 1 h at dusk every other night. The aliquot of zooplankton added to each tank was the same on any given night, though the zooplankton composition and abundance varied due to the natural variability of plankton on the reef. No attempt was made to quantify the zooplankton each night, as the goal was merely to provide adequate feeding opportunities to the corals in the tanks.

Photoautotrophic labeling. *DI-^{13}C pulse-chase:* Four fragments from each colony and treatment were incubated in DI-^{13}C seawater. The DI-^{13}C pulse-labeling incubation was conducted on 18 September (6 d after the heaters were switched off) and 20 September (8 d after the heaters were switched off) for *Montipora capitata* and *Porites compressa*, respectively. Six 40 l glass aquaria were filled with 25 l of seawater at 07:30 h. The glass aquaria were placed in the outdoor flow-through tanks surrounded by flowing seawater to help maintain ambient temperature during the pulse incubation. The coral fragments were placed into 4 of the glass aquaria such that 2 aquaria contained bleached coral fragments and 2 contained non-bleached coral fragments. This was done to allow quantification of changes in seawater DI-^{13}C for bleached and unbleached corals. Aquarium 5 contained half of the reef controls. Aquarium 6 served as a DI-^{13}C control (DI-^{13}C added, no coral). At 08:00 h, 4.5 ml of 0.117 M of 98 at.% ^{13}C $NaHCO_3$ was added to the 5 coral-containing aquaria, thereby increasing the total dissolved inorganic carbon

(DIC) concentration from an average (±1 SE) background level of 2070 ± 17.5 to 2090 µmol kg^{-1}, which is a <0.01% increase. The average (±1 SE) initial aquarium seawater $\delta^{13}C_{DIC}$ value was 878.16 ± 8.72‰. After 8 h, coral fragments were removed from the glass incubation aquaria and returned to unlabeled, natural flow-through seawater. Seawater samples were taken from each aquarium for seawater DI-^{13}C analysis, to determine if there had been a significant change in DIC availability over the course of the incubation. One fragment from each colony, treatment, and control, was removed after chase intervals of 4, 16, 24, and 168 h and immediately frozen at −50°C.

Heterotrophic labeling. *^{13}C-rotifer pulse-chase:* Four fragments from each colony and treatment were incubated overnight with ^{13}C-labeled rotifers. The ^{13}C-rotifer pulse-labeling incubation for *Montipora capitata* and *Porites compressa* was conducted on the nights of 18 and 20 September 2006. Eight 40 l glass aquaria were filled with 16 l of seawater at 07:30 h and placed in outdoor flow-through tanks as indicated above. The coral fragments were placed into 4 of the glass aquaria such that 2 aquaria contained bleached treatment coral fragments and 2 aquaria contained non-bleached coral fragments. Aquarium 5 contained the remaining reef controls. Aquarium 6 served as a ^{13}C-rotifer control (^{13}C-rotifer added, no coral), and the last 2 served as seawater controls (no ^{13}C-rotifer, no coral). At 20:00 h it was dark and labeled rotifers were added to the 5 coral-containing aquaria and the ^{13}C-rotifer control aquarium at a density between 10 and 15 rotifers ml^{-1} of seawater. The rotifers had been ^{13}C-labeled by feeding them ^{13}C-labeled *Nanocropsis* paste for 96 h prior to the pulse incubation. Rotifer $\delta^{13}C$ values were 8267.02 ± 476.34 and 3985.51 ± 756.08 (average ± SE) for the *M. capitata* and *P. compressa* incubations, respectively. The large differences in enrichment between the 2 cultures were a result of natural variability as the rotifer cultures developed. The corals and controls were incubated with ^{13}C-rotifer for 10 h. Prior to dawn, the corals were removed from the glass aquaria and returned to unlabeled, natural flow-through seawater. After a chase interval of 4, 16, 24, and 168 h one fragment from each colony, treatment, and control, was removed from the tanks and immediately frozen at −50°C.

Sample analyses. Coral tissue was removed from half of each coral fragment using an air-brush, separated into the coral host and endosymbiotic algal fractions, and individually loaded onto pre-burned glass fiber filters as described in Rodrigues & Grottoli (2006). During the separation of the host and the endosymbiotic algae, there was unavoidable loss of dissolved organic compounds, which may affect the absolute isotopic enrichment values. However, there was no evidence to suggest that this loss varied significantly with physiological state. Thus, the relative isotopic enrichments are directly comparable between treatments. The exposed skeleton was dried, and the uppermost layer was gently shaved with a Dremel tool fitted with a diamond-tipped drill bit. Since the skeletal sampling technique does produce skeletal material that is a combination of old unlabeled growth and new labeled growth, the reported $\delta^{13}C$ enrichment values are in fact a conservative estimate of the true level of skeletal $\delta^{13}C$ enrichment. The coral host and endosymbiotic algae were analyzed for $\delta^{13}C$ by elemental analyzer–stable isotope ratio mass spectrometer (EA-SIRMS), and the skeletal samples were isotopically analyzed by Kiel-SIRMS. All sample preparation and isotopic analyses were performed in Grottoli's Stable Isotope Biogeochemistry Lab at the Ohio State University. Coral tissue and coral endosymbiotic algal filters were individually combusted in a Costech Elemental Analyzer, and the resulting CO_2 gas was automatically analyzed with a Finnigan Delta IV SIRMS via a Finnigan ConFlow III open split interface. Repeated measurements (n = 56) of internal standards had an SD of ±0.05‰. Each coral skeletal sample was finely ground with a mortar and pestle. A subsample of 80 to 100 µg was analyzed for $\delta^{13}C$ using an automated Carbonate Kiel device coupled to a Finnigan Delta IV SIRMS. Samples were acidified under vacuum with 100% ortho-phosphoric acid, and the resulting CO_2 was cryogenically purified and delivered to the mass spectrometer. Approximately 10% of all samples were run in duplicate. Repeated measurements (n = 40) of an internal standard had an SD of ±0.02‰ $\delta^{13}C$. For both the organic and skeletal analyses, the $\delta^{13}C$ values were reported, i.e. per mil deviation of the ratio of stable carbon isotopes ^{13}C:^{12}C relative to Vienna-Peedee Belemnite Limestone Standard (v-PDB).

Filtered seawater samples were taken and preserved with anhydrous MgCl to establish the $\delta^{13}C_{DIC}$ values at the beginning and end of the incubations according to methods by Raymond & Bauer (2001). In the laboratory, each sample was acidified on the vacuum extraction line under high-purity helium flow, with the resulting CO_2 gas cryogenically isolated under vacuum, and the DIC concentration was determined (McNichol et al. 1994). The CO_2 from each DIC sample was sealed in Pyrex ampoules and introduced into the Finnigan Delta IV SIRMS via an automated 10-port inlet. All $\delta^{13}C$ values were reported as per mil values relative to v-PDB. The SD of replicate analyses of an internal standard was ±0.03‰ (n = 37).

Chl *a* was extracted from ground samples in 100% acetone (Jeffrey & Humphrey 1975) and was used as a measure of bleaching, irrespective of algal symbiont density. The samples were taken from corals frozen 4 h post-labeling. There was no attempt to measure the

recovery in chl *a* during the week-long interval between the reduction in temperature and the pulse-chase labeling. However, the chl *a* measurements do accurately reflect the 'bleached' status of the corals at the time of the pulse-chase labeling, which is critical to the interpretation of the isotope data. Endolithic algae were not visible in the skeleton and were assumed not to have contributed any significant amount of chl *a* to the samples.

Statistical analysis. All $\delta^{13}C$ values were reported as enrichment relative to previously published natural isotopic abundance values provided by Rodrigues & Grottoli (2006), where the same species of corals were reared under virtually identical experimental tank conditions that included both thermally bleached and non-bleached corals. Significant differences in $\delta^{13}C$ enrichment among bleached and non-bleached coral hosts, endosymbiotic algae, and skeletal fractions in the DI-^{13}C and ^{13}C-rotifer pulse-chase experiments were determined using a 3-factor ANOVA with 'treatment' being fixed and orthogonal, having 3 levels (bleached, non-bleached, and reef control), 'tissue' being orthogonal and fixed with 3 levels (coral host, endosymbiotic algae, and skeleton), and 'chase' being orthogonal and random with 4 levels (4, 12, 24, and 168 h). All data were tested for homogeneity of variance using Cochran's test, and any data not meeting this assumption were log-transformed with the addition of a constant to make all data points positive. Probabilities <0.05 were considered significant. When main terms or interactions were significant, post hoc Student-Newman-Keuls were conducted (Underwood 1997). Throughout the experiment, coral fragments were rotated within each tank each day to avoid positional effects within a tank. In addition, all corals were rotated randomly among tanks of similar temperature treatment every 4 d to remove any tank position effects. Due to logistical constraints, it was not possible to pulse-chase both species simultaneously. Therefore, pulse-chase labeling experiments were conducted on each species on sequential days. As such, no statistically direct quantitative comparisons have been drawn between species. However, qualitative comparisons between species were still possible. Differences in chl *a* between bleached and non-bleached coral fragments were determined using a Students' *t*-test using Minitab 14.

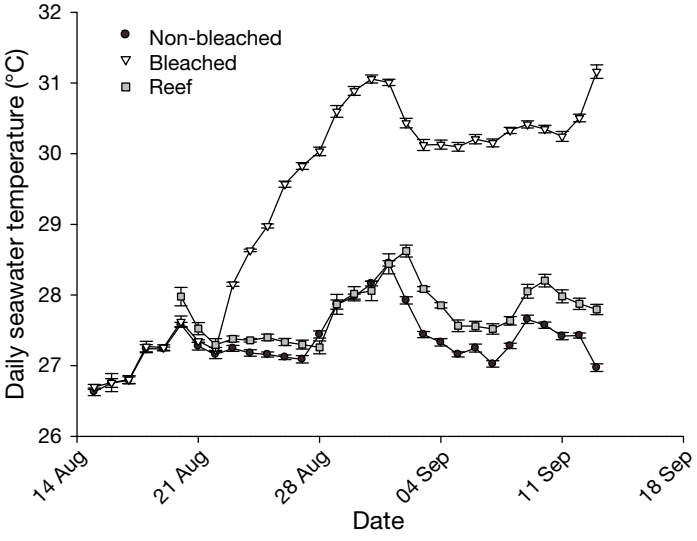

Fig. 1. Daily seawater temperatures (average ± SE) for the 3 different treatments in 2006: non-bleached (●), bleached (▽); and reef control (■)

RESULTS

The water temperature (average ± SE) over the course of the experiment was 30.2 ± 0.20°C for the bleached coral tanks, 27.4 ± 0.08°C for the non-bleached coral tanks, and 27.7 ± 0.07°C for the reef (Fig. 1). At the end of 3.5 wk in the tanks, average chl *a* concentrations of the bleached corals were significantly lower than those of the non-bleached corals (Fig. 2), such that chl *a* was 10.3 and 7.6% of the non-bleached concentrations for *Montipora capitata* and *Porites compressa*, respectively (*t*-test: $F = 188$, $p = 0.001$; $F = 33.1$, $p = 0.001$, respectively).

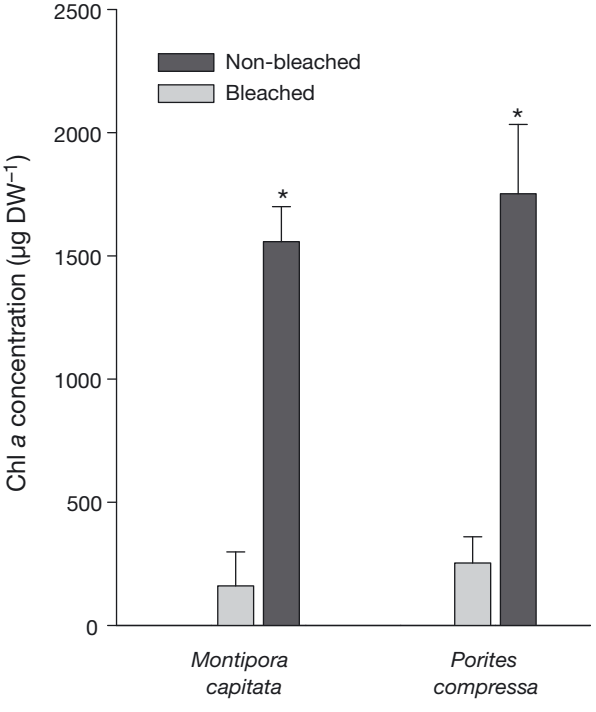

Fig. 2. *Montipora capitata*, *Porites compressa*. Chl *a* concentrations (average ± SE) for bleached and non-bleached corals. *: significant differences between bleached and non-bleached corals within each species; DW: dry weight

Photoautotrophically acquired carbon

Overall, bleached *Montipora capitata* corals were significantly less enriched than the non-bleached corals (Table 1, Fig. 3A,B). There was no significant difference between the non-bleached corals and the reef controls. More specifically, 3 major statistically significant findings were observed. (1) The endosymbiotic algae were more enriched than the coral host tissue in both bleached and non-bleached corals (Table 1, Fig. 3A,B). (2) The skeleton of bleached corals was enriched compared to their endosymbiotic algae (Fig. 3A), but the skeleton and algae did not differ in non-bleached corals (Fig. 3B). (3) Over the course of the chase, enrichment levels were constant in both the coral host and skeleton, but decreased in the endosymbiotic algae at 168 h in both bleached and non-bleached corals (Fig. 3A,B).

Like *Montipora capitata*, bleached *Porites compressa* corals were significantly less enriched than the non-bleached corals overall (Table 2, Fig. 3D,E). However, the reef control corals were significantly less enriched than the non-bleached corals. Specifically, 3 major statistically significant findings were observed that were consistent for both bleached and non-bleached *P. compressa* corals (Table 2, Fig. 3D,E). (1) The coral host tissue was less enriched than either the skeleton or the endosymbiotic algae. (2) Skeleton and endosymbiotic algae did not significantly differ from each other at any time. (3) Skeleton, endosymbiotic algae, and host tissue all decreased over the course of the chase.

Table 1. *Montipora capitata*. Analysis of variance (ANOVA) of the $\delta^{13}C$ enrichment during a 168 h chase following an 8 h incubation with DI-^{13}C-labeled seawater. Data are ln(x+3)-transformed to meet assumptions of homoscedacity (Cochran's test C = 0.1113, not significant). Post hoc Student-Newman-Keuls (SNK) tests were used when terms were significant. Tr: treatment (bleached, non-bleached, reef control); Ti: tissue type (coral host, endosymbiotic algae, skeleton); Ch: chase interval (4, 16, 28, and 168 h); Endo.: endosymbiotic algae

Source	SS	df	*F*-ratio	p
ANOVA				
Treatment	66.20	2	123.74	<0.0001
Tissue	23.91	2	23.41	0.0015
Chase	6.19	3	8.96	<0.0001
Tr × Ti	3.03	4	4.84	0.0147
Tr × Ch	1.60	6	1.16	0.3299
Ti × Ch	3.06	6	2.22	0.0445
Tr × Ti × Ch	1.88	12	0.68	0.7682
Residual	33.14	144		
Total	139.02	179		
Post hoc SNK test of significant terms				
Tissue × Chase				
Coral host	04 = 16 = 28 = 168			
Endo.	04 = 16 = 28 > 168			
Skeleton	04 = 16 = 28 = 168			
04	Coral host < Endo. = Skeleton			
16	Coral host < Endo. = Skeleton			
28	Coral host < Endo. < Skeleton			
168	Coral host = Endo. < Skeleton			
Treatment × Tissue				
Coral host	Temperature < Control = Reef control			
Endo.	Temperature < Control = Reef control			
Skeleton	Temperature < Control = Reef control			
Bleached	Coral host < Endo. < Skeleton			
Non-bleached	Coral host < Endo. = Skeleton			
Reef control	Coral host < Endo. = Skeleton			

Heterotrophically acquired carbon

For *Montipora capitata*, average coral host and endosymbiotic algal enrichment values did not differ from each other, did not differ between bleached and non-bleached corals, and were both more enriched than their skeletons (Table 3, Fig. 4A,B). However, both host tissue and endosymbiotic algae in the reef control corals were significantly more enriched than in both the bleached and non-bleached corals. The skeleton of the bleached corals was less enriched that that of non-bleached and reef control corals. Over the 168 h of the experiment, there was no significant change in enrichment levels in the host tissue, endosymbiotic algae, or skeleton in either bleached or non-bleached corals.

Like *Montipora capitata*, the average coral host and endosymbiotic algal enrichment values of *Porites compressa* did not differ from each other, and were both more enriched than their skeletons in bleached and non-bleached corals (Table 4, Fig. 4D,E). However, the host tissue in the reef control corals was more enriched than in both the bleached and non-bleached corals. The skeleton of the bleached corals was less enriched than that of non-bleached and reef control corals.

DISCUSSION

The relationship between endosymbiotic algae and the coral host is pivotal to the health of each polyp, colony, and ultimately the reef ecosystem. The current study shows that coral bleaching fundamentally disrupts this relationship and alters the way in which photoautotrophic and heterotrophic C is assimilated throughout the holobiont.

Photoautotrophically acquired carbon

In all corals the dramatically elevated $\delta^{13}C$ enrichment values in endosymbiotic algae within the first 4 h of the chase are indicative of a rapid photosynthetic uptake of the DI-^{13}C label by the endosymbiotic algae

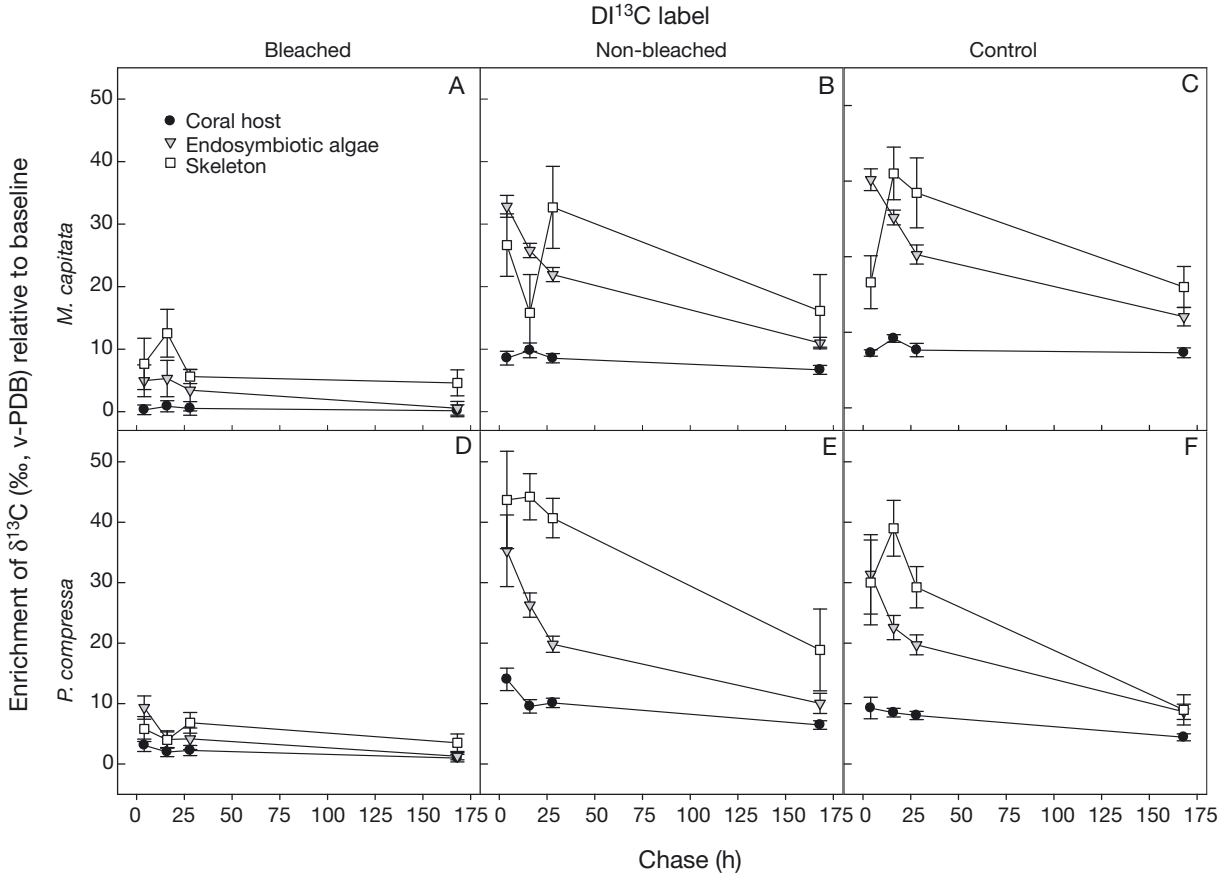

Fig. 3. *Montipora capitata*, *Porites compressa*. $\delta^{13}C$ enrichment (average ± SE) of coral host (●), endosymbiotic algae (▽), and skeleton (□) of (A) bleached, (B) non-bleached; and (C) reef control *M. capitata* corals, and (D) bleached, (E) non-bleached, and (F) reef control *P. compressa* corals during a 168 h chase following an 11 h incubation in $DI^{13}C$-labeled seawater. Values are given as enrichment relative to previously published natural abundance baseline values (Rodrigues & Grottoli 2006). v-PDB: Vienna-Peedee Belemnite Limestone Standard

Table 2. *Porites compressa*. ANOVA of the $\delta^{13}C$ enrichment during a 168 h chase following an 8 h incubation with DI-^{13}C-labeled seawater. Data are ln(x+1)-transformed to meet assumptions of homoscedacity (Cochran's test C = 0.988, not significant). Post hoc SNK tests were used when terms were significant. Abbreviations as in Table 1

Source	SS	df	*F*-ratio	p
ANOVA				
Treatment	94.22	2	170.43	<0.0001
Tissue	28.10	2	27.92	0.0009
Chase	23.09	3	32.25	<0.0001
Tr × Ti	1.81	4	3.12	0.0565
Tr × Ch	1.66	6	1.16	0.3320
Ti × Ch	3.20	6	2.11	0.0557
Tr × Ti × Ch	1.73	12	0.61	0.8334
Residual	34.36	144		
Total	188.00	179		

Post hoc SNK test of significant terms

Treatment
Bleached < Reef control < Non-bleached

Tissue
Coral host < Skeleton = Endo.

Chase
168 < 04 = 16 = 28

Table 3. *Montipora capitata*. ANOVA of the $\delta^{13}C$ enrichment during a 168 h chase following an 11 h incubation with ^{13}C-labeled rotifers. Data are untransformed and meet assumptions of homoscedacity (Cochran's test C = 0.126, not significant). Post hoc SNK tests were used when terms were significant. Abbreviations as in Table 1

Source	SS	df	*F*-ratio	p
ANOVA				
Treatment	361.12	2	12.74	0.0069
Tissue	3454.91	2	76.54	0.0001
Chase	75.16	3	1.03	0.3828
Tr × Ti	130.66	4	9.10	0.0013
Tr × Ch	85.00	6	0.58	0.7454
Ti × Ch	135.42	6	0.92	0.4791
Tr × Ti × Ch	43.07	12	0.15	0.9996
Residual	3514.43	144		
Total	7799.80	179		

Post hoc SNK test of significant terms

Treatment × Tissue

Coral host	Bleached = Non-bleached < Reef control
Endo.	Bleached = Non-bleached < Reef control
Skeleton	Bleached < Non-bleached = Reef control
Bleached	Skeleton < Coral host = Endo.
Non-bleached	Skeleton < Coral host = Endo.
Reef control	Skeleton < Coral host = Endo.

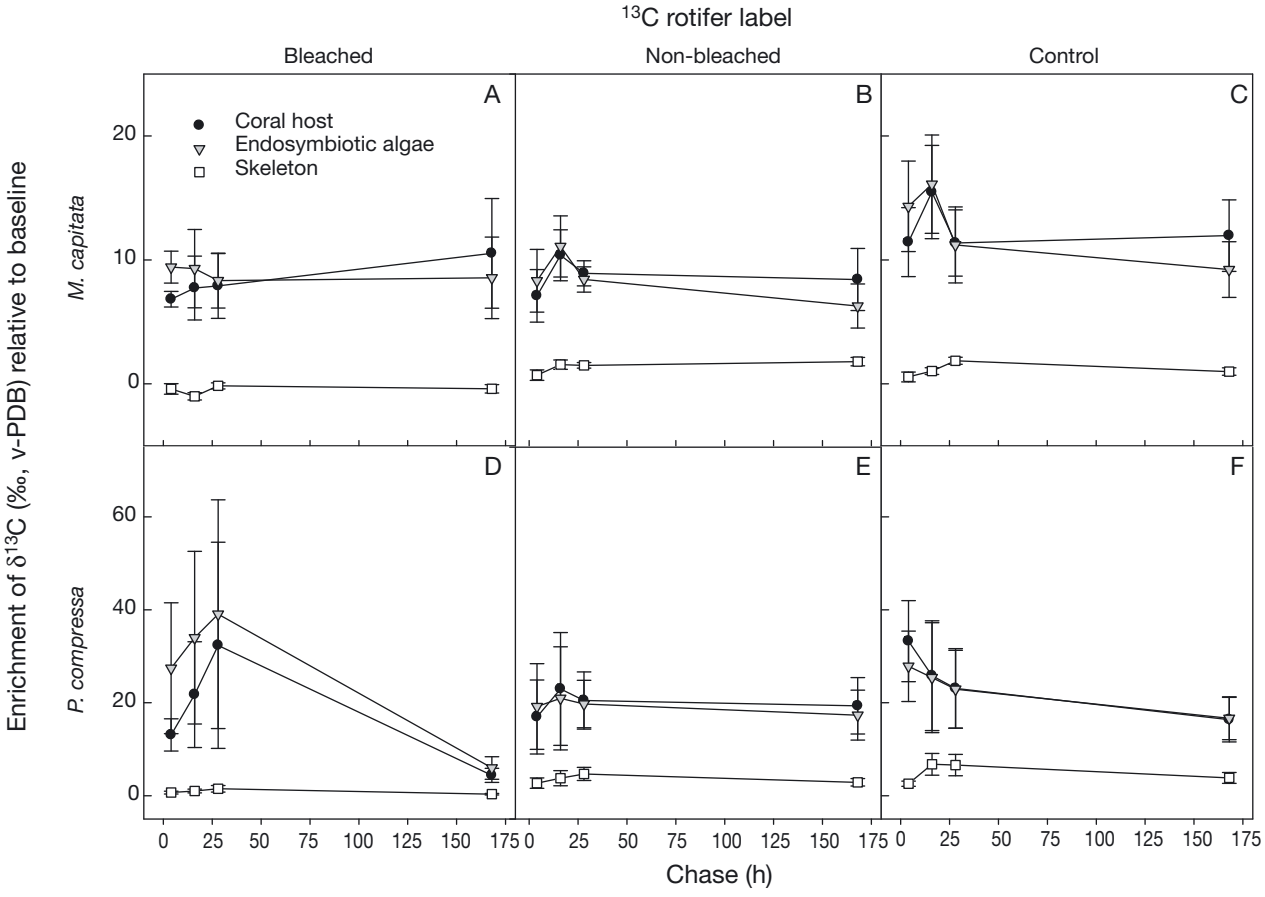

Fig. 4. *Montipora capitata*, *Porites compressa*. $\delta^{13}C$ enrichment of coral host (●), endosymbiotic algae (▽), and skeleton (□) of (A) bleached, (B) non-bleached, and (C) reef control *M. capitata* corals, and (D) bleached, (E) non-bleached, and (F) reef control *P. compressa* corals during a 168 h chase following an 11 h incubation with ^{13}C-labeled rotifers. Values are given as enrichment relative to previously published natural abundance baseline values (Rodrigues & Grottoli 2006). v-PDB: Vienna-Peedee Belemnite Limestone Standard

from the water column (Fig. 3B,C,E,F). There was also modest $\delta^{13}C$ enrichment of all coral hosts except bleached *Porites compressa*. This indicates a rapid translocation of some of this fixed C to the coral host. This is consistent with previous work showing that C translocation occurs within minutes of photosynthetic fixation (Battey & Patton 1984). The levels of enrichment in the host were lower than in the endosymbionts due to the host tissue representing a much larger C pool within which to dilute the labeled C than the endosymbiotic algae. The endosymbiotic algae of both species showed a significant decrease in $\delta^{13}C$ enrichment values over the course of the chase. There are 4 possible mechanisms for this rapid decrease: (1) the rapid translocation of material to the coral host, (2) the respiration of labeled C by the symbiotic algae, (3) the dilution of the labeled C pool by the formation of new non-labeled photosynthate, or (4) some combination of one or more of these mechanisms. Given the rapid decrease over the first 12 h when the corals are in darkness, translocation of material to the coral host and endosymbiont respiration are the most likely principle mechanisms for the reduction in isotopic enrichment of the endosymbiotic algae during the course of the chase.

The lower uptake of C by the endosymbiotic algae via photosynthesis in bleached corals was to be expected, since bleaching reduces photosynthesis rates by 90 and 67%, for *Montipora capitata* and *Porites compressa,* respectively (Rodrigues & Grottoli 2007). There was no evidence of transloction in bleached *M. capitata*, as shown by the close to or below zero values of the coral host. This lack of any translocation of photosynthate from the endosymbiotic algae to coral host may play a role in triggering the enhanced feeding observed in this species during recovery from bleaching (Grottoli et al. 2006, Palardy et al. 2008) and highlights the importance of heterotrophically acquired C for thermally bleached corals.

In the host tissue there was a significant reduction in the isotopic enrichment over the course of the chase in *Porites compressa* (Fig. 3E,F), but not in *Montipora capitata* (Fig. 3B,C). These results indicate that, for

Table 4. *Porites compressa.* ANOVA of the $\delta^{13}C$ enrichment during a 168 h chase following an 11 h incubation with ^{13}C-labeled rotifers. Data are ln(x+1)-transformed to meet assumptions of homoscedacity (Cochran's test C = 0.126, not significant). Post hoc SNK tests were used when terms were significant. Abbreviations as in Table 1

Source	SS	df	*F*-ratio	p
ANOVA				
Treatment	12.80	2	7.65	0.0223
Tissue	94.94	2	171.94	<0.0001
Chase	7.35	3	3.14	0.0272
Tr × Ti	4.14	4	4.89	0.0142
Tr × Ch	5.02	6	1.07	0.3811
Ti × Ch	1.66	6	0.35	0.9064
Tr × Ti × Ch	2.54	12	0.27	0.9927
Residual	112.21	144		
Total	240.65	179		

Post hoc SNK test of significant terms

Treatment × Tissue

Coral host	Bleached = Non-bleached < Reef control
Endo.	Bleached = Non-bleached = Reef control
Skeleton	Bleached < Non-bleached = Reef control
Bleached	Skeleton < Coral host = Endo.
Non-bleached	Skeleton < Coral host = Endo.
Reef control	Skeleton < Coral host = Endo.

Chase

168 < 04 = 16 = 28

P. compressa, the photosynthetically derived C assimilated by the coral host was rapidly respired by the coral host to meet metabolic demands, exuded possibly as mucus, diluted by post-pulse assimilated C, or some combination of these factors. The preferential use of photoautotrophically acquired C for coral host respiration, as opposed to longer term storage, has been reported in another Cnidarian, the symbiotic anemone *Aiptasia* sp. (Bachar et al. 2007). While C loss via mucus secretions by hermatypic corals has been well described (Crossland et al. 1980, Crossland 1987, Ferrier-Pages et al. 1998, Tanaka et al. 2009), the source of this C is less well understood. For *Acropora pulchra*, it has been shown that existing C stores, not newly fixed C, are the C source for the dissolved organic carbon (DOC) secretions in corals (Tanaka et al. 2008). Although mucus is a combination of particulate organic carbon (POC) and DOC, if DOC secretions are indicative of mucus secretions in all corals, it would suggest that organic C loss through mucus production is not the principal cause of the decrease in host $\delta^{13}C$ values over the chase. Since much of the $\delta^{13}C$ reduction occurred predominantly during the first night, when uptake of photosynthetic C was zero (i.e. the first 12 h of the chase), the dilution effect would have been very small. Thus, the observed reduction in the *P. compressa* host $\delta^{13}C$ enrichment values was probably due to the respiration of the labeled C. No such reduction was observed for *M. capitata*, which has a lower respiration rate than *P. compressa* (Coles & Jokiel 1977, Rodrigues & Grottoli 2007). This suggests that, while photoautotrophically acquired C is probably used for short-term metabolic demand in *P. compressa*, the lower metabolic demand of *M. capitata* allows for a greater proportion of photoautotrophically acquired C to be assimilated into storage products in the host.

There are 2 possible sources of C for calcification: seawater DIC and the internal pool of inorganic C produced from respiration (Goreau 1959, Pearse 1970, Erez 1978, Furla et al. 2000, Grottoli 2002). As such, there are 2 pathways in which labeled inorganic C can enter the skeleton: either directly from the labeled seawater DIC or indirectly from the enriched CO_2 liberated as the coral host and endosymbiotic algae respire the isotopically enriched photoautotrophically acquired C. This labeled CO_2 then forms part of the internal pool of DIC and could be cycled back into photosynthesis or used as a source of C for skeletal calcification. By comparing the enrichment in the skeleton of bleached and non-bleached corals, it is possible to make an estimate of the contribution of these 2 sources to skeleton calcification. In bleached corals, photosynthesis is greatly reduced and, consequently, so is the respiration of labeled photosynthate; therefore, the primary source of C for calcification will be the labeled seawater DIC. In non-bleached corals, where there is abundant respiration of labeled photosynthate, C should be assimilated into the skeleton through both pathways. The ratio of the 2 skeletal enrichment values (non-bleached/bleached) can thus provide an estimate of the contribution of the 2 sources to skeleton coral C. For *Montipora capitata* and *Porites compressa*, this calculation reveals that an estimated 30 and 17% of the C for calcification comes from external DIC, respectively. In other words, 70 and 83% of the C for calcification comes from respired C in *M. capitata* and *P. compressa*, respectively. These estimates are in agreement with evidence that respired CO_2 is the principle source of C for skeletogenesis (Erez 1978). Furla et al. (2000) calculated that 75% of skeletal C is metabolically derived, and our estimates are in concordance with this figure, but they do not agree with the conclusions by Al-Horani et al. (2005) that seawater DIC is the main source of C for calcification.

The observed gradual decline in the isotopic skeletal enrichment over the course of the experiment could have 2 possible explanations. Firstly, that the C for calcification came primarily from the internal pool of respired DIC originally derived from photoautotrophically acquired C. As the endosymbiotic algae and the coral host became less labeled, so would their respired CO_2, leading to a reduction in the labeling of the skele-

ton. The second mechanism is dilution of the labeled skeleton by growth-related gains of new non-labeled skeletal C over the course of the chase. Most likely, both mechanisms are involved.

Heterotrophically acquired C in bleached and non-bleached corals

Two principle patterns were observed with respect to the assimilation of heterotrophically acquired C in the host tissue and algal symbionts. (1) The tissue and zooxanthella-enrichment values were always similar and greater than skeletal enrichment values for bleached, non-bleached, and reef control corals. (2) Enrichment levels were consistent over the course of the chase in *Montipora capitata*, but decreased in *Porites compressa*.

For the first principle pattern, the isotopic homogeneity between the coral host and endosymbiotic algae in all corals of both species indicates that once ingested by the coral host, the labeled rotifers are rapidly digested and the labeled C is assimilated by both the animal host and the endosymbiotic algae. There are 2 possible non-mutually exclusive assimilation pathways into the endosymbiotic algae for this heterotrophically acquired C — the assimilation of heterotrophically derived DIC or the assimilation of heterotrophically derived DOC. During the night, respiration of labeled organic rotifer C by the coral host labels the inorganic C pool within the host. As digestion and host respiration continue during the morning, the endosymbiotic algae utilize the internal, now labeled, metabolic DIC for photosynthesis. It has been estimated that metabolically derived DIC accounts for between 60 and 75% of the C fixed during photosynthesis (Muscatine et al. 1989, Furla et al. 2000) — the current results suggest that this value could be higher. The now labeled photosynthetically fixed C is then translocated back to the host, where it is respired once more by the host and continues to internally recycle in this way over the course of the week-long chase period. Thus, in this assimilation pathway, heterotrophic C is delivered from the host to the algal symbionts as DIC via host respiration. This scenario implies that heterotrophically derived C is readily recycled within the coral holobiont.

The second assimilation pathway of heterotrophically acquired C by the endosymbiotic algae involves the transport of organic C. Transport of inorganic nutrients, primarily inorganic nitrogen and phosphorus, from the host to the endosymbiotic algae has been well documented (Szmant-Froelich & Pilson 1984, Muller-Parker et al. 1990) and has been shown to stimulate colony growth and photosynthesis (Ferrier-Pages et al. 2003). Transfer of organic nitrogen has also been demonstrated between Cnidarian hosts and endosymbiotic algae (Piniak et al. 2003, Tanaka et al. 2006). *Symbiodinium* spp. have been characterized as facultative heterotrophs (Steen 1987) and have exhibited the ability to assimilate organic C compounds from their hosts (Carroll & Blanquet 1982, Steen 1986). As such, it is possible that heterotrophically acquired C is being transported directly from the coral host to the endosymbiotic algae in the form of DOC. We can get a better idea of which C, DIC or DOC, is being translocated by the host to the zooxanthellae by examining the skeleton of bleached corals in more detail. Both species of coral calcify immediately following bleaching (Rodrigues & Grottoli 2006). If heterotrophically derived C were being translocated from the host to the endosymbiontic algae in the form of labeled-DIC, we would expect the skeleton of bleached corals to be isotopically enriched (Fig. 4A,D). Since their skeletons are barely isotopically enriched, the primary source of C being translocated from the host to the endosymbiotic algae appears to be predominantly in the form of DOC. A supply of organic C from the host would allow for the maintenance of the remaining facultatively heterotrophic endosymbiotic algal population during bleaching and act as a source of energy for the recovery of the endosymbiotic algae. Either way (i.e. via DIC or DOC), the heterotrophically acquired C is recycled between the animal host and endosymbiotic algae and is an equally important source of fixed C to bleached and non-bleached corals. Interestingly, the levels of heterotrophic C assimilation can be quite variable, as evidenced by the large error bars in the $\delta^{13}C$ enrichment values of bleached *Porites compressa* in the first 24 chase hours (Fig. 4D) and is most likely due to variability in feeding rates among individual coral colonies on any given night.

The second principle pattern observed was that enrichment levels were consistent over the course of the chase in *Montipora capitata*, but decreased in *Porites compressa*. In *M. capitata*, there was no reduction in the levels of enrichment in the skeleton or any of the tissues after the initial assimilation of labeled heterotrophically derived carbon (Fig. 3A–C). This steady state indicates that, once acquired, the heterotrophic carbon was not used to meet metabolic demands, but was assimilated within the coral host and endosymbiotic algae into either storage or structural compounds. A similar pattern was observed in the anemone *Aiptasia* sp., where heterotrophically acquired carbon was stored throughout the organism's body and utilized to build structures such as proteins and membranes (Bachar et al. 2007). However, for *P. compressa*, there was a significant decrease in $\delta^{13}C$ enrichment levels over the course of the chase (Fig. 4D–F). Stored

carbon has been shown to be the primary source of carbon of expelled DOC from corals (Tanaka et al. 2008), and zooplankton fed corals release more carbon as DOC than starved corals (Ferrier-Pages et al. 1998). Thus, the decreases in $\delta^{13}C$ enrichment could be due to a combination of respiration and DOC or mucus secretions in *P. compressa*.

CONCLUSIONS

The present study has shown that the cycling of photoautotrophic and heterotrophic carbon between the components of the holobiont is very different and that the nature of this difference can vary between both species and health status. These differences may play a direct role in a species' ability to recover from bleaching and in its resilience to bleaching events. The present study provides a comprehensive picture of the possible species-specific resilience and adaptive strategies to coral bleaching. Bleached *Montipora capitata* increases heterotrophic carbon acquisition (Grottoli et al. 2006, Palardy et al. 2008) and uses this to maintain energy reserves (Rodrigues & Grottoli 2007), to sustain its host tissue and endosymbiotic algal population (present study), and to sustain reproduction (Cox 2007), but at the expense of calcification (Rodrigues & Grottoli 2006). In contrast, bleached *Porites compressa* does not increase its heterotrophic carbon acquisition (Grottoli et al. 2006, Palardy et al. 2008) and is more reliant on the consumption of stored energy reserves (Grottoli et al. 2006, Rodrigues & Grottoli 2007) to maintain its calcification rates (Rodrigues & Grottoli 2006) and fuel endosymbiotic algal recovery (present study). For species like *M. capitata*, whose recovery from bleaching is fueled by the capture of plankton, recovery will be dependant on the health of the zooplankton community and, hence, the condition of the overlying water column. On reefs with healthy zooplankton populations, coral species that are able to increase feeding when bleached have a competitive advantage over coral species that rely on energy reserves, particularly as the frequency and duration of bleaching events increases in the future (Hoegh-Guldberg 1999). However, which types of species will have a competitive advantage on reefs with declining coastal water quality (Fabricius 2005) is unclear.

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