



# Fatty acid profiles of separated host–symbiont fractions from five symbiotic corals: applications of chemotaxonomic and trophic biomarkers

Taihun Kim<sup>1,2,3</sup> · David M. Baker<sup>1,2</sup> · Se-Jong Ju<sup>4</sup> · Jetty Chung-Yung Lee<sup>1</sup>

Received: 10 February 2021 / Accepted: 7 October 2021 / Published online: 22 October 2021  
© The Author(s), under exclusive licence to Springer-Verlag GmbH Germany, part of Springer Nature 2021

## Abstract

Fatty acids (FAs) are the main components of lipids in corals. We examined FAs profiles from five symbiotic coral species belonging to five different genera (*Acropora*, *Pavona*, *Turbinaria*, *Favites*, and *Platygyra*) and four different families (Acroporidae, Agariciidae, Dendrophyllidae, Faviidae). We separated symbionts from the coral host tissue to investigate the interaction of FA between symbionts and host tissue. After separation, we used FA profiles, in particular specific FAs (e.g. 16:0, 18:0, 18:3n-3, 20:5n-3, 22:6n-3) and their ratios (EPA:DHA, PUFA:SFA) as biomarkers (i.e. signature lipids) to examine chemotaxonomy and trophic level (autotrophy vs. heterotrophy) of each coral species. Gas chromatography–mass spectrometry (GC–MS) was performed to identify and quantify FA. For quantification, the dry weight of total lipids was used to normalize FA concentration ( $\mu\text{g mg}^{-1}$ ). We found that (1) the five different coral species showed define species-specific FA profiles; (2) certain FAs were valuable biomarkers to determine relative trophic strategies (i.e. autotrophy and/or heterotrophy); (3) the application of FA ratios to define trophic level requires caution in research application and data interpretation. Considering the limitations of FA ratios determined herein, we suggest it to be more appropriate to examine response to environmental change within species. Going forward, our study provides important FA baseline data that builds the foundation for future investigations on the impact of environmental changes related to nutrition and metabolism in symbiotic corals.

**Keywords** Coral · Fatty acid · Chemotaxonomy · Trophic level · Fatty acid ratio

## Abbreviations

TL Total lipids  
FA Fatty acid  
SFA Saturated fatty acid

MUFA Monounsaturated fatty acid  
PUFA Polyunsaturated fatty acid  
PA Palmitic acid (16:0)  
SA Stearic acid (18:0)  
OA Oleic acid (18:1n-9)  
LA Linoleic acid (18:2n-6)  
SDA Stearidonic acid (18:4n-3)  
EPA Eicosapentaenoic acid (20:5n-3)  
DPA Docosapentaenoic acid (22:5n-3)  
DHA Docosahexaenoic acid (22:6n-3)  
ALA  $\alpha$ -Linolenic acid (18:3n-3)  
LA Linoleic acid (18:2n-6)  
GLA  $\gamma$ -Linolenic acid (18:3n-6)  
DGLA Dihomo- $\gamma$ -linolenic acid (20:3n-6)  
ARA Arachidonic acid (20:4n-6)  
AdA Adrenic acid (22:4n-6)

Responsible Editor: C. Wild.

✉ Jetty Chung-Yung Lee  
jettylee@hku.hk

- <sup>1</sup> School of Biological Sciences, The University of Hong Kong, Kadoorie Biological Sciences Building, Pokfulam Road, Hong Kong SAR, China
- <sup>2</sup> Swire Institute of Marine Science, The University of Hong Kong, Cape d'Aguilar Road, Shek O, Hong Kong SAR, China
- <sup>3</sup> Jeju Marine Research Center, Korea Institute of Ocean Science and Technology, 2670 Iljudong-ro, Gujwa-eup, Jeju, Republic of Korea
- <sup>4</sup> Global Ocean Resources Research Center, Korea Institute of Ocean Science and Technology, 385 Haeyang-Ro, Yeongdo-Gu, Busan, Republic of Korea

## Introduction

Lipids are essential compounds in marine organisms and source of energy (Bergé and Barnathan 2005; Lee et al. 2006; Parrish 2013). In comparison to other compounds, e.g. macromolecules, such as proteins and carbohydrates, lipids have the highest energy content (approximately  $17 \text{ kJ g}^{-1}$ ,  $18 \text{ kJ g}^{-1}$ , and  $39 \text{ kJ g}^{-1}$ , respectively). Additionally, lipids are important to maintain cell membranes, are components of fat-soluble antioxidants and hormone (e.g. ecdyson), and regulate anti-inflammatory and immune responses (Ackman 1999; Tocher 2003; Lee et al. 2006; Parrish 2013). Thus, lipids play a crucial role in the physiology of marine organisms (e.g. respiration, cell renewal, and reproduction) and also display biochemical and ecological conditions of the marine environment (Arai et al. 1993; Ward 1995; Tarrant 2005; Wang et al. 2013). Lipid biomarkers have the potential to serve as an advanced diagnostic tool for coral health. Subtle changes in physiological condition can manifest as large variations in lipid biomarker concentrations as well as relative ratios of certain classes of lipid. Thus, coral health can be assessed well before changes in mortality, growth rates, occurrence of diseases, and community changes are observed (Rocker et al. 2019). One major component of lipid molecules are fatty acids (FAs), which occur either as free FAs, or chemically bound to different lipid classes. In particular, free fatty acids (hereafter “FA/FAs”) are essential components of lipids in many marine organisms (Bergé and Barnathan 2005; Parrish 2013). They are transferred throughout the food web without experiencing changes in different trophic levels, which encourages their use as effective biomarkers in ecological studies (Parrish et al. 2000; Alfaro et al. 2006). Indeed, many studies consider FAs as signature lipids in trophic biomarkers (Volkman 1999) of various marine organisms including bacteria, diatoms, dinoflagellates, plankton, macroalgae, invertebrates, fishes, and marine mammals (Rajendran et al. 1993; Parrish et al. 2000; Falk-Petersen et al. 2002; Howell et al. 2003; Bergé and Barnathan 2005; Lee et al. 2006; Kelly et al. 2008; Shin et al. 2008; Ju et al. 2011; Sardenne et al. 2017). Furthermore, FA profiles was shown to display a strong taxon specificity (Volkman 1999).

In corals, lipids are one of the main biochemical compounds and a crucial dietary source for energy storage. As such, lipids take up 10–50% of the dried coral tissue (Joseph 1979; Harland et al. 1992, 1993; Al-Lihaibi et al. 1998; Yamashiro et al. 1999, 2005; Oku et al. 2003b; Seemann et al. 2013). Indeed, FA are vital to coral metabolism and stress tolerance (Imbs et al. 2015). Moreover, FA profiles of different coral species have been used as chemotaxonomic biomarkers as they are suggested to be species-specific (Latyshev et al. 1991; Imbs et al. 2007, 2010b, 2016; Lopes et al.

2016). For example, octocorals can be identified by their synthesis of 24:5n-3 and 24:6n-6, as these FA are absent in hexacorals (Svetashev and Vysotskii 1998; Imbs et al. 2010a). Symbiotic corals can be identified by high level of 18:3n-6 (GLA) and 18:4n-3 (SDA), as these FAs are present in symbiotic algae, while they are very low in non-symbiotic corals (Papina et al. 2003; Imbs et al. 2007, 2010b; Imbs 2013; Lopes et al. 2016). Imbs et al. (2007) classified five different families (Acroporidae, Faviidae, Fungidae, Pocilloporidae, and Poritidae) of the scleractinian corals based on their specific polyunsaturated fatty acids (PUFAs), as they found that several FAs can only be detected in certain taxonomic groups of corals. Most scleractinian corals have symbiotic relationships with dinoflagellates, namely the family Symbiodiniaceae (hereafter ‘symbionts’) (LaJeunesse et al. 2018), which are mostly photosynthetic that guides carbon dioxide fixation (Wooldridge 2014). Their photosynthesis contribute significantly to the supply of biomolecules used to feed themselves while the nutrients are also translocated to the coral host for growth, reproduction, and metabolism (Muscatine et al. 1981). The main photosynthates transferred from symbionts to the coral host tissue include lipids, glycerol, glucose, and amino acids (Gates et al. 1995; Papina et al. 2003; Whitehead and Douglas 2003; Reynaud et al. 2009), where excess organic carbon is stored as lipids in the host tissue (Patton et al. 1977; Patton and Burris 1983). Of the lipids, FAs in particular, is one of the most important nutrients transferred across the plant-animal interface in the aquatic food web (Dalsgaard et al. 2003; Allan et al. 2010). It has been reported that photosynthetic organisms (e.g. plants, macro-microalgae, dinoflagellates, etc.) synthesize essential PUFAs, such as 18:2n-6 or 18:3n-3, which are precursors that, in turn, metabolize several n-6 (e.g. 18:3n-6, 20:4n-6) and n-3 PUFAs (e.g. 18:4n-3, 20:5n-3, 22:6n-3) through their respective pathways of FA biosynthesis (Chen et al. 2015; Revel et al. 2016). Animals cannot synthesize these precursor FAs due to the lack of certain metabolic enzymes ( $\Delta^{12}$ ,  $\Delta^{15}$  desaturases) (Papina et al. 2003; Bachok et al. 2006; Revel et al. 2016). Thus, the PUFAs synthesized by symbionts can act as biomarkers to understand the coral-symbiont relationship among coral species.

On the other hand, certain essential FAs cannot be synthesized in higher trophic positions, although they are incorporated into heterotrophic species in the marine ecosystems (Arai et al. 2015). Moreover, FAs mirror nutritional input, making it possible to use them as biomarkers to trace diet and quantify feeding relationships (Dalsgaard et al. 2003; Bay et al. 2013; Mies et al. 2018). For example, 20:5n-3 is abundant in diatoms and 22:6n-3 is abundant in dinoflagellates. Both are highly conserved in the marine food web, which allows food source tracing (Viso and Marty 1993; Scott et al. 2002; Dalsgaard et al. 2003). The ratio of 20:5n-3 to 22:6n-3 (EPA:DHA) has been used to define trophic level

(autotrophy or heterotrophy) and feeding type (herbivore, omnivore, carnivore) in zooplankton (Graeve et al. 1994; Dalsgaard et al. 2003). Additionally, carnivorous zooplankton tend to have higher PUFAs than herbivorous species, hence the PUFA:SFA ratio offers an index of carnivory (Cripps and Atkinson 2000; Dalsgaard et al. 2003; Stevens et al. 2004). A few coral studies successfully applied these FA indices to define trophic level in coral reef ecosystems (Tolosa et al. 2011; Seemann et al. 2013; Salvo et al. 2017; Radice et al. 2019; Rocker et al. 2019).

Although FA profiles have shown to present excellent opportunities for chemotaxonomic and trophic biomarkers in symbiotic corals, applying FA profiles to corals and symbionts separately, as opposed to a two-organism unity, remains understudied. Specifically, symbiotic corals are able to obtain essential FAs either via autotrophy which are being translocated from symbionts, or via heterotrophy through external food sources, e.g. phytoplankton, zooplankton, particulate organic matter (Sebens et al. 1996; Ferrier-Pagès and Gattuso 1998; Anthony and Fabricius 2000; Yahel et al. 2004; Rocker et al. 2019). Furthermore, corals have a high trophic plasticity, switching between autotrophic and heterotrophic feeding strategies to cope with environmental changes (Goreau et al. 1971; Porter 1976; Muscatine et al. 1989; Anthony and Fabricius 2000; Ferrier-Pagès et al. 2003; Fabricius 2005). Therefore, their symbiotic relationship and trophic plasticity provide challenges to the application of FA biomarkers.

In this study, we characterized FA profiles from five symbiotic coral species belonging to five different genera (*Acropora*, *Pavona*, *Turbinaria*, *Favites*, and *Platygyra*) and four different families (Acroporidae, Agariciidae, Dendrophyllidae, Faviidae). To do so, we first separated symbionts from the coral host tissue, and then investigated the interaction between symbionts and the host tissue using FA biomarkers. We then selected these FA biomarkers from the coral host fraction, in particular specific FAs and their ratios (EPA:DHA, PUFA:SFA) to examine chemotaxonomy and trophic level (autotrophy vs. heterotrophy) of each coral species. We tested the following hypotheses: (1) the five coral target species can be classified by distinct FA profiles; (2) symbiotic corals have a relative trophic level (more autotrophic or more heterotrophic) which can be defined by certain FA biomarkers.

## Materials and methods

### Coral sampling

In May 2016, five different symbiotic coral species (*Acropora samoensis* of the family Acroporidae, *Pavona decussata* of the family Agariciidae, *Turbinaria peltata* of the

family Dendrophyllidae, *Favites abdita* and *Platygyra carnosa* of the family Faviidae) were collected from four mesocosm coral tanks at the Swire Institute of Marine Science (SWIMS) located at the marine reserve area of Cape d'Aguilar in Hong Kong SAR. Open circulation flow-through tanks ( $\approx 5 \text{ l min}^{-1}$ ) at SWIMS were used and seawater was supplied directly from the bay after a primary step of physical filtering (sand filter). The seawater included natural food sources such as plankton and particulate organic matter, hence additional feeding was not necessary. The samples from species grown in mesocosm tanks as opposed to species from a natural site were taken to ensure that the corals have not been disturbed by sedimentation or unexpected weather conditions (e.g. heavy rain, typhoon) and all colonies were exposed to the same light conditions and food sources, which enabled to achieve our study purposes. Samples were collected in May 2016. Hong Kong has alternating wet/dry seasons; the wet season is from March to October, characterized by higher temperatures, and the dry season is from November to March. We collected our samples during the transition time between dry and wet season, with an average water temperature of 26.0 °C. Branching and foliaceous corals (*A. samoensis*, *P. decussata*) were collected by scissors, and plate corals (*T. peltata*) or boulder corals (*F. abdita*, *P. carnosa*) were collected by hammer and chisel. Each nubbin was taken from different colonies ( $n=4$ ) in each tank. The collected samples were immediately rinsed with deionized water and kept frozen at  $-70 \text{ °C}$  until further analyses.

### Coral-symbiont separation

For lipid analysis, the coral fragments were rinsed with buffer (5 mM EDTA) with 20  $\mu\text{l ml}^{-1}$  butylated hydroxytoluene in distilled water to prevent FA oxidation and development of artefacts. Coral tissue was extracted from the skeleton using an airbrush with the same buffer solution and collected in 50 ml tubes through a funnel. Slurry tissue, which included coral host tissue and symbionts was homogenized on ice with a tissue grinder for 30 s. The homogenate was centrifuged for 5 min at 4 °C at initially 300 rcf, increasing up to 800 rcf (depending on tissue thickness, amount of mucus, and species) (Table S1) to separate symbionts (pellets) and coral tissue (supernatant). One centrifugation process was difficult to differentiate the two fractions thus further separation was required. The supernatant was transferred to another 50 ml tube and centrifuged in deionized water three more times for 5 min at 4 °C with different velocity of centrifugation depending on the species, to eliminate any remaining symbionts. A drop of supernatant was observed under a microscope to verify no symbiotic cells remained in the coral host tissue sample (Fig. S1); nevertheless minor contamination from symbionts and coral

host fraction may exist in the supernatant. The pellet was re-suspended in 20–30 ml of DI water and centrifuged at 10 rcf for 30 s to remove any carbonate residue. The supernatant was immediately transferred to another tube and then centrifuged three times for 5 min at 4 °C at varying velocity of centrifugation depending on the species (Table S1). After the third centrifugation, the pellet was recollected. Both coral host tissue and symbiont samples were kept in the freezer at – 80 °C. Prior to chemical analyses, the samples were freeze-dried and kept as powder.

### Extraction lipid and fatty acids

Total lipid (TL) was extracted from dried samples of the coral host tissue and symbionts separately by Folch solution (2:1; chloroform:methanol). Folch et al. (1957) method can be applied for a wide range of sample types (Couturier et al. 2020), where the extraction efficiency is high and sample matrix effect is minimal. Extracted TL was dried under nitrogen gas on a 37 °C heating block. The dried TL was weighed to determine TL content as relative proportion (%) of dry weight of symbiont and coral host, respectively. To the dried TL, aqueous 1 M potassium hydroxide (KOH) and internal standard (19:0; nonadecanoic acid, 1 µg l<sup>-1</sup> in dichloromethane) were added, heated for 60 min at 70 °C oven to hydrolyze the esterified FA. The total FAs were then collected by liquid–liquid extraction using hexane:diethyl ether (9:1 v/v), and the solvent was evaporated immediately under nitrogen gas and derivatized with boron trifluoride (BF<sub>3</sub>) at 70 °C for 30 min to form fatty acid methyl esters (FAMES). FAMES were extracted three times with hexane:diethyl ether (9:1 v/v) and then pooled. The extracted FAMES were dried under nitrogen gas and re-suspended in dichloromethane for gas chromatography mass spectrometry (GC–MS) analysis set at electron ionization (EI) mode. A set of external standards (37 Component FAME mix, Supelco, USA) was used to identify and quantify each FAME. GC–MS (Agilent 5977A mass selective detector interfaced with an Agilent 7890B gas chromatograph) was performed using an SP-2560 capillary column (100 m × 0.25 mm, 0.2 µm film thickness, Sigma–Aldrich, USA) to detect the FAs. Helium was used as the carrier gas with a flow rate of 1 ml min<sup>-1</sup>. A volume of 1 µl of the derivatized sample was injected into the GC with a split ratio of 1:100. The oven temperature was programmed to ramp from 100 °C to 140 °C at 10 °C min<sup>-1</sup> and from 140 °C to 240 °C at 4 °C min<sup>-1</sup>. Each FAME was identified using the target and qualifier ion listed in Table S3. FAMES were quantified by relating the peak area of the individual FAME with the peak of the internal standard. For quantification, the dry weight of TL was used to normalize FAME concentration (µg mg<sup>-1</sup>).

### Statistics

ANOVA test was performed using the software SPSS (ver. 19.0, IBM, NY, USA). Statistical differences among the five species were determined for TL and FA ratios by one-way analysis of variance (ANOVA) and Tukey's post hoc test for pairwise comparison. Values of at least  $p < 0.05$  were considered significant in the analysis. Further, we performed multivariate analyses on square root transformed value of the 19 FA profiles using the software PRIMER version 5. Hierarchical cluster analysis was conducted to assess the degree of similarity between symbionts and tissue of their coral hosts. FA profiles were compared among the five coral species by non-metric multidimensional scaling (nMDS) and analysis of similarity (ANOSIM) on the Bray–Curtis similarity index. The contribution of each fatty acid to these clusters was determined by similarity percent (SIMPER) analysis. A principal component analysis (PCA) was performed among coral species to highlight certain FA profiles.

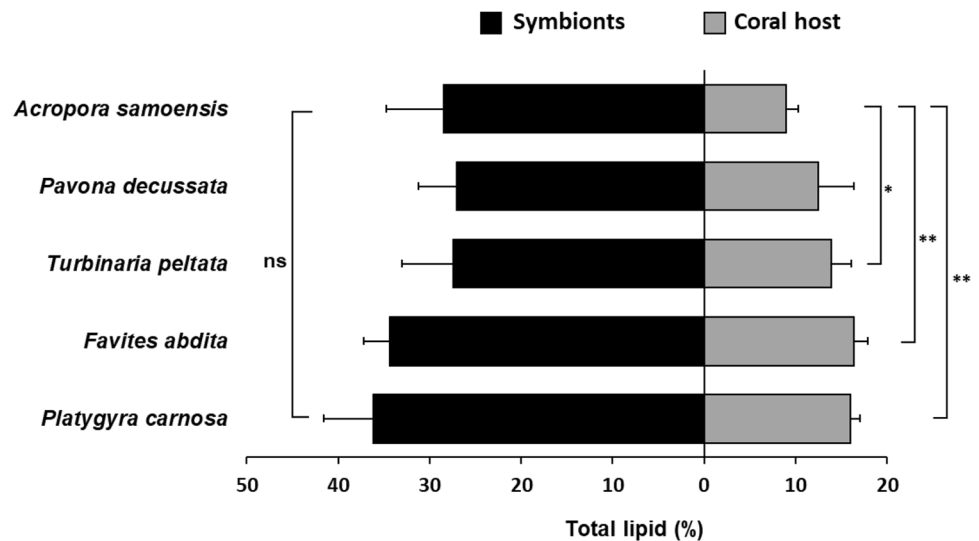
### Results

#### Total lipid and fatty acids of five different coral species

Overall, TL in symbionts showed to be much higher than in host tissue of the five coral species (average  $30.7 \pm 4.2\%$  in symbionts,  $13.5 \pm 3.0\%$  in coral host tissue) (Fig. 1). TL of symbionts in *P. carnosus* showed the highest value ( $36.1 \pm 5.5\%$ ) compared to four other species, but it was not significantly different among the species ( $p > 0.05$ ). There was no significant difference in TL of coral host tissue between *A. samoensis* ( $8.9 \pm 1.4\%$ ) and *P. decussata* ( $12.4 \pm 3.9\%$ ). TL in host tissue of *A. samoensis* was significantly lower compared to *T. peltata* ( $p < 0.05$ ), *F. abdita* ( $p < 0.005$ ), and *P. carnosus* ( $p < 0.005$ ). There were no significant differences between *P. decussata*, *T. peltata*, *F. abdita*, and *P. carnosus*.

Out of the 37 FAMES, we evaluated 26 types of long chain FAs (> 16C) where a total of 19 were detected (Table 1). The most predominant FA in both symbionts and coral host tissue for all species was 16:0. The second most dominant SFA in both symbionts and coral host tissue for all species was 18:0. The 16:0 in symbionts showed to be higher than the host tissue except for those of *P. carnosus*, whereas 18:0 constantly showed to be higher in the host tissue than in symbionts. The major MUFA for all species was 18:1n-9, followed by 16:1. The highest concentration of 18:1n-9 was found in both symbionts and the host tissue of *F. abdita*. *A. samoensis* was characterized by the highest concentration of total PUFA in both symbionts and the host tissue,  $142.0 \pm 38.0$  µg mg<sup>-1</sup> and

**Fig. 1** Total lipid content (%) in symbionts and their host tissue from five different coral species. Statistically significant differences ( $p < 0.05$ ) in each symbiont and coral host tissue are indicated by asterisk (\*): \* indicates  $p < 0.05$ , \*\* indicates  $p < 0.005$ , ns indicates no significance (one-way ANOVA, Tukey's test) between species



$58.1 \pm 11.0 \mu\text{g mg}^{-1}$ , respectively (Table 1). However, *P. decussata* was characterized by the lowest value in both symbionts and the host tissue,  $49.4 \pm 13.7 \mu\text{g mg}^{-1}$  and  $17.4 \pm 1.4 \mu\text{g mg}^{-1}$ , respectively. In general, the major PUFAs were 18:4n-3, 20:5n-3, 22:6n-3, 18:3n-6, and 20:4n-6. PUFAs namely 18:4n-3, 20:5n-3, 22:6n-3, and 18:3n-6 in symbionts for all species showed higher concentrations than the coral host tissue. The highest concentrations of PUFAs (18:4n-3, 20:5n-3, 22:6n-3, 18:3n-6) except for 20:4n-6 in the host tissue were observed in *A. samoensis*. However, the lowest value of 18:4n-3 was found in *F. abdita* ( $0.3 \pm 0.1 \mu\text{g mg}^{-1}$ ), for 20:5n-3 in *P. carnosa* ( $0.6 \pm 0.2 \mu\text{g mg}^{-1}$ ), and for 22:6n-3 and 18:3n-6 in *T. peltata* ( $1.5 \pm 0.2 \mu\text{g mg}^{-1}$ ,  $0.8 \pm 0.1 \mu\text{g mg}^{-1}$ , respectively). The concentration of 18:4n-3, 20:5n-3, and 22:6n-3 in symbionts of *A. samoensis* showed a much higher value ( $23.4 \pm 7.3 \mu\text{g mg}^{-1}$ ,  $30.9 \pm 22.0 \mu\text{g mg}^{-1}$ , and  $44.1 \pm 16.7 \mu\text{g mg}^{-1}$ , respectively) compared to the symbionts of other species (average  $7.1 \pm 2.2 \mu\text{g mg}^{-1}$ ,  $9.9 \pm 2.1 \mu\text{g mg}^{-1}$ , and  $18.4 \pm 3.2 \mu\text{g mg}^{-1}$ , respectively). In addition, 18:3n-3 in symbionts of *A. samoensis* showed to be the highest ( $16.2 \pm 3.7 \mu\text{g mg}^{-1}$ ) whereas it was not detectable in *P. decussata* and *T. peltata*. The concentration of 20:4n-6 in the host tissue showed higher levels than 20:5n-3 and 22:6n-3 among all species. In particular, 20:4n-6 in the host tissue of *T. peltata*, *F. abdita*, and *P. carnosa* had the highest concentration among all PUFAs, where *P. carnosa* had the highest level among all species.

In this study, we applied FA ratios namely 20:5n-3 to 22:6n-3 (EPA:DHA) and PUFA:SFA as biomarkers for coral feeding or determining trophic level. The ratio is an indicator for trophic level (higher value indicates relatively autotrophic, lower value indicates relatively heterotrophic feeding strategies) (Rocker et al. 2019). The ratio of EPA:DHA was significantly higher (one-way ANOVA,

Tukey's test,  $p < 0.05$ ) in *T. peltata* and *A. samoensis* ( $0.9 \pm 0.1$ ,  $0.8 \pm 0.4$ , respectively) compared to the other three species (Table 1). PUFA:SFA has been suggested as indicator for carnivory (higher value indicates relatively carnivorous, lower value indicates relatively herbivorous). PUFA:SFA showed the highest value (one-way ANOVA, Tukey's test,  $p < 0.05$ ) in *A. samoensis* ( $0.9 \pm 0.2$ ), followed by *P. carnosa* ( $0.7 \pm 0.1$ ), *F. abdita* ( $0.5 \pm 0.1$ ), *T. peltata* ( $0.4 \pm 0.1$ ) while *P. decussata* had the lowest value ( $0.3 \pm 0.03$ ).

### Multivariate analysis of coral host and symbiont fatty acid profiles

Cluster analysis was conducted on 19 different FAs (as noted in Table 1) to evaluate whether FA profiles from the symbionts and their coral host tissue are statistically separate. As shown, two groups were formed at 75% similarity level (Fig. 2) where Group I is composed of all coral host tissues except for host tissue from *A. samoensis*, whereas Group II is composed of the symbionts from the five species and including the host tissue of *A. samoensis*.

The nMDS plot based on 19 FA profiles clearly revealed a distinct grouping among the five different species. ANOSIM analysis also confirmed a clear separation among the groups ( $R = 0.999$ ;  $p < 0.05$ ) (Fig. 3). SIMPER analysis showed that each group had high similarity ( $> 90\%$ ) within the group (Table S4). In all species groups, two main FAs namely 16:0 and 18:0 explained 25.1–39.8% of the group similarity. In the *T. peltata*, *F. abdita*, and *P. carnosa* group, 20:4n-6 explained 13.4%, 10.8%, and 13.5%, respectively, of the group similarity. While in the *A. samoensis* group, 18:3n-3 explained 9.5% of the group similarity (Table S5). Dissimilarity between *A. samoensis* and *T. peltata* showed to be the highest (30.3%), which was explained by the contribution of

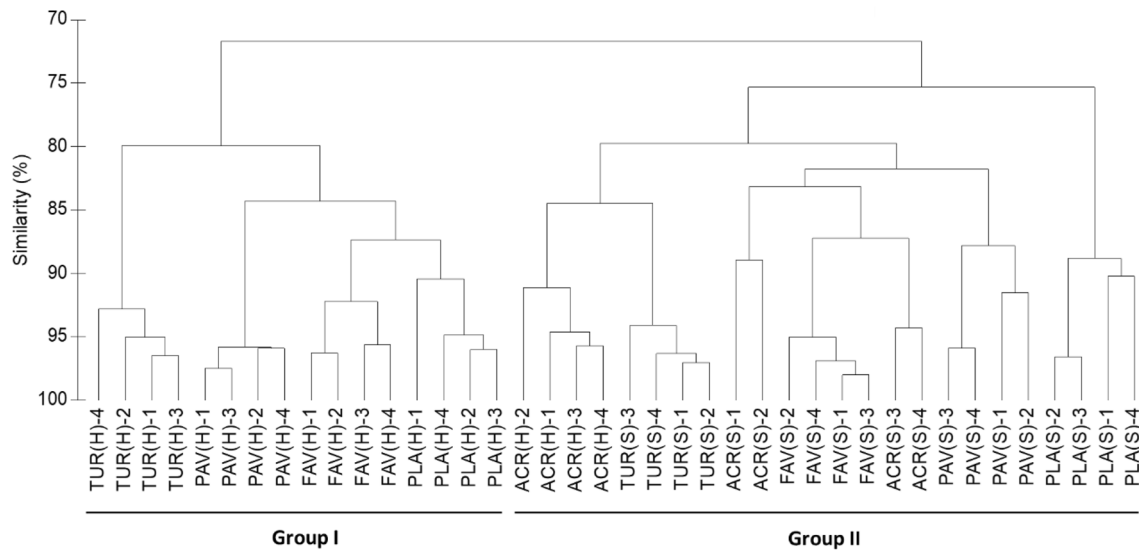
**Table 1** Fatty acid profiles in symbionts and their host tissue from five different coral species

FA	<i>Acropora samoensis</i>		<i>Pavona decussata</i>		<i>Turbinaria peltata</i>		<i>Favites abdita</i>		<i>Platygyra carnosa</i>	
	S	H	S	H	S	H	S	H	S	H
14:0	7.6±1.3	3.6±1.3	7.4±2.6	2.9±0.4	1.5±0.6	0.6±0.1	4.0±0.5	1.0±0.2	2.4±1.0	1.2±0.8
16:0	50.1±9.7	42.0±14.5	63.4±24.0	46.4±2.9	35.4±3.5	20.7±3.1	49.9±2.2	30.9±0.4	23.8±6.1	35.3±4.7
18:0	12.9±5.1	19.4±4.4	15.1±2.4	16.9±1.4	16.1±1.4	23.2±4.3	19.0±1.8	19.7±2.5	8.8±2.3	17.8±2.8
20:0	0.3±0.1	0.8±0.2	0.6±0.1	0.6±0.1	0.2±0.02	0.1±0.02	0.4±0.05	0.3±0.05	0.1±0.02	0.5±0.1
22:0	0.4±0.1	0.2±0.01	0.2±0.04	0.2±0.03	ND	ND	0.1±0.0	ND	0.1±0.0	ND
<b>Σ SFA</b>	71.2±15.6	66.1±20.1	86.7±28.2	67.0±2.1	53.1±4.9	44.6±5.2	73.5±4.2	51.9±2.3	35.2±8.8	54.7±5.0
16:1	5.2±3.0	3.1±0.5	6.5±2.2	3.4±0.3	1.9±0.2	0.3±0.02	2.0±0.2	1.1±0.2	1.8±0.5	2.0±0.8
18:1n-9	5.3±1.2	3.6±0.3	5.5±1.9	3.9±0.4	5.0±0.5	2.0±0.3	6.7±0.3	4.0±0.7	1.4±0.4	2.1±0.4
20:1n-9	1.1±1.3	3.9±0.1	ND	0.2±0.04	1.3±0.1	ND	0.6±0.3	0.5±0.1	ND	0.3±0.04
22:1n-9	0.3±0.1	0.3±0.03	0.2±0.1	0.2±0.05	0.3±0.1	0.2±0.1	0.3±0.03	0.3±0.1	0.1±0.02	0.1±0.04
<b>Σ MUFA</b>	11.9±4.0	11.0±0.6	12.1±4.1	7.7±0.4	8.5±0.7	2.5±0.3	9.5±0.4	6.0±0.9	3.3±0.9	4.5±1.2
18:3n-3 (ALA)	5.3±4.5	16.2±3.7	0.3±0.1	ND	5.8±0.9	ND	2.8±0.6	2.3±0.4	ND	0.9±0.4
18:4n-3 (SDA)	23.4±7.3	1.7±0.7	6.6±2.7	0.6±0.1	7.7±1.3	0.8±0.1	8.4±1.3	0.3±0.1	5.6±3.5	0.7±0.1
20:5n-3 (EPA)	30.9±22.0	7.7±4.2	6.7±2.3	0.7±0.1	17.7±3.5	1.3±0.3	11.1±2.0	0.9±0.3	4.2±0.5	0.6±0.2
22:5n-3 (DPA)	0.9±0.4	1.3±0.5	1.2±0.8	1.2±0.2	2.0±0.2	0.4±0.1	0.4±0.2	0.2±0.1	5.7±2.3	6.3±1.4
22:6n-3 (DHA)	44.1±16.7	9.0±1.3	11.6±2.8	2.4±0.8	22.7±3.7	1.5±0.2	27.2±3.8	2.2±1.1	11.9±2.5	2.1±0.4
<b>Σ n-3 PUFA</b>	104.6±42.5	35.8±9.6	26.4±7.4	4.9±0.8	56.0±9.4	4.0±0.6	49.9±6.9	5.9±0.8	27.4±1.7	10.6±1.0
18:2n-6 (LA)	2.1±0.7	1.1±0.1	3.8±1.7	2.1±0.2	1.4±0.3	0.5±0.1	4.8±0.3	1.8±0.2	1.7±0.4	1.9±0.4
18:3n-6 (GLA)	30.5±10.7	7.4±1.4	14.4±5.2	4.6±0.8	9.7±1.7	0.8±0.1	44.6±6.2	4.8±1.6	19.0±3.9	4.1±1.1
20:3n-6 (DGLA)	0.8±0.4	1.4±0.2	0.7±0.4	0.8±0.1	1.3±0.3	0.5±0.1	1.5±0.1	1.1±0.1	0.2±0.1	0.6±0.1
20:4n-6 (ARA)	2.9±0.6	9.7±1.3	2.8±1.3	2.7±0.3	17.4±3.0	10.7±2.7	9.3±1.1	11.8±4.1	3.7±1.5	18.9±4.1
22:4n-6 (AdA)	1.1±0.2	2.7±0.5	1.2±0.1	2.3±0.5	4.3±0.3	1.9±0.7	2.1±0.3	2.1±0.7	0.7±0.3	2.7±0.3
<b>Σ n-6 PUFA</b>	37.4±11.9	22.3±2.7	23.0±7.6	12.5±1.0	34.0±3.8	14.3±3.4	62.3±7.6	21.6±6.5	25.2±5.9	28.2±5.7
<b>Σ PUFA</b>	142.0±38.0	58.1±11.0	49.4±13.7	17.4±1.4	90.0±12.9	18.3±3.2	112.2±14.2	27.5±7.0	52.7±7.1	38.8±5.7
EPA:DHA	-	0.8±0.4 <sup>a,b</sup>	-	0.3±0.1 <sup>a,c</sup>	-	0.9±0.1 <sup>b</sup>	-	0.5±0.4 <sup>c,d</sup>	-	0.3±0.04 <sup>a,b,d</sup>
PUFA:SFA	-	0.9±0.2 <sup>a</sup>	-	0.3±0.03 <sup>b</sup>	-	0.4±0.1 <sup>c</sup>	-	0.5±0.1 <sup>d</sup>	-	0.7±0.1 <sup>e</sup>

Value given as mean ±SD, n =4. FA concentration: µg mg<sup>-1</sup> of dry TL.

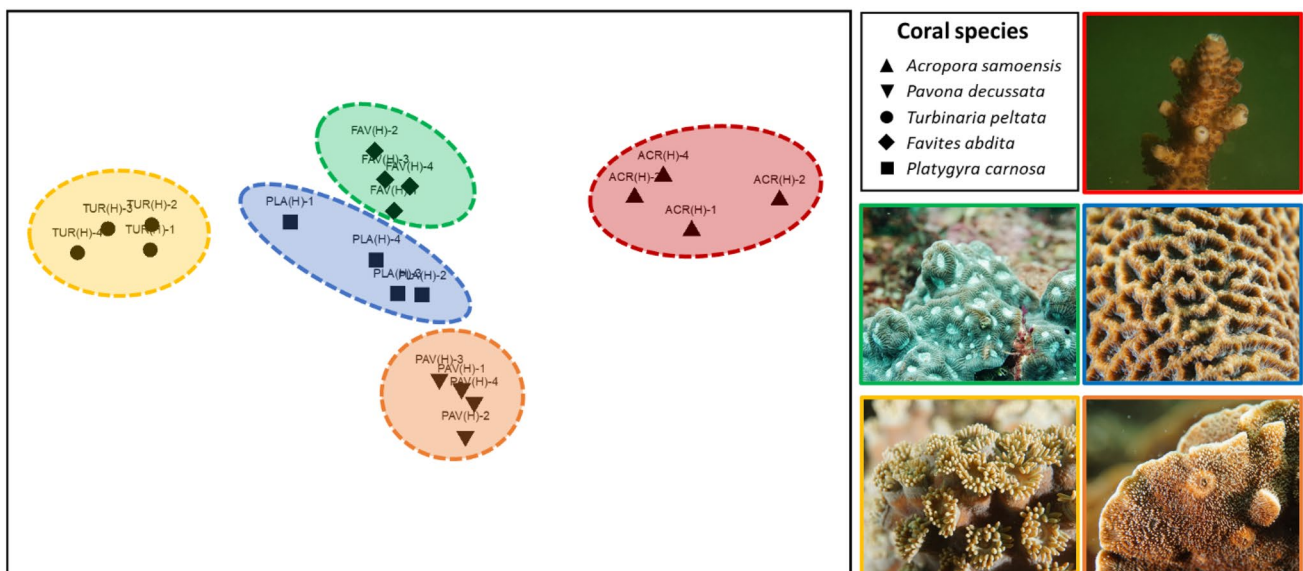
Superscripts sharing different letters indicate significant differences (one-way ANOVA, Tukey's test, p <0.05) among five coral species.

S symbionts, H coral host tissue, ND not detectable.



**Fig. 2** Hierarchical cluster analysis based on a Bray–Curtis similarity matrix of fatty acid profiles ( $\mu\text{g mg}^{-1}$ ) from five different symbiotic coral species ( $n=4$ ): *ACR* *Acropora samoensis*, *PAV* *Pavona decussata*,

*sata*, *TUR* *Turbinaria peltata*, *FAV* *Favites abdita*, *PLA* *Platygyra carnosa*. (S) indicates symbionts, (H) indicates coral host tissue. Group I and Group II derived at 75% similarity level



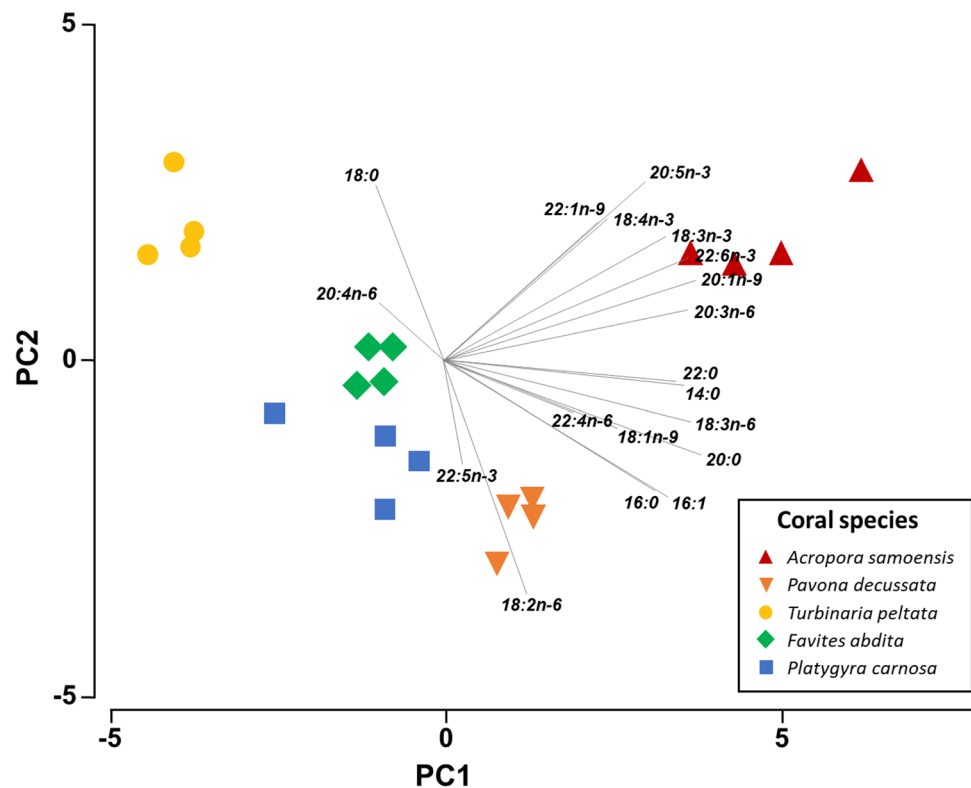
**Fig. 3** Non-metric multidimensional scaling (nMDS) plot of fatty acid profiles of host tissue from five different symbiotic coral species: *ACR* *Acropora samoensis* (Black up-point triangle), *PAV* *Pavona decussata* (Black down-point triangle), *TUR* *Turbinaria peltata*

(black circle), *FAV* *Favites abdita* (black diamond), and *PLA* *Platygyra carnosa* (black square). (H) indicates coral host tissue. Each symbol indicates one coral species. Dotted lines (--) indicate homogeneous groups as determined by ANOSIM ( $R=0.998$ ,  $P<0.05$ )

the following FA, in decreasing order of importance: 18:3n-3, 20:1n-9, 16:0, 18:3n-6, 22:6n-3 (Table S5). Moreover, the dissimilarity between *F. abdita* and *P. carnosa* (same family: Faviidae) was the lowest (12.6%), which is explained by the contribution of the following FAs, in decreasing order of importance: 22:5n-3, 20:4n-6, 18:3n-3, 18:1n-9.

In PCA, PC1, PC2, and PC3 accounted for 48.5%, 17.7% and 13.2%, respectively (only PC1 and PC2 are shown in Fig. 4). PC1 separated *A. samoensis* and *P. decussata* (positive scores) from the other species (negative scores), explaining 48.5% of the variability between the FA profiles of all species. The main FAs driving this distinction for *A. samoensis* were 18:3n-3, 20:5n-3,

**Fig. 4** Principal component analysis (PCA) of fatty acid profiles from five different coral species



22:6n-3, 20:3n-6, and 20:1n-9 (Fig. 4). *A. samoensis* had the highest PC1 scores, whereas *T. peltata* had the lowest. In contrast, PC2 separated *P. decussata* (negative scores) from the other species (positive scores), explaining 17.7% of the variation in FA profiles. In particular, *P. decussata* was characterized by 18:2n-6, *F. abdita* and *P. carnosa* were characterized by 20:4n-6.

## Discussion

Our results showed TL in holobionts (unity of symbionts and coral host tissue) ranged from 19 to 26% in average. Indeed, a similar trend was found in Okinawan corals (14–37%), Caribbean corals (12–32%) and Red Sea corals (12–32%) (Harland et al. 1993; Yamashiro et al. 1999). TL in symbionts separated from coral holobionts were much higher (27–36%) than in coral host tissue (9–16%). Culture experiments on five different marine dinoflagellate taxa have shown that they store 6–16% lipid, in particular *Symbiodinium microdiriatum*, which is one of the symbiotic dinoflagellates associated with corals, contained 15% TL (Mansour et al. 1999). Therefore, it is likely that TL contents in the symbionts and in their coral host separately show distinct different values. However, insofar there are no such studies comparing TL between symbionts and their coral host tissue. TL in corals indicates contribution of energy storage and nutritional status (Harland et al. 1992; Imbs et al. 2010b; Imbs 2013). In

addition, symbionts can translocate lipids to their coral host to maintain physical fitness (Papina et al. 2003; Treignier et al. 2009; Imbs et al. 2014). However, there was no significant variation found in TL of symbionts separated from the five target species, which indicates that symbionts are limited in their ability to store a certain quantity of lipid; this depends on the given environmental condition regardless of the coral host species. According to previous study, it is known that the same Symbiodiniaceae genus (C1) is hosted in all of our target coral species (Wong et al. 2016), which further supports that the environment is the driving variation in TL of symbionts across coral host species. Thus, we hypothesized that lipid production might not be different in symbionts from either coral species. However, TL in the host tissue of *F. abdita* and *P. carnosa* were significantly higher than in the host tissue of other species, which can be explained by the feeding strategy of these two species, i.e. they may feed more than other species. In fact, fed corals showed to have higher lipid contents than unfed corals (Al-Moghrabi et al. 1995; Treignier et al. 2008). On the other hand, TL in *A. samoensis* has shown to be significantly lower than TL in *T. peltata*, *F. abdita*, *P. carnosa*, which reversely may indicate *A. samoensis* relies on relatively less feeding.

Hierarchical cluster analysis confirmed that FA profiles and their abundance in coral host tissue and symbionts are significantly different, with the exception of *A. samoensis* host tissue, which is categorized in the group with symbionts. Indeed, FA profiles in *A. samoensis* host showed



subtle differences compared to its symbionts. Our results have shown that both symbionts as well as the host tissue of *A. samoensis* have considerably higher concentrations of PUFA when compared to other species. Other studies have shown that among symbiotic corals, PUFAs were the major FAs transferred from symbionts to the coral host tissue (Al-Moghrabi et al. 1995; Papina et al. 2003). Although SFAs can be translocated from symbionts to coral host tissue, the variation in concentration of both tissues from the five species was not obvious because corals can synthesize SFAs by themselves, masking a clear distinction (Treignier et al. 2009; Revel et al. 2016). On the other hand, higher PUFA concentrations in the host of *A. samoensis* compared to other species could also mean higher feeding rates on external food sources. However, the *Acropora* species has been found to be relatively autotrophic in studies assessing FA profiles and stable isotopic values (Seemann 2013; Seemann et al. 2013; Conti-Jerpe et al. 2020). In addition, higher concentrations of 18:4n-3 and 18:3n-6 in this species further indicates a stronger reliance on symbionts as these FAs are known to be biomarkers for the presence of symbionts within symbiotic coral species (Imbs et al. 2010b; Imbs 2013). Thus, we suggest that *A. samoensis* relies relatively more on FA from symbionts rather than on external feeding.

Although our target species are associated with the same genus of symbionts (Wong et al. 2016), higher concentration of major PUFAs in symbionts of *A. samoensis* when compared to the other species could potentially be explained by different physiology that depends on the species of coral host due to specific coral morphology (i.e. growth rate, tissue thickness, polyp size) and the absence of disturbances of light penetration (i.e. particulate matter or sedimentation) in our coral tanks, which is beneficial to autotrophic species. Theoretically, symbionts in *A. samoensis* perform the best biochemistry via photosynthesis, where surplus FA can be transferred to the host tissue. In fact, the concentration of each FA in symbionts and the host tissue alike was very similar to each other in this species. Therefore, we confirm that *A. samoensis* is the most autotrophic species among the five target species. However, the PUFA:SFA ratio, which is an indicator of carnivory, was the highest in *A. samoensis*, which contrasts our above interpretation. Although the highest PUFA was observed in *A. samoensis*, SFA did not vary within the five species. This indicates that PUFA is the determinant for the high value of PUFA:SFA ratio in *A. samoensis* which in turn, is responsible for the contradicting indications on trophic level. Therefore, applying FA ratios to determine trophic level in corals has its limitations due to the mixture of possible food sources.

The diversity of FA profiles exhibited by our five target coral species is in accordance with previous coral FA studies (Imbs et al. 2007, 2010b, 2014). These studies confirmed that hard corals can be distinguished on family or subclass

level on the basis of PUFA profiles. In this study, we took this even further and managed to clearly separate all our five corals on genus level by multivariate analyses using 19 FA profiles including not only PUFAs but also SFAs and MUFAs. However, we should be careful when interpreting the data since FA profiles in corals can be modulated by external nutritional input (Parrish et al. 2000; Dalsgaard et al. 2003; Alfaro et al. 2006) as well as by environmental factors, such as season, site, depth, and temperature (Imbs 2013; Seemann et al. 2013; Rocker et al. 2019). Furthermore, our coral samples were collected in flow mesocosm tanks, where sea water comes from the marine protected area of Hong Kong after being primarily filtered through a sand filtration system to remove suspended matter and sedimentation. This implies, coral colonies are exposed to more stable environmental conditions when compared to wild corals in the bay. Regarding season, we sampled in May that falls within the transition period between dry and wet season when it is climatically less extreme. In addition, the average water temperature was 25.7 °C, which is close to the all-year-average water temperature (26.0 °C in 2016). In contrast, Imbs et al. (2007) collected their coral samples in the shallow waters of Vietnam in the South China Sea. Despite the same sampling region, season, depth etc., FAs in corals from natural habitats can be influenced by a variety of food sources (Imbs et al. 2010b). Therefore, we believe that the consistent and stable environmental condition in our coral tanks was the key to distinguish distinct groups among the five coral species.

The five distinct species groups are associated to 16:0 and 18:0 which are known to be transferred from glucose, symbionts and dietary sources (Imbs et al. 2011; Revel et al. 2016). Numerous studies on FA profiles of hard corals have also documented that these FAs are the most abundant FAs since their first report by Meyers (1977) (Latyshev et al. 1991; Harland et al. 1993; Yamashiro et al. 1999; Imbs et al. 2007, 2010b; Imbs 2013). The 16:0 in host cnidarian tissue can be de novo synthesized from symbiont photosynthesis-driven glucose (Oku et al. 2003a; Revel et al. 2016), while a high 16:0 in symbionts is originated from their photosynthesis products. In *P. decussata*, 16:0 in both symbionts and their host tissue showed the highest concentration, suggesting 16:0 in this species relies on symbionts. However, 16:0 in host tissue can be originated from both symbionts and dietary sources, such as zooplankton which is known to have abundant 16:0 (Lee et al. 2006). An elevated concentration of 16:0 in the *P. carnososa* host tissue than in its symbionts, as opposed to the other four species, suggests that *P. carnososa* obtains additional 16:0 from external food sources. Upon obtaining 16:0, the biosynthesis pathway 16:0 → 18:0 → 18:1n-9 can be consistently metabolized in both symbionts and their coral host tissue (Treignier et al. 2009; Revel et al. 2016). However, the coral host cannot

synthesize further from 18:1n-9 to 18:2n-6, which is the precursor of n-6 PUFA pathway, due to the lack of  $\Delta^{12}$ -desaturase enzyme (Dunn et al. 2012; Matthews et al. 2018). Therefore, we assume that the coral host stores energy in the form of 16:0 or 18:0 in lipids, but it is species-specific and depends on the nutrition.

Although, *F. abdita* and *P. carnosa* belong to the same family (Faviidae), their FA profiles differed. The dissimilarity between FA profiles of *F. abdita* and *P. carnosa* was the lowest (12.6%), which indicate that these two species have comparable FA profiles relative to any other combination of species. Both species were also characterized by the highest concentration of 20:4n-6, and further exhibited the highest concentration among all the PUFAs. Unfortunately, there are very few studies on 20:4n-6 in corals. Rocker et al. (2019) documented that symbiont density had a negative correlation with 20:4n-6 concentration, which implies that this FA in the coral host tissue might not be derived from symbionts, hence 20:4n-6 suggests to be a potential indicator of coral feeding. Accordingly, a few studies surmised that 20:4n-6 in corals might originate from external food sources e.g. phytoplankton and/or herbivorous zooplankton (Seemann et al. 2013; Imbs et al. 2016; Kim et al. 2021). Indeed, high 20:4n-6 content in phytoplankton has been documented (Jónasdóttir 2019) Subsequently, higher 20:4n-6 in *P. carnosa*, *F. abdita* and *T. peltata* than in other species show that these species are more heterotrophic. In addition, *P. decussata* is characterized by a negative relationship with 20:4n-6, which confirms the relative autotrophy of this species.

On the other hand, the most dissimilar species coupling with *P. carnosa* and/or *F. abdita* was *A. samoensis*. This species was determined by the predominance of 16:0 and 18:0, followed by 18:3n-3. Especially, 18:3n-3 in *A. samoensis* was the most abundant of all the PUFA, and it should be noted 18:3n-3 plays a key role in further synthesizing essential n-3 PUFA. However, this FA cannot be biochemically synthesized by coral hosts due to their lack of  $\Delta^{15}$ -desaturase enzyme, indicating that 18:3n-3 originates from symbionts via photosynthesis and subsequently transferred to the coral host. Moreover, the high concentration of 18:3n-3 in the host tissue of *A. samoensis* indicates that this species is heavily dependent on autotrophy through its symbionts. The highest dissimilarity (30.3%) of FA profiles among the five coral species was in between *A. samoensis* and *T. peltata*, and 18:3n-3 contributed to this separation the most. In contrast to the high concentration of 18:3n-3 in *A. samoensis*, no 18:3n-3 could effectively be detected in *T. peltata*. In previous studies, on a different species of the same genus, *T. reniformis*, no 18:3n-3 was observed in the representatives under starving conditions, but the concentration was high in corals fed with zooplankton (*Artemia salina* nauplii) (Tolosa et al. 2011). Although we did not quantify the extent of food availability in our coral tanks, a lack of zooplankton is possible.

However, *T. peltata* showed a high concentration of 20:4n-6, which is an indicator of heterotrophic feeding (mainly phytoplankton) as previously discussed. This incompatible result is due to the abundance of different food sources (here, zooplankton vs. phytoplankton), which causes a variation of 18:3n-3 and 20:4n-6 in this species. On the other hand, symbionts of *T. peltata* showed the highest concentration of 18:3n-3 among all five species indicating a low translocation rate from symbionts to the host tissue in this species and might be responsible for our finding, giving the unique characteristic of *T. peltata*. Moreover, exceptional values of 18:3n-3 due to species-specific characteristics have been reported by Papina et al. (2003). They observed low 18:3n-3 in *Montipora digitata* and postulated species-specific differences to be responsible.

Besides a high concentration of 18:3n-3, *A. samoensis* was also characterized by high concentrations of 20:5n-3 and 22:6n-3. These FAs are known to be essential PUFAs in marine organisms, including corals, as they act as functional FAs for growth, reproduction, and physical fitness (Wacker and von Elert 2001; Pernet et al. 2002; Figueiredo et al. 2012). It is known 22:6n-3 is highly conserved through the food web (Scott et al. 2002; Dalsgaard et al. 2003), whereas 20:5n-3 is the dominant FA in symbionts and transferred to the coral host tissue (Revel et al. 2016). Thus, the ratio of 20:5n-3 to 22:6n-3 (EPA:DHA) has been applied to define trophic level (low value means heterotrophy, high value means autotrophy) (Rocker et al. 2019). According to the EPA:DHA ratio, *A. samoensis* and *T. peltata* are defined to be relatively autotrophic, whereas *P. decussata*, *F. abdita*, and *P. carnosa* are relatively heterotrophic species. This ratio confirms our previous conclusions drawn from FA profiles that *A. samoensis* is autotrophic, *F. abdita* and *P. carnosa* are heterotrophic. However, this ratio contrasts with *P. decussata* and *T. peltata* when compared to our findings, as well as to investigation from a previous study (Treignier et al. 2008). We may explain this controversy with the fact that corals are polytrophic, thus they can switch their trophic strategies depending on specific environmental conditions such as food sources and amount or symbiont density influenced by water quality (Dalsgaard et al. 2003; Seemann et al. 2013; Rocker et al. 2019). The most plausible reason is that not only 20:5n-3, but also 22:6n-3 in the coral host tissue can be either transferred from symbionts (Revel et al. 2016) or taken up externally through planktonic food sources (20:5n-3 enriched in diatom, 22:6n-3 enriched in dinoflagellate) (Dalsgaard et al. 2003; Figueiredo et al. 2012; Revel et al. 2016). Although we did not measure FA compositions in our mesocosm tanks, a recent study by Kim et al. (2021) showed that both FAs are enriched in zooplankton from HK waters.

In summary, TL and FA profiles proved to be a powerful tool as chemotaxonomic biomarkers at genus level

of symbiotic corals, especially when the host tissue and symbionts are investigated separately. This confirms our first hypothesis, FA profiles being species-specific, enabling a classification of symbiotic corals when applying coral host tissue from symbionts separately. In addition, our study provides new insights into the interaction of coral-symbionts endosymbiosis and species-specific trophic strategies. We hypothesized that the trophic level of symbiotic corals can be deduced from specific biomarkers. However, we realized that interpretation based on the accepted and widely used FA ratio indicators: EPA:DHA or PUFA:SFA to define specific trophic levels needs to be handled with more caution in research application and data interpretation. Moreover, 20:5n-3/22:6n-3n ratio and PUFA/SFA ratio themselves should not be mistaken for universal indices. Since FA in coral host tissue can be driven by different sources such as symbionts and external food sources, interpretations may not be as straightforward as currently applied. Hence, our second hypothesis is true only when rephrased as certain FA biomarkers giving indications on the relative trophic level of symbiotic corals. Given these limitations of FA ratios, we suggest that it to be more appropriate if these FA ratios were only applied to examine response to environmental change within species. Going forward, our study provides important FA baseline data that builds the foundation to future investigation on impacts of environmental changes on nutrition and metabolism in symbiotic corals.

**Supplementary Information** The online version contains supplementary material available at <https://doi.org/10.1007/s00227-021-03979-9>.

**Acknowledgements** We thank the editor, Prof. Christian Wild, and two anonymous reviewers for their handling of our manuscript and constructive critique on earlier versions. We are grateful for the technical support of HKU and SWIMS staff.

**Author contributions** TK and DMB conceived the study and planned fieldwork. TK and JCYL conducted data analysis. TK, JCYL, and DMB wrote the manuscript with contributions of SJJ. All authors participated and contributed to the final version of the manuscript.

**Funding** This study was supported by General Research Fund No. 17303615 (Research Grants Council Hong Kong).

**Availability of data and material** The datasets for the study are available from the corresponding author upon reasonable request.

**Code availability** Not applicable.

## Declarations

**Conflict of interest** The authors declare that they have no conflict of interest.

**Ethical approval** Hard coral samples were collected and maintained under the permit No. (111) in AF GR MPA 08/9 Pt.16, delivered by

the Agriculture, Fisheries and Conservation Department, Hong Kong SAR. This article does not contain any studies with human participants or animals performed by any of the authors.

**Consent to participate** Authors agree on their participation on this paper.

**Consent for publication** The authors agree to be co-authors on this paper on the order submitted.

## References

- Ackman RG (1999) Comparison of lipids in marine and freshwater organisms. In: Arts MT (ed) Lipids in freshwater ecosystems. Springer Science+Business Media, New York, pp 263–298
- Alfaro AC, Thomas F, Sergeant L, Duxbury M (2006) Identification of trophic interactions within an estuarine food web (northern New Zealand) using fatty acid biomarkers and stable isotopes. *Estuar Coast Shelf Sci* 70:271–286
- Allan EL, Ambrose ST, Richoux NB, Froneman PW (2010) Determining spatial changes in the diet of nearshore suspension-feeders along the South African coastline: Stable isotope and fatty acid signatures. *Estuar Coast Shelf Sci* 87:463–471
- Al-Lihaibi SS, Al-Sofyani AA, Niaz GR (1998) Chemical composition of corals in Saudi Red sea coast. *Oceanol Acta* 21:495–501
- Al-Moghrabi S, Allemand D, Couret JM, Jaubert J (1995) Fatty acids of the scleractinian coral *Galaxea fascicularis*: effect of light and feeding. *J Comp Physiol B* 165:183–192
- Anthony KRN, Fabricius KE (2000) Shifting roles of heterotrophy and autotrophy in coral energetics under varying turbidity. *J Exp Mar Biol Ecol* 252:221–253
- Arai T, Kato M, Heyward A, Ikeda Y, Iizuka T, Maruyama T (1993) Lipid composition of positively buoyant eggs of reef building corals. *Coral Reefs* 12:71–75
- Arai T, Amalina R, Bachok Z (2015) Fatty acid composition indicating diverse habitat use in coral reef fishes in the Malaysian South China Sea. *Biol Res* 48:13
- Bachok Z, Mfilinge P, Tsuchiya M (2006) Characterization of fatty acid composition in healthy and bleached corals from Okinawa, Japan. *Coral Reefs* 25:545–554
- Bay LK, Guerecheau A, Andreakis N, Ulstrup KE, Matz MV (2013) Gene expression signatures of energetic acclimatisation in the reef building coral *Acropora millepora*. *PLoS ONE* 8:e61736
- Bergé JP, Barnathan G (2005) Fatty acids from lipids of marine organisms: molecular biodiversity, roles as biomarkers, biologically active compounds, and economical aspects. *Adv Biochem Eng Biotechnol* 96:49–125
- Chen HK, Song SN, Wang LH, Mayfield AB, Chen YJ, Chen WNU, Chen CS (2015) A compartmental comparison of major lipid species in a Coral-*Symbiodinium* endosymbiosis: evidence that the coral host regulates lipogenesis of its cytosolic lipid bodies. *PLoS ONE* 10:e0132519
- Conti-Jerpe IE, Thompson PD, Wong CWM, Oliveira NL, Duprey NN, Moynihan MA, Baker DM (2020) Trophic strategy and bleaching resistance in reef-building corals. *Sci Adv* 6:5443
- Couturier LIE, Michel LN, Amaro T, Budge SM, da Costa E, De Troch M, Di Dato V, Fink P, Giraldo C, Le Grand F, Loaiza I, Mathieu-Resuge M, Nichols PD, Parrish CC, Sardenne F, Vagner M, Pernet F, Soudant P, Browman H (2020) State of art and best practices for fatty acid analysis in aquatic sciences. *ICES J Mar Sci* 77:2375–2395

- Cripps GC, Atkinson A (2000) Fatty acid composition as an indicator of carnivory in Antarctic krill, *Euphausia superba*. *Can J Fish Aquat* 57:31–37
- Dalsgaard J, John MS, Kattner G, Muller-Navarra D, Hagen W (2003) Fatty acid trophic markers in the pelagic marine environment. *Adv Mar Biol* 46:225–340
- Dunn SR, Thomas MC, Nette GW, Dove SG (2012) A lipidomic approach to understanding free fatty acid lipogenesis derived from dissolved inorganic carbon within cnidarian-dinoflagellate symbiosis. *PLoS ONE* 7:e46801
- Fabricius KE (2005) Effects of terrestrial runoff on the ecology of corals and coral reefs: review and synthesis. *Mar Pollut Bull* 50:125–146
- Falk-Petersen S, Dahl TM, Scott CL, Sargent JR, Gulliksen B, Kwasniewski S, Hop H, Millar R-M (2002) Lipid biomarkers and trophic linkages between ctenophores and copepods in Svalbard waters. *Mar Ecol Prog Ser* 227:187–194
- Ferrier-Pagès C, Gattuso J (1998) Biomass, production and grazing rates of pico- and nanoplankton in coral reef waters (Miyako Island, Japan). *Microb Ecol* 35:46–57
- Ferrier-Pagès C, Witting J, Tambuté E, Sebens KP (2003) Effect of natural zooplankton feeding on the tissue and skeletal growth of the scleractinian coral *Stylophora pistillata*. *Coral Reefs* 22:229–240
- Figueiredo J, Baird AH, Cohen MF, Flot JF, Kamiki T, Meziane T, Tsuchiya M, Yamasaki H (2012) Ontogenetic change in the lipid and fatty acid composition of scleractinian coral larvae. *Coral Reefs* 31:613–619
- Folch J, Lees M, Sloane Stanley G (1957) A simple method for the isolation and purification of total lipides from animal tissues. *J Biol Chem* 226:497–509
- Gates RD, Hoegh-Guldberg O, McFallngai MJ, Bil KY, Muscatine L (1995) Free amino acids exhibit anthozoan host factor activity - they induce the release of photosynthate from symbiotic dinoflagellates in-vitro. *Proc Natl Acad Sci USA* 92:7430–7434
- Goreau TF, Goreau NI, Yonge CM (1971) Reef corals: autotrophs or heterotrophs? *Biol Bull* 41:247–260
- Graeve M, Kattner G, Hagen W (1994) Diet-induced changes in the fatty acid composition of Arctic herbivorous copepods: Experimental evidence of trophic markers. *J Exp Mar Biol Ecol* 182:97–110
- Harland AD, Davies PS, Fixter LM (1992) Lipid content of some Caribbean corals in relation to depth and light. *Mar Biol* 113:357–361
- Harland AD, Navarro JC, Davies PS, Fixter LM (1993) Lipids of some Caribbean and Red Sea corals: total lipid, wax esters, triglycerides and fatty acids. *Mar Biol* 117:113–117
- Howell KL, Pond DW, Billett DSM, Tyler PA (2003) Feeding ecology of deep-sea seastars (Echinodermata: Asteroidea): a fatty-acid biomarker approach. *Mar Ecol Prog Ser* 255:193–206
- Imbs AB (2013) Fatty acids and other lipids of corals: Composition, distribution, and biosynthesis. *Russ J Mar Biol* 39:153–168
- Imbs AB, Demidkova DA, Latypov YY, Pham LQ (2007) Application of fatty acids for chemotaxonomy of reef-building corals. *Lipids* 42:1035–1046
- Imbs AB, Yakovleva IM, Pham LQ (2010a) Distribution of lipids and fatty acids in the zooxanthellae and host of the soft coral *Simularia* sp. *Fish Sci* 76:375–380
- Imbs AB, Latyshev NA, Dautova TN, Latypov YY (2010b) Distribution of lipids and fatty acids in corals by their taxonomic position and presence of zooxanthellae. *Mar Ecol Prog Ser* 409:65–75
- Imbs AB, Yakovleva IM, Latyshev NA, Pham LQ (2011) Biosynthesis of polyunsaturated fatty acids in zooxanthellae and polyps of corals. *Russ J Mar Biol* 36:452–457
- Imbs AB, Yakovleva IM, Dautova TN, Bui LH, Jones P (2014) Diversity of fatty acid composition of symbiotic dinoflagellates in corals: evidence for the transfer of host PUFAs to the symbionts. *Phytochemistry* 101:76–82
- Imbs AB, Dang LP, Rybin VG, Svetashev VI (2015) Fatty acid, lipid class, and phospholipid molecular species composition of the soft coral *Xenia* sp. (Nha Trang Bay, the South China Sea, Vietnam). *Lipids* 50:575–589
- Imbs AB, Demidkova DA, Dautova TN (2016) Lipids and fatty acids of cold-water soft corals and hydrocorals: a comparison with tropical species and implications for coral nutrition. *Mar Biol* 163:1–2
- Jónasdóttir SH (2019) Fatty acid profiles and production in marine phytoplankton. *Mar Drugs* 17:151
- Joseph JD (1979) Lipid composition of marine and estuarine invertebrates: porifera and Cnidaria. *Prog Lipid Res* 18:1–30
- Ju SJ, Ko AR, Lee CR (2011) Latitudinal variation of nutritional condition and diet for copepod species, *Euchaeta* sp. and *Pleuromamma* spp., from the Northwest Pacific ocean using lipid biomarkers. *Ocean Polar Res* 33:349–358
- Kelly JR, Scheibling RE, Iverson SJ, Gagnon P (2008) Fatty acid profiles in the gonads of the sea urchin *Strongylocentrotus droebachiensis* on natural algal diets. *Mar Ecol Prog Ser* 373:1–9
- Kim T, Lee JCY, Kang D-H, Duprey NN, Leung KS, Archana A, Baker DM (2021) Modification of fatty acid profile and biosynthetic pathway in symbiotic corals under eutrophication. *Sci Total Environ* 771:145336
- LaJeunesse TC, Parkinson JE, Gabrielson PW, Jeong HJ, Reimer JD, Woolstra CR, Santos SR (2018) Systematic revision of Symbiodiniaceae highlights the antiquity and diversity of coral endosymbionts. *Curr Biol* 28:2576
- Latyshev NA, Naumenko NV, Svetashev VI, Latypov YY (1991) Fatty acids of reef-building corals. *Mar Ecol Prog Ser* 76:295–301
- Lee RF, Hagen W, Kattner G (2006) Lipid storage in marine zooplankton. *Mar Ecol Prog Ser* 307:273–306
- Lopes AR, Baptista M, Rosa IC, Dionísio G, Gomes-Pereira J, Paula JR, Figueiredo C, Bandarra N, Calado R, Rosa R (2016) “Gone with the wind”: Fatty acid biomarkers and chemotaxonomy of stranded pleustonic hydrozoans (*Veella veella* and *Physalia physalis*). *Biochem Syst Ecol* 66:297–306
- Mansour MP, Volkman JK, Jackson AE, Blackburn SI (1999) The fatty acid and sterol composition of five marine dinoflagellates. *J Phycol* 35:710–720
- Matthews JL, Oakley CA, Lutz A, Hillyer KE, Roessner U, Grossman AR, Weis VM, Davy SK (2018) Partner switching and metabolic flux in a model cnidarian-dinoflagellate symbiosis. *Proc R Soc B* 285:20182336
- Meyers PA (1977) Fatty acids and hydrocarbons of Caribbean corals. *Third Int Coral Reef Symp* 1:529–536
- Mies M, Güth AZ, Tenório AA, Banha TNS, Waters LG, Polito PS, Taniguchi S, Bicego MC, Sumida PYG (2018) In situ shifts of predominance between autotrophic and heterotrophic feeding in the reef-building coral *Mussismilia hispida*: an approach using fatty acid trophic markers. *Coral Reefs* 37:677–689
- Muscatine L, McCloskey LR, Marian RE (1981) Estimating the daily contribution of carbon from zooxanthellae to coral animal respiration. *Limnol Oceanogr* 26:601–611
- Muscatine L, Porter JW, Kaplan IR (1989) Resource partitioning by reef corals as determined from stable isotope composition I.  $\delta^{13}\text{C}$  of zooxanthellae and animal tissue vs depth. *Mar Biol* 100:185–193
- Oku H, Yamashiro H, Onaga K (2003a) Lipid biosynthesis from [ $^{14}\text{C}$ ]-glucose in the coral *Montipora digitata*. *Fish Sci* 69:625–631
- Oku H, Yamashiro H, Onaga K, Sakai K, Iwasaki H (2003b) Seasonal changes in the content and composition of lipids in the coral *Goniastrea aspera*. *Coral Reefs* 22:83–85

- Papina M, Meziane T, van Woesik R (2003) Symbiotic zooxanthellae provide the host-coral *Montipora digitata* with polyunsaturated fatty acids. *Comp Biochem Physiol B Biochem Mol Biol* 135:533–537
- Parrish CC (2013) Lipids in marine ecosystems. *ISRN Oceanography* 2013:1–16
- Parrish CC, Budge TAASM, Helleur RJ, Hudson ED, Pulchan K, Ramos C (2000) Lipid and phenolic biomarkers in marine ecosystems: analysis and applications. In: Wangersky P (ed) *The handbook of environmental chemistry*. Springer, Berlin, pp 193–223
- Patton JS, Burris JE (1983) Lipid synthesis and extrusion by freshly isolated zooxanthellae (symbiotic algae). *Mar Biol* 75:131–136
- Patton JS, Abraham S, Benson AA (1977) Lipogenesis in the intact coral *Pocillopora capitata* and its isolated zooxanthellae: evidence for a light-driven carbon cycle between symbiont and host. *Mar Biol* 44:235–247
- Pernet V, Gavino V, Gavino G, Ancil M (2002) Variations of lipid and fatty acid contents during the reproductive cycle of the anthozoan *Renilla koellikeri*. *J Comp Physiol B* 172:455–465
- Porter JW (1976) Autotrophy, heterotrophy, and resource partitioning in Caribbean reef-building corals. *Am Nat* 110:731–742
- Radice VZ, Brett MT, Fry B, Fox MD, Hoegh-Guldberg O, Dove SG (2019) Evaluating coral trophic strategies using fatty acid composition and indices. *PLoS ONE* 14:e0222327
- Rajendran N, Suwa Y, Urushigawa Y (1993) Distribution of phospholipid ester-linked fatty acid biomarkers for bacteria in the sediment of Ise Bay, Japan. *Mar Chem* 42:39–56
- Revel J, Massi L, Mehiri M, Boutoute M, Mayzaud P, Capron L, Sabourault C (2016) Differential distribution of lipids in epidermis, gastrodermis and hosted *Symbiodinium* in the sea anemone *Anemonia viridis*. *Comp Biochem Physiol Part A Mol Integr Physiol* 191:140–151
- Reynaud S, Martinez P, Houlbrèque F, Billy I, Allemand D, Ferrier-Pagès C (2009) Effect of light and feeding on the nitrogen isotopic composition of a zooxanthellate coral: role of nitrogen recycling. *Mar Ecol Prog Ser* 392:103–110
- Rocker MM, Willis BL, Francis DS, Bay LK (2019) Temporal and spatial variation in fatty acid composition in *Acropora tenuis* corals along water quality gradients on the Great Barrier Reef, Australia. *Coral Reefs* 38:215–228
- Salvo F, Hamoutene D, Hayes VEW, Edinger EN, Parrish CC (2017) Investigation of trophic ecology in Newfoundland cold-water deep-sea corals using lipid class and fatty acid analyses. *Coral Reefs* 37:157–171
- Sardenne F, Kraffe E, Amiel A, Fouché E, Debrauwer L, Ménard F, Bodin N (2017) Biological and environmental influence on tissue fatty acid compositions in wild tropical tunas. *Comp Biochem Physiol Part A Mol Integr Physiol* 204:17–27
- Scott CL, Kwasniewski S, Falk-Petersen S, Sargent JR (2002) Species differences, origins and functions of fatty alcohols and fatty acids in the wax esters and phospholipids of *Calanus hyperboreus*, *C. glacialis* and *C. finmarchicus* from Arctic waters. *Mar Ecol Prog Ser* 235:127–134
- Sebens KP, Vandersall KS, Savina LA, Graham KR (1996) Zooplankton capture by two scleractinian corals, *Madracis mirabilis* and *Montastrea cavernosa*, in a field enclosure. *Mar Biol* 127:303–317
- Seemann J (2013) The use of  $^{13}\text{C}$  and  $^{15}\text{N}$  isotope labeling techniques to assess heterotrophy of corals. *J Exp Mar Biol Ecol* 442:88–95
- Seemann J, Sawall Y, Auel H, Richter C (2013) The use of lipids and fatty acids to measure the trophic plasticity of the coral *Stylophora subseriata*. *Lipids* 48:275–286
- Shin PKS, Yip KM, Xu WZ, Wong WH, Cheung SG (2008) Fatty acid as markers to demonstrating trophic relationships among diatoms, rotifers and green-lipped mussels. *J Exp Mar Biol Ecol* 357:75–84
- Stevens CJ, Deibel D, Parrish CC (2004) Species-specific differences in lipid composition and omnivory indices in Arctic copepods collected in deep water during autumn (North Water Polynya). *Mar Biol* 144:905–915
- Svetashev VI, Vysotskii MV (1998) Fatty acids of *Heliopora coerulea* and chemotaxonomic significance of tetracosapolyenoic acids in coelenterates. *Comp Biochem Physiol B Biochem Mol Biol* 119B:73–75
- Tarrant AM (2005) Endocrine-like signaling in Cnidarians: current understanding and implications for ecophysiology. *Integr Comp Biol* 45:201–214
- Tocher DR (2003) Metabolism and functions of lipids and fatty acids in teleost fish. *Rev Fish Sci Aquac* 11:107–184
- Tolosa I, Treignier C, Grover R, Ferrier-Pagès C (2011) Impact of feeding and short-term temperature stress on the content and isotopic signature of fatty acids, sterols, and alcohols in the scleractinian coral *Turbinaria reniformis*. *Coral Reefs* 30:763–774
- Treignier C, Grover R, Ferrier-Pagès C (2008) Effect of light and feeding on the fatty acid and sterol composition of zooxanthellae and host tissue isolated from the scleractinian coral *Turbinaria reniformis*. *Limnol Oceanogr* 53:2702–2710
- Treignier C, Tolosa I, Grover R, Reynaud S, Ferrier-Pagès C (2009) Carbon isotope composition of fatty acids and sterols in the scleractinian coral *Turbinaria reniformis*: effect of light and feeding. *Limnol Oceanogr* 54:1933–1940
- Viso A-C, Marty J-C (1993) Fatty acids from 28 marine microalgae. *Phytochemistry* 34:1521–1533
- Volkman JK (1999) Australasian research on marine natural products: chemistry, bioactivity and ecology. *Mar Freshw Res* 50:761–779
- Wacker A, von Elert E (2001) Polyunsaturated fatty acids: evidence for non-substitutable biochemical resources in *Daphnia galeata*. *Ecology* 82:2507–2520
- Wang LH, Lee HH, Fang LS, Mayfield AB, Chen CS (2013) Fatty acid and phospholipid syntheses are prerequisites for the cell cycle of *Symbiodinium* and their endosymbiosis within sea anemones. *PLoS ONE* 8:e72486
- Ward S (1995) Two patterns of energy allocation for growth, reproduction and lipid storage in the scleractinian coral *Pocillopora damicornis*. *Coral Reefs* 14:87–90
- Whitehead LF, Douglas AE (2003) Metabolite comparisons and the identity of nutrients translocated from symbiotic algae to an animal host. *J Exp Biol* 206:3149–3157
- Wong JCY, Thompson P, Xie JY, Qiu J-W, Baker DM (2016) *Symbiodinium* clade C generality among common scleractinian corals in subtropical Hong Kong. *Reg Stud Mar Sci* 8:439–444
- Wooldridge SA (2014) Differential thermal bleaching susceptibilities amongst coral taxa: re-posing the role of the host. *Coral Reefs* 33:15–27
- Yahel R, Yahel G, Genin A (2004) Near-bottom depletion of zooplankton over coral reefs: I: diurnal dynamics and size distribution. *Coral Reefs* 24:75–85
- Yamashiro H, Oku H, Higa H, Chinen I, Sakai K (1999) Composition of lipids, fatty acids and sterols in Okinawan corals. *Comp Biochem Physiol B Biochem Mol Biol* 122:397–407
- Yamashiro H, Oku H, Onaga K (2005) Effect of bleaching on lipid content and composition of Okinawan corals. *Fish Sci* 71:448–453