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Special Traits of Tissue Lipids of Whitefish *Coregonus lavaretus* Living in Water Bodies with Different Anthropogenous Load

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Abstract—The impact of water pollution on the content of lipid components of the liver and eggs of whitefish *Coregonus lavaretus* is investigated. The content of triacylglycerols (TAG), sphingomyelin (SPM), phosphatidylinositol (PI), and phosphatidylserine (PS) is higher in the liver of whitefish from polluted waters being a part of adaptogenous reactions (modification of permeability of biomembranes, of activity of membrane enzymes, etc.) providing a possibility of the survival of fish under unfavorable ecological factors. The lipid spectra of whitefish gonads are more stable in comparison with the liver. This may be related to protection of the reproductive system from external influences.

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The water systems of the Kola Peninsula are exposed to intensive pollution from mining and metallurgical enterprises. The wastewater of industrial plants depending on their volume, content of certain substances, and currents create, in a water body, zones with different hydrochemical conditions (Moiseenko, 1997).

Heavy metals belong to the most dangerous waste products, as their toxicity is high, they may be accumulated in tissues and may be included in trophic chains of hydrobionts. They render a high effect on physiologo-biochemical parameters of fish, are cancerogenous, gonadotoxic, and embryotoxic. Under conditions of pollution, the population of whitefish *Coreginus lavaretus* experiences the stress, causing no mass kills but influencing their life cycle and physiological conditions: decrease in the growth rate (dwarf forms are produced), behavioral activity, general endocrine function, shift of the sexual maturity towards younger age groups, as well as increased accumulation of fat (Kashulin et al., 1999; Sharova and Lukin, 2000; Zhou et al., 2001; Moiseenko, 2005). In the fish from water bodies exposed to loads of heavy metals, phosphorus- and nitrogen-containing compounds, clinical and histological manifestations of diseases were found: changes in coloration and structure of the liver (lipoid degeneration), of kidneys (nephrocalcitosis), of gills (hyperemia), and of other organs, excrescence of connective tissue around the vessels (Lukin et al., 2000).

The change in the physiological condition of the organism is preceded by biochemical modifications at the cell and membrane levels. Adaptation of fish to various ecological factors is much related to special traits of lipid composition of cell membrane of tissues which determine their permeability and resistance (Shatunovskii, 1980; Kreps, 1981).

It is known that one of the functions of the liver is a barrier function, including detoxication and the principal function of gonads is a reproductive one. Thus we believed that it was interesting to compare changes in lipid spectra in these organs and the response to impact of industrial emissions containing a complex of inorganic toxicants.

The aim of the present study was the investigation of the lipoid composition of the liver and gonads of whitefish from water bodies polluted by waste products of mining and metallurgical enterprises.

MATERIAL AND METHODS

Lake Imandra is the largest water body in the central Kola Peninsula. On its drainage area there are mining and processing enterprises of apatite–nepheline and copper–nickel profile. In the ichthyofauna of most lakes of the North, the species of the genus *Coregonus* dominate. They are oligotoxobic species, highly sensitive to pollution but having a high plasticity at the same time (Reshetnikov, 1995; Moiseenko, 1997).

The investigations were made on the females of oligorakered whitefish *C. lavaretus* with gonads at maturity stage IV. They are benthophagous and are exposed all year round to intensive impact of industrial pollution accumulated in bottom sediments (Kashulin, 2004).

The whitefish were caught in four water areas differing in the pollution level (Table): in the Kunchast Bay (the western part remote from Lake Imandra not exposed to direct pollution—the conventional control), in the Kislaya Bay (the southern part of Lake Imandra

Principal hydrochemical parameters of polluted zones of Lake Imandra and the Pasvik River (Moiseenko, 1997; Kashulin et al., 1999)

Parameters	Lake Imandra			Vaggetem water area
	Kunchast Bay	Kislaya Bay	Syav Island	the Pasvik River
Silfate, mg/l	9.2	17.5	23.3	–
Total phosphorus, μg P/l	4.0	17.3	45.3	–
Total nitrogen, μg N/l	136.0	213.0	370.0	–
Iron, μg/l	23.0	22.0	32.0	–
Nickel, μg/l	2.0	6.0	10.0	12.8
Copper, μg/l	2.0	3.0	4.0	5.5
Zinc, μg/l	–	–	–	17.0
Manganese, μg/l	–	–	–	13.0

Note: – no data.

principally receiving the effluents of the *Severonikel* integrated works and of Olenegorsk enrichment integrated works), in the area of Syav Island (Lake Imandra where the effluents of the aforementioned works mix, and also *Apatit* production association), and in the system of the Pasvik River (the Vaggetem water area, Norway, influenced by the emissions of OAO *GMK Pechenganikel*).

The industrial waste arrives to Lake Imandra and to the Pasvik system both from drainage areas and with atmospheric precipitation accompanied by acid phase increasing their biological accessibility (Spry and Wiener, 1991; Lukin et al., 1998; Kashulin et al., 1999).

For biochemical analyses, individual samples of the liver and gonads (by 10–15 fish from each zone) were taken. Samples of tissues (500 mg) were homogenized in a 10-fold volume of mixture chloroform–methanol (2 : 1) with an addition of 0.001% antioxidant ionol and kept in a cold place until analysis. Lipids were extracted by the Folch method (Folch et al., 1957). Total lipid (P1) and defatted dry residue (P2) were isolated, dried until permanent weight and weighted. The dry residue comprised protein, carbohydrates, nucleic acids, amino acids, and microelements. Separation to lipid fractions was made on chromatographic plates Silufol (Kavalier, Czechia) in the system of solvents: petroleum ether–sulphuric ether–acetic acid (90 : 10 : 1). The quantity of phospholipids (PL) and triacylglycerols (TAG) was determined by the hydroxamate method (Sidorov et al., 1972), cholesterol (CS)—by the reaction with a colored reagent (Engelbrecht et al., 1974) and expressed as percentage of the dry weight matter (P1 + P2). The composition of individual phospholipids was analyzed by the method of high performance liquid chromatography (Arduini et al., 1996) in a steel column Nucleosil 100-7, (Elsiko, Moscow), mobile phase acetonitrile–hexane–methanol–orthophophoric acid (918 : 30 : 30 : 17.5). Detection was made by the light extinction level at 206 nm. The ratio between phospholipids components was assessed by the values of areas on the chromatogram.

The statistical treatment of the results was made using software packages *Excell* and *Stadia.*

RESULTS AND DISCUSSION

It was found that, in whitefish from the conventionally pure zone (the Kunchast Bay—Control), the content of total lipid in the liver was 18.8% of the dry weight and, in the fish from polluted water bodies (the Kislaya Bay, island Syav), it was higher by 1.2–1.4 times due to a higher level of reserve TAG (Figs. 1, 2). Earlier investigations also revealed a higher level of TAG in the liver of whitefish from the Belaya Bay of Lake Imandra polluted with heavy metals (Smirnov and Kirilyuk, 1994). This agrees with the previously obtained data on high indices of the liver of whitefish from Lake Imandra correlating with the level of toxic load (Moiseenko, 1997). Such accumulation of TAG in the liver was previously found in the experiments on mammals at functional loads of various etiology (diabetes, viral hepatitis, impact of copper ions) (Bozhkov and Dlubovskaya, 1995). Triacylglycerols, which are a universal reserve form of lipids, are used by the organism to a considerable extent as a principal energy source. The increased level of TAG in the liver of whitefish from areas polluted with heavy metals, in comparison with whitefish from a comparatively pure zone, may be related to the decrease in the energy metabolism as a result of inhibition of the activity of acid triacylglycerol lipase in the liver by heavy metals (Tashev, 1980).

The content in the liver of structural lipids (cholesterol and phospholipids) and, respectively, the value of the coefficient CS/PL did not significantly ($p \leq 0.05$) differ in the variants under consideration (Figs. 2, 3). It is known that the values of this coefficient are a parameter characterizing the viscosity of biomembranes influencing the activity of enzymes bound with membranes (Lopukhin et al., 1985).

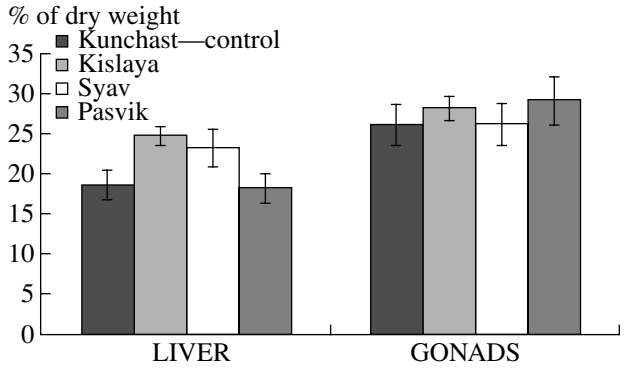

Fig. 1. Content of total lipid in tissues of whitefish *Coregonus lavaretus* from water bodies with different anthropogenous load.

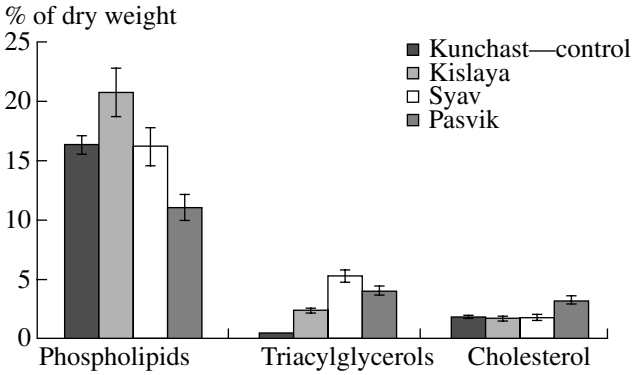

Fig. 2. Content of lipid in the liver of whitefish *Coregonus lavaretus* from water bodies with different anthropogenous load.

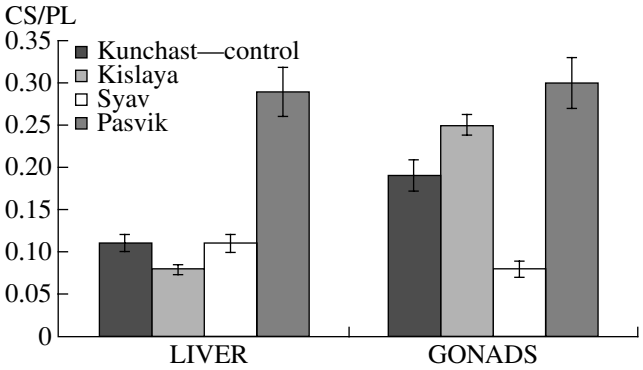

Fig. 3. The coefficient CS/PL in tissues of whitefish *Coregonus lavaretus* from water bodies with different anthropogenous load.

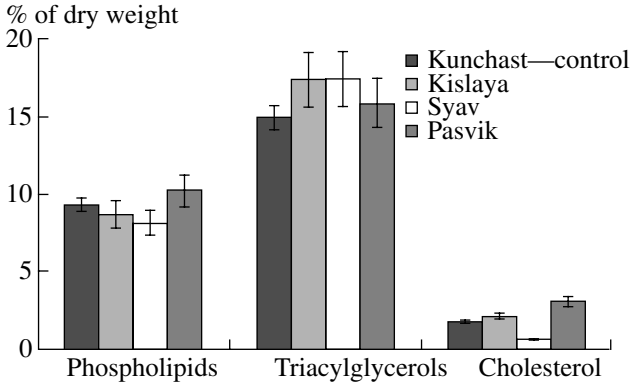

Fig. 4. Content of lipid in the gonads of whitefish *Coregonus lavaretus* from water bodies with different anthropogenous load.

In gonads of whitefish from polluted water bodies, the level of total lipid was identical, including that of phospholipids, triacylglycerols, and cholesterol (except the area of Syav Island) coinciding with the values determined for the control (Figs. 1, 4).

A very low content of cholesterol in gonads of whitefish from the most polluted water area (at Syav Island and, respectively, a ratio CS/PL in comparison with those from Kislaya Bay and Kunchast Bay (control) may indicate to disturbance of lipid–lipid relationships and, consequently, to the loss of ability of cells for adhesion, to the change of microviscosity of the lipid bilayer which influences the activity of lipid-dependent enzymes bound with membranes (Lopukhin et al., 1985; Melkonyan et al., 1989). A considerable decrease in viscosity of biomebranes of eggs may cause its partial resorption. This agrees with histochemical investigations of Moiseenko (1997) demonstrating that the structure of gonads in many female whitefish from areas of Lake Imandra strongly polluted with heavy metals is modified: the eggs are underdeveloped, filled with exudates and their consistency is jellylike.

In spite of the absence of differences in the content of total phospholipids both in the liver and in gonads of whitefish from water areas with different anthropogenous load, the spectra of particular phospholipids fractions are different in type, though have some traits in common. Comparison of spectra of phospholipids fractions in whitefish revealed that there is a direct correlation of the level of phosphatidylinositol (PI), phosphaditylserine (PS), lysophospaditylcholine (LPC), and sphingomyelin (SPM) in the liver and a reverse correlation of PS and LPC in gonads with the level of pollution of water area with heavy metals (Figs. 5–7). The part of phosphatidylcholine (PC) and phosphatidylethanolamine (PEA) in the liver of whitefish from Kislaya Bay is higher than that in the control. In the area of Syav Island, it is significantly lower. A high concentration of LPC and a lowered level of PEA and PC in the liver of whitefish from the area of Syav Island indicate to destructive processes related to oxidation of phospholipids. This may lead to a considerable decrease in the microviscosity of membranes due to disturbance of hydrophobous interaction of fatty acids of lipids with proteins in the structure of membranes. PI is a key

mediator of various signal mechanisms triggering and regulating such cellular processes as differentiation, chemotaxis, adaptation to the environment, etc. (Radchenko et al., 2005). A considerable increase in the concentration of SPM may be attributed to defensive mechanisms directed to the decrease of oxidizability of membrane lipids due to a high saturation of its composition of fatty acids preventing the excessive activation of free-radical oxidation, especially under conditions of deficiency of antioxidants (Barak et al., 1998).

Generally, under conditions of anthropogenous load on water bodies, the phospholipid spectra of the liver of whitefish from comparable water areas demonstrate a lower stability in comparison with the gonads, as the liver is the principal organ of lipid metabolism and of detoxication.

The system of the Pasvik River receives effluents of copper–nickel production with a considerable presence of zinc and manganese (table). It was found that, in the liver and in gonads of whitefish from the Pasvik River, the content of total lipid did not differ from that in the fish from the "control" water body (Kunchast Bay), the level of cholesterol being higher (Figs. 1, 2, 4). This may result from blocking with metals (xenobiotics) of the activity of the monooxigenase enzyme system of the liver containing cytochrome P-450 catalyzing the reactions of oxidation of both cholesterol and of xenobiotics (Rocha et al., 2000). An increased value of the coefficient CS/PL in the liver and gonads of whitefish from the Pasvik River is noticeable (by 2.6 and 1.6 times, respectively), mainly due to accumulation of cholesterol (Fig. 3). Cholesterol is known as a principal stabilizer of biomembranes. Getting between nonsaturated fatty acids of phospholipids, it may inhibit the development of processes of peroxide oxidation (Baraboy et al., 1992; Kolomiytseva et al., 2003).

The content of TAG in the gonads of whitefish corresponded to the control values and in the liver it was considerably higher than that in the control, as noted above for the liver of whitefish from polluted water areas of Lake Imandra (Figs. 2, 4).

The decreased level of total phospholipids in the liver of whitefish is determined (in comparison with the control) due to a lower concentration of principal phospholipids fractions—PEA and PC. The part of PI, PS, and SPM was higher (Fig. 5), similarly to modifications of phospholipids spectra of the liver of whitefish from Syav Island. An increased level of acid phospholipids (possessing functional groups with a negative charge)—PI and PS in the liver of whitefish from polluted water areas may be related to the necessity of acid PL for reactivation of membrane ATPases ensuring the action of ion channels (Fig. 7) (Kreps, 1981; Boldyrev et al., 2006). In the gonads of whitefish, the quantity of total phospholipids does not differ from the control level. However, the spectra of particular phospholipids differed: the level of PS and SPM was higher by two times, and the level of LPC—lower by more than three times (Fig. 6). Thus, differences in lipid in gonads are revealed and are more significant differences—in the liver of whitefish from the system of the Pasvik River in comparison with the control variant.

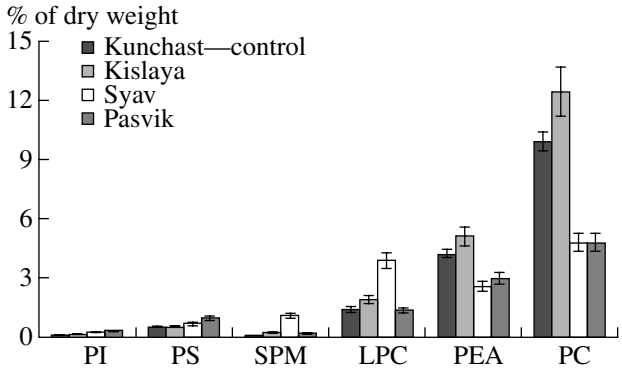

Fig. 5. Content of phospholipid in the liver of whitefish *Coregonus lavaretus* from water bodies with different anthropogenous load.

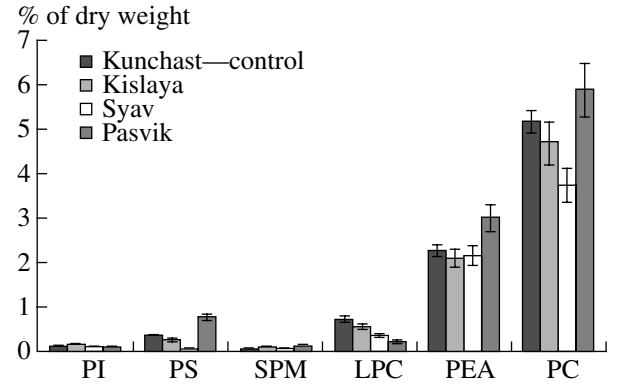

Fig. 6. Content of phospholipid in the gonads of whitefish *Coregonus lavaretus* from water bodies with different anthropogenous load.

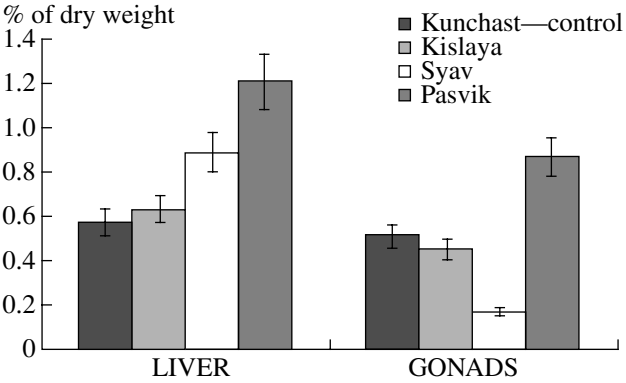

Fig. 7. Content of "acid" phospholipid (PI + PS) in tissues of whitefish *Coregonus lavaretus* from water bodies with different anthropogenous load.

CONCLUSIONS

(1) Accumulation of reserve high-energy triglycerols in the liver of whitefish from all polluted water areas of Lake Imandra and the system of the Pasvik River in comparison with the control (Kunchast Bay) is one of mechanisms of adaptation of fish to changed environmental conditions.

(2) The revealed modifications of phospholipids spectra (the increase in the concentration of highly saturated sphingomyelin, of highly nonsaturated phosphatidylcholine and phosphatidylethanoamine) on the liver of whitefish from polluted water bodies are a part of adaptogenous reactions (modification of permeability of biomembranes; of activity of membrane enzymes, including ATPases responsible for activity of ion channels) which provide a possibility of survival of fish under various combinations of unfavorable ecological factors.

(3) In the liver of whitefish, the shifts of lipid parameters are more significant than in the gonads, which is obviously related to the maximum protection of the system of reproduction against external influences.

(4) Determination of the lipid status of fish may be used for bioindication in ecologo-biochemical monitoring of the state of fish in water bodies exposed to anthropogenous impact.

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REFERENCES

1. A. Arduini, A. Peschechera, S. Dottoris, et al., "High Performance Liquid Chromatography of Long-Chain Acylcarnitine and Phospholipids in Fatty Acid Turnover Studies," J. Lipid Res. **37**, 684–689 (1996).
2. V. A. Baraboi, I. I. E. Brekhman, V. G. Golotin, and Yu. B. Kudryashov, *Lipid Peroxidation and Stress* (Nauka, St. Petersburg, 1992) [in Russian].
3. S. Barak, G. Fischer, and B. Rivney, "On the Mechanism of Sphingomyelin Interaction with Solubilized Membrane Proteins," Membr. Biochem. **7** (3), 153–173 (1998).
4. A. A. Boldyrev, E. I. Kyaivyaryainen, and V. A. Ilyukha, *Biomembranology* (KarNTs RAN, Petrozavodsk, 2006) [in Russian].
5. A. I. Bozhkov and V. L. Dlubovskaya, "Lipid Accumulation in Cytosol of Liver Cells After Local Hypothermia and Partial Hepatectomy," Biokhimiya **60** (11), 1816–1824 (1995).
6. F. M. Engelbrecht, F. Mari, and J. T. Anderson, "Cholesterol Determination in Serum. A Rapid Direction Method," S. A. Med. J. **48** (7), 250–356 (1974).
7. J. Folch, M. Lees, and StanleyG. H. Sloane, "A Simple Method for the Isolation and Purification of Total Lipids from Animal Tissues," J. Biol. Chem. **226** (5), 497–509 (1957).
8. N. A. Kashulin, *Fish of Small Lakes of Northern Fennoscandia Under Conditions of an Aerotechnogenic Pollution* (Kol'sk. NTs RAN, Apatity, 2004) [in Russian].
9. N. A. Kashulin, A. A. Lukin, and P. A. Amundsen, *Fish of Fresh Waters of the Subarctic as Bioindicators of Technogenic Pollution* (Kol'sk. NTs RAN, Apatity, 1999) [in Russian].
10. I. K. Kolomiitseva, N. I. Perepelkina, I. V. Patrushev, and V. I. Popov, "Role of Lipids in the Assembly of Endoplasmic Reticulum and Dictyosomes in Neuronal Cells from the Cerebral Cortex of Yakutian Ground Squirrel *Citellus undulates* During Hibernation," Biokhimiya **68** (7), 954–967 (2003) [Biochemistry (Moscow) **68** (7), 783–954 (2003)].
11. E. M. Kreps, *Lipids of Cellular Membranes* (Nauka, Leningrad, 1981) [in Russian].
12. Yu. M. Lopukhin, A. I. Archakov, Yu. A. Vladimirov, and E. M. Kogan, *Cholesterinosis* (Meditsina, Moscow, 1985) [in Russian].
13. A. A. Lukin, V. A. Dauval'ter, N. A. Kashulin, and N. E. Ratkin, "Effect of Aerotechnogenic Pollution on Catchment Areas of Subartctic Lakes and Fish," Ekologiya, No. 2, 109–115 (1998) [J. Russ. Ecol., No. 2, 92–97 (1998)].
14. A. A. Lukin, V. A. Dauval'ter, and A. P. Novoselov, *The Ecosystem of Pogora Under Modern Conditions* (Kol'sk. NTs RAN, Apatity, 2000) [in Russian].
15. M. M. Melkonyan, K. G. Karagezyan, and S. R. Ovakimyan, "Specific Features of Metabolism of Glyceride Phospholipids in Membranes of Erythrocytes in White Rats Under an Acoustic Stress," Vopr. Med. Khim. **35** (2), 68–71 (1989).
16. T. I. Moiseenko, *Theoretical Foundations of Normalization of Anthropogenic Loads on Subarctic Water Bodies* (Kol'sk. NTs RAN, Apatity, 1997) [in Russian].
17. T. I. Moiseenko, "Effects of Acidification on Aquatic Ecosystems," Ekologiya, No. 2, 120–126 (2005) [J. Russ. Ecol., No. 2, 93–102 (2005)].
18. V. V. Radchenko, M. I. Merkulova, T. M. Shuvaeva, et al., "Functional Expression and Properties of Sec14r-Like Protein with Molecular Mass 45 kD from Rat Olfactory Epithelium," Biokhimiya **70** (12), 1631–1638 (2005) [Biochemistry (Moscow) **70** (12), 1341–631 (2005)].
19. Yu. S. Reshetnikov, "Modern Problems of Study of Coregonids," Vopr. Ikhtiol. **35** (2), 156–174 (1995).
20. M. Rocha, R. Redro, M. A. Reis-Henriques, and J. Coimbra, "Polycyelic Aromatic Hydrocarbons Inhibit in Vitro Ovarian Steroidogenesis in the Flounder (*Platichthys flesus* L.)," Aquat. Toxicol. **48** (4), 549–559 (2000).
21. Yu. N. Sharova and A. A. Lukin, "The Reproduction System of Cisco *Coregonus lavaretus* in the Conditions of

Multifactor Pollution," Vopr. Ikhtiol. **40** (3), 425–428 (2000) [J. Ichthyol. **40** (4), 346–348 (2000)].

22. M. I. Shatunovskii, *Ecological Regularities of Metabolism in Marine Fish* (Nauka, Moscow, 1980) [in Russian].
23. V. S. Sidorov, E. I. Lizenko, O. M. Bolgova, and Z. A. Nefedova, "Lipids in Fish. 1. Methods of Analysis. Tissue Specificity of Lipids in Whitefish *Coregonus albula* L," in *Salmonidae of Karelia* (Kar. Fil. Akad. Nauk SSSR, Petrozavodsk, 1972), No. 1, pp. 152–163.
24. L. P. Smirnov and S. D. Kirilyuk, "Effect of Environmental Pollution on the Fractional Composition of Low-Molecular Peptides from Various Tissues of Whitefish," Izv. Ross. Akad. Nauk., Ser. Biol., No. 4, 617–622 (1994).
25. D. J. Spry and J. G. Wiener, "Metal Bioavailability and Toxicity to Fish in Low-Alkalinity Lakes: A Critical Review," Environ. Pollut., No. 71, 243–304 (1991).
26. T. Tashev, *Inherited and Acquired Enzymopathies* (Meditsina i Fizkul'tura, Sofia, 1980) [in Russian].
27. X.-Q. Zhou, R.-Y. Sun, and C.-J. Niu, "Dongrouxue Yanjiu," Zool. Res. **22** (2), 154–158 (2001).

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