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REPORT

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Characterization of fatty acid composition in healthy and bleached corals from Okinawa, Japan

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Abstract Under bleaching conditions, corals lose their symbiotic zooxanthellae, and thus, the ability to synthesize fatty acids (FAs) from photosynthetically derived carbon. This study investigated the lipid content and FA composition in healthy and bleached corals from the Odo reef flat in Okinawa, southern Japan, following a bleaching event. It was hypothesized that the FA composition and abundance would change as algae are lost or die, and possibly microbial abundance would increase in corals as a consequence of bleaching. The lipid content and FA composition of three healthy coral species (*Pavona frondifera*, *Acropora pulchra*, and *Goniastrea aspera*) and of partially bleached and completely bleached colonies of *P. frondifera* were examined. The FA composition did not differ among healthy corals, but differed significantly among healthy, partially bleached, and completely bleached specimens of *P. frondifera*. Completely bleached corals contained significantly lower lipid and total FA content, as well as lower relative amounts of polyunsaturated FAs and higher relative amounts of saturated FAs, than healthy and partially bleached corals. Furthermore, there was a significantly higher relative concentration of monounsaturated FAs and odd-numbered branched FAs in completely bleached corals, indicating an increase in bacterial colonization in the bleached corals.

Keywords *Acropora pulchra* · Bleached coral · Fatty acid · *Goniastrea aspera* · Lipid · *Pavona frondifera*

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Introduction

Fatty acids (FAs) are the main constituents of lipids as well as essential constituents of cell membrane lipids and precursors of bioactive metabolites (Sargent et al. 1990, 1999). In a healthy symbiotic association, corals derive their FAs endogenously, mainly from the photosynthetic fixation of carbon dioxide (CO_2) by the symbiotic zooxanthellae (Kellogg and Patton 1983; Patton and Burris 1983; Patton et al. 1983; Harland et al. 1993) and from host cell de novo synthesis using glucose derived from zooxanthellae as a major source of carbon (Oku et al. 2003). In addition to endogenous synthesis, corals derive their FAs via heterotrophic feeding on plankton (zooplankton and phytoplankton) and dissolved organic matter (Schlichter 1982; Kellogg and Patton 1983). Phytoplankton, e.g., diatoms and dinoflagellates, are rich sources of many polyunsaturated FAs (PUFAs), in particular 20:5ω3 and 22:6ω3, respectively. They may also be sources of short-chain saturated FAs (SAFAs) and monounsaturated FAs (MUFAs) for corals. Zooxanthellae synthesize FAs in thylakoid membranes located in the algal plastids (Tchernov et al. 2004), whereas the coral host synthesizes FAs in the phospholipids of the endoplasmic reticulum (ER) (Patton et al. 1983). FA synthesis occurs in parallel in both the zooxanthellae and the host (Oku et al. 2003).

Under healthy conditions, FA synthesis within the thylakoid membranes of zooxanthellae involves a stepwise build-up of long-chain acetyl groups from acetyl-CoA to form a 16 carbon SAFA (palmitate) (Kellogg and Patton 1983). Acetyl-CoA carboxylase catalyzes the first step of lipid synthesis, which is both strongly light regulated and rate limiting (Gurr and Harwood 1991; Ohlrogge and Jaworski 1997). Subsequent metabolism and modification of palmitate occurs by membrane-bound enzymes outside the chloroplast in the ER, and consists of chain elongations to produce longer FAs, such as those with 18 carbons (stearate), and a series of desaturation reactions to introduce additional double

bonds (Kellogg and Patton 1983). These FAs (16:0 and 18:0) are key precursors for the biosynthesis of the PUFAs 18:2ω6 and 18:3ω3. The newly biosynthesized FAs are then translocated into the host tissues in the form of "fat droplets" consisting of phospholipids, glycolipids, and neutral lipids (Kellogg and Patton 1983; Patton and Burris 1983; Patton et al. 1983; Harland et al. 1993).

Because animals lack the enzymes (Δ^9 Δ^{12} Δ^{15} desaturases) required to introduce double bonds at carbon atoms at and beyond the Δ^9 position in the FA chain (Dewick 1997), they cannot synthesize 18:2ω6 and 18:3ω3. Nevertheless, this synthesis is necessary to produce ω3 and ω6 PUFAs (Papina et al. 2003). These acids (18:2ω6 and 18:3ω3) must be acquired in the diet or from photosynthetic organisms, and are therefore referred to as essential FAs. Once obtained, 18:2ω6 and 18:3ω3 are then modified in animals using a distinct set of desaturases (Δ^6 and Δ^5) to produce 20:4ω6 and 20:5ω3, and then 20:5ω3 to 22:6ω3, respectively (Sargent et al. 1990). PUFAs, in particular ω3s and ω6s, play important metabolic roles in the regulation of metabolism, such as growth, respiration, energy generation, photosynthesis, production of planula larvae and eggs, formation of biomass, and general enzyme activity (Rinkevich 1989; Harland et al. 1992; Arai et al. 1993; Ward 1995). Most importantly, ω3s improve growth, survival, and resistance to stress (Romdhane et al. 1995).

Bleached corals show decreased algal densities, chl *a* and host tissue biomass (Porter et al. 1989; Szmant and Gassman 1990; Fitt et al. 1993; Allison et al. 1996; Suzuki et al. 2000, 2003; Grottoli et al. 2004) impaired photosynthesis (Tchernov et al. 2004), a net decrease in photosynthesis (Warner et al. 1996; Lesser 1997; Lombardi et al. 2000) and lipid content (Grottoli et al. 2004; Yamashiro et al. 2005), and possibly lower heterotrophy, resulting in the loss of some important nutrition. FA synthesis in corals is directly coupled with photosynthesis (Oku et al. 2003); impaired photosynthesis during bleaching may affect FA metabolism, in particular the FA biosynthetic pathways, resulting in changes in FA composition and reduced lipid production. This may affect the energy stores necessary to support the metabolic needs of corals (Grottoli et al. 2004). The depletion of energy reserves increases the susceptibility to disease and mortality (Meesters and Bak 1993; Mascarrelli and Bunkley-Williams 1999) possibly because of an increase in microbes in the damaged tissues.

Fatty acid composition is often specific to particular groups of organisms (Volkman 1999) and reflects their specific cellular physiological functions and physiological states (Sargent et al. 1990). Given that corals obtain most of their PUFAs from zooxanthellae (Papina et al. 2003), algal loss would cause a significant reduction in PUFAs, particularly in the specific dinoflagellate markers 18:4ω3, 22:5ω3, and 22:6ω3 (Graeve et al. 1994; Zhukova and Aizdaicher 1995). Because corals rely on adequate energy reserves and metabolic precursors for growth, reproduction, and survival under adverse conditions, examining the lipid content and FA composition of healthy and bleached corals is a potential diagnostic indicator of coral health.

The purpose of this study was to investigate the lipid content and FA composition in healthy colonies of *Pavona frondifera*, *Acropora pulchra*, and *Goniastrea aspera* and in bleached colonies of *P. frondifera* from the Odo reef flat in Okinawa, southern Japan, following a bleaching event. It was hypothesized that the FA composition and relative abundance would change as algae are lost or die, and possibly microbial abundance would increase as a consequence of bleaching.

Materials and methods

Collection of coral specimens

In Okinawa, the susceptibility of corals to bleaching has been linked to colony morphology and tissue thickness (Loya et al. 2001), with branched and plate-like corals being more susceptible than massive colonies. As a consequence, three coral species with different morphologies were selected for this study, *P. frondifera* (plate-like), *A. pulchra* (branching), and *G. aspera* (massive), from the Odo reef flat in southern Okinawa, Japan (26°N, 128°E). These species are among the dominant species inhabiting the shallow tidal pools of the Odo reef flat, and were the most affected species during coral bleaching events in the summer of 2003 in Okinawa (personal observation). Coral colonies were collected from tidal pools during low tide (0.2–1.0 m) in August 2003. For *P. frondifera*, healthy, partially bleached, and completely bleached colonies were examined (Fig. 1). Coral colonies were distinguished based on visible signs of bleaching. All specimens were placed in seawater, transferred to the laboratory within 1–2 h of collection, and stored at −20°C until further analysis (within 1 week).

Sample preparation

Coral colonies were rinsed gently with filtered seawater, and tissues were removed from the skeleton using the Water Pik and crush methods. The crush method (whole sample) was used to remove tissues from the healthy and

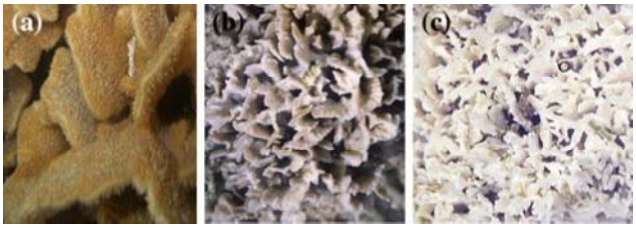


Fig. 1 **a** Healthy, **b** partially bleached, and **c** completely bleached colonies of *Pavona frondifera*

bleached samples of *P. frondifera*, whereas the Water Pik method (using filtered seawater, Whatman GF/F fiberglass filter) was used to remove tissues from healthy samples of *P. frondifera*, *A. pulchra*, and *G. aspera*. The blastate was homogenized and 5 ml subsamples were taken for lipid extraction. Additional 5 ml subsamples were dried overnight at 80°C and washed with distilled water five times until no evidence of salt crystals remained. These samples were re-dried until they reached a constant weight. For each coral species, four to six different samples were prepared for lipid extraction.

For *P. frondifera*, 2–4 cm coral tips (4–6 g) of healthy, partially bleached, and completely bleached colonies were gently rinsed with distilled water and then crushed into 1–2 mm pieces (Latyshev et al. 1991). For each coral condition (healthy, partially bleached, and completely bleached), four different samples were prepared for lipid extraction.

Lipid extraction and FA analysis

Lipids were extracted using a slightly modified method of Bligh and Dyer (1959). Extraction was conducted ultrasonically for 20 min with a mixture of distilled water:methanol:chloroform (1:2:1, 20 cm^3, v:v:v). Lipids were then transferred into the lower chloroform phase which was then centrifuged at 650×*g* for 5 min. Following evaporation of the solvent with nitrogen, extracts were weighed for total lipid content. The lipids were saponified under reflux for 2 h at 100°C, with a 2 mol dm^{-3} NaOH solution in methanol and distilled water (2:1, v:v). After acidification with an ultra-pure HCl solution (37.5%), 2 × 2 cm^3 of chloroform were successively added to recover the lipids. The solvent was then evaporated under a nitrogen stream, and FAs were converted to methyl esters under reflux using 1 ml of 14% BF_3–methanol for 10 min. Saponification and methylation were performed to obtain total FAs (Mfilinge et al. 2005).

Fatty acid methyl esters (FAMEs) were purified by high-performance thin-layer chromatography technique, using Merck plates coated with silica gel (Darmstadt, Germany). The solvents used for developing were a mixture of hexane/diethyl ether/acetic acids (70:30:1). Bands containing FAMEs were scraped and collected in a mixture of chloroform/methanol (2:1, v:v) at 40°C for 60 min. FAMEs were then isolated in the same solution until analysis using gas chromatography. For all samples, a second plate was prepared to estimate the proportion of FAMEs in the total lipids (Yamashiro et al. 1999). After drying, the plate was scanned using a flatbed scanner (Epson GT-9000) and Adobe Photoshop software (Adobe systems, San Jose, CA, USA). The resulting image file was imported into NIH Image version 6 to estimate the relative contribution of the FAs as a proportion of total lipids, by integrating the chromatogram (Meziane and Tsuchiya 2002).

The FAMEs were separated and quantified using a gas chromatography (GC 14.B, Shimadzu, Kyoto, Japan) equipped with a flame ionization detector. Separation was performed with an FFAP-polar capillary column (30 m × 0.32 mm internal diameter, 0.25 µm film thickness) with helium as a carrier gas. After injection at 60°C, the oven temperature was raised to 150°C at a rate of 40°C min^{-1}, then to 230°C at 3°C min^{-1}, and then held constant for 30 min. Flame ionization occurred at 240°C. Most FAME peaks were identified by comparing their retention times with those of authentic standards (Supelco Inc., Bellefonte, PA, USA).

Statistical analyses

One-way analysis of variance (ANOVA) was used to compare the total lipid concentration and FAs of the three physiological conditions of *P. frondifera* (healthy, partially bleached, and completely bleached) and of the three healthy coral species (*P. frondifera*, *A. pulchra*, and *G. aspera*). Any significant species or condition effects were further examined using Fisher's protected least significant difference (PLSD). Data were arcsine *p*-transformed prior to analysis (Zar 1999). Stat View 5 software (SAS Institute Inc., Cary, NC, USA) was used for all statistical analyses. Data for the three physiological conditions of *P. frondifera* (healthy, partially bleached, and completely bleached) and for the three healthy coral species (*P. frondifera*, *A. pulchra*, and *G. aspera*), were further subjected to multivariate analyses using PRIMER software (Clarke and Warwick 2001). This was done to clearly elucidate similarities and differences in FA composition in low-dimensional space. Data matrices (FA composition of healthy and bleached coral samples) were used to create triangular similarity matrices based on the Bray–Curtis similarity coefficient, followed by non-metric multidimensional scaling (n-MDS). Unidentified FAs were not used in these analyses, and data were not transformed. Differences in FA composition among physiological conditions and among species were tested using one-way analysis of similarity (ANOSIM) with 5,000 permutations. For all tests, a criterion of $P < 0.05$ was used to determine statistical significance.

Results

Lipid content and FA composition of healthy and bleached *P. frondifera*

The lipid content did not differ between healthy and partially bleached samples of *P. frondifera*, but both healthy and partially bleached samples differed significantly from the completely bleached samples (Fisher's PLSD, $P < 0.001$; Fig. 2a). The mean percentage of total FA content in healthy and partially bleached corals

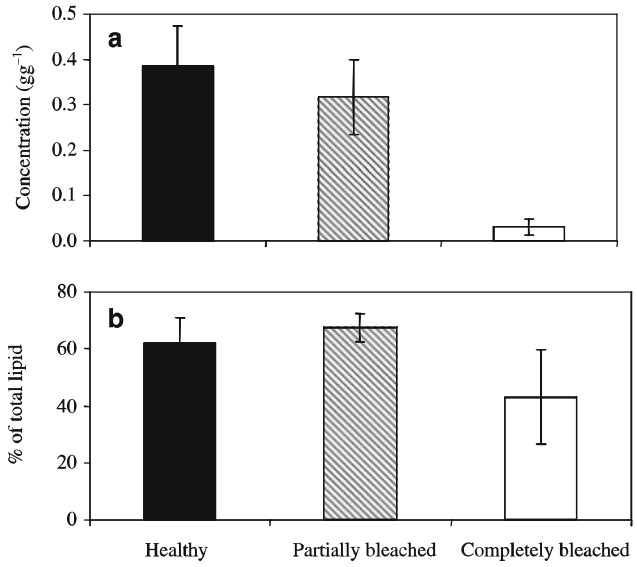


Fig. 2 Comparison of **a** total lipid content (g g^{-1} dry weight) and **b** total fatty acid content (% of total lipid) among healthy, partially bleached, and completely bleached *Pavona frondifera* (mean ± SD), $n = 4$

ranged from 62 to 67% of the total lipid content, whereas that in completely bleached corals was significantly lower (Fisher's PLSD: healthy/partially bleached vs. completely bleached, $P < 0.05$; Fig. 2b).

Fatty acid compositions differed significantly among healthy, partially bleached, and completely bleached *P. frondifera* (ANOVA, $P < 0.05$; Tables 1, 3). Analysis of Bray–Curtis similarities followed by n-MDS ordination revealed clear differences in FA composition between healthy and bleached corals. In particular, there was a distinct dissimilarity in FA composition between completely bleached corals, and healthy and partially bleached corals (Fig. 3). One-way ANOSIM showed a significant difference in FA composition between healthy and completely bleached corals ($R = 0.979$, $P < 0.029$), and between partially bleached and completely bleached corals ($R = 0.990$, $P < 0.029$). However, there was no significant difference in FA composition between healthy and partially bleached corals (Fig. 3).

The relative concentration of PUFAs was lowest in completely bleached corals (Fisher's PLSD: healthy/partially bleached vs. completely bleached corals, $P < 0.01$). In contrast, the relative concentration of SAFA was highest in completely bleached corals (Fig. 4). Completely bleached corals also showed a high diversity of individual PUFAs (similar to healthy and partially bleached corals), but a significantly lower relative mean percentage of important individual PUFAs, e.g., 18:3ω6, 18:4ω3, 20:4ω6, 22:5ω3, 22:5ω6, and 22:6ω3 (Tables 2, 3).

The relative mean percentages of MUFAs and odd-branched FAs (odd-BrFAs; Fig. 4) were significantly higher in completely bleached corals (16 and 0.7%, respectively) than in healthy and partially bleached corals (MUFAs 9–11%, odd-BrFAs <0.1%; Fisher's PLSD: healthy/partially bleached vs. bleached, $P < 0.05$).

In healthy *P. frondifera*, the concentration of lipid and FA components revealed a slight difference between the crush (Table 1) and Water Pik (Table 2) methods. This was likely because of the lower relative mean percentage of SAFAs (45% of total FAs), and consequently a higher percentage of total FAs (62% of total lipid) and PUFAs (45% of total FAs) observed using the crush method. Johannes and Wiebe (1970) found that the Water Pik technique does not remove significant amounts of thick fibrous mesoglea, including zooxanthellae. Therefore, the lower percentages of total FAs and PUFAs in *P. frondifera* tissues collected using the Water Pik method may have been caused by a significant amount of coral tissue remaining in the coral skeleton during tissue removal.

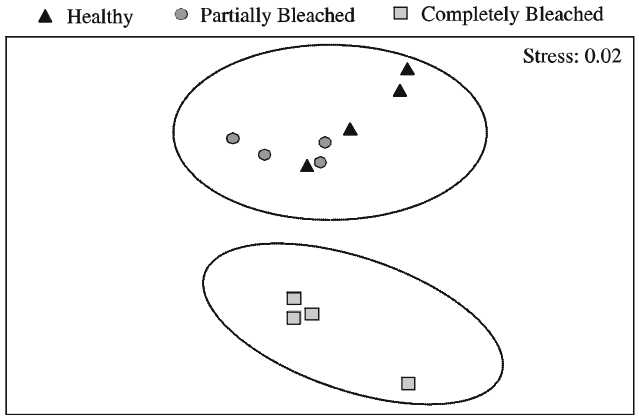


Fig. 3 Non-metric multidimensional scaling plot of the fatty acid composition (% of total fatty acids) of healthy and bleached *Pavona frondifera*

Lipid content and FA composition of healthy *P. frondifera*, *A. pulchra*, and *G. aspera*

The mean total lipid concentration was significantly higher in *G. aspera* (0.51 g g^{-1} tissue dry weight) than in *A. pulchra* and *P. frondifera* (0.31 and 0.26 g g^{-1}, respectively; Fisher's PLSD: *G. aspera* vs. *A. pulchra*, $P < 0.0485$; *G. aspera* vs. *P. frondifera*, $P < 0.0113$; *A. pulchra* vs. *P. frondifera*, $P > 0.5693$; Fig. 5a). In total, 34 FAs were identified in the coral tissues, and the percent concentration of total FAs and FA compositions did not differ significantly among the species (Fig. 5b; Tables 2, 3).

Analysis of Bray–Curtis similarities followed by n-MDS ordination clearly revealed the grouping of FA profiles according to species identity. The FA composition of *P. frondifera* was more similar to that of *A. pulchra* than that of *G. aspera*. One-way ANOSIM

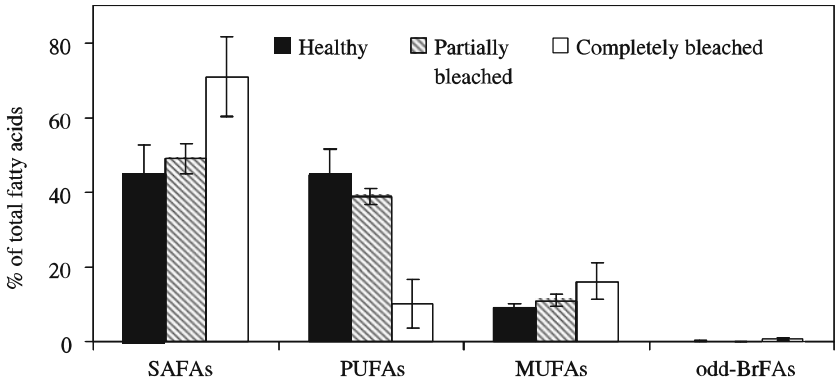


Fig. 4 Comparison of the relative concentrations of fatty acid classes (% of total fatty acids) among healthy, partially bleached, and completely bleached *Pavona frondifera* (mean ± SD), $n = 4$. *SAFAs* saturated fatty acids, *PUFAs* polyunsaturated fatty acids, *MUFAs* monounsaturated fatty acids, *odd-BrFAs* odd-numbered branched fatty acids

Table 1 Total lipid content (g g^{-1} dry wt), total fatty acid content (% of total lipids), and fatty acid compositions (% of total fatty acids) of healthy, partially, and completely bleached *Pavona frondifera* from Odo Coast, Okinawa, Japan in August 2003

Fatty acids	Healthy ($n = 4$)	Partially bleached ($n = 4$)	Completely bleached ($n = 4$)
Total lipid content (g g^{-1} dry wt)	0.38 ± 0.09	0.32 ± 0.08	0.03 ± 0.02
Total fatty acid content (% of total lipids)	61.8 ± 9.2	67.3 ± 4.9	43.1 ± 16.7
12:0	–	–	0.09 ± 0.17
14:0	2.12 ± 0.50	2.25 ± 0.78	4.01 ± 1.11
14:1	0.10 ± 0.12	–	0.33 ± 0.65
15:0 iso	–	–	**0.10 ± 0.11**
15:0	0.04 ± 0.08	–	0.53 ± 0.12
16:0 anteiso	0.06 ± 0.11	–	0.05 ± 0.11
16:0	29.43 ± 7.27	36.63 ± 2.23	53.82 ± 8.51
16:1ω9	0.13 ± 0.15	–	–
16:1ω7	**2.50 ± 0.13**	**2.44 ± 0.42**	**7.27 ± 2.83**
17:0 iso	**0.07 ± 0.08**	**0.05 ± 0.09**	**0.29 ± 0.20**
17:0 anteiso	**0.03 ± 0.06**	–	**0.34 ± 0.11**
16:2ω4	0.09 ± 0.11	–	–
17:0	0.13 ± 0.10	0.06 ± 0.12	0.35 ± 0.06
17:1	0.84 ± 0.80	0.24 ± 0.26	0.11 ± 0.14
18:0 anteiso	0.56 ± 0.34	0.16 ± 0.19	0.05 ± 0.10
18:0	10.68 ± 0.46	7.65 ± 3.99	10.14 ± 1.72
18:1ω9	3.86 ± 0.62	6.13 ± 0.56	6.37 ± 2.45
18:1ω7	**1.18 ± 0.11**	**2.04 ± 1.73**	**1.76 ± 1.07**
18:2ω6	3.09 ± 0.39	5.03 ± 2.13	2.35 ± 1.41
18:3ω6	6.06 ± 1.05	7.39 ± 0.96	1.23 ± 0.82
18:3ω3	0.28 ± 0.12	0.36 ± 0.28	0.25 ± 0.18
18:4ω3	6.15 ± 2.64	3.31 ± 0.75	0.64 ± 0.48
20:0	0.94 ± 0.20	0.76 ± 0.20	0.89 ± 0.18
20:1	0.46 ± 0.40	0.20 ± 0.23	0.22 ± 0.18
20:2	0.56 ± 0.13	0.52 ± 0.15	0.72 ± 1.01
21:0	1.06 ± 0.10	1.30 ± 0.29	0.40 ± 0.29
20:4ω6	12.38 ± 2.17	9.30 ± 1.65	1.51 ± 1.00
20:4ω3	0.31 ± 0.06	0.26 ± 0.07	–
20:5ω3	3.20 ± 1.05	1.49 ± 0.38	0.81 ± 0.66
22:0	0.39 ± 0.09	0.33 ± 0.11	0.45 ± 0.10
22:2	–	0.10 ± 0.20	0.46 ± 0.39
22:4ω3	0.90 ± 1.80	0.08 ± 0.15	0.10 ± 0.21
22:5ω6	4.49 ± 3.23	3.16 ± 0.48	0.55 ± 0.37
22:5ω3	3.84 ± 2.24	4.13 ± 0.88	0.84 ± 0.59
24:0	0.05 ± 0.10	–	0.26 ± 0.04
22:6ω3	3.42 ± 1.19	3.74 ± 1.24	0.75 ± 0.41
Unidentified	0.61 ± 0.31	0.92 ± 0.85	1.99 ± 1.01
ΣSaturated (SAFAs)	44.85 ± 7.63	48.98 ± 3.96	70.94 ± 10.84
ΣOdd-branched (odd-BrFAs)	0.10 ± 0.11	0.05 ± 0.09	0.72 ± 0.31
ΣMonounsaturated (MUFAs)	9.08 ± 0.90	11.04 ± 1.68	16.05 ± 4.90
ΣPolyunsaturated (PUFAs)	44.76 ± 6.83	38.85 ± 2.28	10.21 ± 6.46
Σω3 and ω6 PUFAs	44.11 ± 6.63	38.2 ± 2.07	9.03 ± 5.67
ΣZooxanthellae markers	13.41 ± 2.23	11.18 ± 1.79	2.22 ± 1.35
ΣBacterial markers	3.78 ± 0.15	4.53 ± 1.68	9.75 ± 3.67

Values represent the mean ± SD of four different samples; –: not detected. In bold are fatty acids indicative of microbial presence in the corals

Table 2 Total lipid content (g g^{-1} dry wt), total fatty acid content (% of total lipid), and fatty acid compositions (% of total fatty acids) of *Pavona frondifera*, *Acropora pulchra*, and *Goniastrea aspera* from Odo reef flat in August 2003

Fatty acids	*Pavona* (n = 5)	*Acropora* (n = 4)	*Goniastrea* (n = 6)
Total lipid content (g g^{-1} dry wt)	0.26 ± 0.15	0.31 ± 0.08	0.51 ± 0.16
Total fatty acid content (% of total lipids)	48.05 ± 11.75	48.51 ± 7.33	55.66 ± 7.24
12:0	0.09 ± 0.09	0.03 ± 0.05	–
14:0	3.00 ± 1.57	3.04 ± 1.08	0.95 ± 0.54
14:1	0.03 ± 0.07	–	–
15:0 iso	**0.03 ± 0.06**	**–**	**–**
15:0	0.05 ± 0.12	–	–
16:0 anteiso	0.08 ± 0.18	–	–
16:0	39.46 ± 9.44	34.83 ± 8.74	31.12 ± 3.26
16:1ω9	0.12 ± 0.27	–	–
16:1ω7	**2.67 ± 0.85**	**2.58 ± 0.77**	**1.82 ± 0.51**
17:0 iso	**0.09 ± 0.12**	**0.20 ± 0.26**	**0.18 ± 0.21**
17:0 anteiso	**0.06 ± 0.08**	**0.03 ± 0.06**	**–**
16:2ω4	–	–	0.16 ± 0.05
17:0	0.19 ± 0.07	0.18 ± 0.04	0.09 ± 0.07
17:1	0.09 ± 0.14	0.13 ± 0.18	0.05 ± 0.08
18:0 anteiso	–	0.10 ± 0.07	–
18:0	10.74 ± 3.51	8.62 ± 2.23	9.48 ± 1.98
18:1ω9	5.75 ± 1.12	5.68 ± 1.34	10.20 ± 6.34
18:1ω7	**1.01 ± 0.76**	**1.03 ± 0.72**	**1.82 ± 0.34**
18:2ω6	2.15 ± 0.40	2.85 ± 0.50	3.26 ± 2.10
18:3ω6	7.03 ± 1.80	8.32 ± 0.88	8.98 ± 2.19
18:3ω3	0.09 ± 0.12	0.16 ± 0.12	0.34 ± 0.62
18:4ω3	4.44 ± 4.08	7.58 ± 7.47	0.85 ± 0.54
20:0	0.81 ± 0.22	0.67 ± 0.14	0.91 ± 0.20
20:1	0.96 ± 1.05	0.76 ± 0.87	2.21 ± 0.42
20:2	0.38 ± 0.17	0.33 ± 0.12	0.73 ± 0.11
21:0	0.95 ± 0.16	0.99 ± 0.16	3.21 ± 0.73
20:4ω6	6.73 ± 2.97	6.93 ± 2.34	7.39 ± 1.83
20:4ω3	0.16 ± 0.10	0.23 ± 0.03	0.36 ± 0.08
20:5ω3	2.59 ± 1.91	2.27 ± 1.13	1.62 ± 0.41
22:0	0.29 ± 0.21	0.29 ± 0.11	0.14 ± 0.08
22:1	–	–	0.38 ± 0.16
22:2	0.11 ± 0.24	0.07 ± 0.14	–
22:4ω3	0.05 ± 0.12	0.05 ± 0.09	–
22:5ω6	2.76 ± 1.23	2.62 ± 1.07	4.04 ± 1.26
22:5ω3	2.85 ± 1.67	3.57 ± 1.71	0.60 ± 0.27
24:0	0.03 ± 0.06	0.10 ± 0.12	–
22:6ω3	3.33 ± 2.08	4.21 ± 2.19	6.97 ± 1.50
Unidentified	0.84 ± 0.32	1.56 ± 1.40	2.13 ± 0.97
ΣSaturated (SAFAs)	55.62 ± 11.01	48.74 ± 10.60	45.89 ± 3.41
ΣOdd-branched (odd-BrFAs)	0.17 ± 0.11	0.23 ± 0.32	0.18 ± 0.21
ΣMonounsaturated (MUFAs)	10.63 ± 1.99	10.18 ± 1.75	16.49 ± 6.06
ΣPolyunsaturated (PUFAs)	32.66 ± 11.72	39.18 ± 11.19	35.32 ± 2.34
Σω3 and ω6 PUFAs	29.58 ± 9.87	36.52 ± 10.02	32.81 ± 1.93
ΣZooxanthellae markers	10.62 ± 5.77	15.36 ± 7.89	8.43 ± 1.75
ΣBacterial markers	3.86 ± 1.01	3.84 ± 1.65	3.82 ± 0.44
Ratio of 18:1ω 9/18:4ω 3	1.3	0.7	11.9

Values represent the mean ± SD; –: not detected. In bold are fatty acids indicative of microbial presence in the corals

showed no significant difference in FA composition between *P. frondifera* and *A. pulchra*; however, the FA composition of these two species, differed significantly from that of *G. aspera* (*G. aspera* vs. *P. frondifera*, R = 0.744, P < 0.002; *G. aspera* vs. *A. pulchra*, R = 0.778, P < 0.005; Fig. 6).

The major FAs were SAFAs, which comprised 49–56% of the total FA content (Fig. 7). A preponderance of SAFA, i.e., 16:0 (31–39%), has also been reported in other coral species (Yamashiro et al. 1999). In this study, coral samples also had a high PUFA content (33–39% of total FAs; Fig. 7). The ω3 PUFAs were more dominant than ω6 PUFAs in *A. pulchra* and *P. frondifera*. In *G. aspera*, ω6 PUFAs were predominant (Table 2). The most dominant PUFAs in *A. pulchra* and *P. frondifera* were 18:3ω6, 18:4ω3, 20:4ω6, 22:5ω3, and 22:6ω3 (PUFAs comprising 31 and 24% of the total FA content, respectively), whereas in *G. aspera*, they were 18:2ω6, 18:3ω6, 20:4ω6, 22:5ω6, and 22:6ω3 (comprising 31% of the total FA content). Among these PUFAs, only 22:5ω3 and 22:6ω3 showed significant differences between *G. aspera* and both *A. pulchra* and *P. frondifera* (Fisher's PLSD, P < 0.01; Table 3). The 22:5ω3 and 22:6ω3 are dinoflagellate biomarkers (Graeve et al. 1994; Zhukova and Aizdaicher 1995).

Table 3 Statistical summary from ANOVAs comparing the relative percentages of fatty acids among the coral conditions of *Pavona frondifera* (healthy, partially bleached, and completely bleached) and among the coral species (*Acropora pulchra*, *Goniastrea aspera*, and *Pavona frondifera*)

	Coral conditions			Coral species		
	$F_{2,9}$	P		$F_{2,12}$	P	
Fatty acid composition	5.384	0.0290	*	1.195	0.3363	ns
16:0	13.455	0.0020	**	1.991	0.1792	ns
18:3ω 6	33.558	< 0.0001	***	1.231	0.3265	ns
18:4ω3	12.270	0.0027	**	2.472	0.1262	ns
20:4ω6	48.000	< 0.0001	***	0.063	0.9392	ns
22:5ω 3	6.038	0.0217	*	6.601	0.0117	*
22:5ω 6	4.303	0.0488	*	2.077	0.1680	ns
22:6ω3	11.308	0.0035	**	5.028	0.0259	*
ΣSAFAs	10.068	0.0051	**	1.933	0.1872	ns
ΣOdd-BrFAs	12.811	0.0023	**	1.467	0.2692	ns
ΣMUFAs	5.582	0.0265	*	3.392	0.0680	ns
ΣPUFAs	41.900	< 0.0001	***	0.553	0.5893	ns

SAFAs saturated fatty acids, *odd-BrFAs* odd-numbered branched fatty acids, *MUFAs* monounsaturated fatty acids, *PUFAs* polyunsaturated fatty acids, *ns* not significant
*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$

Discussion

Lipid content and FA composition of healthy and bleached *P. frondifera*

The aim of this study was to test the hypothesis that FA composition and relative abundance in corals change with bleaching as algae are lost or die, and possibly microbial abundance increases. Should such changes occur, then this technique may have the potential as a diagnostic tool of coral health. Results showed that the FA composition of bleached corals differed from that of healthy corals. This difference was indicated by lower lipid and FA levels, suggesting that the corals consumed their own lipid reserves for energy generation (Fig. 2a, b) (Grottoli et al. 2004). The FA composition of completely bleached corals was also very different from that of healthy and partially bleached corals (Fig. 3). FAs have high biological specificity and the ability to signal physiological changes in an organism (Sargent et al. 1990). The near depletion of some of the metabolically important FAs in completely bleached *P. frondifera* indicates a significant deterioration in the health of this coral. Completely bleached samples had relatively lower PUFA and higher MUFA, odd-BrFA, and SAFA levels (Table 1; Fig. 4). However, the similarity in FA composition between healthy and partially bleached corals suggests that the physiological state of partially bleached colonies was still stable (Fig. 3).

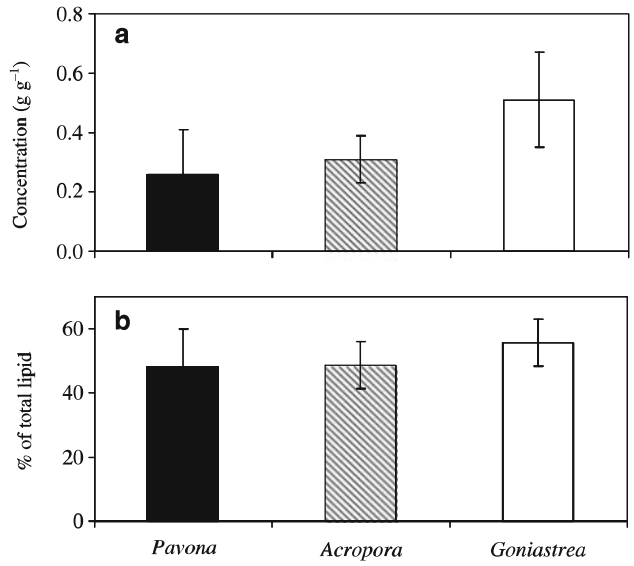


Fig. 5 **a** Total lipid content (g g^{-1} dry weight) and **b** total fatty acid content (% of total lipid) in healthy *Pavona frondifera*, *Acropora pulchra*, and *Goniastrea aspera* (mean ± SD)

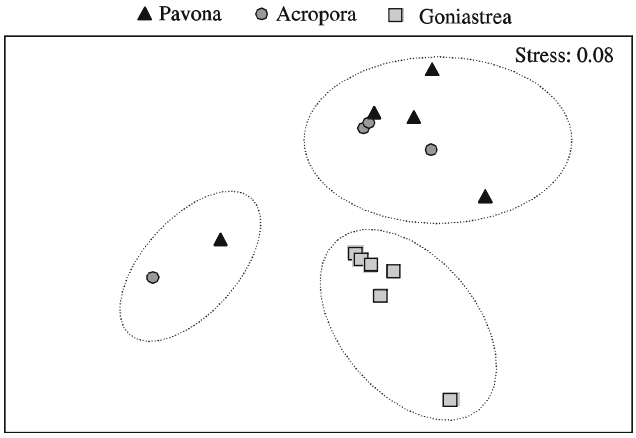


Fig. 6 Non-metric multidimensional scaling plot of the fatty acid composition (% of total fatty acids) of healthy colonies of *Pavona frondifera*, *Acropora pulchra*, and *Goniastrea aspera*

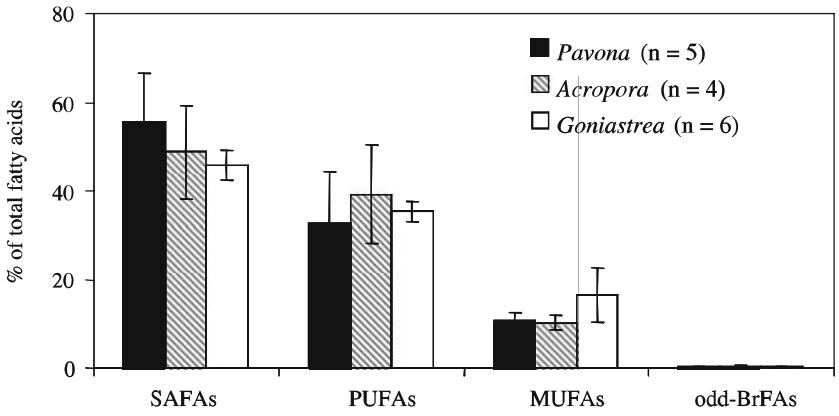


Fig. 7 Comparison of the relative concentrations of fatty acid classes (% of total fatty acids) among healthy *Pavona frondifera*, *Acropora pulchra*, and *Goniastrea aspera* (mean ± SD). *SAFAs* saturated fatty acids, *PUFAs* polyunsaturated fatty acids, *MUFAs* monounsaturated fatty acids, *odd-BrFAs* odd-numbered branched fatty acids

Of particular interest were the significant low levels of the dinoflagellate biomarkers 18:4ω3, 22:5ω3, and 22:6ω3 in the FA composition of bleached samples, which provide evidence of the loss of symbiotic zooxanthellae (Table 1). Because zooxanthellae play an essential role in FA biosynthesis and supply the host cells with metabolites necessary for lipogenesis (Kellogg and Patton 1983; Oku et al. 2003), the low abundance of zooxanthellae in completely bleached samples may have limited the supply of lipids and metabolites to the host. This is supported by a significant correlation between dinoflagellate biomarkers and lipid concentrations (R^2 = 0.766, P < 0.0002) and between dinoflagellate biomarkers and total FA concentrations (R^2 = 0.456, P < 0.0160). Because FA synthesis in coral is directly coupled with photosynthesis (Oku et al. 2003), the low lipid levels in bleached samples could be attributed to low photosynthetic lipid production by zooxanthellae, and the loss of zooxanthellae. Similar low lipid levels in bleached corals in Okinawa (Yamashiro et al. 2005) and Hawaii (Grottoli et al. 2004) have been attributed to the loss of nutritional input from their symbionts.

Apart from de novo synthesis, corals may supplement lipids by heterotrophic feeding on plankton and dissolved organic matter (Schlichter 1982; Kellogg and Patton 1983). This study found no evidence of high phytoplankton markers, e.g., for diatoms (20:5ω3), dinoflagellates (18:4ω3 and 22:6ω3), and algal materials (18:2ω6, 18:3ω3, and 18:3ω6) in bleached corals, which would suggest ingestion of phytoplankton or zooplankton to supplement their dietary lipid. The study also did not find any higher lipid and total FA content in bleached compared to healthy samples, indicating that most lipids in bleached corals originated from endogenous (de novo) synthesis. Thus, it appears that heterotrophy is insufficient to maintain a positive lipid balance during bleaching. In addition, starvation is likely to lead to increases in the levels of FA oxidation enzymes, as well as decreases in FAs and related enzymes involved in FA synthesis. Consequently, substances ingested and metabolized by the bleached corals are not stored; instead, they are rapidly oxidized (e.g., β-oxidation) to generate energy for survival.

The significantly lower amount of PUFAs, in particular, ω3 and ω6, in completely bleached samples (Table 2) correlated significantly with the lower amount dinoflagellate markers (R^2 = 0.982, P < 0.0001). Animal FA biosynthetic pathways are unable to produce ω3 and ω6 PUFAs (Conway and McDowell Capuzzo 1991; Dewick 1997), suggesting that most PUFAs in the coral tissue originate from zooxanthellae biosynthesis (Al-Moghrabi et al. 1995; Papina et al. 2003). Although the nutritional and reproductive requirements for ω3 and ω6 PUFAs are unknown in corals, these PUFAs affect and control many cellular processes in organisms. For example, docosahexaenoic acid (22:6ω3) is required to ensure optimal activity of enzymes associated with the cell membrane (Dratz and Holte 1992; Stubbs 1992). Bishop and Kenrick (1980) and Pernet et al. (2002) reported that zooxanthellae secrete large amounts of eicosapentaenoic acid (20:5ω3) (9–22% of total FAs) and arachidonic acid (20:4ω6). These FAs play essential roles in gonadal maturation in corals; in particular, 20:5ω3 is essential for sperm cell maturation, whereas 20:4ω6 is essential for egg maturation (Pernet et al. 2002). This study found high relative concentrations of 20:4ω6, 20:5ω3, and 22:6ω3 in healthy coral and relatively low concentrations in completely bleached corals, suggesting that the low levels of these PUFAs possibly resulted from the loss of zooxanthellae and high catabolism of these PUFAs during bleaching (Tables 1, 3).

In addition to the loss of symbionts, biochemical factors may explain differences in FA composition and low PUFA levels in bleached corals. This may include substrate limitation for FA synthesis, and a reduced ability for FA chain elongation and desaturation of 18:2ω6 and 18:3ω3. However, further research on FA metabolism is needed, especially regarding the biosynthetic pathways of FA synthesis in bleached corals.

Completely bleached coral samples lost more than 75 and 90% of their original PUFA and lipid concentrations, respectively. These concentrations are critical for normal metabolic function. Given that adequate energy reserves are critical for coral survival under stressful conditions, their depletion may increase the susceptibility of coral to disease and mortality (Meesters and Bak 1993;

Mascarrelli and Bunkley-Williams 1999). However, corals do survive after bleaching because they are able to synthesize PUFAs in the absence of the symbiont. Thus, corals may survive for some time without the symbiotic algae provided that they have enough stored lipids for energy generation. Lipid reserves may also serve as a source of 18:2ω6 and 18:3ω3, which are necessary for the synthesis of ω3 and ω6 PUFAs (Papina et al. 2003).

The high relative percentages of MUFAs and odd-BrFAs in bleached corals suggest an increase in bacterial colonization, as indicated by high relative contributions of bacterial markers, such as 15:0 and 17:0 iso and anteiso, and 16:1ω7 and 18:1ω7 (Jeffries 1972; Volkman et al. 1980) in bleached samples. This study did not characterize the type of bacteria present in bleached corals. However, based on bacterial biomarker characteristics; e.g., the FA biomarkers 15:0 iso, 15:0 anteiso, 15:0 (Findlay and Dobbs 1993), and 17:0 anteiso (Komagata and Suzuki 1987) are characteristic of Gram-positive bacteria; while 17:0 iso, 17:0, 16:1ω7, and 18:1ω7 are characteristic of sulfate-reducing bacteria (Findlay and Dobbs 1993), it is possible that both Gram-positive and sulfate-reducing bacteria were present in healthy corals, but their relative concentrations were highest in completely bleached corals (Tables 1, 2).

FA composition in healthy corals

Unlike bleached corals, the FA composition and total FA content (% of total lipid) did not differ among the healthy coral species. The only major difference occurred in lipid content, with significantly higher levels in *G. aspera* than in *P. frondifera* and *A. pulchra* (Fig. 5a). *P. frondifera* and *A. pulchra* had similar FA compositions, which were distinct from that of *G. aspera* (n-MDS plot, Fig. 6). The similarities and differences in FA composition revealed by n-MDS suggest species-specific differences in the use of lipid reserves (Grottoli et al. 2004), and in the contribution of algae, host biosynthesis, and host diet to the total FA pool (Harland et al. 1993). The variability (i.e., standard deviation) in lipid content and relative percentages of FAs among samples may be partly explained by an uneven distribution of zooxanthellae in a specific region of coral tissue, resulting in differences in photosynthetic rates and lipid production, and by variations in cellular energy demand and use by growing cells in coral tissue (Oku et al. 2002).

FA composition as diagnostic indicator of coral health

The results of this study support the hypothesis that FA composition changes with bleaching as the symbionts are lost or die and bacterial abundance possibly increases. The change in FA composition was mainly characterized by a low PUFA and high bacterial FA levels in bleached corals. Because coral health relies on the presence of adequate energy reserves and metabolic precursors for growth, reproduction, and survival under adverse conditions, the amount of lipids and PUFAs remaining in coral tissues is vital for survival and for maintaining good physiological condition. Therefore, bleached corals that exhibit low levels of PUFAs (especially 18:4ω3, 20:4ω6, 20:5ω3, 22:5ω3, and 22:6ω3), coupled with low lipid levels and high levels of bacterial FAs relative to unbleached samples may be unhealthy.

Polyunsaturated FAs in healthy corals generally range from 15 to 60% (Latyshev et al. 1991; Harland et al. 1993; Al-Moghrabi et al. 1995). A comparison of the PUFA content among healthy (*P. frondifera*, *A. pulchra*, and *G. aspera*; 30–40%) and completely bleached corals (*P. frondifera*; 10.2%) suggests that the relative amount of PUFAs in completely bleached corals was below normal levels required to survive adverse conditions because the bleached corals had lost >75% of their original PUFA content. In contrast, the similarity in the amounts of lipids and total FAs, and FA composition observed in healthy and partially bleached corals, suggests that the partially bleached corals were in a transient phase, and were more likely to recover than the completely bleached corals.

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