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Journal of Experimental Marine Biology and Ecology 358 (2008) 136 – 143

Journal of EXPERIMENTAL MARINE BIOLOGY AND ECOLOGY

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Lipid class composition of bleached and recovering *Porites compressa* Dana, 1846 and *Montipora capitata* Dana, 1846 corals from Hawaii

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Received 30 January 2008; accepted 1 February 2008

Abstract

Corals rely on stored reserves, especially lipids, to survive bleaching events. Lipid class composition reveals the lipid source, and provides evidence of metabolic changes (i.e., photoautotrophic or heterotrophic) during bleaching and recovery. *Porites compressa* Dana, 1846 and *Montipora capitata* Dana, 1846 corals were experimentally bleached in outdoor tanks with seawater temperature elevated to 30 °C (treatment corals). Additional control fragments were maintained in separate tanks at ambient temperatures (27 °C). After one month, all fragments were returned to the reef for 0, 1.5, 4, or 8 months. Lipid class composition was analyzed by Iatroscan (thin layer chromatography-flame ionization detection). In treatment *P. compressa*, triacylglycerol (TG) decreased at 0 and 1.5 months, phospholipid (PL) also decreased at 1.5 months, and both remained lower relative to controls along with wax esters (WE) after 8 months. Neither treatment nor control *P. compressa* had any detectable monoacylglycerol (MG) or diacylglycerol (DG). Overall, *P. compressa* first consumed available storage, then structural lipids, and all lipid classes remained low at the end of the study. In treatment *M. capitata*, TG and PL decreased, while MG increased relative to controls at 0 months. At 4 months, free fatty acid (FFA), sterol (ST), and PL in treatment *M. capitata* were two to ten times higher than controls. Treatment and control lipid class composition were not different from each other at 8 months. In contrast to *P. compressa*, *M. capitata* consumed some lipid classes and augmented others, probably due to sequential metabolism of storage lipids and increased heterotrophy. Overall, lipid class assimilation was more rapid in treatment *M. capitata* corals that switch between heterotrophy and photoautotrophy, than in treatment *P. compressa* corals that rely mostly on photoautotrophy.
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Keywords: Coral bleaching; Energy reserves; Heterotrophy; Lipid classes; Photoautotrophy; Recovery

1. Introduction

Coral bleaching events are primarily caused by increased seawater temperatures and/or solar radiation that result in a reduction of endosymbiotic zooxanthellae and/or photosynthetic pigments giving the colony a white or “bleached” appearance (Brown, 1997; Hoegh-Guldberg, 1999). When healthy, photosynthetically fixed carbon from zooxanthellae can provide the coral host with up to 100% of its daily metabolic energy requirements (Muscatine et al., 1981; Edmunds and Davies, 1986; Davies, 1991; Grottoli et al., 2006). Excess fixed carbon is stored in the host tissue as lipids (Muscatine and Cernichiari, 1969; Patton et al., 1977; Battey and Patton, 1984), representing an important energy reserve (Edmunds and Davies, 1986; Harland et al., 1993). Seasonal variability in total lipid concentration is often related to rates of photosynthesis and light intensity (Stimson, 1987). Total lipid concentrations decline in some (Porter et al., 1989; Fitt et al., 1993; Yamashiro et al., 2005; Rodrigues and Grottoli, 2007) but not all (Grottoli et al., 2004; Rodrigues and Grottoli, 2007) species when zooxanthellae and/or chlorophyll *a* concentrations are low. Total lipid recovery occurred 4 months after bleaching in both *Porites compressa* and *Montipora capitata* corals due to increased photosynthesis and increased heterotrophy, respectively (Rodrigues and Grottoli, 2007), and was verified by $\delta^{13}C$ of the host tissue

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doi:10.1016/j.jembe.2008.02.004

and zooxanthellae (Rodrigues and Grottoli, 2006). Analyses of lipid class composition may provide further evidence of photosynthesis, heterotrophy, and the variation in lipid metabolism after bleaching in these two species.

In healthy corals, triacylglycerol (TG) and wax esters (WE) are the main storage lipids, accounting for 40-73% of total lipids, with sterols (ST) and phospholipids (PL), the main structural lipids making up 9-60% of total lipids (Harland et al., 1993; Yamashiro et al., 1999, 2005; Oku et al., 2002; Grottoli et al., 2004). Levels of the storage lipids (TG and WE) can fluctuate with coral reproductive rate (Stimson, 1987), egg production (Arai et al., 1993), zooxanthellae productivity (Patton et al., 1977; Stimson, 1987; Oku et al., 2003a), metabolic requirements (Crossland et al., 1980; Oku et al., 2002), food availability (Crossland et al., 1980), mucus production (Crossland et al., 1980) and bleaching status (Grottoli et al., 2004). The structural lipids (ST and PL) typically remain stable with short-term changes in nutrient state (Meyers, 1979), though both have been found to fluctuate with short-term sedimentation and heat stress (Niebuhr, 1999) and with *in situ* bleaching (Grottoli et al., 2004).

Changes in lipid class composition in healthy anthozoans occur against background seasonal changes. Free fatty acids (FFA) in a healthy anthozoan increased in the spring and summer months prior to spawning and then subsequently decreased afterwards with lowest levels in the winter (Pernet et al., 2002). In contrast, PL and FFA in healthy *Goniastrea aspera* corals were found to be lowest in the summer and highest in the winter, while TG and WE were highest in the summer and lowest in the winter, suggesting that lipid content was associated with changes in photosynthesis due to changes in light intensity, water temperature or some combination of both (Oku et al., 2003b).

To date, only two studies have investigated the immediate effects of bleaching on lipid class composition in corals. Decreased total lipids, 1.5 months after a natural bleaching event were mostly due to decreased storage lipids in *P. compressa*, while in *M. capitata* some storage lipids decreased but another, diacylglycerol (DG) increased (Grottoli et al., 2004). Similarly, the proportion of storage lipids decreased, resulting in a proportionate increase in some structural lipids in eight coral species approximately one month after a natural bleaching event (Yamashiro et al., 2005). Both studies involved one-time sampling following natural bleaching events. No previous studies have followed lipid class composition in corals over long time periods after a bleaching event.

Here, a detailed investigation of changes in lipid class composition, rather than total lipids, was conducted to investigate detailed changes in lipid metabolism during bleaching and for 8 months after bleaching. Specifically, this study aimed to provide insights into (1) how two coral species manage (by photosynthesis and/or heterotrophy) their lipid reserves, (2) whether management varies between these two species, and (3) whether different management strategies influence resilience after coral bleaching.

To distinguish seasonal changes in lipid class composition from changes due to bleaching, a manipulative experiment was conducted. Corals were bleached with increased seawater temperature in outdoor flow-through tanks for one month followed by eight months on the reef at ambient seawater temperatures. Lipid class concentrations in temperature treated *P. compressa* and *M. capitata* were compared to untreated control corals at 0, 1.5, 4, and 8 months. This design allowed for a quantitative assessment of the following hypotheses, independent of seasonal variation in total lipids or lipid classes: (1) that in both species, storage [TG, WE, DG, monoacylglycerol (MG), and FFA] and structural (ST and PL) lipids decrease during bleaching due to loss or use of specific lipid classes and gradually increase throughout the 8-month period due to assimilation of new lipids; and (2) that in both species, lipid class composition reflects the relative contribution from assimilation of fixed carbon acquired either photosynthetically or heterotrophically.

2. Methods

2.1. Study site

Corals were collected from Kaneohe Bay, Hawaii (21°26.18′N; 157°47.56′W). Seawater temperatures average 27.2 °C ± 1.0 (mean ± SD) from June to October and 24.4 °C ± 1.5 (mean ± SD) from November to May (data from Hawaii Institute of Marine Biology weather station). *P. compressa* is branching, ranging in color from yellow-brown to dark brown. *M. capitata* is plating to branching and medium to dark brown in color. All collected *M. capitata* were branching.

2.2. Experimental design

A detailed description of the tank design can be found in Rodrigues and Grottoli (2006). Briefly, in August 2003, nine fragments were collected from each of twelve large, healthy colonies (genotypes) of *P. compressa* and *M. capitata* at 2 m depth. Eight of the fragments were randomly placed in one of eight tanks filled with filtered seawater that reduced zooplankton and coral heterotrophy. On 4 September 2003, the seawater temperature in four tanks was raised to 30.1 °C ± 1.2 (mean ± SD) for one month, while ambient seawater temperature in the other four control tanks remained at 26.8 °C ± 0.9 (mean ± SD) for the same period. Within each treatment, one fragment from each colony was randomly assigned to each time treatment, of 0, 1.5, 4, or 8 months on the reef under ambient conditions following the one-month period in tanks. All tanks contained 24 fragments, composed of 12 fragments from each species, with each genotype represented once and each assigned time period represented three times. 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). Since the two species are sympatric (colonies are side-by-side across the fringing reef), they would be exposed to the same temperature conditions during natural bleaching events. Therefore, they were exposed to the same conditions during the experiment to accurately assess the

physiological and biochemical consequences of bleaching on each species.

The ninth fragment that was collected from each colony was randomly assigned as a 'reef control' treatment. It was not placed in any tank, but was handled in the same way as all other fragments, then returned to the reef for the one-month period. The reef control allowed for an assessment of whether manipulation and handling of the fragments impacted their overall lipid class composition.

At the end of the one-month tank period, all control corals remained unbleached (dark brown in color) and all treatment corals showed visible signs of bleaching (white in color), with no mortality of any corals during the tank period (Rodrigues and Grottoli, 2007). Visible bleaching was later quantitatively confirmed with analyses of chlorophyll *a* concentration that showed significant decreases in the treatment corals of both species compared to control corals (Rodrigues and Grottoli, 2007). On 4th October 2003, the 0 month group were frozen at -80 °C. All remaining experimental and control fragments were returned to the reef at 2 m depth. At 1.5 months (16th November 2003), 4 months (2nd February 2004), and 8 months (4th June 2004), the respective, pre-assigned corals were recollected and frozen prior to lipid analyses.

2.3. Lipid class analyses

Total lipids were extracted and analysed from ground whole coral samples (reported in Rodrigues and Grottoli, 2007). Extracted lipids were stored in 5 ml of 100% chloroform at -80 °C until lipid classes could be analyzed. Three treatment and three control colonies per species and time period were analyzed for lipid classes. All 12 colonies were not utilized for lipid class analyses because of the time and expense required to conduct the analyses. Each sample was completely dried under ultra-high purity N_2 gas and resuspended in 100% hexane using the following quantities: (1) control *P. compressa* – 200 μl, (2) treatment *P. compressa* – 50 μl, (3) control *M. capitata* – 250 μl, and (4) treatment *M. capitata* – 75 μl (different quantities allowed samples with different total lipid concentrations to be eluted at the same time and in the same run). Total lipids were separated into lipid classes by applying 1 μl of each sample onto thin layer quartz-impregnated rods (Chromarods® Mitsubishi Kagaku Iatron, Inc.) and subjected to a sequential two-step elution scheme. First, hydrocarbon (HC), WE, and fatty acid methyl ester (FAME) fractions were separated from the origin by thin layer chromatography with 99:1:0.05 (v:v:v) hexane:ethyl ether:formic acid for 30 min. After removal of residual solvent, the rods were scanned to 75% of their length using flame ionization detection with an Iatroscan Mark III analyzer. Then, TG, FFA, alcohol (AL), DG, ST, MG, and PL fractions were separated with 80:20:0.1 (v:v:v) hexane:ethyl ether:formic acid for an additional 30 min. Following solvent removal, the entire length of the rods was scanned. Each sample was run in duplicate.

The amount of each lipid class from each sample was determined by comparison to 1 μl of standard mixes with known concentrations, ranging from 0.125 μg μl^{-1} to 1.25 μg μl^{-1}. Standard mixes were composed of 5-α-cholestane for HC, hexadecyl hexadecanoate for WE, methyl stearate for FAME, tripalmitin for TG, stearic acid for FFA, octadecanol for AL, dipalmitin for DG, stigmastanol for ST, DL-α-palmitin for MG, and phosphatidyl dipalmitol for PL. During the second elution, AL and DG separated in the standard mixes, but with one eluted peak at their position in the coral samples. No previous studies on coral lipid class composition have found AL (Niebuhr 1999; Yamashiro et al., 1999, 2005; Oku et al., 2003b; Grottoli et al., 2004); consequently, the peak is referred to as DG although AL may contribute to this peak. Standard curves of concentration versus peak area were calculated for each lipid class and were used to determine the concentrations of lipid classes in each sample. Each lipid class concentration was normalized to the amount of total lipid initially extracted for each sample and standardized to ash-free dry tissue weight (mg gdw^{-1}).

2.4. Statistical analyses

Analyses of variance (ANOVA) were conducted to compare reef control and 0-month control concentrations for each lipid class. The effects of species, type of control (whether reef or 0-month) and genotype were included in the analyses. Effects of species and type of control were fixed and fully crossed. Genotype was a random effect and nested within species. Interaction terms involving genotype were combined with the sum of squares of the error, rather than with the model of the ANOVA. Bonferroni corrections were not used (Quinn and Keough, 2002).

An ANOVA was used to analyze the effects of class, species, genotype, temperature, and time period on lipid class concentration (Table 1). *A posteriori* Tukey tests determined significant differences among means within each effect. *A posteriori* slice tests (e.g., tests of simple effects: Winer, 1971) determined if bleached and control treatment averages significantly differed at each time period within each lipid class and species. Significant p-values are reported with numerical subscripts that refer to the time period that was tested. Power analyses were conducted for all non-significant ANOVAs. Since treatment and control corals were exposed to identical conditions except temperature during the first month, differences were independent of season and could be attributed to bleaching alone.

Data was normally distributed according to plots of residuals versus predicted values. All statistical analyses were generated using SAS software, Version 9.1.3 of the SAS System for Windows. Differences were considered statistically significant at $p<0.05$.

3. Results

Comparison of reef control and 0-month control concentrations revealed no significant differences between groups for most lipid classes. However, power was low (≤0.300) for all analyses. Only the PL class was significantly different between reef and 0-month control groups ($F_{8,2}=31.84$, $p=0.0308$), with a significant species effect (*P. compressa*<*M. capitata*;

Table 1
Results of an ANOVA comparing concentrations of nine lipid classes from two coral species (*Porites compressa* and *Montipora capitata*), at two temperatures (ambient and 30 °C) and 4 time periods after bleaching (0, 1.5, 4, and 8 months) ($F_{153, 269}=7.62$, $p<0.0001$)

Effects [1]	df [2]	SS [3]	F [4]	p [5]
C	8	38057.78	73.59	<0.0001
S	1	889.91	13.77	0.0003
Te	1	430.63	6.66	0.0104
Ti	3	424.68	2.19	0.0896
G	8	1673.38	3.24	0.0016
G in S	2	990.76	7.66	0.0006
C x S	8	7900.45	15.28	<0.0001
C x Te	8	2207.79	4.27	<0.0001
C x Ti	24	4354.41	2.81	<0.0001
S x Te	1	1328.88	20.56	<0.0001
S x Ti	3	715.47	3.69	0.0125
T x Ti	3	995.24	5.13	0.0018
C x S x Te	8	1110.98	2.15	0.0318
C x S x Ti	24	4074.35	2.63	<0.0001
C x Te x Ti	24	3359.21	2.17	0.0017
S x Te x Ti	3	445.45	2.30	0.0780
C x S x Te x Ti	24	2558.59	1.65	0.0315

[1] Effects of class (C), species (S), temperature (Te), and time period (Ti) were considered fixed and fully crossed. Genotype (G) was a random effect and nested within species (G in S). Interaction terms involving genotype were combined with the residual.
[2] df =degrees of freedom.
[3] SS =sum of squares of the effect.
[4] F =F-statistic.
[5] p =p-value.

p=0.0271) and control type effect (reef controls>0-month controls; p=0.0114). These results emphasize the importance of comparing tank controls with tank bleached fragments.

Overall, PL was the most abundant lipid class, followed by both TG and ST (determined by *a posteriori* Tukey tests for both species combined). All other lipid classes did not differ from each other and were the least abundant across all time periods (i.e., PL>TG=ST>FFA=MG=WE=FAME=HC=DG) (Fig. 1). When each species was examined individually, total lipids in *P. compressa* were mainly composed of the structural lipid PL, followed by the storage lipids TG and WE, with trace amounts (<5% of the total) of FFA, ST, HC, and FAME lipid classes across all time periods (PL>TG>WE>FFA=ST=HC=FAME as determined by *a posteriori* Tukey tests; Fig. 1a-i). No DG or MG was extracted from *P. compressa*. In *M. capitata*, total lipids were mainly composed of the structural lipids PL, followed by ST, with trace amounts of WE, DG, HC, and FAME and variable amounts (ranging from trace to ≥20%) of TG, MG, and FFA lipids across all time periods (PL>ST>TG=MG=FFA>WE=DG=HC=FAME determined by *a posteriori* Tukey tests; Fig. 1j-r).

Within each species, differences in lipid classes between treatment and control corals were further examined with *a posteriori* slice tests at each time period following bleaching. For *P. compressa* at 0 and 1.5 months, the concentration of TG in the treatment corals was significantly lowered to 44% and 12% of controls, respectively (Fig. 1a, $p_0=0.0030$, $p_{1.5}<0.0001$), similarly for PL at 1.5 months (41%; Fig. 1f, $p_{1.5}<0.0001$). At 4 months, there were no significant differences between the treatment and control corals for all lipid classes. At 8 months, treatment TG, and PL concentrations were again lowered to 53% and 35% of control corals, respectively (Fig. 1a, $p_8=0.0013$; 1f, $p_8<0.0001$). Also for the first time, WE was significantly reduced, at 34% of control corals (Fig. 1b, $p_8=0.0351$).

In treatment *M. capitata*, the concentration of TG and PL was lower than controls at 0 months (18% and 65% respectively; Fig. 1j, $p_0=0.0331$; 1o, $p_0=0.0343$), while MG was higher (214%; Fig. 1m, $p_0=0.0404$). At 1.5 months and for the remainder of the study, treatment TG and MG were not significantly different from control fragments. Of all lipid classes, only PL was less than control corals at 1.5 months (61%; Fig. 1o, $p_{1.5}=0.0048$). At 4 months, treatment FFA, PL, and ST concentrations were higher than controls by 1102%, 115%, and 575% respectively (Fig. 1n, $p_4=0.0006$; 1o, $p_4<0.0001$; 1p, $p_4<0.0001$). By 8 months, all treatment *M. capitata* lipid classes were not significantly different from controls.

Additional patterns were also detectable from significant interaction effects in the ANOVA (Table 1). The composition of lipid classes differed significantly for both species (C x S, C x S x Te effects). In *P. compressa* total lipids were composed of storage and structural lipid classes, while in *M. capitata* they were mostly composed of structural lipids. The differences between treatment and control corals were greater for *P. compressa* than *M. capitata* for most lipid classes (S x Te effect). Overall, lipid classes tended to be lower in treatment than control corals throughout the 8 month period, except for higher FFA, PL, and ST at 4 months in *M. captitata* (C x Te, C x Ti, Te x Ti, C x S x Ti, C x Te x Ti, and C x S x Te x Ti effects).

4. Discussion

4.1. Storage lipids

In the present study, the reduction of the storage lipid, TG, occurred immediately after experimental bleaching in both *P. compressa* and *M. capitata* (Fig. 1a, j). A similar result was found after a natural bleaching event in these two species (Grottoli et al., 2004), after natural bleaching in eight coral species from Okinawa (Yamashiro et al., 2005), and after short-term experimental sediment stress in *Montastraea* spp. (Niebuhr, 1999). Together these findings suggest utilization of TG storage lipids by corals immediately after bleaching. However, exactly how specific storage lipids are metabolised appears to be species specific in corals. Normally, TG is metabolized sequentially into DG, then MG, as free fatty acids are released at each step and energy is required (Gurr and Harwood, 1991). As expected, TG storage lipids in *M. capitata* appear to have been partially metabolized into more labile forms of MG and then FFA as evidenced by increases in MG immediately following bleaching, followed by increases in FFA at 4 months (Fig. 1j, m-n). Therefore, increased FFA at 4 months probably results as a breakdown product of TG and MG in treatment *M. capitata*. Control corals were not impacted by the stress of bleaching, were not metabolizing their storage lipids to the same extent as treatment corals, and exhibited no increase in FFA

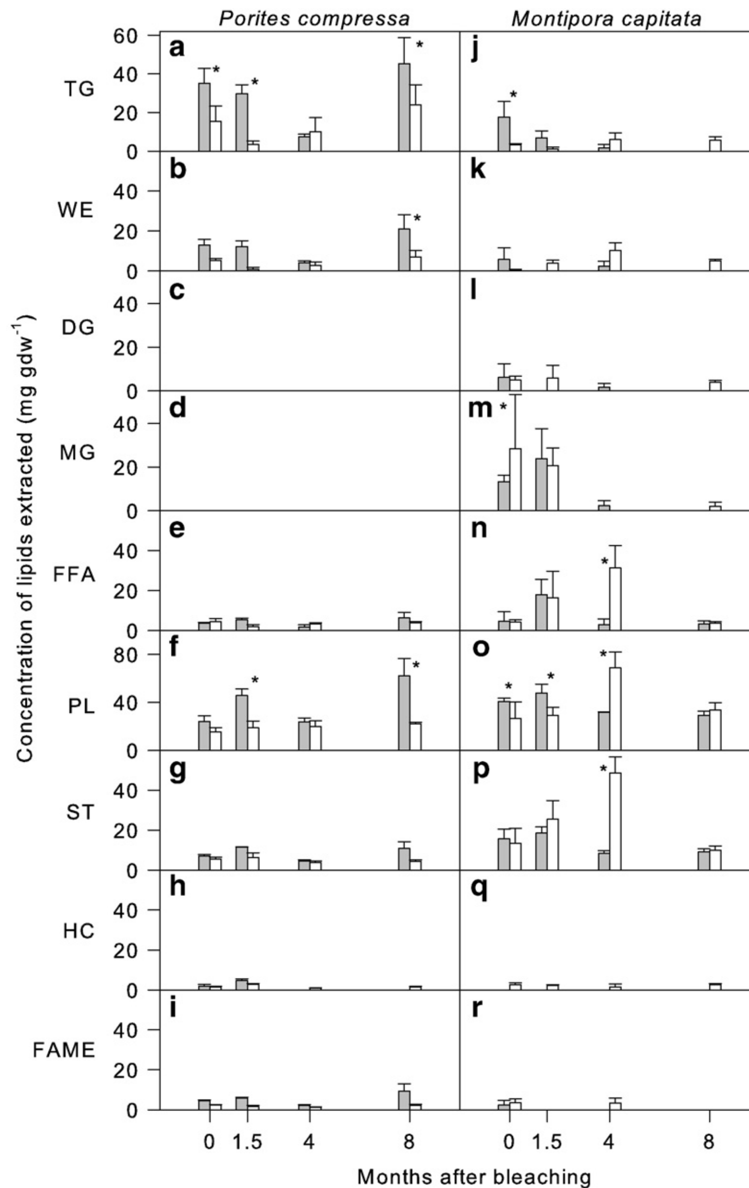

Fig. 1. Concentration of each lipid class eluted: a, j. triacylglycerol (TG); b, k. wax esters (WE); c, l. diacylglycerol (DG); d, m. monoacylglycerol (MG); e, n. free fatty acid (FFA); f, o. phospholipid (PL); g, p. sterol (ST); h, q. hydrocarbon (HC); and i, r. fatty acid methyl ester (FAME) standardized to ash-free dry tissue weight (mg gdw^{-1}) at 0, 1.5, 4, and 8 months after bleaching for control (grey bars) and treatment (white bars) fragments of *Porites compressa* and *Montipora capitata* corals. Note the different concentration scale for phospholipids (panels f and o). Missing bars indicate that the sample was below detection limit. All means are shown ± 1 SE. An * indicates significant differences at $p < 0.05$ between control and treatment means within a single time interval after bleaching by *a posteriori* slice tests. Sample size for each mean is n = 3. Statistical analyses are provided in Table 1.

(Fig. 1n). Likewise, decreased TG and increased FFA concentrations were observed immediately after natural bleaching in *Montipora informis*, *Porites cylindrica*, and *Fungia fungites* (Yamashiro et al., 2005).

In contrast, in *P. compressa* the primary metabolic products of TG (i.e., DG and MG) were not detected in this study (Fig. 1c-d) or in *P. compressa* from the same site three months after natural bleaching (Grottoli et al., 2004; Note: MG was not analyzed in Grottoli et al., 2004). All other lipid class surveys where DG and MG standards were included found that both DG and MG were present in twelve other non-bleached coral species from Okinawa including solitary, plating, branching, and mounding species (Yamashiro et al., 1999; Oku et al., 2002, 2003b). Aside from Grottoli et al. (2004), no other studies on bleached corals have included standards for DG and/or MG, and have not been able to report their presence or absence in other coral species when bleached (Niebuhr, 1999; Yamashiro et al., 2005). It would appear, therefore, that *P. compressa* is unusual in its lack of either DG or MG lipid classes indicating that the pathway used for TG metabolism is different from all other corals surveyed to date. Furthermore, FFA was only present in trace amounts (<5%) throughout the study, suggesting that all three fatty acid chains were cleaved from the glycerol backbone of each TG molecule at once, and any FFA released was immediately metabolized. A species that completely metabolizes TG would release maximum amounts of ATP for daily

metabolic functioning, but would consume lipid reserves more quickly in the process than species that metabolize TG sequentially.

Though low statistical power (p = 0.113) prevents any detailed discussion of the other main storage lipid WE, it is interesting to point out that in this study, in Grottoli et al. (2004) and in 7 of 8 other species for which data is available (Yamashiro et al., 2005) WE decreased following bleaching. Clearly, additional study with higher sample sizes is needed to more rigorously assess how WE varies with bleaching.

4.2. Structural lipids

The structural lipid, PL was significantly lower in treatment than control corals at 1.5 and 8 months after bleaching in *P. compressa* (Fig. 1f), at 0 and 1.5 months after bleaching in *M. capitata* (Fig. 1o), and immediately after short-term experimental exposure to sediment in *Montastraea* spp (Niebuhr, 1999). Therefore, the loss of structural lipid in corals appears to be a response to different types of stressors.

In *P. compressa*, PL was reduced in treatment compared to control corals at 8 months (Fig. 1f), at the time when tissue biomass had fully recovered (Rodrigues and Grottoli, 2007). In *M. capitata*, both structural lipids, PL and ST, were higher in treatment than controls at 4 months (Fig. 1o, p) while full recovery of tissue biomass occurred by 1.5 months (Rodrigues and Grottoli, 2007). The increase in ST at 4 months in *M. capitata* is most likely due to a surge in heterotrophic feeding (see below). Patton et al. (1983) have shown that the incorporation of radioactively labelled carbon into the structural lipids of healthy *Stylophora pistillata* corals increases gradually over 15 d, while activity in storage lipids levels off within the first 2 d, suggesting that assimilation and turn over of structural lipids is slower than that of storage lipids. Slow assimilation of structural lipids may account for observed lags in the time that PL increased compared to when total tissue biomass recovered in both treatment *P. compressa* and *M. capitata*. The lag in treatment *M. capitata* (4 months) compared to that estimated from healthy *S. pistillata* (15 d) investigated by Patton et al. (1983) suggests that the assimilation time for PL of bleached corals is much longer than that of healthy corals. This large difference in timing may also reflect a difference in utilization and maintenance of structural lipids in healthy compared to bleached corals. Healthy corals (like those in Patton et al. 1983) are able to build and maintain structural lipids shortly after assimilation, while bleached *M. capitata* corals appear to be utilizing those structural lipids before being able to build and maintain PL reserves. Although PL in treatment *P. compressa* was not consistent throughout the study relative to control corals, it is likely that complete recovery of PL takes longer than 8 months and may also lag tissue biomass recovery as observed for *M. capitata*.

4.3. Lipid class composition compared to total lipid concentration

Earlier work by Rodrigues and Grottoli (2007) has shown that total lipid concentration was significantly lower in treatment corals for the first 1.5 months after bleaching in both *P. compressa* and *M. capitata*. The results of the current study show that in *P. compressa* decreases in total lipids were driven by decreases in TG and PL, while in *M. capitata* they were due to a combination of decreases in TG and PL and increases in MG (Fig. 1a, f, j, m, o). Therefore, for each species, the first lipid classes to be depleted were those present in the highest concentrations, rather than depleting all classes uniformly. Similarly, Grottoli et al. (2004) and Yamashiro et al. (2005) found that although total lipids decreased immediately after bleaching in corals, not all lipid classes decreased equally. Results of the species investigated here suggest that lipid classes are relied upon differently by these two coral species in the first 1.5 months after bleaching. Storage lipids (TG at 0 and 1.5 months) were relied upon first followed by structural lipids (PL at 1.5 months) for *P. compressa*, while storage and structural lipids (TG and PL at 0 months and PL at 1.5 months) were utilized together by *M. capitata* (Fig. 1). Differences in initial lipid class utilization were also observed for eight coral species immediately after bleaching (Yamashiro et al., 2005).

Total lipid concentrations were not different between treatment and control *P. compressa* at 4 and 8 months (Rodrigues and Grottoli, 2007). This is consistent with the lipid class concentrations of treatment and control *P. compressa* at 4 months (Fig. 1a-i), However, at 8 months, concentrations of WE, TG, and PL were again lower in treatment than control fragments (Fig. 1a-b, f), indicating that lipid classes continued to fluctuate as a result of bleaching. In many lipid classes, there is little change in the concentration of the treatment corals through time, while the control corals fluctuate throughout the 8 months. At 8 months lipid classes in the treatment corals (i.e., TG, WE, PL) did not match the increase observed in the control corals. Therefore, the data suggest that the synthesis of lipid classes remains depressed for at least 8 months following bleaching in *P. compressa*, with only the appearance of recovery in lipid classes at 4 months. An additional reason for this difference between total lipids and lipid classes is that not all lipid classes were eluted from the total lipid samples, while only the most common lipid classes previously reported for corals were targeted. As a result, total lipids are not expected to equal the sum of the lipid classes eluted in this study.

In *M. capitata*, total lipid recovery has previously been demonstrated at 4 months (Rodrigues and Grottoli, 2007). The present study suggests that this is driven by increases in FFA, PL, and ST concentrations seen at this time (Fig. 1n-p). These increases may partly be driven by metabolism of TG (in the case of increased FFA, as previously discussed) and partly driven by heterotrophy (discussed in detail below). Similarly, no significant change in total lipids in *Montastraea annularis* was accompanied by increased FFA concentration following 35 hr of heat stress (Niebuhr, 1999).

These differences between total lipid and specific lipid class recovery have implications for coral host activities that depend on specific classes. For example, coral eggs are made up of 69% to 80% WE, with lesser amounts of TG and PL (Arai et al., 1993). If any of these specific lipid classes, in particular WE, are preferentially depleted, egg production will likely be negatively affected. WE are also preferentially incorporated into coral

mucus (Crossland et al., 1980) used in colony protection and sediment removal. Loss of WE, even if other lipid classes are present in surplus, may decrease mucus production and affect sediment removal from the colony. Since WE was depleted 8 months after bleaching in *P. compressa* (Fig. 1b) both egg and mucus production are likely to be negatively affected. WE were never depleted after bleaching in *M. capitata* (Fig. 1k), and field studies indicate that egg production remains unaffected following bleaching (Cox, 2007). Any other host biological functions that may depend on specific lipid classes, may also be negatively impacted even once total lipid concentrations have recovered.

Differences in lipid class management between species add to the evidence suggesting that *M. capitata* is more resilient after bleaching than *P. compressa* as reported in Grottoli et al. (2006). At the scale of the whole coral reef ecosystem, 'resilience' has been defined as the ecosystem's ability to resist lasting change from disturbance (McClanahan et al., 2002), measurable at the species scale by maintenance of growth, calcification, and/or reproduction. In the case of, *M. capitata*, it may be considered resilient, since it appears to sequentially utilize storage lipids (Fig. 1j, m, n) and increases heterotrophic input when photosynthetic rates are most depressed (Grottoli et al., 2006), thereby maintaining total lipid reserves (Rodrigues and Grottoli, 2007) and reproductive output after bleaching (Cox, 2007). However, other physiological mechanisms (e.g., calcification rate or zooxanthellae physiology) in combination with lipid class management by the host are likely to contribute to coral species resilience after bleaching. A complete assessment of these other physiological mechanisms is required before confirming differences in coral resilience.

4.4. Photoautotrophy versus heterotrophy

$\delta^{13}C$ analyses (Rodrigues and Grottoli, 2006) along with feeding studies (Grottoli et al., 2006) indicated that *P. compressa* is primarily photoautotrophic when non-bleached, when bleached, and for at least 8 months following bleaching. Chlorophyll *a* concentrations fully recovered in treatment fragments at 4 months and surpassed control fragment values at 8 months, while gross photosynthesis fully recovered at 1.5 months (Rodrigues and Grottoli, 2007). Despite fully recovered photosynthetic capacity, TG, WE, and PL lipid classes in treatment *P. compressa* remained low compared to control fragments 8 months after bleaching. The lag between chlorophyll *a* and photosynthesis recovery coupled with the continued changes in lipid class concentrations indicates that time required for bleached *P. compressa* to fully assimilate lipid classes from photosynthetically fixed carbon takes longer than 8 months. Therefore, visual assessments of reef recovery may underestimate the time required for the underlying physiology to recover from bleaching.

In contrast, bleached *M. capitata* can obtain 100% of its daily metabolic requirements from heterotrophy alone (Grottoli et al., 2006). $\delta^{13}C$ analyses of host tissue and zooxanthellae revealed that *M. capitata* is primarily heterotrophic for the first 1.5 months after bleaching, and then switches back to being primarily photoautotrophic by 4 months (Rodrigues and Grottoli, 2006). During the one-month experimental bleaching period in the current study, corals were reared in filtered seawater such that zooplankton and coral heterotrophy were reduced. This accounts for the consumption of storage (TG) and loss of structural (PL) lipids at 0 months in *M. capitata*, indicating that *M. capitata* will depend upon stored reserves whenever both photosynthesis and heterotrophy are not feasible. Once back on the reef with zooplankton available, increased FFA, ST, and PL at 4 months further confirms that bleached *M. capitata* corals were primarily heterotrophic for the first 1.5 to 4 months after bleaching. During those times, photosynthesis rates and chlorophyll *a* concentrations remained low and did not fully recover until 4 and 8 months, respectively (Rodrigues and Grottoli, 2007). Together, these results indicate that lipid class increases were not photosynthetically, but heterotrophically derived during the first 4 months after bleaching, and that both storage and structural lipids were directly obtained or subsequently synthesized from heterotrophically acquired fixed carbon in *M. capitata*.

There was also a lag between maximum heterotrophy (at 1.5 months according to $\delta^{13}C$ analyses: Rodrigues and Grottoli, 2006), total lipid recovery (at 4 months: Rodrigues and Grottoli, 2007), and the increase in the FFA, PL and ST lipid classes at 4 months (Fig. 1n-p). This lag probably represents the time required for bleached *M. capitata* to assimilate lipid classes from zooplankton-derived carbon. Since dark-acclimated *Montipora digitata* have been shown to incorporate more radioactively labelled glycerol into PL, very little into TG, and none into WE, or FFA lipid classes (Oku et al., 2003a), while light-acclimated *M. digitata* incorporated labelled glycerol into PL, TG, WE, and FFA (Oku et al., 2003a), it is likely that mostly structural lipids are built from non-photosynthetic sources. For bleached *M. capitata*, increased PL and ST at 4 months may have resulted from a lag in the assimilation of heterotrophically acquired carbon that is rich in ST, while increased FFA at 4 months likely occurs as the breakdown product of TG, DG and MG (as previously discussed). Therefore, as observed in treatment *M. capitata* the coral host can directly assimilate at least structural lipids independent of zooxanthellae photosynthesis, while storage lipids appear to be more photosynthetically derived.

Overall, lipid reserve management differed among the two coral species investigated. TG, WE, and PL in *P. compressa* were negatively affected for more than 8 months after bleaching, suggesting that this species requires more time than the length of the experiment for lipid class composition to return to pre-bleaching levels. *P. compressa* completely, rather than sequentially, metabolized storage lipids. In contrast, *M. capitata* depleted some and increased other lipid classes, by sequentially metabolizing storage lipids and assimilating structural lipids from increased heterotrophy.

Acknowledgments

We thank the Hawaii Institute of Marine Biology, Department of Land and Natural Resources Hawaii, University of Pennsylvania, P. Jokiel, A. Souza, P. Petraitis, O. Gibb, M. Cathey, L.

Bloch, J. Palardy, and S. Higa. Funding for this study was provided to L.J.R. by a William Penn Fellowship and a Summer Stipend in Paleobiology Research. The majority of the salary, technical, laboratory, and field support was provided by funds awarded to AGG by the Andrew Mellon Foundation and the National Science Foundation Chemical Oceanography (OCE-0610487) and Biological Oceanography (OCE-0542415) programs. Rodrigues was a PhD graduate student in Grottoli's lab during the execution of this research. This is contribution 1305 of the Hawaii Institute of Marine Biology and 1453 of the University of Texas Marine Science Institute. **[SS]**

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