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Effect of symbiotic state on the fatty acid composition of *Anthopleura elegantissima*

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ABSTRACT: *Anthopleura elegantissima*, the most abundant intertidal sea anemone on the Pacific coast of North America, naturally occurs in 3 distinct symbiotic states: zooxanthellate (hosting the dinoflagellate *Symbiodinium muscatinei*), zoochlorellate (hosting the chlorophyte *Elliptochloris marina*), and asymbiotic (lacking symbionts). To document the effect of symbiotic state on host lipids, 10 *A. elegantissima* in each symbiotic state were collected from the same location and habitat. The symbiont and host tissues were separated, and the fatty acid profiles were compared (1) between the 2 symbionts themselves, (2) among anemones in the 3 symbiotic states, and (3) between the symbionts and their host anemones. Significant differences were present in the fatty acid profiles of *S. muscatinei* and *E. marina*, with docosahexaenoic acid (DHA) abundant in *S. muscatinei*, and oleic and α-linolenic acids abundant in *E. marina*. Zooxanthellate anemone tissues had significantly higher total fatty acid content than did tissues of zoochlorellate hosts, supporting suggestions that *S. muscatinei* is more productive and thus a better symbiont. Asymbiotic sea anemones had significantly lower concentrations of total fatty acids than hosts in either symbiotic state and overall lower levels of most fatty acids. Previous research suggests that symbionts translocate specific fatty acids to their coral hosts, but there was no evidence for fatty acid translocation in *A. elegantissima*. Our results support the suggestions that hosting photosymbionts increases the fitness of *A. elegantissima* and that *S. muscatinei* is a better symbiotic partner.

KEY WORDS: *Anthopleura elegantissima* · *Elliptochloris marina* · Fatty acid content · Sea anemone · *Symbiodinium muscatinei* · Symbiosis · Zoochlorellae · Zooxanthellae

INTRODUCTION

Many cnidarians associate symbiotically with dinoflagellates of the genus *Symbiodinium* (commonly called zooxanthellae). The symbionts provide the host with photosynthetic products, including glucose, glycerol, amino acids and possibly lipids (Yellowlees et al. 2008). Some research suggests that the interaction between the host and the endosymbiotic algae is not always mutualistic, but rather a continuum, ranging from parasitism to mutualism (Lesser et al. 2013), and that the nature of the relationship may be determined by the identity of the symbiont. For example, growth, survival, thermal tolerance and, ultimately, fitness of a coral host can be affected by the clade of *Symbiodinium* it hosts (Mieog et al. 2009). Hosting Clade A S*ymbiodinium*, which fixes less carbon than Clade C, may reduce host fitness by not providing enough carbon to meet the host's nutritional requirements (Stat et al. 2008). Loram et al. (2007) found that giant sea anemones *Condylactis gigantea* with Clade A *Symbiodinium* incorporated more photosynthesis-fixed carbon into lipids and less into sugars and amino acids, compared to those hosting Clade B symbionts. Such quantitative and qualitative differences in the symbionts' contributions are believed to affect host lipid levels, in turn influencing growth and reproductive output (Stimson 1987, Ward 1995, Leuzinger et al. 2003).

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A growing body of evidence indicates that lipids play an important role in cnidarian–algal symbioses. For example, *Symbiodinium* cells, and the cnidarian cells that host them, accumulate wax esters, triglycerides, sterols, phospholipids, and fatty acids in organelles called lipid bodies (Peng et al. 2011, Chen et al. 2012, Jiang et al. 2014, Pasaribu et al. 2014). Kopp et al. (2015) found that zooxanthellae in corals turn inorganic bicarbonate and nitrates into lipid droplets and glycogen, which accumulate in the zooxanthellae. The size and density of lipid bodies is positively correlated with solar irradiation (Peng et al. 2012), suggesting that production of the lipids is linked to photosynthesis. Luo et al. (2009) suggest that 'lipid trafficking' between symbiont and host plays a role in regulating the endosymbiosis. Because dinoflagellate fatty acid markers are found in cnidarian host tissue, it is believed that algal symbionts translocate these products to their cnidarian hosts (e.g. Papina et al. 2003, 2007, Treignier et al. 2008, Imbs et al. 2014). Other authors have cited similarities in the relative proportions of fatty acids in zooxanthellae and host tissue as evidence for translocation (Treignier et al. 2008, Mortillaro et al. 2009, Imbs et al. 2010b, 2014). However, it should be noted that fatty acid markers can also be obtained through the diet, so evidence for translocation remains inconclusive.

Anthopleura elegantissima is the most abundant intertidal sea anemone on the Pacific coast of North America and has been extensively used for symbiosis research because it naturally occurs in 3 distinct symbiotic states: zooxanthellate (hosting the dinoflagellate *S. muscatinei*), zoochlorellate (hosting the chlorophyte *Elliptochloris marina*), and asymbiotic (lacking symbionts) (LaJeunesse & Trench 2000, Lewis & Muller-Parker 2004, Letsch et al. 2009). The 2 symbionts are taxonomically, ecologically, morphologically, and metabolically different. *E. marina* is negatively affected by high light and temperatures (Verde & McCloskey 2001, 2002), so zoochlorellate *A. elegantissima* are restricted to cooler, shaded sites, whereas zooxanthellate individuals thrive in areas with more sunlight (Secord & Muller-Parker 2005, Dimond et al. 2011). In symbiosis with *A. elegantissima*, *E. marina* is about 4 times more dense and grows up to 8 times faster than *S. muscatinei* but has about half the volume, half the carbon content, and less chlorophyll per cell (Verde & McCloskey 1996, Bergschneider & Muller-Parker 2008). During the summer months, the productivity of *S. muscatinei* is about 2.5 times greater than that of *E. marina* (Bergschneider & Muller-Parker 2008) and potentially translocates up to 5 times more carbon to the host (Verde & McCloskey 1996). However, due to the greater intracellular density of *E. marina*, the total amount of carbon translocated, at least in the summer months, may be similar for both symbionts (Engebretson & Muller-Parker 1999). There is also evidence that the 2 endosymbionts transfer different photosynthetic products to their hosts, with *E. marina* translocating mainly amino acids (Minnick 1984) and *S. muscatinei* translocating mostly glycerol and sugars (Trench 1971). Hence, whether the quantity and identity of the photosynthates translocated to the host varies between symbionts and how this influences host fitness remain topics of debate.

The goal of our study was to understand how symbiotic state affects fatty acid composition of *A. elegantissima*. Our specific approaches were (1) to compare the fatty acid profiles of the symbionts (*S. muscatinei* and *E. marina*) themselves, (2) to compare the fatty acid profiles of *A. elegantissima* in the 3 symbiotic states, looking for differences in fatty acid concentration and composition that might produce fitness differences, and (3) to compare the fatty acid profiles of the symbionts to their host sea anemones, looking for similarities that could indicate translocation.

MATERIALS AND METHODS

Field sampling

Zooxanthellate, zoochlorellate, and asymbiotic *Anthopleura elegantissima* were collected from Lawrence Point, Orcas Island, Washington, USA, on 26 June 2014 in early summer, when studies demonstrate considerable differences in symbiont productivity due to high solar radiation and temperature (Bergschneider & Muller-Parker 2008). Ten specimens in each of the 3 symbiotic states were collected haphazardly from approximately the same tidal height (0 to 0.5 m mean lower low water [MLLW]) within an area of ~30 m^2. Presumably, sea anemones in this limited area at the same location and tidal height capture similar prey items and might be expected to have similar fatty acid profiles if symbionts have similar effects on the fatty acid composition of their host.

Specimens were removed from the rock surface with a small spatula and transported to Shannon Point Marine Center (SPMC), in Anacortes, WA, USA, where they were placed in individual glass dishes submerged in indoor flow-through sea tables with unfiltered seawater. They were held for 96 h to allow them to empty their gut contents, ensuring that

all fatty acids extracted were from the sea anemones themselves and not from ingested prey.

Preparation of host and symbiont fractions

To permit comparison of the lipid profiles of symbionts and hosts, it was necessary to separate the algal symbionts from the sea anemone gastrodermal cells housing them. The sea anemones were cleared of any attached debris, then placed in a blender cup with 10–15 ml of filtered seawater and homogenized for 30 s. Two 1.5 ml samples of the homogenate from the zooxanthellate and zoochlorellate individuals were placed in microcentrifuge tubes and stored at –80°C until they could be processed to determine symbiont density. The remaining homogenates were centrifuged at maximum speed in a swinging bucket centrifuge for 2 min. This rendered a supernatant containing the host fraction and a pellet comprised of symbiont cells and a small amount of host material. The supernatant was twice transferred to a new test tube and centrifuged again to remove any remaining symbionts. Supernatant purity was verified by placing a sample of the final precipitate under a microscope and confirming that it contained only host cells with no symbionts. The cleaned supernatant, containing only host cells, was stored at –80°C.

A clean symbiont sample was obtained by taking the pellet from the initial centrifugation, resuspending it in filtered seawater, then centrifuging for 2 min at high speed. The supernatant, which contained small amounts of anemone mesoglea, was discarded. This procedure was repeated 3 times to thoroughly wash the symbionts. The pellet was resuspended a final time in filtered seawater then forced through a syringe filter with a 100 µm Nitex mesh to remove any remaining host material. Microscopic examination of the symbiont fraction showed negligible amounts of host material (mostly unfired nematocysts), which were later shown to have no effect on the fatty acid analyses, as lipids that were very concentrated in the hosts (e.g. erucic acid and arachidonic acid) were absent in the symbiont fraction. The clean symbiont fraction was stored at –80°C. Prior to fatty acid extractions, both the cleaned host and cleaned symbiont fractions were freeze dried at –80°C for 48 h.

Fatty acid extraction

Fatty acid extractions were based on a modification of the methods of Folch et al. (1957) and Bligh & Dyer (1959), as described in Malzahn et al. (2010). Preliminary tests showed that ~20 mg of freeze-dried tissue was optimal for fatty acid extraction and analysis. To initiate the extraction, 20–25 mg of freeze-dried sea anemone (from a single individual) or symbionts (extracted from a single anemone) was added to individual test tubes. The exact mass was measured to standardize the fatty acid concentrations in all samples. An internal fatty acid standard of nonadecanoic acid (C19:0) in a 2:1 (vol:vol) dichloromethane/methanol solution was added along with 4 ml of 2:1 dichloromethane/methanol. The mixture was flushed with nitrogen gas and placed in a freezer for 48 h at –80°C.

After 48 h, the samples were thawed, vortexed, and centrifuged at 3500 rpm (2054 × *g*) for 10 min at 5°C to precipitate any sediment. The supernatant was removed and 2 ml of 0.88% KCl buffer was added. The mixture was shaken, vortexed, and centrifuged at 3500 rpm (2054 × *g*) for for 10 min at 5°C. This produced 2 layers: an upper water-soluble fraction containing contaminants, and a lower organic fraction containing fatty acids. The upper layer was discarded, and the lower layer was placed in a nitrogen evaporator (Organomation Associates N-EVAP), where it was completely evaporated using a steady flow of nitrogen gas. Three ml of methanolic-sulphuric acid was added; the samples were flushed with nitrogen gas and then were placed in a heating block at 70°C to esterify the fatty acids. After 60 min, the samples were moved to a cooling rack. Two ml of hexane was added to wash the fatty acids from the methanolic-sulphuric acid, and the samples were vortexed and centrifuged, producing 2 layers. The upper layer, which contained the fatty acid methyl esters (FAMEs), was transferred to the nitrogen evaporator. As those samples were being evaporated, 2 ml of hexane was added to the remaining layer, which was vortexed and centrifuged again. The new upper layer was combined with the previous upper layer, and the solution was evaporated completely. Finally, 100 µl of hexane was added to resuspend the FAMEs, and the sample was flushed with nitrogen gas, closed tightly, sealed with Teflon tape, and stored at –80°C until analysis.

Fatty acid and carbon analyses

All samples were analyzed in a Varian CP3800 gas chromatograph with a Saturn 2000 ion trap mass spectrometer. FAMEs were separated with an Agilent HP-88 capillary column (30 m length, 0.25 mm ID, 0.2 µm film) with helium as the carrier gas. After injection at 60°C, the temperature was held for 1 min

and then increased to 150°C at a rate of 15°C min^{-1}, to 170°C at 3°C min^{-1}, to 190°C at 1°C min^{-1}, to 240°C at 20°C min^{-1}, and finally held constant for 20 min. FAMEs were identified via mass spectrometry by comparing their retention times with a Supelco 37 Component FAME Mix (Sigma-Aldrich) and verified with the NIST Mass Spectral Library (NIST 08). Chromatogram peaks that did not coincide with the FAME Mix retention times and could not be identified with the NIST Library were not included in the analyses. FAME concentrations were calculated by converting the area of the fatty acid peaks in the mass chromatogram to weight using the known nonadecanoic acid internal standard as a reference. Because some peaks did not separate well, the weights of arachidonic acid (C20:4ω6) and erucic acid (C22:1ω9) were pooled for the *Symbiodinium muscatinei* and *Elliptochloris marina* samples. One of the zooxanthellate sea anemone host samples was omitted from the analyses due to unreliable peaks. A 5–8 mg subsample of freeze-dried homogenate from hosts and symbionts was sent to the UC Davis Stable Isotope Facility for ^{13}C analysis. The carbon to dry biomass measurement was used to standardize fatty acid concentrations to carbon content. This was done to eliminate any potential errors generated by different sea anemone sizes or different amounts of minerals in the freeze-dried homogenates.

Symbiont density and protein analyses

To determine symbiont density in the initially collected anemones, 1 of the 2 previously frozen 1.5 ml homogenate samples from each anemone was thoroughly mixed, and symbionts were counted on a hemocytometer. The protein content of the other homogenate sample from the same anemone was determined using the method of Lowry et al. (1951), with bovine serum albumin as the standard. These 2 measurements, which allowed us to express symbiont densities in cells per mg anemone protein, were used to test the possibility that fatty acid content of the host anemones was affected by differences in the densities of their symbionts.

Statistical analyses

The same set of statistical analyses were used for comparisons of fatty acids between the 2 symbiont species (*S. muscatinei* and *E. marina*) and among hosts in the 3 symbiotic states (zoochlorellate vs. zooxanthellate vs. asymbiotic). First, a 1-way ANOVA was used to test for differences in total fatty acid content. Next, multi-dimensional scaling (MDS) analysis (using Euclidean distance as the distance metric) was used to visualize differences in the comparison groups, and ANOSIM was used to test statistically for separation. Data for these analyses were square root-transformed to prevent the most abundant fatty acids from dominating the analyses. Finally, where the ANOSIM was significant, SIMPER was used to determine which fatty acids contributed most to separation of the groups. Because SIMPER does not test for significance, 1-way ANOVA's (or Mann-Whitney *U*-tests if data violated the equal variance assumption) were used to statistically compare the concentrations of the 5 fatty acids that SIMPER indicated were most important in distinguishing groups. MDS, ANOSIM and SIMPER were again used to compare the fatty acid profiles of *E. marina* to their zoochlorellate hosts and of *S. muscatinei* to their zooxanthellate hosts. We used PAST 3.04 (Hammer et al. 2001) for all statistical analyses.

RESULTS

Fatty acid profiles of *Symbiodinium muscatinei* and *Elliptochloris marina*

Contrary to our expectations, *E. marina* had a significantly higher concentration of fatty acids per µg carbon than did *S. muscatinei* ($F_{1,18} = 39.12$, $p < 0.001$, Fig. 1). MDS analysis showed a clear separation of *E. marina* and *S. muscatinei* based on fatty acid profiles (Fig. 2), and ANOSIM showed that the differences

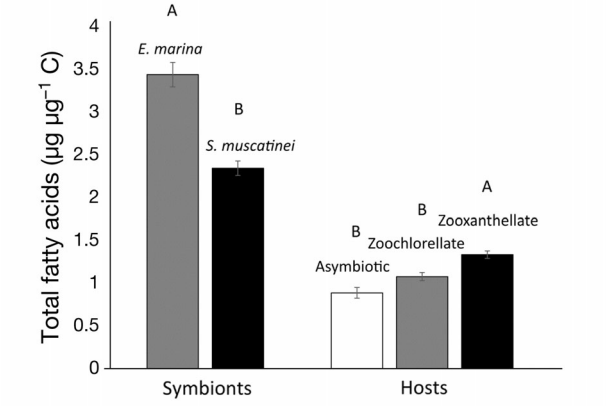

Fig. 1. Total fatty acid concentration in *Symbiodinium muscatinei* and *Elliptochloris marina* extracted from *Anthopleura elegantissima* and in the animal tissue only of zooxanthellate, zoochlorellate, and asymbiotic individuals. Capital letters over the host bars indicate significant differences as indicated by Tukey's HSD comparisons. Standard error bars are shown

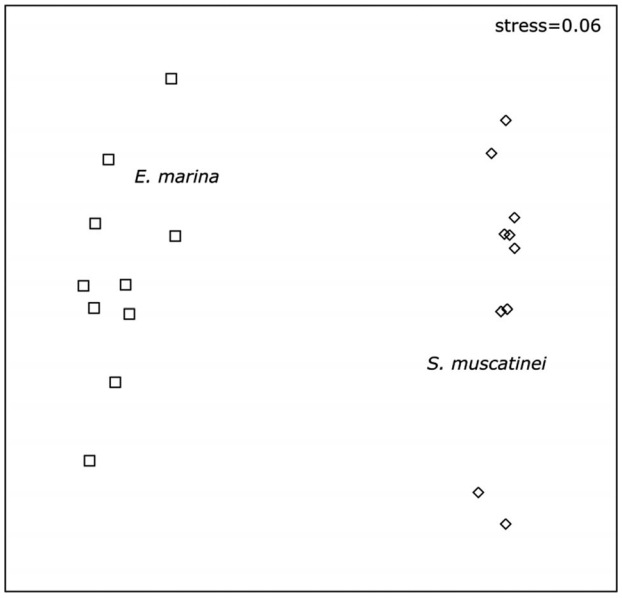

Fig. 2. Non-metric multi-dimensional scaling (MDS) plot of the fatty acid profiles of *Symbiodinium muscatinei* (◇) and *Elliptochloris marina* (□) extracted from *Anthopleura elegantissima*. Low stress value indicates a good fit to the data

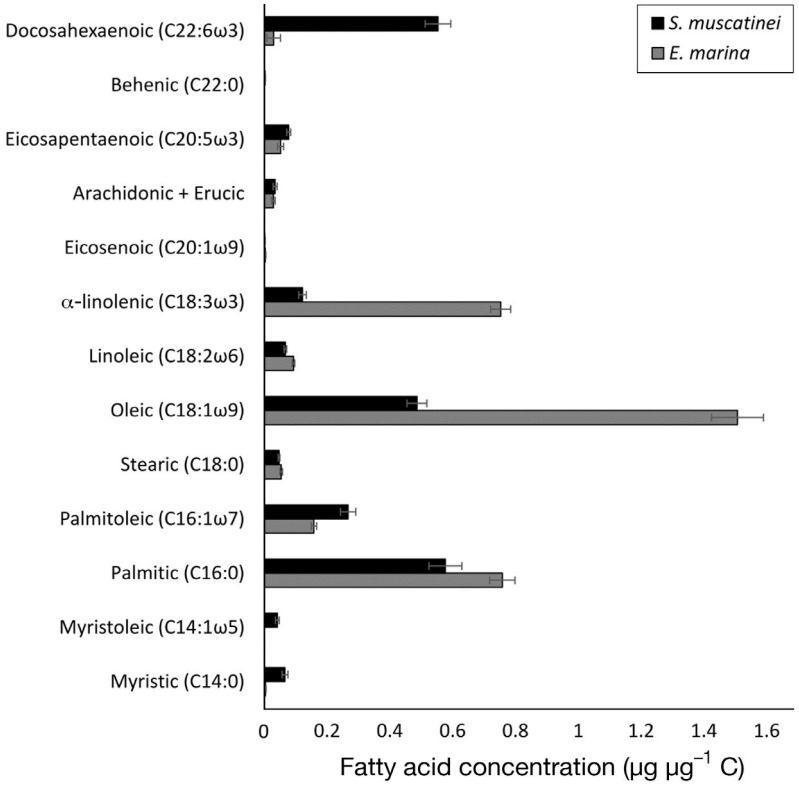

Fig. 3. Fatty acid composition and concentration in *Symbiodinium muscatinei* and *Elliptochloris marina* extracted from zooxanthellate and zoochlorellate *Anthopleura elegantissima*. Fatty acids are ordered by length of the carbon chain. Standard error bars are shown

Table 1. Fatty acids contributing most to differences between *Symbiodinium muscatinei* and *Elliptochloris marina* extracted from *Anthopleura elegantissima*, as indicated by SIMPER analyses. One-way ANOVA *F*-values, or non-parametric Mann-Whitney *U*-values for the comparisons of the 2 species are shown

Fatty acid	Contribution (%)	*F* (p)	*U* (p)	df
DHA	38.6		0 (<0.001)	1,18
Oleic	24.2	132.8 (<0.001)		1,18
α-linolenic	22.6	342.6 (<0.001)		1,18
Myristic	4.3		0 (<0.001)	1,18
Myristoleic	3.4		0 (<0.001)	1,18

were statistically significant (Global R = 1, $p < 0.001$). SIMPER showed that cis-4,7,10,13,16,19-docosahexaenoic acid (C22:6ω3, DHA), oleic acid (C18:1ω9), and α-linolenic acid (C18:3ω3) together accounted for 85.4% of the separation of the 2 symbiont species (Table 1).

The overall most common fatty acids, α-linolenic, oleic and palmitic (C16:0), were all more concentrated in *E. marina* than in *S. muscatinei* (Fig. 3), with α-linolenic over 6 times more concentrated in *E. marina*, and oleic acid over 3 times more concentrated. DHA, in contrast, was over 4 times more concentrated in *S. muscatinei*, being detected in only 2 of 10 *E. marina* samples, but in all *S. muscatinei* samples. Myristoleic acid (C14:1ω5) and behenic acid (C22:0) were detected only in *S. muscatinei*.

Fatty acid profiles of zooxanthellate, zoochlorellate, and asymbiotic hosts

Correlation analysis indicated that symbiont density had no significant effect on fatty acid content in zooxanthellate or zoochlorellate host tissues, allowing us to make direct comparisons of the anemone tissues without the confounding effects of differences in numbers of symbionts. Fatty acid levels were different in tissues of hosts in different symbiotic states ($F_{2,26} = 15.92$, $p < 0.001$), with Tukey's HSD indicating significant differences between zooxanthellate and asymbiotic ($Q = 8.04$, $p < 0.001$), be-

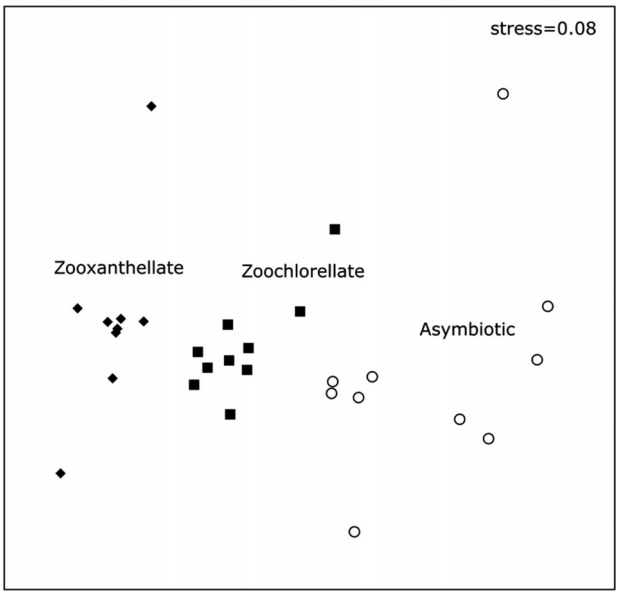

Fig. 4. Non-metric multi-dimensional scaling (MDS) plot of the fatty acid profiles from *Anthopleura elegantissima* in the 3 symbiotic states: zooxanthellate (◆), zoochlorellate (■), asymbiotic (○). Data are from measurements of the anemone tissues alone (symbionts had been removed)

Table 2. Fatty acids contributing to the differences between zooxanthellate, zoochlorellate and asymbiotic *Anthopleura elegantissima*, as indicated by SIMPER analyses. One-way ANOVA *F*-values, or non-parametric Mann-Whitney *U*-values for the comparisons of the symbiotic states are shown

Fatty acid	Contribution (%)	*F* (p)	*U* (p)	df
Zooxanthellate vs. zoochlorellate				
Linoleic	22.4		0 (<0.001)	1,17
Palmitoleic	16.9	108.4 (<0.001)		1,17
Elaidic	9.6	24.3 (<0.001)		1,17
DHA	5.8	0.48 (0.5)		1,17
Oleic	5.5		9 (0.004)	1,17
Asymbiotic vs. zooxanthellate				
Elaidic	27.5	130.7 (<0.001)		1,17
Palmitoleic	15.6		0 (<0.001)	1,17
Linolelaidic	11.6		0 (<0.001)	1,17
Linoleic	9.0		0 (<0.001)	1,17
Oleic	8.1		1 (<0.001)	1,17
Asymbiotic vs. zoochlorellate				
Elaidic	25.9	77.96 (<0.001)		1,18
Linolelaidic	14.5		0 (<0.001)	1,18
DHA	12.4		28 (0.1)	1,18
Eicosenoic	7.5		6 (<0.001)	1,18
Oleic	7.1	22.45 (<0.001)		1,18

tween zooxanthellate and zoochlorellate (Q = 4.62, p = 0.008) but not between zoochlorellate and asymbiotic individuals (Q = 3.42, p = 0.058; Fig. 1). Despite some within-group variability, the MDS plot (Fig. 4) shows separation based on symbiotic state, and ANOSIM confirms the statistical significance of the separation (zooxanthellate vs. zoochlorellate Global R = 0.60, p < 0.001; zooxanthellate vs. asymbiotic Global R = 0.89, p < 0.001; zoochlorellate vs. asymbiotic Global R = 0.57, p < 0.001). The main fatty acids contributing to the differences between hosts in different symbiotic states were linoleic acid (C18:2ω6), palmitoleic acid (C16:1ω7), elaidic acid (C18:1ω9), docosahexaenoic acid (DHA), oleic acid, and eicosenoic acid (C20:1ω9) with statistically significant differences in concentrations of all of these except DHA (Table 2).

With the exceptions of α-linolenic, stearic (C18:0), heptadecanoic (C17:0), and palmitic acids, every fatty acid measured was most concentrated in zooxanthellate hosts and least concentrated in asymbiotic hosts (Fig. 5). α-linolenic acid was only detected in zoochlorellate hosts, and pentadecanoic acid (C15:0) was only detected in zooxanthellate hosts. Myristic (C14:0) and palmitoleic acids were also absent from asymbiotic anemone fatty acid profiles.

Fatty acid profiles of hosts and their algal symbiont

We found significant differences in the fatty acid profiles of zooxanthellate hosts and their symbiont *S. muscatinei* (ANOSIM, Global R = 1, p < 0.001) and between zoochlorellate hosts and their symbiont *E. marina* (ANOSIM, Global R = 1, p < 0.001). The MDS showed much more similarity between zoochlorellate and zooxanthellate host tissues than between the *S. muscatinei* and *E. marina* (Fig. 6). The patterns of fatty acid concentrations were very different between *S. muscatinei* and the hosts from which they had been removed (Fig. 7). DHA, oleic, palmitoleic and palmitic acid were particularly concentrated in *S. muscatinei* but were at much lower concentrations in their host anemones. There was a similar lack of pattern match in the fatty acid profiles of *E. marina* and the hosts from which they had been removed (Fig. 8). The concentrations of α-linolenic, oleic, and palmitic acids in zoochlorellate host tissues, in particular, did not mirror the high concentrations in *E. marina.* Also, although DHA was considerably more concentrated in *S. muscatinei* than in *E. marina*, its levels were almost identical in zooxanthellate and zoochlorellate sea anemones.

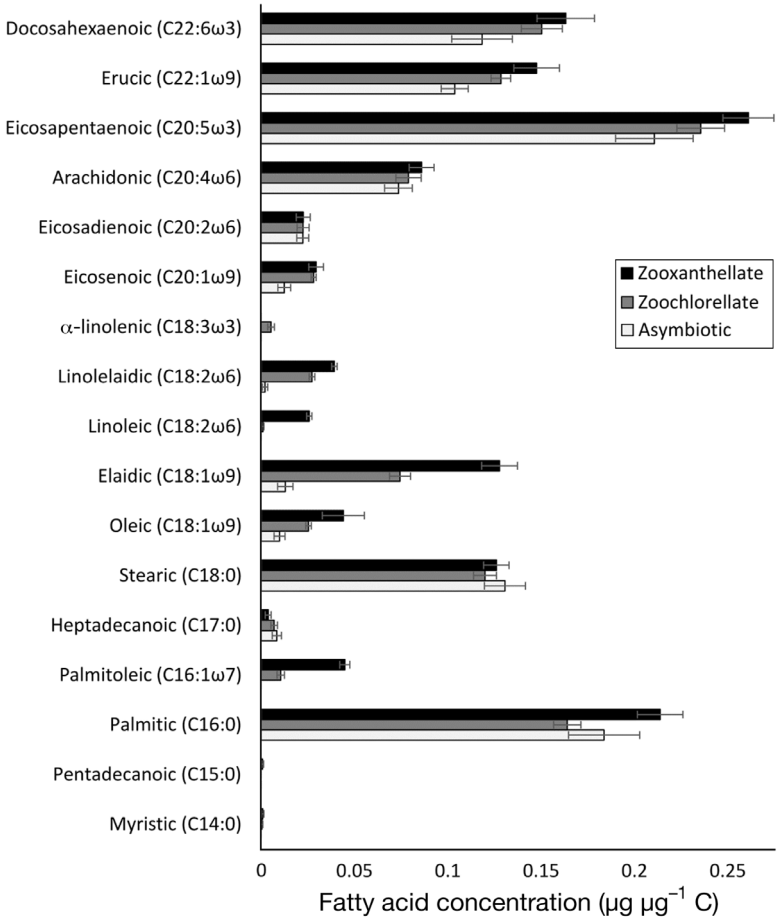

Fig. 5. Fatty acid composition and concentration in animal tissues of zooxanthellate, zoochlorellate, and asymbiotic *Anthopleura elegantissima*. Fatty acids are ordered by length of the carbon chain. Standard error bars are shown

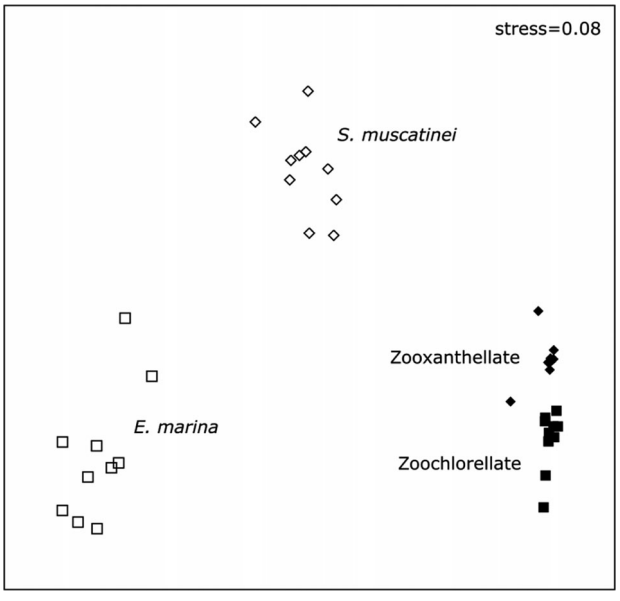

Fig. 6. Non-metric multi-dimensional scaling (MDS) plot of the fatty acid profiles of zooxanthellate (◆) and zoochlorellate *Anthopleura elegantissima* (■) and their symbionts *Symbiodinium muscatinei* (◇) and *Elliptochloris marina* (□)

DISCUSSION

Fatty acid profiles of *Symbiodinium muscatinei* and *Elliptochloris marina*

Our findings that DHA and palmitic acid are the most abundant fatty acids in *S. muscatinei* agree with multiple studies indicating that they are the major fatty acids in *Symbiodinium* isolated from corals (Al-Moghrabi et al. 1995, Papina et al. 2003, Zhukova & Titlyanov 2006, Imbs et al. 2010a). Other fatty acids commonly found in *Symbiodinium* include oleic, stearic, stearidonic (C18:4ω3), palmitoleic, γ-linolenic (C18:3ω6), arachidonic (AA, C20:4ω6), and eicosapentaenoic acids (EPA, C20:5ω3) (Papina et al. 2003, Suzuki et al. 2003, Zhukova & Titlyanov 2006, Díaz-Almeyda et al. 2011, Kneeland et al. 2013, Imbs et al. 2014). Although stearidonic acid, γ-linolenic acid, and EPA are considered marker polyunsaturated fatty acids for dinoflagellates (Imbs et al. 2014), we found little EPA in *S. muscatinei* and no stearidonic or γ-linolenic acid in any of our samples. Octadecapentaenoic (C18:5ω3) and DPA (C22:5ω3) have also been suggested as marker fatty acids, although their concentrations in *Symbiodinium* are generally low (Papina et al. 2003, Imbs et al. 2014); neither was detected in our samples. However, we were unable to identify 2 peaks in the *S. muscatinei* chromatograms. These were absent in *E. marina* chromatograms, suggesting they could have been dinoflagellate marker fatty acids such as stearidonic acid.

Here we present, for the first time, the fatty acid profile of *E. marina*, which has high levels of oleic, α-linolenic, and palmitic acids. As expected, *E. marina* has a fatty acid composition similar to that of other chlorophytes. *Tetraselmis* sp., a marine phytoplankton species, has a fatty acid composition similar to *E. marina*, with oleic acid as the most concentrated

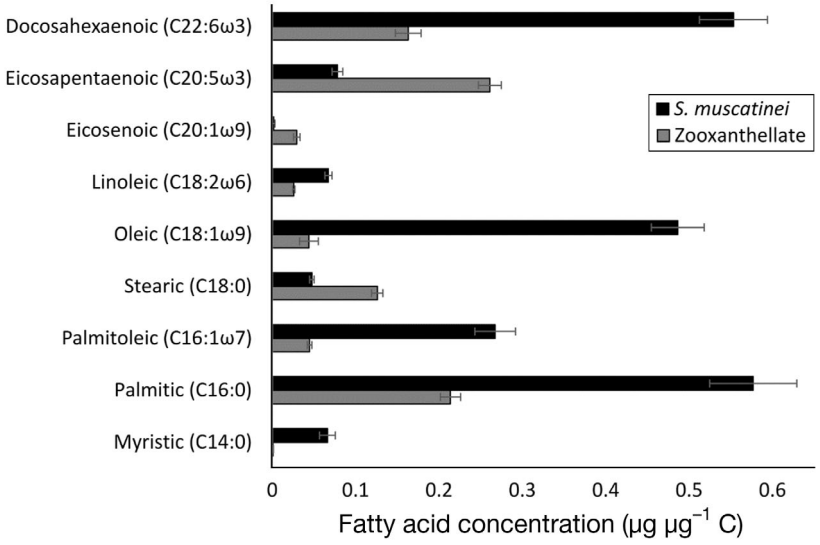

Fig. 7. Fatty acid composition and concentration of *Symbiodinium muscatinei* and zooxanthellate *Anthopleura elegantissima*. Fatty acids are ordered by length of the carbon chain. Standard error bars are shown

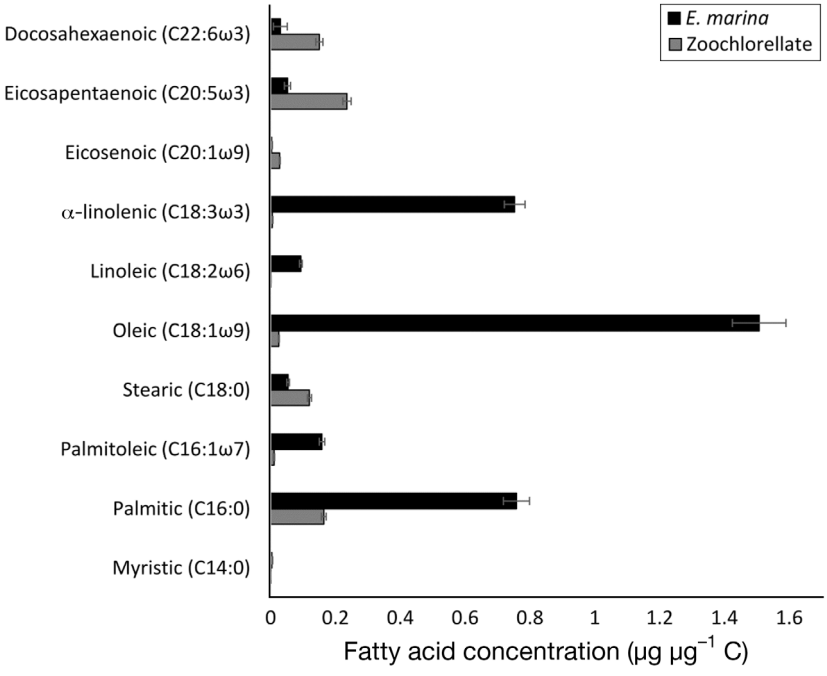

Fig. 8. Fatty acid composition and concentration of *Elliptochloris marina* and zoochlorellate *Anthopleura elegantissima*. Fatty acids are ordered by length of the carbon chain. Standard error bars are shown

form, followed by α-linolenic acid and palmitic acid (Patil et al. 2007). In *Dunaliella* spp., α-linolenic acid and palmitic acid comprise about 60% of the total fatty acid content (Viso & Marty 1993), and *Chlorella* sp. has high concentrations of α-linolenic, palmitic, hexadecatrienoic (C16:3ω3), and linoleic acids (Zhukova & Aizdaicher 1995). However, *Dunaliella* spp. and *Chlorella* sp. both have low levels of oleic acid, which was the most abundant fatty acid in our *E. marina* samples. DHA and palmitoleic acid are absent or in low concentrations in chlorophytes (Viso & Marty 1993, Zhukova & Aizdaicher 1995, Wiltshire et al. 2000, Patil et al. 2007).

The high concentrations of oleic and α-linolenic acids, both unsaturated fatty acids, in *E. marina* may explain why it performs better than *S. muscatinei* at lower temperatures and why zoochlorellate anemones are more abundant in low intertidal habitats and higher latitudes (Secord & Augustine 2000). Cell membrane fluidity decreases at low temperatures, presumably affecting the function of membrane transport proteins and ion gradients (Murata & Los 1997, Reay et al. 1999). The incorporation of lipids that contain unsaturated fatty acids into cell membranes restores membrane fluidity, as 'kinks' in their carbon chains reduce cell membrane density (Hazel 1995). Unsaturated fatty acids in the thylakoid membranes of cyanobacteria protect them from photoinhibition caused by low temperatures (Gombos et al. 1992, 1994) and enhance lipid diffusion across the membrane (Sarcina et al. 2003). Organisms may synthesize unsaturated fatty acids in response to decreasing temperatures. For instance, in Antarctic lakes, a drop in temperature causes the cyanobacterium *Nostoc* sp. to up-regulate the activity of enzymes that desaturate C18 fatty acids (Chintalapati et al. 2007). Lynch & Thompson (1984) found that shifting *Dunaliella salina* cultures from 30° to 12°C led to an increase in their oleic and α-linolenic acid content. Such unsaturated fatty acids are generally more concentrated in macroalgae adapted to temperate waters than in those from warmer waters (De Angelis et al. 2005, Colombo et al. 2006). However, temperature is not the only potential explanation for higher levels of these fatty acids. Studies have shown that chlorophytes may accumulate key fatty acids such as oleic acid and palmitic acid as a

stress response to high irradiance and nitrogen starvation (Mendoza et al. 1996, 1999, Zhekisheva et al. 2002). Whether the high levels of oleic acid we found in *E. marina* reflect their normal fatty acid composition, an adaptation to cold temperatures, or *in hospite* stress is unknown.

Fatty acid profiles of zooxanthellate, zoochlorellate, and asymbiotic hosts

Although *Anthopleura elegantissima* is a temperate sea anemone, its fatty acid profile resembles those of most tropical corals studied to date. Fatty acid content can be influenced by species, location, diet, and seasonality, but most corals have high concentrations of palmitic, stearic, elaidic, oleic, AA, EPA, and DHA (Harland et al. 1993, Al-Moghrabi et al. 1995, Yamashiro et al. 1999, Oku et al. 2002, Bachok et al. 2006, Treignier et al. 2008, Imbs et al. 2010a, Teece et al. 2011, Imbs 2013). Latyshev et al. (1991) analyzed the fatty acid composition of 14 calcifying coral species (with symbionts still in tissues) and found mainly palmitic, stearic, 18:1ω9 (oleic and elaidic), AA, EPA, and DHA. These findings agree well with our own, although most other studies did not detect erucic acid (C22:1ω9) (or found it in very low concentrations) and found much lower concentrations of EPA than we did.

Animals can synthesize saturated fatty acids and monounsaturated fatty acids (MUFAs), from acetyl-CoA, or from precursor fatty acids such as palmitic acid via desaturations (the addition of a double bond to the carbon chain) and elongations (the addition of 2 carbons to the chain), whereas polyunsaturated fatty acids (PUFAs) with ω3 and ω6 desaturations cannot be synthesized de novo by most animals and must be acquired through the diet (Dalsgaard et al. 2003). We found high concentrations of saturated fatty acids and MUFAs such as palmitic, stearic, elaidic, and erucic, and high concentrations of PUFAs including erucic acid, AA, DHA, and especially EPA, in *A. elegantissima*. This sea anemone feeds mainly on zooplankton, larvae, and intertidal invertebrates, including mytilid bivalves (Sebens 1981). Most of the PUFAs and other fatty acids found in high levels in *A. elegantissima*, especially palmitic, palmitoleic, stearic, EPA, and DHA, are also abundant in marine zooplankton (Dalsgaard et al. 2003, Lee et al. 2006, Treignier et al. 2008, Seemann et al. 2013). Mytilid bivalves are also very rich in palmitic, palmitoleic, EPA and DHA (Zandee et al. 1980, Karakoltsidis et al. 1995, Murphy et al. 2002, Passi et al. 2002). We believe the fatty acid profile of *A. elegantissima* can be explained by diet and de novo fatty acid synthesis without requiring direct fatty acid input from their endosymbionts. Erucic acid, which was very concentrated in our samples but not in the corals studied to date, is found in high levels in some species of copepods and present in some mytilids (Karakoltsidis et al. 1995, Dalsgaard et al. 2003). However, it should be noted that invertebrate lipid biosynthesis is not well understood, and there is little information on cnidarian fatty acid synthesis pathways (see Imbs 2013). For further information on animal fatty acid synthesis and the role of fatty acids in food webs see Dalsgaard et al. (2003), Bergé & Barnathan (2005) and Kelly & Scheibling (2012).

Research shows that PUFAs play an important role in invertebrate energetics. AA and EPA accumulate in the eggs of the cnidarian sea pansy *Renilla koellikeri* during months preceding spawning, indicating their likely role in gonad maturation (Pernet et al. 2002). EPA has also been linked to growth and egg production in the freshwater cladoceran *Daphnia magna* (Müller-Navarra et al. 2000) and to egg production in the calanoid copepod *Temora longicornis* (Arendt et al. 2005, Evjemo et al. 2008). The growth of zebra mussel larvae *Dreissena polymorpha* is enhanced by feeding with DHA-enriched algae but not with EPA-enriched algae, suggesting that growth may be limited by the rate of DHA synthesis from EPA when the former is scarce in the diet (Wacker et al. 2002). Postlarval growth of the sea scallop *Placopecten magellanicus* may be affected by dietary supply of DHA, DPA, and AA (Milke et al. 2004). Thus, it seems likely that the greater concentrations of PUFAs in *A. elegantissima* would increase their fitness.

Ponce-McDermott (2012) found that zooxanthellate *A. elegantissima* have higher levels of non-polar lipids, cholesterol, and palmitic acid than do zoochlorellate hosts. Our findings extend that conclusion, showing that, in general, zooxanthellate hosts have greater total fatty acid concentrations, supporting the suggestion that *S. muscatinei* is a better symbiotic partner. Interestingly, we found significantly greater concentrations of palmitoleic, elaidic, oleic, and linoleic acids in zooxanthellate hosts, but no differences in the PUFA DHA. The former fatty acids can be synthesized directly by animals, and their elevated concentrations in zooxanthellate hosts may be simply due to greater contributions of (non-lipid) photosynthetic carbon from the symbionts. Corals are known to synthesize lipids such as triglycerides and wax esters directly from glucose (Oku et al. 2003).

Our results seem to suggest that *A. elegantissima* uses the photosynthates provided by *S. muscatinei* to synthesize fatty acids that are then stored as triglycerides and wax esters, which are mainly composed of palmitic, stearic, and 18:1ω9 (oleic/elaidic) acids in corals (Patton et al. 1983, Yamashiro et al. 1999).

The high concentration of fatty acids in zooxanthellate and zoochlorellate *A. elegantissima* relative to asymbiotic individuals could have resulted from variation in the diets of individuals in different symbiotic states. However, given the restricted area within which the *A. elegantissima* were collected, and reports that symbiotic state has little effect on feeding behavior or efficiency in this species (Hiebert & Bingham 2012), it seems unlikely that differences in heterotrophic feeding created the fatty acid differences we measured. However, this possibility cannot be completely discounted without careful study of anemone feeding under natural field conditions. Though it is possible that starving the sea anemones for 96 h contributed to observed differences in fatty acid concentrations between symbiotic and asymbiotic specimens, it is unlikely that the period was sufficient to significantly change the patterns. Experiments with *A. elegantissima* (Fitt & Pardy 1981) show that, over a month-long starvation period, symbiotic individuals continue to catabolize carbohydrates, presumably originating from their symbionts. Asymbiotic individuals will shift from carbohydrate catabolism to lipid catabolism, but this transition may take several days.

Another possibility is that anemones lacking symbionts simply use the energy available and have little extra to put into storage products. Corals store photosynthetic carbon from their symbionts as lipids that are used to meet metabolic needs (Bachar et al. 2007, Baumann et al. 2014). Under bleaching conditions, the corals begin to deplete their storage lipids (Rodrigues et al. 2008), resulting in lower levels of wax esters and triacylglycerols (Grottoli et al. 2004, Yamashiro et al. 2005). In asymbiotic *A. elegantissima*, the effect may be similar; a lack of photosynthate input from endosymbionts may reduce total energy availability, depressing levels of energy storage products, including fatty acids.

Fatty acid profiles of hosts and their algal symbiont

Several authors have suggested that algal symbionts translocate specific fatty acids directly to their hosts (Papina et al. 2003, 2007, Treignier et al. 2008, Mortillaro et al. 2009, Imbs et al. 2010b), which could also explain the differences in fatty acid profiles we saw between symbiotic and asymbiotic *A. elegantissima*. However, we saw no host/symbiont fatty acid concentration relationships indicative of translocation. For instance, α-linolenic acid and oleic acid were highly concentrated in *E. marina* but scarce in zooxanthellate hosts. DHA is a fatty acid specifically believed to be directly translocated from symbiont to host in corals (Papina et al. 2007, Treignier et al. 2008), but there was little difference in its levels between zooxanthellate and zoochlorellate *A. elegantissima*, even though it was significantly more abundant in *S. muscatinei* than in *E. marina*. These findings suggest that DHA is not translocated by the symbionts but is obtained by the host *A. elegantissima* through diet or by synthesis from precursor fatty acids. Palmitic acid would be another good candidate for translocation because it is abundant in both symbionts and hosts and is the main product of the fatty acid synthesis pathway. However, we again found no evidence that this occurs. Recent research with stable isotopes found no evidence of symbiont-derived fatty acids, including palmitic acid, being used in lipogenesis by the *Symbiodinium*-hosting anemone *Aiptasia pulchella* (Dunn et al. 2012). It appears that the photosynthetic products translocated from symbionts to their anemone hosts may not include fatty acids.

Our results indicate that *A. elegantissima* hosting *S. muscatinei* have greater concentrations of most fatty acids than do anemones hosting *E. marina* and that asymbiotic individuals have lower fatty acid levels than either symbiotic state. This suggests selective advantages related to symbiotic state, with zooxanthellate hosts > zoochlorellate hosts > asymbiotic individuals, a result that aligns well with the conclusions of Bingham et al. (2014) that hosting symbionts increases fitness of *A. elegantissima* and that hosting zooxanthellae or zoochlorellae can fundamentally change life history strategies of this species. The potential adaptive significance of having different fatty acid content and composition should be considered in studies of *A. elegantissima* symbiont dynamics, symbiont shuffling and bleaching, particularly as symbiotic state may contribute to differences in metabolism, growth, reproduction and development. Moreover, further research is needed on the role of fatty acids in cnidarian metabolism, development, and reproduction, to shed light on how hosts are affected by symbiont loss events such as coral bleaching.

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