Lipid and FA Composition of the Pearl Oyster *Pinctada fucata martensii*: Influence of Season and Maturation

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ABSTRACT: The lipid and FA composition of the total lipids of the pearl oyster *Pinctada fucata martensii*, in different seasons and in different areas, were analyzed to clarify its lipid physiology and to estimate the possible influence of its prey phytoplankton. TAG and sterols were the major components in the neutral lipids in all conditions, whereas high levels of phospholipids (PE and PC) were found in the polar lipids. The major FA in the TAG in all samples were 14:0, 16:0, and 18:0 as saturated FA (saturates); 16:1n-7, 18:1n-9, and 18:1n-7 as monoenoic FA (monoenes); and 20:4n-6 (arachidonic acid: AA), 20:5n-3 (EPA), and 22:6n-3 (DHA) as PUFA. The major components found in the polar lipids were 16:0 and 18:0 as saturates; 22:2n-9,15 and 22:2n-7,15 as non-methylene-interrupted dienes (NMID), and AA, 22:3n-6,9,15, EPA, and DHA as PUFA. Although it is a marine animal, characteristically high levels of AA were found in both the TAG and phospholipids. This result suggests that lipids of *P. fucata* may be influenced by those of its phytoplanktonic prey. The increase in levels of NMID from TAG to PE with a decrease in those of monoenes suggests that the tissues of this species are able to biosynthesize only the less unsaturated PUFA, such as NMID. In particular, NMID derivatives are considered to be biosynthesized in the PE; thus, they might play a particular role in the membrane, because NMID were characteristically localized only in the PE.

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The chemical components of pelagic seawater fishes, the FA of fish in particular, have been reported on in detail, with growing recognition of the beneficial uses of dietary fish oils. As for FA determinations of bivalve species, only oyster (Ostreidae), scallop (Pectinidae), and mussel (Mytilidae) lipids have been investigated in detail (for oysters: Refs. 1–3; for scallops: Refs. 4 and 5; for mussels: Refs. 6 and 7). Although the biological and ecological influences on their chemical components are gradually being determined, little information is available as yet on the biochemical constituents of other bivalve species, especially the lipid and FA compositions of bivalves. The pearl oyster, *Pinctada fucata martensii,* is aquacultured in the sea off the coast of Japan and is an important marine resource for pearls in Japan. Although the production of pearls is a key industry in Japan, the other portions of the animals, the soft parts in particular, are mostly thrown away as offal after taking pearls from the shell of the bivalve. Only a few people in a very limited locale in Japan eat the adductor muscle of this bivalve.

On the other hand, recent animal studies have indicated that dietary supplements of n-3 PUFA, such as DHA (22:6n-3), may have beneficial effects on cardiovascular diseases, certain forms of cancer, and aging. Studies of lipids in marine organisms suggest that fish and shellfish can be unique sources of n-3 PUFA such as EPA (20:5n-3) and DHA, which are otherwise not provided in terrestrial oils (8,9).

To clarify the physiology of *P. fucata* and to examine its soft parts as a potential source of n-3 PUFA, the lipid and FA compositions of the total lipids collected in different seasons and in different areas were analyzed. In addition, the relationship between the lipids of this species and those of its prey are discussed (9,10).

MATERIALS AND METHODS

Materials. The samples of *P. fucata* are described in Table 1. Samples 1 and 4 were taken from specimens collected during the spawning season (June and July; seawater temperature, 20 and 23°C), and samples 2 and 3 were from specimens collected during the growing season (November and March; seawater temperature, 11 and 16°C). A total of 41 live specimens of *P. fucata* were obtained from the three different localities and at four different times: Uwajima Bay in the Pacific Ocean (sample 1: latitude 33°19′ N and longitude 132°30′ E in July 1998), the Ago Bay in the Pacific Ocean (samples 2 and 3: latitude 34°22′ N and longitude 136°40′ E in November 1998 and March 1999), and the seashore of Tsushima Island in the Japan Sea (replicate sample 4: latitude 34°22′N and longitude 129°12′E in June 2000). Samples 1 and 4 were from cultivated specimens collected about 1 yr after hatching, and samples 2 and 3 were from cultivated specimens about 1.5 yr after hatching. All the samples were cultivated in the wild and were not provided with artificial food.

Lipid extraction and the analysis of lipid classes. The total soft parts of live samples were immediately immersed in a mixture of chloroform and methanol (2:1, vol/vol) after rinsing them briefly in distilled water and were kept frozen at −40°C under an argon atmosphere for 2 d prior to lipid extraction. Each sample was homogenized in a mixture of chloroform

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Abbreviations: AA, arachidonic acid; CAEP, ceramide aminoethyl phosphonate; DMA, dimethylacetals; DMOX, 4,4-dimethyloxazoline; GE, diacylglyceryl ether(s); NMID; non-methylene-interrupted dienes, PL, phospholipids; SE, steryl ester(s); SFA, saturated fatty acids (saturates); ST, sterols; TFA, total fatty acids; TL, total lipids; WE, wax ester(s).

TABLE 1
Cultivation Locality and Biological Data of the Akoya Pearl Oyster *Pinctada fucata martensii*[a]

Sample no.	Date	Locality				Temperature (°C)	Replicate animals (*n*)	Length (cm)	Width (cm)	Weight (g)
1	July 8, 1998	Uwajima Bay	Pacific Ocean	33°19' N	132°30' E	23	10	5.7 ± 0.2	5.8 ± 0.1	18.6 ± 0.7
2	November 21, 1998	Ago Bay	Pacific Ocean	34°22' N	136°40' E	16	10	7.3 ± 0.2	7.3 ± 0.2	40.0 ± 1.5
3	March 11, 1999	Ago Bay	Pacific Ocean	34°22' N	136°40' E	11	11	7.7 ± 0.2	7.6 ± 0.1	47.1 ± 1.4
4	June 2, 2000	Tsushima Island	Japan Sea	34°22' N	129°20' E	20	10	5.8 ± 0.1	5.5 ± 0.1	28.7 ± 1.3

[a]Results are expressed as mean ± SE (n =10–11). The sex of samples of 2 and 3 was not confirmed, and the sex of samples of 1 and 4 could not be determined because of immaturity.

and methanol (2:1, vol/vol), and a portion of each homogenized sample was extracted according to the procedure of Folch *et al.* (11). The crude total lipids (TL) were separated into classes on silicic acid columns (Kieselgel 60, 70–230 mesh; Merck and Co. Ltd., Darmstadt, Germany), and a quantitative analysis of the lipid constituents was performed using gravimetric analysis of fractions collected by column chromatography. The first eluate (dichloromethane/*n*-hexane, 2:3, vol/vol) was collected as the steryl ester (SE), wax ester (WE), and diacylglyceryl ether (GE) fraction. This was followed by dichloromethane eluting the TAG; dichloromethane/ether (35:1, vol/vol) eluting the sterols (ST); dichloromethane/ether (9:1, vol/vol) eluting the DAG; dichloromethane/methanol (9:1, vol/vol) eluting the FFA; dichloromethane/methanol (1:5, vol/vol) eluting the PE; dichloromethane/methanol (1:20, vol/vol) eluting the ceramide aminoethyl phosphonate (CAEP) with other minor phospholipids (PL); and dichloromethane/methanol (1:50, vol/vol) eluting the PC (12).

Individual lipids from each lipid class, such as PL classes, were identified qualitatively by comparison of the R_f values with standards using TLC (Kieselgel 60, thickness of 0.25 mm for analysis; Merck & Co. Ltd.) and by identifying characteristic peaks using NMR. All sample lipids were dried under argon at room temperature and stored at –40°C.

NMR spectrometry and the determination of lipid classes. Spectra were recorded on a GSX-270 NMR spectrometer (JEOL Co. Ltd., Tokyo, Japan) in the pulsed Fourier transform mode at 270 MHz in a deuterochloroform solution using tetramethylsilane as an internal standard (13).

Some fractions often contained several classes; for instance, the first fraction contained WE, SE, and GE. The molar ratios of WE, GE, and SE were determined by quantitative analysis of NMR results. In NMR, the amount of WE was obtained by the total amount of the combined integrations of the triplet peaks (from 3.90 to 4.20 ppm) as the two protons at the ester alcohol, the amount of GE was obtained by that of the singlet peak (3.50–3.80 ppm) as the two ether protons linked with glycerol carbons, and the amount of SE was obtained by that of the multiplet peak (4.30–4.80 ppm) as one proton at the carbon-linked esterized C-3 alcohol of the sterol. The actual ratio of the WE, SE, and GE in the first fraction was determined as the respective integration divided by the sum of total integrations of the three combined peaks from 3.50 to 4.80 ppm. The actual weight of each class was obtained by calculating the ratio and multiplying by the total weight of the first fraction. Similarly, the third fraction sometimes contained TAG and ST, and had two characteristic peaks: for TAG (3.90–4.40 ppm) an octet-like peak for four protons, and for ST (3.40–3.60 ppm) a multiplet peak for one proton. The actual weights of TAG and ST in the third fraction were obtained by calculating the integration of each divided peak and multiplying by the total weight of the third fraction.

Preparation of methyl esters and GLC of the esters. Individual components of the TAG, PE, and PC fractions were converted to FAME by direct transesterification with boiling methanol containing 1% of concentrated hydrochloric acid under reflux for 1.5 h as previously reported. These methyl esters were purified by using silica gel column chromatography and eluting with dichloromethane/*n*-hexane (2:1, vol/vol).

The composition of the FAME was determined by GLC. Analysis was performed on a Shimadzu GC-8A (Shimadzu Seisakusho Co. Ltd., Kyoto, Japan) and an HP-5890 (Hewlett-Packard Co., Yokogawa Electric Corporation, Tokyo, Japan) gas chromatograph equipped with an Omegawax-250 fused-silica capillary column (30 m × 0.25 mm i.d.; 0.25 μm film; Supelco Japan Co. Ltd., Tokyo, Japan). The injector and column temperatures were held at 230 and 215°C, respectively, and the split ratio was 1:76. Helium was used as the carrier gas at a constant inlet rate of 0.7 mL/min.

Quantification of individual components was performed by means of a Shimadzu Model C-R3A (Shimadzu Seisakusho Co. Ltd.) and an HP ChemStation System (A, 06 revision, Yokogawa HP Co. Ltd., Tokyo, Japan) electronic integrator.

Preparation of 4,4-dimethyloxazoline derivatives (DMOX) and analysis of DMOX by GC–MS. The DMOX derivatives were prepared by adding an excess amount of 2-amino-2-methyl propanol to a small amount of the FAME in a test tube under an argon atmosphere. The mixture was heated at 180°C for 48 h. The reaction mixture was cooled and poured onto saturated brine and extracted three times with *n*-hexane. The extract was then washed with saturated brine and dried over anhydrous sodium sulfate. The solvent was removed under reduced pressure, and the samples were again dissolved in *n*-hexane for analysis by GC–MS (13).

Analysis of the DMOX derivatives was performed on an HP G1800C GCD Series II (Hewlett-Packard Co.) gas chromatograph–mass spectrometer equipped with the same capillary column for determining the respective FA with HP WS software (HP Kayak XA, G1701BA version, PC workstations; Hewlett-Packard Co.). The temperatures of the injector and the column

were held at 230 and 215°C, respectively. The split ratio was 1:75, and the ionization voltage was 70 eV, respectively. Helium was used as the carrier gas at a constant inlet rate of 0.7 mL/min.

FAME were identified (i) using marine lipid methyl esters as standards (Omegawax test mixture No. 4-8476; Supelco Japan Ltd.) and (ii) by comparison of mass spectral data obtained by GC–MS.

RESULTS AND DISCUSSION

Lipid content of P. fucata. Biological data on the *P. fucata* samples are listed in Table 1. The sizes and weights of samples 2 and 3 were markedly greater than those of samples 1 and 4, showing that the *P. fucata* grew during the growing season. The TL content and lipid classes are shown in Table 2. The TL content was 0.4–2.0% in all culture conditions; all the mean lipid contents were very low, similar to those of other oysters (*Crassostrea* spp.; Ref. 2). In addition, the lipid contents were slightly higher (1.0–2.0%) during the spawning season (June and July) than in the growing season (0.4–0.7%, November and March); the comparatively high lipid levels in the spawning season suggest that the lipids may play some role in maturation (14), similar to that in finfishes (15); this phenomenon differed from the tendency toward seasonal variation of glycogen, whose levels are higher in the growing season (16). Otherwise, the higher lipid contents in June and July suggest that the warm seawater might have promoted accumulation of lipids related to a high metabolic activity in this season. The lowest lipid content, seen in sample 3, suggests that the most unfavorable condition, i.e., the low temperature in this season, disturbed the normal lipid metabolism; the most suitable temperature for this bivalve is from 18 to 25°C, and its lowest survival temperature is generally known to be about 13°C.

Lipid classes in the total lipids of P. fucata. The lipid classes of *P. fucata* are shown in Table 2. TAG [7.4–27.3% of the total FA (TFA)] and ST (13.6–39.6% of TFA) were the major components in the neutral lipids in all specimens, with low levels of WE (0.1–0.4% of TFA), SE (0.5–7.4% of TFA), GE (0.9–3.1% of TFA), and DAG (0.3–0.9% of TFA). In contrast, the species had high levels of PL (PE, 20.9–29.9% of TFA; PC, 8.7–13.8% of TFA). In addition, significant levels of CAEP (7.1–11.9% of TFA) were found in the polar lipids with low levels of sphingolipids. The low level of neutral lipids reflected the low lipid content in the species (0.4–2.0%), because it is a lean organism (17,18). In general, the proportions of PL in tissue TL are constant in all animals because they are important as cell membrane lipids, except for the *Euphausia* sp. (*Euphausia pacifica*; Ref. 19), whereas the levels of depot lipids vary (9). The mean PL contents in tissues of all marine organisms can be less than 1% of their tissue weight (17,18). In fact, all of its actual PL levels (0.12–0.76% of total tissue weight) were less than 1% of the tissue weight, similar to those of other marine animals.

The lipid classes in sample 3 differed from those in other samples; in particular, the levels of ST in the sample were highest (7.4 ± 0.9% for SE and 39.6 ± 1.9% for ST). This result may also have been caused by a less favorable condition such as the lower seawater temperature; the high levels of SE and ST might be viewed as the result of low levels of depot TAG.

Similar to data on the Pacific oyster reported by Soudant *et al*. (3), significant levels of FFA (6.9–10.8% of TFA) were found in TL; this may be a result of digested products of glycerol derivatives in its viscera that were degraded by enzymatic metabolism, because the sampling of the whole soft part contained digestive glands that may have contained active enzymes for digestion.

The lipids of *P. fucata* contained mainly glycerol derivatives (TAG, FFA, PE, and PC), and the total proportion of these derivatives reached about 48–73%, with low levels of other depot lipids such as WE and GE. Comparatively high levels of ST (13.2–39.6%) were found, and the ST fractions were considered to contain several kinds of sterols; NMR analysis displayed at least two singlet peaks of C-18-positional methyl moieties of ST.

These lipid classes may be a characteristic of this bivalve species except for the differences in the SE and ST levels in sample 3, which grew under less favorable conditions, all other

TABLE 2
Lipid Contents and Lipid Classes of *Pinctada fucata martensii*[a]

Sample no.	No. of replicates	Lipid contents[b]	WE[c]	SE[c]	GE[c]	TAG[c]	ST[c]
1	9	2.0 ± 0.2	0.1 ± 0.0	0.5 ± 0.2	1.5 ± 0.3	27.3 ± 3.7	13.6 ± 2.2
2	10	0.7 ± 0.0	0.3 ± 0.1	0.9 ± 0.3	1.6 ± 0.4	13.9 ± 1.5	24.7 ± 1.5
3	11	0.4 ± 0.0	0.4 ± 0.2	7.4 ± 0.9	0.9 ± 0.1	7.4 ± 1.0	39.6 ± 1.9
4	10	1.0 ± 0.1	0.2 ± 0.1	0.7 ± 0.2	3.6 ± 0.4	14.0 ± 1.8	22.9 ± 1.5
		DAG[c]	FFA[c]	PE[c]	CAEP[c,d]	PC[c,e]	
1	9	0.9 ± 0.2	6.9 ± 1.4	24.2 ± 4.1	11.9 ± 2.0	13.8 ± 2.9	
2	10	0.7 ± 0.1	10.8 ± 2.5	24.4 ± 1.2	10.9 ± 2.2	12.8 ± 2.1	
3	11	0.8 ± 0.5	10.1 ± 1.5	20.9 ± 2.4	11.2 ± 2.4	8.7 ± 1.9	
4	10	0.3 ± 0.1	9.3 ± 0.6	30.8 ± 1.1	6.3 ± 1.8	12.5 ± 0.9	

[a]Data are mean ± SE (n = 9–11). WE, wax esters; SE, steryl esters; GE, glyceryl ethers; ST, sterols; CAEP, ceramide aminoethyl phosphate.
[b]Results are expressed as weight percent of wet tissue.
[c]Results are expressed as weight percent of total lipids.
[d]CAEP fractions contain small amounts of other minor phospholipids.
[e]PC fractions contain small amounts of sphingomyerlin.

TABLE 3
FA Composition of TAG of *Pinctada fucata martensii* Examined[a]

	1	2	3	4
Entry	Mean SE	Mean SE	Mean SE	Mean SE
Total saturated	30.2 ± 1.9	31.2 ± 1.0	29.8 ± 1.0	28.6 ± 0.6
14:0	5.7 ± 0.6	6.0 ± 0.3	1.5 ± 0.1	2.9 ± 0.1
16:0	15.3 ± 2.0	15.3 ± 0.5	12.4 ± 0.6	15.1 ± 0.5
17:0	1.0 ± 0.2	1.3 ± 0.1	2.2 ± 0.1	1.7 ± 0.1
18:0	5.9 ± 0.3	6.4 ± 0.4	10.1 ± 0.6	7.0 ± 0.2
Total monoenoic	17.5 ± 0.4	23.0 ± 0.5	20.9 ± 0.9	16.6 ± 0.3
16:1n-7	6.3 ± 0.6	8.6 ± 0.3	2.1 ± 0.2	4.1 ± 0.2
18:1n-9	2.1 ± 0.3	3.5 ± 0.1	7.5 ± 1.1	3.7 ± 0.1
18:1n-7	3.9 ± 0.1	5.0 ± 0.2	3.4 ± 0.4	4.1 ± 0.1
18:1n-5	0.2 ± 0.0	0.2 ± 0.0	0.3 ± 0.0	0.2 ± 0.0
19:1n-9	0.4 ± 0.0	0.8 ± 0.0	0.3 ± 0.1	0.2 ± 0.0
20:1n-11	1.5 ± 0.3	1.8 ± 0.1	4.3 ± 0.5	1.1 ± 0.0
20:1n-9	0.9 ± 0.0	1.0 ± 0.0	1.2 ± 0.1	1.1 ± 0.0
20:1n-7	1.3 ± 0.1	1.4 ± 0.1	1.1 ± 0.2	0.8 ± 0.0
Total NMID	3.3 ± 0.5	3.5 ± 0.2	6.7 ± 0.4	3.0 ± 0.1
20:2n-7,15	0.2 ± 0.0	0.2 ± 0.1	0.8 ± 0.3	0.1 ± 0.0
22:2n-9,15	0.9 ± 0.2	0.9 ± 0.1	1.7 ± 0.2	1.2 ± 0.0
22:2n-7,15	1.9 ± 0.3	2.0 ± 0.1	4.0 ± 0.2	1.5 ± 0.1
Total polyenoic	47.2 ± 2.0	40.3 ± 1.3	39.4 ± 1.2	48.9 ± 0.7
Total n-4 polyenoic	2.5 ± 0.2	4.7 ± 0.2	2.5 ± 0.2	1.5 ± 0.1
16:2n-4	0.6 ± 0.1	1.3 ± 0.1	0.2 ± 0.0	0.3 ± 0.0
16:3n-4	0.5 ± 0.1	1.2 ± 0.1	0.4 ± 0.0	0.5 ± 0.0
16:4n-1	0.7 ± 0.3	1.0 ± 0.1	1.1 ± 0.2	0.3 ± 0.0
Total n-6 polyenoic	13.0 ± 0.3	6.4 ± 0.2	11.6 ± 0.4	8.7 ± 0.3
18:2n-6	1.2 ± 0.1	1.6 ± 0.1	1.3 ± 0.1	2.2 ± 0.1
20:2n-6	0.6 ± 0.0	0.7 ± 0.1	1.0 ± 0.1	1.3 ± 0.1
20:4n-6	8.6 ± 0.4	2.1 ± 0.1	5.3 ± 0.4	2.4 ± 0.1
22:3n-6,9,15	1.0 ± 0.1	1.2 ± 0.1	2.3 ± 0.1	1.5 ± 0.1
22:4n-6	0.9 ± 0.1	0.2 ± 0.0	0.6 ± 0.0	0.3 ± 0.0
22:5n-6	0.4 ± 0.0	0.4 ± 0.0	0.7 ± 0.0	0.6 ± 0.1
Total n-3 polyenoic	31.6 ± 2.0	29.2 ± 1.1	25.3 ± 1.2	38.6 ± 0.6
18:3n-3	1.0 ± 0.1	2.4 ± 0.1	0.8 ± 0.1	2.3 ± 0.1
18:4n-3	1.6 ± 0.2	3.7 ± 0.2	0.7 ± 0.1	3.0 ± 0.1
20:4n-3	0.6 ± 0.1	0.7 ± 0.0	0.5 ± 0.1	1.1 ± 0.0
20:5n-3	18.0 ± 1.9	12.3 ± 0.5	8.5 ± 0.7	12.2 ± 0.2
22:5n-3	1.1 ± 0.1	0.8 ± 0.0	1.4 ± 0.1	1.2 ± 0.1
22:6n-3	9.1 ± 0.4	9.0 ± 0.5	13.0 ± 0.5	18.2 ± 0.4
Total FA	94.8 ± 0.4	94.6 ± 0.2	90.0 ± 0.7	94.6 ± 0.1

[a]Data are mean ± SE for several samples (n = 9–29).
[b]In Tables 3–5, the major FA were selected if at least one mean datum was detected at a level of 0.8% or more of total FA.
NMID, non-methylene interrupted diene(s).

samples analyzed which were collected from different areas and in different seasons (samples 1, 2, and 4) were similar to each other (Table 2).

FA composition of TAG depot lipids in P. fucata. FA in the TAG (more than 0.8% of TFA) are shown in Table 3. More than 60 kinds of FA were found in the TAG and PL of *P. fucata*. The wide range of FA detected in the lipids might have been caused by its omnivorous behavior (16), similar to that of higher trophic marine animals (9). Although the FA composition of TAG varied among specimens because of the seasonal and spatial variations of the prey lipids, the major FA were almost the same under the four different conditions. Nine dominant FA (more than about 2% of TFA) in TAG were found among the four different conditions—14:0 (1.5–6.0%), 16:0 (12.4–15.3%), and 18:0 (5.9–10.1%) as saturates; 16:1n-7 (2.1–8.6%), 18:1n-9 (2.1–7.5%), and 18:1n-7 (3.4–5.0%) as monoenes; arachidonic acid (AA) (2.1–8.6%) as n-6 PUFA; and EPA (8.5–18.0%) and DHA (9.0–18.8%) as n-3 PUFA—with significant levels of 11 FA—17:0 as saturates; 20:1n-11, 20:1n-9, and 20:1n-7 as monoenes; 18:2n-6 as dienes; 22:2n-9,15 and 22:2n-7,15 as non-methylene-interrupted dienoic (NMID) FA; 22:3n-6,9,15 as n-6 PUFA; and 18:3n-3, 18:4n-3, and 22:5n-3 (DPA: docosapentaenoic acid) as n-3 PUFA.

In general, both the short-chain saturates (14:0, 16:0, 17:0, and 18:0) and unsaturates (16:1n-7, 18:1n-7, 18:1n-9, 18:2n-6, 18:3n-3, 18:4n-3, and EPA) are also contained in the lipids of

phytoplankton (Bacillariophyceae, Haptophyceae, Cryptophyceae, Eustigmatophytes, and Euglenophyceae: Ref. 20). For example, several species of diatoms (Bacillariophyceae: Refs. 21 and 22) and Eustigmatophytes (23) have markedly high EPA contents (more than 20% of TFA). Furthermore, some phytoplankton lipids often contain significant levels of AA, with high levels of FA, such as 14:0 and 16:0, 16:1n-7, and EPA (Bacillariophyceae, Ref. 24; Rhodophyceae, Refs. 25 and 26; Haptophyceae, Refs. 27 and 28). Taking into account the microalgal biomass and species variety of phytoplankton in the sea, these results suggest that diatoms might be the major prey of *P. fucata* as an EPA and AA source.

On the other hand, it is well known that some phytoplankton, such as dinoflagellates, contain noticeable levels of DHA (more than 3% of TFA), with significant levels of EPA (more than 5% of TFA) (Bacillariophyceae: Refs. 20,22,28; dinoflagellates: Refs. 24,28–30; Haptophyceae: Ref. 27,28,31; Chrysophyceae and Xanthophyceae: Ref. 24). The DHA in the TAG of *P. fucata* might have originated specifically from these phytoplankton classes, mainly supplied by dinoflagellates.

Even though the TAG are only depot lipids, the three PUFA—AA (2.1–8.6%), EPA (8.5–18.0%), and DHA (9.0–18.8%)—in the TAG of *P. fucata* were significantly high. The high EPA levels might be similar to the TAG of other marine bivalves (5,32). Furthermore, the high level of AA in the TAG is an unusual characteristic, because this FA is a minor component in the depot lipids of other marine animals; only trace amounts are usually found in the depot TAG of other marine animals, such as copepods, which are at the same trophic level. Compared with the FA composition of phytoplankton, the high levels of both AA and EPA in TAG of *P. fucata* correspond well with those in some diatom lipids, except for NMID.

FA composition in tissue PL in P. fucata. In contrast to the FA composition of the TAG, the major FA (more than 3% of TFA) of the PL were more limited, as shown in Tables 4 and 5. In the FA composition of PE, low levels of dimethylacetals (DMA), such as DMA 18:0, were included (Table 4). The theoretical values of the FA composition were obtained by deleting the DMA from the TFA of PE. After this treatment, seven major FA were consistently found in the PE of all specimens under the four different conditions (2% or more of the TFA)—18:0 (5.4–7.7%) as saturates; 22:2n-9,15 (7.8–12.9%) and 22:2n-7,15 (2.4–8.2%) as NMID; AA (8.8–11.4%) and 22:3n-6,9,15 (4.3–7.2%) as n-6 PUFA; EPA (7.7–12.8%) and DHA (20.1–33.2%) as n-3 PUFA—with significant amounts of seven other FA—16:0 as saturates; 20:1n-11 and 20:1n-9 as monoenes; 22:4n-6 as n-6 PUFA; and DPA as n-3 PUFA.

The five FA were also the major components in the PC: 16:0 (14.9–20.0%) and 18:0 (3.6–6.9%) as saturates, 22:2n-7,15 (1.1–3.5%) as NMID; AA (3.7–9.5%) as n-6 PUFA; and EPA (7.6–8.8%) and DHA (23.2–40.9%) as n-3 PUFA. Significant amounts of 10 FA—17:0 as saturates; 18:1n-9, 18:1n-7, and 20:1n-11 as monoenes; 22:2n-9,15 as NMID; 22:3n-6,9,15 and 22:5n-6 as n-6 PUFA; and DPA as n-3 PUFA—were found in the PC. Although the detailed FA levels of the PE differed slightly from those of the PC, the profile of these major components of the PE were close to those of the PC. In particular, the total amounts of PUFA in both PE and PC (40.3–60.6% for PE and 51.4–59.0% for PC) were high under all four conditions; therefore, the PE and PC of the *P. fucata* were rich sources of PUFA, similar to those in other marine animals (17,18).

The high levels of total PUFA in the PL suggest the accumulation of PUFA in the tissue, because bivalves may not be able to biosynthesize PUFA such as AA and DHA (10,33). In particular, the high levels of AA, EPA, and DHA might be the result of a concentration of these PUFA in the tissue, because high levels of PUFA were consistently found in the PL, compared with those in the depot lipids. Moreover, significant levels of n-6 PUFA, such as 22:4n-6, which is a the elongation product of AA, were found in the PL, compared with the trace levels found in the TAG. Similarly, the level of AA in the PL increased slightly, compared with that in the TAG. As for n-3 PUFA, the level of DHA increased markedly in both the PE and PC, and DPA increased slightly in the PL, whereas the shorter-chain unsaturates 18:4n-3 and EPA decreased in the PL compared with those in the TAG. The species may selectively concentrate longer and more highly unsaturated FA in its tissues as membrane lipids.

During the spawning season, the DHA content in PC fluctuated between 23.2 and 40.8% of TFA (samples 1 and 4). This amount may be influenced by environmental and seasonal differences in the prey phytoplankton lipids, because the total PUFA levels (NMID, n-4, n-6, and n-3 PUFA: 58.9% of TFA for specimens of sample 1, and 59.0% for those of sample 4) in the spawning season did not differ much from each other. Otherwise, in the spawning season, the fluctuation of its FA composition might be influenced by maturation, because differences in the concentrations of specific chemical components in the tissues were often found between before and after spawning.

Biosynthesis of the NMID in the P. fucata *tissues.* The notable levels of C_{22} NMID in TAG, PC, and in particular PE, suggest that this species may have enzymes in its tissues, for the biosynthetic elongation to 22-carbon chains and for the desaturation to NMID by Δ5 desaturase, similar to the synthetic pathway in other bivalves (10,33–37), because these dienes increased with a decrease in the saturates and monoenes (from TAG to PL). For instance, the levels of long-chain NMID 22:2n-9,15 and 22:2n-7,15 increased markedly, while there were decreases of 14:0, 16:1n-7, 18:1n-9, and 18:1n-7 in the PE, compared with high levels of the shorter-chain and less unsaturated FA in the TAG. This result suggests that these NMID might originate from 18:1n-9 and 18:1n-7. The significantly high level of 22:3n-6,9,15, which is considered to be derived by Δ5 desaturation after the elongation of linoleic acid (18:2n-6,9), also suggests the presence of the desaturase, similar to other NMID derivatives.

All changes in FA from depot to tissue lipids under the four different conditions had the same tendency, in which shorter-chain and less unsaturated FA decreased in the polar lipids. These facts suggest that elongation and desaturation mechanisms exist in *P. fucata*.

TABLE 4
FA Composition of PE of *Pinctada fucata martensii* examined[a]

	1 Mean ± SE	Calcd. 1 Mean ± SE	2 Mean ± SE	Calcd. 2 Mean ± SE	3 Mean ± SE	Calcd. 3 Mean ± SE	4 Mean ± SE	Calcd. 4 Mean ± SE
Total saturated	12.9 ± 1.6	12.8 ± 1.1	7.9 ± 0.4	10.1 ± 0.4	7.7 ± 0.5	9.3 ± 0.3	7.9 ± 0.2	10.7 ± 0.3
14:0	1.4 ± 0.2	1.3 ± 0.1	1.1 ± 0.1	1.4 ± 0.1	0.6 ± 0.1	0.7 ± 0.1	0.7 ± 0.1	0.9 ± 0.1
16:0	2.2 ± 0.5	2.0 ± 0.3	0.9 ± 0.1	1.1 ± 0.1	1.1 ± 0.2	1.3 ± 0.2	0.6 ± 0.0	0.8 ± 0.1
17:0	0.8 ± 0.1	0.9 ± 0.1	0.5 ± 0.1	0.7 ± 0.1	0.5 ± 0.0	0.6 ± 0.0	0.4 ± 0.0	0.6 ± 0.0
18:0	7.2 ± 0.8	7.3 ± 0.6	4.4 ± 0.2	5.6 ± 0.2	4.5 ± 0.3	5.4 ± 0.2	5.7 ± 0.2	7.7 ± 0.2
Total monoenoic	10.1 ± 0.8	10.4 ± 0.5	6.1 ± 0.2	7.8 ± 0.4	4.6 ± 0.3	5.7 ± 0.4	7.6 ± 0.2	10.2 ± 0.2
16:1n-7	0.5 ± 0.2	0.4 ± 0.1	0.5 ± 0.0	0.6 ± 0.1	0.4 ± 0.1	0.5 ± 0.1	0.4 ± 0.0	0.5 ± 0.1
18:1n-9	0.7 ± 0.1	0.7 ± 0.1	0.5 ± 0.1	0.6 ± 0.1	0.2 ± 0.0	0.3 ± 0.0	1.3 ± 0.0	1.8 ± 0.1
18:1n-7	0.8 ± 0.1	0.9 ± 0.1	0.6 ± 0.1	0.8 ± 0.1	0.7 ± 0.2	0.9 ± 0.2	0.5 ± 0.1	0.7 ± 0.1
18:1n-5	0.2 ± 0.0	0.2 ± 0.0	0.3 ± 0.1	0.5 ± 0.2	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.0
19:1n-9	0.2 ± 0.0	0.2 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.4 ± 0.1	0.5 ± 0.1	0.1 ± 0.0	0.1 ± 0.0
20:1n-11	2.5 ± 0.2	2.7 ± 0.1	1.2 ± 0.1	1.6 ± 0.1	0.6 ± 0.1	0.7 ± 0.1	1.7 ± 0.1	2.2 ± 0.1
20:1n-9	2.8 ± 0.3	3.0 ± 0.2	1.6 ± 0.1	2.0 ± 0.1	0.9 ± 0.1	1.1 ± 0.1	2.3 ± 0.1	3.0 ± 0.1
20:1n-7	1.5 ± 0.2	1.5 ± 0.2	0.7 ± 0.0	0.9 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	0.7 ± 0.0	1.0 ± 0.0
Total NMID	19.1 ± 0.9	20.7 ± 0.7	17.6 ± 0.8	22.5 ± 0.8	9.2 ± 0.9	11.0 ± 0.6	14.4 ± 0.3	19.3 ± 0.3
20:2n-7,15	0.4 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	0.6 ± 0.1	0.3 ± 0.0	0.3 ± 0.0	0.5 ± 0.0	0.7 ± 0.0
22:2n-9,15	11.7 ± 0.6	12.6 ± 0.5	10.1 ± 0.6	12.8 ± 0.6	6.5 ± 0.6	7.8 ± 0.5	9.6 ± 0.3	12.9 ± 0.3
22:2n-7,15	6.4 ± 0.3	6.8 ± 0.2	6.4 ± 0.2	8.2 ± 0.2	2.1 ± 0.3	2.4 ± 0.3	3.7 ± 0.1	4.9 ± 0.2
Total polyenoic	46.7 ± 1.2	51.8 ± 1.1	44.6 ± 1.8	56.9 ± 0.6	59.0 ± 2.4	71.8 ± 0.7	42.5 ± 0.7	56.9 ± 0.4
Total n-4 polyenoic	0.3 ± 0.0	0.3 ± 0.0	0.2 ± 0.0	0.3 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.3 ± 0.0
16:2n-4	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
16:3n-4	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
16:4n-1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Total n-6 polyenoic	18.1 ± 0.7	20.2 ± 0.5	14.9 ± 0.7	19.0 ± 0.4	17.5 ± 1.0	21.3 ± 0.7	14.6 ± 0.3	19.6 ± 0.3
18:2n-6	0.3 ± 0.0	0.3 ± 0.1	0.3 ± 0.0	0.4 ± 0.1	0.3 ± 0.0	0.4 ± 0.1	0.3 ± 0.0	0.3 ± 0.0
20:2n-6	1.0 ± 0.2	1.1 ± 0.2	0.5 ± 0.1	0.6 ± 0.1	0.2 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.4 ± 0.0
20:4n-6	8.6 ± 0.6	9.6 ± 0.7	6.9 ± 0.4	8.8 ± 0.3	9.4 ± 0.5	11.4 ± 0.5	6.9 ± 0.2	9.3 ± 0.2
22:3n-6,9,15	4.3 ± 0.2	4.7 ± 0.2	4.2 ± 0.2	5.3 ± 0.2	3.6 ± 0.2	4.3 ± 0.2	5.4 ± 0.1	7.2 ± 0.1
22:4n-6	3.0 ± 0.2	3.4 ± 0.1	1.8 ± 0.1	2.3 ± 0.1	2.5 ± 0.2	3.1 ± 0.2	1.1 ± 0.1	1.5 ± 0.1
22:5n-6	0.8 ± 0.1	1.0 ± 0.1	1.1 ± 0.1	1.4 ± 0.1	1.5 ± 0.1	1.8 ± 0.1	0.5 ± 0.0	0.7 ± 0.0
Total n-3 polyenoic	26.4 ± 2.1	31.3 ± 1.5	29.5 ± 1.2	37.6 ± 0.7	41.3 ± 1.8	50.3 ± 1.2	27.7 ± 0.6	37.0 ± 0.4
18:3n-3	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.3 ± 0.0
18:4n-3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
20:4n-3	0.3 ± 0.0	0.4 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
20:5n-3	6.6 ± 0.5	7.7 ± 0.6	7.0 ± 0.2	9.0 ± 0.4	10.4 ± 0.6	12.8 ± 0.7	7.5 ± 0.2	10.1 ± 0.3
22:5n-3	2.2 ± 0.2	2.6 ± 0.2	2.4 ± 0.1	3.0 ± 0.0	3.1 ± 0.2	3.8 ± 0.1	1.8 ± 0.1	2.5 ± 0.0
22:6n-3	16.7 ± 1.7	20.1 ± 1.1	19.6 ± 1.1	24.8 ± 0.9	27.3 ± 1.4	33.2 ± 1.0	17.7 ± 0.5	23.6 ± 0.4
Total FA	86.8 ± 2.1	95.7 ± 0.3	76.3 ± 2.6	97.3 ± 0.4	80.5 ± 3.6	97.8 ± 0.3	72.5 ± 1.0	97.0 ± 0.2
DMA 15:0	0.0 ± 0.0		0.0 ± 0.0		0.0 ± 0.0		0.1 ± 0.0	
DMA 16:0	0.2 ± 0.1		0.6 ± 0.1		0.5 ± 0.1		0.9 ± 0.1	
DMA 17:0	0.4 ± 0.1		0.9 ± 0.1		0.7 ± 0.1		1.2 ± 0.1	
DMA 17:0i	0.1 ± 0.0		0.0 ± 0.0		0.0 ± 0.0		0.1 ± 0.1	
DMA 18:0	6.6 ± 1.7		17.0 ± 2.1		13.8 ± 3.0		20.1 ± 0.8	
DMA 18:0i	0.3 ± 0.1		0.7 ± 0.1		0.9 ± 0.2		1.1 ± 0.1	
DMA 19:0	0.2 ± 0.0		0.2 ± 0.1		0.0 ± 0.0		0.2 ± 0.0	
DMA 19:0i	0.6 ± 0.1		0.9 ± 0.1		1.1 ± 0.2		1.0 ± 0.1	
DMA 20:0	0.1 ± 0.0		0.1 ± 0.0		0.1 ± 0.0		0.2 ± 0.0	
DMA 18:1	0.8 ± 0.2		0.8 ± 0.1		0.3 ± 0.1		0.2 ± 0.0	
DMA 20:1	0.1 ± 0.0		0.4 ± 0.1		0.2 ± 0.1		0.3 ± 0.0	
Total DMA	9.4 ± 2.2		21.6 ± 2.7		17.7 ± 3.6		25.3 ± 1.0	

[a]Data are mean ± SE for several samples (n = 9–29).
[b]In Tables 3–5, the major FA were selected if at least one mean datum was detected at a level of 0.8% or more of total FA. DMA, dimethyl acetals; i, abbreviation of the "iso"-compound. For other abbreviations see Table 3.

Accumulation of n-3 PUFA in the lipids of P. fucata. In the marine food chain, many pelagic fishes accumulate n-3 PUFA, such as DHA, which may originate from the phytoplankton (8). In general, DHA is the dominant PUFA in both the PE and PC of almost all higher trophic-level marine animals after accumulation in the food chain (17,18,38). Moreover, DHA is required by all marine fish because they are unable to biosynthesize this EFA (8,39,40). Although the high levels of n-3 PUFA in *P. fucata* PL were also similar to the results reported for other marine animals (17), fluctuations of the respective PUFA were

TABLE 5
FA Composition of PC of *Pinctada fucata martensii* Examined[a]

	1	2	3	4
Entry	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE
Total saturated	26.9 ± 0.7	28.1 ± 1.1	23.8 ± 1.1	24.9 ± 0.7
14:0	0.8 ± 0.1	0.5 ± 0.0	0.3 ± 0.0	0.3 ± 0.0
16:0	16.7 ± 2.1	20.0 ± 0.8	14.9 ± 1.2	17.8 ± 0.5
17:0	1.5 ± 0.1	2.1 ± 0.1	2.9 ± 0.2	1.9 ± 0.1
18:0	6.9 ± 1.8	4.2 ± 0.4	4.3 ± 0.4	3.6 ± 0.2
Total monoenoic	12.5 ± 0.5	12.6 ± 1.0	9.9 ± 0.7	8.1 ± 0.2
16:1n-7	1.2 ± 0.1	1.3 ± 0.1	0.6 ± 0.1	0.5 ± 0.0
18:1n-9	2.0 ± 0.3	2.4 ± 0.1	2.8 ± 0.2	1.2 ± 0.2
18:1n-7	2.7 ± 0.2	3.1 ± 0.1	1.4 ± 0.1	2.2 ± 0.1
18:1n-5	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.9 ± 0.2
19:1n-9	0.2 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
20:1n-11	3.2 ± 0.5	3.3 ± 0.6	2.3 ± 0.4	0.8 ± 0.1
20:1n-9	0.9 ± 0.1	0.8 ± 0.1	0.8 ± 0.1	0.6 ± 0.0
20:1n-7	1.1 ± 0.1	0.8 ± 0.1	0.6 ± 0.1	0.4 ± 0.0
Total NMIDs	5.3 ± 1.4	5.0 ± 0.4	5.9 ± 0.7	2.5 ± 0.1
20:2n-7, 15	0.2 ± 0.1	0.2 ± 0.0	0.3 ± 0.0	0.1 ± 0.0
22:2n-9, 15	2.1 ± 0.9	1.3 ± 0.2	1.8 ± 0.3	1.1 ± 0.1
22:2n-7, 15	2.7 ± 0.4	3.4 ± 0.3	3.5 ± 0.5	1.1 ± 0.1
Total polyenoic	53.6 ± 1.5	53.2 ± 2.0	58.0 ± 1.3	62.4 ± 0.7
Total n-4 polyenoic	1.6 ± 0.3	1.4 ± 0.2	1.0 ± 0.1	1.4 ± 0.1
16:2n-4	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.0
16:3n-4	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.3 ± 0.0
16:4n-1	1.1 ± 0.3	0.9 ± 0.2	0.6 ± 0.1	0.9 ± 0.1
Total n-6 polyenoic	16.4 ± 0.5	10.9 ± 0.3	12.8 ± 0.4	8.6 ± 0.1
18:2n-6	0.9 ± 0.1	1.0 ± 0.0	0.6 ± 0.0	1.0 ± 0.0
20:2n-6	0.6 ± 0.0	0.5 ± 0.0	0.4 ± 0.1	0.4 ± 0.0
20:4n-6	9.5 ± 0.4	4.5 ± 0.1	6.1 ± 0.3	3.7 ± 0.1
22:3n-6,9,15	1.5 ± 0.3	1.9 ± 0.0	2.6 ± 0.1	1.3 ± 0.1
22:4n-6	2.2 ± 0.2	0.9 ± 0.1	1.1 ± 0.1	0.8 ± 0.0
22:5n-6	1.4 ± 0.1	1.8 ± 0.1	1.9 ± 0.1	1.4 ± 0.0
Total n-3 polyenoic	35.6 ± 1.1	41.0 ± 1.7	44.1 ± 1.2	52.3 ± 0.7
18:3n-3	0.6 ± 0.1	1.0 ± 0.0	0.5 ± 0.0	0.6 ± 0.0
18:4n-3	0.4 ± 0.1	0.6 ± 0.0	0.2 ± 0.0	0.5 ± 0.0
20:4n-3	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.3 ± 0.0
20:5n-3	8.6 ± 0.6	7.6 ± 0.4	8.4 ± 0.6	7.9 ± 0.2
22:5n-3	2.6 ± 0.1	2.0 ± 0.1	2.5 ± 0.1	2.1 ± 0.1
22:6n-3	23.2 ± 1.1	29.6 ± 1.2	32.3 ± 1.1	40.9 ± 0.8
Total FA	93.0 ± 1.5	93.9 ± 0.5	91.7 ± 0.9	95.4 ± 0.2

[a]Data are mean ± SE for several samples (n = 9–29).
[b]In the Tables 3–5, the major FA are selected, if at least one mean datum was detected at a level of 0.8% or more of total FA.

found in the polar lipids. For example, the DHA in PE fluctuated from 18.4 to 33.2%, and that in PC fluctuated from 23.2 to 40.9%; the AA (9.5% of TFA) seemingly compensated for the lack of DHA (23.2% of TFA) in the FA composition of PC in sample 1. This result suggests that mollusks might not have an absolute requirement for DHA as an EFA, and that *P. fucata* might maintain the higher PUFA only for the fluidity of the cell membranes. This speculation is supported by some reports of extremely low levels of DHA in tissue lipids of some gastropod mollusks (10,34,35,41).

Relationship between prey lipids and the concentration of AA in the lipids of P. fucata. With respect to the level of PUFA, freshwater fish generally take in terrestrial prey rich in n-6 PUFA; consequently, they have comparatively high levels of n-6 PUFA, such as linoleic acid and AA, in their lipids (42). In contrast, marine organisms have mainly n-3 FA as PUFA; therefore, AA is usually undetectable or negligible in marine animal lipids. However, we found that *Siganus canaliculatus*, which is a typical subtropical coral fish, has significant levels of AA with consistently high DHA levels in its lipids (43). It is an herbivorous animal that prefers some seaweeds (macroalgae), such as *Cladosiphon* spp., and accumulates AA that originates from the lipids of brown algae. This species has a vegetarian feeding habit, and its lipids therefore differ from those of carnivorous marine fish species. The high levels of both n-3 and n-6 PUFA in *S. canaliculatus* tissues tended to be similar

to the high PUFA levels in *P. fucata* tissues. As for the difference between *S. canaliculatus* and *P. fucata* in the n-3 and n-6 PUFA levels we found that the levels of DHA in *P. fucata* fluctuated among the different conditions, even though consistently high levels of DHA were observed in *S. canaliculatus* tissues.

Although the role of n-6 PUFA in marine organisms is not clearly determined, *P. fucata* may accumulate AA similarly to n-3 PUFA. Differing from the essentiality of n-3 PUFA for marine subtropical fish, this bivalve may accumulate n-6 PUFA as a substitute for n-3 PUFA to maintain the fluidity of its cell membranes. All marine animals may have a tendency to accumulate PUFA in their tissues, and the difference in n-6 and n-3 PUFA levels among bivalve species may be caused only by their feeding habits.

Although *P. fucata* is omnivorous, similar to many other plankton feeders such as zooplankton, its lipid profile suggests that its prey may differ from those of zooplankton, which are primary consumers on the same trophic level as *P. fucata*. This suggests that *P. fucata* mainly filters specific or limited small phytoplankton, whereas many other animals ingest various prey, such as small animals (copepods, ciliates, and flagellates), phytoplankton, and detritus. In particular, high levels of AA and EPA in its lipids also suggest the effect of the lipids of specific phytoplankton.

Consequently, we determined that the lipids in *P. fucata* tissues contained comparatively high levels of both n-3 and n-6 PUFA, and more specifically, that AA and EPA and their derivatives were characteristic FA in both the depot TAG and the tissue PE and PC. Furthermore, the *P. fucata* may transform biosynthetically from shorter, monoenoic FA to longer NMID localized in the PE; AA may thus be useful as a lipid biomarker of herbivorous animals, because most marine animals do not have high levels of AA.

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