

Heterotrophic and autotrophic assimilation of fatty acids by two scleractinian corals, *Montastraea faveolata* and *Porites astreoides*

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Abstract

We determined the source of fatty acids in scleractinian corals by separately measuring and comparing the $\delta^{13}\text{C}$ values of fatty acids in zooxanthellae and coral hosts from two scleractinian coral species (*Montastraea faveolata* and *Porites astreoides*) from reefs of the Florida Reef Tract. Using an isotopic mass balance approach, we show that zooxanthellae are the dominant source of fatty acids and that in many colonies heterotrophic feeding can be an important and significant source of essential $\omega 3$ fatty acids for corals. There is considerable variability in feeding behavior within species, between species, and within single reef sites. In individual colonies, essential $\omega 3$ fatty acids and energy resources such as triglycerides can be acquired from separate dietary sources, showing the importance of resource partitioning within the coral animal. Consistently, individual coral colonies acquire most of their energy-rich saturated fatty acids from their symbiotic zooxanthellae. The particular feeding mode by which corals acquire their fatty acids appears to be specific to each coral colony. The ability of corals to modulate their feeding modes under different environments may provide an adaptive mechanism to sustain growth under stressful conditions.

Scleractinian corals satisfy their nutritional requirements by a combination of heterotrophic assimilation (feeding) and translocation from autotrophic endosymbiotic dinoflagellates (zooxanthellae) (Muscatine 1990; Houlbrèque and Ferrier-Pagès 2008). Zooxanthellae can supply up to 100% of the animal host's requirements for energy under ideal conditions (Muscatine et al. 1981), whereas during bleaching episodes, the host may acquire all of its metabolic requirements from heterotrophic feeding (Grottoli et al. 2006). Corals require fatty acids to live and reproduce; fatty acids can function (1) as energy reserves (Grottoli et al. 2004), with saturated (SAFAs) and monounsaturated (MUFAs) fatty acids stored as triglycerides and wax esters (Oku et al. 2002; Rodrigues et al. 2008); (2) as membrane constituents in the form of phospholipids; and (3) as polyunsaturated fatty acids (PUFAs) (Tchernov et al. 2004; Imbs et al. 2007; Treignier et al. 2008), which include molecules critical for reproduction and membrane fluidity (Ulrich 1994). PUFAs include a specific subset of fatty acids, known as essential fatty acids (EFAs), that coral hosts cannot endogenously manufacture in appreciable quantities and thus presumably must procure from their diet (Latyshev et al. 1991; Bell et al. 2007). Coral hosts are incapable of constructing EFAs due to an inability to synthesize appreciable quantities of the requisite precursors, linolenic (18:3 $\omega 3$) and linoleic (18:2 $\omega 6$) acids (Latyshev et al. 1991; Bell et al. 2007). Eicosapentaenoic acid (EPA, 20:5 $\omega 3$), docosahexaenoic acid (DHA, 22:6 $\omega 3$), and arachidonic acid (ARA, 20:4 $\omega 6$) are the most important of the EFAs (Ulrich 1994; Sargent et al. 1999; Schleichriem et al. 2006). Corals can acquire EFAs via translocation from zooxanthellae (Papina et al. 2003) or through heterotrophic consumption of zooplankton, pico- and nanoplankton, or dissolved

organic matter (DOM) (Sebens 1987; McAuley 1994; Ferrier-Pagès and Gattuso 1998). Therefore, EFAs can be used as molecular markers of dietary sources for a coral.

The stable carbon isotope ($\delta^{13}\text{C}$) composition of an organic compound such as a fatty acid reflects its synthetic pathway and hence its source (Teece et al. 1999; Hayes 2001). Accordingly, the $\delta^{13}\text{C}$ values of fatty acids in corals will be similar to the $\delta^{13}\text{C}$ values of the same compound in its dietary source. Like other consumers, corals directly assimilate EFAs, and, because the entire carbon skeleton is acquired by the consumer, little or no isotopic fractionation occurs during this process. In the case of nonessential fatty acids, including SAFAs, which can be synthesized de novo by the consumer, the $\delta^{13}\text{C}$ values will reflect the competing processes of assimilation and de novo synthesis (Villinski et al. 2004). The $\delta^{13}\text{C}$ values of fatty acids produced by zooxanthellae and zooplankton differ by more than 5‰ (Treignier et al. 2009), adding to the success of this isotopic approach in elucidating dietary preferences in symbiotic relationships within scleractinian corals (Treignier et al. 2009) and tridacnid clams (Johnston et al. 1995).

The primary objective of our study was to determine the source of fatty acids in scleractinian corals, thereby estimating the extent of heterotrophic feeding of individual corals in situ. We separately measured and compared the fatty acid profiles and $\delta^{13}\text{C}$ values of individual fatty acids in zooxanthellae and coral hosts from the two scleractinian coral species, *Montastraea faveolata* and *Porites astreoides*, from reefs of the Florida Reef Tract. We measured identical parameters in available zooplankton from the same reefs and used an isotopic mass balance approach to determine the proportion of fatty acids that originated from either an autotrophic or a heterotrophic source.

Evaluating the relative contribution of heterotrophic and autotrophic sources of nutrition would provide important

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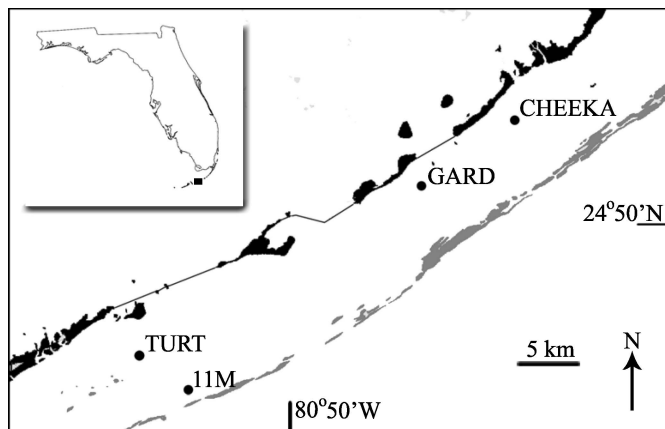


Fig. 1. Locations of reef sites in the Florida Reef Tract. Light gray areas indicate main reef tract and black islands are the Florida Keys. Inset shows black rectangle that is enlarged in main image.

information on the feeding strategies of corals under different environmental conditions and habitats. Feeding behaviors and sources of nutrition of corals have been linked to coral survivorship under thermal stress (i.e., bleaching) (Grottoli et al. 2004; Rodrigues et al. 2008) and will therefore provide important information on the potential resilience of corals to future disturbance scenarios.

Methods

Study site—Corals and zooplankton were collected from reefs of the Florida Reef Tract, Florida (Fig. 1) under permit (FKNMS-2007-055). Fragments (ca. 3 cm²) of *Montastraea faveolata* and *Porites astreoides* were collected by research divers from four or five replicate colonies at four shallow reef sites (GARD, 24°50.245'N, 80°43.768'W; CHEEKA, 24°53.996'N, 80°37.026'W), (TURT, 24°43.903'N, 80°54.037'W; 11M, 24°43.407'N, 80°51.638'W). Samples were frozen for later analysis. Temperature, pH, turbidity, and specific conductivity (converted to salinity) were measured (Yellow Springs Instruments 6600 multiparameter probe) at each site prior to sampling, and water was collected (3 × 800 mL). At these same sites, zooplankton were collected by horizontally towing nets (50 μm) immediately above coral heads, with a flow meter (General Oceanics) used to determine volume sampled with three separate tows performed on a single day. Emerging demersal zooplankton were collected at GARD using similar techniques at dusk on a single night. An aliquot of zooplankton was preserved in ethanol for microscopic examination, and the remainder was frozen in seawater and then lyophilized at −55°C.

Water quality parameters—Water was prefiltered (50 μm) to remove large particles and zooplankton, and then particulates were collected by vacuum filtration onto glass fiber filters (nominal pore size 0.7 μm). Three filters were weighed for total suspended particulate matter (SPM) on an analytical balance (± 0.01 mg). Three additional filters were treated with 1% HCl to remove inorganic carbonate,

and total organic carbon (POC) and nitrogen (PON) content were measured using a Costech Elemental Analyzer (expressed as mass per liter). Atomic C:N ratios were calculated from these data.

Isolation of tissue—Coral tissue was removed from the skeleton using the air-brush technique (Szmant et al. 1990); the resulting slurry was homogenized (Tissue Tearor; 15 s), and an aliquot (0.5 mL) was saved for total lipid analysis. The homogenate was centrifuged (500 × g for 5 min) to pellet the zooxanthellae. The zooxanthellae pellet was then repeatedly washed with filtered seawater (0.2 μm), and pelleted again (3–5 times), with purity being evaluated by microscopy after each wash and sedimentation cycle to ensure less than 5% contamination by animal cells. The original supernatant containing animal cells was repeatedly mixed by shaking and centrifuged (up to 8 times) to remove zooxanthellae until contamination by zooxanthellae was less than 5% by microscopy. Both zooxanthellae and animal fractions were then lyophilized at −55°C.

Total lipid content of corals—A 0.5-mL aliquot of total coral homogenate was lyophilized and then extracted three times (2 mL: 2:1 dichloromethane:methanol) with mixing and sonication. The resulting organic extracts were collected after centrifuging (500 × g; 5 min); all three extracts were combined, dried under a stream of nitrogen gas, and weighed on an analytical balance (± 0.01 mg). Surface areas of the coral pieces were calculated using the foil method (Marsh 1970), and total lipid content was expressed as mg lipid cm⁻².

Fatty acid analysis—Lyophilized aliquots of zooxanthellae, animal, or zooplankton tissue (0.3–2 mg) were separately extracted three times in 2:1 dichloromethane:methanol with mixing and sonication. The resulting organic extracts were collected after centrifugation (500 × g; 5 min). The three extracts were combined and dried under a stream of nitrogen gas, and then the internal standard, tricosanoic acid, was added. Extracts were dissolved in toluene (100 μL) and esterified by adding methanolic hydrochloric acid (500 μL; Sigma-Aldrich) and heating for 60 min at 60°C. The sample was cooled to room temperature, and doubly-distilled deionized water (1 mL) and hexane (2 mL) were added. After thoroughly vortexing the solution (30 s), the resulting fatty acid methyl esters were extracted into hexane three times. The three extracts were combined and evaporated to dryness under a stream of nitrogen, then toluene (500 μL) was added to co-distill any residual water, with the azeotrope being evaporated under a stream of nitrogen. The dry residue was silylated by the addition of *bis*(trimethylsilyl)-trifluoroacetamide (BSTFA, Sigma-Aldrich) and subsequent heating (60 min; 60°C) to protect any alcohol groups; after cooling, surplus BSTFA was evaporated under a stream of nitrogen. The residue was dissolved in dichloromethane (500 μL) and vortex mixed, and the solvent was evaporated under a stream of nitrogen. This step ensured that no residual BSTFA was present prior to gas chromatographic (GC) analysis. Samples were dissolved in 2,2,4-trimethylpentane

and analyzed using a Shimadzu GC 17-A with a flame ionization detector (GC-FID) on an Omegawax column (Supelco; 30 m, inner diameter (i.d.) 0.25 mm, film thickness 0.25 μm). One microliter of sample was injected (split mode, 15:1); the oven was programmed at 60°C for 1 min, then increased to 160°C at 15°C min^{-1} , further increased to 265°C at 5°C min^{-1} , and held at 265°C for 25 min. Injector and detector temperatures were both 250°C, and helium was used as the carrier gas at a flow rate of 1.3 mL min^{-1} . Fatty acids were identified using standard mixtures (Supelco) or by gas chromatography-mass spectrometry (GC-MS) (Shimadzu QP 5050) operating in the electron-impact mode. Concentrations were determined relative to the internal standard (tricosanoic acid) using the response factor for each fatty acid methyl ester (Ackman 2000). Control samples were treated in an identical fashion to determine blank levels, which were always below the fatty acid detection limit of 1 ng mg^{-1} , and the error in overall measurement was 1–7%.

Compound specific stable isotope analysis—The stable isotope composition of individual fatty acids, as methyl esters, was measured by gas chromatography-combustion-isotope ratio mass spectrometry (GC-C-IRMS), using a ThermoTrace Ultra GC coupled via a ThermoFinnigan GC-C III interface to a ThermoFinnigan Delta XL Plus stable isotope mass spectrometer. Identical GC conditions were used for GC-C-IRMS analyses. Correction for the addition of methyl carbon from the methanolic HCl was calculated for each fatty acid by mass balance (Tecece et al. 1999) from analyses of free ($-30.0 \pm 0.1\%$) and methylated ($-31.3 \pm 0.2\%$) tricosanoic acid. All samples were measured in triplicate, and typical precision (1 SD) for individual compounds ranged from 0.2‰ to 0.5‰ for signal sizes 0.3 to 2V. All mass chromatograms and 45:44 traces were manually inspected to ensure that signals were within this linearity range (Sherwood Lollar et al. 2007) and that no co-elution of other lipid compounds occurred with the peaks of interest, thereby altering their isotopic compositions. We identified several sterols in our samples, and our oven was programmed to remain at 265°C for 25 min to ensure that all sterols eluted prior to subsequent runs, as evidenced by GC-MS analyses. No sterols or alcohols were observed in subsequent blank runs. Repeated injections of a series of standard fatty acid methyl esters (C_{16} , C_{17} , C_{18} , C_{19} , C_{20}) showed a precision of $\pm 0.3\%$ and agreed with their off-line values within $\pm 0.5\%$ after correction for the added methyl groups. Daily measurements of a cod oil fatty acid standard showed precision of $\pm 0.5\%$ for $\text{C}_{16:0}$, $\text{C}_{18:0}$, DHA, and EPA. Stable carbon isotope data are presented using the standard ‰ convention:

$$\delta^{13}\text{C} = (R_{\text{sample}}/R_{\text{standard}} - 1) \times 10^3 \quad (1)$$

where R is the $^{13}\text{C}:^{12}\text{C}$ ratio of the sample and standard, respectively, and data are reported relative to the Vienna Pee Dee Belemnite standard.

Fatty acids assimilated from heterotrophic feeding—For each colony, the fraction of coral host fatty acids (DHA,

ARA, and $\text{C}_{16:0}$) derived from heterotrophic feeding on zooplankton (f_{het}) was determined using an isotopic mass balance approach modified from Villinski et al. (2004), assuming that the balance ($1 - f_{\text{het}}$) was acquired from their symbiotic zooxanthellae. By measuring the $\delta^{13}\text{C}$ value of an individual fatty acid from the coral host ($\delta^{13}\text{C}_{\text{host}}$), from the symbiont ($\delta^{13}\text{C}_{\text{sym}}$), and from the zooplankton food source ($\delta^{13}\text{C}_{\text{zp}}$), f_{het} was calculated from the following equation:

$$\delta^{13}\text{C}_{\text{host}} = f_{\text{het}}\delta^{13}\text{C}_{\text{zp}} + (1 - f_{\text{het}})\delta^{13}\text{C}_{\text{sym}} \quad (2)$$

For ease of future discussions, f_{het} was multiplied by 100 to express the amount of fatty acids derived from a source as a percentage. The overall uncertainty in f_{het} expressed as a percentage was $\pm 5\%$ using the propagation of errors formulae of Bevington (1969), based on an uncertainty of $\pm 0.5\%$ in the calculation of the $\delta^{13}\text{C}$ values of fatty acids.

This mass balance model assumes that the transfer of the specific fatty acids from the zooxanthellae via translocation to the host, and from external food sources through heterotrophic feeding, both occur with little ($< 1\%$ propagated experimental error) or no isotopic fractionation and that there is no endogenous synthesis of these particular fatty acids. For EFAs (including DHA, EPA, and ARA) and $\text{C}_{16:0}$ SAFA, minimal isotopic fractionations occur during translocation in symbiotic associations (Johnston et al. 1995; Treignier et al. 2009) and during direct assimilation from external food sources by heterotrophs (Rieley et al. 1999; Jim et al. 2003). The second assumption is also valid for EFAs, as invertebrates are generally unable to endogenously synthesize EFAs in any appreciable quantity (Bell et al. 2007). However, endogenous synthesis of $\text{C}_{16:0}$ fatty acid does occur in invertebrates, although no direct measurements of de novo production have been measured in corals. Invertebrates typically assimilate fatty acids directly from their diet rather than produce them endogenously, if they are present as easily digestible triglycerides rather than polar lipids, which require greater energy to utilize (Gurr et al. 2002). Corals have an overabundant dietary supply of triglycerides from their symbionts (Patton et al. 1977) and dietary zooplankton (Lee and Hirota 1973), and, as such, they would require little endogenous synthesis to fulfill their fatty acid requirements. Similarly, endogenous fatty acid synthesis contributed less than 2% of the total fatty acid pool in daphnids feeding on a triglyceride-rich diet (Goulden and Place 1990). The corals in our study had an abundant supply of fatty acids from triglyceride-rich sources, which would suggest that little endogenous synthesis of $\text{C}_{16:0}$ fatty acids would actually occur, although we do not have a direct measure of the extent of this synthesis. This potential low-level endogenous synthesis of $\text{C}_{16:0}$ ($< 2\%$ cf. daphnids) could affect the value of $\delta^{13}\text{C}_{\text{host}}$ and our subsequent calculation of f_{het} for this particular fatty acid. However, this potential variation is within the overall uncertainty of 5% in our calculation of f_{het} for these corals.

Stable isotope analyses— $\delta^{13}\text{C}$ values of zooplankton, zooxanthellae, animal, and particulate filters were measured at the Environmental Science Stable Isotope Labo-

Table 1. Physicochemical parameters of reef sites from the Florida Keys Reef Tract. (NTU = Nephelometric Turbidity Units; At = atomic).

Site	Depth (m)	Temp. (°C)	pH	Turbidity (NTU)	Salinity	SPM (mg L ⁻¹)	PON (μg L ⁻¹)	POC (μg L ⁻¹)	At C:N	δ ¹³ C _{POC} (‰)
GARD	3.8	29.6	7.4	129.4±1.1	32.9	22.2±1.6	16.3±1.7	126.9±9.9	9.1±0.8	-21.4±1.0
CHEEKA	4.3	29.7	8	100.1±0.8	33.1	24.7±1.6	19.8±0.7	106.4±1.1	6.1±0.4	-21.6±0.3
TURT	5.2	29.4	6.8	99.2±1.1	32.9	22.0±2.4	13.3±1.7	124.9±12.3	11.0±0.7	-22.2±0.7
11M	5.6	29.1	7.9	106.4±9.5	32.9	21.8±1.9	11.8±1.6	93.4±9.9	9.2±0.3	-27.1±1.0

ratory (EaSSIL) at the State University of New York College of Environmental Science and Forestry (SUNY-ESF) using a Costech elemental analyzer linked via a ThermoFinnigan ConFlo III interface to a Finnigan MAT Delta XL Plus stable isotope mass spectrometer (EA-IRMS). All samples were treated with dilute HCl to remove inorganic carbon, which can affect overall δ¹³C values (Teece and Fogel 2004). Samples were analyzed in triplicate, and accuracy and precision of measurement was independently verified using National Institutes of Standards and Technology (NIST) sucrose (δ¹³C = -10.5 ± 0.2‰ [n = 45]), National Bureau of Standards 22 oil (-29.7 ± 0.2‰ [n = 28]), and NIST 1587 peach leaves (-25.8 ± 0.1‰ [n = 110]). Daily precision of the instrument was verified by repeated analyses of internal laboratory standards including acetanilide (-29.9 ± 0.2‰ [n = 34]), fish muscle tissue (-18.1 ± 0.2‰ [n = 32]), and plant tissue (-28.0 ± 0.2‰ [n = 22]), which were analyzed repeatedly during the sample runs. Percentage data was arcsine transformed (Zar 1984), and statistical tests, including *t*-tests, linear regression, and ANOVA followed by Tukey's HSD test, were performed using MiniTab Release 15 software. Comparisons were considered significant at α = 0.05.

Results

Site characteristics—Although the turbidity at GARD was higher than at TURT, CHEEKA, and 11M (Table 1; Tukey's HSD, *p* < 0.05 following ANOVA, *F*_{3,42} = 109.97, *p* = 0.000), SPM concentrations were similar on all reefs (ANOVA, *F*_{3,10} = 2.18, *p* = 0.153). Concentrations of POC were lower at 11M than at GARD and TURT reefs (Tukey's HSD, *p* < 0.05 following ANOVA, *F*_{3,8} = 8.40, *p* = 0.007), and atomic C:N ratios of particulates at TURT were significantly higher than at other reefs (Tukey's HSD, *p* < 0.05 following ANOVA, *F*_{3,8} = 35.60, *p* = 0.000).

Fatty acid content of zooplankton—Zooplankton are a rich source of PUFAs, containing up to 35% DHA and 16% EPA (Fig. 2), and the C_{16:0} SAFA accounted for 20–25% of total fatty acids. There were no significant differences in the amounts of DHA (ANOVA, *F*_{3,4} = 2.84, *p* = 0.170), 16:0 (ANOVA, *F*_{3,4} = 1.36, *p* = 0.374), and 18:0 (ANOVA, *F*_{3,4} = 0.64, *p* = 0.630) in zooplankton from different sites. There were also no differences in amounts of DHA (*t*-test, *t* = 0.83, *df* = 1, *p* = 0.559), 16:0 (*t*-test, *t* = 0.73, *df* = 1, *p* = 0.600), and 18:0 (*t*-test, *t* = 2.47, *df* = 1, *p* = 0.245) in zooplankton collected both

during the day and at night from the GARD site. However, zooplankton collected at night did contain higher amounts of EPA than those collected during the day at the one GARD site (*t*-test, *t* = 13.2, *df* = 1, *p* = 0.048).

Stable isotope composition of zooplankton—Bulk zooplankton from TURT (-16.9‰) and 11M (-17‰) were depleted in ¹³C relative to those from reefs GARD (-15.0‰) and CHEEKA (-14.8‰). All fatty acids in zooplankton from all sites were depleted relative to bulk values and ranged from -28‰ to -18‰ (Table 2). The δ¹³C values of DHA from all zooplankton were similar (ANOVA, *F*_{4,11} = 2.24, *p* = 0.131), and the δ¹³C values of ARA (ANOVA, *F*_{1,5} = 0.43, *p* = 0.543), EPA (ANOVA, *F*_{1,5} = 0.70, *p* = 0.441), and DHA (ANOVA, *F*_{1,5} = 0.46, *p* = 0.528) in demersal zooplankton collected at night (GARDnight) were similar to those of zooplankton collected during the day from this same site (GARD).

Fatty acid content of corals—Corals and their symbiotic zooxanthellae contained a wide range of even-numbered fatty acids including SAFAs, MUFAs, and PUFAs. They ranged from C₁₄ to C₂₂ and contained up to six double bonds (Fig. 3). As a result of the methods used, these fatty

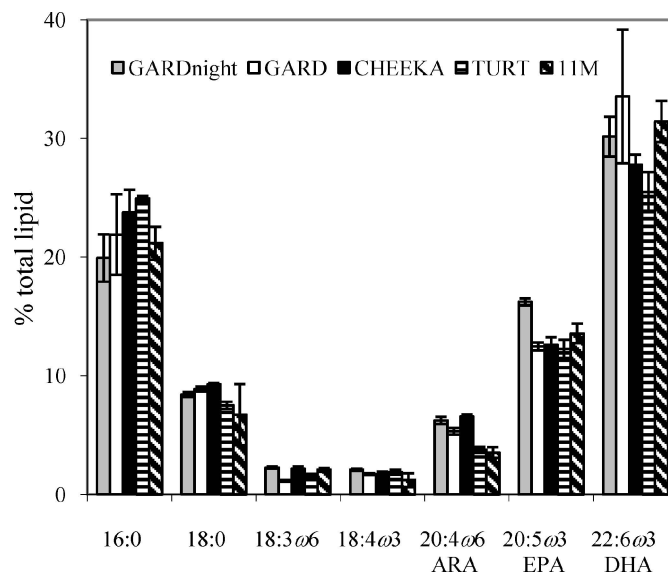


Fig. 2. Composition of the major fatty acids (% total fatty acid) from demersal zooplankton (GARDnight) and daytime-collected zooplankton from four reef sites in the Florida Keys. Bars represent averages (± SD) of separate analyses of three replicate samples from each site.

Table 2. Stable isotope composition of individual fatty acids in zooplankton collected from reefs in the Florida Keys. Samples were collected during daylight, and demersal zooplankton were collected from one site (GARDnight) at dusk. Data represent triplicate measurements of $n = 3$ separate samples from each site.

Fatty acid	GARD ($n=3$)	CHEEKA ($n=3$)	TURT ($n=3$)	11M ($n=3$)	GARDnight ($n=3$)
SAFA					
14:0	-24.9±1.0	-26.2±5.2	-22.6±0.7	-23.7±0.9	-25.2±2.0
16:0	-28.3±2.0	-23.4±4.4	-21.8±0.3	-22.3±0.6	-24.8±2.0
18:0	-25.1±3.4	-22.1±2.6	-23.3±1.9	-23.6±1.9	-22.6±3.5
MUFA					
16:1 ω 7	-22.1±3.4	-20.1±0.6	-21.8±1.0	-22.5±1.0	-20.8±2.4
18:1 ω 9	-20.8±2.1	-19.7±1.3	-21.3±0.6	-21.5±0.2	-19.4±1.3
PUFA					
16:4	-26.9±4.0	-22.8±2.8	-23.9±0.6	-22.7±0.6	-24.1±0.9
18:3 ω 6	-27.2±0.6	-25.4±1.3	-27.8±1.8	-19.0±1.1	-23.2±2.7
18:4 ω 3	-23.4±2.8	-23.0±0.4	-25.0±1.4	-24.8±2.5	-22.0±1.1
20:4 ω 6 ARA	-21.2±1.2	-18.6±2.3	-20.6±1.9	-18.8±2.4	-20.5±1.4
20:5 ω 3 EPA	-23.8±1.3	-21.6±1.8	-23.3±1.4	-24.1±0.5	-22.5±1.0
22:6 ω 3 DHA	-21.2±1.1	-20.1±2.1	-22.3±1.4	-23.2±0.9	-20.4±1.7

acids could be present in vivo as free compounds, as well as those esterified in triglycerides, wax esters, polar lipids (including phospholipids), and other esters. The distributions of fatty acids in zooxanthellae from *P. astreoides* were dominated by SAFAs $C_{16:0}$ and $C_{18:0}$ and by PUFAs 20:4 ω 6 (ARA) and 22:6 ω 3 (DHA). Except for colonies at TURT, zooxanthellae from *M. faveolata* contained significantly lower amounts of ARA (8–9%) than did zooxanthellae from *P. astreoides* (12–27%; t -test, $t = 10.77$, $df = 21$, $p = 0.000$). There was greater variability in the proportions of fatty acids found in symbionts and hosts of *P. astreoides* than for *M. faveolata* from different reef sites. Animal hosts from *P. astreoides* had significantly lower amounts of ARA (Tukey's HSD, $p < 0.05$ following ANOVA $F_{3,13} = 20.05$, $p = 0.000$), and DHA (Tukey's HSD, $p < 0.05$ following ANOVA $F_{3,13} = 32.54$, $p = 0.000$) at TURT than at all other sites. Fatty acid distributions of coral hosts in *P. astreoides* were dominated by $C_{18:0}$ (17–35%), ARA (23–29%), and DHA (12–27%), with the exception of corals from TURT, whose hosts held total SAFA proportions (70%) greater than those of ARA (6%) and DHA (4%). In *M. faveolata* hosts, the amounts of the SAFA $C_{16:0}$ were higher than the amounts of ARA (9–20%) and DHA (6–15%).

Stable isotope composition of fatty acids in symbionts and coral hosts—The $\delta^{13}C$ values of fatty acids in zooxanthellae from individual colonies of *P. astreoides* spanned a large range from -9‰ to -18‰, with average values for specific reef sites shown in Table 3. In general, fatty acids in hosts of *P. astreoides* were similar to, or depleted in ^{13}C relative to, the same compounds in their symbionts, except for DHA in colonies from TURT and 14:0 from GARD colonies. For *M. faveolata*, the average $\delta^{13}C$ values of fatty acids in hosts also spanned a wide range from -10‰ to -20‰ but were not consistently depleted in ^{13}C relative to the same compounds in their symbionts (Table 4). There were no significant differences in the $\delta^{13}C$ values of DHA

(ANOVA, $F_{3,11} = 3.09$, $p = 0.072$) and ARA (ANOVA, $F_{3,12} = 0.04$, $p = 0.990$) in zooxanthellae between reefs for *P. astreoides*. For *M. faveolata*, DHA in zooxanthellae from CHEEKA was significantly depleted relative to that from other colonies from GARD and TURT reefs (Tukey's HSD, $p < 0.05$ following ANOVA $F_{3,13} = 9.22$, $p = 0.002$).

Fatty acids assimilated from heterotrophic feeding—Corals obtain their fatty acids from both heterotrophic feeding (f_{het}) and from their symbionts ($1 - f_{het}$), which are displayed as percentages in Fig. 4 (i.e., $f_{het} \times 100$). There were substantial differences in the amounts of heterotrophically derived fatty acids within, and between, coral species, whether located on the same site or between sites. All colonies of *M. faveolata* obtained less than 50% of their $C_{16:0}$ and ARA from heterotrophic sources, and, except for two colonies (GARD-Colony3 and CHEEKA-Colony1), less than 50% of their DHA was obtained from direct feeding. Individual colonies of *P. astreoides* (GARD-Colony4, CHEEKA-Colony5) and *M. faveolata* (GARD-Colony3, CHEEKA-Colony1) acquired more than 50%, and up to 100%, of their DHA from heterotrophic sources. Single colonies of *P. astreoides* (CHEEKA-Colony3) and *M. faveolata* (CHEEKA-Colony5) obtained all of their $C_{16:0}$ SAFAs and EFAs from their symbionts.

Some colonies obtained their SAFAs and EFAs from different sources: $C_{16:0}$ in *M. faveolata* on TURT was almost entirely derived from its symbiont, whereas, within these same colonies, up to 30% of their DHA was from heterotrophic food sources. The opposite was seen in *P. astreoides* colonies from TURT, in which heterotrophic feeding provided between 10–40% of $C_{16:0}$, whereas more than 90% of the host DHA was from its symbiont.

Total lipid content of corals—Lipid content of individual colonies ranged from 12 to 53 mg cm^{-2} , with highest amounts for both species at CHEEKA and lowest amounts for both at TURT (Fig. 5). At each site there was

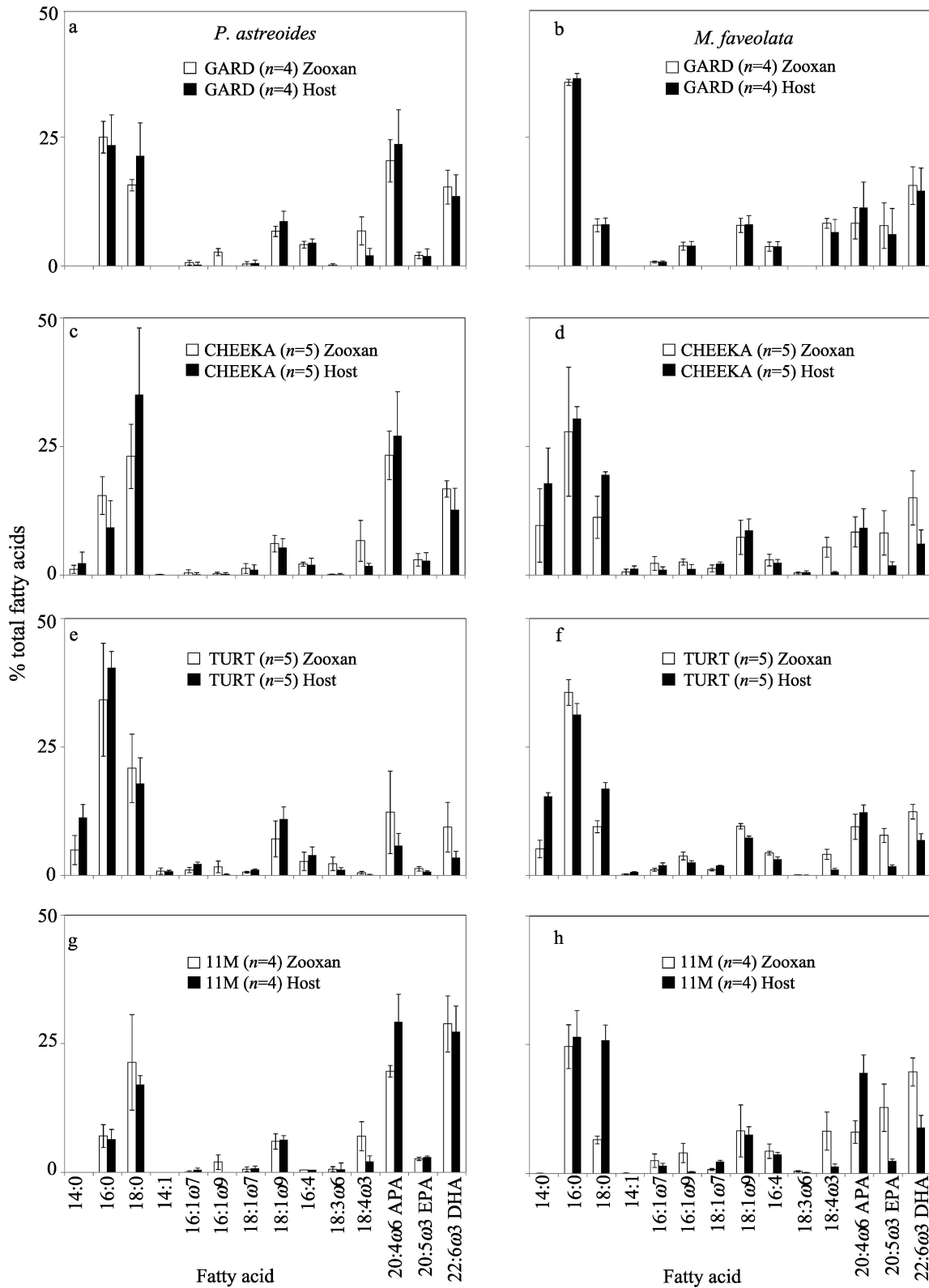


Fig. 3. Fatty acid composition (% total lipid \pm SD) of zooxanthellae and animal hosts for (a, c, e, g) *P. astreoides* and (b, d, f, h) *M. faveolata* from four reefs in the Florida Keys.

Table 3. Stable carbon isotope composition of individual fatty acids in zooxanthellae and coral hosts of *Porites astreoides* from four reef sites in the Florida Keys. Values represent averages (\pm SD) for triplicate measurements of each fraction from n separate colonies. (nd = not determined.)

Fatty acid	Zooxanthellae				Host			
	GARD ($n=4$)	CHEEKA ($n=5$)	TURT ($n=5$)	11M ($n=4$)	GARD ($n=4$)	CHEEKA ($n=4$)	TURT ($n=5$)	11M ($n=4$)
SAFA								
14:0	-13.6 \pm 1.7	-14.1 \pm 1.4	-11.8 \pm 1.2	-14.4 \pm 1.3	-12.4 \pm 1.3	nd	-16.3 \pm 2.6	-14.9 \pm 0.9
16:0	-12.2 \pm 2.0	-14.0 \pm 1.6	-10.6 \pm 0.5	-12.4 \pm 0.9	-13.0 \pm 1.4	-16.2 \pm 4.7	-14.3 \pm 1.1	-13.4 \pm 2.4
18:0	-11.9 \pm 1.9	-13.9 \pm 2.4	-10.8 \pm 0.6	-13.5 \pm 1.3	-12.3 \pm 1.1	-16.1 \pm 3.4	-14.0 \pm 0.6	-13.4 \pm 2.0
MUFA								
16:1 ω 7	-11.5 \pm 0.8	nd	-11.3 \pm 0.3	nd	nd	nd	-14.1 \pm 0.9	nd
18:1 ω 9	-11.4 \pm 1.7	-13.4 \pm 1.0	-10.7 \pm 1.5	-12.6 \pm 1.8	-14.1 \pm 1.4	nd	-13.3 \pm 0.3	-13.3 \pm 2.4
PUFA								
16:4	-12.1 \pm 1.0	-12.0 \pm 1.0	-13.3 \pm 3.4	nd	nd	-14.5 \pm 1.0	-15.1 \pm 1.6	nd
18:3 ω 6	-12.8 \pm 1.2	-13.6 \pm 1.9	-11.9 \pm 2.5	-12.1 \pm 1.5	-14.2 \pm 1.7	nd	-13.8 \pm 1.4	-14.2 \pm 1.7
18:4 ω 3	-12.0 \pm 1.3	-12.3 \pm 2.2	-13.8 \pm 1.7	-11.5 \pm 1.9	-12.2 \pm 1.1	nd	-14.6 \pm 1.2	-15.8 \pm 2.4
20:4 ω 6 ARA	-13.2 \pm 1.3	-12.0 \pm 1.2	-13.1 \pm 1.8	-13.1 \pm 0.9	-13.6 \pm 1.2	-13.9 \pm 1.9	-13.7 \pm 2.7	-13.9 \pm 1.1
20:5 ω 3 EPA	-13.7 \pm 2.0	-15.3 \pm 1.6	-13.2 \pm 3.5	-15.1 \pm 2.4	-16.1 \pm 2.0	nd	nd	-15.6 \pm 2.0
22:6 ω 3 DHA	-11.5 \pm 0.7	-12.8 \pm 1.3	-11.3 \pm 0.8	-11.2 \pm 1.1	-12.2 \pm 1.6	-15.4 \pm 3.7	-9.5 \pm 1.6	-13.1 \pm 1.3

considerable variability in lipid content, with lowest variability at TURT. *P. astreoides* colonies at 11M contained higher amounts of total lipid than those at TURT (t -test, $t = 4.27$, $df = 3$, $p = 0.024$), and for all other sites there were no significant differences in lipid contents of both species (ANOVA, $F_{5,20} = 2.16$, $p = 0.099$). For individual colonies, there was no correlation between total lipid amount and heterotrophic acquisition (f_{het}) of SAFAs ($R^2 = 0.01$, *M. faveolata*; $R^2 = 0.15$, *P. astreoides*), or of EFAs ($R^2 = 0.23$, *M. faveolata*; $R^2 = 0.01$, *P. astreoides*).

Discussion

Coral animals require fatty acids for various cellular functions, including membrane construction, energy meta-

bolism, reproduction, and growth. They can acquire fatty acids from directly consuming prey (i.e., zooplankton and other particles) and via translocation from their autotrophic zooxanthellae. These sources all contain high abundances of fatty acids, and we show that heterotrophic assimilation of fatty acids from zooplankton can be a significant and prevalent source of fatty acids for corals.

Fatty acids in corals—Few studies have separately analyzed the lipid composition of the host and zooxanthellae from the same coral colony (Papina et al. 2003; Papina et al. 2007; Treignier et al. 2008), and this is the first report for Caribbean corals. Zooxanthellae contained amounts of DHA similar to, or slightly higher than, those of their host, suggesting that the symbionts can provide

Table 4. Stable carbon isotope composition of individual fatty acids in zooxanthellae and coral hosts of *Montastraea faveolata* from reef sites in the Florida Keys. Values represent averages (\pm SD) for triplicate measurements of each fraction from n separate colonies. (nd = not determined.)

Fatty acid	Zooxanthellae				Host			
	GARD ($n=4$)	CHEEKA ($n=5$)	TURT ($n=5$)	11M ($n=4$)	GARD ($n=4$)	CHEEKA ($n=4$)	TURT ($n=5$)	11M ($n=4$)
SAFA								
14:0	-17.6 \pm 3.6	-15.2 \pm 0.3	-16.6 \pm 1.6	-15.2 \pm 1.0	-20.2 \pm 1.0	-16.3 \pm 1.0	-14.9 \pm 0.8	nd
16:0	-16.4 \pm 1.9	-13.8 \pm 1.0	-12.6 \pm 1.1	-14.3 \pm 1.2	-18.1 \pm 2.7	-14.3 \pm 0.8	-12.0 \pm 0.7	-15.2 \pm 1.4
18:0	-15.5 \pm 1.5	-15.5 \pm 2.0	-13.6 \pm 1.0	-13.3 \pm 0.8	-17.5 \pm 1.7	-15.4 \pm 1.5	-13.1 \pm 0.8	-15.1 \pm 0.7
MUFA								
16:1 ω 7	-16.9 \pm 0.8	-17.2 \pm 1.0	-13.4 \pm 1.2	-18.7 \pm 2.8	-18.3 \pm 1.0	-13.5 \pm 1.0	-13.9 \pm 0.7	-16.6 \pm 0.5
18:1 ω 9	nd	nd	-11.9 \pm 1.2	-14.2 \pm 1.6	nd	nd	-12.4 \pm 0.8	-15.3 \pm 1.7
PUFA								
16:4	-15.3 \pm 2.7	-11.6 \pm 0.1	-13.1 \pm 1.2	nd	-19.4 \pm 2.1	-13.8 \pm 1.4	-14.6 \pm 0.7	-14.3 \pm 1.0
18:3 ω 6	-13.1 \pm 1.1	-12.5 \pm 0.7	-12.0 \pm 1.1	-13.0 \pm 1.3	-17.7 \pm 0.8	-14.5 \pm 0.9	-13.3 \pm 0.9	-13.9 \pm 1.0
18:4 ω 3	-13.0 \pm 1.2	-13.4 \pm 1.1	-12.7 \pm 1.5	-13.4 \pm 1.6	-18.9 \pm 1.0	-15.7 \pm 2.0	-15.9 \pm 0.3	nd
20:4 ω 6 ARA	-16.3 \pm 1.5	-15.5 \pm 1.1	-14.5 \pm 0.6	-15.4 \pm 1.1	-16.8 \pm 0.7	-15.8 \pm 1.3	-13.6 \pm 0.7	-13.5 \pm 1.1
20:5 ω 3 EPA	-15.9 \pm 0.8	-16.6 \pm 1.2	-13.9 \pm 1.4	-15.1 \pm 0.9	-15.0 \pm 1.0	-18.8 \pm 3.6	-13.7 \pm 1.4	nd
22:6 ω 3 DHA	-13.4 \pm 0.1	-15.4 \pm 0.5	-12.7 \pm 1.2	-14.0 \pm 0.6	-16.6 \pm 1.1	-15.4 \pm 3.0	-14.0 \pm 0.9	-10.1 \pm 1.0

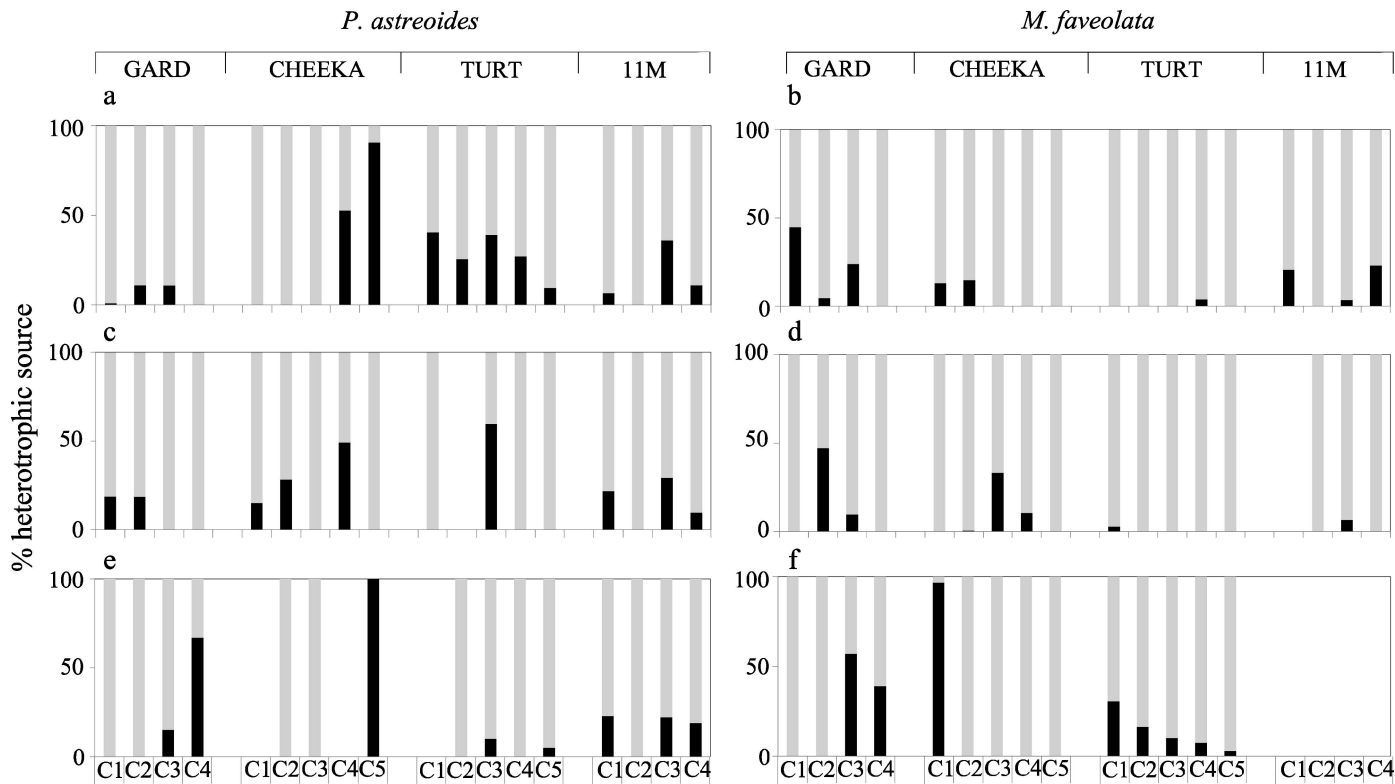


Fig. 4. Relative contribution (%) of heterotrophic sources of fatty acids in coral hosts of (a, b) C_{16:0}, (c, d) ARA, and (e, f) DHA in individual colonies (labeled C1–C5) of (a, c, e) *P. astreoides* and (b, d, f) *M. faveolata* from reefs in the Florida Keys. Dark bars represent % heterotrophic source (f_{het}), and light bars represent % autotrophic source ($1 - f_{het}$). Absence of bars indicates no data were available.

significant quantities of these essential fatty acids to the host. Similarly, the presence of DHA in the coral hosts and symbiotic zooxanthellae of *M. digitata* (Papina et al. 2003) and *T. reniformis* (Treignier et al. 2008) was used as evidence of translocation of this fatty acid from their symbionts. Overall, the animal hosts of *M. faveolata* and *P. astreoides* contained the same fatty acids as did their symbionts, findings similar to those reported for other

intact coral-symbiont associations (Meyers et al. 1978; Bachok et al. 2006; Imbs et al. 2007). The relative amounts of ARA and DHA in corals vary substantially between species (Bachok et al. 2006; Imbs et al. 2007; our study), and DHA represented more than 25% of the fatty acid pool in *P. astreoides* (11M reef site), which is substantially greater than for any other corals reported. DHA is reportedly present as a minor component (< 4%; Bachok

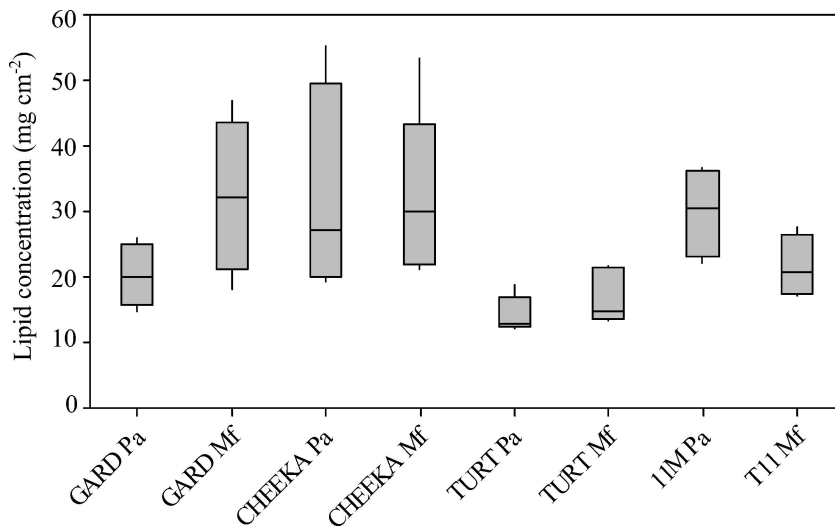


Fig. 5. Box and whisker plot of total lipid content of *P. astreoides* (Pa) and *M. faveolata* (Mf) colonies from different reef sites in the Florida Keys.

et al. 2006; Imbs et al. 2007) in some corals, whereas in others it can represent up to 14% (Imbs et al. 2007).

The fatty acid compositions of the zooxanthellae from *P. astreoides* and *M. faveolata* were dominated by SAFAs ($C_{16:0}$ and $C_{18:0}$) and PUFAs (18:4 ω 3, ARA, and DHA) and were qualitatively similar to those found in other zooxanthellae (Latyshev et al. 1991; Zhukova and Titlyanov 2003; Treignier et al. 2008). We noticed considerable variation in the amount of DHA in zooxanthellae between different reefs for both *P. astreoides* and *M. faveolata*. These variations in lipid composition may reflect environmental parameters that are known to affect lipid compositions of corals and symbionts, including temperature and light intensity (Papina et al. 2007), feeding behavior (Al-Moghrabi et al. 1995; Treignier et al. 2008), and presence of different zooxanthellae clades (Zhukova and Titlyanov 2003). We found no correlation with any of the physicochemical parameters that we measured, although we did not have a direct measure of light intensity or the identity of the specific zooxanthellae clades present. Relative abundances of specific PUFAs and other fatty acids may be a potential indicator of symbiont sensitivity to thermal bleaching (Tchernov et al. 2004), and the wide variations in such abundances between reef sites may ultimately point to potential survivorship of specific reefs over others.

The $\delta^{13}C$ values of coral fatty acids can indicate their dietary sources. Typically, $C_{18:0}$ was similar to or depleted in ^{13}C by less than 1‰ relative to $C_{16:0}$ in coral hosts of both *M. faveolata* and *P. astreoides*, indicating that they are biosynthetically linked via the fatty acid synthetase pathway (Teece et al. 1999; Hayes 2001). These SAFAs can be directly acquired from dietary sources, or they can be synthesized from other biochemicals, including carbohydrates, proteins, and lipids acquired from their diets (Hayes 2001; Treignier et al. 2009). In contrast to SAFAs, EFAs (including DHA, EPA, and ARA) cannot, in general, be produced by the coral animal, so the $\delta^{13}C$ values of these EFAs reflect the dietary source (Hayes 2001). Minimal carbon isotope fractionation occurs during uptake and assimilation of EFAs in symbiotic relationships (Johnston et al. 1995; Villinski et al. 2004; Treignier et al. 2009), as the carbon skeleton of the EFA remains intact during these processes. We observed substantial variations in the $\delta^{13}C$ values of EFAs in individual corals, which showed that corals obtained their EFAs from a combination of heterotrophic feeding and from translocation of these compounds from their symbionts.

Source of fatty acids for corals—Symbiotic zooxanthellae are the dominant source of fatty acids for *M. faveolata* and *P. astreoides* and can supply up to 100% of the host's SAFA ($C_{16:0}$) and EFA (ARA and DHA) requirements (Fig. 4). Zooxanthellae contain an abundance of SAFAs, ARA, and DHA (Fig. 2), and these compounds are translocated to the host, although the proportions that individual colonies derive from the symbiont are highly variable over different reefs and for the two species we studied. The variability in coral acquisition of fatty acids from their symbionts also reflects the wide range of energy or nutrients provided by the zooxanthellae to the host. In

healthy corals, zooxanthellae can provide up to (Muscatine et al. 1981), or even more than, 100% of daily requirements of the host (Grottoli et al. 2006), whereas in low-light or bleaching situations, symbionts provide considerably lower amounts to the metabolic needs of the host (Muscatine 1990). For some coral colonies, heterotrophic feeding can be a significant source of fatty acids and can account for more than 50% of the fatty acids in individual colonies of *M. faveolata* and *P. astreoides*. However, in general, heterotrophic feeding provided considerably fewer fatty acids to these coral hosts than did their symbiotic zooxanthellae.

The particular mode by which corals acquire lipids appears to be specific to each coral colony. Fatty acids in the coral host could be up to 5‰ depleted in ^{13}C relative to the corresponding compounds in the symbiont and more closely matched the $\delta^{13}C$ value of these compounds in zooplankton. This direct indicator of the sources of fatty acids in a symbiotic relationship now provides a molecular marker to determine the extent of heterotrophic feeding of an individual coral. In a laboratory study in which the scleractinian coral *T. reniformis* was directly fed a zooplankton diet, the $\delta^{13}C$ values of the host fatty acids did not show such significant differences relative to those of the symbionts, suggesting that corals obtained their fatty acids from their symbionts and not from their zooplankton diet (Treignier et al. 2009). As pointed out by the authors, the isotopic signatures may not have been so prevalent because (1) the turnover time of animal tissue was probably greater than the term of the experiment (6 weeks), making most of the fatty acids in the host remnants from the symbionts in their condition prior to the experiment and (2) the coral studied, *T. reniformis*, is a strongly autotrophic species. Our field study and this laboratory-based experiment (Treignier et al. 2009) show that individual coral species rely to different extents on heterotrophic feeding to acquire their fatty acids and that $\delta^{13}C$ values of host fatty acids are useful in determining the long-term diet of corals.

The importance of heterotrophic food sources to sustaining coral health is becoming more evident (Muscatine et al. 1989; Grottoli and Wellington 1999; Houlbrèque and Ferrier-Pagès 2008), and here we show direct evidence for the role that heterotrophic feeding can play in the acquisition of organic compounds vital for coral health. We showed that zooplankton are an abundant source of fatty acids, and corals are known to store substantial quantities of lipids and to utilize them for survival during bleaching events (Grottoli et al. 2004; Rodrigues et al. 2008). Several studies suggested that heterotrophic feeding increases total lipid levels (Anthony and Fabricius 2000; Rodrigues and Grottoli 2007); however, we found no correlation between total lipid amount and heterotrophic acquisition of SAFAs or EFAs for our two species.

Spatial variability in feeding modes of corals—We observed substantive differences in the feeding behaviors of *P. astreoides* and *M. faveolata* colonies that were living within 20 m of each other. On single reefs, some colonies of *P. astreoides* acquired more than 50% of their fatty acids from heterotrophic feeding, whereas other neighboring

colonies acquired 0% from direct feeding. For *M. faveolata*, similar behavior was observed, although heterotrophic feeding rarely provided more than 40% of fatty acids. This intra-reef or colony-specific variability in acquisition of fatty acids suggests that the immediate area surrounding a particular coral head likely dictates its feeding mode and that corals are opportunistic feeders. Coral feeding rates may be proportional to zooplankton abundance (Sebens et al. 1998; Palardy et al. 2008), water flow (Sebens et al. 1997; Piniak 2002), and light availability (Treignier et al. 2008). On individual reefs, there can be significant spatial variations of these factors, including zooplankton abundance (Alldredge and King 1977; Yahel et al. 2005; Heidelberg et al. 2010), water flow and boundary layers (Falter et al. 2004; Reidenbach et al. 2006), and light availability and photosynthetic performance of individual polyps (Ralph et al. 2002; Hill et al. 2004; Ulstrup et al. 2006). A combination of these factors likely explains the differences of feeding patterns among colonies within a single reef system. There can be variability in the clades of zooxanthellae present within a single colony, and *M. faveolata* harbors multiple clades of zooxanthellae (Kemp et al. 2007) that vary spatially or relative to light availability.

Switching from autotrophic to heterotrophic carbon sources appears to be species-specific, and some corals exhibit great plasticity in their feeding modes (Anthony and Fabricius 2000; Piniak 2002; Houlbrèque and Ferrier-Pagès 2008). During bleaching events, *M. capitata* increased its feeding rate to acquire more than its daily requirements from heterotrophic sources, whereas *P. compressa* was largely dependent on autotrophic carbon sources (Rodrigues and Grottoli 2007). Our study contends that *M. faveolata* and *P. astreoides* have great plasticity in their feeding behaviors and that individual colonies rely on heterotrophic sources to a varying extent that cannot be correlated to a specific factor.

The recent drastic deterioration of coastal habitats worldwide has been well documented. Concurrent with declines in habitat condition, significant declines in the abundance, diversity, and spatial distribution of coral reefs and neighboring seagrasses have been recorded along with associated losses in the ecological and economic services that these ecosystem engineers contribute (Gardner et al. 2003; Waycott et al. 2009). While the classical paradigm of corals only being able to thrive in areas with high water quality may apply for most areas of the world, recent research has highlighted the presence of thriving coral communities on nearshore marginal areas with increased sedimentation, higher nutrient loads, and reduced light availability (Anthony and Fabricius 2000; Lirman and Fong 2007). Moreover, the vulnerability of corals to disturbance can be influenced by their energetic status, and the lipid reserves stored by corals may allow them to increase their resistance and resilience to stress. The increased availability of heterotrophic energy and nutrient sources in nearshore coastal habitats has been linked to higher coral growth, increased energy storage, and increased resilience to disturbances such as coral bleaching (Edinger et al. 2000; Anthony 2006; Rodrigues and

Grottoli 2007). For example, in Australia's Great Barrier Reef, corals in high-turbidity and high-nutrient habitats are able to thrive due to heterotrophic feeding and nutrient assimilation (Anthony 2000, 2006; Anthony and Fabricius 2000). Our study shows that individual coral colonies can acquire their essential biochemicals such as fatty acids from both autotrophic and heterotrophic sources, and the feeding mode of individual corals is specific to each colony. The demonstrated ability of corals to modulate their main feeding mode, from autotrophy to heterotrophy, under marginal conditions (i.e., high turbidity, sedimentation, high nutrient levels) may provide an adaptive mechanism for sustained growth over the short term that may be fundamental to corals exposed to multiple stressors.

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