Thermal Acclimation and Dietary Lipids Alter the Composition, But Not Fluidity, of Trout Sperm Plasma Membrane

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ABSTRACT: The effect of a long-term adaptation of rainbow trout to 8 and 18°C combined with a corn oil- or a fish oil-supplemented diet on the characteristics of the spermatozoan plasma membrane was investigated. The experiment lasted up to 22 mon during which spermatozoa were collected from the mature males. Spermatozoan plasma membranes were isolated by nitrogen cavitation, and the cholesterol content, phospholipid composition and fatty acid pattern were investigated. Membrane viscosity was assessed on whole cells by electron spin resonance using spin-labeled phospholipids. Neither diet nor rearing temperature influenced the cholesterol content of the plasma membrane nor the phospholipid class distribution. The rearing temperature of the broodstock only slightly affected the phospholipid fatty acids. A minor decrease in 18:0 and increase in monounsaturated fatty acids was observed for the cold-adapted fish. These modifications were not sufficient to affect membrane fluidity, and we conclude that trout spermatozoa do not display any homeoviscous adaptations in these conditions. On the contrary, the dietary fatty acid intake greatly modified the fatty acid profile of plasma membrane phospholipids. The fish oil-fed trout displayed a much higher n-3/n-6 fatty acid ratio than did the corn oil-fed ones, but the 22:6n-3 levels remained unchanged. Modifications in plasma membrane composition by the diet were obtained although neither of the two diets was deficient in essential fatty acids. The enrichment in n-3 fatty acids, however, did not affect plasma membrane fluidity which was unchanged by the diets.
Lipids 30, 23–33 (1995).

The quality of the spermatozoan plasma membrane in rainbow trout is a key factor in protecting the cell from the extracellular environment, be it the hypo-osmotic water into which milt is ejaculated or the media and extenders used in artificial fertilization. The importance of trout sperm plasma membrane integrity in resistance to stress such as osmotic shock or cold shock was confirmed by Maléjac *et al.* (1). Lipids, particularly phospholipids and cholesterol, are important structural elements of the cell membrane. It has been demonstrated in many vertebrate species that the resistance of sperm to cold shock depends on their cholesterol content (2–4), the cellular level of unsaturated fatty acids (5), and the phase transition temperature of spermatozoan glycolipids (6). In artificial membrane vesicles, the fatty acid composition has been shown to affect bilayer permeability and the ability of the membrane to fuse (7,8). Fatty acid composition also influences the osmotic fragility of erythrocytes, which additionally depends on the nature of phospholipid polar head groups (9,10). Several reviews have extensively covered the relationships between membrane composition (i.e., fatty acid unsaturation and acyl chain length, cholesterol, phospholipids, and proteins) and the physical parameter often referred to as "membrane fluidity" (11–13).

In mammals, changes in plasma membrane composition through dietary manipulation are well documented (reviewed in Ref. 11). In fish, many studies have shown that the fatty acid composition of the diet influences tissue lipids, particularly the fatty acid pattern in phospholipids (for review, see Refs. 14,15). The lipid composition of the broodstock diet has been reported to affect the fatty acid profile of trout eggs and spermatozoa (16,17) and of gilthead sea bream eggs (18). These studies, however, compared essential fatty acid (EFA)-deficient and non EFA-deficient diets and concerned whole cells. To our knowledge, the only data available in fish membranes concern the brush border membrane of trout and carp intestine (19–21).

The temperature at which fish are reared also affects the lipid composition of the cells. Cold acclimation of these poikilothermic animals has been shown to increase the proportions of unsaturated fatty acids and phosphatidylethanolamine and to decrease the proportions of saturated fatty acids, phosphatidylcholine, sphingomyelin, and cholesterol (13). These

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Abbreviations: BCA, bicinchoninic acid; BHT, butylated hydroxytoluene; DFP, diisopropyl fluorophosphate; EFA, essential fatty acid; ESR, electron spin resonance; HPLC, high-performance liquid chromatography; MUFA, monounsaturated fatty acid; SE, standard error; SL-PC, spin-labeled phosphatidylcholine; SL-PS, spin-labeled phosphatidylserine; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid; UFA, unsaturated fatty acid.

modifications were assumed to increase membrane fluidity and to maintain normal cellular functions at low environmental temperature.

It is not known how the rearing temperature and changes in the dietary fatty acid profiles affect the lipids in the membranes of rainbow trout spermatozoa. The present study was undertaken to investigate the combined effects of temperature and dietary lipids on the cholesterol content, phospholipids, and fatty acid composition of phospholipid classes in the plasma membranes of rainbow trout spermatozoa. To this end, broodstock were reared at two water temperatures (8 and 18°C), and the diets were enriched with either linoleic acid or n-3 polyunsaturated fatty acids (PUFAs). For each set of rearing conditions, the biophysical state of the spermatozoan membrane was assessed by electron spin resonance (ESR) to estimate membrane fluidity.

MATERIALS AND METHODS

Animals, rearing temperatures and diets. One-year-old juvenile rainbow trout (*Oncorhynchus mykiss*) specimens of a winter strain, weighing about 100 g and reared at the INRA experimental farm at Donzacq, France (18°C water), were divided into four groups. Two groups were transferred to the INRA experimental farm at Lees-Athas, France (8°C water). In each farm, the trout were fed one of two experimental isoenergetic diets, in the form of pellets. Diet compositions are shown in Table 1. The relative percentages of n-3 fatty acids (0.5% in the corn oil diet and 1.1% in the cod liver oil diet) and n-6 fatty acids (3.7 and 1.36% respectively) met the known EFA requirements of broodstock fish. The corn oil diet was enriched in linoleic acid (three times more than in the cod liver oil diet), the cod liver oil diet in n-3 fatty acids (2.3-fold with respect to the former). The ratio of highly PUFA (at least four unsaturations) to saturated fatty acids was 2-fold lower in the corn oil diet (0.21) than in the cod liver oil diet (0.40). To obtain the same growth rate at both rearing temperatures, the fish reared at 18°C were rationed.

The first sexual maturity of the fish occurred 9 mon after the beginning of the experiment. Their mean weight was 1 kg and they were then two years old. Sperm was collected by hand stripping. For each rearing condition, sperm from six to nine fish were pooled. Each fish contributed the same sperm volume in the pool. This year, we obtained one to two pools of sperm, depending on the group. These pools were used for the determination of the lipid composition of plasma membrane. After another year under these rearing conditions (total of 22 mon), fish reached their second sexual maturity as they became three years old. Sperm was collected again and pooled in the same conditions. The two pools obtained this year were used for both the lipid composition and the fluidity studies on the plasma membrane. Since no difference in the lipid composition appeared between the two breeding seasons, the corresponding data were not separated in Tables 3 to 7.

Membrane preparation. Pure spermatozoan plasma membranes were prepared as previously described (22). Briefly, washed spermatozoa were subjected to a 900 psi nitrogen pressure for 20 min. Sperm extrusion lasted one minute, and a crude membrane preparation was collected after centrifuging (500 × *g* for 20 min) on a 1M sucrose cushion. Plasma membranes were separated from contaminating nuclear membranes, mitochondria, and flagella by sucrose density ultracentrifugation (54,000 × *g* for 2 h) on a subcontinuous gradient (1.8 to 0.25 M). The purity of the plasma membrane fraction was assessed by electron microscopy and by enzyme marker assays (22). The plasma membrane fraction showed a 4- to 5-fold enrichment in 5′-nucleotidase (EC 3.1.3.5; plasma membrane marker) activity, and no succinic dehydrogenase (EC 1.3.99.1; mitochondrial marker) activity was detected.

TABLE 1
Composition of Experimental Diets

Ingredients (wt%)[a]	Corn oil diet	Cod liver oil diet
Fish meat	45	45
Wheat starch (native)	20	20
Soybean LTI	18	18
Pre-gelatinized starch	5	5
Mineral mix (INRA[b], France)	2	2
Vitamin mix (INRA[b], France)	2	2
Sodium alginate	2	2
Corn oil	6	0
Cod liver oil	0	6
Antioxidant (ethoxyquin, ppm)	100	100
Protein (%)	43	43
Lipid (%)	11	11
Digestible energy (kJ/g)	15.5	15.5
Major fatty acids (molar % of the total)		
14:0	2.0	6.0
16:0	17.6	24.6
16:1	2.2	8.1
18:0	2.9	4.0
18:1	23.8	22.3
18:2n-6	44.8	15.2
18:3n-3	1.7	2.2
18:4n-3	0.3	1.3
20:1	0.2	2.3
20:4n-6	0.2	0.4
20:4n-3	—	0.3
20:5n-3	2.0	5.5
22:1	—	1.6
22:5n-3	0.2	0.5
22:6n-3	2.0	5.8
Total n-3	6.3	15.5
Total n-6	45.0	15.6
n-3/n-6	0.1	1.0
UI	139.3	128.6

[a]LTI, Low Trypsin Inhibiteur extruded full fatty soybean; UI, unsaturation index = relative percentage of each unsaturated fatty acid times the number of double bonds for that particular fatty acid.
[b]Mineral mix contained the following ingredients (g/kg mix):calcium carbonate, 215; magnesium hydroxide, 124; KCl, 90; ferric citrate, 20; KI, 0.4; NaCl, 40; calcium hydrogen phosphate ($CaHPO_4$), 500; copper sulfate, 3; zinc sulfate, 4; cobalt sulfate, 0.2; manganese sulfate, 3.
[c]Vitamin mix contained the following diluted in cellulose (g/kg mix):vitamin A (500,000 IU/g), 1.5; vitamin D_3 (100,000 IU/g), 1.5; vitamin E (500 IU/g), 6; vitamin K, 0.25; thiamine, 0.75; riboflavin, 1.5; pyridoxine, 0.75; nicotinic acid, 8.75; vitamin C, 25; folic acid, 0.25; vitamine B_{12} (1000 mg/kg), 2.5; inositol, 50; biotin (2%), 6.25; calcium pantothenate, 2.5; choline (50%), 200.

Lipid extraction and analysis. One volume of plasma membrane suspension was vigorously mixed with four volumes of chloroform/methanol (2:1, vol/vol) (23) containing 0.02% butylated hydroxytoluene (BHT) as an antioxidant. The lower phase was carefully separated, dried, and dissolved in chloroform with 0.02% BHT. Total cholesterol was determined enzymatically (Biochemica test combination; Boehringer, Mannheim, Germany) in 96-well plates after lipid extraction. Phospholipids were quantitated by colorimetric determination of phosphorus according to Bartlett (24) after hydrolysis with sulfuric acid. Phospholipids were separated from neutral lipids and glycolipids according to Linard (25). After adsorption of the sample to the Sep-Pak silica columns (Waters Associates, Millipore Corporation, Milford, MA), 20 mL chloroform were pushed through the cartridge, followed by 10 mL acetone and 20 mL methanol. Phospholipid classes were separated by high-performance liquid chromatography (HPLC) on a Zorbax silica column (250 mm length, 4.6 mm internal diameter, 5 μm particle size, Societe Francaise de Chromatographie, Eragny, France) on line with a LCD/Milton Roy 3000 detector (Riviera Beach, CA) at 205 nm. The mobile phases were: Solvent A, *n*-hexane/2-propanol (3:2, vol/vol), and Solvent B, *n*-hexane/2-propanol/water (3:2:0.275, by vol) at a flow rate of 1.5 mL/min. After injection of phospholipids, the column was eluted for 9 min with 50% A:50% B. The proportion of B was increased in 5 min to 78%, maintained for 9 min, increased to 100% in 2 min, and maintained for another 14 min. Finally, the proportion of B was decreased to 50% in 4 min, maintained for 9 min, and elution was stopped. Under these conditions, the various classes of phospholipids eluted within 50 min. Quantitation of these classes was performed by phosphorus assay.

Fatty acid distribution in the phospholipid classes was determined by gas–liquid chromatography of the fatty acid methyl esters obtained after transmethylation (26). For each class, 1 mg phospholipid in 1 mL methanol and 8 mL 12 N hydrochloric acid chloroform/2,2-dimethoxypropane (9.87:86.25:3.95, by vol) with 0.02% BHT were mixed in screw-cap vials which were then closed under a flush of nitrogen and heated at 65°C. The reaction was carried out for 3 h for total phospholipids, 4 h for phosphatidylethanolamine and phosphatidylcholine, 4.5 h for phosphatidylinositol, and 5 h for phosphatidylserine. After the tubes had cooled, fatty acid methyl esters were extracted in 4 mL *n*-hexane, dried and dissolved in isooctane.

Á Chrompack CP 9000 gas chromatograph (Les Ullis, France) equipped with a splitter injector, a flame-ionization detector, and a CP wax 52 CB bonded fused silica capillary column (50 m ¥ 0.25 mm internal diameter) was used for fatty acid methyl ester analysis. The carrier gas was hydrogen, at a flow rate of 1.5 mL/min. The injector and detector were set at 250 and 270°C, respectively, and the oven temperature was increased from 180 to 225°C at a rate of 4°C/min. Peaks were identified by comparing their retention times with those of authentic fatty acid methyl esters, and areas were calculated on a Spectra-Physics integrator (SP4400, Chrompack, Les Ullis, France).

Spermatozoan spin-labeling. The spin-labeled analogues of phospholipids used in these experiments are 1-palmitoyl-2-(4-doxylpentanoyl)phosphatidylcholine (SL-PC) and -phosphatidylserine (SL-PS) (see Ref. 27 for their synthesis description). The required amount of spin-labeled analog (corresponding to 1.6% of total membrane phospholipids) from a chloroform solution was deposited in a tube and dried under nitrogen. The dried film was resuspended in one volume of seminal-fluid-like mineral medium (28) consisting of 110 mM NaCl, 28.3 mM KCl, 1.1 mM $MgSO_4$, 1.8 mM $CaCl_2$ and 20 mM tris buffer (pH 9). One volume of spermatozoa in suspension ($2 \cdot 10^{10}$ cells/mL) and one volume of diisopropyl fluorophosphate (DFP) 5 mM were mixed with the spin-labeled phospholipid. Cell suspension was incubated for 30 min at room temperature to allow SL-PS to equilibrate in the membrane inner leaflet. ESR spectra were recorded at 4, 8, 13, 18 and 24°C after re-oxidation of nitroxides by 12 mM potassium ferricyanide. All ESR measurements were performed with a Varian E-109 spectrometer (Palo Alto, CA) equipped with a temperature control device. To compare the mobility of the spin probe in the different conditions, the apparent rotational correlation time was estimated as described (29).

Protein content. Membrane suspensions were digested in 1 M sodium hydroxide overnight at room temperature, and the protein content was determined according to Smith *et al.* (30) using the Pierce bicinchoninic acid (BCA) protein assay reagent (Rockford, IL).

Statistical analysis. Sperm from six to nine fishes were pooled for each individual analysis. Unless otherwise mentioned, the results shown are the means of three to four of these analyses ± standard error (SE). The statistical significance of differences between different groups was determined by the Mann-Whitney test (rank-sum test) (31). This test was performed by comparing the two diets, irrespective of the rearing temperature, and the two temperatures, irrespective of the diet in order to increase the sample number (from 3–4 to 6–8) and to allow this kind of analysis. The total number of fish involved in each group varied from 17 to 34.

RESULTS

Neither the lipid content of the two diets nor the rearing temperature had any significant effect on the phospholipid or cholesterol contents (relative to protein) of the spermatozoan plasma membrane (Table 2). The high SEs are due to high individual variations and made it difficult to assess the statistical significance of small differences between groups.

The different broodstock rearing conditions did not result in significantly different phospholipid class distributions in spermatozoan plasma membranes (Table 3). Whatever the diet or rearing temperature, phosphatidylcholine was the major phospholipid (46.5 to 54%), followed by phosphatidylethanolamine (29.5 to 33%) and phosphatidylserine (9.5 to 10.3%). Sphingomyelin was present in minor proportions (1.0 to 2.7%), and diphosphatidylglycerol was hardly detected (1.0%).

TABLE 2
Lipid Composition of Spermatozoan Plasma Membrane from Trout Reared at 8 or 18°C and Fed with Either a Corn Oil-Supplemented or a Cod Liver Oil-Supplemented Diet Before Spawning[a]

	8°C Reared		18°C Reared	
	Corn oil diet	Cod liver oil diet	Corn oil diet	Cod liver oil diet
Phospholipid (μmol/mg protein)	3.30 ± 0.59	2.81 ± 0.48	2.66 ± 0.31	2.93 ± 0.36
Cholesterol (μmol/mg protein)	1.06 ± 0.21	0.99 ± 0.28	0.92 ± 0.12	0.74 ± 0.09
Cholesterol/phospholipid (molar ratio)	0.33 ± 0.08	0.35 ± 0.06	0.35 ± 0.04	0.26 ± 0.06

[a]Values are means of three to four pools ± SE of six to nine fish sperm. No statistical significance was observed between the groups (Mann-Whitney test).

TABLE 3
Phospholipid Class Distribution (% of total phospholipids) of Spermatozoan Plasma Membrane from Trout Reared at 8 or 18°C and Fed with Either a Corn Oil-Supplemented or a Cod Liver Oil-Supplemented Diet Before Spawning[a]

	8°C Reared		18°C Reared	
	Corn oil diet	Cod liver oil diet	Corn oil diet	Cod liver oil diet
Phosphatidylcholine	48.67 ± 2.52	51.24 ± 4.15	46.51 ± 3.89	54.00 ± 5.66
Phosphatidylethanolamine	31.33 ± 3.06	31.43 ± 3.18	32.99 ± 1.90	29.50 ± 3.54
Phosphatidylserine	10.33 ± 1.53	9.89 ± 0.65	10.01 ± 2.48	9.50 ± 2.12
Phosphatidylinositol	3.33 ± 1.53	2.73 ± 0.53	4.50 ± 1.27	2.50 ± 0.71
Lysophosphatidylcholine	3.00 ± 0.00	2.97 ± 1.15	3.51 ± 1.03	2.50 ± 0.71
Sphingomyelin	2.67 ± 1.53	0.99 ± 0.02	1.00 ± 0.82	1.00 ± 0.00
Diphosphatidylglycerol	0.67 ± 0.58	0.74 ± 0.50	1.50 ± 1.29	1.00 ± 0.00

[a]Values are means of three to four pools ± SE of six to nine fish sperm. No statistical significance was observed between the groups (Mann-Whitney test).

We investigated the fatty acid profiles of the four main phospholipid classes: phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, and phosphatidylinositol (Tables 4 to 7). Palmitic acid was present in high amounts in trout spermatozoan plasma membrane, accounting for 40% of the total fatty acids in phosphatidylcholine (Table 4), 20% in phosphatidylethanolamine (Table 5), and over 10% in phosphatidylserine (Table 6) and phosphatidylinositol (Table 7). Oleic acid was also a major fatty acid in all four classes. In phosphatidylserine, 25% of the total fatty acids was 18:1n-9, and in the remaining classes the percentage was 12 to 20%. Phosphatidylserine was characterized by high proportions of 22:6n-3 (25%), and this fatty acid accounted for 10 to 15% of the total fatty acids in phosphatidylinositol, phosphatidylcholine and phosphatidylethanolamine. Of the four classes, phosphatidylcholine, the major phospholipid, had the lowest unsaturated fatty acid (UFA) to saturated fatty acids (SFA) ratio and the lowest unsaturation index. The high UFA to SFA ratio in phosphatidylserine is due to the fact that half of the phosphatidylserine fatty acids were 18:1n-9 and 22:6n-3 (Table 6).

The four phospholipid classes all exhibited the same general pattern of fatty acid change in response to reduced water temperature and to dietary modifications. In phosphatidylcholine, the proportion of 18:0 was lower at a rearing temperature of 8°C while the proportion of monounsaturated fatty acids (MUFA) was higher (Table 4). This slightly but significantly increased the MUFA to PUFA ratio but did not influence the UFA to SFA ratio or the unsaturation index. The effect of diet on phosphatidylcholine was as follows: the high amounts of 18:2n-6 in corn oil increased not only the plasma membrane 18:2n-6 levels (2-fold) but also the proportions of all n-6 PUFA (20:2n-6, 20:4n-6, and 22:5n-6 2-fold), the greatest difference between the two dietary groups being the 4-fold enrichment in 20:3n-6 in corn oil-fed trout (Table 4). The high proportions of 18:2n-6 and 20:4n-6 in trout fed corn oil as compared to trout fed cod liver oil resulted in a lower MUFA to PUFA ratio in the former. The corn oil diet had only one-third as much 14:0 and one-fourth as much 16:1 as did the cod liver oil diet (Table 1), but the plasma membrane phosphatidylcholines in corn oil-fed trout contained two-thirds as much 14:0 as found in that from fish oil-fed trout. The high proportion of n-3 PUFAs in the cod liver oil diet (two times more 20:5n-3 and 22:5n-3 than in the corn oil diet) resulted in a similar enrichment of spermatozoan membrane phosphatidylcholine in these fatty acids. Surprisingly, the 22:6n-3 levels were the same in both groups, although the cod liver oil-coated pellets were 3-fold richer in this fatty acid. Thus, phosphatidylcholine is the phospholipid class for which the difference in the n-3/n-6 ratio between the two diets is the highest and for which the UFA/SFA ratio is most affected by the diets. The resulting unsaturation index was slightly higher in the cod liver oil-fed fish, mainly due to the proportion of 20:5n-3, but this difference was observed mainly in fish reared at 18°C and was not significant.

Phosphatidylethanolamine was more unsaturated than phosphatidylcholine, the UFA/SFA ratio being about two times higher in phosphatidylethanolamine. The effects of di-

TABLE 4

Fatty Acid Composition of Phosphatidylcholines in Spermatozoan Plasma Membrane from Trout Reared at 8 or 18°C and Fed with Either a Corn Oil-Supplemented or a Cod Liver Oil-Supplemented Diet Before Spawning[a]

Fatty acid[b]	8°C Reared		18°C Reared	
	Corn oil diet	Cod liver oil diet	Corn oil diet	Cod liver oil diet
14:0	1.65 ± 0.35[c]	2.47 ± 0.09[d]	1.30 ± 0.12[c]	2.09 ± 0.48[d]
15:0	0.51 ± 0.09[c]	0.69 ± 0.03[d]	0.39 ± 0.05[c]	0.54 ± 0.11[d]
16:0	38.31 ± 2.68	38.89 ± 0.81	37.94 ± 1.65	38.57 ± 3.62
17:0	0.23 ± 0.02[c]	0.26 ± 0.01[d]	0.19 ± 0.01[c]	0.27 ± 0.02[d]
18:0	2.50 ± 0.13[a]	2.06 ± 0.13[a]	3.52 ± 0.43[b]	3.57 ± 0.22[b]
16:1n-9	1.21 ± 0.25[a,c]	1.13 ± 0.15[a,d]	1.00 ± 0.13[b,c]	0.40 ± 0.45[b,d]
16:1n-7	1.37 ± 0.15[a,c]	2.04 ± 0.16[a,d]	0.78 ± 0.11[b,c]	1.75 ± 1.00[b,d]
18:1n-9	13.20 ± 1.52	12.12 ± 0.43	12.34 ± 0.95	11.25 ± 0.74
18:1n-7	7.14 ± 0.92[a]	8.85 ± 1.49[a]	5.02 ± 0.86[b]	7.78 ± 1.55[b]
20:1n-9/7	0.29 ± 0.03	0.41 ± 0.09	0.38 ± 0.24	0.34 ± 0.02
18:2n-6	6.51 ± 0.45[c]	3.22 ± 0.33[d]	10.13 ± 0.49[c]	3.63 ± 0.27[d]
18:3n-3	0.20 ± 0.08	0.19 ± 0.07	0.24 ± 0.12	0.26 ± 0.02
20:2n-6	1.46 ± 0.28[c]	0.68 ± 0.16[d]	1.76 ± 0.28[c]	0.63 ± 0.01[d]
20:3n-6	1.20 ± 0.20[c]	0.32 ± 0.08[d]	2.08 ± 0.17[c]	0.42 ± 0.05[d]
20:4n-6	4.20 ± 0.77[c]	2.44 ± 0.18[d]	5.04 ± 0.75[c]	2.99 ± 0.52[d]
20:5n-3	6.16 ± 1.38[c]	10.41 ± 1.64[d]	4.45 ± 0.36[c]	10.84 ± 2.15[d]
22:3n-9	0.35 ± 0.18	0.33 ± 0.06	0.29 ± 0.09	0.41 ± 0.01
22:5n-6	0.43 ± 0.11[c]	0.24 ± 0.02[d]	0.57 ± 0.10[c]	0.27 ± 0.04[d]
22:5n-3	0.28 ± 0.07[c]	0.40 ± 0.03[d]	0.23 ± 0.04[c]	0.48 ± 0.14[d]
22:6n-3	11.62 ± 2.61	11.85 ± 0.48	10.87 ± 1.52	12.77 ± 3.55
Sum SFA	43.29 ± 3.01	44.41 ± 0.85	43.46 ± 1.44	45.09 ± 3.95
Sum MUFA	23.94 ± 2.61[a]	25.27 ± 1.79[a]	20.31 ± 1.56[b]	21.91 ± 2.76[b]
Sum PUFA	32.77 ± 5.24[c]	30.31 ± 2.15[d]	36.23 ± 2.98[c]	32.99 ± 6.71[d]
Sum n-3	18.37 ± 3.93[c]	23.00 ± 2.06[d]	15.89 ± 1.84[c]	24.50 ± 5.86[d]
Sum n-6	13.97 ± 1.44[c]	6.97 ± 0.58[d]	19.88 ± 1.25[c]	8.04 ± 0.85[d]
n-3/n-6	1.30 ± 0.14[c]	3.32 ± 0.38[d]	0.80 ± 0.06[c]	3.02 ± 0.41[d]
MUFA/PUFA	0.75 ± 0.17[a,c]	0.84 ± 0.12[a,d]	0.57 ± 0.09[b,c]	0.69 ± 0.22[b,d]
UFA/SFA	1.32 ± 0.17[c]	1.25 ± 0.04[d]	1.30 ± 0.08[c]	1.23 ± 0.19[d]
UI	167.17 ± 24.38	172.53 ± 9.28	165.39 ± 13.20	181.23 ± 32.96

[a,b]Significant effect ($P < 0.05$) of the rearing temperature between a and b inside one diet (Mann-Whitney test); [c,d]significant effect ($P < 0.05$) of the diet between c and d inside one rearing temperature (Mann-Whitney test).

[a]Values are means of three to four pools ± SE (molar %) of six to nine fish sperm.

[b]SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; UI, unsaturation index; PUFA, polyunsaturated fatty acid; UFA, unsaturated fatty acid.

etary n-3 and n-6 enrichment were about the same as for phosphatidylcholine. The proportion of 22:6n-3 was not affected. In phosphatidylethanolamine, however, the high proportion of 20:5n-3 in cod liver oil-fed trout resulted in an increased unsaturation index despite the high levels of 20:4n-6 and 18:2n-6 in corn oil-fed groups. Rearing at 18°C resulted in higher 18:0 and lower 16:0 levels than observed in 8°C reared trout, leading to a lower MUFA/PUFA ratio in the latter.

Phosphatidylserine is the phospholipid class in which fatty acids, excluding 18:0, were the most strongly influenced by the rearing temperature (Table 6). Amounts of all SFA possessing 14 to 17 carbons were higher in 18°C acclimated trout, resulting in a lower UFA/SFA ratio in this group. Palmitoleic acid levels were higher in the 18°C reared groups. The two diets affected the fatty acid profile of phosphatidylserine as described for phosphatidylcholine, with 22:6n-3 unchanged whatever the rearing conditions. Contrary to phosphatidylcholine, phosphatidylserine exhibited a lower MUFA/PUFA ratio in the cod liver oil-fed groups. The unsaturation index was unaffected by the rearing conditions.

In phosphatidylinositol, the five fatty acids, 16:0, 18:1n-9, 20:4n-6, 20:5n-3, and 22:6n-3, were present in proportions approaching 10% or more. The high levels of 16:0 and 18:0 made this phospholipid class very saturated, a fact reflected in the low unsaturation index. The diet mainly affected 20:4n-6 and 20:5n-3 levels, the 22:6n-3 level remaining unchanged. As observed for phosphatidylcholine and phosphatidylethanolamine, 18:0 levels were higher in the fish reared at 18°C, leading to a lower UFA/SFA ratio. As in phosphatidyl-serine and phosphatidylcholine, the unsaturation index was not affected by the rearing conditions.

The fact that 20:3n-9 was not detectable in all four phospholipid classes confirms that neither of the diets was EFA-deficient (32).

Membrane viscosity. Membrane viscosity was measured on sperm from each of the four experimental groups. ESR spectra were recorded with SL-PC and SL-PS at five different temperatures, namely at 4°C, the temperature at which spermatozoa are stored after collection; 8 and 18°C, the two rearing temperatures before spawning; 13°C, the temperature at which trout are usually reared in our laboratory; and at 24°C, the temperature used for thawing and fertilization after cryopreservation. The SL-PC reflected the microviscosity in the outer monolayer, and the SL-PS reflected that of the inner

TABLE 5
Fatty Acid Composition of Phosphatidylethanolamines in Spermatozoan Plasma Membrane from Trout Reared at 8 or 18°C and Fed with Either a Corn Oil-Supplemented or a Cod Liver Oil-Supplemented Diet Before Spawning[a]

Fatty acid[b]	8°C Reared		18°C Reared	
	Corn oil diet	Cod liver oil diet	Corn oil diet	Cod liver oil diet
14:0	1.32 ± 0.16	0.93 ± 0.37	0.79 ± 0.39	0.78 ± 0.01
15:0	0.67 ± 0.19	0.68 ± 0.34	0.49 ± 0.27	0.39 ± 0.03
16:0	21.50 ± 2.17[a]	20.22 ± 0.58[a]	19.10 ± 2.95[b]	17.87 ± 1.66[b]
17:0	0.32 ± 0.02[c]	0.38 ± 0.08[d]	0.28 ± 0.06[c]	0.31 ± 0.02[d]
18:0	3.48 ± 0.75[a]	2.94 ± 0.24[a]	4.69 ± 0.17[b]	4.88 ± 0.08[b]
16:1n-9	2.04 ± 0.64	1.50 ± 0.68	1.48 ± 0.92	1.00 ± 0.12
16:1n-7	0.48 ± 0.07[c]	0.86 ± 0.05[d]	0.30 ± 0.03[c]	0.62 ± 0.01[d]
18:1n-9	18.45 ± 3.62	17.88 ± 0.85	12.90 ± 1.96	18.55 ± 1.82
18:1n-7	1.51 ± 0.18[c]	2.24 ± 0.12[d]	1.28 ± 0.02[c]	2.38 ± 0.03[a]
20:1n-9/7	0.32 ± 0.05[c]	0.39 ± 0.05[d]	0.25 ± 0.05[c]	0.51 ± 0.15[d]
18:2n-6	9.68 ± 0.68[c]	6.68 ± 0.13[d]	13.04 ± 0.87[c]	7.32 ± 0.63[d]
18:3n-3	0.29 ± 0.04[c]	0.46 ± 0.11[d]	0.35 ± 0.10[c]	0.47 ± 0.05[d]
20:2n-6	0.77 ± 0.16[c]	0.46 ± 0.05[d]	1.16 ± 0.16[c]	0.50 ± 0.00[d]
20:3n-6	1.45 ± 0.51[c]	0.40 ± 0.14[d]	2.40 ± 0.35[c]	0.42 ± 0.01[d]
20:4n-6	8.84 ± 1.23[c]	5.17 ± 0.36[d]	12.88 ± 0.50[c]	6.13 ± 0.11[d]
20:5n-3	10.65 ± 2.04[c]	18.70 ± 0.92[d]	9.51 ± 0.85[c]	17.43 ± 0.65[d]
22:3n-9	0.28 ± 0.15	0.21 ± 0.08	0.17 ± 0.06	0.16 ± 0.02
22:5n-6	0.46 ± 0.06[c]	0.26 ± 0.04[d]	0.71 ± 0.14[c]	0.29 ± 0.06[d]
22:5n-3	0.24 ± 0.02[c]	0.43 ± 0.02[d]	0.28 ± 0.03[c]	0.52 ± 0.09[d]
22:6n-3	15.02 ± 1.68	16.85 ± 0.83	15.85 ± 1.80	17.55 ± 2.75
Sum SFA	27.65 ± 2.32	25.49 ± 1.48	25.87 ± 3.17	24.83 ± 1.60
Sum MUFA	24.21 ± 3.18[a]	24.25 ± 1.25[a]	17.18 ± 2.28[b]	23.78 ± 1.55b
Sum PUFA	48.14 ± 4.51	50.26 ± 1.79	56.95 ± 3.44	51.40 ± 3.15
Sum n-3	26.31 ± 3.07[c]	36.73 ± 1.53[d]	26.09 ± 2.07[c]	36.28 ± 3.56[d]
Sum n-6	21.33 ± 1.90[c]	13.09 ± 0.36[d]	30.35 ± 1.86[c]	14.81 ± 0.46[d]
n-3/n-6	1.23 ± 0.10[c]	2.81 ± 0.05[d]	0.86 ± 0.06[c]	2.45 ± 0.32[d]
MUFA/PUFA	0.51 ± 0.11[c]	0.48 ± 0.04[a]	0.30 ± 0.05[b]	0.46 ± 0.06[b]
UFA/SFA	2.64 ± 0.30	2.93 ± 0.22	2.90 ± 0.46	3.04 ± 0.26
UI	234.82 ± 18.35[c]	262.53 ± 8.77[d]	255.32 ± 14.94[c]	265.59 ± 18.50[d]

[a,b]Significant effect ($P < 0.05$) of the rearing temperature between a and b inside one diet (Mann-Whitney test); [c,d]significant effect ($P < 0.05$) of the diet between c and d inside one rearing temperature (Mann-Whitney test).
[a]Values are means of three to four pools ± SE (molar %) of six to nine fish sperm.
[b]See Table 4 for abbreviations.

monolayer. This distribution was determined by their properties of transmembrane movement. For a given sample, the apparent correlation times calculated from the ESR spectra revealed that the higher the recording temperature, the lower the membrane microviscosity surrounding the spin label. The rearing temperature, however, did not affect membrane viscosity. The correlation times recorded at a given temperature (8 or 18°C) were the same for fish reared at 8 or 18°C (Fig. 1). This means that no adaptation of spermatozoan membranes of cold-acclimated trout compensated for the cold-induced increase in viscosity. Dietary lipids also had no effect on plasma membrane microviscosity as assessed by ESR. In addition, for any sperm cell sample, the microviscosity of the inner monolayer was always lower than that of the outer monolayer since the apparent correlation time of SL-PS was shorter than that of SL-PC.

DISCUSSION

Plasma membrane characteristics. Teleostean spermatozoa have no acrosome, and fertilization of the eggs occurs after the spermatozoa have passed through the micropylar canal to reach the egg plasma membrane. Prior to this study, no information was available on the phospholipid composition of the plasma membranes of such spermatozoa. Our results show that by comparison with mammals (33–37), levels of sphingomyelin in trout spermatozoan plasma membrane are very low. This low sphingomyelin content was also observed in trout erythrocyte plasma membranes (2.8%) (38). Phosphatidylserine levels, on the contrary, are higher in trout spermatozoan plasma membrane than in mammalian ones. The distribution of phosphatidylcholine and phosphatidylethanolamine in trout spermatozoan membrane is quite similar to that found in vertebrate membranes of other cell types, where these classes predominate. Phosphatidylethanolamine is more abundant, however, in trout spermatozoa than in mammalian ones. One could expect that the high phosphatidylethanolamine levels in trout plasma membranes would contribute to enhancing their fusion capacity as unsaturated phosphatidylethanolamines are thought to show a tendency toward forming nonbilayer-type configurations due to their "cone-shaped" structure (39,40). Surprisingly, we observed

TABLE 6

Fatty Acid Composition of Phosphatidylserines in Spermatozoan Plasma Membrane from Trout Reared at 8 or 18°C and Fed with Either a Corn Oil-Supplemented or a Cod Liver Oil-Supplemented Diet Before Spawning[a]

Fatty acid[b]	8°C Reared		18°C Reared	
	Corn oil diet	Cod liver oil diet	Corn oil diet	Cod liver oil diet
14:0	1.30 ± 0.10[a]	1.43 ± 0.70[a]	1.94 ± 0.25[b]	2.09 ± 0.33[b]
15:0	0.69 ± 0.04[a]	0.90 ± 0.35[a]	1.15 ± 0.20[b]	1.40 ± 0.16[b]
16:0	8.18 ± 1.47[a]	9.76 ± 2.78[a]	9.65 ± 1.92[b]	10.86 ± 2.27[b]
17:0	0.32 ± 0.03[a]	0.39 ± 0.05[a]	0.37 ± 0.05[b]	0.45 ± 0.14[b]
18:0	7.50 ± 1.90	7.20 ± 1.34	7.87 ± 1.14	8.18 ± 1.43
16:1n-9	2.69 ± 0.93[a]	3.03 ± 0.98[a]	3.64 ± 1.06[b]	3.47 ± 2.25[b]
16:1n-7	0.24 ± 0.21	0.47 ± 0.13	0.42 ± 0.10	0.41 ± 0.28
18:1n-9	27.58 ± 2.04	24.75 ± 1.35	25.36 ± 3.84	24.04 ± 0.38
18:1n-7	0.99 ± 0.06[c]	1.26 ± 0.12[d]	0.80 ± 0.19[c]	1.41 ± 0.09[d]
20:1n-9/7	0.45 ± 0.22	0.39 ± 0.21	0.25 ± 0.08	0.54 ± 0.35
18:2n-6	6.28 ± 0.96[c]	7.50 ± 1.60[c]	4.69 ± 0.98[c]	8.14 ± 0.45[d]
18:3n-3	0.61 ± 0.57	0.45 ± 0.32	0.34 ± 0.24	0.71 ± 0.45
20:2n-6	0.89 ± 0.76[c]	0.24 ± 0.09[d]	0.74 ± 0.13[c]	0.35 ± 0.21[d]
20:3n-6	0.87 ± 0.16[c]	0.26 ± 0.11[d]	1.22 ± 0.19[c]	0.42 ± 0.29[c]
20:4n-6	5.33 ± 1.45[c]	3.08 ± 1.06[d]	7.10 ± 1.41[c]	3.03 ± 0.45[d]
20:5n-3	4.38 ± 1.33[c]	6.68 ± 1.57[d]	3.53 ± 0.99[c]	6.19 ± 0.24[d]
22:3n-9	0.23 ± 0.12	0.25 ± 0.20	0.18 ± 0.12	0.07 ± 0.07
22:5n-6	0.75 ± 0.23[c]	0.46 ± 0.25[d]	1.35 ± 0.11[c]	0.41 ± 0.12[d]
22:5n-3	0.99 ± 0.20[c]	1.76 ± 0.73[d]	0.94 ± 0.04[c]	1.25 ± 0.86[d]
22:6n-3	24.74 ± 0.72	25.86 ± 3.00	24.58 ± 2.85	23.39 ± 2.65
Sum SFA	18.73 ± 3.04[a]	20.16 ± 3.16[a]	24.48 ± 3.43[b]	23.91 ± 2.63[d]
Sum MUFA	35.20 ± 4.07[a,c]	32.40 ± 1.81[a,d]	32.91 ± 4.01[b,c]	30.96 ± 2.18b,[d]
Sum PUFA	46.07 ± 1.09	47.45 ± 3.24	45.61 ± 2.29	45.14 ± 4.53
Sum n-3	30.83 ± 1.61[c]	34.95 ± 2.62[d]	29.44 ± 2.20[c]	31.73 ± 3.74[d]
Sum n-6	14.81 ± 0.47[c]	11.93 ± 2.06[d]	15.70 ± 0.99[c]	12.95 ± 1.42[d]
n-3/n-6	2.08 ± 0.17[c]	2.99 ± 0.50[d]	1.88 ± 0.21[c]	2.46 ± 0.27[d]
MUFA/PUFA	0.77 ± 0.11[c]	0.69 ± 0.07[d]	0.72 ± 0.12[c]	0.69 ± 0.11[d]
UFA/SFA	4.44 ± 0.97[a]	4.04 ± 0.69[a]	3.74 ± 0.71[b]	3.22 ± 0.46[b]
UI	258.09 ± 8.15	265.49 ± 17.72	257.01 ± 10.60	246.56 ± 21.75

[a,b]Significant effect ($P < 0.05$) of the rearing temperature between a and b inside one diet (Mann-Whitney test); [c,d]significant effect ($P < 0.05$) of the diet between c and d inside one rearing temperature (Mann-Whitney test).

[a]Values are means of three to four pools ± SE (molar %) of six to nine fish sperm.

[b]See Table 4 for abbreviations.

that trout spermatozoan plasma membranes contain about 1% of diphosphatidylglycerol. This phospholipid is thought to be almost exclusively localized in the inner mitochondrial membrane (41). Such a contamination in our plasma membrane fraction is unlikely since no succinic dehydrogenase (mitochondrial marker) activity was detected (22). A mitochondrial contamination leading to add 1% of diphosphatidylglycerol to the total plasma membrane phospholipids would surely be detectable by enzymatic marker assay. We can then reasonably assume that trout spermatozoan plasma membrane contains diphosphatidylglycerol. In mammals, some authors have reported its presence in plasma membranes of ram epididymal or ejaculated spermatozoa (42,37).

Of the four phospholipid classes studied here, phosphatidylcholine was the most saturated, due to very high levels of 16:0. Such a preeminence of 16:0 in phosphatidylcholine is often described, whether in whole cells or in plasma membrane. Examples include cod (*Gadus morhua*) erythrocytes (43), cod and trout muscle, liver, gill, heart, kidney, and spleen (44,45), buffalo fish (*Jotiobus cyprinellus*) sperm (46), and brush border membrane from rainbow trout intestine (20). In the present study, phosphatidylinositol also displayed very saturated profiles as compared to phosphatidylethanolamine and phosphatidylserine, which contained more PUFAs. The plasma membranes of mammalian spermatozoa differ markedly from trout spermatozoan membranes in regard to fatty acid saturation in the various classes of phospholipids. In goat and boar caudal epididymis, phosphatidylethanolamine fatty acids are more saturated than those of phosphatidylcholine (35,34). In these species, furthermore, phosphatidylinositol and phosphatidylserine are the most saturated phospholipids (SFA percentages for boar caudal epididymis are respectively 98 and 64%). In phosphatidylinositol of trout spermatozoan plasma membrane, arachidonic and stearic acids predominate. This is also true in trout gill, spleen, and kidney (47) and in mammals. Phosphatidylinositol may be the primary source of arachidonate for lipoxygenase and cyclooxygenase reactions in fish sperm as postulated for gonad cell lines (48), since prostaglandins have been identified in the testes and milt of numerous fish, including trout (14,15).

Dietary effects. The dietary fats affected the various phospholipid classes differently. The n-3/n-6 ratio was much less affected in phosphatidylserine (1.5-fold) than in the other phospholipids (2.5 to 2.7-fold). This was due, however, to the

TABLE 7

Fatty Acid Composition of Phosphatidylinositols in Spermatozoan Plasma Membrane from Trout Reared at 8 or 18°C and Fed with Either a Corn Oil-Supplemented or a Cod Liver Oil-Supplemented Diet Before Spawning[a]

Fatty acid[b]	8°C Reared		18°C Reared	
	Corn oil diet	Cod liver oil diet	Corn oil diet	Cod liver oil diet
14:0	1.36 ± 0.40	2.49 ± 0.57	1.54 ± 0.92	1.53 ± 0.69
15:0	0.09 ± 0.02	0.17 ± 0.05	0.10 ± 0.05	0.09 ± 0.05
16:0	11.20 ± 1.13	15.25 ± 2.04	10.76 ± 2.47	12.67 ± 2.94
17:0	0.68 ± 0.25	0.94 ± 0.14	0.64 ± 0.11	0.81 ± 0.08
18:0	19.16 ± 1.95[a]	19.12 ± 2.35[a]	23.79 ± 3.13[b]	24.32 ± 0.25[b]
16:1n-9	2.25 ± 0.44	4.04 ± 1.57	2.81 ± 1.40	2.91 ± 1.58
16:1n-7	0.20 ± 0.24	0.58 ± 0.37	0.39 ± 0.27	0.39 ± 0.42
18:1n-9	21.97 ± 1.10[c]	15.10 ± 1.86[d]	17.38 ± 0.96[c]	12.87 ± 3.77[d]
18:1n-7	1.12 ± 0.22	1.17 ± 0.25	0.80 ± 0.19	1.17 ± 0.12
20:1n-9/7	0.34 ± 0.29	0.27 ± 0.18	0.20 ± 0.11	0.31 ± 0.15
18:2n-6	6.27 ± 0.92[c]	3.63 ± 0.61[d]	5.27 ± 0.92[c]	3.66 ± 0.67[d]
18:3n-3	1.13 ± 0.97	1.06 ± 0.73	0.56 ± 0.34	0.62 ± 0.18
20:2n-6	0.91 ± 0.77	0.32 ± 0.18	0.41 ± 0.16	0.27 ± 0.08
20:3n-6	0.88 ± 0.43[c]	0.22 ± 0.19[d]	1.43 ± 0.22[c]	0.44 ± 0.11[d]
20:4n-6	13.22 ± 1.68[c]	8.75 ± 0.76[d]	13.43 ± 1.12[c]	7.28 ± 0.87[d]
20:5n-3	6.43 ± 2.33[c]	13.18 ± 2.18[d]	7.38 ± 1.55[c]	14.86 ± 1.39[d]
22:3n-9	0.20 ± 0.15	0.25 ± 0.14	0.21 ± 0.07	0.30 ± 0.05
22:5n-6	0.61 ± 0.44[c]	0.21 ± 0.24[d]	0.27 ± 0.06[c]	0.16 ± 0.04[d]
22:5n-3	0.74 ± 0.59	0.30 ± 0.15	0.21 ± 0.05	0.38 ± 0.06
22:6n-3	6.78 ± 0.65	8.11 ± 1.55	7.78 ± 1.04	9.29 ± 1.42
Sum SFA	32.93 ± 1.49	38.50 ± 1.91	37.30 ± 2.57	39.97 ± 3.60
Sum MUFA	28.59 ± 0.75[c]	24.84 ± 2.55[d]	24.99 ± 3.32[c]	22.30 ± 1.82[d]
Sum PUFA	38.47 ± 1.51	36.67 ± 2.72	37.70 ± 2.99	37.73 ± 1.82
Sum n-3	15.25 ± 1.25[c]	22.68 ± 3.44[d]	15.94 ± 2.64[c]	25.19 ± 2.66[d]
Sum n-6	22.77 ± 1.94[c]	13.63 ± 1.00[d]	21.20 ± 0.40[c]	12.12 ± 1.02[d]
n-3/n-6	0.68 ± 0.10[c]	1.69 ± 0.38[d]	0.75 ± 0.11[c]	2.10 ± 0.40[d]
MUFA/PUFA	0.74 ± 0.04[a]	0.68 ± 0.11[a]	0.67 ± 0.13[b]	0.59 ± 0.02[b]
UFA/SFA	2.04 ± 0.14[a]	1.60 ± 0.12[a]	1.69 ± 0.20[b]	1.52 ± 0.24[b]
UI	185.33 ± 6.95	191.13 ± 13.43	184.74 ± 14.73	197.32 ± 12.25

[a,b]Significant effect ($P < 0.05$) of the rearing temperature between a and b inside one diet (Mann-Whitney test);[c,d]significant effect ($P < 0.05$) of the diet between c and d inside one rearing temperature (Mann-Whitney test).

[a]Values are means of three to four pools ± SE (molar %) of six to nine fish sperm.

[b]See Table 4 for abbreviations.

constant high level of 22:6n-3 in this phospholipid, irrespective of the diet. This persistence of 22:6n-3 raises questions as to its physiological importance for trout spermatozoa. Yu and Sinhuber (49) showed that an EFA-deficient diet in trout induced only a 30% decrease in 22:6n-3 content of body phospholipids over a 3.5-month period. Casteldine and Buckley (32,50) reported the same phenomenon, i.e., maintenance of the 22:6n-3 levels in trout that received diets devoid of EFAs. This could be linked to slow catabolism of long-chain PUFAs or to an efficient system for recycling 22:6n-3 during lipid metabolism (32). The rate of ß-oxidation of 22:6n-3 in the mitochondria of different fish tissues is limited or null (15). This, coupled with the apparent retention of 22:6n-3 during lipid mobilization, might ensure that this fatty acid is specifically retained to fulfill its role as a major constituent of biomembrane phospholipids (15). In whole trout spermatozoa, however, Watanabe *et al.* (16) have shown that when 18:2n-6 was available in an (n-3)-deficient diet, a 50% drop in 22:6n-3 was compensated by a 4-fold increase in 20:4n-6 and 22:5n-6. Whole spermatozoa from trout fed an EFA-deficient diet for one year displayed a decrease in 22:6n-3 levels although these levels were maintained in erythrocytes (17). It has been proposed that this PUFA could play a role in controlling membrane fluidity (51) or could be a substrate of lipoxygenases (15). Our study suggests a major role for 22:6n-3 in the phospholipids of trout spermatozoan plasma membrane, since levels remain constant even when most of the available fatty acids in the diet are of the n-6 series.

Temperature effect. The temperature at which the fish were reared appears to affect neither the cholesterol and phospholipid contents of spermatozoan plasma membrane nor its phospholipid class distribution. Maintenance of cholesterol levels was somewhat surprising, considering that a correlation between cholesterol content and temperature is often described in membranes of poikilothermic animals (13). However, most of these studies dealt with short-term adaptation of the fish (less than one month), and mitochondria were the structures described to be most commonly affected by temperature. Membranes of cold-adapted poikilotherms usually possess higher proportions of phosphatidylethanolamine and, less commonly, lower proportions of phosphatidylcholine than membranes formed at higher growth temperatures. This increases the proportion of nonbilayer-forming lipids at cold temperatures. Like the cholesterol decrease, this adaptation is

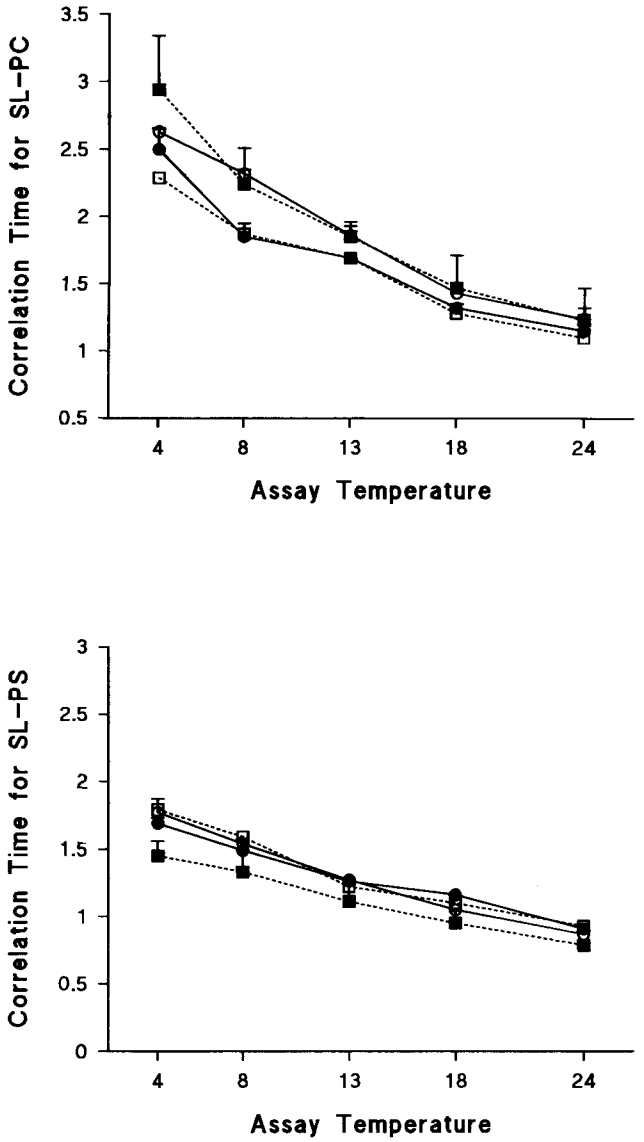

FIG. 1. Correlation time (in ns) as a function of temperature (in °C) for spin labeled-phosphatidylcholine (SL-PC) and -phosphatidylserine (SL-PS) incorporated in trout spermatozoan plasma membrane; ○, 8°C-reared trout fed with corn oil diet; ●, 8°C-reared trout fed with cod liver oil diet; □, 18°C-reared trout fed with corn oil diet; ■, 18°C-reared trout fed with cod liver oil diet. Values reported are the result of two different pools (± SE) of five to eight individual sperms. No significant differences appeared between the two diets and the two rearing temperatures.

believed to maintain a constant viscosity of the membrane. From the insignificant variations obtained for each phospholipid class in spermatozoan plasma membranes from trout reared at 8 or 18°C, it appears that this type of adaptation to low temperature did not operate in these cells. This lack of change with long-term adaptation to a different temperature is in agreement with previous observations on flounder (*Platichthys fletus*) erythrocyte plasma membranes (52). If changes in the cholesterol and phospholipid contents are merely short-term responses to thermal stress, the fact that the fish were transferred to the colder temperature prior to spermatogenesis and remained at that temperature until the process was complete could explain the apparent lack of thermal adaptation in trout spermatozoa.

A nearly ubiquitous response to exposure to a cold environment, as judged from most studies, is a drop in the proportion of SFAs and a corresponding rise in the proportion of UFAs. The introduction of double bonds in the phospholipid fatty acids disturbs the molecular packing of the membrane. Upon adaptation to a warmer temperature, conversely, an increase in the saturation level of fatty acids increases membrane viscosity. This "homeoviscous adaptation" (53) is thought to maintain a constant fluidity in biological membranes whatever the rearing temperature, in order to maintain membrane functions. We, conversely, have shown that long-term adaptation of trout to two very different rearing temperatures has little effect on the phospholipid fatty acids of trout spermatozoan plasma membranes. This is at variance with most studies on short-term adaptation of fish tissues (13): trout hepatocytes, liver plasma membranes, and mitochondria, carp liver and muscle mitochondrial membranes, and cod and carp erythrocytes (43,54). In all these cases, production of UFAs was stimulated by a sharp drop in the rearing temperature. A long-term adaptation of the fish does not always result in an acclimatory response. Although a long-term temperature adaptation resulted in slight but significant changes of the fatty acid composition in flounder erythrocyte plasma membrane (45,52), it was not the case in Atlantic salmon (*Salmo salar*) liver (55). The margin of 10°C between the two acclimation temperatures employed in our study is somewhat smaller than is commonly employed in such experiments (margins of 15 to 20°C). However, Malak *et al.* (56) showed that a six-week acclimation of trout to 11 and 21°C notably affected the fatty acid pattern of several tissues. This allows us to think that the rearing temperatures we used are of sufficient importance to trigger an acclimation response in trout. The *in vitro* experiments on trout and carp hepatocytes have shown that prolonged exposure to cold diminished the capacity for UFA production and that the total capacity to produce saturated or unsaturated species clearly did not correlate with growth temperatures (57). These observations could be one explanation for the apparent lack of response to a long-term temperature adaptation we observed in trout sperm.

The viscosity of trout spermatozoan plasma membranes as assessed by ESR appeared unaffected by the rearing temperature of the broodstock. In a previous paper (58), we showed that phosphatidylcholine is located mainly on the outer leaflet of the plasma membrane while most of the phosphatidylserine is inside. The two spin-labels used here should thus provide information on microviscosity in each bilayer leaflet. That the viscosity of neither leaflet is affected by the rearing temperature of the broodstock is in good agreement with the very slight variations observed in the fatty acid profile of each phospholipid class. Such a lack of a homeoviscous adaptation has been described for the sarcoplasmic reticulum of goldfish white muscle (59) and for the brush border membrane of carp intestinal mucosa (60). Homeoviscous adaptation is not a

property of all cellular membranes, but occurs selectively in situations where compensations maintaining membrane order serve to maintain membrane function and hence, to some extent, tissue function as well (60). The trout spermatozoan is a highly differentiated cell, but one whose metabolic activity is low and whose viability in the genital tract before spawning does not seem to depend on the molecular order of its plasma membrane. Membrane viscosity assessed by ESR with spin-labeled phospholipid gives information at the interfacial level of the membrane, since the doxyl group is located at the third carbon level. Adjustments of viscosity in the hydrophobic core cannot be detected by this probe. Hazel *et al.* (61) observed in trout hepatic plasma membranes that compensatory fluidity changes in response to temperature are more important in the hydrophobic moiety of the membrane than at the interface.

In conclusion, the fatty acid profile of phosphatidylcholines, phosphatidylethanolamines, phosphatidylinositols, and phosphatidylserines in trout spermatozoan plasma membranes was affected by the fatty acid profiles of the broodstock diet. This indicates the ability of the plasma membrane to incorporate most available fatty acids. This ability is, however, limited by the need for n-3 fatty acids as shown by the higher n-3/n-6 ratio in the plasma membrane than in the food pellets and by the constancy of the 22:6n-3 levels. As judged from the results of long-term adaptation to two different temperatures before and during spermatogenesis, the rearing temperature had little effect on the lipid composition of the plasma membranes of mature spermatozoa. This agrees with the fact that no difference in membrane viscosity was observed between the two groups and supports the hypothesis that rainbow trout spermatozoa do not display any homeoviscous adaptation.

ACKNOWLEDGMENTS

This work was supported by the Institut National de la Recherche Agronomique, by the Centre National de la Recherche Scientifique (URA 256), and by a grant from the "Région Bretagne" (Développement de Biotechnologies appliquées à l'aquaculture). K. Müller was a recipient of a short-term EMBO fellowship. We are grateful to Drs. G. Corraze, L. Frémont and X. Pelletier for kind and helpful discussions and advice. We thank Mr. Monasz for care with the fish and Mrs. Heydorff for skillful assistance.

REFERENCES

1. Malejac, M.L., Loir, M., and Maisse, G. (1990) *Aquat. Living Resour. 3*, 43–54.
2. Quinn, P.J., and White, I.G. (1967) Aust. J. Biol. Sci. 20, 1205–1215.
3. Darin-Bennett, A., and White, I.G. (1977) *Cryobiology 14*, 466–470.
4. Ansah, G.A., and Buckland, R.B. (1982) *Poult. Sci. 61*, 623–637.
5. Poulos, A., Darin-Bennett, A., and White, I.G. (1973) *Comp. Biochem. Physiol. 46B*, 541–549.
6. Parks, J.E., and Lynch, D.V. (1992) *Cryobiology 29*, 255–266.
7. Ehringer, W.D., Belcher, D., Wassall, S.R., and Stillwell, W. (1991) *Chem. Phys. Lipids 57*, 87–96.
8. Stillwell, W., Ehringer, W., and Jenski, L.J. (1993) *Lipids 2*, 103–108.
9. Salte, R., Thomassen, M.S., and Wold, K. (1988) *Bull. Eur. Ass. Fish Pathol. 8*, 63–66.
10. Hagve, T.A., Johansen, Y., and Christophersen, B. (1991) *Biochim. Biophys. Acta 1084*, 251–254.
11. Stubbs, C.D., and Smith, A.D. (1984) *Biochim. Biophys. Acta 779*, 89–137.
12. Quinn, P.J., Joo, F., and Vigh, L. (1989) *Prog. Biophys. Mol. Biol. 53*, 71–103.
13. Hazel, J.R., and Williams, E.E. (1990) *Prog. Lipid Res. 29*, 167–227.
14. Greene, H.S.D., and Selivonchick, D.P. (1987) *Prog. Lipid Res. 26*, 53–85.
15. Henderson, R.J., and Tocher, D.R. (1987) *Prog. Lipid Res. 26*, 281–347.
16. Watanabe, T., Takeushi, T., Saito, M., and Nishimura, K. (1984) *Bull. Jap. Soc. Scient. Fish. 50*, 1207–1215.
17. Leray, C., and Pelletier, X. (1985) *Aquaculture 50*, 51–59.
18. Mourente, G., and Odriozola, J.M. (1990) *Fish Physiol. Biochem. 8*, 93–101.
19. Di Costanzo, G., Duportail, G., Florentz, A., and Leray, C. (1983) *Molecular Physiology 4*, 279–290.
20. Pelletier, X., Duportail, G., and Leray, C. (1987) *Membrane Biochem. 7*, 55–66.
21. Behar, D., Cogan, U., Viola, S., and Modaky, S. (1989) *Lipids 24*, 737–742.
22. Labbe, C., and Loir, M. (1991) *Fish Physiol. Biochem. 9*, 325–338.
23. Folch, J., Lees, M., and Stanley, G.M.S. (1957) *J. Biol. Chem. 226*, 497–509.
24. Bartlett, G.R. (1959) *J. Biol. Chem. 254*, 466–468.
25. Linard, A. (1988) *Cah. Techn. INRA 20*, 41–48.
26. Berry, J.F., Cevallos, W.H., and Wade, R.R. (1965) *J. Am. Oil Chem. Soc. 42*, 492–500.
27. Fellmann, P., Zachowski, A., and Devaux, P. (1994) in *Methods in Molecular Biology* (Graham, J.M., and Higgins, J.A., eds.) Vol. 27 II, pp. 161–175, Humana Press Inc., Totowa.
28. Billard, R., and Jalabert, B. (1974) *Ann. Biol. Anim. Biochim. Biophys. 14*, 601–610.
29. Keith, A., Bulfield, G., and Snipes, W. (1970) *Biophys. J. 10*, 618–629.
30. Smith, P.K., Krohn, R.I., Hermanson, G.T., Mallia, A.K., Gartner, F.H., Provenzano, M.D., Fujimoto, E.K., Goeke, N.M., Olson, B.J., and Klelk, D.C. (1985) *Anal. Biochem. 150*, 76–85.
31. Schwartz, D. (1986) *Collection Statistique en Biologie et en Médecine*, 3rd edn., pp. 246–247, Flammarion Medecine Sciences, Paris, France.
32. Casteldine, A.J., and Buckley, J.T. (1982) *Comp. Biochem. Physiol. 71B*, 119–126.
33. Parks, J.E., Arion, J.W., and Foote, R.H. (1987) *Biol. Reprod. 37*, 1249–1258.
34. Nikolopoulou, M., Soucek, D.A., and Vary, J.C. (1985) *Biochim. Biophys. Acta 815*, 486–498.
35. Rana, A.P.S., Majumber, G.C., Misra, S., and Ghosh, A. (1991) *Biochim. Biophys. Acta 1061*, 185–196.
36. Stojanoff, A., Bourne, H., Andrews, A.G., and Hyne, R.V. (1988) *Gamete Res. 21*, 297–311.
37. Hinkovska, V.T., Dimitrov, G.P., and Koumanov, K.S. (1986) *Int. J. Biochem. 18*, 1115–1121.
38. Bolis, L., and Fange, R. (1979) *Comp. Biochem. Physiol. 62B*, 345–348.
39. Mantsch, H.H., Martin, A., and Cameron, D.G. (1981) *Biochemistry 20*, 3138–3145.
40. Lindblom, G., and Rilfors, L. (1989) *Biochim. Biophys. Acta 988*, 221–256.

41. Ioannou, P.V., and Golding, B.T. (1979) *Prog. Lipid Res. 17*, 279–318.
42. Parks, J.E., and Hammerstedt, R.H. (1985) *Biol. Reprod. 32*, 653–668.
43. Lie, O., Lied, E., and Lambertsen, G. (1989) *Aquaculture 79*, 137–144.
44. Lie, O., and Lambertsen, G. (1991) *J. Chromatogr. 565*, 119–129.
45. Sorensen, P.G. (1993) *Comp. Biochem. Physiol. 106B*, 907–912.
46. Drokin, S. (1993) *Aquat. Living Resour. 6*, 49–56.
47. Turner, M.R., Leggett, S.L., and Lumb, R.H. (1989) *Comp. Biochem. Physiol. 94B*, 575–579.
48. Tocher, D.R. (1990) *Fish Physiol. Biochem. 8*, 239–249.
49. Yu, T.C., and Sinhuber, R.O. (1972) *Lipids 7*, 450–454.
50. Casteldine, A.J., and Buckley, J.T. (1980) *J. Nutr. 110*, 675–685.
51. Farkas, T., Csengeri, I., Majoros, F., and Olah, J. (1980) *Aquaculture 20*, 29–40.
52. Sorensen, P.G. (1990) *Comp. Biochem. Physiol. 96B*, 571–576.
53. Sinensky, M. (1974) *Proc. Natl. Acad. Sci. USA 71*, 522–525.
54. Dey, I., Szegletes, T., Buda, Cs., Nemcsok, J., and Farkas, T. (1993) *Lipids 28*, 743–746.
55. Skuladottir, G.V., Schiöth, H.B., Gemundsdottir, E., Richards, B., Gardarsson, F., and Jonsson, L. (1990) *Aquaculture 84*, 71–80.
56. Malak, N.A., Brichon, G., Meister, R., and Zwingelstein, G. (1989) *Lipids 24*, 318–324.
57. Hazel, J.R. (1984) *Am. J. Physiol. 246*, R460–R470.
58. Müller, K., Labbe, C., and Zachowski, A. (1994) *Biochim. Biophys. Acta 1192*, 21–26.
59. Cossins, A.R., Christiansen, J., and Prosser, C.L. (1978) *Biochim. Biophys. Acta 511*, 442–454.
60. Lee, A.C., and Cossins, A.R. (1990) *Biochim. Biophys. Acta 1026*, 195–203.
61. Hazel, J.R., McKinley, S.J., and Williams, E.E. (1992) *J. Comp. Physiol. 162*, 593–601.

[Received February 19, 1994, and in revised form December 6, 1994; Revision accepted December 12, 1994]