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Lipid-enriched diets reduce the impacts of thermal stress in corals

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ABSTRACT: Coral species are better able to survive bleaching events with heterotrophic feeding, which raises the level of lipid provisions and coral resilience against stress episodes. We report that a lipid-enriched diet allowed 2 coral species (*Duncanopsammia axifuga* and *Acropora millepora*) to resist an experimental bleaching event that involved exposure to temperatures of 32°C for 14 d. The diet, containing *Artemia* enriched with omega-3 polyunsaturated fatty acids (PUFAs), increased the proportions of essential fatty acid within coral tissue at normal temperatures, and maintained normal levels of these fatty acids under high temperatures. Neither of the 2 species significantly bleached when fed an enriched diet, and *D. axifuga* also grew faster, and increased colour, chlorophyll, symbiont density and PUFA proportions in the enriched diet compared to controls. Overall, this study sheds new light on the role of heterotrophic feeding in coral resilience to bleaching and provides a novel approach for bleaching prevention, reef restoration and improved coral aquaculture.

KEY WORDS: Lipid-enriched *Artemia* · Omega-3 fatty acids · Polyunsaturated fatty acids · Coral feeding · *Acropora millepora* · *Duncanopsammia axifuga*

INTRODUCTION

Although it is well established that corals are vulnerable to rising sea temperatures (Hughes et al. 2003, 2017, Palumbi et al. 2014), ongoing research is revealing various adaptations that allow corals to cope with temperature stress (Logan et al. 2014, Boulotte et al. 2016, Hoey et al. 2016). However, the rate of ocean warming may be progressing faster than corals can evolve adaptive responses (Eakin 2014, Hughes et al. 2017), with an increased frequency of bleaching events and reduced time for coral recovery (Hughes et al. 2017). Human intervention via reef restoration may be an important measure for allowing coral ecosystems to survive (Rinkevich 2014). Here, we explore the potential of artificial heterotrophic feeding in facilitating resistance to thermal stress in corals as an adjunct to reef restoration endeavours.

Corals depend on energy reserves, and particularly on their nutritional lipid stores, to survive bleaching events (Rodrigues et al. 2008, Grottoli & Rodrigues 2011, Baumann et al. 2014). This is because stress induces metabolic alterations that activate mechanisms to catabolize lipid reserves (Hillyer et al. 2017). Species that can utilize or increase heterotrophic feeding in the absence of symbiont-derived nutrition are better adapted to survive and will recover faster after a bleaching event (Rodrigues & Grottoli 2007, Grottoli et al. 2014, Towle et al. 2015), by consuming a diverse array of organisms from bacteria to mesozooplankton, as well as dissolved and particulate organic matter (Anthony 1999, Houlbrèque & Ferrier-Pagès 2009, Levas et al. 2016). Even corals receiving abundant light still obtain additional essential nutrients through plankton consumption (Iluz & Dubinsky 2015). By contrast, corals subject to food limitation show increased symbiont degradation due

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to autophagy (Bodemann et al. 2011), as well as disruption in division cycle synchrony between the host and symbiont due to host-regulated controls in the supply of nutrients to the symbiont (McAuley 1985).

There are clearly positive effects from increased heterotrophy during bleaching events (Rodrigues et al. 2008, Grottoli & Rodrigues 2011), because it allows the coral host to maintain carbon supplies during stressful environmental conditions (Forsman et al. 2012). Bleached corals can rely on lipids obtained through heterotrophy for at least 1 yr (Baumann et al. 2014) and help to maintain the symbiont thylakoid membrane integrity (Tchernov et al. 2004). For example, up to 100% of the nutrients acquired by bleached corals come from heterotrophy, compared to 15 to 35% in healthy corals (Houlbrèque & Ferrier-Pagès 2009). Furthermore, those species adapted to raise their energy reserves by increased heterotrophy under stressful conditions should have long-standing ecological advantages (e.g. more energy to support higher metabolic rates under stressful conditions, and faster recovery after bleaching) (Grottoli et al. 2006).

Bleaching can reduce the level of lipids and the probability of coral survival (Tolosa et al. 2011, Imbs & Yakovleva 2012), with polyunsaturated fatty acids (PUFAs) being the most oxidatively unstable under elevated temperature conditions (Porter 2013). Moreover, coral thermal sensitivity is correlated with the degree of saturation of lipids from the symbiont thylakoid membrane (Tchernov et al. 2004), and Lim et al. (2017) recently demonstrated that supplementary feeding with lipid-enriched *Artemia* can increase the levels of PUFAs in corals. For this reason, we tested the hypothesis that 2 coral species (*Duncanopsammia axifuga* and *Acropora millepora*) would survive a high temperature episode (2 wk at 32°C) and/or improve their overall health condition (at 26°C), when continuously fed with a diet based on *Artemia salina* enriched with marine-derived lipids, with a high proportion of PUFAs (particularly omega-3 fatty acids, docosahexaenoic [DHA] and eicosapentaenoic [EPA]), relative to a control diet of unenriched *A. salina* and corals that were not fed.

MATERIALS AND METHODS

Coral fragments and experimental setup

Eight healthy colonies of *Duncanopsammia axifuga* (large polyp size ~10 mm) and 6 of *Acropora millepora* (small polyp size ~2 mm) were collected from the Great Barrier Reef, adjacent to Cairns (Queensland, Australia). Each colony was packaged in a plastic bag full of seawater, covered inside an insulated cooler and transported by air overnight to the National Marine Science Centre (NMSC) (Coffs Harbour, northern NSW, Australia). Once they arrived, coral colonies were acclimated for at least 1 mo in 1200 l outdoor tanks with flow-through filtered seawater maintained at 26°C (to match the ambient temperature at time/point of collection) using a heat pump (2100 l h^{-1}; EVO-F5). Every mother colony was fragmented into similar nubbin pieces (≤5 cm), using a cordless Dremel® rotary tool (model 8220), and fixed to glass tile mounts using cyanoacrylate glue (Gel control, Loctite®). Nubbins were acclimated in the same tank for at least one further month until all injured tissue was completely regenerated. A total of 72 healthy fragments (36 per species) were randomly selected for the experiment. All corals were ranked medium-high in colour (4 to 5) at the start of the experiment, as compared to the Coral Health Monitoring Chart (Siebeck et al. 2006).

Nubbins were transferred into independent vials of 250 ml, each one with air supply (from an air compressor), and independent irrigation dripper valves (~0.6 ± 1.2 ml s^{-1}) delivered flow-through seawater from Charlesworth Bay, Solitary Islands Marine Park, adjacent to the NMSC (30° 16′ 0.91″ S, 153° 8′ 25.27″ E). Water was filtered through a gravel filter, sand filter and 10 µm cartridge filter. Photoperiod was set at 12 h d^{-1} by 4 overhead metal halide lamps at an average of 280 ± 50 µmol m^{-2} s^{-1} (see Fig. S1 in the Supplement at www.int-res.com/articles/suppl/m573p129_supp.pdf).

Using 12 replicates per species for each of the 3 different treatments: (1) unfed (*U*), (2) 2 d old normal (unenriched) *Artemia* (*N*) and (3) lipid-enriched 2 d old *Artemia* (*E*); corals were initially kept for 10 d under ambient temperature conditions (26 ± 0.5°C) and fed every day at 17:00 h for 1 h using an optimum feeding rate of 40 ind. ml^{-1} for *A. millepora* and 90 ind. ml^{-1} for *D. axifuga*. This rate was previously calculated using the density at which half-saturation occurs (K_m) (Anthony 1999), and considering the feeding rate at 6 different *Artemia* concentrations, by the Michaelis-Menten model application of SigmaPlot 13 (Systat software) (A. Tagliafico et al. unpubl. data). After the 10 d period, water temperature for half of the corals within each feeding treatment (n = 6 replicates per species) was elevated to 32°C (1°C rise per day), using heater-chiller units (Aquahort) to manipulate and maintain both ambient and elevated temperatures. Corals were maintained under the same daily feeding regime, and the experiment was stopped after 14 d.

Coral growth rate was calculated by measuring buoyant weight (Jokiel et al. 1978) using an analytical balance (0.001 g resolution) with a weighing hook (Ohaus; model PA213), at the beginning and at the end of the experiment. Water volume and temperature were kept constant throughout the buoyant weight measurements, and the tiles on which the corals were attached were cleaned to prevent any biofouling adding to weight gain. Growth rate was expressed in mg of mass (skeleton and tissue) increment per gram of initial weight per day (Movilla et al. 2012). Additionally, colour was measured at Day 1 and Day 14 of the experiment, using the Coral Health Monitoring Chart (Siebeck et al. 2006). Coral tissue was removed with compressed air, immediately frozen in liquid nitrogen and stored at −80°C until laboratory analysis.

Lipid enrichment of *Artemia*

Beginning 2 d prior to the start of the experiment and then every day, 10 g of *A. salina* cysts ('AAA' grade GSL *Artemia* cysts; INVE Belgium) were incubated in a 10 l cone tank with high aeration and light, and harvested after 20 h at 28°C. A magnetised cyst collector tube (Sep-Art^{TM}; INVE Belgium) was used during the harvesting, to avoid collecting any unhatched cysts or shells. Nauplii were maintained at a density of 400 to 600 ind. ml^{-1}. After 24 h, water was exchanged and half of the nauplii were enriched using 2 g of a soluble commercial product (Selco® S.presso, INVE Belgium) containing high levels of essential PUFAs (C22:6n-3 = 260 mg g^{-1}; C20:5n-3 = 25 mg g^{-1}), previously mixed for 2 min in a blender with 0.5 l of freshwater. A 2-stage enrichment process was used whereby the 2-d-old *Artemia* nauplii were allowed to feed in 8 l of filtered and aerated seawater containing the enriched emulsion for 4 h, then the seawater was renewed and another 2 g of the mixed enriched emulsion was added and left for another 4 h.

In order to confirm that the diet was effectively enriched with lipids, prior to starting experiments ~180 mg of non-enriched *Artemia* versus lipid-enriched *Artemia* (n = 3) were extracted using analytical grade chloroform:methanol (1:1; Sigma), then derivatised into fatty acid methyl esters (FAMEs) using 2 M sodium hydroxide in methanol followed by 14% boron trifluoride in methanol (Sigma-Aldrich) and re-extracted in analytical grade hexane by phase partition with saturated sodium chloride solution according to Valles-Regino et al. (2015). The extracts were analysed by gas chromatography/mass spectrometry (GC/MS; Agilent 6890, coupled with an Agilent 5973 mass selective detector). The mass spectra were recorded at 70 eV ionization voltage over the mass range of 35 to 550 Da. Identification of peaks was based on comparison to a marine lipid mixture (PUFA No.1 Marine Source, Analytical Standards, Sigma-Aldrich) and matched to a mass spectral library (WILEY 275 and NIST98). A comparison of the lipid profile of the enriched and non-enriched *Artemia* diets is provided in Fig. 1, with mean percent composition based on the integrated area under the curve in Table S1 in the Supplement.

Laboratory analyses

Chlorophyll *a* and c_2 were calculated using the Jeffrey & Humphrey (1975) equation, while host soluble proteins were obtained through the Bradford (1976) method and symbiont density following the protocol provided by Hill & Scott (2012) (n = 72). Lipids from the coral host and zooxanthellae (n = 48) were extracted together based on a modified version (Valles-Regino et al. 2015) of the Folch method (Folch et al. 1957). Lipid yield and protein values were standardized by the weight of coral tissue that was extracted (mg g^{-1}). Chlorophyll and symbiont density were standardized taking into account the tissue yield and coral surface area (Veal et al. 2010).

Coral tissue lipid extracts were derivatized into FAMEs and analysed by flame ionisation detection coupled to a gas chromatographer (Agilent 6890N) (Valles-Regino et al. 2015). Standard FAMEs (SUPELCO 37-Component FAME Mix CRM47885) and marine lipid mixtures (PUFA No.1 Marine Source, Analytical Standards, Sigma-Aldrich) were used to calibrate retention times.

Statistical analyses

Permutational multivariate analysis of variance (PERMANOVA) was used to test hypotheses about the effects of feeding regimes and temperatures on each univariate response variable (chlorophyll, symbiont density, proteins, growth, final colour, each type of identified FA, and the percent composition of total FAs). Multivariate PERMANOVAs were used to test hypotheses about differences in major FA composition among treatments. All PERMANOVA analyses used similarity matrices based on Euclidean distances and 9999 permutations (Anderson et al.

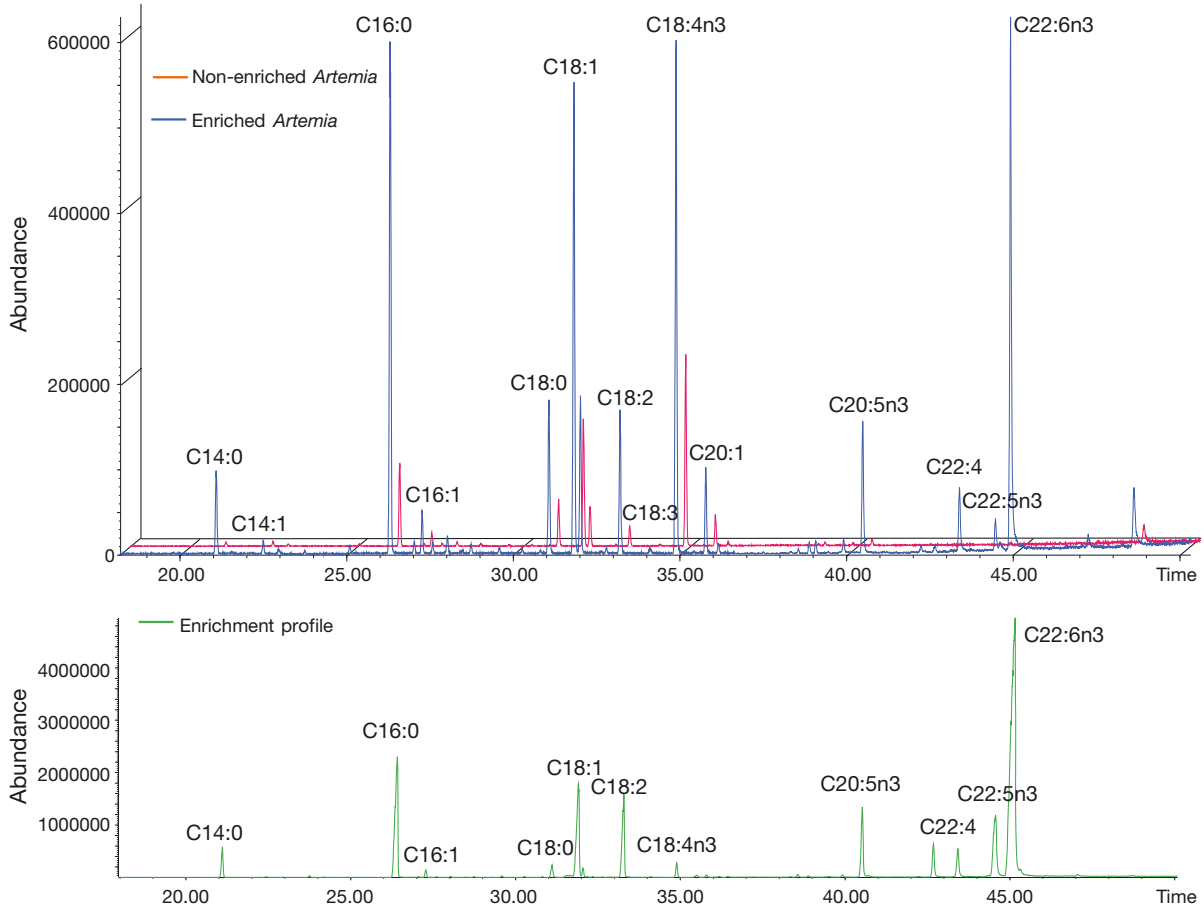

Fig. 1. Coral feeding diets: non-enriched *Artemia* versus lipid-enriched *Artemia* fatty acid profiles and a commercial enrichment diet profile (major fatty acid peaks are labeled)

2008). When significant differences were found in PERMANOVA analyses, post hoc pair-wise tests were undertaken when appropriate.

RESULTS

Corals fed with the lipid-enriched diet had significantly higher colour, and for both species, most (except one *Acropora millepora* replicate) did not bleach after 14 d at extreme elevated temperature (Fig. 2). By contrast, corals in the unfed treatment at 26°C remained in the same colour range or one step lower, but were bleached at 32°C.

The enriched diet for *Duncanopsammia axifuga* resulted in higher growth, chlorophyll and symbiont density compared to unfed coral and corals fed with the normal or unenriched *Artemia*. However, elevated temperature caused a significant reduction in growth, symbiont density and total chlorophyll (Table 1, Fig. 3). There was no significant effect of temperature or feeding on protein and the total lipid yield extracted from the coral tissue of each species (Tables 1 & 2, Figs. 3 & 4). However, diet resulted in a significant change in the relative proportion of major FA classes, with an alteration in the percent composition of saturated fatty acids (SFAs) and unsaturated acids of *D. axifuga* (Table 1, Fig. 4). When fed the enriched diet, *D. axifuga* had a significantly lower proportion of SFAs and significantly higher PUFAs relative to corals that were unfed or fed with unenriched *Artemia* (Fig. 4, Table 3). Omega-3 and -6 FAs were significantly higher in both fed treatments compared to unfed controls (Fig. 4, Table 3). Temperature had no significant effect on the SFAs or PUFAs in *D. axifuga,* but caused a slight significant drop in monounsaturated fatty acids (MUFAs) (Fig. 4, Table 3). For *A. millepora*, MUFAs were significantly higher in unfed compared to the normal (unenriched) *Artemia* diet (Fig. 4, Table 3). In comparison, diet had no significant effect on the relative proportions of SFAs or PUFAs in *A. millepora* (Fig. 4, Table 3). However, in this coral species temperature resulted in a significant increase in SFAs and a decrease in PUFAs

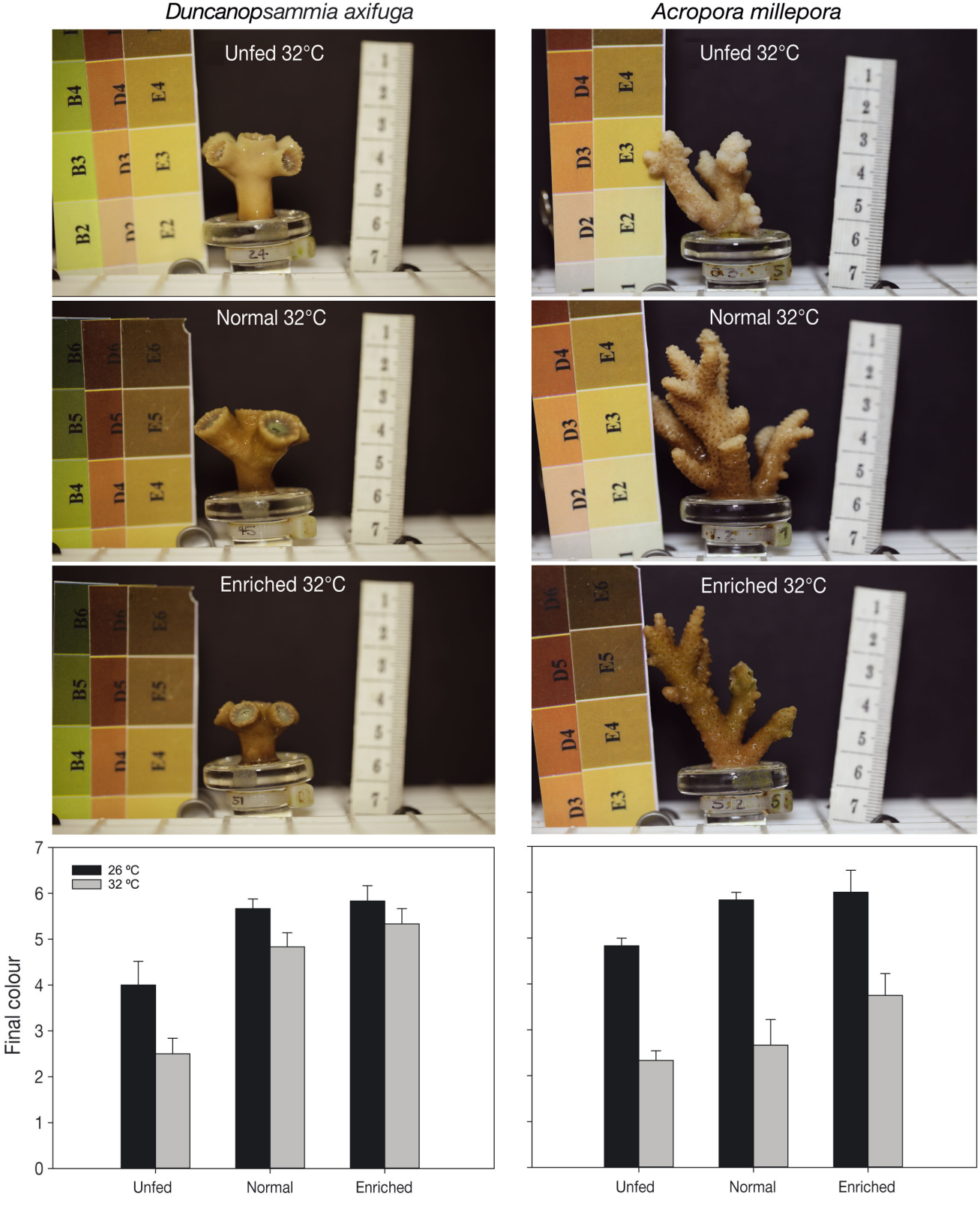

Fig. 2. Lipid-enriched diet enhances coral resilience against bleaching: images are representative overall final coral colour (higher number = darker colour) for *Duncanopsammia axifuga* and *Acropora millepora* after 14 d under 2 different temperatures (26 and 32°C). Coral pictures were selected from the 32°C treatment. Bars: means ± SE

Table 1. Statistical outcomes for *Duncanopsammia axifuga*. PUFA: polyunsaturated fatty acids; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids. Significant effects in **bold**

	Feeding (FED)		Temperature (TEMP)		FED × TEMP	
	Pseudo-*F*	p-value	Pseudo-*F*	p-value	Pseudo-*F*	p-value
UNIVARIATE						
Growth	4.8469	**0.017**	18.994	**0.0001**	0.0228	0.9777
Protein	0.0114	0.9897	1.3983	0.2518	0.0555	0.946
Total chlorophyll ($a + c_2$)	6.1943	**0.0067**	12.683	**0.0014**	2.2511	0.1206
Symbiont density	3.5202	**0.042**	55.711	**0.0001**	0.7185	0.4895
Final colour	28.908	**0.0001**	12.143	**0.003**	1.1765	0.3168
Total lipid yield	0.1294	0.8778	0.0176	0.906	0.1801	0.8484
PUFA	18.104	**0.0001**	1.1805	0.2984	0.5067	0.6302
Omega-6	7.6456	**0.0034**	0.2059	0.6565	0.6645	0.5306
Omega-3	12.285	**0.0005**	2.3284	0.1426	1.0429	0.3768
SFA	12.096	**0.0002**	3.4836	0.0733	0.6991	0.5259
MUFA	4.2064	**0.0332**	5.0993	**0.0388**	0.1712	0.8385
C14:0	1.5603	0.2332	0.0419	0.84	0.8874	0.4259
C16:0	15.495	**0.0002**	22.262	**0.0002**	3.6029	**0.0479**
C18:0	7.715	**0.0024**	0.0913	0.7694	0.0840	0.9225
C20:0	8.2151	**0.0001**	0.4042	0.5646	1.1627	0.3492
C16:1n-7	0.1382	0.8775	8.6585	**0.0066**	0.0923	0.9168
C18:1n-9	5.0819	**0.0182**	0.0780	0.7902	0.2455	0.7879
C18:1n-7	7.2143	**0.0037**	3.299	0.0859	0.2472	0.7899
C20:1n-9	3.9733	**0.0414**	14.414	**0.0015**	1.488	0.2539
C22:1n-9	0.4001	0.6957	0.0002	0.9887	0.1753	0.8406
C18:3n-3	14.014	**0.0003**	4.385	0.0508	1.0881	0.3514
C18:4n-3	20.675	**0.0001**	14.644	**0.0011**	3.9199	**0.0431**
C20:5n-3	39.814	**0.0001**	17.567	**0.0004**	0.8441	0.4566
C22:5n-3	1.318	0.3078	0.1498	0.7011	0.7122	0.5049
C22:6n-3	1.3398	0.2937	0.01199	0.9194	0.7144	0.5211
C18:2n-6	3.5898	**0.0488**	12.914	**0.0015**	4.8237	**0.017**
C18:3n-6	4.3564	**0.0298**	0.14686	0.7087	0.4879	0.6175
C20:3n-6	0.2108	0.9829	0.00309	0.9959	1.3914	0.2178
C20:4n-6	1.7153	0.2172	0.81878	0.3881	1.9536	0.1746
C22:4n-6	4.2185	**0.0224**	0.55463	0.4707	1.171	0.3454
C20:2n-6	29.641	**0.0001**	0.34708	0.5196	0.6162	0.5477
C20:2	5.4979	**0.0138**	5.3376	**0.035**	0.3937	0.6761
MULTIVARIATE						
Major fatty acids (n = 21)	6.9203	**0.0001**	2.9363	**0.0437**	0.8451	0.5137

and omega-3 (Table 3, Fig. 4). Elevated temperature also significantly reduced the total chlorophyll and zooxanthellae density in *A. millepora*, but these parameters were not affected by diet. Similarly, growth was not significantly affected by diet or temperature in *A. millepora* (Table 3, Fig. 4).

Multivariate analysis demonstrated that temperature had a significant effect on the major FA composition for both species (Tables 1 & 2). Additionally, of the 21 specific FAs assessed for *D. axifuga*, 3 exhibited significant feeding × temperature interactions (C16:0, C18:4n-3 and C18:2n-6). Post hoc tests investigating these significant interaction terms did not provide clear patterns and were associated with inconsistent trends among feeding treatments at different temperatures (Table 4). Another 10 FAs showed a significant effect with feeding (e.g. C20:5n-3 and C22:4n-6 were significantly higher in the enriched diet treatment compare to the other treatments). Additionally, 4 FAs in *D. axifuga* (C16:1n-7, C20:1n-9, C20:5n-3 and C20:2) significantly decreased with temperature (Table 3). For *A. millepora*, 4 FAs differed significantly among feeding treatments, with C18:1n-9 and C18:0 having the highest values in the unfed treatment; whereas C22:6n-3 and C20:3n-6 showed significantly higher values in the lipid-enriched *Artemia*. Furthermore, 7 FAs showed significant differences with temperature, including C14:0 and C16:0 increasing; and C20:1n-9, C20:5n-3, C22:5n-3, C22:6n-3 and C20:3n-6 decreasing at higher temperatures, respectively (Table 3). Complete FA profiles across treatments for both species are provided in Tables S2 & S3 in the Supplement.

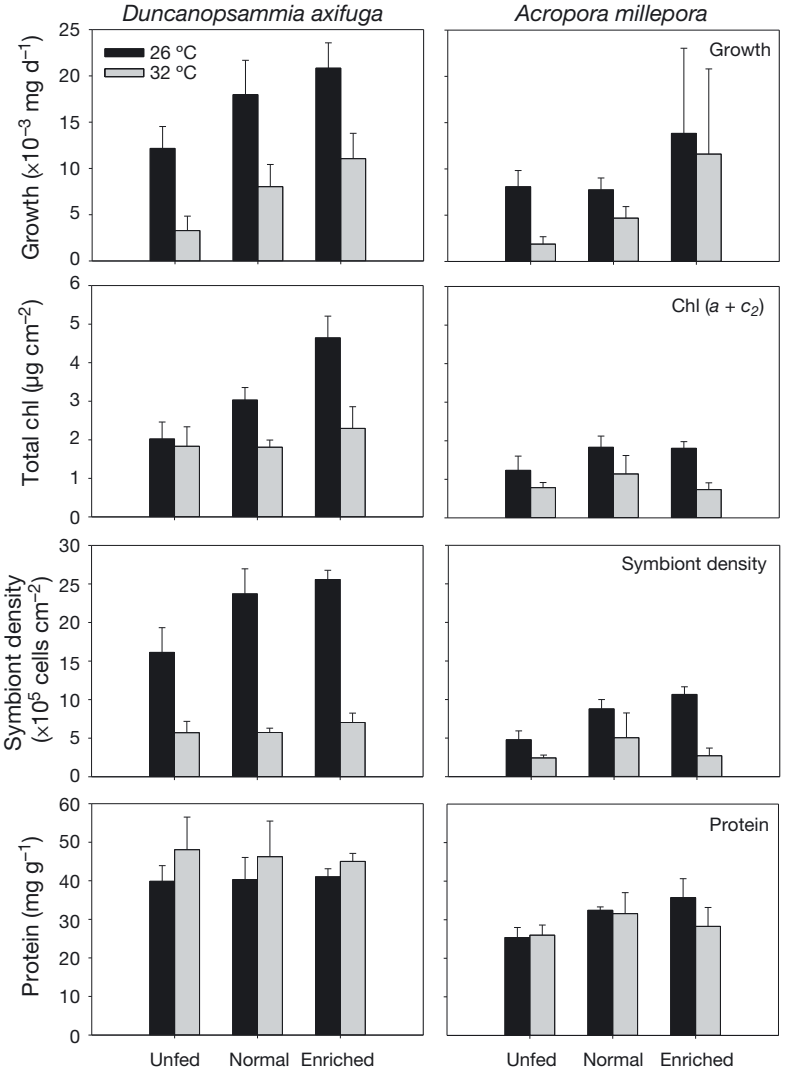

Fig. 3. Influence of a lipid-enriched diet on the responses of *Duncanopsammia axifuga* and *Acropora millepora* to extreme temperatures. Growth, total chlorophyll ($a + c_2$), symbiont density and protein content are shown after 14 d under 2 different temperatures (26°C and 32°C) and 3 different treatments: unfed, normal *Artemia* and enriched *Artemia*. Bars: means ± SE of the main effects

DISCUSSION

Thermal stress in corals results in bleaching due to the loss of symbiotic algae. It can also simultaneously impact the lipid composition and particularly the PUFAs, which are susceptible to peroxidation (Porter 2013). Our study confirms that corals can assimilate high levels of essential PUFAs from lipid-enriched diets, and this can help compensate against the effects of thermal stress, providing a buffer for maintaining a relatively high proportion of PUFAs in their lipid composition when exposed to elevated temperatures. Corals consuming the lipid-enriched diet had improved colour, which for *Duncanopsammia axifuga* is supported by higher chlorophyll levels and greater symbiont densities. This positive effect of heterotrophy on coral symbiont autotrophy is consistent with a recent study by Lim et al. (2017), which found elevated photosynthetic rates due to higher symbiont densities in *Galaxea fascicularis* fed with a PUFA-enriched diet. In our study, neither diet nor temperature had significant impacts on protein and total lipids, but they did alter the FA composition, with temperature significantly decreasing and diet significantly increasing the levels of certain essential FAs (e.g. C22:6n-3 in *Acropora millepora* and C20:5n-3 in *D. axifuga*). Although there were few significant interactions between diet and temperature, the independent but opposite effects of these factors on a number of parameters clearly points to an overall improved condition of corals fed with a lipid-enriched diet, irrespective of temperature stress. The higher growth rates observed in both coral species suggests enriched feeding might represent a useful technique for coral aquaculture.

Bleached corals in natural ecosystems consistently show higher amounts of SFAs and MUFAs, as well as a lower amount of PUFAs (Bachok et al. 2006). In our study, a similar outcome was observed for *A. millepora*, which showed poorer colour, reduced chlorophyll and lower zooxanthellae density at higher temperatures, coincident with significantly higher SFAs and lower PUFAs in the FA composition. Bleaching reduces FA content, particularly n-3 PUFA concentrations, but this effect can be alleviated in fed corals, or exacerbated in starved coral under thermal stress (Tolosa et al. 2011). As shown in our experiments on *D. axifuga*, the acquisition of essential FAs through diet might mitigate the temperature effect on the relative proportions of SFAs and PUFAs in the holobiont. This may help shield the coral from total bleaching and keep the symbiosis stable, as supported by increased symbiont densities in *D. axifuga* fed with the enriched diet relative to unfed coral, irrespective of temperature stress. By comparison, the slower feeding species, *A. millepora*, showed no significant effects of feeding on the ratio of SFAs:PUFAs, along with no changes in chlorophyll or zooxanthellae. Lim

Table 2. Statistical outcomes for *Acropora millepora*. PUFA: polyunsaturated fatty acids; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids. Significant effects in **bold**

	Feeding (FED)		Temperature (TEMP)		FED × TEMP	
	Pseudo-*F*	p-value	Pseudo-*F*	p-value	Pseudo-*F*	p-value
UNIVARIATE						
Growth	1.4261	0.2657	0.9043	0.4421	0.0894	0.8945
Protein	1.9041	0.1681	0.7068	0.401	0.6647	0.52
Total chlorophyll ($a + c_2$)	0.9539	0.4039	6.7863	**0.0131**	0.4097	0.6839
Symbiont density	2.0398	0.1465	9.7516	**0.0022**	1.2657	0.309
Final colour	7.82	**0.003**	97.885	**0.0001**	1.0521	0.3585
Total lipid yield	0.6984	0.5092	0.0105	0.917	0.9499	0.4039
PUFA	2.0674	0.1551	8.8414	**0.0016**	0.3131	0.7349
Omega-6	1.2405	0.3166	1.0476	0.3197	0.3521	0.7005
Omega-3	2.5335	0.1089	18.752	**0.0008**	0.4519	0.6474
SFA	1.2504	0.3145	18.834	**0.0008**	0.4233	0.6574
MUFA	4.2778	**0.017**	0.2068	0.6866	0.3092	0.7724
C14:0	1.8215	0.1917	92.392	**0.0001**	1.3575	0.2751
C16:0	0.2258	0.8068	6.5297	**0.02**	0.3677	0.7084
C18:0	4.4287	**0.0209**	1.1384	0.3073	0.1357	0.8836
C20:0	2.1978	0.1375	1.1018	0.3071	0.1227	0.884
C14:1	1.6461	0.224	0.0647	0.8077	0.1812	0.8467
C16:1n-7	0.5639	0.5812	0.2605	0.6156	0.1357	0.8705
C18:1n-9	4.635	**0.0202**	0.3999	0.5528	0.0872	0.9184
C18:1n-7	2.5962	0.1063	1.7918	0.2017	0.4834	0.626
C20:1n-9	1.5158	0.2412	5.4704	**0.0246**	0.5750	0.5967
C18:4n-3	2.2122	0.1287	1.8363	0.1999	0.4283	0.6632
C20:5n-3	1.3132	0.2949	7.2495	**0.0153**	0.3603	0.7077
C22:5n-3	0.7537	0.5022	6.9951	**0.014**	0.3323	0.7264
C22:6n-3	4.2036	**0.0371**	80.051	**0.0001**	0.2525	0.7825
C18:2n-6	0.3426	0.7346	3.6154	0.0693	0.6537	0.5445
C18:3n-6	0.5551	0.5871	0.6483	0.432	0.0181	0.9824
C20:3n-6	4.1669	**0.0331**	27.988	**0.0001**	0.0327	0.9701
C20:4n-6	0.2619	0.7823	1.0407	0.3212	0.0840	0.9207
C22:4n-6	0.2310	0.8005	0.1955	0.6723	0.1015	0.9042
C20:2n-6	0.4493	0.6464	0.1424	0.6987	0.8677	0.4253
C20:2	0.5730	0.5762	0.1835	0.6676	0.0226	0.9764
MULTIVARIATE						
Major fatty acids (n = 20)	1.3843	0.2417	5.684	**0.0046**	0.2868	0.9397

et al. (2017) suggested that some species might require a period of adjustment to assimilate PUFA diet supplements. Nevertheless, the different outcomes for the 2 species in our study highlights the potential for species-specific positive feedback between heterotrophy, endogenous PUFAs and autotrophy; an effect that has been recently corroborated in another coral species with high ingestion rates when fed PUFA-enriched *Artemia* (Lim et al. 2017).

The variation in the FA proportions from 'optimum conditions' (enriched diet at 26°C) to 'extreme conditions' (unfed at 32°C) can be attributed to some of their properties and functional roles. FAs are important structural components of cell membranes and the viscosity of the lipid bilayer is influenced by the melting temperature of FAs, which decreases with the number of double bonds (Nelson et al. 2008). Under increasing temperatures, the relative proportion of SFAs in cell membranes increases as a natural adaptive response to thermal stress, to regulate cell membrane permeability and preserve correct physiological functions, i.e. homeoviscous adaptation (Hazel 1995). In addition, FAs are stored as triglycerides which can be metabolised as a source of energy (Nelson et al. 2008). Metabolic rates are higher at elevated temperatures and PUFAs stored as triglycerides can become susceptible to oxidation (Porter 2013) and depletion via gluconeogenesis (Nelson et al. 2008). This may explain why higher SFAs and lower PUFAs were found in *A. millepora* under temperature stress. However for *D. axifuga*, heterotrophic feeding on the PUFA-enriched diet appears to have helped maintain high PUFA levels, even after 2 wk of exposure to elevated water temperatures capable of causing bleaching.

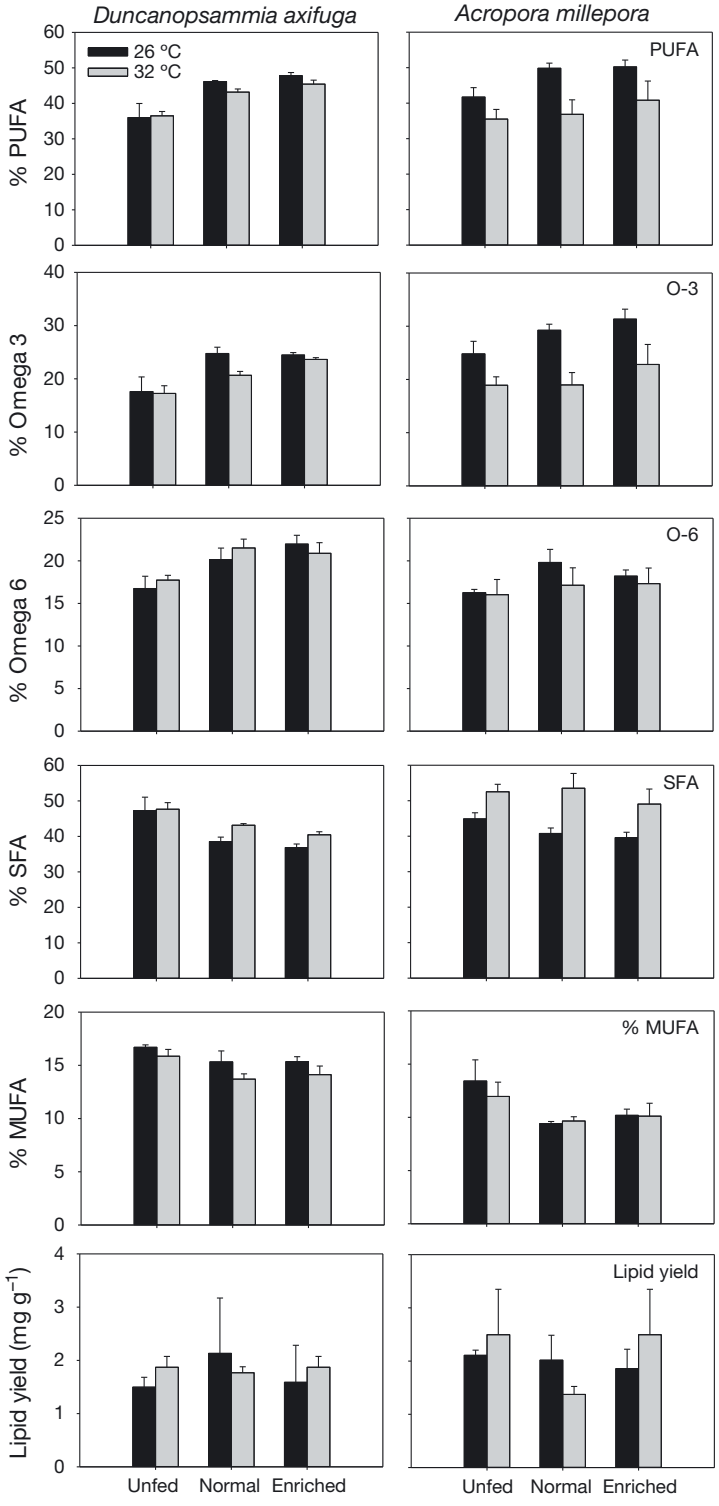

Fig. 4. Influence of a lipid-enriched diet on the lipid composition of *Duncanopsammia axifuga* and *Acropora millepora* exposed to extreme temperatures. The proportions of polyunsaturated fatty acids (PUFA), omega-3 fatty acid, omega-6 fatty acid, saturated fatty acid (SFA), monounsaturated fatty acid (MUFA) and lipid yield are shown after 14 d under 2 different temperatures (26°C and 32°C) and 3 different treatments: unfed, normal *Artemia* and enriched *Artemia*. Bars: means ± SE

The exchange of metabolites plays an essential role in the ecological success of the coral–zooxanthellae symbiosis (Muscatine 1990). Under normal conditions, the symbiont provides necessary PUFAs (C18:4n-3 and C22:5n-3) to the coral host (Papina et al. 2003), C18:4n-3 being a necessary precursor to other n-3 PUFAs (Guil-Guerrero 2007) and C22:5n-3 participating in many biological roles, such as cell signalling regulation and cell proliferation (Salem et al. 2001). However, both FAs were components of the enrichment diet, with C18:4n-3 boosted for *D. axifuga* and C22:5n-3 for *A. millepora* at normal temperatures. Yet corals also shape the FA profile of the symbiont by transferring FAs, such as C20:4n-6 and C22:4n-6, which are not typically found in dinoflagellates (Imbs et al. 2014). In the present study, we were not able to separate symbiont FAs from the coral host (due to small coral tissue samples), and most of the specific FA markers that can be used to confirm PUFA exchange between symbiont and coral (Tolosa et al. 2011, Imbs et al. 2014) were present in the diet. Specifically, C22:4n-6 was boosted in *D. axifuga* fed with the enrichment diet, even though this n-6 PUFA could be synthesized from other n-6 precursors, such as C18:2n-6 and C20:4n-6 that were also present in the diet. Similarly, zooxanthellae PUFA biomarkers and the critical FA C22:6n-3 (Volkman et al. 1998, Bachok et al. 2006, Imbs et al. 2010) usually tend to decrease during thermal stress (Kneeland et al. 2013), but instead were increased by the enriched diet. Additionally, the level of C20:5n-3, a major component of the lipid enrichment diet and considered an essential FA to coral–symbiont metabolism (Imbs et al. 2014), was also raised in both species due to its high concentration in the diet. A previous study also demonstrated the increase of coral PUFAs (e.g. C18:4n-3 and C20:4n-6) after the use of *Artemia* lipid enrichment diet under normal temperatures (Lim et al. 2017). Regardless of the source, elevated proportions of specific PUFAs in both species might enhance thermal stability of membranes and decrease vulnerability to attack by reactive oxygen species (Tchernov et al. 2004).

A considerable amount of work has focused on the assimilation of carbon and nitrogen to elucidate symbiotic contributions (Nahon et al. 2013, Kopp et al. 2015), but more emphasis

Table 3. Post hoc pair-wise test analysis for the feeding and temperature main effects in *Duncanopsammia axifuga* and *Acropora millepora*. PUFA: polyunsaturated fatty acids; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids. (<) mean significantly smaller at $p < 0.05$; (>) mean significantly greater at $p < 0.05$; (=) no significant differences detected

	Unfed vs. normal	Unfed vs. enriched	Normal vs. enriched	26 vs. 32°C
Duncanopsammia axifuga				
PUFA	U < N	U < E	N < E	26 = 32
Omega-6	U < N	U < E	N = E	26 = 32
Omega-3	U < N	U < E	N = E	26 = 32
SFA	U > N	U > E	N > E	26 = 32
MUFA	U > N	U > E	N = E	26 > 32
Growth	U = N	U < E	N = E	26 > 32
Total chlorophyll ($a + c_2$)	U = N	U < E	N < E	26 > 32
Symbiont density	U = N	U < E	N = E	26 > 32
Colour	U < N	U < E	N = E	26 > 32
Stearic (C18:0)	U > N	U > E	N = E	26 = 32
Arachidic (C20:0)	U > N	U > E	N = E	26 = 32
Palmitoleic (C16:1n-7)	U = N	U = E	N = E	26 > 32
Oleic (C18:1n-9)	U > N	U > E	N = E	26 = 32
cis-Vaccenic (C18:1n-7)	U = N	U < E	N < E	26 = 32
Eicosenoic (C20:1n-9)	U = N	U > E	N = E	26 > 32
α-Linolenic (C18:3n-3)	U < N	U < E	N < E	26 = 32
EPA (C20:5n-3)	U < N	U < E	N < E	26 > 32
γ-linoleic (C18:3n-6)	U < N	U < E	N = E	26 = 32
Docosatetranoic (C22:4n-6)	U = N	U < E	N < E	26 = 32
Eicosadienoic (C20:2)	U > N	U > E	N = E	26 > 32
Acropora millepora				
Total chlorophyll ($a + c_2$)	U = N	U = E	N = E	26 > 32
Symbiont density	U = N	U = E	N = E	26 > 32
Colour	U < N	U < E	N = E	26 > 32
PUFA	U = N	U = E	N = E	26 > 32
Omega-3	U = N	U = E	N = E	26 > 32
SFA	U = N	U = E	N = E	26 < 32
MUFA	U > N	U = E	N = E	26 = 32
Myristic (C14:0)	U = N	U = E	N = E	26 < 32
Palmitic (C16:0)	U = N	U = E	N = E	26 < 32
Stearic (C18:0)	U = N	U > E	N = E	26 = 32
Oleic (C18:1n-9)	U > N	U = E	N = E	26 = 32
Eicosenoic (C20:1n-9)	U = N	U = E	N = E	26 > 32
EPA (C20:5n-3)	U = N	U = E	N = E	26 > 32
DPA (C22:5n-3)	U = N	U = E	N = E	26 > 32
DHA (C22:6n-3)	U = N	U < E	N = E	26 > 32
Dihomo-γ-linoleic (C20:3n-6)	U = N	U < E	N = E	26 > 32

is needed to understand the functional role of lipids, and the transfer of nutrients from *Symbiodinium* across the gastroderm (Peng et al. 2011). Only recently have entire genomes become available for some coral species (Shinzato et al. 2011) and their specific *Symbiodinium* (Lin et al. 2015). With a better understanding of the pathways contained within each contributing genome and the contribution of a heterotrophic diet, more information on nutrient assimilation can be extracted to better understand lipogenesis, lipid transportation and storage within the symbiotic relationship.

The precise way by which corals obtain lipids may vary among coral colonies (Teece et al. 2011). However, overall, the lipid-enriched diet raised the proportions of PUFAs and omega-3 FAs for both coral species (>20% for *D. axifuga* and 13% for *A. millepora* at 32°C, and even more at 26°C). Yet for *A. millepora*, PUFA levels were significantly lower at higher temperatures, and can be explained by the dissimilar feeding rates of each species (40 art ml^{-1} for *A. millepora* and 90 art ml^{-1} for *D. axifuga*). This supports the hypothesis that there will be species-specific winners and losers during recovery from extreme environmental events for corals (Loya et al. 2001, Grottoli et al. 2014), even after using artificial feeding assistance. Nevertheless, our findings provide a basis to optimise the diet for enhancement of PUFA reserves to in-

Table 4. Post hoc pair-wise test of the *Duncanopsammia axifuga* fatty acids focusing on feeding (top) and temperature (bottom) following significant feeding × temperature interactions. (<) mean significantly smaller at $p < 0.05$; (>) mean significantly greater at $p < 0.05$; (=) no significant differences detected

Factor: feeding	Unfed vs. normal	Unfed vs. enriched	Normal vs. enriched
Palmitic (C16:0) at 26°C	U = N	U > E	N > E
Palmitic (C16:0) 32°C	U = N	U > E	N > E
Stearidonic (C18:4n-3) at 26°C	U < N	U < E	N = E
Stearidonic (C18:4n-3) at 32°C	U = N	U < E	N = E
Linoleic (C18:2n-6) at 26°C	U = N	U = E	N = E
Linoleic (C18:2n-6) at 32°C	U < N	U = E	N = E
Factor: temperature	**Unfed 26 vs. 32°C**	**Normal 26 vs. 32°C**	**Enriched 26 vs. 32°C**
Palmitic (C16:0)	26 = 32	26 < 32	26 < 32
Stearidonic (C18:4n-3)	26 = 32	26 = 32	26 > 32
Linoleic (C18:2n-6) at 26°C	26 = 32	26 < 32	26 = 32

crease the resilience of small polyp species, such as the Acroporidae family. Lipid-enriched *Artemia* may be suitable for large heterotrophic species such as *D. axifuga*, and the use of smaller zooplankton species, such as the common rotifer *Brachionus plicatilis* (also suitable for the lipid enrichment process), may be more appropriate for corals with smaller polyps, such as some *Acropora* species.

Outside of a controlled experimental setting, essential FAs are generated by planktonic sources in the ocean, affecting heterotrophs and food web dynamics (Galloway & Winder 2015). Under larger scale abiotic stress, alterations in planktonic communities are magnified up the food chain (Edwards et al. 2013). When considering the major changes in phyto- and zooplankton communities already detected in the last few decades (Edwards et al. 2013), as well as the predicted shift in planktonic communities to lower nutritional food sources by the effects of ocean climate change (Przytulska et al. 2015), our observed enhancement of coral bleaching resilience through diet might indicate further physiological and ecological implications.

A lipid-enriched *Artemia* diet increases FA content, zooxanthellae density and chlorophyll levels in corals (Lim et al. 2017). In this context, lipid-enriched feeding can be included amongst other novel management approaches currently available to improve the resilience and recovery of coral reefs following bleaching (Hoey et al. 2016, Jin et al. 2016). Such a dietary intervention has limitations, especially considering the geographic extent of many coral reefs. Nonetheless, it might be considered for small sites with high conservation status.

Overall, we have shown the importance of heterotrophic feeding in coral resilience to bleaching. These findings have significant implications for understanding how rising temperatures may affect species that strongly rely upon non-symbiont derived lipids as a source of energy reserves. The lipid-enriched feeding, used as a diet enhancement tool, could improve survival rates during coral relocation in restoration projects, in aquarium trade shipments, and is likely to increase coral growth rates in aquaculture. Furthermore, under predicted stress episodes, the lipid-enriched diet could also be used as a localized solution for targeted endangered species and to improve coral resilience against bleaching.

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