FATTY ACID AND PHOSPHOLIPID PROFILE OF FRESHWATER SARDINE *MIROGREX TERRAESANCTAE* FROM THE SEA OF GALILEE

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ABSTRACT

*Fatty acid and lipid compositions from different tissues of Galilee Sardine important in the diet of East Mediterranean inhabitants were examined. More than 50 fatty acids were detected in freshwater fish sardines (*Mirogrex terraesanctae*). The muscles of this fish contained a high level of omega-3 fatty acids.*

PRACTICAL APPLICATIONS

Sardines are an important commercial freshwater fish species. The lipids of different sardine tissues, fished in Lake Kinneret (the Sea of Galilee), were examined. The benefits of including omega-3 fatty acids in the diets of humans are well documented. Fatty acids play a major role in the functioning of the immune system and the maintenance of all hormonal systems in the body. Sardines (*Mirogrex terraesanctae*) are an excellent source of both docosahexaenoic acid and eicosapentaenoic acid. Sardines are one of the most popular treats in Israeli homes.

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INTRODUCTION

Fish meat is a nutritious food for humans. It includes oil containing a high percentage of n-3 polyunsaturated fatty acids (PUFAs), such as eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) (Kobatake *et al.* 1984; Suzuki *et al.* 1995), preventing cardiovascular diseases (Illingworth and Ullmann 1990; Brown and Hu 2001; Iso *et al.* 2001), and improving learning ability (Yonekubo *et al.* 1994; Suzuki *et al.* 1998) and visual function (Carlson and Werkman 1996; Birch *et al.* 2000). Furthermore, taurine, also present in fish meat in a relatively high concentration, acts as an antioxidant (Aruoma *et al.* 1988), and its deficiency results in the deterioration of eyesight (Hayes *et al.* 1975). Therefore, regular ingestion of fish is important in the maintenance of human health (Ackman 1967, 2000; Hornstra 2000; Rissanen *et al.* 2000; Yamada *et al.* 2000; Arts *et al.* 2001; Lauritzen *et al.* 2001; Sugano 2001).

Mirogrex terraesanctae (*Acanthobrama terraesanctae*), known as the lavnun or Galilee sardine-like cyprinid fish, has been popular in the diets of many people in Bible Land since ancient ages. The lavnun has been familiar to fishermen for ages as a substrate spawner in both deep and shallow water, but Gafny *et al.* (1992) and Yaron *et al.* (1980) recognize only inshore spawning. The receding lake was expected to reduce the sardine population. Lipids and fatty acids of *Mirogrex terraesanctae* have not yet been studied.

MATERIALS AND METHODS

Fish Sample

Adult fish, *Mirogrex terraesanctae* (*A. terraesanctae*), were collected in September 2004 from the Sea of Galilee (Lake Kinneret). The muscle, gills, brain and eyes were pooled from at least 100 samples of 15-cm size and were stored at −4C. The samples of tissues were kept in liquid nitrogen for 1 day before shipment to Jerusalem for analysis.

Extraction and Separation of Lipids

Lipids were extracted according to the method of Bligh and Dyer (1959) using chloroform and methanol as solvents. Total lipids (TLs) were separated into neutral and polar lipid fractions with the help of column chromatography with silica gel G, and chloroform and methanol as eluents. Neutral lipids (NLs) and phospholipids (PLs) were detected using thin layer chromatography as described previously (Rezanka *et al.* 2003). TLs in chloroform were separated into NLs and PLs by passing through a glass column (20 mm

diameter × 30 cm) packed with a slurry of activated silicic acid (70–230 mesh, Merck, Darmstadt, Germany) in chloroform (1:5, v/v). The eluting solvents for NLs, glycolipids (GLs) and PLs were chloroform, acetone and methanol, respectively. The solvents were evaporated by using a rotary evaporator, and the percentage of each fraction was determined gravimetrically. Residue was dissolved in chloroform and then stored at −20C as lipid classes. The fatty acid profile of two major individual classes was estimated by gas chromatography-mass spectrometry (GC-MS). Polar lipids were separated into phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI), and phosphatidylserine, and PI, sphingomyeline, lyso-PE and lyso-PC using two-dimensional (2-D) TLC on glass plates. Silica gel G plates (20 × 20 cm, Macherey-Nagel GmbH & Co., Düren, Germany) were prewashed in the developing solvent system: isooctane/isopropyl alcohol/acetic acid (95:5:1, v/v/v), air dried for 0.5 h and activated by heating for 1 h at 120C under vacuum (15 mm). From a stock solution of 0.5 mg/mL in chloroform/methanol (1:1, v/v), 20–30 mg of sample was spotted onto silica gel plates using 2-mL Microcap pipettes (Blaubrand, Wertheim, Germany). Before development, the plates were dried using a hand dryer on a cool setting for 5 min. The chromatography chamber was saturated with vapor from the solvent system for 30 min before the development of plates. The plates were allowed to develop until the solvent front was about 2 cm from the top, were removed and air-dried for half an hour. The plates were visualized in iodine vapor. The solvent systems for 2D TLC were used as first dimension: chloroform–methanol–25% aqueous ammonium (65:35:5, v/v/v), and second dimension: chloroform–methanol–acetic acid–water (35:15:4:2, v/v/v/v).

Preparation of Fatty Acid Methyl Esters (FAMEs)

Neutral and polar lipid samples were transesterified according to Christie (1982). A 10-mg sample was dissolved in 1 mL diethyl ether and 20 mL methyl acetate. Two hundred fifty milliliters of 1 M sodium methoxide in methanol was added. The sample was worked up and left for 5 min at room temperature. Saturated oxalic acid solution (35 mL) was added, with brief agitation, to neutralize the solution. The solvent was removed under nitrogen, and an appropriate volume of hexane was added to bring the sample to the concentration required for analysis.

GC-MS Analysis

Analysis of FAMEs from sardines was carried out using the Hewlett-Packard 5890 (series II) gas chromatograph (Palo Alto, CA) equipped with a 5971B mass selective detector. FAMEs were analyzed by GC-MS using an RTX-l capillary column: length, 60 m; internal diameter, 0.32 mm; and film

thickness, 0.25 μm (Restek, Bellefonte, PA). The GC oven program had an initial temperature of 40C for 2 min, a 2C/min run to 300C and a final hold at 300C (20 min). The injector temperature was kept at 180C (splitless), and the carrier gas (helium) flow rate was 25 cm/s. The MS detector was operated at 194C, and the scan range was from 30 to 650 *m/z* at 0.9 scan/set scan rate. The solvent delay was 3 min (Dembitsky *et al.* 1999; Rezanka *et al.* 2003).

RESULTS AND DISCUSSION

TL contents and lipid classes in tissues and organs are presented in Table 1. Among the NLs in muscles, triacylglycerols (83%) dominated. Low levels of NLs and triacylglycerols were detected in the whole eyes. The low TLs and the phospholipids of the muscle were thus typically more important dietary sources of these functional fatty acids than the triacylglycerols.

The GC-MS analysis of FAMEs from neutral and polar lipid fractions is shown in Tables 2–4. Among the saturated fatty acids, palmitic (16:0, 4.91–11.90%), stearic (18:0, 1.01–2.01%) and miristic (14:0, 0.90–1.94%) acids (Table 2) dominated. The total content of saturated fatty acids varied from 8.56% in gill polar lipids to 20.35% in brain NLs.

For lipid analysis, only samples of 15-cm fish size were used even when the other size categories of fish were available. Thus, acoustic monitoring of pelagic fish in the Sea of Galilee is based on counts of fish, divided into size categories. The most important category is 10- to 20-cm fish size. Biomass is estimated by multiplying the number by the mean weight in each category.

TABLE 1.
LIPID CLASSES COMPOSITION OF DIFFERENT ORGANS AND TISSUES

Organs	Muscle	Gills	Brain	Eyes
Total lipids (% fresh weight)	16.3	2.3	5.5	4.1
Neutral lipids	88.4	45.2	16.9	11.2
Phospholipids (PLs)	5.9	22.4	41.4	63.7
Other lipids	5.7	32.4	41.7	25.1
Phosphatidylcholine (PC)	59.8 ± 2.1	46.7 ± 2.4	38.9 ± 1.8	32.1 ± 1.6
Lyso-PC	2.2 ± 0.3	6.2 ± 0.8	0.8 ± 0.1	1.3 ± 0.3
Phosphatidylethanolamine (PE)	19.7 ± 1.4	23.2 ± 1.5	31.5 ± 1.3	42.4 ± 2.1
Lyso-PE	1.4 ± 0.3			
Phosphatidylserine	3.6 ± 0.5	4.4 ± 0.3	13.6 ± 1.1	15.1 ± 0.6
Phosphatidylinositol	4.1 ± 0.4	11.6 ± 0.8	3.8 ± 0.5	0.6 ± 0.2
Sphingomyeline	4.8 ± 0.5	2.1 ± 0.3	6.4 ± 0.3	5.9 ± 0.2
Other PLs	4.4 ± 0.5	5.8 ± 0.6	5.0 ± 0.5	2.6 ± 0.1

Values are mean ± SD; $n = 3$.

TABLE 2.
DISTRIBUTION OF SATURATED FATTY ACIDS (FAs)

FA	MNL	MPL	GNL	GPL	BNL	BPL	ENL	EPL
14:0	1.94	1.32	1.80	1.25	2.19	1.82	1.06	0.90
iso-15:0	0.03	0.01	0.01	0.00	0.33	0.07	0.00	0.00
anteiso-15:0	0.04	0.02	0.00	0.00	0.00	0.00	0.00	0.00
15:0	0.49	0.19	0.41	0.18	0.38	0.10	0.21	0.08
iso-16:0	0.17	0.10	0.08	0.03	0.14	0.12	0.05	0.01
16:0	9.27	7.67	7.15	4.91	11.90	8.80	8.65	7.16
iso-17:0	0.06	0.04	0.02	0.00	0.01	0.00	0.08	0.03
anteiso-17:0	0.11	0.07	0.06	0.01	0.03	0.00	0.03	0.00
17:0	0.49	0.28	0.28	0.16	0.56	0.42	0.19	0.13
18:0	1.41	1.04	2.11	1.36	2.56	2.01	1.01	0.91
19:0	0.67	0.36	0.28	0.20	0.41	0.32	0.22	0.15
20:0	0.77	0.33	0.60	0.28	0.82	0.71	0.39	0.28
21:0	0.12	0.07	0.11	0.09	0.28	0.22	0.07	0.05
22:0	0.16	0.12	0.11	0.08	0.69	0.50	0.12	0.08
23:0	0.02	0.10	0.04	0.01	0.08	0.07	0.01	0.00
24:0	0.07	0.03	0.02	0.00	0.35	0.31	0.06	0.04
Total	15.82	11.75	13.08	8.56	20.35	15.47	12.15	9.82

NL, neutral lipids; PL, polar lipids; M, muscle; G, gills; B, brain; E, eyes.

While the technology of acoustic counting is discussed at length by Walline *et al.* (1992), the equally important parameter, mean weight, is not estimated. In the 10- to 20-cm category, a mean weight of 30 g is applied to all the years from 1981 to 1990. For the 10- to 20-cm category, a mean weight of <15 g can be deduced from 1981 size-distribution data on commercial lavnun catches (Davidoff 1982; Landau 1991). The Sea of Galilee research acknowledged a rise in sardine abundance in the early 1990s, and supports the "Dilul" project as a remedy to the drastic changes in the lavnun stock in 1992 (Ostrovsky and Walline 1999).

The omega-3 and omega-6 fatty acids make up two families that are absolutely essential to human life (Ackman 2000). These essential fatty acids are important because they form the important components of cell membranes. They also regulate the body's use of cholesterol and the production of substances that regulate nearly all other body processes (Innis and Hansen 1996).

Omega-3 fatty acids in their long-chain forms, EPA and DHA, are both found almost exclusively in deep, coldwater fish. EPA is needed to create health-promoting prostaglandins that support cardiovascular health and cell membranes throughout the body. DHA is vital for a healthy brain, eyes and reproductive system.

Fish and seafood from cold waters characteristically and uniquely contain significant quantities of long-chain omega-3 polyunsaturated. Although there

TABLE 3.
COMPARATIVE DISTRIBUTION OF OMEGA-11, 9, 8, 7, 5 AND FOUR FATTY ACIDS (FAs)

FA	MNL	MPL	GNL	GPL	BNL	BPL	ENL	EPL
Total n-11	3.94	2.84	3.78	2.55	7.16	8.61	4.75	3.25
18:1n-11	0.89	0.74	1.02	0.75	1.11	1.17	0.68	0.59
20:1n-11	2.86	1.95	2.57	1.64	4.32	6.13	3.79	2.46
22:1n-11	0.16	0.13	0.18	0.16	0.53	0.37	0.21	0.18
24:1n-11	0.03	0.02	0.01	0.00	1.20	0.94	0.07	0.02
Total n-9	14.46	23.41	26.53	23.48	34.73	35.03	22.46	18.17
14:1n-9	0.16	0.09	0.24	0.13	0.18	0.08	0.17	0.12
15:1n-9	0.32	0.15	0.22	0.16	0.31	0.25	0.00	0.00
16:1n-9	0.51	0.43	0.52	0.55	1.12	0.80	0.34	0.27
18:1n-9	12.93	9.43	11.81	9.35	15.38	14.15	8.23	7.98
20:1n-9	13.39	12.29	12.17	11.68	16.09	18.14	12.50	8.64
20:3n-9	0.31	0.44	0.69	0.91	0.16	0.24	0.63	0.75
22:1n-9	0.60	0.43	0.57	0.45	0.86	0.84	0.44	0.31
24:1n-9	0.17	0.15	0.31	0.25	0.63	0.53	0.15	0.10
Total n-8	0.54	0.34	0.38	0.23	0.74	0.66	0.21	0.09
17:1n-8	0.31	0.19	0.26	0.18	0.35	0.33	0.12	0.06
19:1n-8	0.16	0.11	0.10	0.05	0.28	0.23	0.07	0.03
21:1n-8	0.07	0.04	0.02	0.00	0.11	0.10	0.02	0.00
Total n-7	12.95	9.81	14.85	12.54	18.67	16.28	10.31	7.91
14:1n-7	0.32	0.25	0.28	0.19	0.12	0.05	0.15	0.09
15:1n-7	0.75	0.40	0.59	0.20	0.55	0.33	0.23	0.14
16:1n-7	7.72	5.89	9.17	8.12	11.07	9.18	6.19	4.70
16:2n-7	0.11	0.13	0.08	0.15	0.03	0.02	0.12	0.19
18:1n-7	3.28	2.59	3.96	3.36	6.21	5.88	3.28	2.60
20:1n-7	0.77	0.55	0.77	0.52	0.69	0.82	0.34	0.19
Total n-5	0.49	0.52	0.92	0.93	0.97	1.33	0.79	0.92
16:1n-5	0.34	0.36	0.61	0.54	0.28	0.37	0.64	0.66
18:1n-5	0.15	0.16	0.31	0.39	0.69	0.96	0.15	0.26
Total n-4	0.85	1.00	0.60	0.80	0.24	0.18	0.52	0.86
16:2n-4	0.34	0.37	0.24	0.39	0.09	0.08	0.14	0.35
16:3n-4	0.51	0.63	0.36	0.41	0.15	0.10	0.38	0.51

NL, neutral lipids; PL, polar lipids; M, muscle; G, gills; B, brain; E, eyes.

is some evidence that fish can elongate and desaturate the shorter-chain omega-3 polyunsaturated, the current opinion is that most of the long-chain omega-3 polyunsaturated are formed in the microscopic algae, plankton and planktonic crustacea at the bottom of the marine food chain. They then pass up the food chain into the higher fish and, of course, ultimately to humans. There are three significant members of the omega-3 group, all with 20 or more carbon atoms and all with five or more double bonds.

The most widely researched 5c,8c,11c,14c,17c-eicosapentaenoic acid (20:5n-3), usually referred to as EPA, is sometimes called timnodonic acid and is the major omega-3 polyunsaturated in most seafood. It is capable of being

TABLE 4.
DISTRIBUTION OF OMEGA-6 AND OMEGA-3 FATTY ACIDS (FAs)

FA	MNL	MPL	GNL	GPL	BNL	BPL	ENL	EPL
Total n-6	6.15	8.76	6.00	8.81	3.93	5.11	6.70	8.48
16:3n-6	0.07	0.11	0.14	0.15	0.07	0.05	0.12	0.14
18:2n-6	2.69	2.94	1.81	2.06	1.22	1.60	2.35	2.06
18:3n-6	0.23	0.63	0.31	0.45	0.08	0.12	0.56	0.77
20:2n-6	0.32	0.43	0.31	0.46	0.16	0.20	0.61	0.74
20:3n-6	0.25	0.50	1.00	1.11	0.17	0.27	0.46	0.88
20:4n-6	1.50	2.53	1.30	2.48	1.29	1.38	1.12	1.26
22:2n-6	0.16	0.14	0.14	0.17	0.26	0.24	0.15	0.13
22:3n-6	0.11	0.17	0.22	0.28	0.24	0.31	0.21	0.26
22:4n-6	0.26	0.46	0.24	0.64	0.26	0.51	0.29	0.88
22:5n-6	0.56	0.85	0.53	1.01	0.18	0.43	0.83	1.36
Total n-3	44.80	41.57	33.86	42.10	13.21	17.33	42.11	50.50
16:3n-3	0.25	0.28	0.18	0.33	0.08	0.12	0.63	0.92
16:4n-3	0.28	0.47	0.41	0.46	0.10	0.17	0.64	0.87
18:3n-3	2.89	3.72	2.57	4.13	1.33	1.77	5.93	6.67
18:4n-3	1.80	2.11	1.77	2.71	0.87	1.54	1.56	1.99
20:3n-3	0.34	0.52	0.26	0.59	0.16	0.27	0.00	0.00
20:4n-3	2.74	0.92	1.20	0.28	0.31	0.55	0.83	1.14
20:5n-3	7.99	8.11	5.46	5.85	1.29	1.73	9.36	10.04
22:3n-3	0.21	0.32	0.18	0.29	0.07	0.09	0.15	0.29
22:4n-3	0.90	1.42	0.71	1.30	0.16	0.25	0.77	1.09
22:5n-3	1.98	3.09	3.07	6.56	0.61	1.61	5.62	7.79
22:6n-3	25.40	20.61	18.05	19.60	8.23	9.23	16.62	19.70
n-6/n-3 EPA/DHA	0.14	0.21	0.17	0.21	0.30	0.29	0.16	0.17

NL, neutral lipids; PL, polar lipids; M, muscle; G, gills; B, brain; E, eyes; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.

elongated to 7c,10c,13c,16c,19c-docosapentaenoic acid (DPA; 22:5n-3), which in turn can be converted to 4c,7c,10c,13c,16c,19c-docosahexaenoic acid (22:6n-3), usually called DHA but also known as clupadonic acid. EPA or 20:5 n-3 is also capable of being metabolized to a range of biologically active substances referred to generically as eicosanoids. Prostaglandins and leukotrienes are important members of this group. They are produced locally and are powerful regulators of biological activity. A parallel series of eicosanoids can also be produced from 5c,8c,11c,14c-eicosatetraenoic acid (20:4n-6), called arachidonic acid, which tends to have an even more potent biological activity. Because the n-6 family tends to dominate the human food (by a factor of eight times or more) compared with the n-3 family, most eicosanoids produced by the human body tend to be of the n-6 type.

Increasing the dietary intake of n-3 polyunsaturated alters this balance, and this is thought to be in part responsible for the beneficial health impact of n-3 polyunsaturated from seafood.

The second most abundant long-chain n-3 polyunsaturated is 22:6 n-3 or DHA. It is actually the most abundant n-3 polyunsaturated in certain fish, such as tuna, but in most fish, it is present to a lesser extent than EPA. It is not thought to be metabolized directly to eicosanoids, but because it can be retroconverted to EPA, it is possible that a high DHA intake could also affect the eicosanoid balance. The most significant aspect of DHA, from the human nutrition point of view, is its role as a major structural component of brain, nerve and retinal membranes. In these membranes, it can form up to 60% of the polyunsaturated present, and recent research is leading to the view that functional abnormalities can result from the depletion of membrane DHA levels. DHA plays a unique role in the building of these tissues in the fetus, and such is its importance, especially during the first few months of life, that breast milk supplies 0.1–0.4% of fatty acids as DHA, while there is almost no EPA present in breast milk. Breast milk DHA can be augmented by a dietary intake of fish and fish oils, but the EPA level does not vary too much.

All *cis*-7,10,13,16,19-docosapentaenoic acids (22:5n3), sometimes called clupanodonic acid, are a minor component of most fish, present to the extent of 1–3% of the total fatty acids. Little is known of any specific physiological effects of this polyunsaturated, although of course it is in principle converted either to 20:5n-3 or to 22:6n-3, and as such could augment their available supplies. The distribution fatty acids in two major individual phospholipids, PC and PE, are shown in Tables 5 and 6, respectively.

All *cis*-5,8,11,14-eicosatetraenoic acids (20:4n-6) are a minor component of some fish lipids. Fish from tropical waters can have significant amounts of 20:4n-6, but analytical information is not readily available. Small amounts of short-chain omega-3 polyunsaturates are also present in fish lipids, chiefly all *cis*-9,12,15-octadecatrienoic acid (18:3n-3) (α-linolenic acid) and all *cis*-6,9,12,15-octadecatetraenoic (stearidonic) acid (18:4n-3), but the amounts rarely exceed 0.1-0.2% of all fatty acids, except in PE.

The difference between the fatty acid compositions of marine and freshwater fish has been noted by several authors (Yamada 1972; Reichwald 1976; Corraze and Kaushik 1999; Kolakowska *et al.* 2003). Some examples of fatty acid patterns are given in Tables 2–4. Although these fish lipids are higher in n-3 fatty acids, it is clear that freshwater fish have higher levels of n-6 fatty acids than marine species. The average n-6/n-3 ratios are 0.37 and 0.16 for freshwater and marine fish, respectively. Fish, in general, contain more n-3 than n-6 PUFA, and should have a higher dietary requirement for n-3 PUFA. Thus, the dietary essential fatty acid requirement of marine fish for n-3 PUFA may be higher than that of freshwater fish. The same type of difference in the n-6/n-3 ratio between freshwater and seawater is seen when some species of fish migrate from oceans to streams, or vice versa. The PUFA ratio of sweet

TABLE 5.
DISTRIBUTION OF FATTY ACIDS (FAs) IN PHOSPHATIDYLCHOLINE (PC)

FAs in PC	Muscle	Gills	Brain	Eyes
Total	20.38	27.14	18.22	15.38
14:0	2.34	3.87	1.55	1.28
iso-15:0	0.46	0.26	0.11	0.22
anteiso-15:0	0.52	0.63	0.23	0.13
15:0	1.68	1.33	0.93	0.88
iso-16:0	0.97	1.11	0.67	1.36
16:0	10.71	16.35	9.50	7.59
iso-17:0	0.21	0.03	0.05	0.16
anteiso-17:0	0.33	0.05	0.07	0.12
17:0	0.51	0.07	0.12	0.22
18:0	2.14	3.26	4.34	2.19
19:0	0.16	0.06	0.21	0.37
20:0	0.14	0.08	0.31	0.42
21:0	0.08	0.04	0.09	0.12
22:0	0.03	0.00	0.04	0.08
23:0	0.04	0.00	0.00	0.04
24:0	0.06	0.00	0.00	0.02
Total n-11	0.42	0.87	0.74	1.08
18:1n-11	0.12	0.34	0.41	0.52
20:1n-11	0.21	0.29	0.27	0.44
22:1n-11	0.09	0.21	0.06	0.08
24:1n-11	0.00	0.03	0.00	0.04
Total n-9	6.41	4.09	8.22	3.35
14:1n-9	0.00	0.01	0.00	0.00
15:1n-9	0.09	0.04	0.00	0.00
16:1n-9	0.07	0.06	0.12	0.07
18:1n-9	2.14	1.74	3.68	1.35
20:1n-9	3.68	2.12	4.37	1.87
20:3n-9	0.32	0.08	0.05	0.06
22:1n-9	0.07	0.04	0.00	0.00
24:1n-9	0.04	0.00	0.00	0.00
Total n-8	0.30	0.05	0.31	0.45
17:1n-8	0.17	0.00	0.19	0.22
19:1n-8	0.09	0.03	0.12	0.14
21:1n-8	0.04	0.02	0.00	0.09
Total n-7	5.68	7.41	4.95	2.39
14:1n-7	0.05	0.03	0.06	0.00
15:1n-7	0.07	0.05	0.11	0.00
16:1n-7	3.28	5.81	2.19	1.38
16:2n-7	0.88	0.15	0.22	0.05
18:1n-7	1.17	1.29	2.14	0.96
20:1n-7	0.23	0.08	0.23	0.00
Total n-5	0.38	0.30	0.02	0.10
16:1n-5	0.27	0.18	0.00	0.08
18:1n-5	0.11	0.12	0.02	0.02

TABLE 5. CONTINUED

FAs in PC	Muscle	Gills	Brain	Eyes
Total n-4	0.40	0.48	0.16	0.17
16:2n-4	0.24	0.32	0.09	0.11
16:3n-4	0.16	0.16	0.07	0.06
Total n-6	3.87	4.82	6.04	18.56
16:3n-6	0.00	0.00	0.27	0.38
18:2n-6	0.11	1.14	0.45	4.33
18:3n-6	0.17	0.68	0.51	0.78
20:2n-6	0.15	0.46	0.62	0.64
20:3n-6	0.26	0.21	0.37	0.32
20:4n-6	2.93	1.98	2.51	7.86
22:2n-6	0.07	0.14	0.66	0.65
22:3n-6	0.09	0.11	0.33	1.11
22:4n-6	0.05	0.07	0.21	2.26
22:5n-6	0.04	0.03	0.11	0.23
Total n-3	67.16	54.03	61.34	58.52
16:3n-3	0.04	0.68	0.22	1.17
16:4n-3	0.06	0.35	0.46	2.14
18:3n-3	0.87	4.12	2.15	8.45
18:4n-3	0.44	1.78	0.67	4.17
20:3n-3	1.91	0.44	0.96	1.66
20:4n-3	4.21	9.18	7.16	4.88
20:5n-3	15.22	18.16	21.16	18.98
22:3n-3	1.87	0.87	1.17	1.62
22:4n-3	3.51	3.12	2.11	2.33
22:5n-3	7.56	2.36	1.14	6.12
22:6n-3	31.98	12.97	24.14	7.00
n-6/n-3	0.06	0.09	0.10	0.32

smelt (*Plecoglosus altivelis*) changes drastically in only 1 month as they migrate from the sea to a freshwater river. A similar but reverse change occurs in the masu salmon (*Oncorhynchus masu*) as they migrate from freshwater to seawater. Even within the same species of fish, the salinity of the water seems to cause a dramatic change in the fatty acid pattern.

The difference between marine and freshwater fish may be simply because of differences in the fatty acid content in the diet or it may be related to a specific requirement of fish related to physiological adaptations to the environments (Ackman 2002; Miyashita 2002; Kondo and Yanagisawa 2005; Mayer *et al.* 2006). The PLs are generally considered to be structural or functional lipids, being incorporated to a larger extent in the membrane structure of cell and subcellular particles. The triacylglycerols are more often storage lipids and reflect the fatty acid composition of the diet to a greater extent than do the phospholipids. In Table 3, the fatty acid compositions of the neutral (triacylglycerols mainly) and polar (PLs and glycolipids) fractions of

TABLE 6.
DISTRIBUTION OF FATTY ACIDS (FAs) IN PHOSPHATIDYLETHANOLAMINE (PE)

FAs in PE	Muscle	Gills	Brain	Eyes
Total	15.22	14.66	14.43	18.78
14:0	1.24	2.81	1.63	1.75
iso-15:0	0.11	0.09	0.00	0.23
15:0	1.13	0.98	0.87	1.34
iso-16:0	0.78	1.11	0.54	0.88
16:0	9.16	6.23	8.13	10.22
17:0	0.22	0.11	0.09	0.21
18:0	2.21	3.12	2.89	4.03
19:0	0.14	0.07	0.06	0.04
20:0	0.23	0.14	0.22	0.08
Total n-9	0.46	0.67	0.56	0.91
16:1n-9	0.12	0.22	0.09	0.23
18:1n-9	0.25	0.34	0.44	0.68
20:1n-9	0.09	0.11	0.03	0.00
Total n-7	4.16	2.69	2.41	2.09
16:1n-7	0.66	0.35	0.53	0.78
16:2n-7	0.34	0.23	0.12	0.42
18:1n-7	3.16	2.11	1.76	0.89
Total n-6	8.43	4.42	5.23	10.06
18:2n-6	2.23	1.38	2.11	6.12
18:3n-6	0.44	0.33	0.18	0.14
20:2n-6	0.48	0.24	0.08	0.10
20:3n-6	0.23	0.22	0.09	0.16
20:4n-6	3.68	1.19	2.11	3.12
22:2n-6	0.67	0.33	0.44	0.23
22:3n-6	0.32	0.25	0.11	0.15
22:4n-6	0.21	0.35	0.08	0.03
22:5n-6	0.17	0.13	0.03	0.01
Total n-3	71.73	77.56	77.37	69.16
18:3n-3	0.58	3.28	0.44	1.09
18:4n-3	0.34	1.16	0.03	3.29
20:3n-3	0.24	0.66	0.36	0.19
20:4n-3	1.19	8.38	15.25	12.56
20:5n-3	12.91	12.77	13.18	19.98
22:3n-3	0.68	1.14	0.93	0.78
22:4n-3	1.38	1.08	0.65	0.66
22:5n-3	2.14	0.77	0.24	0.97
22:6n-3	52.27	48.32	46.29	29.64
n-6/n-3	0.12	0.06	0.07	0.15

fish lipids are presented. It can be seen that the effect of changing environment on the fatty acid composition of the PL is as great as in the case of salmon, and considerably greater in the case of sweet smelt, than it is on the triacylglycerol composition (Kondo and Yanagisawa 2005).

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