**Fatty acid composition and lipid quality indices of bream**

*Abramis brama* (Linnaeus, 1758) *of Lake Kotokel* (Western Transbaikalia)

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**Abstract.** This paper describes the study of fatty acid (FA) composition and lipid quality indices of bream *Abramis brama* (Linnaeus, 1758) from Lake Kotokel (Western Transbaikalia). In the muscle tissues of bream sampled in 2009 and 2019, high levels of polyunsaturated fatty acids (PUFA), including essential docosahexaenoic (DHA), eicosapentaenoic (EPA), and arachidonic acids were measured. Indicators of nutritional quality based on the fatty acid composition showed that the values of the hypocholesterolemic/hypercholesterolemic (HH) ratio indices were sufficiently high. The atherogenicity (AI) and thrombogenicity (TI) indices, which are indicators for the nutritional value, were less than 1 in the studied fish. In terms of flesh-lipid quality (FLQ), bream had the highest proportion of total EPA + DHA. According to the obtained data for the composition of fatty acids in the muscle tissue of the studied fish from Lake Kotokel, the anthropogenic load on Lake Kotokel has not yet had a statistically significant effect on the fish muscle quality.

**1. Introduction**

Lake Kotokel is located within the “Baikal Harbor”, a special economic zone of tourist and recreational type, and two kilometres from the eastern shore of Lake Baikal, between the delta of the Selenga river and the Barguzin Bay. The lake is a popular recreation area among residents of Republic of Buryatia. Lake Kotokel is a fishery reservoir where inhabited by roach, pike, bream, dace and ide [1]. The shallow depths of the lake make it warm in summer, reaching up to 25°C. In the summer, the lake has always attracted a huge number of vacationers both from Buryatia and from other regions of the country.

In July 2008, a situation developed in the recreational area of Lake Kotokel that many experts regarded as an ecological disaster. As a result, a series of poisoning occurred among residents of coastal villages, one of whom died. Decree No. 4 (10 June 2009) of the Head State Sanitary Doctor of Buryatia imposed a ban on usage of Lake Kotokel for recreational, drinking and household purposes. The reason for the ban was an outbreak of a disease caused by enterovirus infection, Gaff disease, associated with swimming in the lake and eating contaminated fish caught in Lake Kotokel.

One hypothesis about the cause of Gaff disease, related to arachidonic acid, was put forward in 1984 by scientists of the Novosibirsk Institute of Bioorganic Chemistry of Siberian Branch of the USSR Academy of Sciences [2]. Among the various available bioindicators of surface water contamination, fish are one the most suitable objects for assessing the quality of aquatic systems. Assessments of water quality and the health of hydrobionts, including fish, which are regarded as human food items, point to the need for ongoing environmental monitoring. The toxic effects can be assessed at organ, tissue, cellular,
subcellular, genetic, and other levels. As a result, protein, lipid, and fat metabolism are significantly disrupted [3]. The most important physiological and biochemical indicators of the state of organisms and populations under different habitat conditions are lipid indicators. Lipids are the main source of energy for organisms, and functioning of any ecosystem is largely associated with their biosynthesis and transport in food chains [4-6]. The main type of fat that play a role in the dietary patterns of modern people is \( n-6 \) PUFA from terrestrial mammals, but the consumption of \( n-3 \) PUFAs is inadequate, even in highly developed countries [7-9]. Consumption of fish is known to provide many benefits for human health due to its high content of essential PUFA of the \( n-3 \) family, namely EPA and DHA (22:6n3). Regular consumption of essential fatty acids prevents cardiovascular diseases and neural disorders [10-13]. Thus, this determines the importance of biochemical studies of fish in the Baikal region, not only in terms of studying ecology and taxonomy but also for clarifying their nutritional value.

Thus, the aim of the study was to determine the fatty acid composition and lipid quality indices of the muscle tissue of bream *Abramis brama* (Linnaeus, 1758) of Lake Kotokel (Western Transbaikalia).

2. Materials and Methods

2.1. Field sampling

Sampling of bream from the water of Lake Kotokel (Figure 1) was conducted in July 2009 \( (n=12) \) and in July 2019 \( (n=12) \) using a trap net at a depth of about 2–8 m. Adult fish whose length (including caudal fin) was between 21 and 25 cm for bream were analysed. The collected fish were stored at \(-18^\circ\text{C}\) for less than seven days before laboratory analyses.

![Figure 1](image1.jpg)

**Figure 1.** The studied area was located in Western Transbaikalia, Russia; a – Lake Baikal; b – Lake Kotokel (geographical coordinates: 52°45'54.7''N; 108°05'13''E).

2.2. Collection site

Lake Kotokel is located on the eastern coast of Lake Baikal from 2 km from it, between the mouths of the Turka and Kika rivers. It reaches 15 km in length and about 5 km in width. The lake surface area is 68.9 km\(^2\) and the catchment area is 183 km\(^2\). The height of the lake above the level of Lake Baikal is 5 m, and the average depth is 3.5 m (with a maximum depth of 14 m). The lake belongs to reservoirs with a very small specific catchment area. Several streams and more than ten springs flow into the lake and one river (the Istok) flows out, which is actually a channel connecting Kotokel with the Kotochik river, left tributary of the Turka river. The lake Kotokel is connected to Baikal through the Istok, Kotochik and Turka rivers. It is a low-flowing accumulative reservoir with a slowed down water exchange. Average annual and intra-annual fluctuations in the water level in the lake are insignificant and do not exceed 1 m.
2.3. Sample derivatisation
The FA content was determined in subsamples of the tissues weighing approximately 0.5-1.0 g. The subsample was cut from the white dorsal muscle, from the side about 2/5 of the distance from snout to tail, carefully avoiding red muscle, skin, and bone. The weighed and homogenized subsamples of fish muscle tissues were transferred to 15 mL thick-walled glass tubes. After the addition of 1 mL of anhydrous methanol containing 2M HCl in the nitrogen atmosphere, the tubes were securely closed with Teflon-lined screw caps and placed in an oven for 2 h at 90°C for complete methanolysis [14, 15]. After cooling to room temperature, the tubes were opened and the methanol evaporated down to about 0.5 mL under a stream of nitrogen gas, and 0.5 mL distilled water was then added to reduce the solubility of the formed FA methyl esters (FAME), which were extracted with 2 × 1 mL n-hexane. All chemicals were of high purity.

2.4. Fatty acid analysis
FAs were analysed by gas chromatography–mass spectrometry (GC-MS) as described in [21]. Methyl esters of FAs were examined by the gas chromatography-mass-spectrometry method using Agilent 6890B gas chromatograph with a 5973N quadrupole mass spectrometer as the detector and an HP-5MS capillary column (30 m x 0.25 mm x 0.2 μm; Hewlett-Packard, Palo Alto, CA, USA). Helium (99.9999% purity) was used as the carrier gas at a flow rate of 1.5 mL/min. The oven temperature was programmed as follows: it was kept at a constant temperature of 125°C for 0.5 min, from 125 to 320°C (at the rate of 7°C/min), and kept constant at 320°C for 0.5 min. The injector and detector temperatures were set to 280 and 250°C, respectively. The split ratio was adjusted to 40:1.

For quantitative analysis, the characteristic ions and retention times of all analytes were determined in full-scan (m/z 50 to 550) using electron ionization (electron energy – 70 eV). The MS data were acquired in scanning mode at a speed of 2.5 s per scan.

The percent composition of the lipid fraction derivatives was calculated from the GC peak areas relative to the total peak area based on the GC-MS analyses of the lipid fraction. Qualitative analysis was based on comparing the retention times and total mass spectra of the corresponding pure compounds using NIST14.L and standard mixtures of Bacterial Acid Methyl Esters (CP Mix, Supelco, Bellefonte, PA, USA) and Fatty Acid Methyl Esters (Supelco, 37 compounds, FAME Mix, 10 mg/mL in CH2Cl2). The relative amount of each FA in a sample was expressed as a percent of the sum of all fatty acids in the sample.

2.5. The lipid Quality Indices
The fatty acid composition was used to determine several nutritional parameters of lipids in fish muscles. The lipid quality indexes were calculated using the following equations.

2.5.1. Index of Atherogenicity. The index of atherogenicity (AI) is the correlation between the total main saturated fatty acids and the main classes of unsaturated fatty acids. The former is considered to be proatherogenic as it provides the adhesion of lipids to cells of the immunological and circulatory system. The latter are antiatherogenic as they inhibit the aggregation of plaques and diminish the levels of esterified fatty acid, cholesterol, and phospholipids, thereby preventing the appearance of micro and macro coronary diseases [16-20].

\[
AI = \frac{C_{12:0} + (4 \times C_{14:0}) + C_{16:0}}{(n-3)PUFA + (n-6)PUFA + MUFA}
\]

where PUFA – polyunsaturated fatty acids; MUFA – monounsaturated fatty acids; C12:0 – lauric acid, C14:0 – myristic acid, C16:0 – palmitic acid.

2.5.2. Index of Thrombogenicity. Thrombogenicity index (TI) is defined as the relationship between the pro-thrombogenetic (saturated) and the anti-thrombogenetic fatty acids (MUFA, n-6 PUFA, and n-3PUFA) [16, 19].

\[
TI = \frac{C_{14:0} + C_{16:0} + C_{18:0}}{0.5 \times C_{18:1} + 0.5 \times \text{sum of other MUFA} + 0.5 \times (n-6)PUFA + 3 \times (n-3)PUFA + (n-3)PUFA + (n-3)PUFA + 1}
\]
2.5.3. Flesh-Lipid Quality. The flesh-lipid quality (FLQ) is the percentage correlation between the main $n$-3 PUFA (EPA + DHA) and the total lipids. Thus, the higher the value of this index, the better the quality of the dietary lipid source [19]:

$$FLQ = \frac{100 \times EPA + DHA}{\% \text{ of total fatty acids}}$$

(3)

where hypocholesterolemic fatty acids (OFA): \(OFA = C_{12:0} + C_{14:0} + C_{16:0}\); hypercholesterolemic fatty acids (DFA): \(DFA = C_{18:0} + \text{UFA}\); EPA – eicosapentaenoic acid (C20:5); DHA – docosahexaenoic (C22:6); UFA – unsaturated fatty acids (MUFA + PUFA); C18:0 – stearic acid.

2.6. Statistical analysis

The results are presented as arithmetic mean ± standard deviation (SD). The calculation of mean, SD and Fisher’s LSD test was carried out by the Microsoft Excel software. Differences were found to be significant at \(p \leq 0.05\).

3. Results and Discussion

Because the arachidonic hypothesis had been proposed by scientists of the Novosibirsk Institute of Bioorganic Chemistry of the Siberian Branch of the USSR Academy of Sciences [2], we investigated the fatty acid composition of the bream from Lake Kotokel, sampled in 2009 and 2019. A total of 47 fatty acids were found in the muscle tissue of the studied bream sampled in 2008 and in 2019. Acids in relative proportions higher than 0.1% are presented in Table 1. Palmitic 16:0 and stearic 18:0 acids were dominant among the saturated fatty acids (SFA). Such saturated fatty acids with odd-numbered carbon atoms such as 15:0, iso15:0, isoo17:0, and aiso17:0 were found in fewer amounts. Oleic 18:1n9 acid was the dominant MUFA in muscle tissue of bream sampled in 2008 and in 2019. The content of oleic acid was found to be negatively correlated with the content of linoleic and \(\alpha\)-linolenic acids. DHA 22:6n3, arachidonic 20:4n6 and EPA 20:5n3 acids were dominant among the PUFA.

The processes of tissue metabolism can serve as the fastest and most effective assessment of toxicant effects. Intensification of free radical oxidation of lipids and dysfunctions of antioxidant systems place an important role in the reaction of a living organism to the toxic effect of toxicants. In many studies of lipid peroxidation affected by xenobiotics, as well as thermal pollution of water bodies, it was confirmed that the main reaction of aquatic organisms has been shown in the enhancement of free-radical lipid oxidation [22, 23]. The values \(n\)-3 PUFA in muscle tissue of bream from 2019 (20.8%) were higher than in muscle tissue of bream from 2009 (16.1%) (\(p \leq 0.05\)). It was revealed that among other polyunsaturated FAs, the content of linolenic and linoleic acids was not significantly differ (\(p>0.05\)). Polyunsaturated \(n\)-3 fatty acids are essential for fish. It has been established as a result of numerous experiments, including those on freshwater species [24]. An increased content of \(n\)-6 and \(n\)-3 polyunsaturated fatty acids identified in this research in the muscles of the studied fish determines their high nutritional value. The ratio of fatty acids \(n\)-3/\(n\)-6 in the muscle tissue of the studied fish was 1.4 and 1.9. The \(n\)-3/\(n\)-6 ratios are typical for freshwater fish, where the ratio lies in the interval 0.5–3.8, compared to the interval 4.7–14.4 for marine fish [25]. From a nutritional point of view, the ratio is close to the recommended ideal value of between 0.5 and 1 [26]. Therefore, freshwater fish are currently recognized as a valuable dietary component of human nutrition, as valuable as sea fish [27].

It is well known that an important place in the reaction of a living organism to the toxic effect of toxicants is hold by the intensification of the processes of free radical lipid peroxidation and dysfunction of antioxidant systems. The regulation of lipid peroxidation is carried out by the coordination of antioxidant defence systems, which ensure the removal of hazardous compounds with a high destructive potential. Cellular metabolism, as well as the reactions of lipid metabolism to toxic effects of various etiologies in fish has not studied well enough.
Therefore, indicators of nutritional quality based on the FA composition were determined. The HH index provides insight into the effect of FA on blood cholesterol level. A higher value of HH index is preferable. The content of hypocholesterolemic and hypercholesterolemic fatty acids in the muscles of bream sample was investigated as their effect on the frequency of pathogenic events such as atheroma and/or thrombus formation differs from the formation of single FAs. The atherogenic and thrombogenicity indexes were not significantly different, and their values were 0.47, 0.44, 0.37, and 0.38, respectively. Lipids with AI <1 and TI <1 are suggested to be beneficial for human health. Tonial et al. (2014) considered that MUFA and PUFA are more beneficial for health as they prevent coronary heart disease [28]. The muscles of bream sampled in 2019 showed significantly higher FLQ (14.4) (p ≤ 0.05) than those of bream sampled in 2009 (10.0).

Table 1. Fatty acid composition of muscle tissue of bream Abramis brama L. from Lake Kotokel, mean±SD (number of samples: n=12 in 2009, and n=12 in 2019).

| Fatty acid | Date of sampling | Fatty acid | Date of sampling |
|------------|-----------------|------------|-----------------|
|            | 2009            | 2019       | 2009            | 2019       |
| 12:0       | 0.10±0.02a      | 0.11±0.02a | 16:2n6          | 0.22±0.09a | 0.25±0.03a |
| 14:0       | 2.74±1.30a      | 1.05±0.25a | 16:3n4          | 0.47±0.64a | 0.62±0.08a |
| i15:0      | 0.62±0.18a      | 0.10±0.02a | 18:4n3          | 0.50±0.20a | 0.07±0.01a |
| a15:0      | 0.49±0.17a      | 0.05±0.01a | 18:2n6          | 4.35±1.02a | 2.91±1.84a |
| 15:0       | 0.49±0.13a      | 0.46±0.06a | 18:3n6          | 0.19±0.06a | 0.26±0.11a |
| 16:0       | 20.58±5.01a     | 23.57±5.26a| 18:3n3          | 3.52±1.38a | 3.05±1.09a |
| i17:0      | 1.00±0.12a      | 0.72±0.11a | 20:4n6          | 3.62±1.87a | 5.72±1.99b |
| a17:0      | 0.92±0.25a      | 0.55±0.08a | 20:5n3          | 4.81±1.54a | 7.37±1.39b |
| 17:0br     | 0.16±0.12a      | 0.08±0.01a | 20:3n3          | 0.34±0.11a | 0.27±0.10a |
| 17:0       | 0.65±0.15a      | 0.77±0.12a | 20:4n3          | 0.47±0.15a | 0.99±0.12a |
| 18:0       | 4.64±1.58a      | 7.64±1.69a | 20:2n6          | 0.44±0.07a | 0.48±0.09a |
| 20:0       | 0.11±0.04a      | 0.07±0.01a | 21:5n3          | 0.28±0.15a | 0.41±0.05a |
| 14:1n5     | 0.23±0.12a      | 0.05±0.01a | 22:5n6          | 1.24±2.87a | 1.0±0.13a  |
| 16:1n9     | 0.76±0.28a      | 0.31±0.10a | 22:6n3          | 5.19±2.08a | 7.04±1.14b |
| 16:1n7     | 9.34±2.69a      | 6.46±1.34a | 22:4n6          | 1.23±2.65b | 0.45±0.05a |
| 16:1n5     | 0.35±0.27a      | 0.45±0.09a | 22:5n3          | 0.94±0.36a | 1.55±0.20a |
| 17:1n9     | 1.21±0.96a      | 0.55±0.10a | SFA             | 32.4±5.9a  | 35.4±5.9a  |
| 18:1n9     | 20.64±6.77b     | 17.25±3.89a| UFA             | 67.6±6.6a  | 64.6±7.1a  |
| 18:1n7     | 5.69±1.02a      | 6.55±1.15a | MUFA            | 39.7±8.8b  | 32.1±5.4a  |
| 18:1n5     | 0.20±0.05a      | 0.04±0.01a | PUFA            | 27.9±7.6a  | 32.5±5.0b  |
| 20:1n11    | 0.19±0.07a      | 0.09±0.01a | n-3PUFA         | 16.1±1.9a  | 20.8±2.3b  |
| 20:1n9     | 0.92±0.35a      | 0.45±0.05a | n-6PUFA         | 11.3±1.8a  | 11.1±1.5a  |
| n-3/n-6    | 1.4±0.2a        | 1.9±0.3a   |                 |            |            |

Note: Calculation of mean, standard deviation and Fisher’s LSD-test was performed using Microsoft Excel software. a, b – significant differences between fish samples; identical letter (in rows) indicates no statistically significant difference (p > 0.05).
Table 2. Values for lipid quality indices of muscle tissue for the muscles of bream *Abramis brama* from Lake Kotokel, mean±SD.

| Date of sampling | 2009          | 2019          |
|------------------|---------------|---------------|
| Number of samples| n=12          | n=12          |
| AI               | 0.47±0.07<sup>a</sup> | 0.44±0.06<sup>a</sup> |
| TI               | 0.37±0.05<sup>a</sup> | 0.38±0.04<sup>a</sup> |
| FLQ              | 10.0±1.5<sup>a</sup> | 14.4±1.9<sup>b</sup> |
| OFA              | 23.3±2.1<sup>a</sup> | 25.0±2.8<sup>a</sup> |
| DFA              | 72.2±4.9<sup>a</sup> | 72.2±5.7<sup>a</sup> |
| HII              | 3.1±0.4<sup>a</sup> | 2.9±0.6<sup>a</sup> |

**Note:** Calculation of mean, standard deviation and Fisher’s LSD-test was performed using Microsoft Excel software. <sup>a</sup>, <sup>b</sup> – significant differences between fish samples; identical letter (in rows) indicates no statistically significant difference (p > 0.05).

4. Conclusion
The fatty acid profiles of bream sampled in 2019 are similar to the ones sampled in 2009, which probably indicates that fish species of Lake Kotokel are not influenced by significant anthropogenic impact. The arachidonic hypothesis, despite its biochemical validity (and above all, the fact that arachidonic acid accumulates in hydrophobic tissues of the body; which according to some data from medical histories corresponds to the accumulation of an unknown toxin in adipose tissue, in fish brain, etc.) still has no perfect toxicological confirmation. Most importantly, the hypothesis has no explanation for the appearance of arachidonic acid (a fairly common substance in plant and some animal tissues) in elevated amounts in certain representatives of natural biogeocenoses or in humans and domestic animals.

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