Aquat. Living Resour., 1993, **6**, 49-56

Phospholipid distribution and fatty acid composition of phosphatidylcholine and phosphatidylethanolamine in sperm of some freshwater and marine species of fish

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Received March 31, 1992; accepted October 8, 1992.

Drokin S. I., *Aquat. Living Resour.*, 1993, **6**, 49-56.

Abstract

The phospholipid composition and fatty acid characteristics of diacyl and plasmalogen forms of phosphatidylcholine and phosphatidylethanolamine of sperm of five marine and five freshwater species of fish have been determined. There was considerable variation in composition within each group of fish, which obscured any differences which are related to the osmotic environment. Since such factors as temperature, nutrition, etc. probably greatly influence the lipid composition, we failed to determine a relationship between osmolality and phospholipid composition. We have also found certain phospholipid peculiarities, characteristic of sperm of various species of fish.

Nevertheless, as we have found previously, the molar ratio of cholesterol to phospholipids in fish sperm is determined by the salinity of the habitat.

Keywords: Phospholipid, fatty acid, osmolality, fish sperm.

Composition en acides gras et phospholipides de la phosphatidylcholine et de la phosphatidyléthanolamine dans le sperme de quelques poissons marins et d'eau douce.

Résumé

La composition en phospholipides et les caractéristiques en acides gras des formes diacyl et plasmalogène de la phosphatidylcholine et de la phosphatidylethanolamine du sperme de 5 espèces de poissons marins et de 5 espèces de poissons d'eau douce ont été déterminées. Il y a de considérables variations dans la composition à l'intérieur de chaque groupe de poissons, ce qui occulte les différences liées au milieu osmotique. Les facteurs, telles que la température, la nutrition, etc., influencent probablement la composition en lipides de façon importante. Nous n'avons pas pu déterminer une relation entre la pression osmotique et la composition en phospholipides. Nous avons trouvé des particularités aux phospholipides du sperme de quelques espèces de poissons. Néanmoins, comme nous l'avions trouvé précédemment, le rapport molaire du cholestérol aux phospholipides, dans le sperme de poisson, est déterminé par la salinité du milieu.

Mots-clés : Phospholipide, acide gras, pression osmotique, sperme de poisson.

INTRODUCTION

The fact that sperm of one species of fish exists under hypertonic environmental conditions, while the sperm of other species exists under hypotonic conditions, illustrates the adaptive abilities of organisms and their cells, which they have developed in the course of evolution.

The necessity for fish sperm to exist in water with a certain tonicity is largely determined by those membrane properties which are responsible for cell osmoregulation. One of the most important structural elements involved in the regulation of these properties is the phospholipid and fatty acid composition (Aloia and Boggs, 1985). The phospholipid composition of milt of some species of fish has been described elsewhere (Ackman, 1967; Jaugaard *et al.*, 1967; Sidorov, 1983; Labbé and Loir, 1991). However, these works have not described composition as a function of osmoregulation status and, hence, give no physiological understanding.

In this respect we thought it necessary to perform a comparative study of phospholipids and fatty acids of sperm of some freshwater and marine species of fish.

MATERIAL AND METHODS

The fish to be studied were systematically chosen from taxonomically different groups. All the fish belong to different families, with the exception of carp (*Cyprinus carpio*) (spawning temperature is 20°C) and grass carp (*Ctenopharyngodon idella*) (spawning temperature is 26°C). We also harvested milt from Mozambique bream (*Sarotherodon mossambicus*) (spawning temperature is 26°C), flier (*Centrarchus macropterus*) (spawning temperature is 25°C), and buffalo (*Jotiobus cyprinellus*) (spawning temperature is 26°C). In our experiments we used milt of marine fish: Black sea turbot (*Psetta maeotica*) (spawning temperature is 7.5-11°C), blunt-snouted mullet (*Mullus barbatus ponticus*) (spawning depth is 50 m), round goby (*Neogobius maelonostomus* (spawning temperature is 10-16°C), annular bream (*Diplodus annularis*) (spawning temperature is 15-20°C), and

Table I. – Phospholipid composition of the sperm of some Black Sea species (mean ± SE; $n = 6$ to 8; $p \leqslant 0.05$).

Phospholipids	Black Sea turbot		Annular bream	
	µg P/10^9 sperm	% form the sum	µg P/10^9 sperm	% from the sum
SM	2.59 ± 0.47	6.83	2.60 ± 0.21	14.86
PtdSer + PtdIns	2.42 ± 0.21	6.39	0.67 ± 0.11	3.81
PPtdCho	4.62 ± 0.71	12.37	1.85 ± 0.12	10.53
DPtdCho	16.05 ± 1.32	42.30	7.39 ± 0.30	42.19
PPtdEth	5.60 ± 0.62	14.78	1.47 ± 0.12	8.39
DPtdEth	6.54 ± 0.53	17.26	3.54 ± 0.2	20.22
Σ	37.87	100	17.52	100
P/D PtdCho	0.06	–	0.14	–
P/D PtdEth	0.86		0.42	

Phospholipids	Blunt-snouted mullet		Round goby		Long-striped wrasse	
	µg P/10^9 sperm	% from the sum	µg P/10^9 sperm	% from the sum	µg P/10^9 sperm	% from the sum
SM	1.45 ± 0.40	8.55	1.95 ± 0.25	9.13	1.93 ± 0.16	9.30
PtdSer + PtdIns	1.55 ± 0.62	9.13	1.03 ± 0.03	9.08	1.04 ± 0.08	5.02
PPtdCho	1.12 ± 0.55	6.63	1.10 ± 0.07	5.14	1.18 ± 0.1	5.68
DPtdCho	4.32 ± 1.05	25.48	11.29 ± 0.13	52.86	11.2 ± 2.34	53.95
PPtdEth	2.70 ± 0.43	15.93	1.18 ± 0.12	5.52	1.16 ± 0.06	5.56
DPtdEth	5.81 ± 1.04	34.28	4.82 ± 0.09	22.56	4.25 ± 0.14	20.49
Σ	16.93	100	21.35	100	20.76	100
P/D PtdCho	0.26		0.09		0.11	
P/D PtdEth	0.47		0.46		0.27	

SM: sphingomyelin; PtdSer: phosphatidylserine; PtdIns: phosphatidylinositol; DPtdCho: phosphatidylcholine; DPtdEth: phosphatidylethanolamine; PPtdCho: plasmalogenic phosphatidylcholine; PPtdhEth: plasmalogenic phosphatidylethanolamine. P: plasmalogen; D: diacyl.

Tableau 2. – Phospholipid composition of the sperm of some freshwater species (mean ± SE; $n = 6$ to 8; $p \leq 0.05$).

Phospholipids	Mozambique bream		Flier		Buffalo	
	µg P/10^9 sperm	% from the sum	µg P/10^9 sperm	% from the sum	µg P/10^9 sperm	from the sum
SM	2.06 ± 0.3	6.67	6.16 ± 0.17	17.96	8.28 ± 0.25	18.6
PtdSer	2.48 ± 0.4	8.03	4.04 ± 0.3	11.78	3.93 ± 0.44	8.84
PtdIns	1.35 ± 0.33	4.37	2.37 ± 0.4	7.96	2.72 ± 0.56	6.11
PPtdCho	3.50 ± 0.49	11.33	2.17 ± 0.18	6.33	6.69 ± 1.36	15.0
DPtdCho	10.15 ± 0.66	32.8	7.94 ± 1.08	23.15	9.75 ± 1.77	21.9
PPtdEth	4.97 ± 0.54	16.09	3.99 ± 0.25	11.64	4.08 ± 1.7	9.17
DPtdEth	5.74 ± 0.35	18.6	8.27 ± 0.16	24.11	8.30 ± 0.17	18.66
DPtdG		–	–		–	–
Lyso-PtdCho	0.61 ± 0.17	2.0	–	–	0.73 ± 0.14	1.6
Σ	30.88	100	34.29	100	44.48	100
P/D PtdCho	0.34		0.27		0.69	
PD PtdEth	0.87		0.48		0.49	

	Grass carp		Carp	
	µg P/10^9 sperm	% from the sum	µg P/10^9 sperm	% from the sum
SM	6.63 + 0.34	20.9	3.32 ± .71	9.96
PtdSer	2.97 + 0.21	9.37	1.78 ± .22	5.35
*PtdIns	–	–	1.51 + .18	4.54
**PPtdCho			1.92 ± .13	5.76
DPtdCho	10.81 + 0.22	34.1	10.14 ± .51	30.4
PPtdEth	1.70 + 0.20	5.36	3.19 ± .55	9.58
DPtdEth	8.19 + 0.34	25.8	7.44 ± .96	22.3
DPtdG	1.43 + 0.19	4.51	–	–
LysoPtdCho	–	–	–	–
Σ	31.7	100	33.32	100
P/D PtdCho	–		0.19	
P/D PtdEth	0.2		0.42	

* – were not identified; ** – were not determined. DPtdG: Diphosphatidylglycerol; LysoPtdCho: Lysophosphatidylcholine.

long-striped wrasse (*Symphodus tinca*) (spawning temperature is 15-20°C).

The sperm of freshwater fish was harvested with the help of injections of wild carp pituitary body. The sperm of marine species of fish was harvested during their natural spawning without hormonal stimulation.

Following 15 min centrifugation of sperm at 1 500 g the supernatant was isolated, and lipids were extracted from sperm (Bligh and Dyer, 1958).

Aiming at complete extraction of lipids, we additionally sonicated spermatozoa in the mixture of chloroform-methanol (1:2) with the frequency of 22 kHz for 3 min on ice.

In experiments we used a two-dimensional thin-layer chromatography, by means of which phospholipids were separated in the alkaline mixture (horizontal plane) – chloroform: methanol: 28%-ammonium (65:25:5 v/v, respectively). Following oxidation in HCl vapour, we separated phospholipids in another dimension (vertical) in acidic mixture – chloroform: methanol: acetone: vinegar acid: water (50:10:20:10:5 v/v, respectively) (Horrocks and Sun, 1972). The spots were removed, lipids were mineralized, and the amount of the inorganic phosphorus was determined (Fiscke and Subbarow, 1925).

For gas chromatography the plates from thin-layer chromatography were sprayed with dichlorofluorescein. The spots, containing phosphatidylcholine and phosphatidylethanolamine, were transferred into vials where methylation was performed by 0.21 N NaOH on absolute methanol.

Gas-chromatography analysis of fatty acid methyl ethers of phospholipids was conducted on a Pye Unicam and a Khrom-5 chromatographs, using flame ionization detector. Separation was performed in a liquid phase (diethyleneglycol succinate) on a chromosorb w (80-120 mesh) which was flushed by acid and silanized. Helium flow rate was 50 ml/min, hydrogen was 50 ml/min, and that of air was 400 ml/min. The oven temperature was 200°C. Fatty acids were identified by comparison with authentic standards, and by the logarithm of the relative captured volume of

Table 3. – Fatty acid composition of phosphatidylcholine in the sperm of freshwater fish. The data are given in percentage from the sum (D: diacyl; P: plasmalogen).

Fatty	Flier		Carp		Mozambique bream		Buffalo		Grass carp	
	D	P	D	P	D	P	D	P	D	P*
14:0	1.6	3.5	–	1.2	5.7	8.7	0.6	4.2	1.7	
15:0	3.2	6.9	–	3.9	–	1.7	0.4	0.4	–	
15:1	–	–	–	0.9				–	–	
16:0	27.7	7.4	24.1	9.4	29.9	21.0	55.5	43.9	29.5	
16:1 ω7	10.0	11.0	2.6	1.0	3.8	5.6	3.6	2.1	5.8	
17:0	1.0	–	0.6	–	3.4	1.8	2.0	5.2		
17:1ω 8	–	–	–	–	–	–	–	–		
18:0	4.6	11.0	6.6	12.6	13.7	29.2	8.3	5.4	5.5	
18:1 ω9	3.5	10.3	19.0	12.3	6.4	4.9	6.0	6.3	13.7	
18:2 ω6	2.6	1.5	5.1	16.7	6.6	2.5	4.0	6.5	7.1	
18:3 ω6	4.8	1.0		0.4	3.7	4.1	0.4	–	0.9	
18:3 ω3	–	–	–	0.9	–					
20:1 ω9	3.9	10.6	3.5	14.2	1.2	3.9	0.3	–	3.6	
20:2 ω9	4.0	–	1.1		1.1	1.4		–		
20:2 ω6	–	–	0.8	–	1.6	0.6	–	–		
20:3 ω6	4.1	8.4	5.1	1.1	0.5	1.0	1.6	2.4	–	
20:4 ω6	9.9	11.1	28.9	13.5	13.1	6.3	11.1	13.2	17.1	
20:4 ω3	–	–	–	–	2.1	0.4	0.4	0.9	–	
20:5 ω3	3.5	5.4	–	–	0.4	1.3		0.6	8.4	
22:4 ω6	2.3	3.6	0.6	–	1.4	0.8	0.7	1.0	2.9	
22:5 ω6	4.0	3.9	1.3	9.8	2.5	0.8	2.3	2.7	–	
22:5 ω3	1.8	2.1	–	–	1.3	1.7	0.9	0.5	0.6	
22:6 ω3	7.6	2.2	–	1.4	1.8	2.3	4.6	4.9	3.2	
S	38.1	28.8	31.3	27.1	52.7	62.4	66.8	59.1	36.7	
PN	44.6	39.2	42.9	43.8	36.1	23.2	26.0	32.7	40.2	
PN/S	1.2	1.4	1.4	1.6	0.7	0.4	0.4	0.6	1.1	
ω3/ω6	0.77	0.32	–	0.06	0.36	0.35	0.31	0.27	0.48	

S: saturated fatty acids; PN: polyunsaturated fatty acids. *: not determined.

methyl ethers of saturated and unsaturated fatty acids. The content of each acid was determined after calculating peak areas.

Statistical analysis

The data obtained were statistically processed on PC according to the program, based on the methods of variation statistics, described elsewhere (Zaks, 1976).

RESULTS

Nine classes of phospholipids were identified in the sperm of fish species under study. The basic phospholipid in the quantitative aspect is phosphatidylcholine. However, its content in sperm of flier (*table* 1) and blunt-snouted mullet (*table* 2) does not significantly exceed the content of phosphatidylethanolamine, which in sperm of the rest of the fish species is second to phosphatidylcholine. With the exception of blunt-snouted mullet sperm, the content of phosphatidylcholine is higher than those of other phospholipids in sperm of marine fish as compared with freshwater ones. The sperm of freshwater fish, with the exception of Mozambique bream, is rich in sphingomyelin, though, which is the most unsaturated phospholipid. The content of acid phospholipids is also higher in freshwater fish sperm.

We found traces of lysophosphatidylcholine in the sperm of Mozambique bream and buffalo.

Diphosphatidylcholine was detected only in the sperm of grass carp.

While studying plasmalogens of phosphatidylcholine and phosphatidylethanolamine (and the data on their analysis in fish sperm are scarce in the literature reviewed), we found that the plasmalogen/diacyl (P/D) ratio varies considerably both in marine and freshwater fish. It should be noted, however, that the sperm of all the studied fish species contains less plasmalogens than diacyl (*tables* 1, 2).

We identified 23 fatty acids when studying fatty acid composition of phosphatidylcholine, phosphati-

Table 4. – Fatty acid composition of phosphatidylethanolamine in the sperm of freshwater fish. The data are given in percentage from the sum (D – diacyl; P – plasmalogen).

Fatty acids	Flier		Carp		Mozambique bream		Buffalo		Grass carp	
	D	P	D	P	D	P	D	P	D	P*
14:0	1.0	3.3	–	2.1	7.3	6.1	–	1.1	0.9	
15:0	2.5	7.0	..	3.2	–	1.4	–	0.3	..	
15:1	–	–	0.4	–		–	-	–	–	
16:0	24.6	15.2	16.1	8.6	12.6	5.9	22.7	27.0	13.2	
16:1 ω7	5.4	11.6	0.7	11.5	4.1	1.8	0.4	0.7	–	
17:0	0.3		0.8	0.5	–	1.8	0.6	5.3	–	
17:1 ω7	–	–	1.2	.	–	–	1.0	–	–	
18:0	3.7	7.6	23.7	10.1	36.5	19.9	25.1	20.3	23.8	
18:1 ω9	3.5	11.8	5.8	6.0	11.5	5.4	8.8	6.5	10.9	
18:2 ω6	4.7	2.9	1.5	21.0	3.3	0.7	4.9	3.4	2.1	
18:3 ω6	2.5	1.3	0.8	3.9	2.0	0.5	–	1.6	–	
18:3 ω3	–	–	–	–	2.3	2.2	–	–	–	
20:1 ω9	2.8	11.7	3.7	11.1	1.3	3.2	0.7	0.6	1.4	
20:2 ω9	3.2	–	0.8	–	2.2	0.8	0.6	0.3	–	
20:2 ω6		–	0.7	–	–	–	–	-	–	
20:3 ω6	1.2	–	1.6		-	1.1	2.0	1.6	–	
20:4 ω6	17.3	13.1	40.6	6.7	7.1	22.9	15.9	14.8	25.4	
20:4 ω3			.	–	1.7	8.5	–	1.7	-	
20:5 ω3	1.9	6.4	–	–	–	0.7	0.3	–	5.9	
22:4 ω6	3.5	6.0	–	4.5	1.9	2.1	1.3	1.5	4.6	
22:5 ω6	1.6	2.1	–		2.5	6.2	5.2	4.5	1.4	
22:5 ω3	5.7	–	1.6	9.8	1.0	2.9	0.5	–	–	
22:6 ω3	14.8			-	2.8	4.3	10.0	9.2	8.7	
S	32.1	33.1	40.6	24.5	56.4	35.1	48.4	54.0	37.9	
PN	56.4	31.8	47.6	45.9	25.8	52.9	40.7	38.6	48.1	
PN/S	1.76	0.96	1.17	1.87	0.46	1.51	0.8	0.7	1.3	
ω3/ω6	0.80	0.25	0.08	0.38	0.46	0.56	0.37	0.39	0.44	

* not determined.

dylethanolamine, and their plasmalogens (*tables* 3, 4, 5, 6). The most important fatty acids were palmitic (16:0), stearic (18:0) acids, oleic (18:1), eicosaenoic (20:1) acids, linoleic (18:2), arachidonic (20:4 ω6), eicosapentaenic (20:5 ω3), and docosohexanoic (22:6 ω3) acids. The basic indispensible fatty acids for fish are linoleic acid (18:2 ω6) and linoleneic acid (18:3 ω3). These acids occupy a large portion of sperm of both marine and freshwater fish (*tables* 3-6). However, the content of linoleic acid is higher in phospholipids of sperm of marine fish species than in that of freshwater fish. The latter contains a higher amount of metabolites of linoleic acids, in particular, those of 20:3 ω6 and 20:4 ω6.

There are certain exceptions, for instance carp among freshwater fish, and annular bream gilthead and blunt-snouted mullet among marine fish (*tables* 3-6). Not infrequently the content of linoleic fatty acid, as is observed in freshwater fish sperm, is almost the same as the content of oleic acid, while in sperm of marine fish the content of linoleic fatty acid is greater.

It should be emphasized that in every group of fish fatty acid composition of the phospholipids varied greatly, and certain tendencies were found only by classifying fatty acids according to their certain properties.

A distinguishing feature of the fatty acid composition of phospholipids of marine fish tissue is the ratio of ω3/ω6 fatty acids (Ackman, 1967).

It follows from the results obtained (*tables* 3-6) that carp sperm contains the smallest amount of the ω3 fatty acids, and flier sperm is similar by this index to the sperm of marine fish, e.g. blunt-snouted mullet and long-striped wrasse. That means that there is no characteristic dependence of the ω3/ω6 ratio of fatty acids in sperm of freshwater and marine fish.

A comparison of the phospholipid fatty acid composition of sperm of freshwater and marine fish as regards the ratio of polyunsaturated to polysaturated fatty acids may be useful. This ratio in plasmalogen and diacyl forms of phosphatidylcholine is practically the same in sperm of both marine and freshwater fish. However, the ratio of polyunsaturated fatty acids to saturated ones in phosphatidylethanolamine is higher in sperm of marine fish than in that of freshwater fish. Nevertheless, the maximum unsaturated fatty acid composition (in freshwater fish) of

Table 5. – *Fatty acid composition of phosphatidylcholine of the sperm of marine fish. The data are given in percentage from the sum (D – diacyl; P – plasmalogen).*

Fatty acids	Black sea turbot		Round goby		Annular bream gilthead		Long-striped wrasse		Blunt-snouted mullet	
	D	P	D	P	D	P	D	P	D	P
14:0	1.6	3.8	0.8	0.9	1.4	1.3	1.4	0.6	2.5	1.6
15:0	0.6	1.8	0.9	1.5	0.7	3.2	1.3	0.7	3.2	2.9
15:1		-	–	0.6	0.7		–	0.4	–	–
16:0	49.2	30.3	35.3	47.0	29.8	7.6	43.3	35.2	8.0	8.0
16:0 ω7	2.6	2.5	1.4	1.6	4.9	6.9	6.9	0.3	2.6	
17:0		1.5	0.8	1.0	–	1.1	0.9	0.4	–	–
17:1 ω8	–	–			–	–			–	
18:0	2.1	6.4	2.1	8.5	3.9	10.9	2.4	3.3	11.7	12.6
18:1 ω9	1.8	6.5	1.5	4.0	3.4	11.1	4.0	9.1	10.2	14.1
18:2 ω6	20.2	20.0	25.1	14.6	9.2	12.5	13.6	28.0	17.9	12.8
18:3 ω 6	0.3	1.5	4.0	1.4	14.1	2.7	0.5	0.5	1.3	–
18:3 ω3	1.1	0.9	0.3	-	1.3	0.8		0.9	1.4	–
20:1 ω9	4.5	7.3	1.6	1.5	2.1	10.5	0.7	8.1	11.3	12.3
20:2 ω9			0.6	–	–			–	–	–
20:2 ω6	–	–	0.5		3.0	0.7	–			-
20:3 ω6	1.3	1.1	0.6	–	1.8	7.3	–	1.0	–	-
20:4 ω6	1.5	2.1	2.2	0.7	4.3	5.7	3.5	2.0	8.7	9.1
20:4 ω3	0.3	1.1	0.7		–	1.5	–	0.9	0.8	
20:5 ω3	1.2	0.4	8.3	1.5	9.7	14.2	11.7	1.0	7.4	7.8
22:4 ω6	0.9	1.1	0.5	2.2	–	–	0.7	0.6	5.8	12.7
22:5 ω6	0.9	2.9	1.0	-	8.0	2.2	1.0	1.4	3.1	6.3
22:5 ω3	2.5	2.9	3.0	6.1	0.7	-	2.3	1.2	1.7	–
22:6 ω3	7.1	6.0	8.7	7.2	0.9	-	5.8	4.4	2.3	
S	53.5	43.4	39.9	58.9	35.8	24.1	49.3	40.2	25.4	25.1
PN	37.3	40.0	55.5	33.7	53.0	47.6	39.1	41.9	30.4	48.7
PN/S	0.7	0.9	1.4	0.6	1.48	1.98	0.8	1.0	2.0	1.9
ω3/ω 6	0.49	0.39	0.62	0.78	0.47	0.53	1.03	0.25	0.37	0.19

phosphatidylethanolamine of flier and carp sperm (*tables* 3-6) is similar to the minimum unsaturated fatty acid composition (in marine fish) of phosphatidylethanolamine of Black Sea turbot sperm. Phosphatidylethanolamine of Black Sea turbot sperm contains the highest amount (as compared with other fish under study) of the most unsaturated long-chain fatty acid – 22:6 ω 3 (21.6%). This may to some extent compensate for a surprisingly low unsaturation of fatty acids of Black Sea turbot sperm phospholipids. If we consider the ratio of polyunsaturated fatty acids to saturated ones with regard to the spawning temperature, it turns out that sperm phospholipids of the most warmth requiring-species (from the species under study), Mozambique bream, has the lowest unsaturation, while sperm phospholipids of the most cold-spawning (plus high pressure), blunt-snouted mullet, has the highest unsaturation. There is no striking difference between the other species, although buffalo and grass carp have similar spawning temperatures, and the ratios of polyunsaturated to saturated fatty acids differ greatly. Thus, according to the most unsaturated phospholipid-phosphatidylethanolamine, the index of the ratio of polyunsaturated to saturated fatty acids is higher in the sperm of marine fish species than freshwater fish. Certain exceptions were evident, though.

It should be noted that plasmalogen forms of phosphatidylcholine and phosphatidylethanolamine do not have a higher extent of saturation than diacyl forms.

DISCUSSION

The differences in the lipid composition of the sperm of blunt-snouted mullet and the sperm of the rest of the fish under study are related, apparently, to their differing spawning conditions. Blunt-snouted mullet is the only fish (among those under study) which spawns at a depth of down to 50 m and a temperature no higher than 10°C.

The fact that phosphatidylcholine dominates over other phospholipids in the sperm of marine fish as compared with freshwater fish is very important. It is known that phosphatidylcholine has a great affinity with membrane (Evans and Settchell, 1978). Phosphatidylcholine also protects sperm from osmotic and

Table 6. – Fatty acid composition of phosphatidylcholine in the sperm of marine fish. The data are given in percentage from the sum (D – diacyl; P – plasmalogen).

Fatty acids	Black sea turbot		Round goby		Annular bream gilthead		Long-striped wrasse		Blunt-snouted mullet	
	D	P	D	P	D	P	D	P	D	P
14:0	2.4	3.0		0.8	0.7	2.2	0.4	0.3	0.6	1.3
15:0	1.3	1.1	–	1.1	1.6	2.0	0.3	1.0	2.2	2.3
15:1	–	0.6	0.3	1.5	–	–	0.4	0.7	–	–
16:0	21.9	24.6	12.5	12.4	7.2	3.7	23.2	19.5	5.5	5.6
16:1 ω7	2.4	5.7	0.7	1.3	4.1	2.0	0.8	0.9	7.6	0.6
17:0	0.5	1.2	1.3	2.0		–	1.0	1.0	1.2	–
17:1 ω8	–	–	–	–	–	–	–		–	–
18:0	5.4	7.2	7.7	12.9	6.8	7.0	3.5	4.9	8.8	8.9
18:1 ω9	7.0	7.0	10.7	13.8	7.3	13.1	9.1	9.4	7.7	12.7
18:2 ω6	15.9	14.1	36.8	19.9	9.8	8.5	18.3	27.7	13.8	9.0
18:3 ω6	0.8	1.4	2.7	–	5.5		0.5	1.3	1.2	–
18:3 ω3	–	0.5	0.7	–	0.5	–	0.8		–	–
20:1 ω9	7.7	8.2	3.4	4.5	6.1	6.4	4.6	9.8	9.0	8.3
20:2 ω9	–	–	–	–	2.0	1.6	–	–	–	–
20:2 ω6			–	–	–	–	–	–	–	–
20:3 ω6	0.6	–	0.4	–	5.8	4.8	1.2	1.2	–	6.0
20:4 ω6	4.7	3.2	2.6	2.1	4.4	2.4	4.9	3.5	8.2	6.7
20:4 ω3	0.6	4.9	1.0	3.7	2.6	4.1	1.2	2.4	1.7	1.3
20:5 ω3	1.5	1.1	4.5	4.5	30.4	37.4	6.8	1.4	6.3	5.9
22:4 ω6	1.1	1.1	0.3	-	–	–	1.2	1.1	17.2	27.6
22:5 ω6	2.0	3.8	1.2	6.7	2.4	2.6	2.6	4.3	2.5	1.2
22:5 ω3	2.9	2.4	2.1	3.7	2.2	2.1	4.4	2.1	4.1	2.6
22:6 ω3	21.6	9.2	10.9	9.4	0.8		14.2	7.5	3.1	–
S	31.5	35.9	21.5	29.2	16.8	14.9	27.4	26.7	17.1	18.1
PN	51.7	41.7	62.8	50.0	66.4	63.5	56.1	52.5	58.1	60.3
PN/S	1.64	1.16	2.92	1.71	3.95	4.26	2.05	1.97	3.4	3.33
ω3/ω6	1.06	0.77	0.44	0.74	1.31	3.42	0.96	0.34	0.35	0.19

cold stress (Simpson *et al.*, 1986), and, if it were not for certain exceptions, this fact may be viewed as one of the reasons for cryoresistance of marine fish sperm, which we described previously (Drokin *et al.*, 1989).

Peculiarities in phospholipid composition of sperm of marine and freshwater fish are related, apparently, to the permeability of their membranes. It is known, for instance, that acid phospholipids act as ionophores in the cell (Ivkov and Berestovsky, 1982). Phosphatidylserine is a phospholipid which disorders or fluidizes membrane (Davis and Burne, 1980), while phosphatidylinositol actively participates in the regulation of cell function. It is important for activation of membrane enzymes, motility and performance of the sperm capacitation reaction (Aloia and Boggs, 1985). Sphingomyelin is the most saturated phospholipid, and, apparently, an increase in its content should make the sperm membrane more rigid. On the contrary, phosphatidylethanolamine is the most unsaturated phospholipid, but its concentration is more or less similar in the sperm of the fish under study. Lysophosphatidylcholine affects membranes of spermatozoa (with the exception of acrosomal reaction), that is why its presence is normally unfavourable and regarded as pathological (Lizenko *et al.*, 1985). Diphosphatidylglycerol is, mainly, a mitochondrial phospholipid. The weight of mitochondrial membranes of sperm is very small in comparison with the weight of plasma membrane, that is why diphosphatidylglycerol comprises only a minor portion of the total weight of the phospholipid, and its amount cannot be measured by the given method of measurement.

Let us consider our fatty-acid analysis of sperm phospholipids and, in the first place, the absence of the dependence of the ratio of ω3/ω6 fatty acids on the environment (sea- or fresh water). It has been reported elsewhere (Ackman, 1967), that this ratio is much higher in brain and other tissues of marine fish as compared with freshwater species. However, this ratio has been shown to be strongly dependent on temperature (Akulin, 1975), and on rearing conditions, as has been shown for fish sperm (Leray and Pelletier, 1985). As far as the ratio of polyunsaturated to saturated fatty acids is concerned, we do not have a clear pattern, and only for phosphatidylethanolamine is this index generally higher in sperm of marine fish than that of freshwater species.

Apparently, the absence of the dependence of the peculiarities of fatty acid composition of sperm phospholipids of the fish species under study on the environmental conditions (sea- or freshwater) is a consequence of the effect of numerous factors on phospholipid fatty acids. Spawning temperature, feeding, time of activity, etc., are probably among these factors.

However, in the previous study we have found that the value of the molar ratio of cholesterol to phospholipids is much higher in the sperm of marine fish than that of freshwater species (Drokin *et al.*, 1989). Besides, the sperm of marine fish species turned out to be much more cryoresistant than that of freshwater species. This correlates with the role of cholesterol described elsewhere (Ivkov and Berestovsky, 1982), which may perform similar function in fish sperm.

The ratio of plasmalogen to diacyl forms, as has been described for marine fish, directly reflects the temperature of their habitat (Akulin, 1975). However, we have failed to find such a dependence in sperm phospholipids. A low level of plasmalogen than diacyl testifies, apparently, to the fact that sperm phospholipids of the studied fish species undergo poor peroxidation.

Acknowledgements

This paper was presented to the workshop "Gamete and embryo storage and cryopreservation in aquatic organisms", 30 March-2 April 1992, Marly-le-Roy, France. Financial support: E.C., Programme FAR.

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