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Effects of feeding on production, body composition and fatty acid profile of scleractinian coral *Galaxea fascicularis*

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ABSTRACT

To meet the emerging demand of corals in various industries, it is necessary to develop an optimal technique to improve coral growth and health. Since heterotrophy provides a vital source of nutrients for corals, it is possible to implement a cultivation pattern combining regular methods with artificial feeding for corals. To this end, this study investigated the effects of feeding (supplemented with *Artemia salina* nauplii) on the production (growth and budding rate), body composition (tissue protein, crude lipid, and ash contents), and fatty acid profile (total fatty acid and polar lipid fatty acid profiles) of scleractinian coral *Galaxea fascicularis* in polyp morphology. Our results showed that feeding increased the production of *G. fascicularis*, with fed corals exhibiting a 51.4% higher growth rate and a double budding rate. Meanwhile, feeding changed the body composition of *G. fascicularis*, with increased tissue protein and crude lipid contents by 69.8%, and 31.0%, respectively, which indicated a good assimilation of supplementary proteins and lipids via feeding. Moreover, feeding also significantly changed the total FA profile of *G. fascicularis*, as it improved the levels of monounsaturated (MUFAs) and polyunsaturated fatty acids (PUFAs) (mainly n-3 PUFAs and n-6 PUFAs), but decreased the proportion of saturated fatty acids (SFAs) (mainly 16:0). However, there was no significant difference in the polar lipid FA composition of *G. fascicularis* between the two feeding regimes. This study highlights substantial improvements in the physiological state and health condition of corals under artificial feeding, and demonstrates that *A. salina* nauplii can serve as a suitable nutrient source for *G. fascicularis* in aquaculture.

1. Introduction

Coral reefs are among the most diverse and productive ecosystems globally (Brandl et al., 2019). Serving as the framework for these ecosystems, corals are facing severe degradation owing to increased sea-surface temperatures, ocean acidification, and other threats associated with climate change (Hoegh-Guldberg et al., 2017; Hughes et al., 2018). Meanwhile, the demand for corals in reef rehabilitation and other uses such as marine ornamental trade, the pharmaceutical industry, and scientific research, is rapidly increasing (Leal et al., 2016). Consequently, efforts are currently underway to develop *ex situ* and *in situ* coral aquaculture (Leal et al., 2018). Compared to *in situ* aquaculture, the advantage of *ex situ* aquaculture lies in its ability to artificially manipulate culture conditions (Barton et al., 2017), as vital abiotic and biotic factors can be manipulated to guarantee the successful husbandry of corals (Leal et al., 2018). The primary goal of *ex situ* coral aquaculture

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is to maximize production, which makes branching corals such as *Acroporidae* and *Pocilloporidae* species a good candidate as they have higher growth rates (Leal et al., 2016). Compared with branching corals, massive species such as *Poritidae* and *Galaxea* with lower growth rates are less popular in culture activities (Barton et al., 2017), leading to a lack of variation of coral species supplied to various industries.

Reef-building corals are mixotrophic feeders, that is, they can not only obtain the energy required for basic life activities through photosynthesis, but also assimilate nutrients through heterotrophic (requiring exogenous complex organic compounds of nitrogen and carbon, such as those obtained from plant or animal matter, for metabolic synthesis) predation (Houlbrèque and Ferrier-Pagès, 2008). It is generally believed that partnerships with endosymbiotic algae enable corals to flourish in nutritionally poor environments (Muscatine and Porter, 1977). However, the contribution of heterotrophic nutrition to reef-building corals has been underestimated (Houlbrèque and Ferrier-Pagès, 2008). Indeed, heterotrophy plays a much more important role than what is generally believed, as it has been clearly demonstrated that feeding can promote photosynthesis (Houlbrèque et al., 2004; Lyndby et al., 2018), calcification (Drenkard et al., 2013; Ferrier-Pagès et al., 2003; Houlbrèque et al., 2003), budding (Rodolfo-Metalpa et al., 2008), and tissue growth (Anthony and Fabricius, 2000; Houlbrèque et al., 2003, 2004) in some coral species. Likewise, compared with unfed corals, fed corals are proved to better resist temperature stress (Ferrier-Pagès et al., 2010; Baumann et al., 2014; Tremblay et al., 2016; Tagliafico et al., 2017) and irradiation stress (Levy et al., 2016; Treignier et al., 2008). It is believed that massive corals with large polyps such as *Galaxea* are ideally more suitable for plankton capture (Palardy et al., 2005, 2006). This provides the possibility of implementing a coral cultivation pattern that combines regular methods with artificial feeding.

To date, studies on coral feeding have mainly focused on its role in ecology, physiology, and stress resistance. To better evaluate the status of corals in culturing processes, it is important to detect the productivity of corals. Unfortunately, there is very limited information related to coral feeding that aims to improve coral production (Tagliafico et al., 2018). Moreover, previous studies are mostly limited to the addition of indiscriminate feeds (Hii et al., 2009; Lyndby et al., 2019; Toh et al., 2013;), with little consideration of the nutritional profile of these diets or the biochemical response of the coral consumer. To achieve high production, it is more important to maintain high quality. As body composition, which refers to the proportion of fat, protein, and ash in organisms, can reflect the overall health and fitness of corals, more attention should be paid to the body composition of corals during culturing processes. However, there is little published information that focuses on the effect of feeding on corals from the perspective of body composition (Conlan et al., 2018a).

Lipid, also considered as energy reserves (mainly refers to lipids and fatty acids (FAs) in this study), accounts for much of the coral body composition and is involved in a majority of physiological processes in corals (Conlan et al., 2017; Imbs et al., 2010). As FA composition is often species-specific, the FA profiles of different coral species have been used as valid chemotaxonomic indicators (Imbs et al., 2010; Lopes et al., 2016). Moreover, as certain FAs cannot be synthesized by marine consumers, FA profiles can be used as biomarkers to trace the nutritional input of corals (Mies et al., 2018). In addition, FAs (mainly polar lipid FAs) are the primary constituents of the cells and subcellular organelle membranes of corals; by changing their FA composition, corals can adapt to various environmental conditions (Liu et al., 2020). As changes in the FA composition can reflect changes in the ecology of corals, as well as their health and nutrition (Imbs et al., 2010; Rocker et al., 2019), it is useful and necessary to study the FA profiles of corals during the culturing process.

Although *Galaxea fascicularis*, a typical massive coral, shows a lower growth rate than branching species, it is an important reef coral that is widely distributed in the Indo-Pacific region. *Artemia salina* nauplii, rich in nutrients that are suitable for consumption by corals, is commonly used as live prey for the artificial feeding of corals (Lim et al., 2017). Therefore, in the present study, the massive-shaped reef-building coral *G. fascicularis*, which is dominant in the Luhuitou area, China, was chosen for *ex situ* culture under two feeding regimes (with or without *A.* nauplii supplementation, respectively). We assessed the production (growth and budding rate) and body composition (tissue protein, crude lipid, and ash contents) of *G. fascicularis*, and explored the effect of heterotrophy on coral physiology from the perspective of energy reserves (the total FA and polar lipid FA profiles). The main objective of this study was to better assess the physiological effects of feeding on *G. fascicularis*, as well as to provide a practical basis for the improvement of the production of corals in *ex situ* culture conditions, especially for slow-growing and heterotrophic species.

2. Material and methods

2.1. Coral sampling and maintenance

Ten small colonies of *G. fascicularis* were collected randomly at 3 m depth from Luhuitou reef, Hainan province, China (N18°12′, E109°28′) by SCUBA diving on October 6, 2018. In order to improve the utilization while minimizing the damage to donor corals, single polyps of *G. fascicularis* were adopted in this study. The collected corals were fragmented to single polyps using a bone cutter. Each polyp was then attached to a ceramic chip (2.2 ×2.2 cm) using animal glue (GEL-10, Aron Alpha, Japan). Note that only mature and healthy polyps were selected in this study, and the surface area of single polyps could be considered the same (2.131 ± 0.092 cm^2).

The polyps of *G. fascicularis* were then equally separated and maintained (36 polyps in each tank) in four in-door 40-L plastic tanks. They were allowed healing for two weeks prior to the experiment. The tanks were filled with 0.5-μm-filtered and UV-sterilized seawater, which was pumped from the Luhuitou reef area. Half of the seawater was changed daily, and the tanks were cleaned twice per week to avoid algal growth. Internal water in the tanks was continuously circulated using a submerged pump (AT101S, Atman, Beijing, China) at a rate of 600 L h^{-1}. Salinity was maintained at 34 ± 0.5 psu. Water temperature was controlled by digital aquarium heaters (MX-1019, Weipro, China) at a targeted value of 27 ± 0.5 °C, and each tank was illuminated with full-spectrum fluorescent light (Giesemann, Germany) at around 200 μmol photons m^{-2} s^{-1} on a 12 h light/12 h dark cycle.

2.2. Experimental design

Artemia salina nauplii were prepared at 36 h before the experiments as described by Hii et al. (2009). by culturing the shrimp in an inverted 2 L clear glass container with 0.5-μm-filtered, UV-sterilized seawater and vigorous aeration. Feeding experiments were conducted after the acclimation period (two weeks). Four tanks were randomly divided into two treatments: corals that were fed *A. salina* nauplii (Fed), and corals without feeding (Control). For the fed group, each polyp was given 80–100 A. *salina* nauplii (Aquamaster, China) every day following, which were pipetted directly over the oral disc of polyps; for the control group, no food was supplied during the whole experiment. All submerged pumps were closed during the daily feeding period until all polyps finished eating, and excess *A. salina* nauplii were removed to avoid water pollution.

Polyps of *G. fascicularis* were maintained under these two feeding regimes for four weeks (record the first day of the experiment as Day0, and the last day as Day28). Growth rate (n = 12) and budding rate (n = 24) were measured at Day28, and all polyps were then frozen for later determination of tissue protein content (n = 6), crude lipid content (n = 6), ash content (n = 6), total FAs and polar lipid FAs composition (n = 6), as described below.

2.3. Measurement of coral growth rate, budding rate, and body composition

The growth rate of corals in this study was measured by buoyant weight technique (Davies, 1989). Using precision electronic balance with an accuracy of 0.1 mg, the skeletal weight of *G. fascicularis* were measured from their buoyant weight in seawater, and the growth rate of corals were reflected by the difference of buoyant weight between Day0 and Day28.

Scleractinian corals have the ability to reproduce asexually through budding. In contrast to branching species, bud formation on *G. fascicularis* is easily distinguishable and can thus be considered as an indicator of the reproduction and growth status of *G. fascicularis* (van Os et al., 2012). At Day28, occurrence of buds on polyp tissue was recorded for each individual, and the budding rate was calculated by cumulative number per polyp per week.

The body composition can indicate the health status of corals. Body composition analyses of *G. fascicularis* and *A. salina* nauplii were conducted at the end of the experiment. For protein content, coral tissue was detached using an airbrush, and the slurry was homogenized using a handheld homogenizer. Then the homogenate was suspended in 0.2 μm filtered and sterilized seawater (FSW), and the tissue protein content of *G. fascicularis* was measured using the BCA assay kit (W041-1-1, Nanjing Jiancheng) and expressed as mg per polyp. While the protein content of *A. salina* nauplii was estimated indirectly using Dumas method (nitrogen × 6.25) (Ebeling, 1968). Other samples of *G. fascicularis* and *A. salina* nauplii were freeze dried for the further detection of crude lipid content and ash content. Crude lipid content was measured by the petroleum ether extraction method using Soxhlet extractor (AG-SXT-06, Shanghai Ouge), and ash content was determined following combustion at 450 °C for 6 h. The data of crude lipid content and ash content were reported as the percentage of dry weight.

2.4. Total FAs and polar lipid FAs analyses

Samples of *G. fascicularis* collected from each group (n = 6) were used to detect total FAs and polar lipid FAs composition, and samples of *A. salina* nauplii (n = 3) were used to detect total FAs composition. As previously described by Liu et al. (2020), each sample (0.1 g) was homogenized and the lipid fraction was extracted using chloroform/methanol (2:1, v/v) containing 0.01% butylated hydroxytoluene as antioxidant. The chloroform layer was separated from the methanol and dried to a constant weight under a stream of nitrogen to obtain lipids. Polar lipid FAs were separated on one-dimensional thin-layer hybrid silica gel plates (100 ×50 mm) (Yinlong Company, Yantai, China) with N-hexane/ether/acetic acid (84:15:1, v/v/v) as the developing solvent according to the method described by Liu et al. (2018). FA methyl esters (FAMEs) were obtained by esterification with 2 mL methyl esterification reagent (hydrochloric acid/methanol, 1:5, v/v) at 90 °C for 3 h, and the upper phase was dried under nitrogen and resuspended in hexane.

FAMEs were quantified by injecting 1 μl of sample into a gas chromatograph (GC-2010 Plus; Shimadzu, Kyoto, Japan) equipped with a flame-ionization detector (GC-2010; Shimadzu) and an RTX-WAX fused-silica capillary column (length, 30 m; internal diameter, 0.25 mm; thickness, 0.25 mm; Phenomenex, Torrance, CA, United States). The gradient temperature program was set as follows: (i) initial temperature of 60 °C for 1.0 min; (ii) increase at a rate of 10 °C min^{-1} to 190 °C, (iii) increase at 2.0 °C min^{-1} to 260 °C; (iv) hold at 260 °C for 0.6 min. FAME identification and quantification were performed by comparing the retention times (identification) and peak areas (quantification) with 37-FAME Mix calibration solution (Supelco, Bellefonte, PA, United States).

2.5. Statistical analyses

All data were evaluated for normality using the Kolmogorov-Smirnov test ($P > 0.05$), and Levene's test ($P > 0.05$) was used to assess the homogeneity of variance. Then the significance of differences in growth rate, budding rate, tissue protein content, crude lipid content, ash content, total FAs and the polar lipid FA composition of the fed and unfed corals were tested using the independent samples *t*-test. Statistical significance was set at $P < 0.05$. Principal component analysis (PCA) was used to identify significant differences in the FA profiles of fed and unfed corals. The FAs of *G. fascicularis* that were in great significant difference ($P < 0.01$) under the different feeding regimes were identified, and the relative FA compositions were calculated using the following equation:

$$R_{FAs}\ (\%\ \text{Fed over Unfed}) = P_{Fed} - P_{Unfed}$$

where R_{FAs} refers to the relative compositions of a certain FA; P_{Fed} and P_{Unfed} refer to the proportion of a certain FA for the fed and unfed groups, respectively.

All statistical analyses were conducted using SPSS Statistics for Windows, version 20.0 (SPSS Inc., Chicago, IL, USA) and R with the devtools package, and data were reported as mean value ± standard deviation (mean ± SD).

3. Results

3.1. Effect of feeding on the production and body composition of G. fascicularis

Feeding improved the production of *G. fascicularis* (Fig. 1). The growth rate of fed corals was 51.4% higher than that of the unfed group (Unfed group: 0.701 ± 0.081 mg $polyp^{-1}$ d^{-1}; Fed group: 1.060 ± 0.123 mg $polyp^{-1}$ d^{-1}; $P = 0.023$) (Fig. 1a). Subsequently, the budding rate of fed corals was more than twice that of the unfed group (Unfed group: 0.504 ± 0.051 polyps $polyp^{-1}$ $week^{-1}$; Fed group: 1.222 ± 0.076 polyps $polyp^{-1}$ $week^{-1}$; $P < 0.001$) (Fig. 1b-c).

Table 3 lists the nutritional composition of *A. salina* nauplii. The total energy value of *A. salina* nauplii used in this study was 8.50 ± 0.10 MJ/kg. The protein (21.42 ± 2.57% of dry weight), crude lipid (8.45 ± 1.84% of dry weight), and ash (55.66 ± 1.57% of dry weight) contents are shown in Table 3. Notably, feeding changed the body composition of *G. fascicularis* (Fig. 2). Feeding significantly increased the tissue protein content of *G. fascicularis*, which was 69.8% higher than that of unfed corals (Unfed group: 2.258 ± 0.267 mg $polyp^{-1}$; Fed group: 3.834 ± 0.291 mg $polyp^{-1}$; $P = 0.003$) (Fig. 2a). In the fed group, the crude lipid content increased significantly and was 31.0% higher than that in the unfed group (Unfed group: 4.39 ± 0.45%; Fed group: 5.75 ± 0.55%; $P < 0.001$) (Fig. 2b). In contrast, the ash content of *G. fascicularis* in the fed group was slightly lower than that in the unfed group (Unfed group: 88.93 ± 1.11%; Fed group: 86.27 ± 1.88%; $P = 0.03$) (Fig. 2c).

3.2. FA profiles of G. fascicularis and A. salina nauplii

Twenty-one FA species were identified in the total FAs of *G. fascicularis*, including seven saturated FAs (SFAs), six monounsaturated FAs (MUFAs), and eight polyunsaturated FAs (PUFAs) (Table 1); twenty FA species were identified in the polar lipid FAs of *G. fascicularis*, including seven SFAs, four MUFAs, and nine PUFAs (Table 2); twenty-seven FA species were identified in the total FAs of *A. salina* nauplii, including ten SFAs, seven MUFAs, and ten PUFAs (Table 3). Eight major FAs were recurrently found in *G. fascicularis*, including myristic acid (14:0), palmitic acid (16:0), stearic acid (18:0), palmitoleic acid (16:1n7), oleic acid (18:1n9), arachidonic acid methyl ester (20:4n6), EPA (20:5n3), and DHA (22:6n3). Seven major FAs were recurrently found in *A. salina* nauplii, including palmitic acid (16:0), stearic acid (18:0), palmitoleic acid (16:1n7), oleic acid (18:1n9), linoleic acid (18:2n6), linolenic acid methyl ester (18:3n6), and EPA

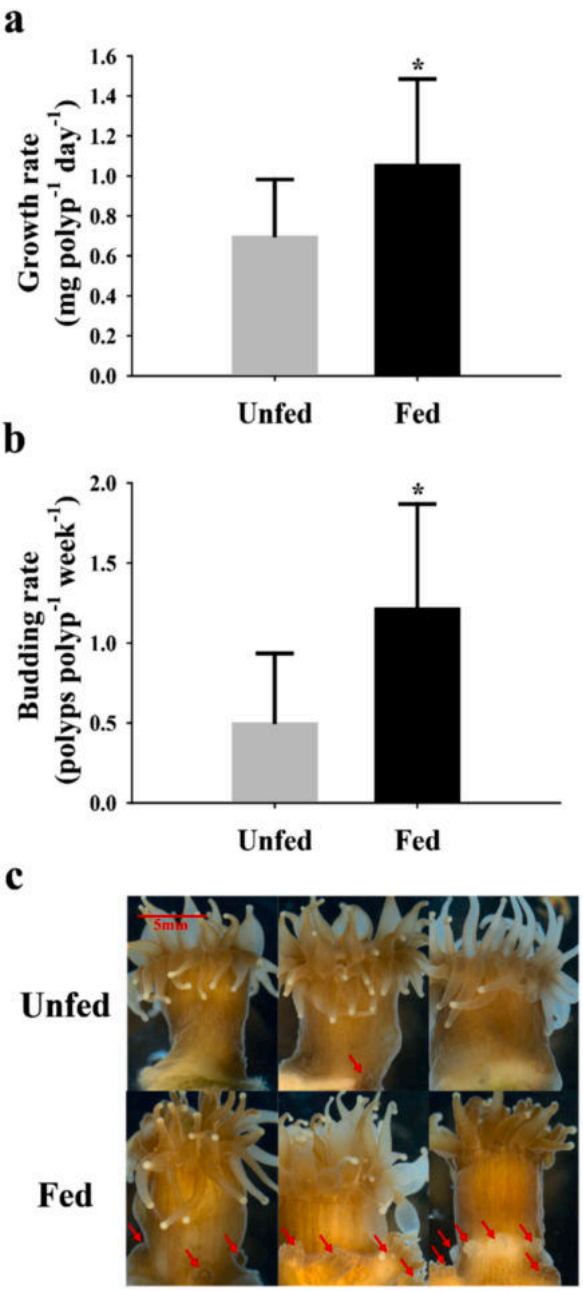

Fig. 1. Effects of feeding on the production of G. fascicularis. (a) Growth rate. (b) Budding rate. (c) Budding morphology. Red arrows indicate the newly-born coral buds. The asterisks indicate significant difference between the Fed group and the Unfed group ($P < 0.05$).

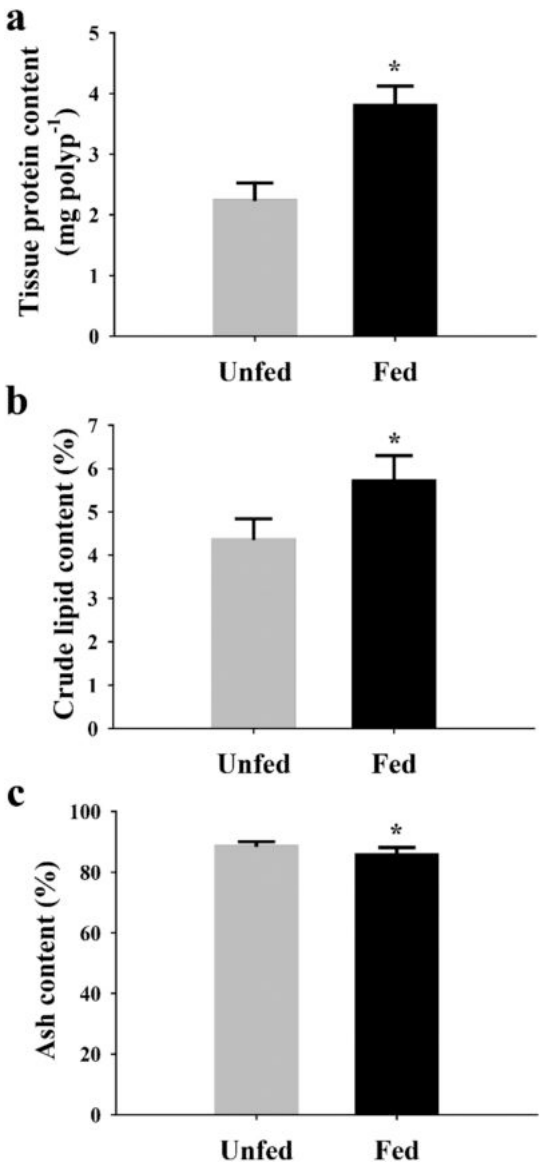

Fig. 2. Effects of feeding on the body composition of *G. fascicularis*. (a)Tissue protein content. (b) Crude lipid content. (c)Ash content. The asterisks indicate significant difference between the Fed group and the Unfed group ($P < 0.05$).

(20:5n3).

SFA made up the highest proportion of the total FAs in *G. fascicularis*, especially in the unfed group, as it accounted for more than 60% of the total FAs. On the contrary, While the MUFA and PUFA contents were relatively low, as, combined, they accounted for approximately 20% of the total FAs (Table 1). However, in *A. salina* nauplii, the MUFA and PUFA contents (accounting for 41.24 ± 1.30% and 41.18 ± 0.67% of the total FAs, respectively) were much higher than the SFA content (accounting for 17.57 ± 0.63%) (Table 4).

3.3. Effect of feeding on the FA profile of G. fascicularis

The total FA and polar lipid FA profiles of *G. fascicularis* under the two feeding regimes are shown in Table 1 and Table 2, and the data matrix of the total FAs and polar lipid FAs in the coral samples analyzed was subjected to PCA to decrease the number of descriptors associated with the data set (Fig. 3).

Overall, feeding on *A. salina* nauplii changed dramatically the FA composition of *G. fascicularis* (Table 1, Fig. 3a). As is shown in Fig. 3a, the first principal component (PC1) and the second principal component (PC2) accounted for 45.6% and 22.5%, respectively, of the variability in the data set. PC1 had positive loadings for stearic acid (0.32) and palmitic acid (0.30) and negative loadings for linolenic acid methyl ester (−0.32), EPA (−0.31), oleic acid (−0.3), linoleic acid (−0.29), and DHA (−0.21); PC2 showed positive loadings for trianoic acid (0.40) and negative loadings for palmitoleic acid (−0.28). In contrast, in the polar lipid FA compositions of *G. fascicularis*, neither SFA nor unsaturated fatty acids (UFAs), showed significant changes (Table 2, Fig. 3b). As is shown in Fig. 3b, there was no significant difference in the polar lipid FA profiles of *G. fascicularis* under different feeding regimes.

To better visualize the FA changes of *G. fascicularis* under the two feeding regimes, the FAs with great significant changes ($P < 0.01$) are presented in Fig. 4. We found that the SFA levels in the fed corals

Table 1
Effects of feeding on the total fatty acid (FA) compositions of *G. fascicularis* (% total FA).

	Unfed	Fed	*P* value
Saturated fatty acids (SFAs)			
C14:0	5.08 ± 0.36	5.28 ± 0.20	0.31
C16:0	44.88 ± 1.02	35.32 ± 0.81	< 0.001
C17:0	0.14 ± 0.01	0.13 ± 0.01	0.07
C18:0	5.76 ± 0.22	4.20 ± 0.19	< 0.001
C20:0	0.48 ± 0.04	0.48 ± 0.06	0.91
C22:0	0.21 ± 0.03	0.24 ± 0.05	0.37
C23:0	3.76 ± 0.43	3.63 ± 0.24	0.56
∑SFAs	60.32 ± 0.71	49.28 ± 0.65	< 0.001
Monounsaturated fatty acids (MUFAs)			
C14:1n5	0.31 ± 0.03	0.37 ± 0.03	0.02
C16:1n7	13.73 ± 1.09	14.83 ± 0.37	0.06
C17:1n7	0.11 ± 0.04	0.12 ± 0.01	0.72
C18:1n9	3.93 ± 0.30	6.88 ± 0.27	< 0.001
C20:1n9	0.13 ± 0.03	0.20 ± 0.05	0.02
C22:1n9	0.29 ± 0.14	0.20 ± 0.04	0.19
∑MUFAs	18.5 ± 0.77	22.59 ± 0.56	< 0.001
Polyunsaturated fatty acids (PUFAs)			
C18:2n6	2.43 ± 0.22	3.67 ± 0.21	< 0.001
C18:3n3	2.11 ± 0.14	2.10 ± 0.19	0.94
C18:3n6	0.56 ± 0.06	3.74 ± 0.17	< 0.001
C20:2n6	0.59 ± 0.08	0.60 ± 0.08	0.85
C20:3n3	1.12 ± 0.03	1.22 ± 0.09	0.03
C20:4n6	5.30 ± 0.70	4.54 ± 0.33	0.05
C20:5n3	3.41 ± 0.23	6.27 ± 0.20	< 0.001
C22:6n3	5.65 ± 0.14	6.00 ± 0.23	0.01
∑PUFAs	21.18 ± 0.56	28.13 ± 0.27	< 0.001
n-3 PUFAs	12.30 ± 0.44	15.58 ± 0.48	< 0.001
n-6 PUFAs	8.88 ± 0.97	12.55 ± 0.22	< 0.001

Table 2
Effects of feeding on the polar lipid fatty acid (FA) compositions of *G. fascicularis* (% polar lipid FA).

	Unfed	Fed	*P* value
Saturated fatty acids (SFAs)			
C14:0	1.37 ± 0.21	1.00 ± 0.55	0.16
C16:0	22.71 ± 1.15	22.48 ± 1.07	0.73
C17:0	0.14 ± 0.15	0.06 ± 0.12	0.35
C18:0	11.74 ± 1.44	12.21 ± 0.99	0.52
C20:0	0.45 ± 0.49	0.91 ± 0.47	0.13
C22:0	0.33 ± 0.36	0.42 ± 0.35	0.67
C23:0	13.90 ± 1.47	15.69 ± 0.58	0.03
∑SFAs	50.64 ± 2.41	52.77 ± 1.83	0.12
Monounsaturated fatty acids (MUFAs)			
C16:1n7	2.22 ± 0.24	2.06 ± 0.21	0.36
C17:1n7	0.36 ± 0.43	0.46 ± 0.46	0.71
C18:1n9	2.11 ± 0.30	1.96 ± 0.13	0.29
C22:1n9	2.57 ± 2.85	2.30 ± 1.90	0.85
∑MUFAs	7.25 ± 3.02	6.79 ± 2.10	0.76
Polyunsaturated fatty acids (PUFAs)			
C18:2n6	1.79 ± 0.17	1.55 ± 0.06	0.02
C18:3n3	1.49 ± 0.12	1.38 ± 0.20	0.27
C18:3n6	0.19 ± 0.20	0.07 ± 0.13	0.25
C20:2n6	1.06 ± 0.27	1.16 ± 0.16	0.48
C20:3n3	0.38 ± 0.42	0.97 ± 0.37	0.03
C20:4n6	21.20 ± 1.20	20.00 ± 1.54	0.16
C20:5n3	8.96 ± 0.42	8.30 ± 0.45	0.03
C22:2n6	0.30 ± 0.33	0.21 ± 0.26	0.62
C22:6n3	6.75 ± 0.34	6.80 ± 0.22	0.76
∑PUFAs	42.11 ± 1.02	40.44 ± 2.15	0.12
n-3 PUFAs	17.57 ± 0.44	17.45 ± 0.87	0.80
n-6 PUFAs	24.54 ± 0.95	22.99 ± 1.50	0.058

decreased significantly (Fed over Unfed: −11.04 ± 0.80%), primarily due to the decreased 16:0 (Fed over Unfed: −9.56 ± 0.99%) and 18:0 (Fed over Unfed: −1.56 ± 0.31%) levels (Fig. 4). On the contrary, there was a significant improvement in the MUFA content (Fed over Unfed: 4.09 ± 0.75%) of the fed group, which was mainly due to the increase in the 18:1n9 (Fed over Unfed: 2.95 ± 0.51%) level (Fig. 4). In a similar manner, the PUFA levels in the fed corals increased significantly (Fed over Unfed: 6.95 ± 1.12%), with a similar trend observed in n-3 PUFA (Fed over Unfed: 3.29 ± 0.45%) and n-6 PUFA (Fed over Unfed: 3.67 ± 0.91%). This general increase in PUFA was associated with a specific increase in 18:2n6 (Fed over Unfed: 1.24 ± 0.34%), 18:3n6 (Fed over Unfed: 3.18 ± 0.15%), and 20:5n3 (Fed over Unfed: 2.85 ± 0.34%) (Fig. 4).

4. Discussion

The results of the present study strongly suggest that heterotrophic feeding can promote the overall skeletal growth and asexual reproduction of *G. fascicularis*; in other words, food intake could accelerate the productivity of *G. fascicularis* (Fig. 1). Likewise, several studies have found that feeding induces an increase in the overall skeletal growth rate (Conlan et al., 2018a; Drenkard et al., 2013; Huang et al., 2020) and bud formation rate (Rodolfo-Metalpa et al., 2008; Maier et al., 2020) of multiple reef coral species. However, the results of a study on *G. fascicularis* did not show changes in the rate of new polyp production between the fed and unfed group (van Os et al., 2012). As there is little difference in the type of food used and feeding amount the corals were fed between our study and the study of vas Os et al. (2012), any discrepancies in the results may be explained by different feeding frequencies. The feeding frequency in our study was once a day, while the feeding frequency in the aforementioned study did not exceed three times a week. Two main explanations may account for this feeding-mediated growth enhancement. First, many nutrients that cannot be supplied by photosynthesis can be acquired through heterotrophic feeding (Houlbrèque and Ferrier-Pagès, 2008), thus the additional nutrients could stimulate the growth and asexual reproduction of corals. Additionally, feeding can enhance productivity indirectly by increasing the photosynthetic rate (Ferrier-Pagès et al., 2010). It has been reported that feeding greatly improves the zooxanthellae density and photosynthetic rate of *G. fascicularis*; this finding helped expand the main nutrient sources of corals and indicated a higher organic carbon accumulation rate of holobionts (Yu et al., 2019). For the industry of coral aquaculture, a higher productivity induced by feeding will be of great value, to aquarium trade for its market price, to scientific purposes for its sample volume, as well as to restoration projects as larger colonies have greater survival chances.

Body composition, growth, nutrient utilization, and resource allocation are interrelated and change considerably during the lifespan of animals (Dumas et al., 2010). Interestingly, the body composition of *G. fascicularis* was significantly affected by the feeding regimes. The tissue protein and lipid contents of *G. fascicularis* increased significantly when food was introduced, while the ash content decreased slightly (Fig. 2). The higher tissue protein and lipid content in the fed group than in the unfed group likely reflects a good assimilation of proteins and lipids, particularly considering that *A. salina* nauplii is rich in proteins and lipids (Table 3). Similarly, the promotive effect of feeding on the protein (Conlan et al., 2018a; Ferrier-Pagès et al., 2003; Houlbrèque et al., 2004) and lipid (Al-Moghrabi et al., 1995; Conlan et al., 2018a; Lim et al., 2017; Tolosa et al., 2011; Treignier et al., 2008) contents of corals has also been observed in several previous studies. In scleractinian corals, proteins play vital roles in most biological processes, such as enzymatic catalysis, material transportation and storage, immunity, and growth (Diniz et al., 2014), while lipids provide bulk energy, yielding at least one-third more energy relative to proteins or carbohydrates (Parrish, 2013). In our study, the rapid growth of *G. fascicularis* in the fed group was mirrored with higher tissue protein and lipid contents. This conforms to the widely-accepted theory that high protein levels are characteristic of active growth, where protein synthesis and retention facilitate calcification, tissue synthesis, and the polyp production of corals (Conlan et al., 2017). In contrast, others posit that high growth rates in corals could reduce lipid concentrations, possibly due to the catabolism of high-energy lipids to support cell proliferation and

Table 3
Total energy value (MJ/kg), body composition (% dry weight) and total fatty acid (FA) compositions (% total FAs) of *A. salina* nauplii.

Total energy value and body composition		Saturated fatty acids (SFAs)		Monounsaturated fatty acids (MUFAs)		Polyunsaturated fatty acids (PUFAs)	
Total energy value	8.50 ± 0.10	C6:0	0.17 ± 0.09	C14:1n5	0.09 ± 0.02	C18:2n6c	7.69 ± 0.42
		C12:0	0.08 ± 0.01	C15:1n5	0.15 ± 0.01	C18:2n6t	0.13 ± 0.01
		C14:0	1.48 ± 0.06	C16:1n7	13.54 ± 0.30	C18:3n3	0.24 ± 0.01
Proteincontent	21.42 ± 2.57	C15:0	0.32 ± 0.02	C17:1n7	0.46 ± 0.04	C18:3n6	9.24 ± 0.06
		C16:0	9.90 ± 0.08	C18:1n9	26.10 ± 0.84	C20:2n6	0.09 ± 0.01
		C17:0	0.72 ± 0.11	C20:1n9	0.72 ± 0.19	C20:3n3	0.12 ± 0.01
Crude lipid content	8.45 ± 1.84	C18:0	4.20 ± 0.33	C22:1n9	0.18 ± 0.04	C20:3n6	2.32 ± 0.05
		C20:0	0.13 ± 0.06			C20:5n3	20.57 ± 0.79
		C22:0	0.30 ± 0.16			C22:2n6	0.14 ± 0.00
Ash content	55.66 ± 1.57	C23:0	0.27 ± 0.04			C22:6n3	0.64 ± 0.28
		∑SFAs	17.57 ± 0.63	∑MUFAs	41.24 ± 1.30	∑PUFAs	41.18 ± 0.67

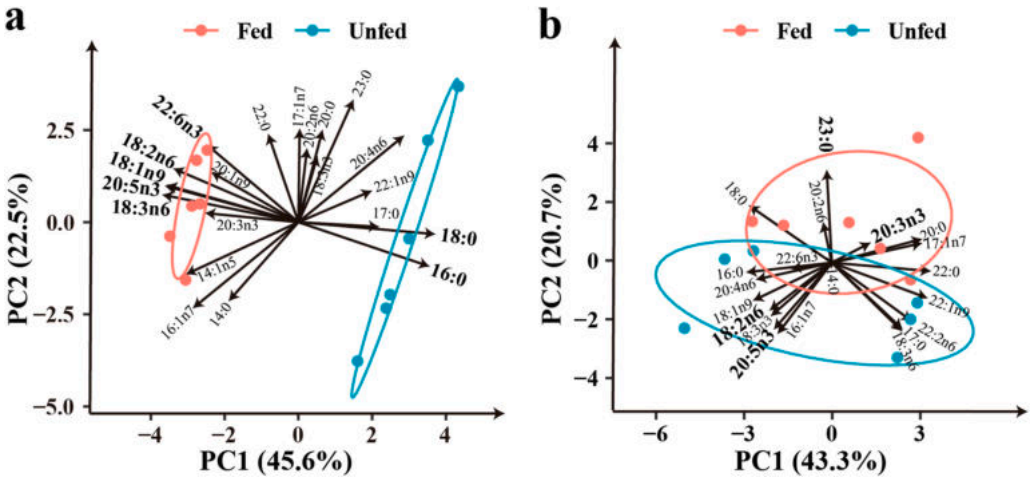

Fig. 3. Principal component analysis of *G. fascicularis* FA compositions under two feeding regimes. (a) Total FAs. (b) Polar lipid FAs. The FAs in bold indicate significant differences between the Fed group and the Unfed group.

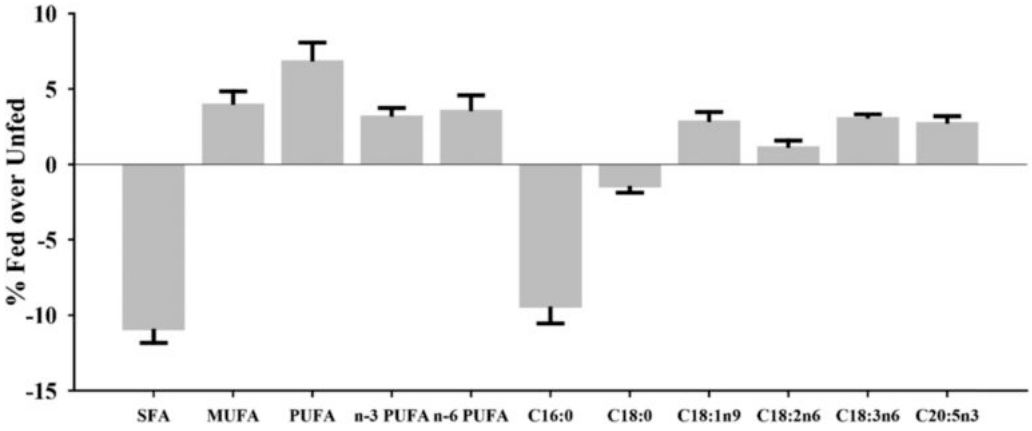

Fig. 4. Changes in relative FA compositions of *G. fascicularis* under two feeding regimes. All data displayed are in great significant difference ($P < 0.01$). SFA: Saturated fatty acid; MUFA: Monounsaturated fatty acid; PUFA: Polyunsaturated fatty acid.

calcification (Conlan et al., 2018b; Denis et al., 2013;). Thus, the results of our study are highly suggestive of the fact that heterotrophic intake could compensate for the loss of lipids caused by the rapid growth of corals. Given that the lipid content of *A. salina* nauplii used in this study is relatively low (Tagliafico et al., 2017), a lipid-enriched diet could be chosen for future coral culturing processes.

FAs are the main constituents of total lipids (Imbs and Yakovleva, 2012). The FA patterns of *G. fascicularis* were similar to those of this species (Lim et al., 2017; Radice et al., 2019) (Table 1). The SFAs (mainly 16:0) were the most prevalent fatty acids in both the unfed (over 60%) and fed corals (approximately 50%). As 16:0 is largely derived from zooxanthellae (Figueiredo et al., 2012; Papina et al., 2003), the bulk storage of 16:0 may explain the importance of zooxanthellae to *G. fascicularis*. The MUFA and PUFA contents in *G. fascicularis* were relatively low, and, combined, accounted for approximately 20% of the total FAs. With regard to MUFAs, the high abundance of 16:1n7 and 18:1n9 indicated the diversity of food sources for *G. fascicularis*, because the former is likely derived in substantial quantities from photosynthetic organisms and the latter is a major FA in most marine animals (Dalsgaard et al., 2003; Schukat et al., 2014). Likewise, a high abundance of

several PUFAs (such as 20:4n6, 20:5n3, and 22:6n3) was found. This is probably due to the fact that symbiotic zooxanthellae in cnidarians contain a lot of PUFAs and symbionts transfer PUFAs to host tissues (Figueiredo et al., 2012).

Significant changes in the total FA composition of *G. fascicularis* were observed between the fed and unfed groups (Fig. 3a, Table 1). Corals that were fed *A. salina* nauplii contained significantly lower proportions of SFAs, but higher proportions of MUFAs and PUFAs (Fig. 4, Table 1). The changes in the total FA composition of the fed group suggested that corals could assimilate high levels of FAs from diets. Specifically, the proportion of SFAs (mainly 16:0) in fed corals dropped significantly, indicating a decline in the proportion of photosynthetic-derived FAs in fed corals. SFAs are known to be readily catabolized and are the preferred sources of metabolic energy in marine organisms (Figueiredo et al., 2012). Although the relative proportion of SFAs dropped, feeding significantly increased the lipid content of corals (Teece et al., 2011). Thus, the decline in the proportion of SFAs does not mean that the energy storage of fed corals has decreased. This result is consistent with those of a previous study, which also found a decreased proportion of SFAs in fed *Turbinaria reniformis* (Tolosa et al., 2011).

In contrast to SFAs, feeding significantly increased the proportion of MUFAs and PUFAs (especially n-3 and n-6 PUFAs) (Fig. 4, Table 1). Among the MUFAs, 18:1n9, which is a necessary precursor for the synthesis of n-6 PUFAs, showed the largest increase in fed corals (Imbs et al., 2010). For PUFAs, fed corals presented high relative concentrations of 18:2n6 and 18:3n6, which are known eicosanoid precursors and are critical for numerous physiological processes, including pigmentation and immune function (Imbs et al., 2010; Papina et al., 2003). The level of 20:5n3 (EPA), considered to play an essential role in the reproduction processes and commonly reported as one of the dominant PUFAs in healthy corals (Papina et al., 2007), was also present at a significantly higher proportion in the fed corals because of its high concentration in the diet. Additionally, the 18:2n6, 20:3n3, and 22:6n3 (DHA) contents of fed corals increased as well; these are important for improving the health of *G. fascicularis*. As the content of these FAs in diets is relatively low, whether these were sourced from heterotrophic input or increased through *de novo* synthesis attributable to higher zooxanthellae densities is uncertain. A higher proportion of UFAs in fed corals have been consistently reported in other studies (Conlan et al., 2018a; Lim et al., 2017; Treignier et al., 2008), and researchers have confirmed that higher levels of UFAs are benefit for corals to resist environmental stress (Tolosa et al., 2011; Tagliafico et al., 2017), such as coral bleaching. Therefore, the higher levels of UFAs, particularly n-3 and n-6 PUFAs observed in fed corals, which represents a substantial improvement in health conditions, suggested that enriched feeding might represent a useful technique in coral aquaculture.

No significant differences in the polar lipid FA compositions were detected between the two feeding regimes for *G. fascicularis* (Fig. 3b, Table 2). As polar lipid FAs are the structural basis of cell membranes in corals and their symbionts (Imbs and Yakovleva, 2012), this suggests that the input of heterotrophic nutrients has little effect on the FA composition of the holobiont membrane system, and it also reflects the stability of the membrane structure of *G. fascicularis* under heterotrophic nutrition limitation. A previous study suggested that the changes in nutritional structure on a short time scale will not affect the polar lipid compositions of *Xenia* sp. (Meyers, 1979); the results of our study, which was undertaken over a longer time scale (4 weeks), corroborated this notion. Interestingly, recent studies have found that environmental stress can cause species-specific changes in the content and composition of polar lipid FAs in corals (Imbs and Yakovleva, 2012; Sikorskaya et al., 2020). Considering this, future research should be conducted to examine whether feeding could help corals resist environmental stress from the perspective of polar lipid FAs.

5. Conclusions

In conclusion, the present study showed that, by promoting its growth and bud formation rate, feeding on *A. salina* nauplii could increase the production of scleractinian coral *G. fascicularis* in aquaculture. Meanwhile, feeding changed the body composition of *G. fascicularis*, with fed corals exhibiting higher tissue protein and crude lipid contents, thereby indicating good assimilation of supplementary proteins and lipids via feeding. Moreover, heterotrophy significantly changed the total FA profile of *G. fascicularis*. Fed corals had higher levels of UFAs (particularly n-3 and n-6 PUFAs) and lower proportions of SFAs than the unfed corals, suggesting a substantial improvement in the physiological state and health condition of corals during culturing processes. However, no obvious changes were found in the polar lipid FA compositions between of the fed and unfed corals. These results provide important insights into the dietary requirements of corals, and indicate that *A. salina* nauplii could serve as a suitable feeding regime for *G. fascicularis* and slow-growing but heterotrophic preferred corals in aquaculture.

CRediT authorship contribution statement

Xiaolei Yu: Conceptualization, Methodology, Investigation, Writing – original draft, preparation. **Lei Jiang**: Conceptualization, Methodology, Writing – review & editing. **Jianfeng Gan**: Investigation. **Yuyang Zhang**: Conceptualization, Methodology. **Yong Luo**: Investigation. **Chengyue Liu**: Conceptualization, Supervision, Writing – review & editing. **Hui Huang**: Conceptualization, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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