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Lipid Profile of the Freshwater Pearl Mussel *Margaritifera margaritifera* Inhabiting Different Biotopes of the Lake-River System of the Kem River, White Sea Basin

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Abstract: The freshwater pearl mussel *Margaritifera margaritifera* is considered to be the most rapidly declining species of freshwater bivalve, whereas its colonies in rivers of the White Sea Basin remain the most numerous in the world. The lipid profiles of mantle, muscle (foot), and digestive gland tissues of the freshwater pearl mussel from the Kem, Ukhta, and Vozhma Rivers in autumn (end of September, early October) were studied using high performance thin-layer chromatography. The highest total lipid [TL] content was found in the digestive gland. Cholesterol esters, non-esterified fatty acids, phospholipids, and cholesterol were the dominant lipids in all studied tissues. The reduced triacylglycerol content in the mussels was associated with its utilization during the spawning period. The colony of the freshwater pearl mussel inhabiting the Vozhma River was distinguished by higher TL content in the mantle and digestive gland. Data on the size-age characteristics of mollusks from the Kem, Ukhta, and Vozhma Rivers and the relationship between the structural and storage lipid content and size-age parameters are discussed. The results are important for different conservation strategies of endangered species, such as the freshwater pearl mussel, especially in ecological monitoring based on evaluation of the physiological and biochemical state of mollusks and rare natural colonies.

Keywords: *Margaritifera*; freshwater pearl mussel; lipids; biochemical adaptations; nature protection and conservation

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1. Introduction

The freshwater pearl mussel *Margaritifera margaritifera* (Linnaeus, 1758) is a bivalve in the family Margaritiferidae of the order Unionoida. *M. margaritifera* has a number of unique features, including the maximum lifespan among freshwater invertebrates (up to 200 years) [1–3] together with high reproductive potential even in polluted waters and in extreme old age [4]. The freshwater pearl mussel lives only in the rivers and streams of the northern hemisphere with low levels of lime and nutrients [4], and therefore can be considered an indicator species of oligotrophic waters [5].

The freshwater pearl mussel was widespread in the rivers of North America and Europe until the beginning of the 20th century. It is now considered an endangered species [6,7]. Currently, the number of freshwater mollusks is decreasing worldwide [8,9]. *Margaritifera margaritifera*, along with *Margaritifera auricularia*, are considered to be the most rapidly declining species [10]. Populations of the freshwater pearl mussel are found along the northeastern coast of North America, Western and Northern Europe, and Northern European Russia [9,11–18], while they have almost completely disappeared in Belarus, Denmark, Lithuania, and Poland [19]. The main reasons for the formation of island populations of the freshwater pearl mussel [2] and species extinction are considered to be excessive commercial fishing until the beginning of the 20th century, violations of the

hydrological regime due to a construction of dams for hydroelectric power stations on rivers, and, as a result, the siltation of watercourses and pollution [4,8,20–24]. Moreover, the freshwater pearl mussel has a complex life cycle with an obligate parasitic stage on salmonids [4,22,23,25–33]. The disappearance of the host fish species in rivers is one of the factors endangering the freshwater pearl mussel. The freshwater pearl mussel successfully metamorphoses only on the gills of Atlantic salmon (*Salmo salar*), sea trout (*Salmo trutta f. trutta*), and brown trout (*S. trutta f. fario*) in its European distribution [12,20,31,32,34–38].

The freshwater pearl mussel colonies in the rivers of the White Sea Basin are the most numerous in the world [2,21,39–41] due to the large area of spawning–growing grounds for salmonids [40]. The Keret River is considered to have the one of the largest freshwater pearl mussel colonies, estimated at approximately 2 million individuals [39]. Most *M. margaritifera* in Northern European Russia remain poorly studied, including colonies in the Kem River Basin. The Kem River, the largest lake-river system in the Republic of Karelia, includes more than 11 thousand lakes and their numerous tributaries. The construction of a dam on the Kem River led to declining numbers of the freshwater pearl mussel; however, some mollusks survived and the population started to recover. At the same time, there are biotopes in the Kem River Basin with the freshwater pearl mussel colonies in which the ages of some individuals may reach 100 years following favorable living conditions. The study of the freshwater pearl mussel in the watercourses of the Kem River Basin makes it possible to perform a comparative study of the freshwater pearl mussel colonies of uneven ages in biotopes with different anthropogenic loads.

Information about the distribution and functioning of the freshwater pearl mussel under various environmental conditions is actively used for the conservation or restoration of its natural populations; however, this information is insufficient for the determination of the negative impact of environmental factors on the metabolic processes of the organism at the initial stages. Lipids are considered to be biochemical indicators of the physiological state of animals [42]. These biomolecules are characterized as biological effectors, regulators, and mediators of many processes in living organisms, in addition to their main structural and energetic roles [43–45]. Many studies have explored the lipid content of tissues of freshwater [46–48] and marine bivalves [49–52]. However, the lipid content of the freshwater pearl mussel has not yet been studied, even though the genome of this species has been deciphered [53].

This study aimed to analyze the total lipid content and levels of individual classes of lipids in certain functional tissues—the mantle, muscle (foot), and digestive gland—of *Margaritifera margaritifera* from uneven-aged colonies inhabiting different biotopes of the Kem River Basin, including the Kem River estuary and the Ukhta and Vozhma Rivers.

The contribution of the tissue-specificity of the lipid content of the freshwater pearl mussel to the stage of the reproductive cycle was hypothesized. We assume that the lipid status may reflect the influence of environmental factors (including anthropogenic) at the initial biochemical stage, prior to changes at the physiological level of the organism. Direct correlations between the lipid content and the size-age parameters of the freshwater pearl mussel were also hypothesized.

The obtained results facilitate the discussion of the role of lipids in the adaptation of freshwater mollusks to environmental factors. The results are useful for the artificial breeding of endangered species of mollusks to restore their natural populations, particularly for the selection of potent compositions (based on the combinations of lipids) of the artificial environment for growing glochidia [54,55]. The obtained data are important for different conservation strategies of endangered species, such as *M. margaritifera*, especially in ecological monitoring activities based on evaluation of the physiological and biochemical state of mollusks and rare natural colonies.

2. Materials and Methods

2.1. Sampling

The freshwater pearl mussel specimens were collected in different rivers of the Kem lake-river system located in the Republic of Karelia (Northern European Russia) during the post-spawning period (27 September and 3 October 2019). The studied biotopes—the Kem River (estuary), Ukhta River, Vozhma River, and Tetri River (in order of mention by geographic location from south to north) are located within the Kem River Basin in the White Sea Basin (Figure 1). The water temperature during the sampling period ranged from 3 to 5 °C (Table 1). In all rivers, the host for the freshwater pearl mussel larvae (glochidia) was the Atlantic salmon *Salmo salar* L. Data on the sampling locations and their individual characteristics are presented in the Table 1. The map of the sampling locations is shown in Figure 1. A number of individuals were caught in accordance with permission number 116 of 07/04/2019 of the Federal Service for Supervision of Consumer Rights Protection and Human Welfare of the Russian Federation.

Table 1. The characteristics of sampling of *Margaritifera margaritifera* and the studied biotopes of the lake-river system of the Kem River, White Sea Basin.

River	Sampling Date	Water Temperature	Number of Samples (*n*)	Water System	Features
Kem (stream of the estuary zone)	27 September 19	5 °C	15	White Sea (mouth)	A young colony of the freshwater pearl mussel, recovered after construction of the Kem hydroelectric power station cascade. Oil products and heavy metals as pollutants were determined in the estuary of the Kem River [56]. Close proximity to the city of Kem.
Ukhta	27 September 19	5 °C	15	Middle Kuito (mouth) Lake → Luusalmi strait → Lower Kuito Lake → Kem River → White Sea	A young colony of the freshwater pearl mussel, recovered after construction of the dam 47 years ago. Close proximity to the city of Kalevala.
Vozhma	3 October 19	3 °C	15	Pistarvi Lake (mouth) → Pista River → Uper Kuito Lake → Elman River → Middle Kuito Lake → Luusalmi strait → Lower Kuito Lake → Kem' River → White Sea	Individuals over 100 years old are found in the colony.
Tetri	3 October 19	3 °C	2	Vozhma River (mouth) → Pistarvi Lake → Pista River → Upper Kuito Lake → Elmane River → Middle Kuito Lake → Luusalmi → Lower Kuito Lake → Kem River → White Sea	A young colony of the freshwater pearl mussel.

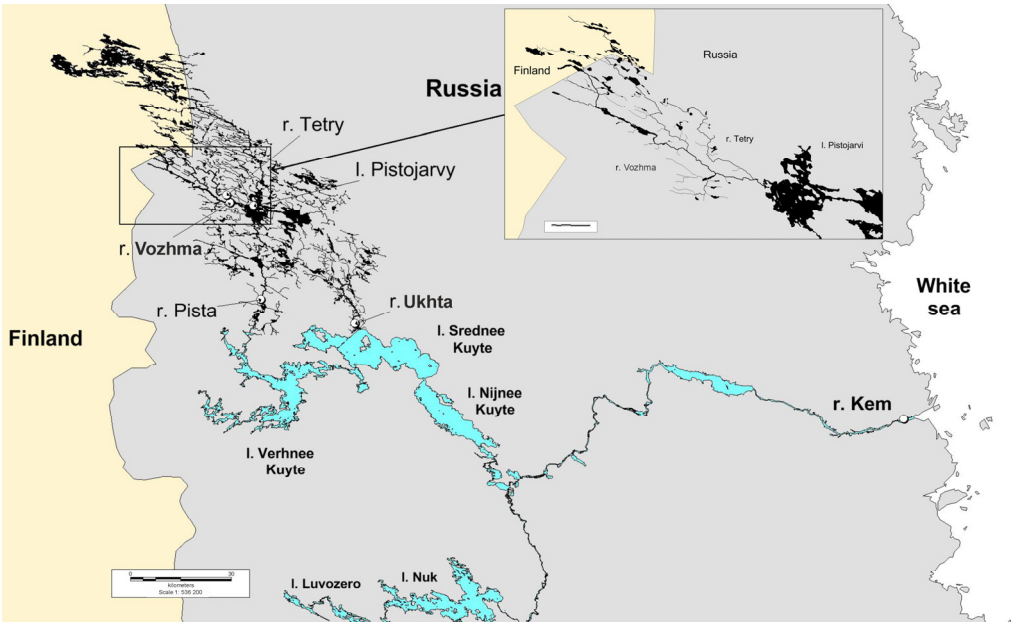

Figure 1. Map of the sampling locations of *Margaritifera margaritifera* on the Kem, Ukhta, Vozhma and Tetri Rivers. Notes: r.—river; l.—lake.

The mantle, muscle (the foot), and digestive gland tissues of the FPM were collected for biochemical analysis (15 samples, of each tissue (1 g weight) of mollusks from each studied colony) from the Kem, Ukhta, and Vozhma Rivers. It is worth noting that only two individuals were collected from the Tetri River. The samples from the Tetri River and the obtained data were not used in the statistical analysis but were mentioned in the text where appropriate.

2.2. Lipid Analysis

Total lipids [TL] were extracted with chloroform/methanol (2:1 by volume) following the method described by Folch et al. (1957) [57] using a Hei-VAP Advantage ML/G3 rotary evaporator (Heidolph Instruments, Schwabach, Germany). The % dry weight (dw) of TL and the individual lipid classes of structural (phospholipids [PL], cholesterol [CHOL]) and storage lipids (triacylglycerols [TAG], cholesterol esters [ECHOL]), as well as intermediate products of their metabolism (monoacylglycerols [MAG], diacylglycerols [DAG], and non-esterified fatty acids [NEFA]) were studied by high-performance thin-layer chromatography (HPTLC). Individual lipid classes were detected using HPTLC Silicagel 60 F254 Premium Purity glass plates (Merck, Darmstadt, Germany). CAMAG HPTLC equipment (CAMAG, Muttenz, Switzerland) was used, including a Linomat 5 semi-automatic applicator for applying samples to the plate, an automatic chamber ADC 2 for separating TL into individual classes, a derivatizer for automatic plate spraying, a TLC Plate Heater 3 for developing the lipid class spots, and a Camag TLC Scanner spectrodensitometer for spot scanning, calculation, and measurement procedures. The lipid classes were identified according to the reference standards of the studied components (Sigma-Aldrich, USA; Avanti Polar Lipids, Inc., USA). Unidentified lipid substances (unknown 1, Rf = 0.47 and unknown 2, Rf = 0.59) were revealed during the analysis. A detailed description of the analytical procedures was presented in our previous studies [58–60].

2.3. Determination of Size and Age Parameters of the Freshwater Pearl Mussel

Determination of the size and age of the freshwater pearl mussel was carried out for individuals collected from colonies in the Kem, Ukhta, and Vozhma Rivers. From each colony, 15 specimens of mussels were manually collected and analyzed. The upper conchiolin layer was removed by boiling the shells in a 1 M KOH solution for 10 min,

and the annual rings on the middle prismatic layer became clearly visible. Shell images were obtained by scanning them with an HPScan Jet5400c scanner (HP, Beijing, China). The length of each intact annual ring was measured with an accuracy of 0.1 mm using Excel. The data were approximated by the Bertalanffy equation and its recurrent form using previously described approaches [61–63]. The rationale for this method for age determination was that almost all shells had a corroded apical zone and some annual rings were undetectable. Obtained data for individual speciments are presented in Table S1 (Supplementary Materials). The size-age characteristics of mollusks from the Tetri River were not considered in the comparative analysis with other rivers due to the small sample size (2 specimens).

2.4. Statistical Analysis

Statistical processing of the obtained data, as well as construction of the linear regression models, was performed using R programming language (version 4.2.2) in the RStudio development environment with additional packages: "readxl" (version 1.4.1), "tidyverse" (version 1.3.2), "factoextra" (version 1.0.7), and "ggpmisc" (version 0.5.2). The significance of differences in biochemical data was determined using the nonparametric Wilcoxon-Mann-Whitney test. Multivariate analysis using principal component analysis [PCA] was performed. Correlation analysis was carried out using the nonparametric Spearman coefficient. Differences between lipid values were considered significant at $p \leq 0.05$. Biochemical analysis was performed at the Environmental Biochemistry Laboratory using the equipment of the Core Facility of the Karelian Research Centre of the Russian Academy of Sciences.

3. Results

3.1. The Total Lipid Content and Levels of Individual Lipid Classes

The highest TL content was found in the digestive gland of the freshwater pearl mussel compared to that in the other tissues (Figure 2). ECHOL, as well as NEFA, CHOL, and PL were the dominant lipid classes in all studied tissues (Tables 2–4). A significantly higher TL content was found in the mantle and digestive gland of *M. margaritifera* from the Vozhma River compared to that in the freshwater pearl mussel tissues from the other studied rivers.

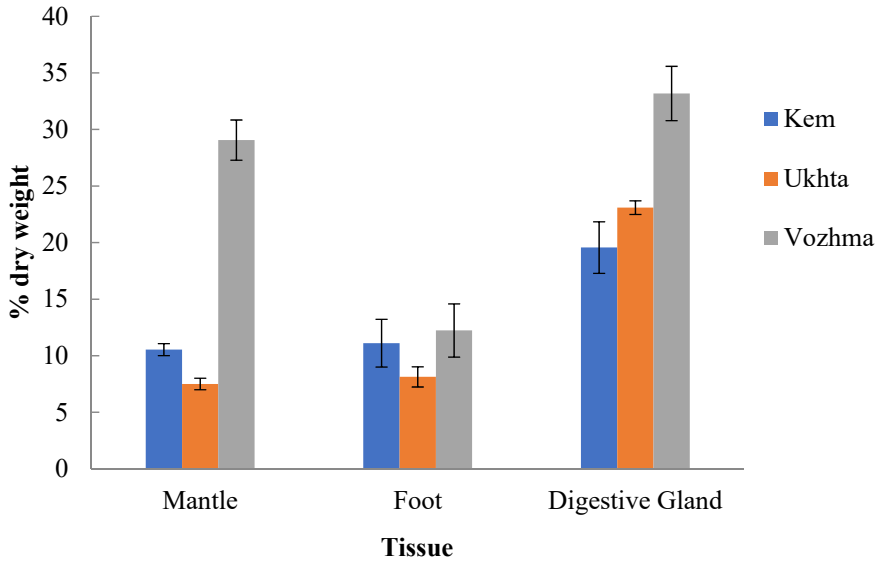

Figure 2. The total lipid content (% dry weight) in the mantle, foot, and digestive gland of *M. margaritifera* from the studied biotopes of the lake-river system of the Kem River, White Sea Basin.

Table 2. The total lipid content and levels of individual lipid classes (% dry weight) in the mantle tissue of *Margaritifera margaritifera* from the studied biotopes of the lake-river system of the Kem River, White Sea Basin.

	Kem (Estuary)	Ukhta	Vozhma
TL	10.54 ± 0.53	7.5 ± 0.51 a	29.6 ± 1.78 ab
PL	1.11 ± 0.08	0.7 ± 0.05 a	4.08 ± 0.33 ab
CHOL	1.29 ± 0.07	1.01 ± 0.07 a	4.21 ± 0.25 ab
ECHOL	4.59 ± 0.28	2.83 ± 0.2 a	13.37 ± 0.9 ab
TAG	0.09 ± 0.01	0.08 ± 0.01	0.18 ± 0.03 ab
DAG	0.17 ± 0.01	0.16 ± 0.02	0.38 ± 0.02 ab
MAG	0.41 ± 0.03	0.31 ± 0.03 a	0.99 ± 0.05 ab
NEFA	2.03 ± 0.08	1.57 ± 0.12 a	4.82 ± 0.24 ab
Unknown 1	0.05 ± 0.01	0.11 ± 0.01 a	0.22 ± 0.02 ab
Unknown 2	0.81 ± 0.05	0.72 ± 0.07	1.35 ± 0.12 ab
CHOL/PL	1.16 ± 0.05	1.44 ± 0.06	1.03 ± 0.09

Notes: TL, total lipids; PL, phospholipids; CHOL, cholesterol; ECHOL, cholesterol esters; TAG, triacylglycerols; DAG, diacylglycerols; MAG, monoacylglycerols; NEFA, non-esterified fatty acids; Unknown 1,2—unidentified lipids; a—differences were significant ($p \leq 0.05$) compared to the Kem River, b—differences were significant ($p \leq 0.05$) compared to the Ukhta River.

Table 3. The total lipid content and levels of individual lipid classes (% dry weight) in the foot tissue of *Margaritifera margaritifera* from the studied biotopes of the lake-river system of the Kem River, White Sea Basin.

	Kem (Estuary)	Ukhta	Vozhma
TL	11.11 ± 2.11	8.13 ± 0.89	12.23 ± 2.35
PL	1.26 ± 0.23	0.76 ± 0.08 a	1.68 ± 0.4
CHOL	1.6 ± 0.32	1.1 ± 0.12 a	1.93 ± 0.38
ECHOL	4.94 ± 0.91	2.85 ± 0.27 a	5.01 ± 1.04
TAG	0.24 ± 0.14	0.15 ± 0.04	0.14 ± 0.02
DAG	0.18 ± 0.04	0.14 ± 0.02	0.17 ± 0.03
MAG	0.45 ± 0.06	0.38 ± 0.04	0.47 ± 0.08
NEFA	1.69 ± 0.34	1.64 ± 0.22	1.9 ± 0.31
Unknown 1	0.04 ± 0.01	0.13 ± 0.03 a	0.13 ± 0.06
Unknown 2	0.71 ± 0.09	0.99 ± 0.15 a	0.81 ± 0.1
CHOL/PL	1.28 ± 0.35	1.45 ± 0.1	1.15 ± 0.08

Notes: TL, total lipids; PL, phospholipids; CHOL, cholesterol; ECHOL, cholesterol esters; TAG, triacylglycerols; DAG, diacylglycerols; MAG, monoacylglycerols; NEFA, non-esterified fatty acids; Unknown 1,2—unidentified lipids; a—differences were significant ($p \leq 0.05$) compared to the Kem River.

Table 4. The total lipid content and levels of individual lipid classes (% dry weight) in the digestive gland of *Margaritifera margaritifera* from the studied biotopes of the lake-river system of the Kem River, White Sea Basin.

	Kem (Estuary)	Ukhta	Vozhma
TL	19.56 ± 2.28	23.09 ± 0.6	33.18 ± 2.4 ab
PL	1.98 ± 0.34	1.66 ± 0.06	3.18 ± 0.35 ab
CHOL	1.95 ± 0.25	1.96 ± 0.05	3.16 ± 0.23 ab
ECHOL	5.59 ± 0.72	5.77 ± 0.17	9.1 ± 0.9 ab
TAG	0.28 ± 0.03	0.21 ± 0.02	0.41 ± 0.05 ab
DAG	0.28 ± 0.03	0.38 ± 0.01	0.5 ± 0.04 ab
MAG	0.68 ± 0.06	1.01 ± 0.05	1.11 ± 0.05 ab
NEFA	3.51 ± 0.37	4.5 ± 0.16	5.68 ± 0.3 ab
Unknown 1	0.45 ± 0.04	1.1 ± 0.02	0.86 ± 0.06 ab
Unknown 2	4.84 ± 0.48	6.49 ± 0.19	9.07 ± 0.59 ab
CHOL/PL	0.98 ± 0.12	1.18 ± 0.05	0.99 ± 0.08

Notes: TL, total lipids; PL, phospholipids; CHOL, cholesterol; ECHOL, cholesterol esters; TAG, triacylglycerols; DAG, diacylglycerols; MAG, monoacylglycerols; NEFA, non-esterified fatty acids; Unknown 1,2—unidentified lipids; a—differences were significant ($p \leq 0.05$) compared to the Kem River, b—differences were significant ($p \leq 0.05$) compared to the Ukhta River.

3.1.1. Mantle

It was found that the mantle of the freshwater pearl mussel from the Vozhma River had a significantly higher TL content (29.6% dw) than that of mollusks from the other rivers

(Table 2). The TL content in the mantle of the freshwater pearl mussel from the Ukhta River was the lowest among the studied rivers (7.5%). In terms of the quantitative content, ECHOL, NEFA, PL, and CHOL (listed in decreasing order of content) were the dominant lipid classes, with the highest levels in mollusks from the Vozhma River and decreased levels in individuals from the Uhta River (Table 2). The level of storage lipids (TAG) was no more than 0.2% dw. The ratios of CHOL/PL, reflecting the fluidity state of biological membranes, were 1.44, 1.16, and 1.03 for the freshwater pearl mussel from the Ukhta, Kem, and Vozhma Rivers, respectively (Table 2).

According to the multivariate statistical analysis performed by PCA, 99.6% of the total variance was described (PC1—99% and PC2—0.6%) and differences between the lipid profiles of the freshwater pearl mussel from the different rivers were observed (Figure 3). Mollusks from the Kem and the Ukhta Rivers were more similar in their lipid composition compared to mollusks from the Vozhma River. The greatest contribution to the formation of PC1 was made by ECHOL (PC1 = 0.88), PL (PC1 = 0.29), CHOL (PC1 = 0.27), and NEFA (PC1 = 0.26). It was shown that these lipid classes were more intensely accumulated in the freshwater pearl mussel from the Vozhma River than in molluscs from the other rivers.

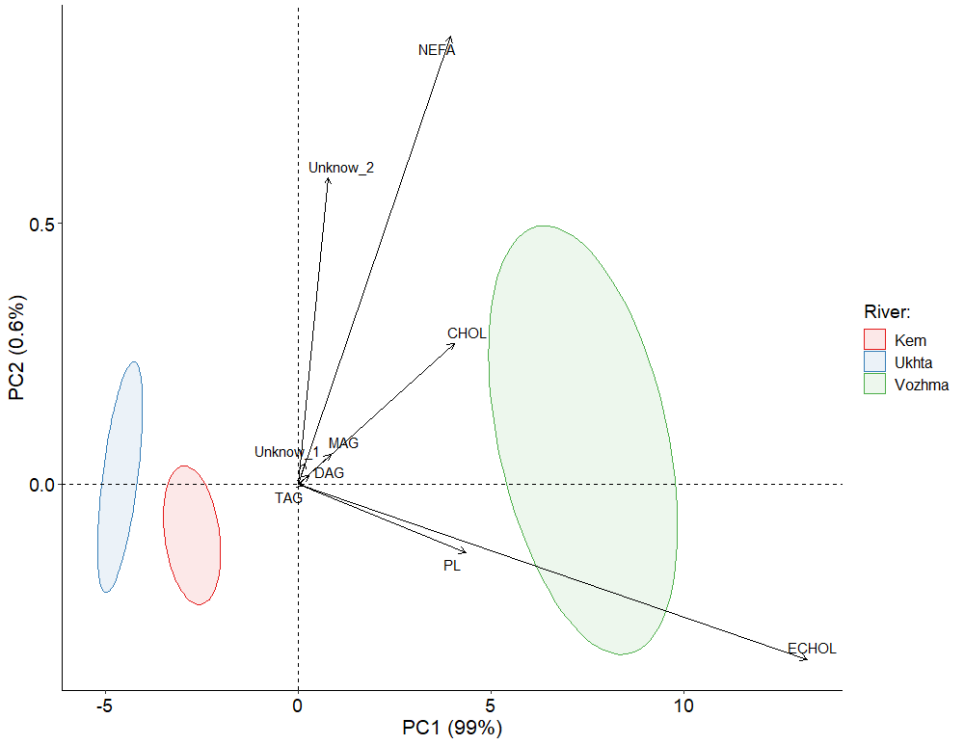

Figure 3. Principal component analysis of individual lipid classes in the mantle tissue of *Margaritifera margaritifera* from the studied biotopes of the lake-river system of the Kem River, White Sea Basin.

3.1.2. Foot (Muscle Tissue)

The TL content in the foot of the freshwater pearl mussel was not significantly different between the mollusks from the studied rivers and accounted for 8–12% dw. In terms of the quantitative content, ECHOL, NEFA, PL, and CHOL were the dominant lipid classes (Table 3). The studied lipid classes were not significantly different in the foot of the freshwater pearl mussel from the different rivers. The only exception was the mollusks from the Ukhta River, which were distinguished by the lowest ECHOL, PL, and CHOL levels. The ratios of CHOL/PL were highest in the foot of the freshwater pearl mussel from the Ukhta (1.45) and Tetri Rivers (1.52) and were lowest in the foot of mollusks from the Vozhma River (1.15).

3.1.3. Digestive Gland

The digestive gland of the freshwater pearl mussel from the Vozhma River had the highest TL content (33.18% dw) due to all dominant lipid classes—ECHOL, NEFA, PL, and CHOL. Meanwhile, the digestive gland of the freshwater pearl mussel from the Kem (19.56) and Tetri (12.76) had the lowest TL content (Table 4). The TAG level varied in the digestive gland of the freshwater pearl mussel in the studied rivers from 0.12% to 0.41%. A high content of one of the unknown component (2) accounted for the highest level (9.07%) in mollusks from the Vozhma River, and the lowest levels were found in mollusks from the Kem (4.84%) and Tetri Rivers (3.43%). The ratio of CHOL/PL in the digestive gland of the freshwater pearl mussel ranged from 0.98 to 1.19.

3.2. Individual Size, Age, and Growth Characteristics of M. margaritifera

The studied colonies of the freshwater pearl mussel had pronounced differences in size and age characteristics (Figure 4). Individual size, age, and growth characteristics of *M. margaritifera* are presented in the Table S1 (Supplementary Materials).

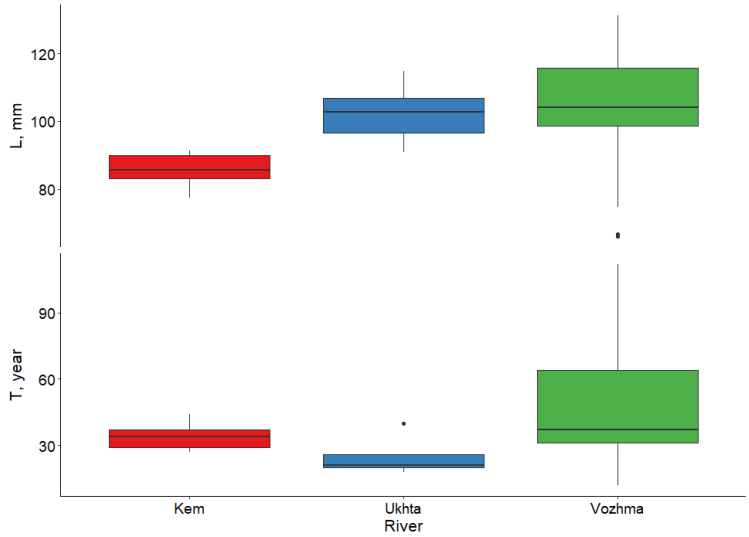

Figure 4. Values of shell length and age of *Margaritifera margaritifera* from the studied biotopes of the lake-river system of the Kem River, White Sea Basin. Notes: L—length of the shell, mm; T—age, years.

The average length of the shell of the freshwater pearl mussel from the Vozhma River was 103.4 mm. The average length of the shell of mollusks from the Ukhta River was larger than that of individuals from the Kem River (102.8 vs. 85.7 mm, respectively). The average age of mollusks from the Kem, Ukhta, and Vozhma Rivers was 34, 25, and 50 years, respectively.

The age of molluscs ranged from 27 to 44 years from the Kem River, from 19 to 40 years from the Ukhta River, and from 13 to 112 years from the Vozhma River. At the same time, the maximum shell length of individuals from the Vozhma River reached 131.2 mm and corresponded to the highest age for individuals from this colony.

The relationship between the shell size and age was found for the freshwater pearl mussel from the Ukhta and Vozhma Rivers. This relationship was not shown for mollusks from the Kem River (estuary) (Figure 5).

The relationships between the TL content, PL, CHOL, TAG, and ECHOL levels, and the size-age characteristics were studied.

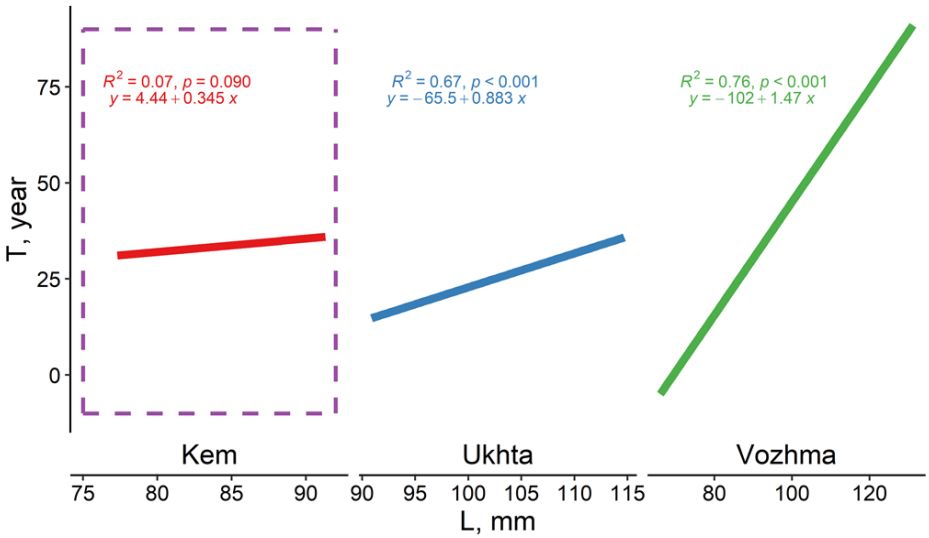

Figure 5. Linear dependence between the shell length and age of *Margaritifera margaritifera* from the studied biotopes (the Kem, the Ukhta, and the Vozhma Rivers) of the lake-river system of the Kem River, White Sea Basin. Notes: R^2—coefficient of determination, *p*—level of significance, L—shell length.

3.2.1. Vozhma River

In the mantle, the TL content and ECHOL, PL, and CHOL levels had significant negative regression values with both the shell length and age (Figures 6 and 7). A negative dependence on the TAG level was only found for the length of shell. At the same time, according to the correlation analysis using Spearman coefficients, dependences between the TAG level and both age (p = 0.34) and shell length (p = 0.20) were not shown; whereas correlations between other classes of lipids—TL (p = 0.007), ECHOL (p = 0.02), PL (p = 0.02), and CHOL (p = 0.02)—with both shell length and age were shown.

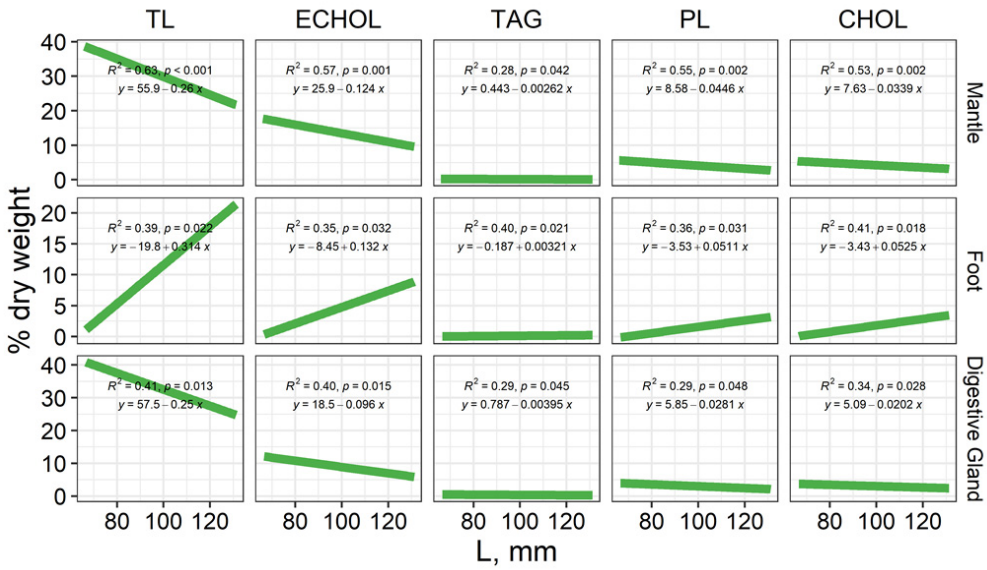

Figure 6. Linear models of the relationship between the total lipid content and levels of individual lipid classes and the shell length of *Margaritifera margaritifera* from the Vozhma River. Notes: R^2—coefficient of determination, *p*—level of significance, L—shell length; TL, total lipids; PL, phospholipids; CHOL, cholesterol; ECHOL, cholesterol esters; TAG, triacylglycerols.

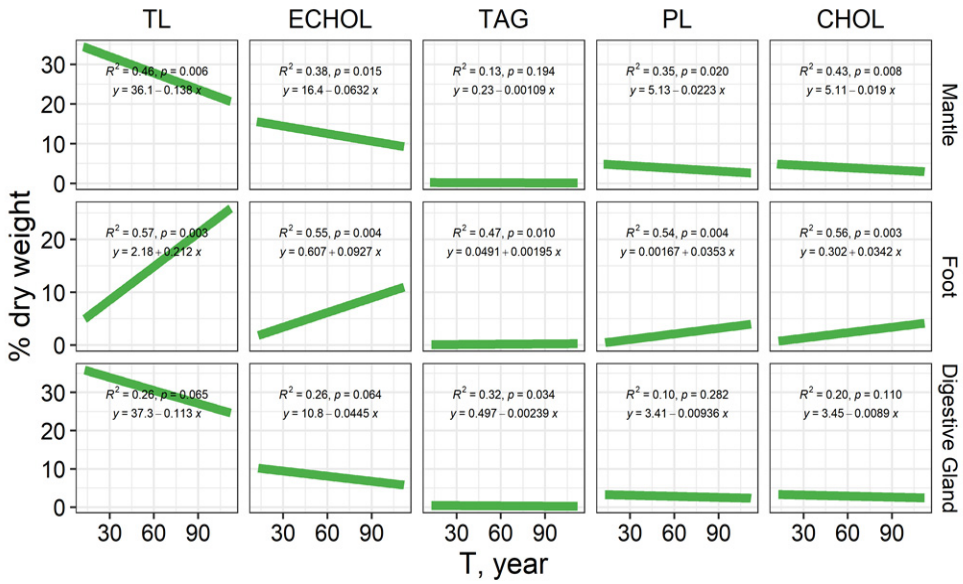

Figure 7. Linear models of the relationships between the total lipid content and levels of individual lipid classes and the age of *Margaritifera margaritifera* from the Vozhma River. Notes: R^2—coefficient of determination, *p*—level of significance, T—age; TL, total lipids; PL, phospholipids; CHOL, cholesterol; ECHOL, cholesterol esters; TAG, triacylglycerols.

For the foot, the TL content and TAG, ECHOL, PL, and CHOL levels had significant positive regression values with the shell length and age of the mollusks. According to the correlation analysis, this dependence was found only between TL content ($p = 0.04$), TAG level ($p = 0.02$), CHOL level ($p = 0.03$), and age.

For the digestive gland, significant negative regression values were shown between the TL content, ECHOL, TAG, PL, and CHOL levels, and shell length. The relationship with age was shown only for TAG levels. The correlation analysis showed the dependence between the TAG level ($p = 0.02$), ECHOL level ($p = 0.04$), and age.

3.2.2. Ukhta River

Significant regression values were not shown between the lipid components and size-age parameters of the freshwater pearl mussel from the Ukhta River. According to the correlation analysis, negative correlations were shown between the TL content ($p = 0.04$), ECHOL level ($p = 0.009$), PL level ($p = 0.03$), and age in the mantle of mollusks from the Ukhta River.

3.2.3. Kem River

Significant regression values were not shown between the lipid components and the size-age parameters of the freshwater pearl mussel from the Kem River. According to the correlation analysis, negative correlations were found between the TAG level and shell length ($p = 0.002$) for the mantle of mollusks from the Kem River.

4. Discussion

It was found that the TL content of the freshwater pearl mussel from all studied rivers was highest in the digestive gland compared to that in the mantle and foot, indicating that the TL content of the mollusks was tissue specific. The digestive gland and gonads in bivalves are usually distinguished by the highest lipid content [64–67]. In the current study, ECHOL was the prevalent lipid component in all the studied tissues of the freshwater pearl mussel. This storage lipid along with TAG, is an important source of energy in the organism, and its structural elements can be used for the synthesis of other biologically active substances in the cell [42,68,69]. NEFA, PL, and CHOL present in the tissues of the freshwater pearl mussel were also dominant lipid classes in the freshwater pearl mussel,

while MAG, DAG, and TAG were minor components. The identification of NEFA is characteristic for molluscs. For example, sterol esters, TAGs, free fatty acids, carotenoids, sterols, and polar lipids were shown in the lipid profile of the mussel *Perna canaliculus* [70]. Free fatty acids were present in the body of freshwater gastropods *Lymnaea stagnalis* and *Lymnaea stagnalis* in the amount of 5.2% and 5.5% of total lipids, respectively [71]. It is known that there are differences in the qualitative composition of dominant lipids in bivalve species. Thus, PL and CHOL were prevalent in the mantle, gills, and foot of the White Sea littoral mussel *Mytilus edulis* [72] in comparison with the content of storage lipids ECHOL and TAG [72]. In the mussel *M. galloprovincialis* cultivated in southern Spain, PL and TAG were the dominant lipid components in the mantle and digestive gland of females and males [73,74]. The bivalve *Modiolus modiolus* (family Mytilidae), a typical inhabitant of the sublittoral zone of the White Sea, was characterized by high concentrations of storage lipids TAG and ECHOL and a low concentration of structural CHOL [72].

A comparative study of the lipid status of different colonies of the freshwater pearl mussel showed that mollusks from the Vozhma River had a significantly higher TL content in the mantle (in two times) and digestive gland. The multivariate statistical analysis performed by PCA confirmed the differences between levels of lipid components of the freshwater pearl mussel from the Vozhma River and those of mollusks from the other rivers. The lipid composition of hydrobionts largely depends on the influence of environmental factors. The studied the freshwater pearl mussel from different rivers was collected in the same period (post-spawning) at a water temperature of 3 to 5 °C. All rivers belong to the Kem River Basin and are characterized by the presence of Atlantic salmon, which is a host for developing larvae (glochidia) of the freshwater pearl mussel. At the same time, the freshwater pearl mussel of the studied colonies differed in size-age characteristics; thus, among the factors that determine such differences in lipid status may be the anthropogenic influence. It known that the lipid content of mollusks may be age-related. Thus, levels of CHOL were higher in mussels from the White Sea aged 1+ years, while individuals aged 2+ to 5+–6+ years were characterized by increased levels of storage lipids, such as TAG and ECHOL [72].

In connection with the biochemical analyses conducted for the freshwater pearl mussel, a study of the dependence between the size-age characteristics and the lipid class content was performed. The presence of recording structures in the form of annual rings on the surface of the shell of the freshwater pearl mussel makes it possible to estimate the age-growth parameters of mollusks [60–62]. The long lifespan of the freshwater pearl mussel (more than 100 years) leads to the formation of a colony within a limited part of the river, represented by individuals of different ages living in similar conditions. Thus, there is a representative opportunity to obtain comparative data on the biochemical features of mollusks with different size-age characteristics. The freshwater pearl mussel colonies from the Kem (the estuary) and the Ukhta Rivers are known to be under permanent anthropogenic influence (hydro construction, household wastewater). The freshwater pearl mussel individuals collected from the Vozhma River were 13 to 112 years old. Individuals over 100 years old distinguished this colony as being the oldest, while the colonies were relatively young in other studied rivers (i.e., the Kem and Ukhta Rivers) and "reborn" after the construction of a dam on the river. It was observed that the TL content was higher in mollusks inhabiting the Vozhma River along with age—these mollusks were older in comparison with specimens from the other studied biotopes. Both physiological and biochemical characteristics may point to the quality of the inhabited environment of the river (low anthropogenic influence); thus, the discussed parameters can be used as additional assessment criteria of the condition of the freshwater pearl mussel colonies and the ecological state of the biotope.

The freshwater pearl mussel from the estuary of the Kem River did not show a significant relationship between age and shell length compared to mollusks from other rivers. This colony of *M. margaritifera* may be considered particularly oppressed due to the influence of pollution and catastrophic changes in the water level of the studied area of the

Kem River (estuary). The absence of dependences between most biochemical parameters and shell size or age of the freshwater pearl mussel from the Ukhta and the Kem Rivers may reflect the stress of the mollusk organism in these biotopes. The significant dependences between the lipid content and the size-age parameters established mainly for mollusks from the Vozhma River may be a reflection of the normal physiological state and development of these organisms caused by living under favorable conditions. Established positive regression values between the TL content, levels of individual lipid classes in the foot of the freshwater pearl mussel, and the size and age of the mollusks from this river may be associated with the growth of the foot throughout the life of the mollusk. Meanwhile, the growth of the shell and, respectively, the mantle of the mollusk slow down with age, which may be reflected in the negative regression values for both the shell length and age.

Seasonal fluctuations in the lipid content are usually observed primarily in storage lipids and not in PL due to the function of the latter as a structural component in the biomembrane. It is known that changes in the ratio of structural lipids (CHOL/PL) involved in regulating the fluidity of the biomembrane follow only after changes in the fatty acid composition [69,75,76]. In all the studied tissues of the freshwater pearl mussel, this ratio ranged from 1.0 to 1.5, which corresponded to normal, age-appropriate values. It was reported that the ratio of CHOL/PL was 1.0–1.2 in sublittoral mussels of the White Sea aged 4–6 years, while it was less than 1.0 in young mussels. In littoral mussels aged 4–8 years, which are more exposed to temperature changes, this ratio was 0.6 [50].

The current study found a low level of TAG in the freshwater pearl mussel tissues at the end of September in the post-spawning period. It is known that the fertilization of the freshwater pearl mussel occurs in July-August. In mid-August, numerous (up to 3 million per individual) larvae of very small sizes (50–70 μm) appear on the valves of the freshwater pearl mussel. In late August–early September, an adult mussel throws larvae (glochidia) into the water column [21,26,28–30,77]. Mollusks can actively accumulate lipids during gametogenesis [78,79]. The lipid content in the mantle of the female bivalve *M. galloprovincialis* increases during the reproductive cycle [73,74]. Elevated TAG levels were observed in the mature female mollusc *Pecten maximus* [80]. Storage TAGs are able to quickly mobilize in the organism and provide 2.5 times more energy per unit mass than carbohydrates [42,68]. The sagittal part of the mussel's mantle, where the maturation of reproductive products occurs, is distinguished by a high TAG content as well as the accumulation of nutrients necessary for the development of the gonads [46]. The oocytes of bivalve mollusks store a significant amount of lipids, thus ensuring the further development and viability of their larvae [81]. An increase in the TAG content was shown in the pre-spawning period in the bivalve mollusks *Diplodom patagonicus* and *Chlamys tehuelcha*, and a further decrease in the TAG level corresponded with the release of the reproductive products into the environment [46,82]. Immature mussels showed elevated levels of structural lipids compared to other periods of the reproductive cycle [72].

The glochidia, after separation from the mother's body, are unable to eat because they do not yet have a mouth, anus, or digestive tract; therefore, they must have the lipid reserves needed to support their metabolic processes. the freshwater pearl mussel glochidia must attach themselves to the gill filaments of the host fish and then undergo metamorphosis [2,29,33]. TAGs play an important role in the development of the freshwater pearl mussel larvae. In previous studies [30,83], increased TAG and CHOL levels were found in the freshwater pearl mussel glochidia, which was associated with their sensitivity and determined their resistance to temperature stress and hypoxia. After attaching to the gills of the host fish, the glochidia are gradually surrounded by a cyst, which is formed by the epithelial tissues of the gills of the host and receives nutrients through the bloodstream, primarily glycogen and lipids [83], which are used for the development of the young organism [84]. It should be noted that in a previous study [30], increased TAG levels (21.74% dw) were found in the gills of salmon heavily infected with the freshwater pearl mussel glochidia (>70) compared to that in gills with a lower level of infection with glochidia (1–30) (9.08%).

5. Conclusions

This study presents data on the TL content and levels of individual lipid classes in the mantle, muscle (foot), and digestive gland of *M. margaritifera* collected in rivers of Northern European Russia (the Kem, Ukhta, and Vozhma Rivers) at the end of September, early October. Tissue specificity was observed in the lipid profile: the TL content was higher in the digestive gland of the freshwater pearl mussel from all studied rivers. It was detected that ECHOL, NEFA, PL and CHOL were the dominant lipid classes in all the studied tissues of mollusks. The reduced TAG level probably indicates its use in the growth and development of larvae (glochidia) and its utilization during the spawning process of mollusks, which usually takes place at the end of August and early September.

The study presents data on the size-age characteristics of the freshwater pearl mussel from the Kem, the Ukhta, and the Vozhma Rivers. The relationship between the lipid content (structural and storage) and the age and shell length of the mollusks was shown. For the freshwater pearl mussel from the Vozhma River, a significant relationship was shown mainly between the biochemical parameters and size of the shell of the mollusks, while a relationship between the biochemical parameters and age was undetected in some cases. The relationship between the age and shell size of the freshwater pearl mussel from the estuary of the Kem River was not found, nor was the relationship between the biochemical parameters and the size-age characteristics of the mollusks from this biotope. This may indicate a suppression of the freshwater pearl mussel colony due to anthropogenic influence. At the same time, the freshwater pearl mussel from the Vozhma River was distinguished by a higher content of total lipids (especially in the mantle) and significant correlations between the biochemical parameters and shell length and age. Further additional research is needed.

The obtained results provide perspective biochemical indicators for studying the compensatory reactions of the freshwater pearl mussel to the impact of environmental factors and, as a result, are important for different conservation strategies of endangered species such as *M. margaritifera*.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/d15020293/s1, Table S1: Individual size, age, and growth characteristics of *M. margaritifera*.

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