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Indexes of Lipid Metabolism in Fish from the Zaporizke Reservoir

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Abstract. The total lipid contents and iodine value of fats had been determined in fish from two sections of the Zaporizke Reservoir (Ukraine) with different contamination levels. Research was conducted using the muscle and liver tissue samples from pike-perch (Sander lucioperca), european perch (Perca fluviatis), prussian carp (Carassius gibelio), roach (Rutilus rutilus) and rudd (Scardinius erythrophthalmus). Obtained data showed that at the contaminated zone, the total lipid contents were significantly ($p \le 0.05$) reduced in muscle tissue of pike-perch and european perch in comparison with the samples from "conventionally clean" lower section of the reservoir. Increased iodine value of fats in muscle tissue and liver tissue were detected in the predatory fish and both fish groups respectively. The research results could be used for estimation of the adaptation processes in freshwater fish as well as for indication of environmental contamination level in the natural and artificial reservoirs.

1. Introduction

It is well known that the aquatic organisms have many types of biochemical adaptation mechanisms to varying difficulty, which allow them to adapt successfully to adverse environmental conditions. One of those mechanisms is the alteration of lipid metabolism. Lipids have structural functions in cellular membranes. They are precursors for eicosanoids and act as carriers for lipidsoluble substances. Also, they are used for energy production in the cells. A number of authors had confirmed the individual parts of lipid metabolism, which formed strategies for metabolic response to xenobiotic compounds and toxic resistance of aquatic organisms, especially fish [1–5]. Lipids being one of the main components of biological membranes affect their permeability. They take part in the processes of nerve impulse transmission and intercellular contact creating. Lipids serve as second messengers in the cell signaling processes [5].

Fish lipids contain numerous unsaturated double bonds in the fatty acid structures, and iodine value is integral index of unsaturation degree of the fish fats. Lipid content and fatty acid composition differ in fish under the various environmental conditions, different temperatures and salinity levels [4]. Fish in general contain more ω 3 than ω 6 polyunsaturated fatty acids (PUFA), but the average ω 6/ ω 3 ratios are 0.37 and 0.16 for freshwater and marine fish, respectively [6]. Seasonal changes have been observed in the both total lipid contents and iodine values of fats in some fish species. For example, the iodine value, or degree of unsaturation of the herring fats was minimal in April and maximal in June [6].

It had been revealed that the common compensatory adaptation mechanism due to changes in PUFA concentrations in lipids has been established [7]. Reduced PUFA contents lead to disruption of membrane fluidity, lowering dive integrated in the lipid matrix proteins, changes in enzyme activity, and reduced intensity of ATP synthesis. Given that fish lipids contain high levels of PUFA, it follows that the tissues of fish are extremely sensitive to lipid peroxidation (LPO) processes. Some researchers consider the LPO products as unique biomarkers of water environment pollution and fish tissue disorders [7; 8].

In that connection, the aim of our study was to investigate the total lipid contents and their unsaturation degree in muscle and liver tissues of fish species with various ways of existence and nutrition under the conditions of the Zaporizke Reservoir zones with varying levels of anthropogenic pollution.

2. Materials and Methods

The species of the study were pike-perch (*Sander lucioperca*, Linnaeus, 1758), european perch (*Perca fluviatis*, Linnaeus, 1758), prussian carp (*Carassius gibelio*, Bloch, 1782), roach (*Rutilus rutilus*, Linnaeus, 1758) and rudd (*Scardinius erythrophthalmus*, Linnaeus, 1758) captured in autumn period.

Subjects were selected by standard methods of research control catches at the lower section of the Zaporizke Reservoir area near the Viyskove village (48°22'30.75"N; 35°20'80.05"E), which is characterized by environmental conditions as "conventionally clean", and Samara Bay (48°53'40.21"N; 35°18'73.20"E) as technogenically contaminated zone [9–11].

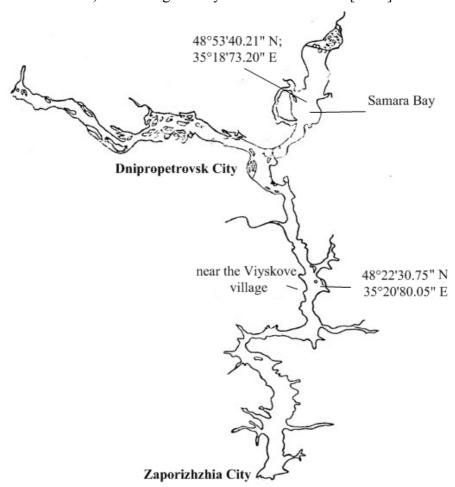


Figure 1. The Zaporizke Reservoir map and the sites of samples location [11].

Totally, 60 specimens of investigated fish were used, 120 samples of muscle and liver tissues were collected for biochemical assays.

The content of total lipids in biological samples was measured gravimetrically by the Folch method after extraction with 2:1 chloroform-methanol mixture during 12 hours [12]. One mass of tissue added to 20 parts chloroform-methanol mixture and left for extraction. Non lipid impurities were removed with 1 % KCl solution [12–14].

The iodine value is a measure of the unsaturation of fats and hence of their potential to become oxidized. A sample was treated with an excess of iodine monochloride solution in acetic acid. The amount of iodine absorbed was determined by back-titration with standard sodium thiosulphate solution [15]. Results are expressed in terms of the gram amounts of iodine absorbed per 100 g of sample (% iodine absorbed).

The resulting digital data were analyzed mathematically by standard methods of variation statistics, significant differences between the means were evaluated using Student t-test at a significance level of $p \leq 0.05$.

3. Result and Discussion

Fat is a source of energy and performs the structural functions in organisms [4, 5]. It is the building material and involved into the transport of various substances in the tissues. Lipids as compounds of cell membranes, support protein balance.

On the fat amounts in muscles, the fishes are divided into three groups, low-fat or lean (cod, 0.3–0.8 %), middle-fat (carp, 3–5 %), and high-fat (herring, salmon, sturgeon 13–15 %). The main fat depots in the body of the fish are subcutaneous fat, abdominal, muscles, liver, caviar (in sturgeon) [16; 17].

As a result of our research it had been obtained data on the lipid contents in biological tissues of fish from different habitats at the Zaporizke Reservoir.

Analyzing the results of biochemical assays of the samples from lower section of the reservoir, the higher rates of the total lipid levels were found in the muscles of predatory fish (pikeperch, european perch) compared to those contents in muscle of benthophage (prussian carp). Roach and rudd showed the same fat content level, which means 13.9 mcg/g on the average (Fig. 2).

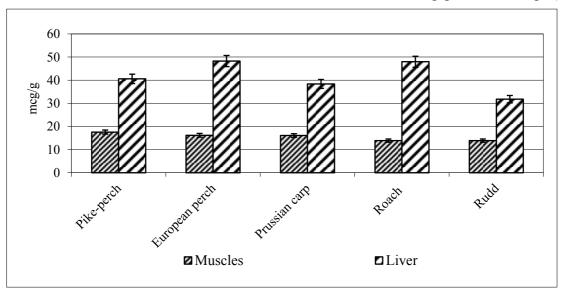


Figure 2. The contents of total lipids in the tissues of fish from the lower section of the Zaporizke Reservoir (mcg/g of dry weight, $M \pm m$, n = 6).

As a specific tissue for the storage of fats, liver samples showed the higher lipid levels in the both predatory and peaceful fish from the lower section of the reservoir. The most important lipid content was observed in the liver tissue of roach (48.0 mcg/g), the lowest lipid content was observed in rudd (30.3 mcg/g). Thus, the lipid contents apparently had been caused by individual characteristics of fish species.

It has been reported that in different species and ecological groups of fish, the lipid distribution in tissues and organs depend on environmental conditions, physical activities, and age etc. Placement of the main fat reserves in muscle tissue is characteristic of predatory species and probably due to their greater mobility as predatory fishes [15; 16].

The muscle tissue of predatory fish species from the Samara Bay showed significantly reduced contents of total lipids in pike-perch and european perch respectively on 16.0 % and 34.5 % comparing to "conventionally clean" zone of the reservoir (Fig. 3). The muscle samples of peaceful

fish revealed increased total lipid content by 10.0 % in prussian carp and by 18.0 % in roach. The increase in total lipid level of rudd muscle tissue was not significant.

In the liver of all investigated fish species, the lipid contents generally decreased. This reduction was most observed in the roach. The total lipid level indicates the activity of anabolic processes and utilization of lipids as an energy source in adaptive and metabolic rearrangements of structural components of cells [7; 8].

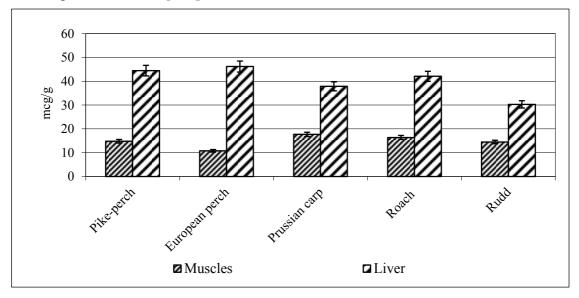


Figure 3. The contents of total lipids in the tissues of fish from the Samara Bay $(mcg/g \text{ of dry weight}, M \pm m, n = 6).$

Fat deficiency as well as its excessive accumulation is a negative factor satisfied to disorders in lipid metabolism in fish and/or adaptation to extreme conditions, exceptionally anthropogenic pollution of the reservoir zone observed. Samara Bay is characterized with a weak strength, extensive areas of shallow water, and by results of the many years research it has considered to be ecologically critical area [9; 10]. By the reason of emissions of coal industry and municipal wastewater into the Samara River near the cities of Novomoskovsk and Pavlograd, the hydrochemical regime of Samara Bay is characterized with high contents of mineral substances, inorganic toxicants, nitrates, ammonium nitrogen, and deficiency of dissolved oxygen in certain seasons, those affect negatively the physiological state of the fish.

Iodine value is an important indicator of the physical and chemical properties of the fats. The mean of iodine value characterizes quantitatively unsaturated fatty acid composition of lipids located in the body.

Polyunsaturated fatty acids are the most labile chemical acid groups in the fats of fish. Thus, the dynamics of their contents in fats can give a mode of the qualitative lipid changes in the fish tissues due to environmental features.

Our research resulted in determination of the iodine values of lipids in biological tissues of investigated fish species from the Zaporizke Reservoir. Obtained data showed that amounts of iodine value had not differed with statistical significance in predatory and peaceful fishes from the lower section of the reservoir (Fig. 4). Generally, iodine value depends on environmental conditions. This is because fishes living in cold waters have iodine values higher than that of fishes living in warm waters [4; 6].

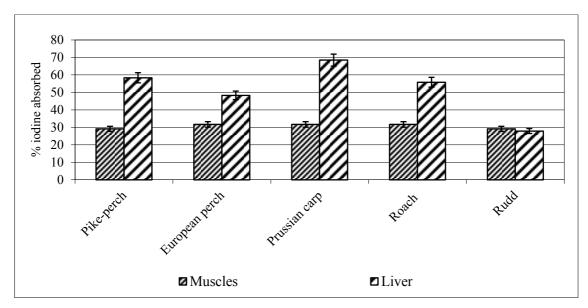


Figure 4. The iodine value of lipids in fish tissues from the lower section of the Zaporizke Reservoir (% iodine absorbed, $M \pm m$, n = 6).

In the liver tissue of fish from the lower section of the reservoir we observed minor variations of iodine values of predatory and peaceful fishes. Among peaceful species investigated, the maximal iodine value of liver lipids was observed in prussian carp, and the minimal value was in rudd.

At the Samara Bay, there were significant increases in the means of iodine value in the muscle tissue of predatory fish species of pike-perch and european perch, respectively by 65.0 % and 40.0 % (Fig. 5). In roach, the iodine value of muscle lipids was higher by 20.1 % than one at lower section of the reservoir. Furthermore, in the muscle tissue of predatory fish species the iodine values exceed significantly those data in peaceful fishes by 31.5 % on average.

Obtained data satisfied to the structural changes in lipids to upward with unsaturated fatty acids in their composition. Also, the more activity of fish species with increasing complexity of food chains conditioned the high-quality muscle tissue occupation with unsaturated fats. Comparing the means of iodine value and decrease in total lipid contents it could be concluded that lipids were spent at a faster rate due to the content of polyunsaturated fatty acids, which are more available for recycling.

In the liver lipids the iodine values were increased by 17.3 % in pike-perch and by 13.3 % in the both european perch and rudd. On our opinion, that could be explained as an adaptive response aimed to increasing of the lipid availability as meaningful energy substance. In the benthopage of prussian carp the iodine values of liver lipids were reduced by 30.3 % that indicated to prevailed amount of saturated fatty acids in the lipid composition under the conditions of chronic environmental intoxication.

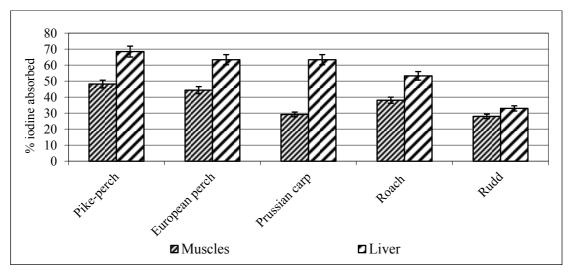


Figure 5. The iodine values of lipids in fish tissues from the Samara Bay (% iodine absorbed, $M \pm m$, n = 6).

Thus, on the obtained data it could be concluded that the levels of polyunsaturated fatty acids were the largest in predatory fish species (pike-perch, european perch), and the lowest level was in rudd.

4. Conclusion

According to greater mobility of predators, the content of total lipids in their muscle was higher in comparison with the benthophages. At the contaminated zone of Samara Bay the total lipid content was significantly reduced in muscle tissue of predatory fish than at the "conventionally clean" area of the Zaporizke Reservoir. The concentration of lipids in liver tissue of predators was higher than in peaceful fish that was obviously due to individual and species characteristics.

The iodine value of muscle lipids did not differ in both groups of investigated fish species at the "conventionally clean" area of the reservoir. But at the contaminated zone, a significantly increased iodine value was observed in muscle tissue of predatory fish. Predators being the final link in the food chain had the much more unsaturated lipids in their tissues than benthophages and herbivorous fish species. However, the iodine value of liver lipids was evenly increased in both groups of predatory and peaceful fishes under the environmental pollution compared to "conventionally clean" area.

The obtained research results could be used for estimation of the adaptation processes in freshwater fish as well as for indication of environmental contamination level in the natural and artificial reservoirs.

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