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Seasonal Changes in Eicosapentaenoic and Arachidonic Acid Contents in Bivalves and Plankton Collected from Lake Hamana†

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Bivalves and plankton samples were collected monthly for one year from brackish Lake Hamana in Japan, and contents of 5,8,11,14,17-eicosapentaenoic acid (EPA) and 5,8,11,14-eicosatetraenoic acid (arachidonic acid, AA) and their weight percentage to total fatty acids were determined by fluorescence high-performance liquid chromatography. The results regarding bivalves showed that the EPA and AA levels are approximately ten-times higher in liver than in adductor on the average for one year, and are remarkably dependent upon the season. The seasonal changes of EPA and AA contents in bivalve liver are seemingly related to those of plankton, which are the diet of bivalves. From these facts, it can be seen that plankton is the main origin of EPA and AA in bivalve bodies and supply these fatty acids all the way up to humans through a food chain.

Fatty acids are one of the important substances constituting living cells; especially, 5,8,11,14,17-eicosapentaenoic acid (EPA) and 5,8,11,14-eicosatetraenoic acid (arachidonic acid, AA) of C_{20} serve as precursors of prostaglandins (PG) and thromboxanes (TX). In human blood vessels, EPA changes to PGI_3 and TXA_3, and AA to PGI_2 and TXA_2. Both PGI_2 and PGI_3 have the ability to suppress platelet aggregation and vascular smooth muscle contraction; whereas TXA_2 has a strong action on platelet aggregation and vascular smooth muscle contraction, TXA_3 shows almost no such action.[1-3] In addition, EPA can block TXA_2 synthesis;[4] thus, EPA is effective in preventing thrombosis and myocardial infraction.

The fatty acid content or composition in a human body depends upon both nutritional and physiological conditions. The EPA contents in human blood and plasma are much lower than AA contents, and the relative content of EPA can be increased by dieting on fish rich in EPA.[5,6] Thus, it is suggested that the main source of EPA in humans is marine organisms such as fish.

The food chain in marine environments has been an interesting subject: e.g., since fish cannot biosynthesize linoleic acid ($C_{18}^{2=}$) and linolenic acid ($C_{18}^{3=}$),[7] they obtain these fatty acids from phytoplankton or algae. In addition, a freshwater fish can produce long-chain fatty acids of C_{20} and C_{22} from the shorter chain precursors, C_{18} fatty acids, contained in diets, whereas marine fish cannot.[8-10] In contrast, bivalves cannot biosynthesize such long-chain fatty acids as EPA and AA.[11,12]

Thus, in a marine system, plankton plays a role of primary producer for long-chain fatty acids. The fatty acid content or composition in plankton varies in relation to such environmental conditions as temperature and light intensity.[13,14] Therefore, an investigation on the behavior of these long-chain fatty acids in plankton and the other marine organisms under the natural conditions is important for a better understanding of the food chain in marine environments.

The present authors have already established a fluorescence high-performance liquid chromatographic (HPLC) method using 9-anthryldiazomethane(ADAM) as a pre-labeling reagent,[15] and have determined the fatty acids of C_{18} and C_{20} in various marine organisms.[16,17] Although the free fatty acid level is much lower than the esterified fatty acid level, a highly sensitive and selective determination is possible by using this method. These studies have shown that the EPA contents and the EPA/AA ratios in plankton and Mollusca, such as cuttlefish, are higher than those in marine fishes.

We have studied C_{18} and C_{20} fatty acids in bivalves, a kind of Mollusca, and plankton collected monthly from brackish Lake Hamana, Japan, during 1985. Here, we present data on seasonal changes of the fatty acid contents and discuss the relationship between the EPA and AA contents in bivalves and those in plankton from the viewpoint of a food chain.

Experimental

Standards and Reagents. All standard fatty acids of $C_{18}^{0-3=}$ and $C_{20}^{0-5=}$ were purchased from Sigma (St. Louis, MO, USA), and ADAM was purchased from Funakoshi Chemical (Tokyo, Japan). All other reagents were of commercially analytical grade. The preparation techniques of a standard solution of fatty acids and an ADAM solution have been previously described in detail.[15-17]

Samples. Clam(*Tapes (Amygdala) japonica*) (3.5—4 cm long) and oyster (*Crassostrea gigas*) (8—12 cm long) were collected monthly during the year of 1985 from the southern inshore part (water depth: about 1 m) of brackish Lake Hamana. On the same sampling date, plankton samples of different size were collected by horizontal hauls (0.5—1 m layer from the surface) with 20, 40, and 94 μm mesh-sized nets from the same area, and separated into plankton A (20 μm), B (40 μm), and C (94 μm). These plankton samples were filtrated on a Millipore filter (pore size: 0.45 μm). All samples were stored in a freezer (−10 °C) before use.

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As the representative organs to be examined in bivalve body, liver and adductor were selected from parts of viscera and muscle, respectively. Bivalve liver acts as a digestive organ, different from that of Vertebrate. Moreover, whole bodies were examined.

Analytical Procedure. Bivalve liver and adductor were isolated from bodies with a small knife. The homogenate of a whole body was obtained by grinding in a mortar. The liver, adductor and homogenate of a whole body of bivalve samples (10—20 mg) were weighed as wet weight in a glass homogenizer (volume: 1 ml). One ml of a mixture of chloroform and methanol (2:1) was added, and the sample was homogenized. The homogenate was transferred into a graduated centrifugal tube with a stopper, and diluted to 1 ml with the above-mentioned solvent since some part of the solvent evaporated during the homogenization. After centrifuging at 3000 rpm for 5 min, the supernatant was filtrated through a disposable filter (pore size: 0.45 μm).

An aliquot of the sample filtrates was derivatized with an ADAM and analyzed by the HPLC method using a Shimadzu Model LC-4A liquid chromatograph (Zorbax C_8 column) equipped with a Shimadzu Model RF-510LC fluorescence spectromonitor and a Shimadzu Model C-R1A(S) chromatopac as described in the previous papers.[16,17] In the determination of EPA and AA in living-body samples, the recovery was 90—100% and the coefficient of variation by repeated experiments was about 7%. The detection limit was 1—2 ng for $C_{18}^{0-3=}$ and $C_{20}^{0-5=}$ fatty acids.

Plankton samples (10—50 mg), which remained on the filter were homogenized after adding about 1 ml of the mixture of chloroform and methanol (2:1). The homogenates were transferred into the centrifugal tube, diluted with the same solvent up to 3 ml and then vortexed for 3 min. The procedure from centrifugation was the same as that mentioned above. The contents of fatty acids in plankton were obtained on a dry-weight basis, by determining the moisture in the sample.

Measurements of Chlorophylls, Carbon and Nitrogen in Plankton. For the extraction of pigments, the plankton together with the filter was ground in a mortar with 3—5 ml of acetone and water (9:1) and then transferred into a graduated centrifugal tube. After centrifugation at 3000 rpm for 5 min, the absorbance of the supernatant was measured at 630, 647, 664, and 750 nm. The contents of chlorophyll *a*, *b*, and *c* were calculated, without standards, according to the equation of Jeffrey and Humphrey.[18]

The carbon and nitrogen contents were measured using a Yanagimoto Model MT-500 CN corder with hippuric acid as a standard.

Results and Discussion

Figure 1 shows the seasonal changes of the EPA and AA contents in bivalves. In both clam and oyster, the EPA and AA contents in the liver are higher than those in the adductor and homogenate of a whole body; the ratio of EPA or AA content in the liver to that of the adductor was approximately ten on the average for one year. The contents of EPA and AA show seasonal fluctuations. In the case of liver, the EPA and AA contents remarkably increase in the late spring when bivalves breed, and again in the early winter. In the adductor and homogenate of a whole body, the variations of these fatty acid levels are not significant. Differences between clam and oyster were not significant regarding these results.

On the other hand, the EPA and AA contents in plankton increase during midsummer and early winter for plankton A or B, and in the above-mensioned seasons and late spring for plankton C as shown in Fig. 2(a). Since sand and mud in water may have been collected together with plankton by the plankton net, the fatty acid content per carbon weight was calculated in order to exclude any error due to sand or mud contamination. The peaks during midsummer (Fig. 2(b))

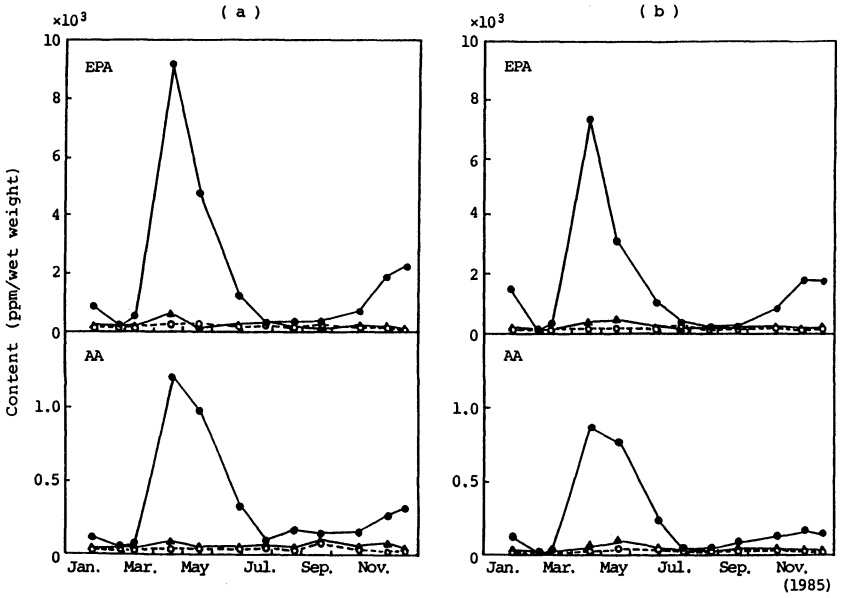

Fig. 1. Seasonal changes of EPA and AA contents in (a) clam and (b) oyster. ●—●: liver, ○--○: adductor, ▲—▲: whole body.

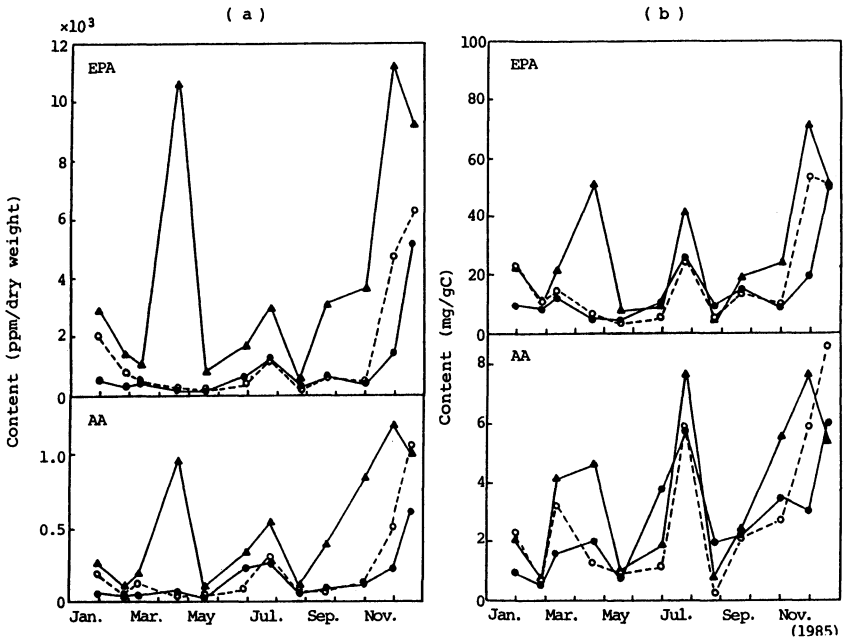

Fig. 2. Seasonal changes of EPA and AA contents per (a) dry weight and (b) carbon weight in plankton. ●—●: plankton A (mesh size of the used plankton net: 20 μm), ○--○: plankton B (40 μm), ▲—▲: plankton C (94 μm).

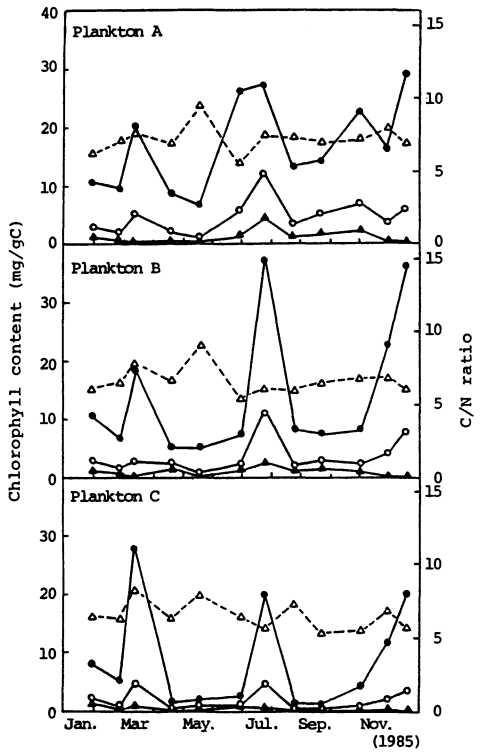

Fig. 3. Characteristics of plankton A, B, C and their seasonal changes. ●—●: chlorophyll *a*, ▲—▲: chlorophyll *b*, ○—○: chlorophyll *c*, △--△: C/N ratio.

are clear in comparison with those of Fig. 2(a).

Since collected plankton consists of various species, plankton A, B, and C were characterized by the chlorophyll content and C/N ratio (Fig. 3). On the average, the chlorophyll content, which is an index of

Table 1. Relation of Chlorophyll Contents to EPA and AA Contents in Plankton

Combination	Correlation coefficient		
	Plankton A	Plankton B	Plankton C[a)]
Chlorophyll *a*-EPA	0.687	0.764	0.527
Chlorophyll *b*-EPA	0.056	−0.386	−0.285
Chlorophyll *c*-EPA	0.484	0.590	0.556
Chlorophyll *a*-AA	0.893	0.927	0.611
Chlorophyll *b*-AA	0.512	0.249	−0.157
Chlorophyll *c*-AA	0.814	0.813	0.673

a) The correlation coefficients for the data except for April were calculated, since the data in April for plankton C were apparently predicted to deviate from the relationship of the data in other months.

phytoplankton, becomes higher in the order plankton C<B<A; thus, the size becomes smaller, since the size of phytoplankton is generally smaller than zooplankton.

The C/N ratio corresponds to adipose/protein ratio in the body. As can be seen in Fig. 3, the C/N ratio in plankton A and B in May is approximately 9, which is significantly high for a plankton value. This high C/N ratio could be due to the resuspension of bottom sediments (C/N ratios of which are higher than those of plankton) into overlying water. Lower EPA and AA contents in the sample of May (Fig. 2) seem to be consistent with higher C/N ratios.

In a comparison between Fig. 2(b) and Fig. 3, the peaks in the seasonal changes of the chlorophyll contents of plankton appear in roughly the same months as the peaks of EPA or AA contents. Then, their corre-

lation coefficients were calculated (Table 1). The seasonal changes of chlorophyll *a* and *c* contents were considerably related to that of the EPA and AA contents in Fig. 2(b), except that the EPA and AA contents in plankton C increase in April. This increase of EPA and AA is probably attributable to zooplankton, since the chlorophyll content in plankton C (94 μm) was very low during April. It is likely that zooplankton breeds by feeding on phytoplankton, which proliferates in March, as suggested from the significant increase in chlorophyll *a* and *c* contents (Fig. 3); consequently, zooplankton accumulates EPA and AA in its body.

When considering plankton as a diet of bivalve, the unit (ppm/dry weight) for fatty acid content in Fig. 2(a) is more adequate, rather than the unit (mg/gC) in Fig. 2(b) since bivalve cannot selectively feed on only plankton. Comparing Fig. 1 with Fig. 2(a), it is evident that when the EPA and AA contents increase in the bivalve liver, those in plankton C, which is the main diet for bivalve, also increase. This suggests that EPA and AA in bivalves are obtained from relatively large phytoplankton, zooplankton and their fecal pellets.

The averaged fatty acid composition and its degree of variation during one year, as a coefficient of the variation (the percentage of standard deviation to average), are given in Table 2 for the bivalve liver and plankton. The fatty acid compositions of clam and oyster were almost the same and the degree of year-round variation in the composition of the bivalve liver was smaller than that of plankton. This result can be explained by the fact that the dominant plankton species change depending on the season and that these plankton are different regarding fatty acid composition. In each plankton sample the variation of the $C_{18}^{1=}$ weight percentage was remarkable. Consequently, in Lake Hamana, it is considered that some plankton species, of which populations vary during the year, show $C_{18}^{1=}$ weight percentages different from

Table 2. Averaged Fatty Acid Composition in Bivalves and Plankton for a Year (1985)

Sample	Weight percentage[a] (Coefficient of variation(%))								
	$C_{18}^{0=}$	$C_{18}^{1=}$	$C_{18}^{2=}$	$C_{18}^{3=}$	$C_{20}^{1=}$	$C_{20}^{2=}$	$C_{20}^{3=}+C_{16}^{0=}$	$C_{20}^{4=}$ (AA)	$C_{20}^{5=}$ (EPA)
Clam liver	13.4	18.0	8.2	9.5	4.1	1.4	20.4	4.3	20.7
	(21.7)	(28.0)	(31.1)	(23.8)	(21.7)	(26.1)	(19.5)	(30.6)	(38.8)
Oyster liver	8.6	20.7	10.1	10.9	7.3	0.8	17.0	3.3	21.3
	(25.5)	(12.3)	(16.4)	(20.2)	(24.2)	(28.8)	(19.2)	(35.6)	(36.0)
Plankton A	13.1	13.6	3.4	24.7	ND	ND	20.1	3.5	21.6
	(52.5)	(115.7)	(73.1)	(37.3)			(19.6)	(31.9)	(49.3)
Plankton B	10.6	19.7	4.2	20.9	0.7	ND	18.6	3.4	21.9
	(43.0)	(89.9)	(76.7)	(40.4)	(47.5)		(20.9)	(34.3)	(49.5)
Plankton C	9.5	12.7	4.2	21.1	0.9	0.4	19.0	3.8	28.4
	(35.0)	(60.4)	(46.2)	(37.6)	(74.2)	(76.4)	(16.8)	(27.1)	(30.7)

a) Weight percentage to total fatty acids($C_{16}^{0=}$, $C_{18}^{0-3=}$, and $C_{20}^{0-5=}$)
$C_{20}^{0=}$ was not detected in all samples.

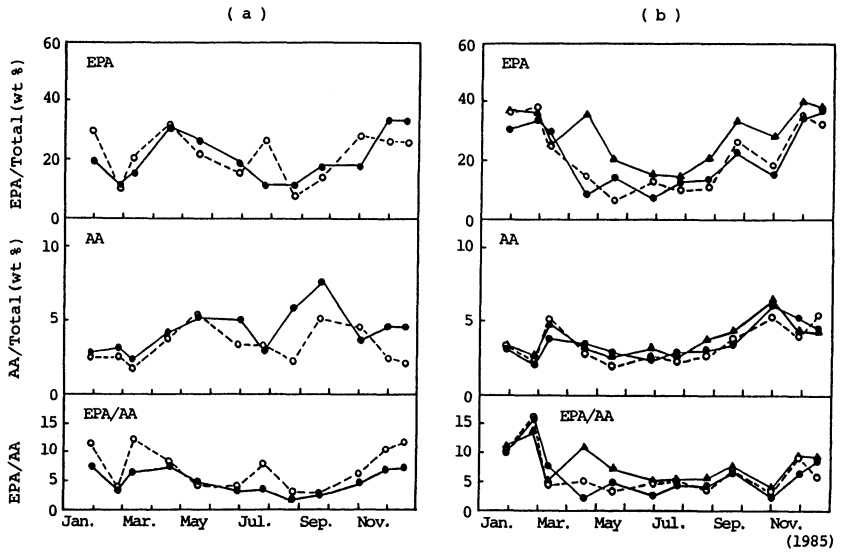

Fig. 4. Seasonal changes of EPA and AA weight percentage to total fatty acids ($C_{16}^{0=}$, $C_{18}^{0-3=}$, and $C_{20}^{0-5=}$) and EPA/AA ratio in (a) bivalve liver and (b) plankton. (a) ●—●: clam, ○--○: oyster, (b) ●—●: plankton A, ○--○: plankton B, ▲—▲: plankton C.

each other. In the bivalve liver, the variations of the EPA and AA weight percentages were larger than those of the other fatty acids.

Figure 4 shows the seasonal changes of the EPA and AA weight percentage to the total fatty acids ($C_{16}^{0=}$, $C_{18}^{0-3=}$, and $C_{20}^{0-5=}$) and the EPA/AA ratio for bivalve liver and plankton. The seasonal changes of the EPA weight percentage in individual samples were different from those of AA, whereas there was no significant difference in the behavior between clam and oyster, or among plankton A, B, and C. Between bivalve liver and plankton, a clear relationship was not found for the each seasonal change of the EPA and AA weight percentage.

Since all trends regarding the fatty acids for clam and oyster in this experiment were closely similar to each other, they are considered to be just as adequate for discussion as the typical data of bivalves.

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