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Lipid Composition of the Tissues of *Coregonus lavaretus* L. of the Kola Peninsula

Lyudmila Mishanina[1] and Anna Malaeva[2]

[1]Department of Microbiology and Biochemistry, Institute of Nature and Technology, Murmansk State Technical University, Murmansk, Russia
[2]Department of Foreign Languages, Institute "Maritime Academy", Murmansk State Technical University, Murmansk, Russia

Abstract

The article presents the results of the investigation of the lipid composition of the muscle, liver and eggs of the whitefish *Coregonus lavaretus* L. of different age groups harvested from lakes Lovozero and Umbozero of the Kola Peninsula. The study of the lipid composition of the tissues to some extent allows evaluating the features of lipid metabolism and the physiological state of an organism. Lipids were extracted from the tissues applying Bligh -- Dyer method. Fractional composition of lipids was determined using one-dimensional thin layer chromatography. Individual lipid fractions were determined by densitometry. The content of total lipids in the muscle tissue of mature whitefish from Umbozero Lake was lower than that of the whitefish from Lovozero Lake. With ageing, the content of phospholipids in the muscle tissue of the whitefish from Umbozero unlike the whitefish from Lovozero increases, but the content of triacylglycerols decreases, due to the lack of nutrition and active swimming of the whitefish in cold water of Umbozero Lake. The portion of triacylglycerols in the liver of the whitefish from Lovozero is higher than in the whitefish from Umbozero, which is probably related to the diet of the whitefish from Lovozero. In the whitefish eggs from the two reservoirs structural lipids are dominant and high content of cholesterol was identified. Molar ratio of CHS/PHL and PHCH/PHEA is lower in the whitefish from Umbozero compared to the whitefish from Lovozero, which should lead to an increase in the functional activity of membrane structures, lipid matrix fluidity and metabolism in the fish from Umbozero.

Keywords: whitefish, Umbozero Lake, Lovozero Lake, total lipids, phospholipids, cholesterol, triacylglycerols.

Corresponding Author:
Lyudmila Mishanina
ludapoh@yandex.ru

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1. Introduction

Nowadays, it is relevant to study both physiological and biochemical mechanisms of fish adaptation to the ecological conditions of the environment, and the functional role of various classes of organic substances in adaptive mechanisms [1--5].

The study of the commercially valuable whitefish *Coregonus lavaretus* L., which is ubiquitously declining, is of great scientific interest.

The purpose of the study is to analyze the biochemical composition of whitefish *Coregonus lavaretus* L. from lakes Umbozero and Lovozero of the Kola Peninsula, namely, to study the lipid composition of the muscle tissue, liver and eggs of these objects to assess the characteristics of metabolism and the development of fish.

2. Methods and Equipment

The object of the study was whitefish *Coregonus lavaretus* L. aged 3+ (before puberty) and 5+ (mature) from lakes Lovozero and Umbozero of the Kola Peninsula.

The muscle tissue and liver of the whitefish (3+ and 5+), as well as the eggs of mature whitefish (5+) were used as the material for the study. The whitefish were caught in summer (late June -- early July). The samples were consisted of tissue samples taken from 2--4 specimens of the same age.

Total lipids from the tissues were isolated according to Bligh -- Dyer method [6].

The fractional composition of lipids was determined by the method of one-dimensional thin-layer chromatography. Silica gel plates on aluminum substrate of the company ``Merck'' (Germany) were used for the analysis.

Fractional composition of lipids was studied in the system n-hexane-sulfuric ether-glacial acetic acid (90: 20: 1 (v / v / v)); phospholipids - chloroform-methanol-water (65: 25: 4 (v / v / v)) [6].

Lipid fractions were identified using CS-9000 unit from ``Shimadzu'' (Japan) using the standards from Sigma (USA).

Statistical processing of the measurement results was carried out by generally accepted methods with statistical reliability of the results at $p \leq 0.05$ [7].

3. Results and discussion

The content of total lipids of the whitefish from Lovozero and Umbozero lakes is presented in Table 1. The liver and eggs do not differ significantly in the lipid content.

TABLE 1: Lipid content in muscle tissue and liver of whitefish of different age,%.

Name of Lake	Content of lipids, % of the chemical composition				
	3+		5+		
	muscle tissue	liver	muscle tissue	liver	eggs
Lovozero	12.6±0.8	28.5±1.4	16.2±0.8	31.4±1.2	35.1±3.3
Umbozero	10.7±0.9	31.0±1.3	11.6±1.0	33.2±1.2	36.2±3.2

The eggs accumulate up to 36.2 % of the total lipids, up to 33.2 % are found in the liver and up to 16.6 % are in the muscles. High lipid content in the gonads reflects the process of their accumulation in the oocytes for the subsequent development of the embryos. Higher lipid content in the liver compared to the muscle tissue features liver's depository function in the body.

The content of total lipids in the tissue muscle varies: the level of total lipids of the mature whitefish from Umbozero is lower (11.6 %) than in the whitefish from Lovozero (16.2 %) (differences are significant at $p \leq 0.05$). It is probably resulted from different fish diets and the peculiarity of the temperature regimen in the lakes.

Umbozero is a deep and slightly warmed lake, and is inhabited by sparsely rakered whitefish (with the number of gill rakers from 16 to 30). In a warmer and shallower reservoir (Lovozero Lake) both sparsely rakered whitefish and multirakered whitefish (with the number of gill rakers from 30 to 60). In this regard, there is a great difference in the diet of the whitefish. Whitefish with a large number of gill rakers have an increased ability to filter plankton with the gill apparatus and therefore larger forms of benthos and small fish in are avoided. Sparsely rakered whitefish prefer feeding on benthos [8, 9].

Deep water, predominance of rocky shores, the poor vegetation, low water temperature, salinity and concentrations of nutrients in Umbozero led to the low quantitative indicators of planktonic and benthic communities. Umbozero is characterized as ultra-oligotrophic. Zooplankton refers to rotifer-copepodite type, which is poor forage, and is represented by copepodes, cladoceran crustaceans, rotifers and bivalve molluscs. The average biomass of zooplankton is 0.4 g/m^3. The average biomass of benthofauna is 0.85 g/m^3, at least 50 % of them are chironomids. Unlike Umbozero Lovozero is an oligotrophic lake. The zooplankton of Lovozero Lake is dominated by rotifers and cladocerans; its average biomass is 0.8 g/m^3. Lovozero Lake is characterized by a relatively large diversity of invertebrate organisms. Caddisfly larvae and mollusks are widely presented in the lake.

Larval chironomids dominate in the benthos in amount, biomass and species composition. The average biomass of benthos is 2.10 g/m^3, the maximum rate being12.36 g/m^3 [10, 11].

Triacylglycerols, phospholipids, free fatty acids, diacylglyceride, sterols and esters of sterols are the main lipid fractions (Table 2). Phosphatidylcholine and phosphatidylethanolamine preveil among phospholipid fractions.

The results from Table 2 show that the content of phospholipids in the muscle tissue of whitefish from Lovozero Lake decreases 1.4 times with age, while in the fish of Umbozero Lake the increase in the proportion of phospholipids in 1.3 times and sterols 1.7 times

Table 2: Fractional composition of lipids in muscle tissue and liver of whitefish of different ages, % of total lipids.

Fraction	3+		5+		
	muscle tissue	liver	muscle tissue	liver	eggs
Lovozero Lake					
Phospholipids, including	40.5±2.8	37.8±2.6	33.7±2.0	32.2±2.0	40.8±2.8
Phosphatidylcholine	19.9±1.2	20.2±1.6	17.2±1.2	18.3±1.3	23.4±2.1
Phosphatidylethanolamine	10.8±0.6	9.1±0.7	9.5±0.5	8.5±0.6	10.3±0.6
Other phospholipids (total)	7.8±0.5	8.5±0.6	7.0±0.4	5.4±0.4	7.1±0.2
Diacylglycerols	4.1±0.3	5.8±0.5	5.7±0.3	7.2±0.5	2.0±0.1
Sterols (cholesterol)	12.2±0.5	16.6±0.7	11.3±0.5	18.5±1.2	32.5±0.4
Free fatty acids	13.6±1.0	17.7±1.3	3.7±0.5	17,4±1.3	2.3±0.2
Triacylglycerols	30.9±2.2	15.8±1.4	46.3±2.0	17.3±1.5	19.6±1.5
Sterol esters	0.7±0.1	6.3±0.4	1.3±0.1	7.4±0.6	2.8±0.2
Umbozero Lake					
Phospholipids, including	35.5±2.0	44.4±3.6	47.9±2.1	43.8±2.5	42.9±2.9
Phosphatidylcholine	14.3±0.7	20.3±1.6	23.3±1.0	21.2±1.3	20.2±2.0
Phosphatidylethanolamine	12.4±0.5	15.5±0.6	17.5±0.6	14.7±0.5	13.3±0.9
Other phospholipids (total)	7.7±0.4	8.6±0.5	7.1±0.5	7.9±0.7	9.4±0.2
Diacylglycerols	6.6±0.5	6.3±0.3	6.9±0.4	5.3±0.3	1.7±0.1
Sterols (cholesterol)	6.2±0.4	15.1±0.7	9.4±0.4	15.8±1.1	29.2±0.4
Free fatty acids	12.1±1.0	22.6±1.3	12.7±1.0	21.5±1.3	2.1±0.2
Triacylglycerols	36.1±2.7	10.9±0.4	28.5±2.4	12.7±0.6	22.7±2.3
Sterol esters	3.5±0.3	0.7±0.1	2.6±0.2	0.9±0.1	1,4±0.1

was noted. The results can be explained by lower water temperature in Umbozero Lake. It was found that, one of the variants of temperature adaptation of coldwater fish is the synthesis of phospholipids (PHL) and cholesterol (CHS), resulting in their increased amount in fish tissues, which raises the resistance of individuals to low temperatures [12].

The portion of triacylglycerols in the muscle tissue of whitefish of Lovozero Lake, increased 1.5 times with age, and on the contrary, in the fish of Umbozero Lake decreased 1.2 times, which is apparently connected with the outlaying of stored lipids for active swimming and constructive metabolism in cold water considering the lack of food for the whitefish from Umbozero. The increase in the level of triacylglycerols in the whitefish from Lovozero was synchronous with the changes in the portion of total lipids, which is particularly evident in the muscles, being the main depot of reserve lipids.

In the whitefish muscles from different lakes, the molar ratio of CHS/PHL varies. Within certain limits this ratio is known to vary and indicate the degree of functional activity

of membrane-bound enzymes. A lower CHS/PHL index (up to 0.19) is characteristic of the whitefish muscle tissue from Umbosero (table 3), which is associated with high locomotion of the whitefish, and, therefore, with the processes increasing the functional activity of membrane structures and, consequently, metabolism [13].

Table 3: Molar ratio of CHS/PHL for whitefish muscle tissue.

Lake	Age	
	3+	5+
Lovozero	0.25	0.34
Umbozero	0.17	0.19

The main lipid components of the whitefish liver from the two lakes are structural lipids - phospholipids. In the whitefish from Lovozero, the portion of triacylglycerides in the liver is higher (up to 17.3 %) than in the fish from Umbozero (up to 12.7 %) (differences are significant at $p \leq 0.05$), which is probably due to the intensive feeding of the whitefish from Lovozero.

A higher CHS/PHL index was established for the liver of the whitefish from Lovozero and Umbozero (Table 4) than for the muscle tissue (up to 0.57), since the cholesterol content in the membranes of hepatocytes is higher than in myocytes, which indicates greater stabilization of the membranes of hepatocytes. However, for the liver of the whitefish from Umbozero, this index is lower than for the liver of the whitefish from Lovozero.

Table 4: Molar ratio of CHS/PHL for whitefish liver.

Lake	Age	
	3+	5+
Lovozero	0.44	0.57
Umbozero	0.34	0.36

Structural lipids prevail in the eggs of mature whitefish from the two lakes, besides a high content of cholesterol (up to 32.5 %), stored in oocytes during vitellogenesis and were discovered. A higher level of CHS/PHL (Table 5) was established for the whitefish eggs from Lovozero and Umbozero, than for the liver -- up to 0.80. For the gonads of the whitefish from Lovozero, this index is lower than for the whitefish eggs from Umbozero. Membrane viscosity optimal for the necessary components to enter the oocyte during the growth of the gonads is achieved by changing the cholesterol level and the ratio of CHS/PHL.

Table 5: The molar ratio of CHS/PHL for whitefish caviar.

Lake	Age 5+
Lovozero	0.80
Umbozero	0.68

The main components of phospholipids of the whitefish muscle tissue, liver and eggs were phosphatidylcholine (PHCH) and phosphatidylethanolamine (PHEA). Phosphatidylcholine and phosphatidylethanolamine differ in the degree of unsaturation of their fatty acid radicals, namely, phosphatidylcholine is more saturated than phosphatidylethanolamine. Muscle tissue, liver and gonads of the whitefish from Umbozero are characterized by a decrease in the ratio PHCH/ PHEA in contrast to the whitefish from Lovozero, which should lead to the increase of liquidity of the lipid matrix and, consequently, to the acceleration of metabolism (Tables 6, 7, 8) [14, 15].

Table 6: Ratio of PHCH/PHEA for muscle tissue of whitefish.

Lake	Age	
	3+	5+
Lovozero	1.84	1.81
Umbozero	1.15	1.33

Table 7: PHCH/PHEA ratio for whitefish liver.

Lake	Age	
	3+	5+
Lovozero	2.22	2.15
Umbozero	1.31	1.44

Table 8: Ratio of PHCH/PHEA for eggs of mature whitefish.

Lake	Age 5+
Lovozero	2.27
Umbozero	1.57

4. Conclusion

1. The level of total lipids in the muscle tissue of mature whitefish from Umbozero Lake is lower than that of *Coregonus lavaretus* L. from Lovozero Lake, which is associated with different feeding of fish and the peculiarities of the temperature regime in the water bodies.

2. With age, the content of phospholipids in the muscle tissue of epy whitefish from Umbozero, unlike the whitefish from Lovozero, increases, but the content of triacylglycerols decreases. This can be explained by malnutrition and intensive swimming of the whitefish in the colder water of Umbozero Lake.

3. For the whitefish from Lovozero, the proportion of triacylglycerols in the liver is higher (up to 17.3 %) than for the fish from Umbozero (up to 12.7 %), which is probably resulted from the intensive feeding of the whitefish from Lovozero.

4. Structural lipids prevail in the whitefish eggs from the two lakes, and a high content of cholesterol (up to 32.5 %), stored in oocytes during vitellogenesis are found.

5. The molar ratio of CHS/PHL and PHCH/PHEA are lower for the whitefish from Umbozero compared to the whitefish from Lovozero, which should lead to an increase in the functional activity of membrane structures and the liquidity of the lipid matrix, and, accordingly, to the acceleration of metabolism in the fish from Umbozero.

6. The main role of lipids and lipid components in the organs and tissues of the whitefish is to participate in the mechanisms of the adaptive response which develops during the adaptation of this species to various existence conditions.

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