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Energy reserves and metabolism as indicators of coral recovery from bleaching

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Abstract

With reduced zooxanthellae, chlorophyll *a* (Chl *a*), or both, concentrations, bleached corals rely on some combination of energy reserves (i.e., lipid, carbohydrate, protein) and heterotrophy to survive and recover. To understand the dynamics of energy reserves and metabolism during long-term recovery, *Porites compressa* and *Montipora capitata* corals were experimentally bleached in outdoor tanks for 1 month (treatment corals). Additional corals were maintained in separate tanks at ambient temperatures (control corals). Recovery occurred on the reef for 0, 1.5, 4, or 8 months. At 0 months all treatment corals were white in color, with lower Chl *a*, lipid, carbohydrate, protein, tissue biomass, and photosynthesis than control corals. During recovery, *P. compressa* replenished energy reserves and tissue biomass at 8 mo, long after photosynthesis and Chl *a* had recovered at 1.5 and 4 months, respectively. *M. capitata* replenished energy reserves at 1.5 months, despite decreased Chl *a* and photosynthesis levels. *P. compressa* depends on photosynthetically fixed carbon for recovery from bleaching, whereas *M. capitata* does not. Overall, *M. capitata* had a faster recovery rate than *P. compressa* for all measured variables except Chl *a* concentration. With intensifying bleaching, coral diversity on future reefs may favor species with faster recovery rates.

Coral bleaching is primarily caused by elevated seawater temperatures, ultraviolet radiation, or both, resulting in decreased endosymbiotic zooxanthellae or photosynthetic pigments and a pale to white coral colony (e.g., Brown 1997). Bleaching severity and mortality varies among individuals, species, depths, and locations (e.g., Loya et al. 2001). Research on the underlying mechanisms driving variability has focused on zooxanthellar temperature constraints (e.g., Smith et al. 2005) and genetic variation (e.g., Baker 2001) to explain bleaching susceptibility. The few studies that investigated the role of the coral host either examined a single time period (Porter et al. 1989; Grottoli et al. 2004, 2006) or were unable to control for seasonal effects over long-term observations of recovery (Fitt et al. 1993, 2000). No studies have both distinguished seasonal from bleaching effects on the host *and* followed these effects over long-term recovery.

In healthy corals, photosynthetically fixed carbon is translocated from zooxanthellae to host, providing up to 100% of daily metabolic requirements (Muscatine et al. 1981; Grottoli et al. 2006). Excess is stored in the host as lipids at concentrations of 10–40% of total biomass (Stimson 1987; Porter et al. 1989; Grottoli et al. 2004) and represents a significant energy reserve in corals (Edmunds and Davies 1986; Harland et al. 1993).

In bleached corals, decreased zooxanthellae densities or chlorophyll *a* (Chl *a*) levels result in decreased net photosynthesis (Porter et al. 1989; Lesser 1997; Grottoli et al. 2006). Photosynthetically fixed carbon translocated to the host decreases, leaving bleached corals to rely on stored lipid, carbohydrate, or protein reserves to survive and recover. As expected, these reserves and tissue biomass decrease in most (Porter et al. 1989; Fitt et al. 1993, 2000; Grottoli et al. 2004, 2006), but not all (Grottoli et al. 2004, 2006), bleached corals. Bleached *Montipora capitata* maintained energy reserves by increasing heterotrophy (Grottoli et al. 2006).

To investigate long-term dynamics in coral host physiology during recovery from bleaching, *Porites compressa* and *M. capitata* were bleached with elevated seawater temperatures for 1 month in outdoor tanks and compared with control (nonbleached) corals. Afterward, all corals recovered on the reef under natural conditions. Throughout 8 months of recovery, mortality rate, Chl *a*, zooxanthellae, lipid, carbohydrate, protein, tissue biomass, photosynthesis, and respiration of temperature-treated corals were compared with untreated control corals. This design allowed for a quantitative assessment of the hypotheses that (1) Chl *a,* zooxanthellae, and photosynthesis decrease after bleaching and gradually increase during recovery; (2) during recovery, the coral host will (a) consume stored energy reserves (i.e., lipids, carbohydrates, protein, and tissue biomass), (b) reduce metabolic rate (i.e., respiration) to conserve stored energy reserves, or (c) maintain energy reserves and respiration,

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indicating that heterotrophy is supplementing stored reserves.

Materials and methods

Study site—Corals were collected from Kaneohe Bay, Hawaii (21°26.18′N, 157°47.56′W). Seawater temperatures average 27°C ± 1.0°C (1 SE) from June to October and 24.5°C ± 1.5°C from November to May (from Hawaii Institute of Marine Biology weather station). *P. compressa* is branching and yellow-brown to dark brown in color. *M. capitata* is plating to branching and medium to dark brown in color. All collected fragments of *M. capitata* were branching.

Experimental design—The experimental design is described in detail in Rodrigues and Grottoli (2006). Briefly, 8 5-cm-tall fragments from 12 large colonies of each species were collected at 2 m depth in August 2003. One fragment from each colony was randomly placed in each of eight tanks fitted with a 50-μm filter that reduced zooplankton and coral heterotrophy in the tanks. For 1 month starting 04 September 2003, seawater temperature in four tanks was raised to 30.1°C ± 0.05°C using aquarium heaters (treatment), while seawater temperature was ambient (26.8°C ± 0.04°C) in the other four control tanks. Within each treatment and control group, one fragment from each colony was randomly assigned to 0, 1.5, 4, or 8 months of recovery. Corals were rotated within and among tanks of the same treatment to minimize any positional and tank effects. The experiment mimicked the timing, duration, and temperature of a 1996 natural bleaching event in Kaneohe Bay (Jokiel and Brown 2004).

On 04 October 2003, the condition (nonbleached, bleached, partially bleached, dead, or partially dead) and photosynthesis (P) and respiration (R) rates (described below) of each fragment in the 0-month recovery group were determined. They were then frozen at −80°C for further laboratory analyses. Remaining fragments were placed at 2-m depth for recovery. The same procedure was repeated after 1.5 months (16 November 2003), 4 months (02 February 2004), and 8 months (04 June 2004) of recovery.

Metabolic analyses—P and R rates were measured on at least three whole fragments from each temperature and recovery group within 2-liter plastic chambers using a YSI 550 dissolved oxygen meter (Lesser 1997). Fragments were analyzed within 4 d of each other to minimize any effects of changing day length, weather, and days of recovery. P and R were standardized to coral ash-free dry tissue biomass. P was measured under ambient daylight between 11:00 h and 14:00 h. R was measured in a darkened chamber immediately after P analyses during the day and 2 h after sunset at night. Dissolved oxygen was recorded every 3 min for 30 min (P) or 40 min (R). Chamber seawater was continuously stirred with a magnetic propeller and stirring plate. The entire apparatus, except the stirring plate, was placed inside a flow-through seawater tank to minimize coral disturbance and maintain a constant temperature. Chambers were rinsed with fresh seawater between trials.

Laboratory analyses—All measurements were made on whole coral samples (skeleton + animal tissue + zooxanthellae) ground with a mortar and pestle and normalized to total ash-free dry tissue biomass of the organic fraction (animal tissue + zooxanthellae) according to Grottoli et al. (2004). Only tissue biomass was standardized to surface area. For *P. compressa*, sample surface area was calculated from branch tips using a cork borer of known area. Because of the irregular surface of *M. capitata*, sample surface area was calculated for a cone or cylinder depending on shape of the branch tips.

Chl *a*, zooxanthellae, lipid, carbohydrate, and protein concentrations were measured on five separate branch tips from each fragment. Chl *a* and total lipids were extracted according to Grottoli et al. (2004), without the methanol wash in the lipid procedure (T. Pease pers. comm.). Zooxanthellae were separated from the animal tissue by centrifugation. Three subsamples were counted with a hemacytometer and light microscope. Total carbohydrate was extracted using the phenol-sulfuric acid method (Dubois et al. 1956) with glucose as a standard. Total protein was extracted using the bicinchoninic acid method (Smith et al. 1985) with bovine serum albumin as a standard (Pierce BCA Protein Assay Kit). Sample sizes differed for each extraction because of partial death during recovery and limited material for all extractions.

Statistical analyses—Analysis of variance (ANOVA) compared the effects of species, genotype, temperature, and recovery interval on Chl *a*, zooxanthellae, lipid, carbohydrate, protein, and tissue biomass (Table 1). Because of lower sample size, P, day R, and night R were analyzed for each species separately (Table 2). A posteriori slice tests (i.e., tests of simple effects, Winer 1971) determined if treatment and control averages significantly differed within species and recovery interval. Bonferroni corrections were not used (Quinn and Keough 2002). Replicate genotypes across temperature treatments and recovery intervals reduced overall variation between treatments. Since treatment and control corals were exposed to identical conditions except temperature during the first month, differences during recovery were independent of season and could be attributed to bleaching alone. When treatment values were not statistically different from control values at a single recovery interval, they are referred to as "fully recovered" throughout the text.

All data were normally distributed according to plots of residuals versus 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.) Values of $p \leq 0.05$ were considered significant.

Results

Mortality—At the start, all fragments of *P. compressa* and *M. capitata* were nonbleached (dark brown color).

Table 1. Results of five four-way ANOVAs for average chlorophyll *a* ($F_{37,103}=8.21$, $p<0.0001$), zooxanthellae ($F_{33,46}=1.27$, $p=0.2260$), lipid ($F_{37,116}=4.17$, $p<0.0001$), carbohydrate ($F_{37,115}=3.24$, $p<0.0001$), protein concentrations ($F_{37,115}=3.11$, $p<0.0001$), and tissue biomass ($F_{37,120}=26.67$, $p<0.0001$), comparing two species (*Porites compressa* and *Montipora capitata*) from 12 colonies or genotypes, at two temperatures (ambient and 30°C), and four recovery intervals (0, 1.5, 4, and 8 months). Effects of temperature (T), recovery interval (R), and species (S) were fixed and fully crossed. Genotype was a random effect, nested within species (G in S). Because of reduced sample size, interaction terms involving genotype were combined with the residual.

Variable	Effect	df	SS	*F*-statistic	*p*-value
Chlorophyll *a*	T	1	12,916,054	71.17	<0.0001
	R	3	17,490,479	32.13	<0.0001
	S	1	380,368	2.10	0.1507
	T × S	1	4,246,141	23.40	<0.0001
	R × S	3	1,750,755	3.22	0.0260
	T × R	3	6,465,556	11.88	<0.0001
	T × R × S	3	1,357,109	2.49	0.0642
	G in S	22	4,075,457	1.02	0.4468
Zooxanthellae	T	1	1.3×10^{15}	7.38	0.0093
	R	3	6.7×10^{14}	1.29	0.2907
	S	1	5.0×10^{14}	2.90	0.0956
	T × S	1	1.2×10^{15}	7.05	0.0108
	R × S	3	4.1×10^{14}	0.79	0.5080
	T × R	3	5.7×10^{14}	1.09	0.3644
	T × R × S	3	4.3×10^{14}	0.82	0.4888
	G in S	22	1.1×10^{15}	0.35	0.9910
Lipids	T	1	0.200	30.56	<0.0001
	R	3	0.034	1.71	0.1689
	S	1	0.262	40.04	<0.0001
	T × S	1	0.015	2.37	0.1265
	R × S	3	0.036	1.84	0.1429
	T × R	3	0.078	3.98	0.0097
	T × R × S	3	0.003	0.15	0.9326
	G in S	22	0.184	1.28	0.1997
Carbohydrates	T	1	0.015	9.63	0.0024
	R	3	0.038	8.28	<0.0001
	S	1	0.023	14.77	0.0002
	T × S	1	0.002	1.39	0.2402
	R × S	3	0.013	2.85	0.0406
	T × R	3	0.012	2.64	0.0528
	T × R × S	3	0.004	0.84	0.4762
	G in S	22	0.035	1.05	0.4157
Proteins	T	1	4.262	3.95	0.0491
	R	3	23.823	7.37	0.0001
	S	1	36.173	33.56	<0.0001
	T × S	1	0.878	0.81	0.3687
	R × S	3	5.925	1.83	0.1452
	T × R	3	5.177	1.60	0.1931
	T × R × S	3	3.863	1.19	0.3151
	G in S	22	17.875	0.75	0.7742
Tissue biomass	T	1	79.904	23.09	<0.0001
	R	3	93.305	8.99	<0.0001
	S	1	2,263.616	654.16	<0.0001
	T × S	1	11.232	3.25	0.0741
	R × S	3	44.074	4.25	0.0069
	T × R	3	25.146	2.42	0.0693
	T × R × S	3	38.726	3.73	0.0132
	G in S	22	89.171	1.17	0.2863

df, degrees of freedom; SS, sum of squares of the effect.

Control corals remained nonbleached, with no mortality throughout the study. Treatment *M. capitata* and *P. compressa* showed signs of visible bleaching (pale to white color) after 8 d and 14 d, respectively, of increased seawater temperatures. Total plus partial mortality of treatment fragments primarily occurred in the first 1.5 months of recovery with rates of 78% and 61% for *P. compressa* and *M. capitata*, respectively (Fig. 1A,B). Visible recovery (brown color) started after 1.5 and 4 months for *P. compressa* and *M. capitata*, respectively.

Table 2. Results of six three-way ANOVAs for average photosynthesis rate (*Porites compressa*: $F_{14,14}=1.33$, $p=0.3000$; *Montipora capitata*: $F_{15,22}=1.84$, $p=0.0946$), day respiration rate (*P. compressa*: $F_{14,14}=1.56$, $p=0.2083$; *M. capitata*: $F_{15,22}=1.34$, $p=0.2591$), and night respiration rate (*P. compressa*: $F_{14,14}=0.74$, $p=0.7113$; *M. capitata*: $F_{15,22}=0.75$, $p=0.7100$), comparing 12 colonies or genotypes, at two temperatures (ambient and 30°C), and four recovery intervals (0, 1.5, 4, and 8 months) within two species (*P. compressa* and *M. capitata*), separately. Effects of temperature (T) and recovery interval (R) were fixed and fully crossed. Genotype (G) was a random effect. Interaction terms involving genotype were combined with the residual.

Variable	Effect	df	SS	*F*-statistic	*p*-value
Porites compressa					
Photosynthesis rate	T	1	0.3616	1.54	0.2355
	R	3	0.4362	0.62	0.6148
	T × R	3	1.2527	1.77	0.1981
	G	7	1.2123	0.74	0.6460
Day respiration rate	T	1	0.0861	2.82	0.1151
	R	3	0.1052	1.15	0.3634
	T × R	3	0.0916	1.00	0.4209
	G	7	0.2506	1.17	0.3763
Night respiration rate	T	1	0.0135	1.28	0.2764
	R	3	0.0216	0.69	0.5757
	T × R	3	0.0090	0.29	0.8337
	G	7	0.0460	0.63	0.7269
Montipora capitata					
Photosynthesis rate	T	1	3.5093	7.29	0.0131
	R	3	1.6181	1.12	0.3625
	T × R	3	4.6937	3.25	0.0413
	G	8	3.0487	0.79	0.6159
Day respiration rate	T	1	0.0986	1.84	0.1884
	R	3	0.0729	0.45	0.7167
	T × R	3	0.3015	1.88	0.1627
	G	8	0.5033	1.18	0.3566
Night respiration rate	T	1	0.0104	0.52	0.4794
	R	3	0.1478	2.46	0.0897
	T × R	3	0.0386	0.64	0.5963
	G	8	0.0948	0.59	0.7745

df, degrees of freedom; SS sum of squares of the effect.

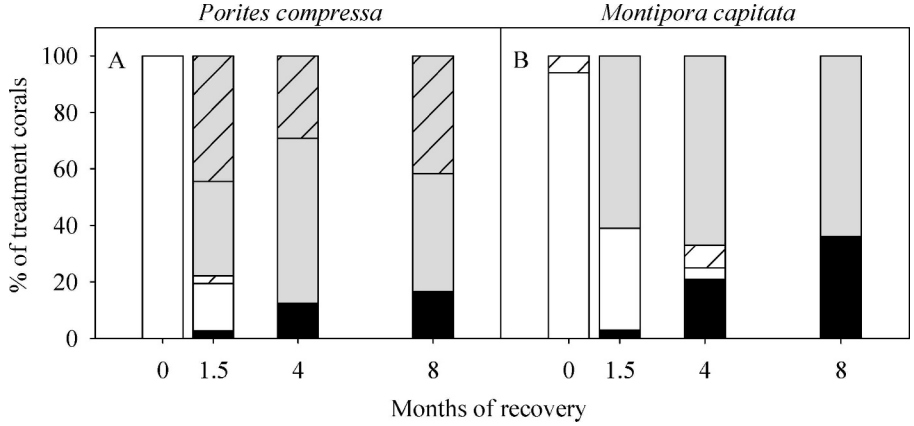

Fig. 1. Percentage of nonbleached, bleached, partially bleached, dead, and partially dead (A) *Porites compressa* treatment corals and (B) *Montipora capitata* treatment corals at 0, 1.5, 4, and 8 months of recovery. Nonbleached fragments were dark brown in color, not significantly different in appearance from control fragments, and completely covered by living tissue (black bars). Bleached fragments were white in color and completely covered by living tissue (white bars). Partially bleached fragments were pale brown in color and completely covered by living tissue (white hatched bars). Dead fragments were completely covered by filamentous or encrusting algae (or both), with no living tissue remaining (gray bars). Partially dead fragments were partially covered by filamentous or encrusting algae (or both) and partially covered by patches of living tissue that varied in color from light to dark brown (gray hatched bars). There is no partially dead category for *M. capitata*.

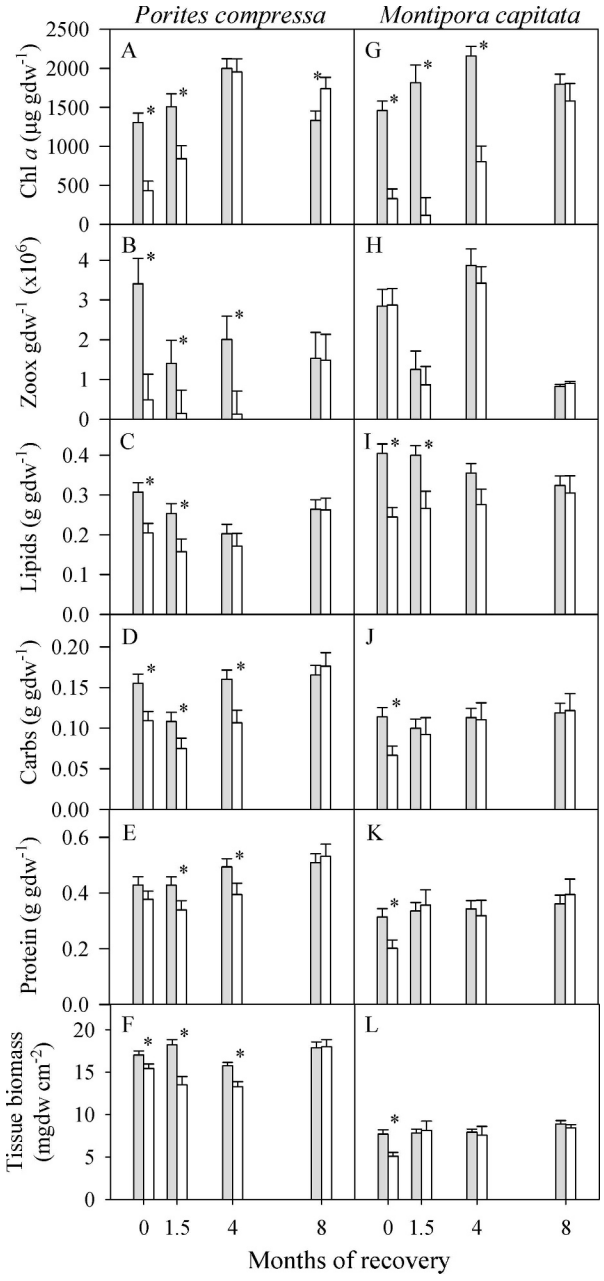

Fig. 2. Average (A, G) Chl *a* concentrations, (B, H) zooxanthellae (zoox) concentrations, (C, I) lipid concentrations, (D, J) carbohydrate (carbs) concentrations, (E, K) protein concentrations, and (F, L) tissue biomass in control (gray bars) and treatment (white bars) *Porites compressa* and *Montipora capitata* at 0, 1.5, 4, and 8 months of recovery. All averages are standardized to grams of ash-free dry tissue weight (gdw) and are shown ± 1 SE. Symbols (*) indicate significant differences at $p < 0.05$ between means within a single time interval by a posteriori least-squares mean slice tests. Sample sizes range between 4 and 12. Statistical analyses are in Table 1.

Total plus partial mortality was 88% and 83% for treatment *P. compressa* at 4 and 8 months, respectively, whereas no additional *M. capitata* corals died after 1.5 months. Treatment *M. capitata* experienced no partial mortality, with tissue either completely dying or surviving. After 8 months, 17% of *P. compressa* and 36% of *M. capitata* had visibly recovered.

P. compressa—At 0 months, Chl *a* concentration in treatment *P. compressa* decreased to 33% of control fragments (Fig. 2A). At 1.5 months, Chl *a* in treatment fragments increased to 56% of control corals, was fully recovered at 4 months, and exceeded that of the controls at 8 months. Meanwhile, zooxanthellar concentration decreased to 14%, 11%, and 6% of controls at 0, 1.5, and 4 months, respectively, and fully recovered at 8 months (Fig. 2B). Therefore, Chl *a* cell^{-1} increased while zooxanthellar density was low.

Energy reserves also fluctuated throughout recovery. At 0 and 1.5 months, lipid concentrations of treatment *P. compressa* decreased to 67% and 62% of control levels, respectively, then fully recovered by 4 and 8 months (Fig. 2C). Treatment carbohydrate concentrations were 70%, 69%, and 67% of control levels at 0, 1.5, and 4 months, respectively, and fully recovered by 8 months (Fig. 2D). Treatment protein concentrations were not significantly different from control levels at 0 months, but were 79% and 80% of control levels at 1.5 and 4 months, respectively, before full recovery by 8 months (Fig. 2E). Treatment tissue biomass was 91%, 74%, and 84% of control levels at 0, 1.5, and 4 months, respectively, before full recovery by 8 months (Fig. 2F).

These physiological changes were mirrored in *P. compressa* metabolism. At 0 months, treatment gross P rates and day R rates were 33% and 55% of control rates, respectively, and fully recovered throughout 1.5, 4, and 8 months (Fig. 3A,B). Night R rates were not significantly different for treatment and control fragments throughout recovery (Fig. 3C).

M. capitata—In treatment *M. capitata,* Chl *a* concentration decreased more dramatically than *P. compressa* to 23% of control levels at 0 months and continued to decrease to just 6% at 1.5 months (Fig. 2G). There were signs of recovery at 4 months when treatment concentrations were 37% of controls, and full recovery occurred by 8 months. Despite changes in Chl *a*, treatment zooxanthellar density was not significantly different from controls throughout recovery (Fig. 2H).

Similar to *P. compressa*, lipid concentrations in treatment *M. capitata* decreased to 61% and 67% of controls at 0 and 1.5 months, respectively, before full recovery at 4 and 8 months (Fig. 2I). At 0 months, treatment carbohydrate, protein concentrations, and tissue biomass decreased to 58%, 64%, and 66% of controls, respectively (Fig. 2J–L). All three variables were fully recovered at 1.5, 4, and 8 months.

Some metabolic changes also occurred in treatment *M. capitata*. At 0 months, gross P and day R rates were not different from controls, but were 10% and 39% of control levels, respectively, at 1.5 months (Fig. 3D,E). Both P and day R fully recovered at 4 and 8 months. Like *P. compressa*, night R rates were not significantly different for treatment and control fragments throughout recovery (Fig. 3F).

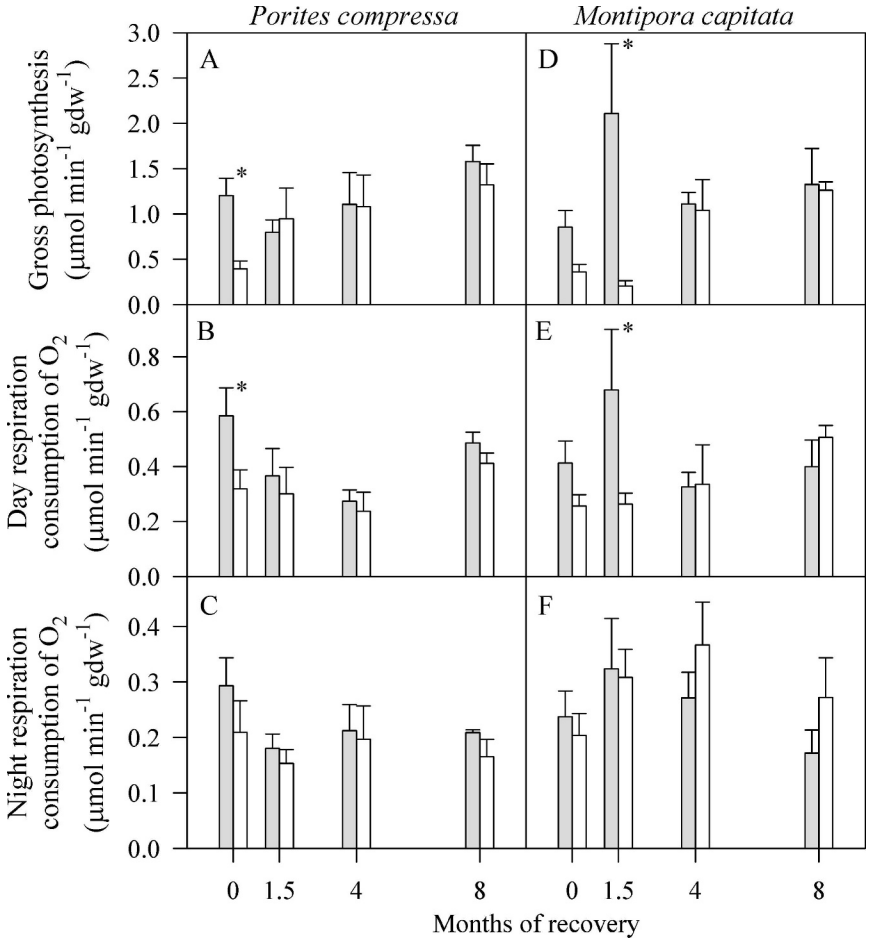

Fig. 3. Average (A, D) photosynthesis rate, (B, E) day respiration rate, and (C, F) mean night respiration rate in control (gray bars) and treatment (white bars) *Porites compressa* and *Montipora capitata* at 0, 1.5, 4, and 8 months of recovery. All averages are rates (per min) standardized to grams of ash-free dry tissue weight (gdw) and are shown ±1 SE. Symbols (*) indicate significant differences at $p < 0.05$ between means within a single time interval by a posteriori least-squares mean slice tests. Sample sizes range between 2 and 7. Statistical analyses are in Table 2.

Interaction effects—Some interactions among temperature treatment (T), recovery interval (R), and species (S) effects were significant (Tables 1, 2). This was because (1) there was a greater difference between treatment and control Chl *a* levels in *M. capitata* than *P. compressa*, and zooxanthellae density in *P. compressa* compared with *M. capitata* (T × S effects); (2) Chl *a*, carbohydrate concentrations, and tissue biomass in treatment fragments recovered at different times for each species (R × S effects, and T × R × S effect for tissue biomass only); and (3) Chl *a*, lipid concentrations, and gross P rates for *M. capitata* differed between treatment and controls as recovery progressed (T × R effects).

Discussion

Mortality due to bleaching—Mortality rates were similar to those of several Hawaiian corals maintained at 31°C for 30 d (Jokiel and Coles 1977). Similar partial mortality of *P. compressa* also occurred after freshwater inundation to Kaneohe Bay (Jokiel et al. 1993). Overall, *M. capitata* fragments were twice as likely as *P. compressa* to fully recover from bleaching after 8 months (Fig. 1). This is consistent with findings that calcification rates in *M. capitata* fully recovered by 8 months, while those of *P. compressa* took longer (Rodrigues and Grottoli 2006).

Chl a and zooxanthellae—Chl *a* concentrations were similar to those after a natural bleaching event in the same species and location (Grottoli et al. 2004). Therefore, this experimental study is probably a good reflection of a natural bleaching event in Hawaii. In *P. compressa*, Chl *a* and zooxanthellae concentration initial decrease and gradual recovery (Fig. 2A,B) are consistent with observed P rates (Fig. 3A) and findings in other species after natural (e.g., Porter et al. 1989; Fitt et al. 1993, 2000) and experimental (Hueerkamp et al. 2001) bleaching. In *P. compressa,* Chl *a* recovered (Fig. 2A) while zooxanthellae

density was low (Fig. 2B), suggesting that it is more cost-effective (for the host or the zooxanthellae or both) to increase Chl *a* per cell than regrow zooxanthellae, and that photosynthetic efficiency per cell is high.

In contrast, Chl *a* concentration in *M. capitata* continued to decrease after returning to ambient seawater temperatures (Fig. 2G), similar to *Pavona clavus* after experimental bleaching (Hueerkamp et al. 2001). *M. capitata* zooxanthellae density was constant despite decreased Chl *a* concentration after experimental bleaching with increased temperature (Fig. 2H) and ultraviolet radiation (Grottoli-Everett and Kuffner 1995). Therefore, this species has the same bleaching response for different stressors. Zooxanthellae density in *Montipora monasteriata* was constant after short-term temperature-induced bleaching, despite decreased levels of photosynthetic pigments (Dove et al. 2006). Therefore, maintaining zooxanthellar density when stressed while depleting pigment levels may be restricted to the genus *Montipora*.

Of the two species, only *P. compressa* may be able to acquire new zooxanthellar type(s) as required by the adaptive bleaching hypothesis (Buddemeier and Fautin 2002). Further research is needed to determine if zooxanthellar type changes in response to bleaching stress in *P. compressa*, and what the ecological advantages are for corals like *M. capitata* that do not lose zooxanthellae when bleached.

Energy reserves and tissue biomass—For treatment *P. compressa*, recovery of lipids (4 months), carbohydrates (8 months), protein (8 months), and tissue biomass (8 months) coincided with or followed recovery of gross photosynthesis (1.5 months) and Chl *a* (4 months). Therefore, treatment *P. compressa* relied on stored reserves when bleached, and was dependent on photosynthetically fixed carbon for survival and recovery. This is consistent with the generalized pattern that Chl *a* and zooxanthellae concentrations recover first, lagged by energy reserves and tissue biomass (Fitt et al. 1993, 2000).

In treatment *M. capitata*, recovery of lipids (4 months), carbohydrates (1.5 months), protein (1.5 months), and tissue biomass (1.5 months) preceded or coincided with recovery of gross photosynthesis (4 months) and Chl *a* (8 months). This is probably because bleached *M. capitata* can meet 100% of daily metabolic requirements from heterotrophy (Grottoli et al. 2006). $\delta^{13}C$ analyses of host tissue and zooxanthellae indicated that fixed carbon was heterotrophically acquired at 1.5 months in treatment *M. capitata* before resuming photoautotrophic acquisition at 4 and 8 months (Rodrigues and Grottoli 2006). Tank seawater was filtered during bleaching, reducing zooplankton and coral heterotrophy, and accounting for decreased energy reserves and tissue biomass at 0 months. Therefore, *M. capitata* depends upon stored reserves whenever both photosynthesis and heterotrophy are not feasible. Under natural bleaching conditions with zooplankton available, no change in energy reserves or tissue biomass would be expected. In the field, *M. capitata* bleached with increased ultraviolet radiation maintained lipid reserves (Grottoli-Everett 1995). Here, increased heterotrophy between 0 and 1.5 months had the significant and long-term effect of restoring and maintaining energy reserves throughout recovery.

The order that reserves recovered differed between the species. *P. compressa* recovered lipids, then carbohydrates and protein, while *M. capitata* recovered carbohydrates and protein, then lipids. Two years after natural bleaching, *Montastraea annularis* had increased carbohydrates during the first 10 months, lipids during the last 14 months, and protein during the whole 2-yr period (Fitt et al. 1993). Therefore, energy reserves are differentially metabolized and synthesized by different species throughout recovery. In the case of *P. compressa*, reliance on photosynthesis indicates that carbohydrates would be translocated, whereas lipids and proteins must be synthesized. However, lipids recovered first in *P. compressa*, providing further evidence that photosynthetically derived carbohydrates are immediately stored in the host as lipids (Muscatine and Cernichiari 1969; Patton et al. 1977). For *M. capitata*, heterotrophy provided lipids, carbohydrates, and proteins, so reserves were rebuilt faster and more efficiently than in *P. compressa*.

Metabolism—For both species, decreased P : R ratios after bleaching are consistent with other Hawaiian species (Coles and Jokiel 1977). P rates fully recovered in both species when Chl *a* was 50% recovered, indicating that P rates were not dependent upon total Chl *a* available in nonbleached corals. Furthermore, zooxanthellae density was less than 15% recovered in *P. compressa* when P rates fully recovered. Dubinsky et al. (1990) found P rates per cell inversely correlated with zooxanthellar density, and attributed this to competition among zooxanthellae for available CO_2. Light absorption was also inversely correlated with zooxanthellar pigments because of mutual shading (Stambler and Dubinsky 2005), suggesting that Chl *a* or zooxanthellae in nonbleached corals is present in surplus.

M. capitata had lower R rates compared with other species (Coles and Jokiel 1977), possibly allowing bleached colonies to better conserve energy reserves. Here, nonbleached *M. capitata* only had lower R rates than *P. compressa* at 0 months, coinciding with the timing of the earlier study in August to September 1974 (as reported in Coles et al. 1976). In treatment fragments of both species, day R only decreased when P rates and Chl *a* were at their lowest levels (Fig. 3), possibly indicating reduced metabolic activity of remaining zooxanthellae. Night R rates and therefore overall demand for stored energy reserves by the host were the same irrespective of bleaching status in both species.

Recovery strategies—*P. compressa* tolerated temperature stress, taking 6 d longer to visibly bleach than *M. capitata* during experimental (this study) and 35 d longer during natural (Grottoli et al. 2004) bleaching events. Once bleached, *P. compressa* had a higher mortality and lower probability of full recovery than *M. capitata* (Fig. 1). Of those that survived early recovery, *M. capitata* was more likely to fully recover than *P. compressa*.

Therefore, surviving bleaching appears to be a trade-off between initial temperature tolerance and long-term energy reserve maintenance. Although *P. compressa* was more tolerant of initial temperature stress, once bleached it was dependent on stored reserves. In contrast, *M. capitata* was more susceptible to initial temperatures stress, but once bleached it increased heterotrophy (Grottoli et al. 2006) and maintained stored reserves. By 8 months, the proportion of nonbleached *M. capitata* was more than double that of *P. compressa* (Fig. 1). Although less temperature tolerant initially, bleached *M. capitata* appeared more resilient than *P. compressa* in the long term.

In the future, species like *P. compressa* that tolerate temperature stress will dominate reefs after short events (less than 1 month). However, once bleached or after longer events, maintaining or replenishing energy reserves (like *M. capitata*) may better predict reef composition. Corals that acquire fixed carbon predominantly from photosynthesis (like *P. compressa*) can only survive while energy reserves are available and death will occur once they are depleted. Consecutive events would further hamper recovery, since it took 8 months to fully replenish carbohydrate and protein stores (Fig. 2D,E) and longer for calcification (Rodrigues and Grottoli 2006). Corals that acquire significant fixed carbon from photosynthesis and heterotrophy (like *M. capitata*) could survive regardless of bleaching duration or frequency. With bleaching events expected to increase in duration and frequency, future reefs will likely be dominated by species that maintain energy reserves and have faster recovery rates, like *M. capitata*, rather than species with greater temperature tolerance but slower recovery rates, like *P. compressa*.

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