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REPORT

Seasonal variation in the lipidome of two species of *Millepora* hydrocorals from Vietnam coastal waters (the South China Sea)

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Abstract In shallow tropical seas, hydrocoral colonies are locally abundant and important reef-framework builders. Hydrocorals are rich in lipids, which play an essential role in their physiology, but no data on seasonal variations in lipids of hydrocorals are available to date. In the present study, the profiles of total lipids, fatty acids (FA), storage and structural lipids classes, and molecular species of structural lipids in symbiotic dinoflagellates and the host of the hydrocorals *Millepora dichotoma* and *M. platyphylla* were analyzed each month throughout the year. In both hydrocoral species, the twofold increase in total lipids in the summer coincided with the water temperature rise and was caused primarily by the considerable accumulation of storage lipids with insignificant variations in structural lipids. The FA markers confirmed the year-round hydrocorals' predation on zooplankton and indicated that *M. dichotoma* can consume more diatoms than *M. platyphylla* during October–January. The polar lipidomes of the two *Millepora* species were similar. The remodeling of alkyl and sphingosine groups in three structural lipid classes rather than unsaturation degree of their FA groups may be considered as a seasonal adaptive response of host biomembranes in *Millepora*. The symbiotic dinoflagellates of the hydrocorals kept the galactolipid ratio stable throughout the year but significantly increased the levels of sulfoquinovosyldiacylglycerol and betaine lipid in winter. The FA markers of the symbionts correlated negatively with galactolipids, thus, indicating a higher transfer of the symbiotic FA to the host in winter. Throughout the year, symbionts controlled their lipid class composition, but their lipid molecular species composition varied in a random manner. The considerable amounts of digalactosyldiacylglycerols with $C_{20–22}$ polyunsaturated FA distinguished *M. platyphylla* from *M. dichotoma* and suggested that different hydrocorals contain different symbiont species. Data on seasonal variations of the lipidomes provide new background information for future studies of ecology, physiology, and biochemistry of hydrocorals.

Keywords *Millepora* · Fatty acids · Lipids · Lipidomic analysis · Seasonal variation · Coral reef ecology

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Introduction

Coral colonies with a hard exoskeleton, such as scleractinian and hydrozoan corals, constitute the structural base of tropical coral reefs, and are, therefore, of particular interest for the studies of coral reef ecology. Many of these studies consider data on the content and composition of

lipids, which make up to 30% of dry tissues in hard coral (Imbs 2013). Lipids play an essential role in coral physiology (Treignier et al. 2008; Lim et al. 2017). Lipid and fatty acid (FA) analyses are often applied to assess the coral health (Yamashiro et al. 2001; Rocker et al. 2019), energy expenditure (Anthony et al. 2009; Drenkard et al. 2018), feeding strategy (Teece et al. 2011; Seemann et al. 2013; Naumann et al. 2015), and reproductive strategy of corals (Ward 1995; Viladrich et al. 2016; Grinyó et al. 2018). Lipid indices are used to study the symbiont–host association (Tchernov et al. 2004; Oakley and Davy 2018), species-species interaction (Fleury et al. 2004), bleaching and recovery of coral reefs (Yamashiro et al. 2005; Bachok et al. 2006; Rodrigues and Grottoli 2007; Rodrigues et al. 2008; Wall et al. 2019), and influence of environmental factors (Ben-David-Zaslow and Benayahu 1999; Oku et al. 2003).

A short review on the role and high importance of lipids and their FA in biochemistry, physiology, and ecology of corals has been recently made by Rocker et al. (2019). Total lipids are divided into storage lipids, which are long-term energy storage of corals, and structural lipids that compose coral cell membranes. In general, the storage lipid content and storage/structural lipid ratio indicate the nutrition status and energy balance of coral species (Naumann et al. 2015). Structural lipid compositions of the marine invertebrates are similar within large taxa, such as class or phylum, adapt to environmental temperature, and determine cell membrane properties (e.g., membrane fluidity). FA obtained by chemical hydrolysis of lipid molecules are mainly applied as markers of food sources (Imbs and Latyshev 2012; Mies et al. 2018). Most coral species contain symbiotic dinoflagellates of the family Symbiodiniaceae (LaJeunesse et al. 2018) referred to as zooxanthellae. Both zooxanthellae and a coral host have specific lipid and FA markers (Kneeland et al. 2013; Imbs et al. 2015c), which allows a monitoring of each symbiotic partner in the holobiont (Garrett et al. 2013; Sikorskaya et al. 2020).

Coral lipid profiles reflect short-term events and vary throughout the seasonal cycle, but not so many year-round studies of coral lipid variations have been conducted to date. For symbiotic coral species, these variations were described for *Heteroxenia fuscescens* (Ben-David-Zaslow and Benayahu 1999), *Goniastrea aspera* (Oku et al. 2003), *Acropora digitifera* (Hinrichs et al. 2013), *Pseudoplexaura crucis* (McCauley et al. 2018) and are related with changes in light intensity, water temperature, and coral nutrition. At the same time, the lipid indices were stable throughout the year in *Acropora spicifera* (Hinrichs et al. 2013) and *Eunicea tourneforti* (McCauley et al. 2018). The seasonal variations in polyunsaturated FA (PUFA) were described for *Mussismilia hispida* (Mies et al. 2018) and *Acropora tenuis* (Rocker et al. 2019) as indicators of coral feeding strategy and holobiont health. Studies of year-round lipid variations were also performed on several asymbiotic anthozoans, which do not have autotrophic mode of nutrition. Seasonal variations in the lipid indices were observed in the boreal soft coral *Gersemia rubiformis* (Imbs et al. 2006), gorgonian *Paramuricea clavata* (Rossi et al. 2006), red coral *Corallium rubrum* (Rossi and Tsounis 2007), sea pen *Veretillum cynomorium* (Baptista et al. 2012), antarctic pennatulid *Malacobelemnon daytoni* (Servetto et al. 2017), and mesophotic gorgonian *Paramuricea macrospina* (Grinyó et al. 2018). These variations were caused by food availability, spawning, and oogenesis. No clear seasonal pattern of variations in wax esters, triacylglycerols, FA, and fatty alcohols was observed in the deep-water hard coral *Lophelia pertusa* (Dodds et al. 2009). All year-round studies of lipid dynamics were carried out on anthozoan but not hydrozoan corals.

All such studies were based on the integral lipid indices, e.g., the content of total lipids, composition of their FA, or lipid class ratio. However, total lipids and each lipid class are known to be a mixture of certain molecules referred to as "lipid molecular species," and their full range is defined as lipidome of certain biological objects (Spener et al. 2003). Lipidomics of marine organisms has rapidly developed in recent years (Rezanka et al. 2018). Profiles of molecular species of polar lipids were determined for several species of tropical and cold-water soft corals (Imbs et al. 2015a, b; Imbs and Dang 2017; Dang et al. 2018). Choline glycerophospholipid molecular species of the reef-building coral *Seriatopora caliendrum* were analyzed (Tang et al. 2019). Recently, the lipidomes of the alcyonarian *Sinularia siaesensis* (Sikorskaya and Imbs 2018) and the zoantharian *Palythoa* sp. (Sikorskaya 2020) have been described. Due to the rapid development of methods in lipidome analysis, we believe that the lipidomic approach can be a new efficient tool for coral ecology studies (Rosset et al. 2019) in addition to the integral lipid indices.

Hydrozoan corals (or hydrocorals) of the genus *Millepora* contain zooxanthellae and are common members of coral reef communities in the Indo-Pacific and Atlantic Oceans (Lewis 2006) (Rodríguez et al. 2019). Total lipid and FA compositions of several *Millepora* species were described earlier (Latyshev et al. 1991; Yamashiro et al. 1999; Imbs et al. 2010c). The composition of phospholipid classes and their molecular species has recently been determined in two *Millepora* species (Imbs et al. 2019). To date, there is a lack of data on annual cycles of lipid, FA, and lipid molecular species content in hydrocoral species. A study of biochemical changes in hydrocorals is important to understand the key factors influencing a coral reef community. Data on seasonal changes in the lipid profile

will help identify the range of natural variations in the hydrocoral health indices, which are necessary for the coral reef management and comparative studies on corals (Dodds et al. 2009; Hinrichs et al. 2013).

In the present study, the content of total lipids, total FA, and lipid classes was analyzed in the hydrocorals *Millepora dichotoma* and *M. platyphylla* collected from coral reefs of Vietnam (the South China Sea) on a monthly basis. The seasonal variations in storage and structural lipids classes, as well as major FA, in *M. dichotoma* were compared to those in *M. platyphylla*. Then, the seasonal variations in the molecular species of structural lipids of these hydrocorals were studied. The annual cycle of molecular species profiles of major phospholipid, phosphonolipid, glycolipid, and betaine lipid classes was determined. The key components in the polar lipidome of the symbionts and the host were identified. Possible causes of the observed variations were suggested.

Materials and methods

Collection of specimens and extraction of total lipids

In the present work, year-round variations in the levels of hydrocoral lipid compounds measured every month were briefly mentioned as seasonal variations. December–February and June–August were considered as the winter and summer periods, respectively. Colonies of the hydrocorals *Millepora dichotoma* Forskål, 1775 and *M. platyphylla* Hemprich & Ehrenberg, 1834 (Cnidaria: Hydrozoa: Anthoathecata: Milleporidae) were collected by SCUBA divers at a depth of 4–6 m in the Nha Trang Bay, the South China Sea (12°17′N and 109°14′E) from January to December 2019. Seven fragments (about 10 × 10 cm) of different colonies of each hydrocoral species were sampled monthly. A total of 84 fragments of each species were collected during the year. Branched fragments of *M. dichotoma* with 16–20 top branches and plate fragments of *M. platyphylla* were cut out from upper parts of colonies. The fragments were transported in seawater, shaded to prevent overexposure to solar radiation and maintain temperatures relatively constant, and brought to the laboratory within 1 h after being collected. In the laboratory, polyps were immediately washed from each colony with water under pressure, suspended and divided into two equal parts. One part of the suspension was dried at 150 °C to a constant dry weight (dr.w.). To obtain total lipids, the second part of the suspension was extracted with a mixture of chloroform and methanol according to Folch et al. (1957), with modifications as described previously (Imbs et al. 2019). Total lipids were evaporated under reduced pressure, weighed, dissolved in chloroform, and stored at −80 °C.

Lipid analysis

The classes of total lipids and polar lipids were separated by thin-layer chromatography (TLC) and quantified by densitometry of TLC plates as described previously (Imbs et al. 2019). For full description of the experiments, see the electronic supplementary material (ESM).

Fatty acids (FA) in the form of methyl esters were analyzed by gas chromatography with a flame ionization detector according to Imbs et al. (2019). The structures of FA as 4,4-dimethyloxazoline (DMOX) derivatives were confirmed by gas chromatography–mass spectrometry as described previously (Imbs et al. 2019). A complete description of all conditions of FA analysis is provided in ESM.

Total lipids were separated by HPLC and lipid molecular species were identified by high-resolution tandem (ion trap–time of flight) mass spectrometry in both positive and negative modes with electrospray ionization (ESI) as described previously (Imbs et al. 2019). A detailed description of the analytical conditions is also provided in ESM. The total abundance of each glycolipid and betaine lipid class and the relative abundance of certain lipid species within each lipid class were calculated according to Rosset et al. (2019).

Statistical analysis

Differences in the mean concentrations of total lipids, lipid classes, and FA were examined with a two-factor fully orthogonal analysis of variance (ANOVA). The factors were month (twelve levels) and species (two levels). Both factors were fixed. In addition, differences in the mean concentrations of lipid molecular species between months for each hydrocoral species were analyzed by one-way ANOVA. Differences in the mean percentage of each lipid molecular species (n = 84) between *M. dichotoma* and *M. platyphylla* were also analyzed by one-way ANOVA. The raw data were used following evaluation of the homogeneity of variances (Levene's test) and the normality of data distribution (Shapiro–Wilk's test). Significant differences between levels within the factors were examined post hoc with Tukey HSD test. Significance of the relationship between the lipid classes of symbiotic dinoflagellates (zooxanthellae) was assessed with Spearman's rank correlation. All statistical analyses were performed using STATISTICA 5.1 (StatSoft, Inc., USA). A probability level of $p < 0.05$ was considered statistically significant. Values are presented as the mean ± standard error.

Results

Total lipids, lipid classes, and fatty acids of holobiont

Data on the monthly content of the total lipids, lipid classes, and FA in both *Millepora* species are presented in the electronic supplementary material (ESM) and visualized in Fig. 1. The average lipid contents in winter (December–February) and summer (June–August) periods and the effects of two factors (hydrocoral species and months of the year) on the lipid levels are summarized in Table 1. Total lipids (TL) of both species contained three storage lipid classes (waxes (WX), triacylglycerols (TG), and monoalkyldiacylglycerols (MADAG)) as well as polar lipids (PL) and sterols (ST), which are structural components of biomembranes. Free fatty acids (FFA) were detected in *M. dichotoma* and *M. platyphylla* in minor quantities, 2.9 ± 0.2 and 1.5 ± 0.1 mg g^{-1}, respectively. The TL content in *M. dichotoma* and *M. platyphylla* was significantly (HSD test, $p < 0.05$) peaking in May–June and June–July (Table 1, ESM Table S1). The content of each lipid class (Fig. 1, ESM Table S2) significantly varied throughout the year and between species (Table 1).

In *M. dichotoma*, amounts of the lipid classes increased from January to June, and the storage classes dominated TL from March to June (Table 1, Fig. 1a). After July, the level of PL remained quite stable and finally exceeded WX and MADAG, which continued to decline until December. By

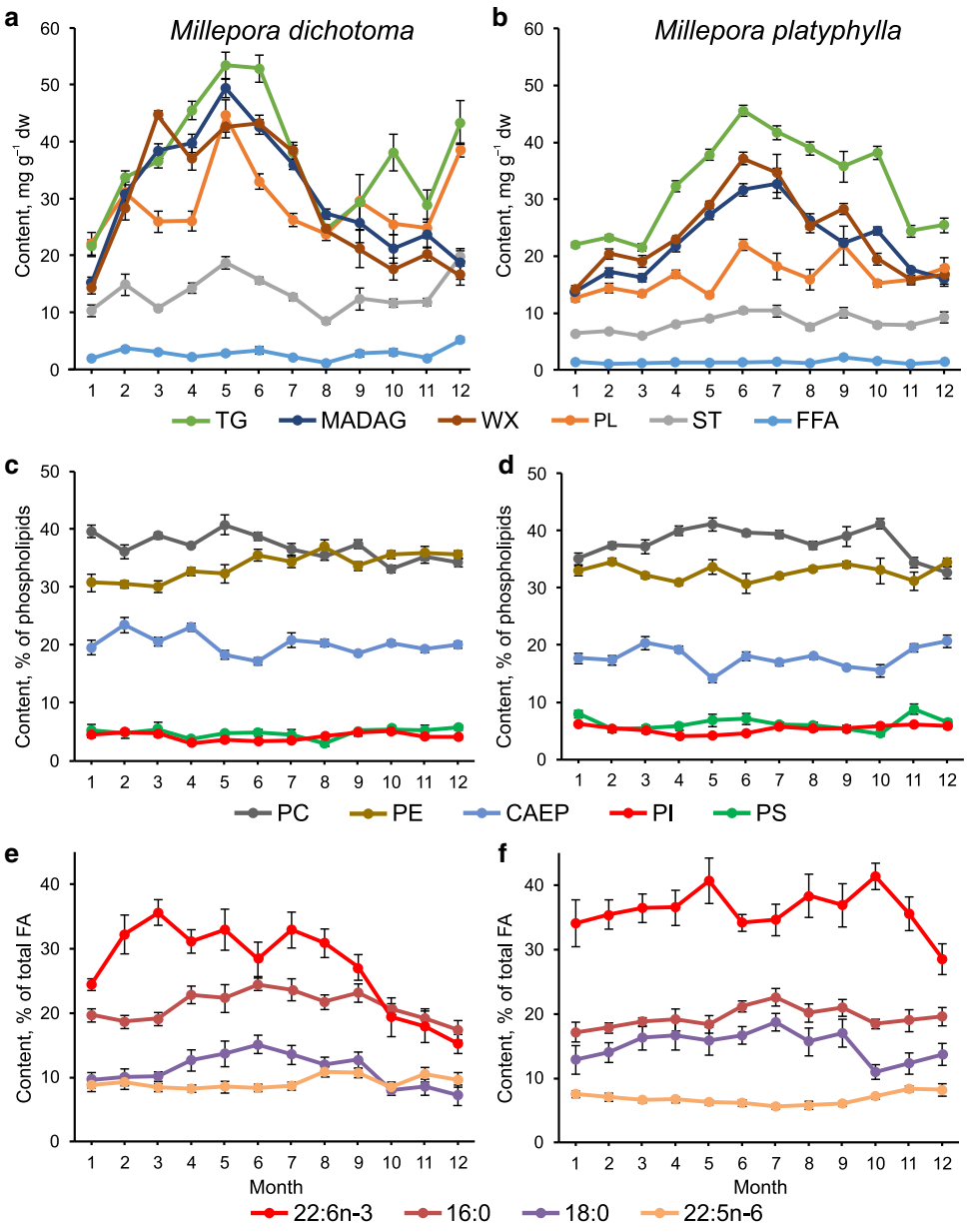

Fig. 1 Variations in contents of (**a**, **b**) total lipid classes (mg g^{-1} d.w.), (**c**, **d**) polar lipid classes (% of polar lipids), and (**e**, **f**) four major fatty acids (% of total FA) in the hydrocorals *Millepora dichotoma* and *M. platyphylla* from January to December. Values are mean ± SE, $n = 7$. For abbreviations, see the text

Table 1 Average contents of total lipids, lipid classes, and selected fatty acids of two *Millepora* hydrocorals in winter and summer, their seasonal peaks, and the results of two-way ANOVA comparing lipid and fatty acid contents among months (twelve levels) and species (two levels)

Lipids	Average seasonal content				Peak	Factors					
	M. dichotoma		*M. platyphylla*			Month		Species		Month × Species	
	Winter	Summer	Winter	Summer		$F_{11,144}$	*p*	$F_{1,144}$	*p*	$F_{11,144}$	*p*
	Content, mg g^{-1} dry tissue										
TL	126.2	140.1	81.5	135.8	S	5.01	***	36.86	***	1.65	ns
WX	19.7	35.5	17.1	32.4	S	75.18	***	91.19	***	19.60	***
MADAG	21.6	35.4	15.6	30.2	S	47.27	***	53.04	***	20.12	***
TG	32.9	38.5	23.6	42.1	S	51.59	***	197.51	***	17.77	***
ST	15.1	12.3	7.5	9.5	M	17.31	***	302.07	***	10.33	***
PL	30.6	27.7	15.0	18.7	M	10.61	***	388.99	***	8.27	***
	Content, % of PL										
PE	32.4	35.7	34.0	32.1	M	3.10	***	8.93	***	3.96	***
PC	36.7	36.9	35.1	38.8	M	10.21	***	7.89	***	6.68	***
PS	5.2	4.2	6.5	6.3	M	2.86	***	22.71	***	1.63	ns
PI	4.5	3.7	5.7	5.2	M	7.20	***	63.42	***	1.99	*
CAEP	21.1	19.5	18.6	17.7	M	6.58	***	40.38	***	3.93	***
	Content, integrated peak values, a.u. 10^8										
MGDG	0.14	0.42	0.20	0.29	S	19.07	***	2.91	ns	2.08	*
DGDG	0.19	0.38	0.46	0.49	S	7.08	***	43.65	***	2.62	***
SQDG	3.57	1.44	1.93	1.88	W	5.11	***	13.74	***	3.85	***
DGCC	2.09	0.83	1.35	0.70	W	17.36	***	48.38	***	2.36	**
	Content, % of total FA										
16:0	18.5	23.2	18.2	21.3	S	3.36	***	8.70	***	0.84	ns
18:0	8.9	13.5	13.5	17.0	S	3.95	***	36.08	***	0.41	ns
18:4n-3	9.2	9.3	7.6	5.8	W	10.81	***	175.99	***	1.16	ns
18:5n-3	23.9	30.7	32.7	35.7	S	18.83	***	33.26	***	1.92	*
20:5n-3	6.9	4.3	3.7	1.6	W	8.01	***	11.50	***	3.66	***
22:5n-6	1.0	0.5	1.3	0.4	W	1.94	*	68.99	***	1.59	ns
22:6n-3	3.0	0.5	1.9	0.8	W	5.16	***	67.87	***	2.94	***

S a summer rise, *W* a winter rise, *M* multidirectional changes, *TL* total lipids, *WX* waxes, *MADAG* monoalkyldiacylglycerols, *TG* triacylglycerols, *ST* sterols, *PL* polar lipids, *MGDG* monogalactosyldiacylglycerol, *DGDG* digalactosyldiacylglycerol, *SQDG* sulfoquinovosyldiacylglycerol, *DGCC* diacylglyceryl-3-*O*-carboxyhydroxymethylcholine

Asterisks indicate significant difference: *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; *ns* not significant

the end of the year, TG again significantly (HSD test, $p < 0.05$) accumulated in *M. dichotoma* tissues after a decrease in August. Multidirectional variations in the ST content were observed throughout the year.

M. platyphylla showed a simpler pattern of lipid dynamics than *M. dichotoma* (Table 1, Fig. 1b). The storage lipid classes dominated TL throughout the year. Each storage lipid class had a clear maximum in summer (HSD test, $p < 0.05$). The levels of structural lipids changed slightly and showed weak maxima in July and September.

Polar lipids of both hydrocoral species include one phosphonolipid class (ceramidaminoethylphosphonate (CAEP)) and four phospholipid classes: ethanolamine, choline, serine, and inositol glycerophospholipids (PE, PC, PS, and PI, respectively) (ESM Table S2). Percentages of PL classes significantly varied throughout the year and between species (Table 1), but their dynamics did not show any clear turning points (Fig. 1c, d). The level of PC was higher (HSD test, $p < 0.05$) than the level of PE in *M. dichotoma* colonies in January–June (Fig. 1c). The same dominance of PC was observed in *M. platyphylla* in February–October (Fig. 1d). No significant (HSD test, $p > 0.05$) differences were observed between the PC and PE levels in other months. The interaction between the

factors (month and species) was not significant for PS (Table 1). Compared to the storage lipids, the PL composition of both hydrocorals was relatively stable throughout the year.

Two saturated FA (16:0 and 18:0) and two PUFA (22:6n-3 and 22:5n-6) were the major components in total FA obtained by the hydrolysis of TL of both hydrocoral species (ESM Tables S3, S4). The level of 22:6n-3 was very high in *M. dichotoma* and *M. platyphylla* (up to 49.7 and 58.0% of total FA, respectively). Amounts of essential C_{20} PUFA, such as 20:4n-6 and 20:5n-3, were small (about 3% of total FA).

In *M. dichotoma*, a difference in 16:0 was found only between June and December; the level of 18:0 significantly (HSD test, $p < 0.05$) differed between June and October–December periods (ESM Table S3). The highest proportion of 22:6n-3 in *M. dichotoma* was recorded between March and August and then, a twofold reduction in 22:6n-3 (Fig. 1e), accompanying a fivefold increase in 20:5n-3 (Fig. 2a) was observed between October and December (HSD test, $p < 0.01$) (ESM Table S3). In *M. platyphylla*, the level of 22:5n-6 in July was higher than that in November and December (HSD test, $p < 0.05$) (ESM Table S4, Fig. 1f). The highest proportion of 20:5n-3 was recorded in winter (HSD test, $p < 0.01$) (Fig. 2b). The interaction between factors (month and species) was not significant for 16:0, 18:0, and 22:5n-6 (Table 1).

In *M. dichotoma* colonies, the ratio 16:0/16:1n-7 was lowest from October to January (4.9–15.2) and significantly (HSD test, $p < 0.05$) differed from that in June–August (110.6–132.5) (ESM Table S3). Despite the ratio 16:0/16:1n-7 varied in *M. platyphylla* from 50.5 in January to 233.9 in July (ESM Table S4), the differences were not significant (HSD test, $p > 0.05$) throughout the year.

Fatty acids and lipid classes of symbiotic dinoflagellates (zooxanthellae)

Hydrocorals of the genus *Millepora* contain symbiotic dinoflagellates. Therefore, the specific lipid markers of these symbionts such as two fatty acids (18:4n-3 and 18:5n-3) (ESM Tables S3, S4), three glycolipids (monogalactosyldiacylglycerol (MGDG), digalactosyldiacylglycerol (DGDG), and sulfoquinovosyldiacylglycerol

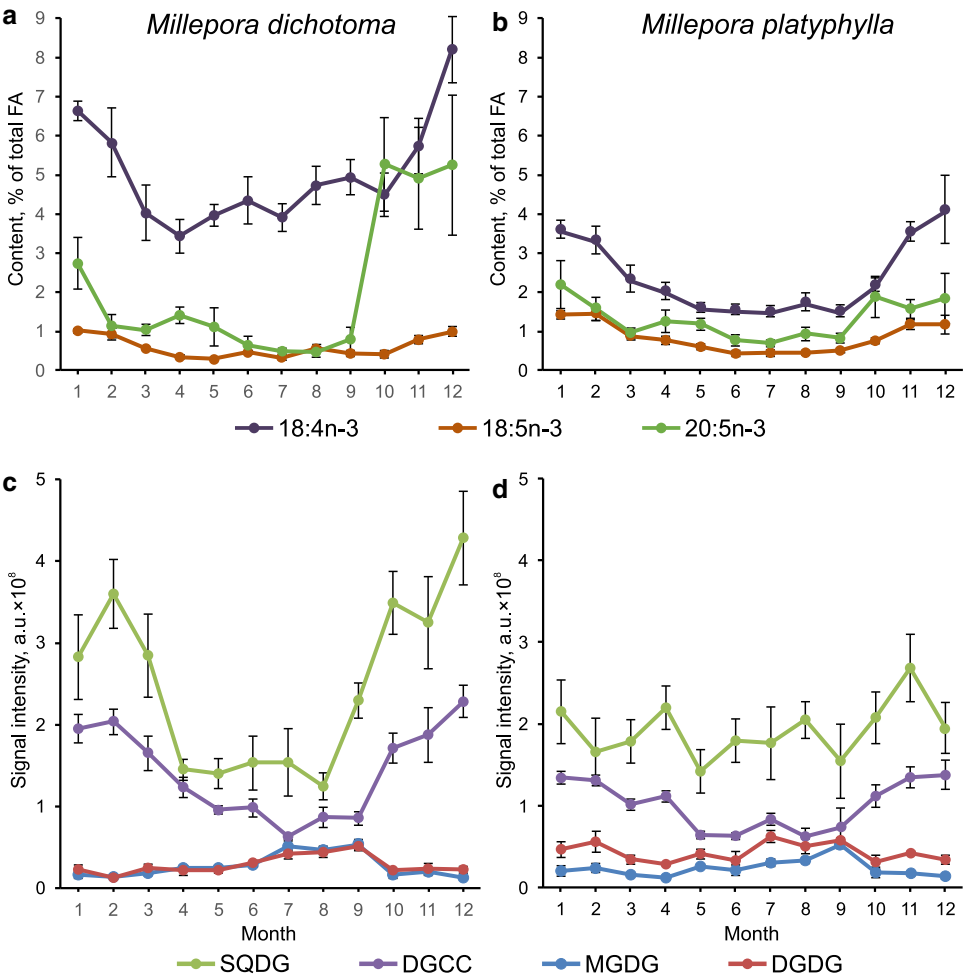

Fig. 2 Variations in contents of (**a**, **b**) fatty acids (18:4n-3, 18:5n-3, and 20:5n-3) (% of total FA), (**c**, **d**) glycolipids (MGDG, DGDG, and SQDG) and betaine lipid DGCC in the hydrocorals *Millepora dichotoma* and *M. platyphylla* from January to December. Values are mean ± SE, $n = 7$. Amounts of MGDG, DGDG, SQDG, and DGCC measured in hydrocoral lipids are expressed as the integrated peak values in the HPLC–MS chromatograms. For abbreviations, see the text

(SQDG)), and one betaine lipid (diacylglyceryl-3-*O*-carboxyhydroxymethylcholine (DGCC)) (ESM Tables S5, S6) were detected in the total lipids and FA of *Millepora*. The study revealed clear seasonal trends in the symbiont markers (Fig. 2).

The percentage of the PUFA markers significantly varied throughout the year and between species (Fig. 2a, b, Table 1), with a peak recorded in winter and the lowest levels in summer (ESM Tables S3, S4). The average levels of 18:5n-3 were similar in both species, whereas the average level of 18:4n-3 in *M. dichotoma* was twice as high as that in *M. platyphylla* (Fig. 2a,b).

Except for MGDG, the lipid markers of symbionts significantly varied throughout the year and between species (Table 1). MGDG and DGDG were highest from July to September, while DGCC was highest from November to February (HSD test, $p < 0.05$) (Fig. 2c,d). The marked (HSD test, $p < 0.05$) depletion of SQDG was observed in *M. dichotoma* in summer (Fig. 2c). MGDG correlated positively with DGDG ($r_s = 0.77$) and negatively with DGCC ($r_s = -0.74$) and SQDG ($r_s = -0.43$) in *M. dichotoma*. Only DGDG clear correlated with MGDG ($r_s = 0.71$) in *M. platyphylla*. The latter hydrocoral species contained approximately two times more DGDG than *M. dichotoma*.

A comparison between lipidomes of two hydrocoral species

The chemical structure and content of 152 molecular species of phosphorus-containing lipids (PE, PC, PS, PI, CAEP), glycolipids (MGDG, DGDG, SQDG), and betaine lipid DGCC were determined in hydrocoral TL by high-resolution mass spectrometry (ESM Table S7). Profiles of DGCC and glycolipids were analyzed for the first time, whereas the structures of PL molecular species had been preliminary described in our recent study (Imbs et al. 2019). Profiles of the molecular species (with the concentration more than 1% of each lipid class) are shown in Figs. 3 and 4. The dominance of PE 16:1e/22:5, PE 18:1e/22:5, PC 16:0e/22:6, PC 16:0e/22:5, PS 20:0/22:4, PI 20:0/22:4, CAEP 18:2b/16:0, and CAEP 18:1b/16:0 should be regarded as characteristic of the lipidome of the genus *Millepora*.

FA are unevenly distributed among the lipid classes. Molecules with 22:4 dominated PS and PI, while molecules with 22:5 dominated PE. Both 22:5 and 22:6 formed the majority of PC molecules, and DGCC molecules were mainly formed by 22:6. Most of MGDG and DGDG molecules contained 18:4 and 18:5. Saturated and monounsaturated $C_{14\text{-}18}$ FA were mainly included in SQDG and CAEP. Very-long-chain PUFA, 28:7 and 28:8, were detected only in DGCC. An identical FA distribution among the lipid classes was found in both hydrocorals.

Significant differences in the percentage of most molecular species were found between *M. dichotoma* and *M. platyphylla* (ESM Table S7); nevertheless, the lipidomic patters of the two *Millepora* species were visually similar (Figs. 3, 4). The *M. dichotoma* lipids contained more PE 16:1e/22:5 and CAEP 18:2b/16:0, but less PE 19:1e/22:5 and CAEP 18:1b/16:0 (Fig. 3). MGDG 18:4/18:4 reached higher level in *M. dichotoma*, whereas 18:4/18:5 dominated MGDG in *M. platyphylla* (Fig. 4). A high level of DGDG molecules with $C_{20\text{-}22}$ FA in *M. platyphylla* (40.8%) distinguished this species from *M. dichotoma* (5.4%) (Fig. 4). For *M. dichotoma*, DGDG with $C_{20\text{–}22}$ FA was absent from most specimens but detected in some specimens at a concentration reaching 46% of total DGDG. In contrast, all the *M. platyphylla* specimens contained DGDG with $C_{20\text{–}22}$ FA, which ranged from 6.0 to 73.7% of total DGDG.

Seasonal variations in lipid molecular species

The monthly levels of each lipid molecular species in *M. dichotoma* and *M. platyphylla* are provided in ESM Table S8 and ESM Table S9, respectively. Most of these molecular species significantly varied throughout the year, but mainly without any clear patterns (ESM Tables S8, S9). The annual dynamics of the abundant molecular species are shown in Figs 5, 6, 7. The average levels of several major molecular species of phospholipids in winter and summer periods are presented in Table 2.

In both hydrocorals, maximum concentrations of PE 18:1e/22:5 were recorded from May to September (HSD test, $p < 0.05$) (Fig. 5a,d). A significant (HSD test, $p < 0.05$) increase in the total PE with C_{18} alkyl groups from 48.0 ± 1.2% in January to 65.6 ± 1.1% in July was observed in *M. platyphylla*. In *M. dichotoma*, the level of PE with C_{18} alkyl groups raised from 48.1 ± 1.3% in January to 54.9 ± 2.0% in June and then dropped to 36.4 ± 1.4% in December. Simultaneously, the level of PE with other alkyl groups decreased in summer.

In both hydrocorals, maximum concentrations of alkylacyl PC 16:0e/22:5 and 16:0e/22:6 were recorded from May to September (HSD test, $p < 0.05$) (Fig. 5b,e). The summer increase in alkylacyl PC coincided with a twofold reduction in diacyl PC compared to the winter period. The main diacyl PC was highly unsaturated PC 22:6/22:6, which reduced to a third of the value recorded in February by July or September in *M. dichotoma* or *M. platyphylla*, respectively (ESM Tables S8, S9). Like C_{18} alkyl PE and alkylacyl PC, the maximum concentration of CAEP 18:1b/16:0 was observed in both hydrocorals from May to September and it was accompanied by a decrease in CAEP 18:2b/16:0 with more unsaturated sphingosine base (Fig. 6b,e).

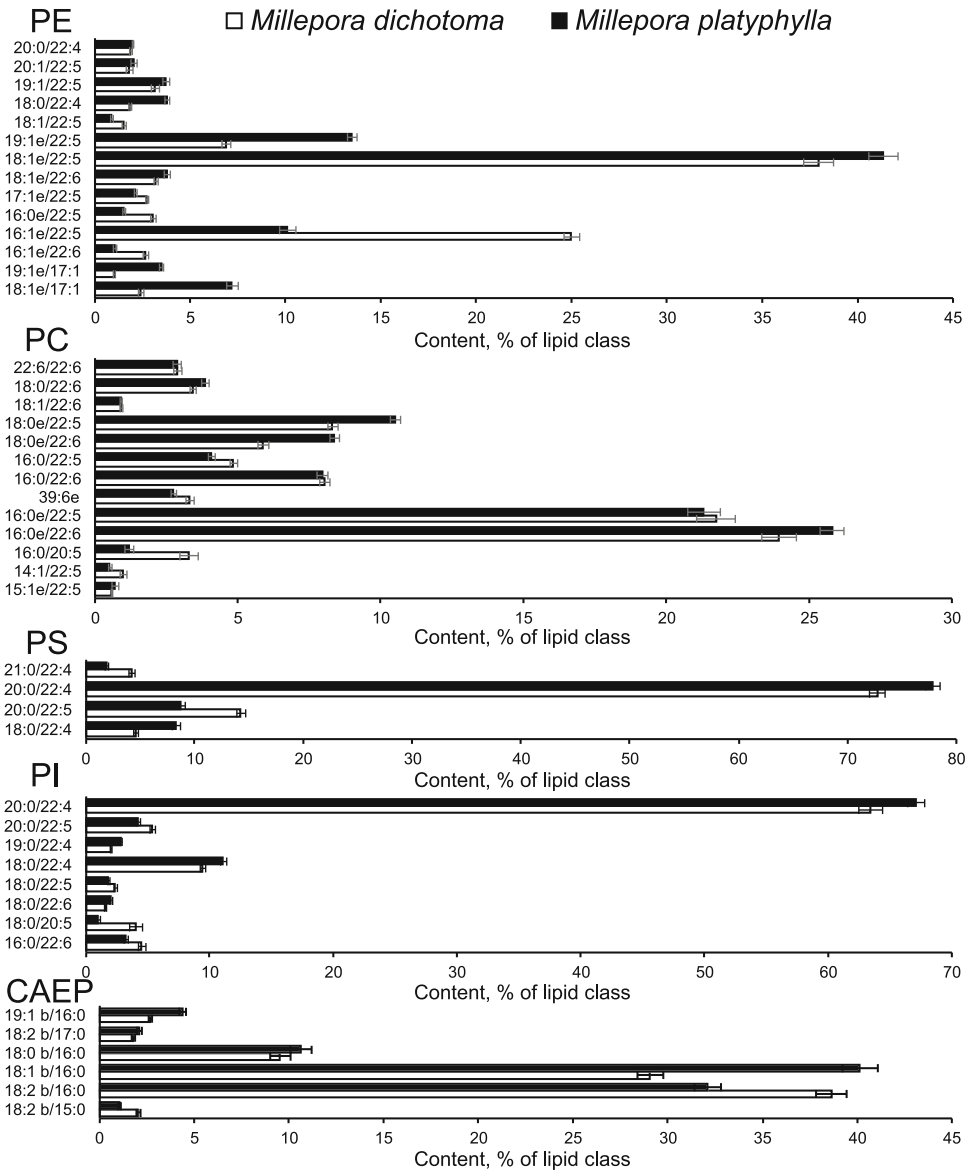

Fig. 3 A comparison of contents (% of each lipid class) of the molecular species of phosphorus-containing lipids (PE, PC, PS, PI, and CAEP) in the hydrocorals *Millepora dichotoma* (white bars) and *M. platyphylla* (black bars). Values are mean ± SE, $n = 84$. For abbreviations, see the text

The levels of the abundant PS 20:0/22:4 and PI 20:0/22:4 significantly varied throughout the year (Table 2) but these variations had no clear patterns (Fig. 6a,d). The level of PI 18:0/20:5 significantly increased (HSD test, $p < 0.05$) in *M. dichotoma* after September (Fig. 6a).

The study revealed clear seasonal trends in the major molecular species of three structural lipids (PE, PC, and CAEP) of the *Millepora* host tissues (Table 2), but the trends in lipid molecular species of symbiotic dinoflagellates were not as obvious.

The level of the main betaine molecular species, DGCC 16:0/22:6, had local peaks in May and September (Fig. 6c,f). In *M. platyphylla* colonies, both MGDG and DGDG contained more 18:4/18:5 than 18:4/18:4 throughout the year (Fig. 7d,e). The same dominance was observed in *M. dichotoma* colonies from April to December (Fig. 7a,b). In contrast to the level of each glycolipid classes, which fluently changed throughout the year (Fig. 2c,d), the proportions of glycolipid molecular species showed sharp and random variations (Fig. 7, ESM Tables S8, S9). Moreover, the level of each glycolipid molecular species varied greatly between the seven repetitions collected in the same month. For example, the percentage of MGDG 18:4/18:5 ranged from 9.0 to 62.0%, MGDG 18:4/18:4—from 9.0 to 61.7%, DGDG 18:4/18:5—from 1.2 to 51.4%, and DGDG 18:4/18:4—from 2.4 to 79.2% in the *M. dichotoma* samples. In general, the proportions of most molecular species of glycolipids fluctuated randomly from colony to colony and had no clear seasonal pattern.

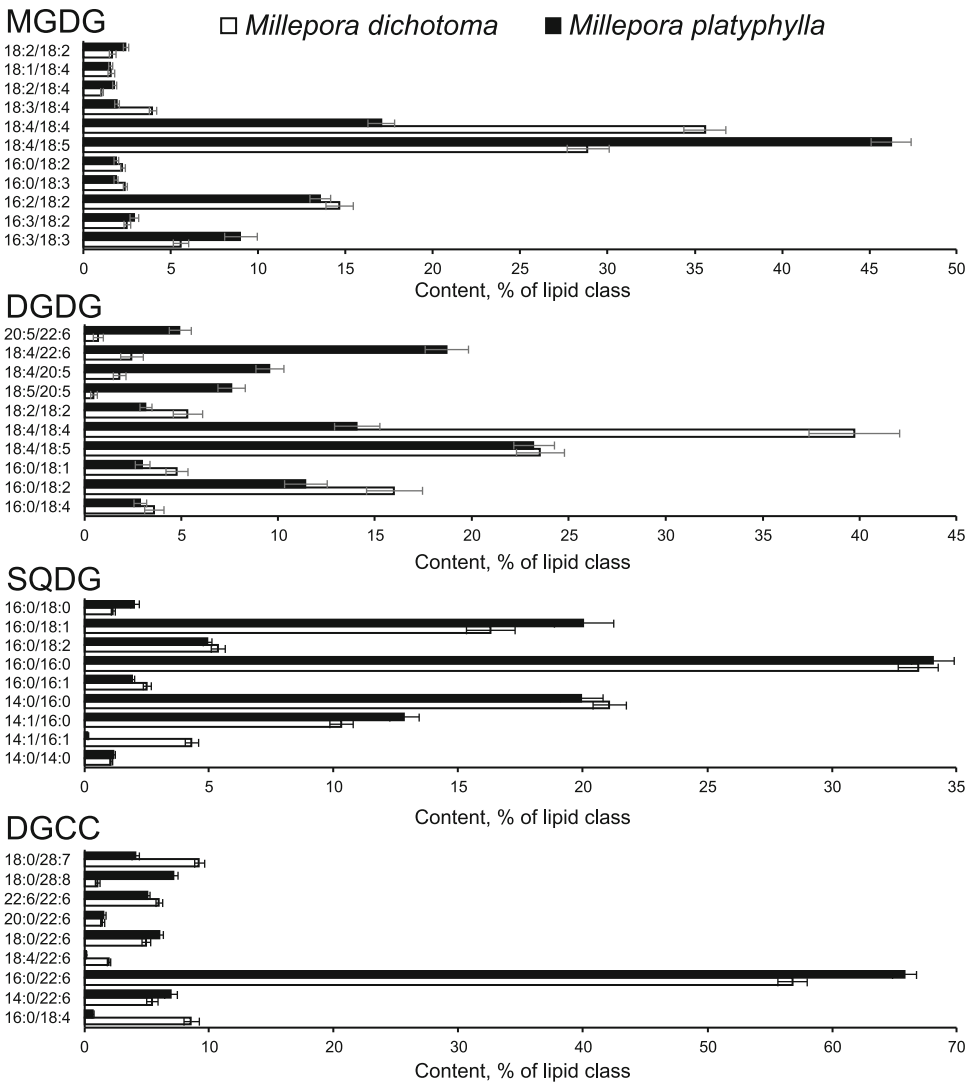

Fig. 4 A comparison of contents (% of each lipid class) of the molecular species of glycolipids (MGDG, DGDG, and SQDG) and betaine lipid DGCC in the hydrocorals *Millepora dichotoma* (white bars) and *M. platyphylla* (black bars). Values are mean ± SE, $n = 84$. For abbreviations, see the text

Discussion

Total lipids, lipid classes, and fatty acids of holobiont

Symbiotic coral colonies are known to depend on water temperature and solar radiation (Dubinsky and Stambler 2011). In the habitat of the hydrocoral species studied (the Nha Trang Bay, the South China Sea, Vietnam), water temperature reaches 28–30 °C in April–September and drops to 24–25 °C in December–January, while maximum of solar radiation is observed between February and April (Weather Spark 2020). In both hydrocoral species studied, the content of total lipid had maximum values in the summer and coincided only with the maximum of water temperature. The similar increase in total lipids in summer was recorded for several symbiotic coral species, as well as for shallow-water asymbiotic species, and was possibly associated with higher food availability (Ben-David-Zaslow and Benayahu 1999; Oku et al. 2003; Rossi et al. 2006; McCauley et al. 2018). The accumulation of total lipids by *Millepora* in the summer may be related to feeding on zooplankton (Lewis 1992), which become more abundant in summer after the spring outbreak of phytoplankton.

Storage and structural lipids play different roles in the total lipid dynamics of *Millepora*. The seasonal increase in total lipids occurred primarily due to the accumulation of the storage lipids against a background of the relatively stable year-round level of the structural lipids, which form a lipid bilayer of cell membranes (Dowhan et al. 2008). This observation confirms that the ratio of storage to structural lipids (Saunders et al. 2005; Hinrichs et al. 2013) is more preferable than the amounts of total lipids (Ben-David-Zaslow and Benayahu 1999; Rossi et al. 2006; Rossi and Tsounis 2007; McCauley et al. 2018) to assess the energy status of *Millepora*. PUFA predominantly concentrated within the polar lipids and therefore, the seasonal

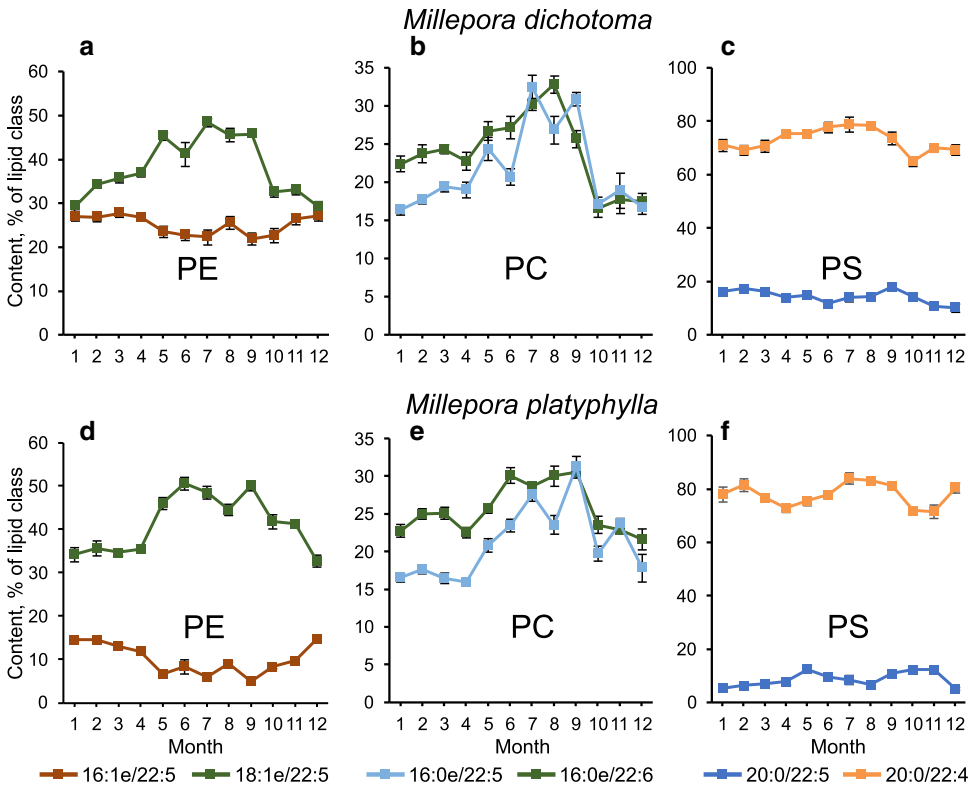

Fig. 5 Variations in contents (% of each lipid class, mean ± SE, $n = 7$) of major phospholipid molecular species such as (**a**, **d**) PE, (**b**, **e**) PC, and (**c**, **f**) PS in the hydrocorals *Millepora dichotoma* and *M. platyphylla* from January to December. For abbreviations, see the text

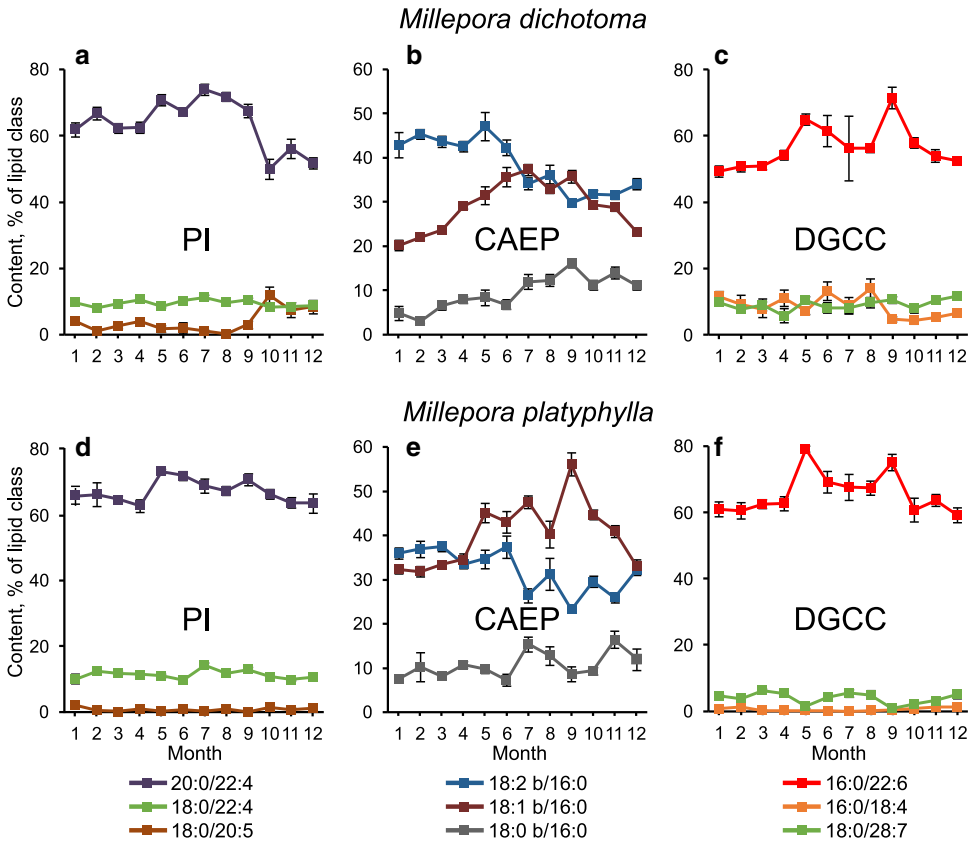

Fig. 6 Variations in contents (% of each lipid class, mean ± SE, $n = 7$) of major molecular species of (**a**, **d**) phospholipid PI, (**b**, **e**) phosphonolipid CAEP, and (**c**, **f**) betaine lipid DGCC in the hydrocorals *Millepora dichotoma* and *M. platyphylla* from January to December. For abbreviations, see the text

increase in saturated 16:0 and 18:0 may reflect the summer rise of the storage lipid level in *Millepora*.

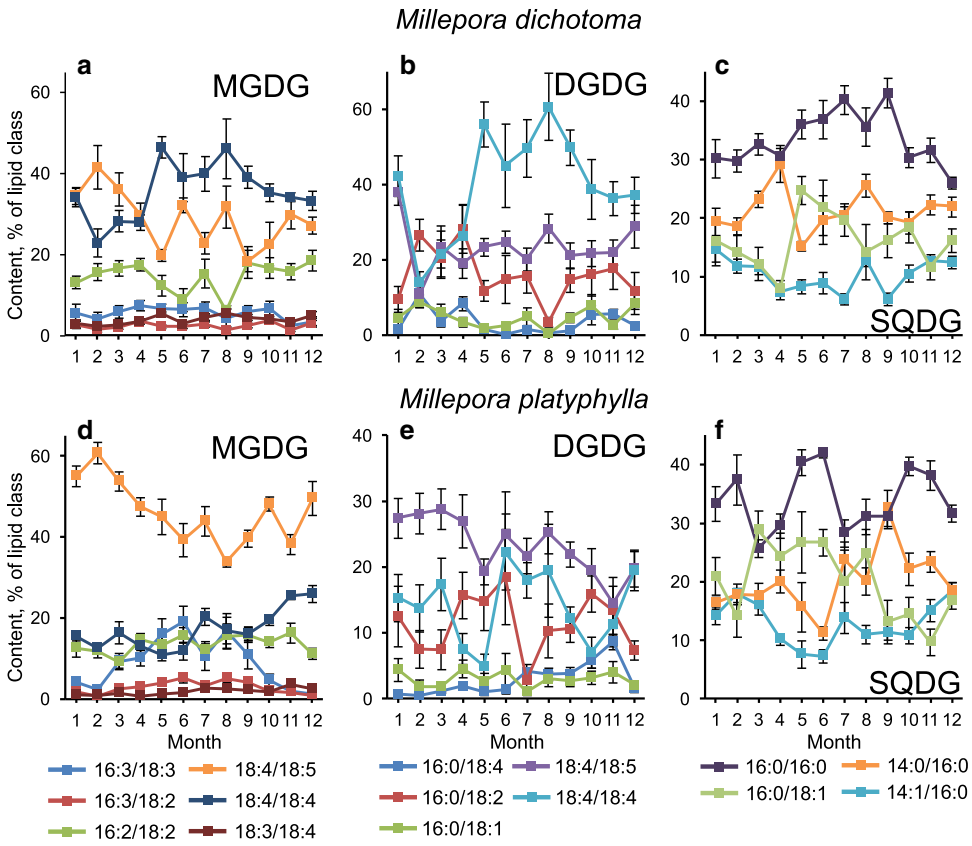

Fig. 7 Variations in contents (% of each lipid class, mean ± SE, $n = 7$) of major molecular species of glycolipids (**a**, **d**) MGDG, (**b**, **e**) DGDG, and (**c**, **f**) SQDG in the hydrocorals *Millepora dichotoma* and *M. platyphylla* from January to December. For abbreviations, see the text

Table 2 Average contents (% of the polar lipid class) of selected lipid molecular species of two *Millepora* species in winter and summer, their seasonal peaks, and the results of one-way ANOVA comparing contents of molecular species among months (twelve levels) throughout the year

Lipid molecular species	*Millepora dichotoma*					*Millepora platyphylla*				
	Seasonal values		Peak	$F_{11,72}$	p	Seasonal values		Peak	$F_{11,72}$	p
	Winter	Summer				Winter	Summer			
PE 18:1e/22:5	30.9	45.0	S	31.17	***	34.2	47.9	S	26.11	***
PC 16:0e/22:6	21.2	30.1	S	17.71	***	23.2	29.6	S	13.16	***
PS 20:0/22:4	69.8	78.2	M	4.67	***	79.9	81.6	M	5.44	***
PI 20:0/22:4	60.1	70.9	M	2.37	*	65.1	69.4	M	2.80	**
CAEP 18:2b/16:0	40.8	37.6	W	12.35	***	35.0	31.7	W	7.37	***
CAEP 18:1b/16:0	21.8	35.3	S	21.57	***	32.4	43.6	S	17.45	***

S a summer rise, *W* a winter rise, *M* multidirectional changes, PE, PC, PS, and PI, ethanolamine-, choline-, serine-, and inositol glycerophospholipids, respectively, *CAEP* ceramidaminoethylphosphonate. Asterisks indicate significant difference: *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$

In corals, lipids are accumulated during gonad maturation and spent intensively in the spawning period to cover energy demands of invertebrates (Ward 1995; Leuzinger et al. 2003; Viladrich et al. 2016; Grinyó et al. 2018). Moreover, reproductive materials released from coral colonies are very rich in lipids, primarily WX or TG (Arai et al. 1993; Figueiredo et al. 2012). Hence, a release of reproductive materials can reduce the content of lipids such as, first of all, storage lipids after spawning. The sexual reproduction in *Millepora* is seasonal, beginning with the appearance of ampullae and ending with the release of planktonic medusae in April and May (Taiwan), or in April–July (Barbados), or in June–March (Curacao) (Lewis 2006). In the previous study on *M. dichotoma*, the reduction in the content of storage lipids in August and the change in the PE/PC ratio in July were explained by maturation and spawning (Imbs et al. 2020). In the present study, the same changes in the storage and structural lipids

of *M. dichotoma* were observed in the same months. In *M. platyphylla* colonies, the same lipid changes began in November. The energy demands and the release of planktonic medusae can cause the loss of storage lipids. Possibly, the changes in the ratio of main structural lipids (PE/PC) of *Millepora* can be attributed to remodeling of cell membranes during the formation of reproductive materials.

The FA composition of total lipids is used to identify the major type of food ingested (Dalsgaard et al. 2003; Arts et al. 2009; Naumann et al. 2015) and to estimate the impact of heterotrophic and autotrophic nutrition in corals (Teece et al. 2011; Seemann et al. 2013). Animals having a 16:0/16:1n-7 ratio of about 1 and a high concentration of 20:5n-3 feed predominantly on diatoms (Kharlamenko et al. 1995). High values of the 16:0/16:1n-7 ratio (up to 200) and low concentrations of 20:5n-3 (about 2% of total FA) in *Millepora* show that diatoms are a minor food source for these hydrocorals throughout the year. Nevertheless, the rise of 20:5n-3 level (up to 5% of total FA) and the considerable depletion of the 16:0/16:1n-7 ratio (5–15) in *M. dichotoma* during October–January indicate that this hydrocoral can shift its food profile and consumes more diatoms than *M. platyphylla*, when water temperature is relatively low.

The considerable level of 22:6n-3 in *Millepora* distinguishes them from other corals and hydrocorals (Imbs et al. 2019). Symbiotic dinoflagellates (zooxanthellae) contribute both 22:6n-3 and 18:4n-3 to total FA of *Millepora*. The levels of 22:6n-3 and 18:4n-3 in pure zooxanthellae are close (Treignier et al. 2008; Imbs et al. 2010a). We found that total FA of *Millepora* contained six times more 22:6n-3 than 18:4n-3, and therefore, zooxanthellae cannot be responsible for the high level of 22:6n-3 observed in *Millepora*. Zooplankton is characterized by high amounts of 22:6n-3 (Treignier et al. 2008). In some cases, 22:6n-3 is used as a marker for carnivores (Kharlamenko et al. 1995). Large amounts of 22:6n-3 in *Millepora* formally confirm the hydrocorals' predation on zooplankton (Lewis 1992, 2006). The summer rise of 22:6n-3 level coincided with the increase in total lipids, which had been previously explained by feeding on zooplankton.

On the other hand, we assume that 22:6n-3 is mainly synthesized in hydrocoral tissues. It has been recently supposed that *Millepora* readily converts 20:4n-6 into 22:5n-6 and 20:5n-3 into 22:6 n-3 (Imbs et al. 2019). Therefore, $C_{20–22}$ PUFA cannot be reliable markers for identifying food of *Millepora*. The fast metabolism of dietary FA by consumer (Iverson 2008) may be a serious obstacle to use C_{20-22} PUFA as trophic markers in case of *Millepora*.

Lipid molecular species of host tissues

The composition of polar lipid molecular species of *Millepora* have been described recently (Imbs et al. 2019). Polyps of *Millepora* are inhabited by endocellular symbiotic dinoflagellates, and, thus, holobiont lipids include both symbiont and host lipids. It is possible to recognize host's polar lipids in total lipids the holobiont without separation of symbionts (Garrett et al. 2013; Sikorskaya and Imbs 2018) due to the fundamental differences in the profile of polar lipids classes between cnidarians (Joseph 1979) and microalgae (Li-Beisson et al. 2019). Phosphorus-containing lipids, such as PC, PE, PI, PS, and CAEP, are the major structural lipids of the host tissues in hydrocorals and corals (Imbs 2013; Imbs et al. 2019). Diacyl PC was found in zooxanthellae (Awai et al. 2012), but alkylacyl PC (about 70% of total PC of *Millepora*) cannot be synthesized by zooxanthellae, and hence, characterize only the host's polar lipidome. The lipidome profiles of the host tissues in the two *Millepora* species were quite similar. The difference in the content of PE 16:1e/22:5, PE 19:1e/22:5, CAEP 18:2b/16:0, and CAEP 18:1b/16:0 is species specific. The possible causes of the seasonal variations in the dominant molecular species are considered below.

We supposed that seasonal variations in several molecular species of the host's lipidome may be caused by seasonal variations in dietary PUFA, which then are incorporated into polar lipid molecules of the consumer. Such intensive incorporation was earlier reported for nudibranch molluscs that fed on corals (Imbs and Grigorchuk 2019). Most phospholipid molecular species of *Millepora* contain C_{22} PUFA, such as 22:4n-6, 22:5n-6, and 22:6n-3. The latter acid can be partly obtained by feeding on zooplankton (Treignier et al. 2008). Diatoms are the main source of 20:5n-3 (Kharlamenko et al. 1995). If dietary 22:6n-3 and 20:5n-3 are incorporated into structural lipid molecules, the increase in PC 16:0e/22:6 in summer and PI 18:0/20:5 in October–January may be explained by feeding on zooplankton, which is partly substituted by diatoms in the cold period.

Fatty acids 22:4n-6 and 22:5n-6 are almost absent from food sources of hydrocorals and are synthesized by the host from 20:4n-6 (Imbs et al. 2019). Invertebrates can synthesize C_{20} PUFA (Monroig et al. 2013), but the origin of dietary 20:4n-6 in corals still remains unclear (Imbs and Latyshev 2012). The seasonal changes in the level of 20:4n-6, which are essential for animals, may be responsible for dynamics of PE 16:1e/22:5, PE 18:1e/22:5, PC 16:0e/22:5, and PI 20:0/22:4.

The fluidity of biomembranes is largely determined by the degree of membrane FA unsaturation. An increase in the proportion of unsaturated FA is a well-known adaptive response of marine organisms to a decrease in water

temperature (Hazel 1995; Sargent et al. 2002). The insignificant reduction in the proportion of total saturated FA in one of the hydrocoral species in winter followed this adaptive response, but the proportion of PE and PC with highly unsaturated acyl groups slightly reduced, when water temperature dropped.

Most molecules of hydrocoral phospholipids contain one acyl group (or FA group) and one alkyl group. We found that a seasonal change affected rather the chemical structure of alkyl groups of phospholipid molecules than the unsaturation degree of their acyl groups. PE with C_{18} alkyl groups and alkylacyl PC increased, whereas PE with C_{16} alkyl groups and diacyl PC declined in both *Millepora* species in the summer. Such observations were earlier reported by Latyshev et al. (1986), who found that *Lobophytum* soft corals in summer contained PE and PC in plasmalogen form, which was not detected in winter. Molecules of CAEP are composed of a sphingosine base and an acyl group. CAEP molecules of both hydrocoral species showed a considerable summer increase in the degree of saturation of the sphingosine bases in contrast to the stable FA composition. We suppose that the seasonal optimization of structural lipids in *Millepora* occurs through adjustment of the acyl chain length of PE, the alkylacyl/diacyl ratio of PC, and the saturation degree of sphingosine bases of CAEP. The other two phospholipids (PS and PI) contained only diacyl molecules, and their seasonal changes were multidirectional and species specific.

Thus, lipidomic approach helps clarify the changes that occur in structural lipids and biomembranes at the molecular level. Remodeling of the three major classes of structural lipids (PE, PC, and CAEP) may be considered as an adaptive response of *Millepora* to seasonal variations in environmental factors (Ernst et al. 2016).

Lipid molecular species of zooxanthellae

Diacyl PC is the most abundant phospholipid of cultured strains of symbiotic dinoflagellates (Awai et al. 2012). Up to 50% of acyl groups of PC in dinoflagellates are comprised of 22:6n-3 (Leblond and Chapman 2000; Awai et al. 2012), which is considered as a FA marker of dinoflagellates (Dalsgaard et al. 2003). We assume that the highly unsaturated molecular species diacyl PC 22:6/22:6 detected in total lipids of *Millepora* originated from zooxanthellae lipids. The PC 22:6/22:6 was not detected in the asymbiotic hydrocoral species (Imbs et al. 2019) and therefore can be suggested as an additional lipidomic marker of symbiotic dinoflagellates in hydrocorals.

Glycolipids (MGDG, DGDG, and SQDG) and betaine lipids constitute the principal lipid classes in plants including, in particular, zooxanthellae. A lipidomic analysis of *Millepora* confirmed that 18:4n-3 and 18:5n-3 concentrate in two glycolipids, MGDG and DGDG (Bishop and Kenrick 1980; Leblond and Chapman 2000; Leblond et al. 2015). The inverse correlation between these C_{18} PUFA and two glycolipids observed in both *Millepora* species indicates that a part of C_{18} PUFA does not belong to zooxanthellae glycolipids but associated with storage lipids of the holobiont (Sikorskaya and Imbs 2018). It was suggested that zooxanthellae can transfer their PUFA to the host coral tissues (Papina et al. 2003; Imbs et al. 2010b). More intensive transfer of PUFA from symbionts to the host in winter, when dietary PUFA from plankton are more limited, may lead to the accumulation of C_{18} PUFA in storage lipids of *Millepora* and increase the level of C_{18} PUFA, when the level of MGDG + DGDG is lowest.

Glycolipids mainly perform a function of structural lipids of chloroplasts, whereas a part of glycolipids is important in the binding and stabilization of proteins associated with photosystem II (PSII) (Kern et al. 2009; Mizusawa and Wada 2012). In the winter, both *Millepora* species showed an increase in DGCC and PC 22:6/22:6; the level of SQDG considerably increased in *M. dichotoma*. The seasonal increase in the content of charged bilayer-forming lipids (SQDG, DGCC, and PC) may result from the increase in the packing density of lipid molecules in symbiont's membranes as the water temperature dropped. MGDG molecules cannot form lipid bilayers and were identified in the protein–cofactor complex PSII from thylakoid membranes (Mizusawa and Wada 2012). The stable MGDG/DGDG ratio throughout the year and the higher level of MGDG + DGDG in both *Millepora* species in the summer can be explained by the increase in the PSII complex content.

The higher content of DGDG and large amounts of DGDG molecules with $C_{20\text{-}22}$ PUFA distinguished *M. platyphylla* from *M. dichotoma*. As was shown earlier, DGDG with C_{20} PUFA (e.g., 20:5/18:5 and 20:5/18:4) is abundant in many marine dinoflagellate strains, but some strains lack these DGDG molecular species (Gray et al. 2009; Leblond et al. 2010). Furthermore, DGDG with C_{22} PUFA was not earlier described from marine dinoflagellates. If the presence of DGDG with $C_{20\text{-}22}$ PUFA is species specific for dinoflagellates, the presence of different zooxanthellae species in *M. dichotoma* and *M. platyphylla* should be recognized. The difference in photosynthetic community between two hydrocoral species is also confirmed by different 18:4n-3 levels, MGDG/DGDG ratios, and annual dynamics of SQDG.

Despite the smooth seasonal variations in the lipid classes of zooxanthellae, the variations in their major molecular species proved to be surprisingly chaotic. Leblond et al. (2015) showed that the proportion between molecular species of MGDG and DGDG is largely

dependent on the temperature during growth and other factors. In the case of *Millepora*, sharp differences in the proportions of molecular species of zooxanthellae lipids were observed between colonies collected at the same place and time. It seems likely that *Millepora* precisely controls the content of each lipid class of the holobiont and lipid molecular species of the host. Nevertheless, the content of each lipid molecular species of symbionts seems to be not as important for *Millepora*.

In contrast to cultured dinoflagellates, symbiotic dinoflagellates are located inside host's gastrodermal cells, forming symbiosomes (Davy et al. 2012). It is possible that each hydrocoral host creates specific environmental conditions inside the symbiosoms. These local environmental conditions may be unique for each colony and determine the proportion of symbiont's lipid molecular species that is specific for this colony. This hypothesis about a "mosaic" symbiotic lipidome within a hydrocoral population can be verified by comparing symbiotic lipidomes between parts of the same colony and between parts of different colonies.

Thus, the year-round study of the lipid molecular species and the integral lipid indices elucidated the range and possible causes of seasonal lipid variations in *Millepora* and may be useful for future analysis of trophic and symbiont–host interactions of these hydrocorals.

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Compliance with ethical standards

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

Ethical approval This article does not contain any studies with human participants or vertebrate animals performed by any of the authors. All the experiments on invertebrate animals were reviewed and approved by the Ethics Committee of the A.V. Zhirmunsky National Scientific Center of Marine Biology, Far Eastern Branch, Russian Academy of Sciences, and conducted in agreement with the principles expressed in the Declaration of Helsinki.

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