Phospholipids of the Rotifer, Prawn, and Larval Fish^{*1}Shin-ichi Teshima,^{*2} Akio Kanazawa,^{*2} Koji Horinouchi,^{*2}
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In order to define the nutritional role of dietary phospholipids (PL) in larval fish, the PL classes and possible fatty acid combinations of phosphatidylcholine (PC) were investigated on the food organisms such as the micro-algae and rotifers *Brachionus plicatilis*, post-larval prawns (P-30) *Penaeus japonicus*, and larval fish. PC and phosphatidylethanolamine were the major PL classes in a marine *Chlorella* (CH), *Tetraselmis* (TS), rotifers cultured with either CH or TS, red sea bream (40-days old) *Chrysophrys major*, knife jaw (30 days-old) *Oplegnathus fasciatus*, Ayu (74-days old) *Plecoglossus altivelis*, and prawn. The major PC in the larval fish and prawn were those having fatty acid combinations of $C_{16}C_{20}$ (mainly $C_{16:0}C_{20:5\omega3}$), $C_{16}C_{22}$ (mainly $C_{16:0}C_{22:6\omega3}$), and $C_{18}C_{22}$ (mainly $C_{18:0}C_{22:6\omega3}$ and/or $C_{18:1\omega9}C_{22:6\omega3}$). Because larval fish are incapable of *de novo* synthesis of $\omega3$ - and $\omega6$ -series of fatty acids and also possibly have a limited ability for the synthesis of some PC, a part of these PC occurring in the larval fish may be derived from food organisms such as the rotifers which contained PC with fatty acid combinations of $C_{16}C_{20}$ (mainly $C_{16:0}C_{20:5\omega3}$).

Recent our work has shown that some dietary phospholipids (PL) are necessary for good growth and survival of the larval fish such as the red sea bream *Chrysophrys major*,^{1,2)} the Ayu *Plecoglossus altivelis*,^{3,4)} the starry flounder *Paralichthys olivaceus* (Unpublished data), and the knife jaw *Oplegnathus fasciatus*.²⁾ These findings are unusual because animals in general can synthesize phospholipids from dietary or endogenous fatty acids, diglycerides, and triglycerides. Kanazawa *et al.*⁵⁾ found that phosphatidylcholine (PC) and phosphatidylinositol (PI) containing unsaturated fatty acids such as linoleic acid (18:2 $\omega6$), docosahexaenoic acid (22:6 $\omega3$), etc. as fatty acid moieties were effective in enhancing growth and survival rates of the larval Ayu.

The importance of inclusion of dietary PL in diets has also been shown on the crustaceans such as the prawn *Penaeus japonicus*⁶⁻⁷⁾ and the lobster *Homarus americanus*.^{8,9)} Recently, we have demonstrated that the retardation of growth in the prawn *P. japonicus* receiving a PL-deficient diet is probably attributable to the insufficient transport of dietary lipids, especially cholesterol which is one of essential nutrients.¹⁰⁻¹³⁾ Regarding the larval fish, however, little is known on the mecha-

nism by which dietary PL exert a growth-enhancing effect.

Generally, larval fish grow well on a live food such as the rotifers.^{14,15)} The manifestation of PL components of food organisms and predators may provide an understanding of the modification, transport, and storage of dietary PL in the larval fish. As part of defining the nutritional role of supplemental PL in diets, we investigated the compositions of PL classes and molecular species of PC in several food organisms, larval fish, and post-larval prawn.

Materials and Methods

Culture of Food Organisms

The micro-algae, *Chlorella saccharophila* (a marine *Chlorella*) and *Tetraselmis tetrathele*, were cultured outdoors at $28 \pm 2^\circ\text{C}$ in 500 l-round transparent tanks containing 20‰ or 35‰ sea water with the following fertilizers(g/t)¹⁶⁾: Ammonium sulfate, 5; super phosphate lime, 15; urea, 5; Clewat-32 (Teikoku-kagaku Co.), 5. The algae were harvested in the end of logarithmic growth-phase (population density: *Chlorella*, 10.3×10^6 cells/ml; *Tetraselmis*, 0.26×10^6 cells/ml).

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Three types of the algae were used for the culture of rotifers *Brachionus plicatilis*: (1) *Chlorella* culture in 20‰ sea water [CH(20‰)]; (2) *Chlorella* culture in 35‰ sea water [CH(35‰)]; (3) *Tetraselmis* cultured in 20‰ sea water [TS(20‰)]. When *Chlorella* and *Tetraselmis* bloomed up to the densities of 15×10^6 cells/ml and 0.16×10^6 cells/ml, respectively, the rotifers were inoculated at the concentration of 5 individuals/ml water. The rotifers were cultured outdoors in 1 ton-polycarbonate tanks at 21–29°C for 4 days until they were increased to the population density of 30–40 individuals/ml. In this paper, the rotifers cultured with CH (20‰), CH(35‰), and TS(20‰) were abbreviated as CH(20‰)-rotifer, CH(35‰)-rotifer, and TS(20‰)-rotifer, respectively.

Larval Fish and Post-larval Prawn

The red sea bream (30-days old), knife jaw (30-days old), and Ayu (74-days old) were obtained from the Fisheries Experimental Station of Oita Prefecture. After hatching, the red sea bream and knife jaw were maintained on the rotifers cultured with a marine *Chlorella* (CH-rotifer), whereas the Ayu received a combination of CH-rotifer, *Artemia*, and a commercial diet. The post-larval prawn (P-30) was produced in our laboratory by feeding a combination of micro-particulate diets¹⁷⁾ and *Artemia* nauplii.

Separation and Analysis of Lipid Components

Total lipids (TL) were extracted with chloroform-methanol-water¹⁸⁾ and separated into neutral lipids (NL) and PL by column chromatography on Kieselgel 60 as described previously.¹⁰⁾ PL were further separated into PL classes by thin-layer chromatography (TLC) on Kieselgel H containing sodium carbonate with chloroform-methanol-acetic acid-water (25:15:2:0.5) and then quantitated photometrically.¹⁰⁾ After TLC, PC fraction was eluted from the TLC plates with chloroform-methanol-14N ammonia (56:48:2) for the estimation of molecular species.¹⁰⁾

Gas-liquid Chromatography (GLC)

GLC was conducted using a Shimadzu GC-8A with a hydrogen flame ionization detector. Fatty acid compositions (%) of PC before and after catalytic hydrogenation with a platinum black were determined by GLC on 5.0% Shinchrom E-71 (column temp. 215°C).¹⁰⁾ The hydrogenates of diglycerides (DG) were converted to trimethylsilyl (TMS) derivatives with hexamethyldisilazane and trimethylchlorosilane. The TMS derivatives

of DG were subjected to GLC on 1.5% OV-1 on Shimalite W (AW, DMCS, 80–100 mesh) (glass column 50 cm $\times$ 3 mm i.d., column temp. 230°C to 300°C at an increasing rate of 2°C/min) for determination of total carbon numbers of fatty acyl chains in PC.²⁰⁾ Dipalmitin (Sigma Chem. Co.), distearin (Sigma Chem. Co.), diarachidin (Nu Chek Prep. Inc.), and dibehenin (Nu Chek Prep. Inc.) were used as standards.

Estimation of Possible Fatty Acid Combinations in PC

The possible fatty acid combinations in PC were estimated by the method of Ohshima *et al.*²⁰⁾ PC fraction was converted to 1,2-diglycerides (1,2-DG) by hydrolysis with phospholipase C (EC 3.1.4.3). The resultant 1,2-DG were extracted with chloroform-methanol and purified by TLC on Kieselgel G with hexane-ether-acetic acid (80:30:1). Possible fatty acid combinations of PC were estimated from the fatty acid compositions (%) before and after hydrogenation and the PC compositions (%) based on total carbons in fatty acyl chains.

Results

Lipid Contents and PL Class Compositions

Table 1 shows the TL, NL, and PL contents, and PL class compositions of the marine *Chlorella*, *Tetraselmis*, rotifers, larval fish, and prawn (P-30). The *Chlorella*, *Tetraselmis*, and rotifers contained TL ranging from 8.06% to 12.13% of dry matters and PL amounting to 28.4–37.5% of TL. The PL of the micro-algae and rotifers were composed of PC (41.7–47.1% of total PL), phosphatidylethanolamine (PE) (34.5–39.0%), PI (7.3–11.1%), and phosphatidylserine (PS) (1.6–6.7%) as the prominent PL classes. The proportions of PL classes of the micro-algae and rotifers thus resembled each other.

The larval fish such as the red sea bream, knife jaw, and Ayu, and the post-larval prawn contained TL ranging from 0.96% to 3.5% and PL amounting to 34.7–43.4% of TL. These animals gave similar PL class compositions each other and contained PC (43.3–48.8%) and PE (30.5–32.6%) as the major PL classes as observed in the CH(20‰), CH(35‰), and TS(20‰)-rotifers.

Fatty Acid Compositions of PC

Table 2 shows the fatty acid compositions of soybean lecithin and PC from the *Chlorella*, *Tetraselmis*, rotifers, larval fish, and prawn.

Table 1. Total lipid, neutral lipid, and phospholipid class contents of the food organisms, larval fish, and post-larval prawn *P. japonicus*

Organism* ¹	Content (% of dry or fresh wt.)* ²			Composition (% of total phospholipids)* ³						
	TL	NL	PL	PE	PS	PI	PC	SM	LPC	UK
Marine <i>Chlorella</i> [CH(20‰)]	8.06	5.77	2.29(28.5%) ^{*4}	34.5	1.6	11.1	47.1	1.2	1.6	2.9
<i>Tetraselmis</i> [TS(20‰)]	8.35	5.97	2.37(28.4%)	38.6	2.1	9.1	45.7	1.0	1.3	2.2
CH(20‰)-rotifer	10.75	6.72	4.03(37.5%)	37.4	5.0	11.1	41.9	3.4	1.2	—
CH(35‰)-rotifer	12.13	7.64	4.49(37.0%)	39.0	3.4	10.4	42.1	2.9	2.2	—
TS (20‰)-rotifer	11.41	7.11	4.30(37.7%)	37.3	6.7	7.3	41.7	2.7	4.3	—
Red sea bream (30 days old)	1.85	1.18	0.67(36.3%) ^{*4}	32.6	10.1	5.2	43.3	4.1	4.7	—
Knife jaw (30 days old)	1.70	0.97	0.73(42.8%)	32.8	3.6	9.8	44.0	5.3	2.6	1.9
Ayu (74 days old)	3.53	2.00	1.53(43.4%)	32.6	4.7	3.3	48.8	4.1	6.5	—
Prawn (Post-larvae P-30)	0.96	0.63	0.33(34.7%)	30.5	4.3	10.9	45.4	4.8	2.1	2.0

*1 CH (20 ‰) and TS (20 ‰) indicate marine *Chlorella* and *Tetraselmis* cultured in a water (20 ‰ salinity), respectively. CH (20 ‰)-rotifer means *B. plicatilis* reared with marine *Chlorella* [CH (20 ‰)].

*2 TL, total lipids; NL, neutral lipids; PL, phospholipids. TL, NL, and PL contents of the food organisms were expressed as % of dry weight, and those of the larval fish and post-larval prawn were shown as % of fresh weight.

*3 PE, phosphatidylethanolamine; PS, phosphatidylserine; PI, phosphatidylinositol; PC, phosphatidylcholine; SM, sphingomyelin; LPC, lysophosphatidylcholine; UK, unknown phospholipids.

*4 Per cent of total lipids.

Soybean lecithin comprised $C_{18:2\omega6}$ as the major fatty acids. The major fatty acids of *Chlorella* PC were $C_{18:0}$, $C_{18:1\omega7}$, and $C_{20:5\omega3}$, whereas those of *Tetraselmis* PC were $C_{18:0}$, $C_{18:1\omega9}$, and $C_{18:3\omega3}$. The PC of CH(20‰)- and CH(35‰)-rotifers involved $C_{18:0}$, $C_{18:2\omega6}$, and $C_{20:5\omega3}$ as the major fatty acids, whereas that of TS(20‰)-rotifer did $C_{18:0}$, $C_{18:3\omega3}$, and $C_{20:4\omega3}$. The occurrence of high levels of $C_{20:4\omega3}$ in the PC of TS(20‰)-rotifer suggests the conversion of exogenous $C_{18:3\omega3}$ to $C_{20:4\omega3}$ in the rotifers fed TS(20‰) containing abundance of $C_{18:3\omega3}$. Fukusho *et al.*²¹⁾ also reported that the rotifer cultured with *Tetraselmis* contained $C_{18:3\omega3}$ as the major fatty acids (22.4% of total fatty acids) and lesser amounts of highly unsaturated fatty acids (HUFA) such as $C_{20:4\omega6}$ (5.7%) and $C_{20:5\omega3}$ (5.8%).

The PC of the red sea bream, knife jaw, Ayu, and prawn contained $C_{18:2\omega6}$, $C_{20:5\omega3}$, and $C_{22:6\omega8}$ in addition to $C_{18:0}$, $C_{18:1}$, and $C_{18:1\omega9}$ as the prominent fatty acids.

Possible Fatty Acid Combinations in PC

Table 4 shows the possible fatty acid combinations in PC which were estimated from both the fatty acid compositions of hydrogenated PC and the PC compositions based on total carbons in fatty acyl chains (Table 3). Soybean lecithin was composed of five components with the fatty acid combinations of $C_{16}C_{18}$, $C_{18}C_{18}$, $C_{14}C_{22}$, $C_{16}C_{20}$, and $C_{18}C_{18}$, the most prominent combinations being $C_{16}C_{18}$ (33.7% of total PC) and $C_{18}C_{18}$ (59.9%). Because the major fatty acids in the

C_{18} and C_{18} fatty acids were $C_{18:0}$ and $C_{18:2\omega6}$ (Table 4), the major fatty acid combinations of $C_{16}C_{18}$ and $C_{18}C_{18}$ were conceived to be those of $C_{18:0}C_{18:2\omega6}$ and $C_{18:2\omega6}C_{18:2\omega6}$, respectively. In the similar manner, the major fatty acid combinations of PC from the food organisms, larval fish, and prawn were estimated (Table 5).

The PC of CH(20‰) was mainly composed of three components with the fatty acid combinations of $C_{16}C_{18}$ (22.9%), $C_{14}C_{20}$ (24%), and $C_{16}C_{20}$ (39.6%). The major fatty acid combinations of $C_{16}C_{18}$ and $C_{14}C_{20}$ could be those of $C_{18:0}C_{18:1}$ and $C_{14}C_{20:5\omega3}$, respectively. The PC with $C_{16}C_{20}$ moieties might be those containing $C_{18:0}C_{20:5\omega3}$ and/or $C_{18:1}C_{20:5\omega3}$. The PC of TS(20‰) consisted of mostly those with fatty acid combinations of $C_{16}C_{18}$ (52.6%; mainly $C_{16:0}C_{18:3\omega3}$), $C_{16}C_{20}$ (14.2%; mainly $C_{18:0}C_{20:5\omega3}$), and $C_{18}C_{18}$ (16.3%; mainly $C_{18:1\omega9}C_{18:2\omega6}$ and/or $C_{18:1\omega9}C_{18:3\omega3}$). Since the PC of marine organisms generally contain unsaturated fatty acids at position 2 much more than saturated fatty acids,²²⁻²⁶⁾ the PC of *Chlorella* and *Tetraselmis* possibly possess $C_{20:5\omega3}$ and $C_{18:3\omega3}$ as the fatty acid moieties at position 2.

The CH(20‰)-rotifer contained the PC with fatty acid combinations of $C_{16}C_{18}$ (13.1%; mainly $C_{16:0}C_{18:1}$), $C_{14}C_{20}$ (11.8%; mainly $C_{14:0}C_{20:5\omega3}$), $C_{16}C_{20}$ (24.6%; mainly $C_{18:0}C_{20:5\omega3}$), $C_{18}C_{18}$ (11.8%; mainly $C_{18:1\omega9}C_{18:2\omega6}$ and/or $C_{18:0}C_{18:2\omega6}$) and $C_{16}C_{22}$ (13.7%; mainly $C_{16:0}C_{22:6\omega8}$ and/or $C_{18:0}C_{22:5\omega3}$). The CH(35‰)-rotifer gave similar fatty acid combinations in PC to the CH(20‰)-rotifer, but the former possessed higher levels of

Table 2. Fatty acid compositions (%) of phosphatidylcholines from the food organisms, larval fish, and post-larval prawn *P. japonicus*

Fatty acid	Soybean lecithin	Marine <i>Chlorella</i>	<i>Tetraselmis</i>	CH (20‰)-rotifer	CH (35‰)-rotifer	TS (20‰)-rotifer	Red sea bream	Knife jaw	Ayu	Prawn (P-30)
14:0	0.13	7.94	0.86	3.51	3.20	2.14	0.96	0.71	0.57	1.05
14:1	—	0.53	—	—	—	—	0.26	0.25	—	0.69
14:2	—	—	—	0.89	0.43	0.32	—	—	0.10	0.44
14:3	—	1.39	0.30	1.48	0.49	0.84	0.21	0.40	0.18	—
(Sum)	0.13	9.84	1.16	5.88	4.12	3.30	1.43	1.39	0.85	2.18
16:0	16.44	18.73	26.64	18.17	20.73	26.11	23.80	19.58	20.92	16.81
16:1 ω 7	—	22.53	5.10	9.09	8.69	4.36	4.60	3.58	2.64	2.62
16:2 ω 6	—	0.78	0.99	—	1.08	—	—	—	—	—
16:3 ω 6	—	0.57	1.34	0.29	0.23	0.12	1.01	1.17	0.83	0.91
16:3 ω 3	0.23	0.49	0.99	—	—	0.40	0.57	0.92	0.26	0.24
16:4 ω 3	—	0.17	9.58	—	0.57	1.25	0.31	—	0.19	0.48
(Sum)	16.67	43.27	44.64	27.55	31.30	32.24	30.29	25.25	24.84	21.06
18:0	4.14	0.30	0.94	4.15	4.06	4.47	11.24	10.83	5.81	6.78
18:1 ω 9	12.99	2.54	11.29	5.10	8.49	6.58	14.53	13.35	14.91	14.59
18:2 ω 6	56.35	2.53	7.71	11.05	21.40	4.38	6.37	9.15	5.52	11.26
18:3 ω 6	8.30	0.41	0.17	—	—	0.16	—	—	—	—
18:3 ω 3	—	0.11	22.47	—	1.54	19.50	0.17	0.31	0.54	1.27
18:4 ω 3	—	—	2.27	0.66	0.10	1.17	—	—	0.13	—
(Sum)	81.78	5.89	44.85	20.90	35.59	36.26	32.31	33.64	26.91	33.90
20:0	—	—	—	—	—	—	0.18	0.35	—	0.22
20:1 ω 9	0.19	—	2.16	3.52	3.18	3.65	1.16	1.15	0.57	1.34
20:2 ω 6	—	—	—	0.42	1.07	—	0.55	0.50	0.17	0.56
20:3 ω 6	—	0.53	0.18	2.81	1.97	0.99	0.65	0.49	0.18	0.46
20:3 ω 3	—	5.13	0.64	3.86	2.74	2.40	3.53	2.57	3.76	2.82
20:4 ω 6	—	—	0.89	4.26	2.71	8.17	0.44	0.56	0.55	0.90
20:5 ω 3	0.50	34.05	4.26	14.72	8.52	5.12	6.48	6.01	11.18	13.87
(Sum)	0.69	39.71	8.13	29.59	20.19	20.33	12.99	11.63	16.41	20.17
22:0	—	—	0.01	—	—	—	—	—	—	—
22:1 ω 9	—	0.10	0.42	4.63	—	3.41	0.97	0.74	0.51	1.10
22:2 ω 6	—	—	—	—	2.81	—	0.06	—	—	—
22:3 ω 6	—	0.13	—	0.57	0.29	0.34	—	—	0.11	—
22:3 ω 3	—	—	—	0.45	—	0.12	0.35	0.19	0.33	—
22:4 ω 3	—	0.41	0.49	2.98	1.74	2.21	1.16	0.90	0.66	1.07
22:5 ω 3	0.11	0.12	0.18	5.12	2.89	0.88	4.20	1.41	2.00	0.28
22:6 ω 3	0.62	0.54	0.13	2.26	1.07	0.91	16.24	24.85	27.38	20.25
(Sum)	0.73	1.30	1.22	16.01	8.80	7.87	22.98	28.09	30.99	22.70

the PC having $C_{16}C_{18}$ (32.9%, mainly $C_{16:0}C_{18:2\omega6}$) moieties and lower levels of the PC having $C_{16}C_{20}$ (12.3%) and $C_{14}C_{20}$ moieties than the latter. The TS(20‰)-rotifer involved mainly the PC with fatty acid combinations of $C_{16}C_{18}$ (43.8%; mainly $C_{16:0}C_{18:3\omega3}$) and $C_{18}C_{18}$ (14.7%; mainly $C_{18:0}C_{18:3\omega3}$ and/or $C_{18:1\omega9}C_{18:3\omega3}$). As the fatty acid moieties at position 2 of PC, the TS-rotifer was thus likely to contain $C_{18:3\omega3}$ in contrast to the CH-rotifer containing $C_{18:2\omega6}$ and $C_{20:5\omega3}$.

The PC of red sea bream consisted of mainly the components with fatty acid combinations of

$C_{18}C_{18}$ (19.4%; mainly $C_{18:0}C_{18:1\omega9}$), $C_{16}C_{20}$ (15.5%; mainly $C_{18:0}C_{20:5\omega3}$), $C_{16}C_{22}$ (21.9%; mainly $C_{16:0}C_{22:0\omega3}$), and $C_{18}C_{22}$ (14.8%; mainly $C_{18:0}C_{22:0\omega3}$ and/or $C_{18:1\omega9}C_{22:0\omega3}$). The knife jaw gave similar fatty acid combinations in PC to the red bream, although the former contained higher levels of the PC having $C_{18}C_{22}$ (31.0%) than the latter. The PC of Ayu was composed of largely the components with fatty acid combinations of $C_{16}C_{22}$ (21.0%; mainly $C_{16:0}C_{22:0\omega3}$) and $C_{18}C_{22}$ (28.5%; mainly $C_{18:1\omega9}C_{22:0\omega3}$). The prawn revealed similar fatty acid combinations in PC to the larval fish

Table 3. Total carbon numbers in fatty acyl chains of phosphatidylcholines (PC) from the food organisms, larval fish, and post-larval prawn *P. japonicus*

Organism*	Total carbon number (% of total fatty acyl chains of PC)						
	C ₃₂	C ₃₄	C ₃₆	C ₃₈	C ₄₀	C ₄₂	C ₄₄
Soybean lecithin	4.3	33.7	62.0	—	—	—	—
Marine <i>Chlorella</i> [CH (20‰)]	22.9	24.3	42.6	5.7	4.5	—	—
<i>Tetraselmis</i> [TS (20‰)]	3.8	52.6	30.4	9.8	3.4	—	—
CH (20‰)-rotifer	13.1	18.7	36.4	22.6	5.6	2.5	1.3
CH (35‰)-rotifer	10.4	32.9	33.4	16.5	3.7	2.5	0.7
TS (20‰)-rotifer	6.3	50.7	29.3	11.4	2.0	0.2	—
Red sea bream	6.5	19.4	25.9	24.0	12.8	7.8	3.5
Knife jaw	2.0	14.2	20.5	28.2	31.0	2.5	1.6
Ayu	0.7	5.7	14.7	30.0	37.0	7.9	4.1
Prawn	6.5	11.0	21.7	25.2	25.3	6.6	3.6

* See Table 1 for abbreviations.

examined, but it contained considerably large proportions of the PC having fatty acid combinations of C₁₈C₂₀ (22.7%; mainly C_{18:1ω9}C_{20:5ω8} and/or C_{18:2ω6}C_{20:5ω8}) besides C₁₆C₂₀ (20.1%) and C₁₈C₂₂ (25.3%).

Discussion

The addition of PL, specifically PC and PI, has been shown to be indispensable for sustaining good growth and survival of the larval fish.¹⁻⁴⁾ In the case of the larval Ayu, the incidence of malformation, especially scoliosis and twist of jaw, was also markedly reduced by the supplement of PL such as PC to diets.⁴⁾ But, a basic question to be answered is why dietary PL are required for growth and survival of the larval fish in contrast to the case of grown fish. We thought that the detailed analysis of PL components of both food organisms and larval fish might give some clue to the mechanism by which dietary PL enhance the growth and survival of larval fish, because the practical seed production of fish has been successfully performed using the live food such as the rotifer and *Artemia* nauplii.

The results of the present study showed that the compositions (%) of PL classes were not notably different among the animals examined. PC and PE were the major PL classes in the rotifers, larval fish, prawn, and micro-algae, *Chlorella* and *Tetraselmis*. But, the fatty acid compositions and molecular species of PC differed among the organisms examined (Tables 4 and 5). The larval fish such as the red sea bream, knife jaw, and Ayu contained the PC including ω3-highly unsaturated fatty acids (HUFA) as the fatty acid moieties; the major PC being those with fatty

acid combinations of C_{18:0}C_{20:5ω3}, C_{18:0}C_{22:6ω3}, C_{18:0}C_{22:6ω3}, and/or C_{18:1ω9}C_{22:6ω3}. The prawn also possessed the PC including C_{20:5ω3} or C_{22:6ω3} as the major fatty acid moieties. On the other hand, the rotifers contained the PC with fatty acid combinations of C_{18:0}C_{20:5ω8}, C_{18:0}C_{18:2ω6}, C_{18:0}C_{18:2ω6}, and/or C_{18:1ω9}C_{18:2ω6} besides C_{18:0}C_{18:1} as the prominent components. Since fish are incapable of *de novo* synthesis of ω6- and ω3-series of fatty acids,^{26,27)} the PC including ω3-HUFA moieties in the larval fish are conceived to be derived from dietary sources. Therefore, the PC including ω3-HUFA moieties may be necessary for the larval fish if the rate of biosynthesis of such a PL from free fatty acids does not satisfy metabolic demands. In fact, Kanazawa *et al.*²³⁾ have shown that the PC fraction containing abundance of ω3-HUFA from the bonito eggs was highly effective in improving the growth and survival of the larval Ayu. Considering these data and information, we assume that the PC containing ω3-HUFA occurring in the larval fish examined may originate mainly from the PC having fatty acid combination of C_{18:0}C_{20:5ω3} which was found in the rotifers.

The following may be postulated for the reason why dietary PL enhance the growth and survival of larval fish: (1) Dietary PL may accelerate the assimilation of dietary lipids such as triglycerides in the larval fish having an undeveloped digestive system; or (2) the larval fish may possess a limited ability for the biosynthesis of some PL which are important in the formation of cellular components and lipoproteins responsible for the lipid transport in the body, then require dietary sources of specific PL. Further work is required to clarify the nutritional role of dietary PL in larval fish.

Table 4. Possible fatty acid combinations in phosphatidylcholines from the food organisms, larval fish, and post-larval prawn *P. japonicus*

Organisms*	Total carbon number and possible combination of fatty acids (mol.%)												
	C ₃₂		C ₃₄		C ₃₆			C ₃₈		C ₄₀		C ₄₂	C ₄₄
	C ₁₄ C ₁₈	C ₁₀ C ₁₈	C ₁₄ C ₂₀	C ₁₀ C ₁₈	C ₁₄ C ₂₂	C ₁₆ C ₂₀	C ₁₈ C ₁₈	C ₁₆ C ₂₂	C ₁₈ C ₂₀	C ₁₈ C ₂₂	C ₂₀ C ₂₀	C ₂₀ C ₂₂	C ₂₂ C ₂₂
Soybean lecithin	—	4.3	—	33.7	1.2	1.0	59.9	—	—	—	—	—	—
Marine <i>Chlorella</i> [CH(20‰)]	—	22.9	24.3	—	—	39.6	4.4	2.2	5.7	—	4.5	—	—
<i>Tetrasalmis</i> [TS(20‰)]	1.8	2.0	—	52.6	—	14.2	16.3	2.2	7.7	—	3.3	—	—
CH(20‰)-rotifer	—	13.1	11.8	6.9	—	24.6	11.8	13.7	8.9	—	5.8	2.5	1.3
CH(35‰)-rotifer	—	10.4	—	32.9	6.4	12.3	14.7	8.2	8.3	—	3.7	2.5	0.7
TS(20‰)-rotifer	—	6.3	7.0	43.8	—	14.6	14.7	10.9	0.5	2.0	—	0.3	—
Red sea bream	3.0	3.6	—	19.4	—	15.5	10.4	21.9	2.2	14.8	—	7.8	3.6
Knife jaw	2.2	0.1	—	14.2	—	11.5	9.0	20.3	8.0	31.0	—	2.5	1.6
Ayu	1.5	—	—	5.7	—	8.3	5.2	21.0	9.0	28.5	5.3	7.9	4.1
Prawn	2.8	3.8	—	11.0	—	20.1	3.5	5.5	22.7	25.4	—	6.6	3.6

* See Table 1 for abbreviations.

Table 5. Presumed molecular species of the main PC in the food organisms, larval fish, and post-larval prawn *P. japonicus*

Organism	Molecular species
Soybean lecithin	C _{16:0} C _{18:2ω6} ; C _{18:2ω6} C _{18:2ω6}
Marine <i>Chlorella</i> [CH (20%)]	C _{16:0} C _{18:1} ; C _{14:0} C _{20:5ω3} ; C _{16:0} C _{20:5ω3} and/or C _{16:1} C _{20:5ω3}
<i>Tetraselmis</i> [TS (20%)]	C _{16:0} C _{18:3ω3} ; C _{16:0} C _{20:5ω3} ; C _{18:1ω9} C _{18:2ω6} and/or C _{18:1ω9} C _{19:3ω3}
CH (20%)—rotifer	C _{16:0} C _{18:1} ; C _{14:0} C _{20:5ω3} ; C _{16:0} C _{20:5ω3} ; C _{18:1ω9} C _{18:2ω6} and/or C _{18:0} C _{18:2ω6}
CH (35%)—rotifer	C _{16:0} C _{18:1} ; C _{16:0} C _{18:2ω6} ; C _{16:0} C _{20:5ω3} ; C _{18:1ω9} C _{18:2ω6} and/or C _{18:0} C _{18:2ω6}
TS (20%)—rotifer	C _{16:0} C _{18:3ω3} ; C _{16:0} C _{20:4ω6} and/or C _{16:0} C _{20:5ω3} ; C _{18:0} C _{18:3ω3} and/or C _{18:1ω9} C _{18:3ω3}
Red sea bream	C _{16:0} C _{18:1ω9} ; C _{16:0} C _{20:5ω3} ; C _{16:0} C _{22:6ω3} ; C _{18:0} C _{22:6ω3} and/or C _{18:1ω9} C _{22:6ω3}
Knife jaw	C _{16:0} C _{18:1ω9} ; C _{16:0} C _{20:5ω3} ; C _{16:0} C _{22:6ω3} ; C _{18:0} C _{22:6ω3} and/or C _{18:1ω9} C _{22:6ω3}
Ayu	C _{16:0} C _{22:6ω3} ; C _{18:1ω9} C _{22:6ω3}
Prawn	C _{16:0} C _{18:1ω9} ; C _{16:0} C _{20:5ω3} ; C _{18:1ω9} C _{20:5ω3} and/or C _{18:2ω6} C _{20:5ω3} ; C _{18:1ω9} C _{22:6ω3} and/or C _{18:2ω6} C _{22:6ω3}

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