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Monthly variation in the lipid composition and content of Pacific oysters, *Crassostrea gigas*, cultured in Van Don, Quang Ninh

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ABSTRACT

We conduct a study to investigate the year-round fluctuation of lipid composition and content in Pacific oysters (Crassostrea gigas) cultured in Van Don, Quang Ninh, for the first time. Our results showed that the total lipid content of oysters ranged from 1% to 1.6%, with the highest levels observed during their pre-reproductive period (July and December). Polyunsaturated fatty acids were the most abundant type of lipids in oysters, ranging from 41.66% to 53.36%. We identified six lipid classes in Pacific oysters, with the three dominant classes being PoL, ST, and TG, which exhibited significant variation, with the highest variability observed in May and June and the lowest in July. The primary fatty acids in oyster lipids were 14:0, 16:0, 18:0, 18:1n-7, 20:4n-6, 20:5n-3, and 22:6n-3. In summary, our study provides novel insights into the lipid composition and content of Pacific oysters cultured in Van Don, Quang Ninh. The results demonstrate the temporal variability in lipid classes and fatty acid composition throughout the year, with the highest lipid content observed during the pre-reproductive period. These findings could contribute to better understanding the nutritional value of Pacific oysters and inform future aquaculture practices.

Keywords: Lipid, fatty acids, lipids class, EPA, DHA, Oysters, Van Don.

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INTRODUCTION

Lipids are natural organic compounds abundant in the living cells of animals, plants, and microorganisms. These organic compounds play an essential role in the physiology and pathophysiology of living organisms. They are part of the structure of cell membranes and are stored in tissues as a reserve of energy. They are also a medium for dissolving fat-soluble vitamins and an important energy source in the daily diet [1–3]. According to research by Kathleen M. E., several compounds belonging to different lipid classes act as signaling molecules and cellular messengers. These compounds include sphingosine 1-phosphate, diacylglycerol, and phosphatidylinositol phosphate; prostaglandins; steroid hormones such as estrogen, testosterone, and cortisol; and oxysterols [4].

The Pacific oyster (*Magallana gigas* or *Crassostrea gigas*) belongs to the animal kingdom, phylum Mollusca, class Bivalvia, order Ostreoida, family Ostreide, genus Magallana [5–7]. *M.gigas* are found primarily in the Pacific Ocean, but they are temperature and salt tolerant (-2)–36^{o}C and 5–45^{o}F; as such *M.gigas* oysters can and are being farmed in many countries around the world, including Vietnam [8, 9].

Oysters are high in protein, amino acids, and fatty acids, particularly those that help the body's metabolism. It is a food with high nutritional and pharmacological value due to its preventive and curative effects on many diseases, especially cancer. Oleic and linolenic fatty acids, mainly monounsaturated, can help the body fight breast cancer metastasis. Unsaturated fatty acids, especially ω3 and ω6, can treat cardiovascular diseases and high blood pressure, and control cholesterol. Oysters also contain other substances such as vitamins and essential trace elements: Cu, Zn, Fe, Mn, Se, and the iodine content in oysters is 200 times higher than that of cow's milk and egg white. In particular, oysters have a high zinc content. For every 100g of fresh oysters, the oyster's soft tissue contains up to 47.8 mg of zinc [10, 11].

Since the second half of the twentieth century, researchers have studied oyster species' lipids and fatty acids, particularly Pacific oysters. In 1974, Watanabe *et al*. studied the total lipids and fatty acids of two species of American (*Crassostrea virginica*) and European (*Ostrea edulis*) oysters [12]. In 2004, the seasonal variation of total lipid and fatty acid composition in the oyster *Crassostrea rhizophora* collected in Rio de Janeiro, Brazil, was researched. The results show that, in the same year, the total lipid content of the studied samples according to different seasons had no statistical difference ($P > 0.05$), with the content reaching 1.7% [13]. Another study on farmed and wild *Crassostrea madrasensis* collected in Gangewadiya, Sri Lanka, was conducted in 2019 and found an average total lipid content of 1.28 ± 0.02%. Variations in the total lipid content of these two species were also observed [14].

In 1999, lipid and fatty acid compositions of polar and neutral lipids in the muscle, digestive glands, and gonads of the Pacific oyster *Crassostrea gigas* were analyzed. The results indicated that the polar lipids of all three components under artificial culture conditions were similar to those in natural conditions. The polar lipids fluctuated slightly, with the mean value of phosphatidylcholine (PC), phosphatidyl ethanolamine (PE), phosphatidyl inositol (PI), ceramide amino ethyl phosphonate (CAEP), and phosphatidyl serine (PS) being 34%, 25%, 11%, and 9.5% respectively [15].

After investigating the total lipids, phospholipids, fatty acids, and sterols of *Crassostrea gigas* in the Bay of Bourgneuf, France, for four consecutive seasons, Flora Dagorn et al., (2016) showed that the total lipid content (% dry weight) varied from 7.1% (winter) to 8.6% (spring). Polar lipids (PLs) accounted for 28.1% (spring) to 50.4% (winter). Phosphatidylcholine was the predominant PL throughout the year (up to 74% of total PL in winter). Thirty-seven fatty acids were identified in PLs. Twenty free sterols were identified, including cholesterol accounting for 29.9% of total sterols and

phytosterols, accounting for about 33% [16]. Eicosapentaenoic acid (20:5n-3 EPA/7.53% to 14.5%) and docosahexaenoic acid (22:6n-3 DHA/5.51% to 9.5%) were dominant polyunsaturated FAs in all seasons.

In Vietnam, the current research on oysters only focuses on biological research characteristics, reproductive characteristics, and adaptability, while studies on lipids and lipid composition are still limited. For the first time, Le Thi Thanh Tra et al., (2021) studied the lipid, phospholipid, and fatty acid class composition of oyster *Crassostrea lugubris* (Sowerby, 1871) from Lang Co Beach, Hue Province, Vietnam. Phosphatidylglycolic acid (PGA) was the new phospholipid class first identified in marine species in general and *Crassostrea lugubris* in particular. The main eight classes of PL were determined in PoL fraction: diphosphatidylglycerol (DPG), phosphatidylethanolamine (PE), phosphatidylcholine (PC), phosphatidylinositol (PI), phosphatidylserine (PS), ceramide aminoethylphosphonate (CAEP), CAEP with hydroxylated FAs (CAEP-OH), and lysophosphatidylcholine (LPC). PE and PC accounted for approximately 63% of the total known PL. Polyunsaturated FAs accounted for more than 30% of TL [17].

Therefore, in this study, we studied the changes in the content and composition of lipids and fatty acids of Pacific oysters, *Crassostrea gigas*, cultured in Van Don island, Quang Ninh province, for twelve consecutive months (from December 2018 to November 2019).

MATERIAL AND METHODS

Material

The oysters, *Crassostrea gigas*, were collected from December 2018 to November 2019 in Bai Tu Long bay, Van Don district, Quang Ninh province, Vietnam, and brought to the Institute of Natural Products Chemistry, Vietnam Academy of Science and Technology shortly after. Soft tissues of oysters were then separated and crushed.

Lipid extraction

The crushed soft tissue was extracted for TL following a modified Bligh–Dyer extraction procedure (Bligh & Dye, 1959). 30 mL of chloroform/methanol solution (1:2, v:v) was used to extract 10 g of soft oyster tissue in 6 hours at 30^{o}C to obtain the homogenate, which was then filtered to obtain the residue. This residue was extracted several times in 20 mL of chloroform over 6 hours at 30^{o}C. The homogenates were combined and mixed with 20 mL of H_2O to separate the mixture into layers. After evaporating the lower layer, the TL was dissolved in chloroform. Total lipid was extracted with seven repetitions and stored at -5^{o}C.

Lipid class analysis

The pre-coated silica gel plates (6 cm × 6 cm) Sorbfil PTLC-AF-V (Sorbfil, Krasnodar, Russia) were prepared to determine lipid class compositions. These classes of TL were determined by comparison with standards. The plate was developed in two steps: full-length development using n-hexane/diethyl ether/acetic acid (85:15:1, v:v:v) was performed first, followed by redevelopment with chloroform/methanol (2:1, v:v) for 5% length. Afterwards, air drying commenced on the plates, spraying with 10% H2SO4 methanol and heating at 240^{o}C for 10 minutes (Imbs et al., 2015).

Gray scale chromatograms were obtained using a flatbed scanner (Epson Perfection 2400 PHOTO), and their band intensities were evaluated with software (Sorbfil TLC Video densitometer, Krasnodar, Russia) to determine the quantitation of lipid classes.

Fatty acids analysis

Gas chromatography (GC) and gas chromatography-mass spectrometry (GC–MS) equipment were used to analyze FAs. The instruments used for performing GC–MS analysis consisted of a gas chromatograph (Shimadzu GCMS-QP5050A, Kyoto, Japan) (electron impact at 70 eV) equipped with a MDN-5s (Supelco, Bellefonte, PA, USA)

capillary column (30 m × 0.25 mm ID) using helium as the carrier gas at 30 cm/s. GC-MS provided exact structures for fatty acids, while GC gave fatty acid contents. To perform GC analysis, a Shimadzu GC-2010 chromatograph (Kyoto, Japan) equipped with a flame ionization detector and a capillary column with dimensions of 30 m × 0.25 mm × 0.25µm (SUPELCOWAX 10, Supelco, Bellefonte, PA, USA) was employed in conjunction with helium as the carrier gas (at 30 cm/s).

Lipid and polar lipids were first treated with 2% H2SO4 in methanol, which commenced in 2 hours at 80^{o}C in a screw-top vial, followed by purification by TLC development in hexane–diethyl ether (95:5, v:v). Injector and detector temperatures were 240^{o}C. GC analysis was employed to analyze fatty acid methyl esters (FAME) at a column temperature of 210^{o}C. FA was identified by comparing the results to authentic standards and reporting equivalent chain lengths (Christie et al., 1988).

FAs were structurally determined by performing GC–MS against corresponding FAME and, subsequently, matching the obtained spectra with the NIST library and FA mass spectra archive (Mass Spectrometry of Fatty Acid Derivatives, 2020; Harrabi et al., 2009). The thermal profile of the column initiated at 160^{o}C, followed by acceleration at 2 ^{o}C/min to 240^{o}C that lasted for 20 min. The injector temperature was set at 250^{o}C.

RESULTS AND DISCUSSIONS

Total lipids

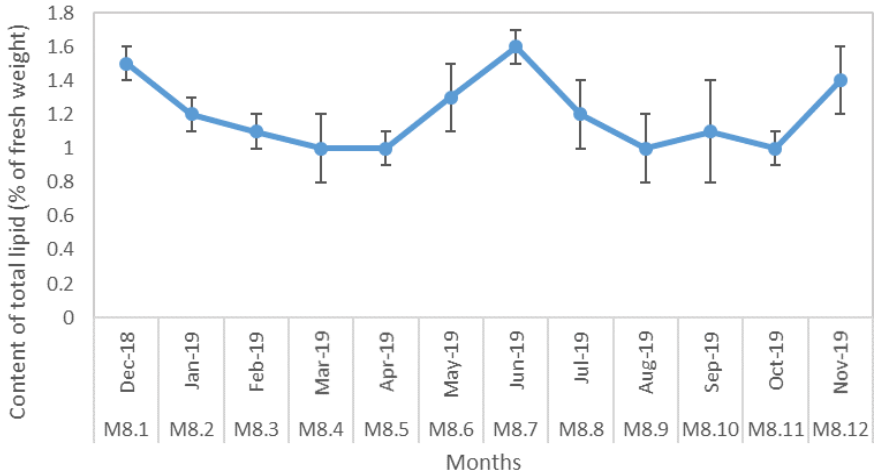

Figure 1. The total lipid content of *C. gigas* in 12 months

Table 1. Changes in total lipid content by 12 months

No.	Sample	Collection time	Content (% of fresh weight)	No.	Sample	Collection time	Content (% of fresh weight)
1	M8.1	12/2018	1.5 ± 0.1	7	M8.7	6/2019	1.6 ± 0.1
2	M8.2	1/2019	1.2 ± 0.1	8	M8.8	7/2019	1.2 ± 0.2
3	M8.3	2/2019	1.1 ± 0.1	9	M8.9	8/2019	1.0 ± 0.2
4	M8.4	3/2019	1.0 ± 0.2	10	M8.10	9/2019	1.1 ± 0.3
5	M8.5	4/2019	1.0 ± 0.1	11	M8.11	10/2019	1.0 ± 0.1
6	M8.6	5/2019	1.3 ± 0.2	12	M8.12	11/2019	1.4 ± 0.2

The pre-reproductive period of oysters every year (two main reproductive periods: March to May and August to October). The total lipids were 1.0–1.6% of the fresh wet weight of the oysters (Fig. 1 and Table 1). The total lipid (TL) is highest in December (M8.1)

and July (M8.7). During the reproductive phase, the TL of oysters decreased sharply, reaching the year's lowest level. Thus, TL that was heavily accumulated before the reproductive period is consumed during the reproductive period.

Lipid class composition

The composition and content of different classes of substances in TL over 12 months are presented in Fig. 2 and Table 2.

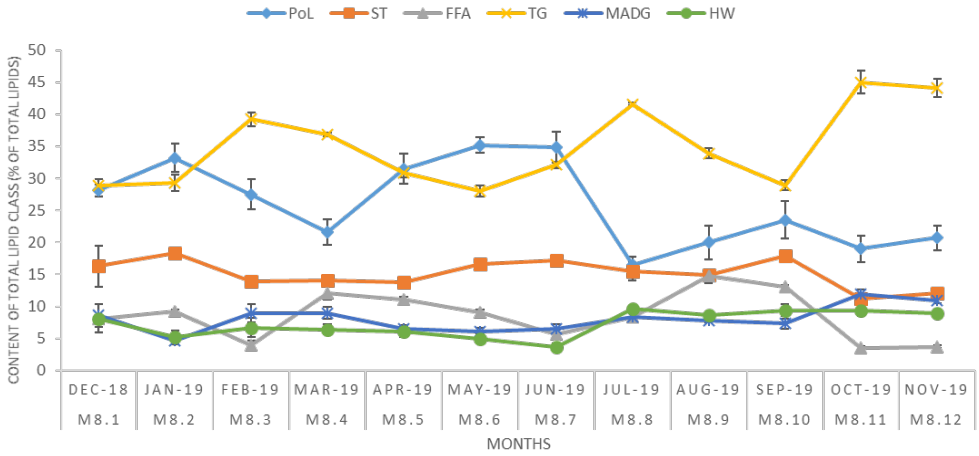

Figure 2. The lipid classes content of *C. gigas* in 12 consecutive months

Table 2. Changes in the composition and content of lipid classes by 12 months

Class	Content (% of total lipid)					
	M8.1	M8.2	M8.3	M8.4	M8.5	M8.6
PoL	28.1 ± 0.9	33.2 ± 2.2	27.5 ± 2.3	21.6 ± 2.0	31.5 ± 2.4	35.2 ± 1.2
ST	16.3 ± 3.2	18.4 ± 0.4	13.9 ± 0.9	14.1 ± 0.5	13.8 ± 0.8	16.6 ± 0.6
FFA	8.1 ± 0.6	9.2 ± 0.3	3.9 ± 0.8	12.0 ±1.0	11.1 ± 0.4	9.1 ± 0.4
TG	28.8 ± 1.0	29.3 ± 1.3	39.2 ± 1.1	36.8 ± 0.3	30.9 ± 0.7	28.0 ± 0.8
MADG	8.6 ± 1.8	4.7 ± 0.6	8.9 ± 1.4	9.0 ± 0.9	6.5 ± 0.6	6.1 ± 0.6
HW	8.1 ± 2.2	5.2 ± 1.0	6.7 ± 1.5	6.4 ± 0.8	6.1 ± 0.8	5.0 ± 0.8
Class	Content (% of total lipid)					
	M8.7	M8.8	M8.9	M8.10	M8.11	M8.12
PoL	34.8 ± 2.4	16.5 ± 1.3	20.0 ± 2.6	23.5 ± 2.9	19.0 ± 2.1	20.4 ± 1.9
ST	17.2 ± 0.5	15.5 ± 1.4	14.9 ± 0.4	17.9 ± 0.3	11.2 ± 0.1	12.1 ± 0.1
FFA	5.7 ± 0.6	8.3 ± 0.8	14.7 ± 1.0	13.0 ±0.3	3.5 ± 0.3	3.7 ± 0.2
TG	32.1 ±0.5	41.6 ± 0.2	33.9 ± 0.8	28.9 ± 0.8	45.0 ± 1.8	44.1 ± 1.4
MADG	6.5 ±0.7	8.4 ± 0.9	7.8 ± 0.3	7.3 ± 0.8	11.9 ± 0.8	10.9 ± 0.4
HW	3.7 ± 0.5	9.7 ± 0.3	8.6 ± 0.3	9.4 ± 0.9	9.4 ± 0.9	8.7 ± 0.6

The lipid composition of oysters can be broken down into six main classes: polar lipid (PoL), sterol (ST), free fatty acids (FFA), triglyceride (TG), monoalkyl diacyl glyceride (MADG), hydrocarbon and wax (HW), as shown in the figure. While the composition of lipid classes varies throughout the year, PoL, ST, and TG. PoL consistently the three main classes present. PoL levels fluctuate significantly, peaking in May and June and reaching their lowest point in July. This is likely due to a large amount of PoL being consumed from cell membranes before the reproductive season, which occurs from August to October. TG also exhibits dramatic fluctuations, with high levels in October and November when oysters are harvested. Peaks of TG were observed in January and September,

while low levels were recorded in May, September, and December. FFA levels are inversely related to TG, likely due to continuous metabolism between the two forms. ST, MADG, and HW classes remain relatively stable over time, with ST levels fluctuating between 11.2% and 18.4%, MADG ranging from 4.7–11.9%, and HW varying from 3.7–9.7%.

Overall, the changes in lipid composition during the year are closely linked to the oyster spawning season. The high PoL content, ranging from 19.0–35.2%, suggests that oysters are a rich source of polar lipids, especially PL, which could be a valuable raw materials for the food industry.

Fatty acid composition

Table 3. Changes in the composition and content of fatty acids by 12 months

FFA	Content (% of total FFA)											
	M8.1	M8.2	M8.3	M8.4	M8.5	M8.6	M8.7	M8.8	M8.9	M8.10	M8.11	M8.12
14:0	3.00	4.40	2.02	3.40	2.52	3.28	3.87	4.43	2.20	2.52	4.47	3.28
i-15:0	0.98	1.11	1.19	0.94	1.15	1.06	0.91	0.93	1.11	1.15	0.93	1.06
16:3n-3	2.68	2.16	2.37	3.45	3.34	3.32	4.27	4.82	3.00	3.34	3.21	3.32
16:1n-7	0.24	1.02	0.26	0.65	0.49	0.33	0.35	0.43	0.34	0.49	0.35	0.33
16:1n-5	0.19	0.09	0.20	0.31	0.16	-	0.12	0.16	0.25	0.16	0.14	-
16:0	24.69	25.07	24.30	24.24	23.04	24.73	23.14	25.54	24.80	23.04	27.95	24.72
i-17:0	0.48	0.30	0.66	0.33	0.34	0.29	0.24	0.23	0.72	0.34	0.33	0.29
17:0	1.79	1.73	2.66	-	2.37	1.67	1.45	1.62	2.44	2.37	1.91	1.67
18:3n-3	2.74	7.10	3.46	2.11	5.18	5.13	4.38	2.65	2.94	5.18	1.70	5.13
18:4n-3	0.52	0.13	0.24	0.12	0.27	0.23	0.40	0.15	0.24	0.27	0.11	0.23
18:2n-6	-	1.67	-	1.71	1.15	0.97	1.03	1.20	1.61	1.15	1.60	0.97
i-18:0	1.30	1.91	1.63	-	1.56	-	-	-	-	1.56	-	-
18:3n-3	1.78	1.27	2.50	2.23	1.85	1.78	1.73	1.88	2.34	1.85	2.06	1.78
Phytanic	-	0.23	-	-	0.18	0.86	-	1.00	-	0.18	1.56	0.86
18:1n-9	2.95	2.99	6.89	5.45	1.22	3.07	2.58	3.44	6.39	1.22	3.29	3.07
18:1n-7	6.71	4.12	4.39	5.58	6.78	5.12	5.51	5.90	5.03	6.78	4.29	5.12
18:1n-5	0.25	1.15	0.29	0.96	0.52	0.24	0.23	0.20	0.36	0.52	0.32	0.24
18:0	9.14	4.71	5.52	5.65	5.75	5.64	4.91	5.98	5.56	5.75	5.83	5.64
20:4n-6	3.90	2.81	3.46	2.38	3.27	4.14	4.79	3.70	2.84	3.27	3.22	4.14
20:5n-3	11.71	13.03	8.50	11.05	14.08	11.36	15.20	13.39	8.80	14.08	11.36	11.36
20:3n-6	0.69	0.68	0.66	0.94	0.99	0.26	0.40	0.25	0.76	0.99	0.70	0.26
20:2n-6	0.49	-	-	-	-	0.32	0.28	-	-	-	-	-
20:1n-11	3.64	0.75	0.55	0.50	0.43	0.36	0.52	0.46	0.51	0.43	0.39	0.36
20:1n-9	-	1.90	3.77	2.77	3.38	3.33	2.54	2.87	3.93	3.38	2.48	3.33
20:1n-7	2.98	2.91	2.14	4.00	3.28	2.71	2.33	2.52	2.69	3.28	2.58	2.71
21:5n-3	0.60	0.90	0.49	0.76	0.80	0.58	0.65	0.57	0.15	0.80	0.64	0.58
22:2-nmi	0.76	1.00	1.82	0.85	0.94	1.00	0.86	0.72	1.64	0.94	0.84	1.00
22:6n-3	9.35	8.60	12.10	6.90	5.93	9.74	9.81	8.54	10.44	5.93	8.91	9.74
22:4n-6	1.15	1.19	1.19	1.15	1.11	1.43	1.34	1.08	1.04	1.11	1.34	1.43
22:5n-3	0.70	2.20	1.95	3.33	3.23	2.92	2.26	2.46	2.66	3.23	2.71	2.92
Other	4.59	2.87	4.81	8.23	4.69	4.17	3.92	2.88	5.22	4.69	4.77	4.48
SFA	41.38	39.22	37.98	34.55	36.73	36.67	34.52	38.73	36.82	36.73	41.42	36.67
UFA	58.62	60.78	62.02	65.45	63.27	63.33	65.49	61.27	63.18	63.27	58.58	63.33
MUFA	16.96	14.93	18.47	20.22	16.26	15.16	12.12	15.98	19.49	16.26	13.85	15.16
PUFA	41.66	45.85	43.55	45.22	47.01	48.17	53.36	45.29	43.69	47.01	44.73	48.17
ω-3	30.08	35.38	31.61	29.96	34.68	35.04	30.51	34.46	30.57	34.68	30.71	35.04
ω-6	6.23	6.36	5.31	6.18	6.52	7.11	7.84	6.23	6.26	6.52	6.86	6.79
ω-9	2.95	4.89	10.66	8.22	4.59	6.40	3.10	6.31	10.32	4.59	5.77	6.40

The fatty acid composition and content of oysters varied over the course of 12 months, as shown in Table 3. The data reveal that there no clear pattern in the changes in fatty acid composition and content throughout the year. However, the main fatty acids present are consistently 14:0, 16:0, 18:0, 18:1n-7, 20:4n-6, 20:5n-3, and 22:6n-3. Unsaturated fatty acids make up the majority of the total fatty acid content, with a total unsaturated to saturated fatty acid ratio of upto 2:1. Polyunsaturated acids make up the majority of the saturated fatty acid, ranging from 41.66–53.36% of the total fatty acid content. The w-3, 6 and 9 content is high, ranging from 39.26–48.55%. The total content of EPA and DHA, two long-chain fatty acid, fall within the range of 17.95–25.01%.

The content of long-chain fatty acids, including AA, EPA, and DHA, is the highest in June (M8.7), accounting for 29.79% of the total fatty acid content. The lowest levels of these fatty acids were observed in March (M8.4) and August (M8.9), at 20.34% and 22.08%, respectively. The lower levels of long-chain fatty acids in March and August may be due to the start of the breeding season, which consumes a significant amount of these fatty acids.

CONCLUSION

This study investigated the change in lipid content and composition of Pacific oyster *C. gigas* cultured in Van Don, Quang Ninh Province, Vietnam over 12 months. The results showed that the total lipid content of the oysters ranged from 1.0 to 1.6% of fresh weight, with the highest content is detected in July and December, which corresponds to the pre-reproductive period. The total lipid was found to consist of six main classes, including polar lipid (PoL), sterol (ST), free fatty acid (FFA), triglyceride (TG), monoalkyl diacyl glyceride (MADG), hydrocarbon, and wax, with PoL (19.0-35.2%), ST (11.2-18.4%), and TG (28-45%) being the predominant classes throughout the study period. The major fatty acids in the oyster lipids were 14:0, 16:0, 18:0, 18:1n-7, 20:4n-6, 20:5n-3, and 22:6n-3. Furthermore, the oyster lipids were found to contain a high amount of polyunsaturated fatty acids (41.66–53.36%).

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