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# IMPACT OF ENVIRONMENTAL FACTORS ON THE STRUCTURE OF FISH COMMUNITIES, ISOTOPIC RATIO IN HYDROBIONTS AND TROPHIC LEVELS IN THE RIVERS ECOSYSTEM OF DIFFERENT ECOLOGICAL STATE

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VILNIAUS UNIVERSITETAS

GAMTOS TYRIMŲ CENTRAS

LAURYNAS ČIVAS

# APLINKOS VEIKSNIŲ ĮTAKA ŽUVŲ BENDRIJŲ STRUKTŪRAI, HIDROBIONTŲ IZOTOPINIAM SANTYKIUI IR MITYBOS LYGMENIMS SKIRTINGOS EKOLOGINĖS BŪKLĖS UPIŲ EKOSISTEMOSE

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# INTRODUCTION

## Relevance of the study

River ecosystems are significant reservoirs of biodiversity and constitute the principal source of renewable water supply for humans and freshwater ecosystems. However, nowadays rivers are under unprecedented anthropogenic stress worldwide and, according to the Millennium Ecosystem Assessment, may be among the most endangered ecosystems on the planet.

The Water Framework Directiveadopted by the European Union introduced a new legal water management and protection method based not on national or political boundaries, but on natural geographical and hydrological formations – river basins. In accordance with the Water Framework Directive, a good ecological state of all water bodies had to be achieved by 2015. (European Commission, 2000). However, the achievement of these objectives has been postponed due to technical, financial possibilities, and natural conditions. River Basin District (RBD) management plans are being implemented from 2015 to 2021, and after that they will be updated. Results of this research will help to upgrade RBD management plans and programs of measures (Environmental Protection Agency, 2010). According to the European Union Water Framework Directive, fish are one of the biological quality elements that reflect human-induced changes in the ecological state of rivers and lakes. Fish community indices (species diversity, population structure, growth, community composition, abundance, biomass, long-term dynamics of indicators) enable researchers to fairly accurately determine factors affecting water bodies, and to develop measures for the management and protection of fish communities (European Commission, 2000; Ministry of Environment of the Republic of Lithuania 2007). Studies into benthic invertebrates and fish are among the most informative ones used in the impact assessment of complex agricultural pollution on rivers, because these organisms are permanently resident in water bodies. These may include species that are involved in water self-cleaning processes, and individual invertebrate species are good indicators of water quality (Ministry of Environment of the Republic of Lithuania, 2010, Government of Lithuania, 2011). As fish occupy different positions in the food chain and are relatively long-lived

organisms, they indirectly reflect not only short-term but also long-term changes in their food items and the living medium. The functioning, structure, and diversity of foodwebs are of critical importance for the functioning of ecosystems (Thebault and Loreau, 2003; 2005; Duffy et al., 2005; Tylianakis et al., 2007). Biodiversity and food webs are closely related to ecosystem stability, with some debate relative to the exact nature of the relationships (McCann, 2000; Dunne et al., 2002; Loreau and de Mazancourt 2013; Galiana et al., 2014). Food-chain length represents an important measure of food-web structure and exerts a strong influence on community composition, species diversity, and ecosystem functioning (Post and Takimoto, 2007; Sabo et al., 2010). Thus, food-web dynamics can help understand functional diversity in river ecosystems (Thompson et al., 2012). The stable isotope method allows determining the source of nutrients, trophic levels in the food chain and its length, as well as the anthropogenic impact on river ecosystems. Quite a number of similar studies have been carried out in recent years in the USA (Finlay, 2001; Power and Dietrich, 2002; Thorp and Delong, 2002; Rybczynski et al., 2008; Duda et al., 2011; Sullivan, 2013), Canada (Anderson and Cabana, 2005; Romanuk et al., 2006), Australia (Bunn and Winning., 2003; Melville and Cannolly, 2003), Great Britain (Barth et al., 2003; Bearhop et al., 2004), France (Pasquaud et al., 2008), Italy (Camusso et al., 1999) and in some other countries covering different ecoregions, but in the 15$^{th}$ ecoregion (FAME Consortium, 2004), to which the Lithuanian territory belongs, such studies have not been conducted, so there is no possibility for comparisons. In Lithuania, the stable isotope analysis of aquatic ecosystems was carried out only in the Curonian Lagoon (Lesutienė et al., 2007, 2008; Lesutienė, 2009) and in some lakes (Rakauskas et al., 2013; Rakauskas, 2014) for the purpose of ascertaining the role of alien crustaceans in food webs. Metabolism of allochthonous and autochthonous organic matter in food webs of Lithuanian rivers has not been studied yet. Little is known about the the peculiarities of food chains in Lithuanian rivers and the impact of ecological conditions on them.

**Thesis Aim**

The aim of this thesis is to investigate the influence of abiotic factors on fish communities structure and isotopic ratio in hydrobionts in Lithuanian rivers of different ecological state.

**Tasks of this thesis**

- To assess the ecological state of the Nevėžis and Žeimena Rivers employing fish-based biological assessment methods.
- To determine and compare physical and chemical parameters of the rivers and carbon (C) and nitrogen (N) isotope ratios in aquatic organisms (hydrobionts).
- To assess the direct and indirect environmental impact on isotopic ratio in hydrobionts and trophic levels.
- To investigate the origin of the dominant organic matter (autochtonous or allochtonous) and to assess the impact of hydromorphological and hydrochemical factors on the organic matter distribution within food webs of river ecosystems.
- To determine and compare the isotopic niche structure of macrozoobenthos and fish communities in rivers of different ecological state and to assess the impact of environmental factors on isotopic niche parameters of the aforesaid communities.

**Defended statements**

- Hydrological conditions of rivers affect changes in ecological fish groups and communities thereby inducing changes in their ecological state.
- Trophic levels of macrozoobenthos and fish and the proportion of autochtonous/allochtonous organic matter vary depending on hydromorphological river characteristics and the impact of other environmental factors.
- The altered (regulated) riverbed morphology leads to the following changes in the diet of hydrobionts: an increase in $\delta^{15}N$ values and autochtonous organic matter amount, and a decrease in $\delta^{13}C$ values, trophic levels and the amount of allochtonous organic matter.
- Decrease in $\delta^{13}C$ values and an increase in $\delta^{15}N$ values in hydrobiont organisms is related to an increase in the allochtonous primary production. An increase in the autochtonous primary production leads to the lowering of hydrobionts‘ trophic levels and, herewith, to the shortening of a river food chain.

- Isotopic niche parameters of hydrobiont communities depend on hydromorphological and hydrochemical environmental factors.

**Scientific novelty**

This is the first study to have been made into trophic webs of the Nevėžis and Žeimena Rivers from an ecological viewpoint and the first assessment of the anthropogenic impact on the ecological state of river ecosystems employing the mehod of stable isotopes analysis.This is the first study to have:

- investigated food chains in Lithuanian rivers of different ecological state using the method of stable isotopes analysis;
- assessed the impact of environmental factors on river food webs based on the example of the Nevėžis ir Žeimena Rivers;
- determined the source of the dominant organic matter (autochtonic vs. allochtonic) in food webs of hydrobiont communities in rivers of different ecological state;
- determined the isotopic niche structure of macrozoobenthos and fish communities in rivers of different ecological state and to assess the impact of environmental factors on the isotopic niche parameters of the aforesaid communities.

**Scientific and practical significance**

For this study, we chose catchments of Lithuanian rivers of the same type (according to the catchment area and riverbed inclination) but of different ecological state. The Nevėžis River catchment is particularly anthropogenized and highly affected by agriculture and urbanization. The Žeimena River is one of the cleanest Lithuanian rivers with its catchment almost unaffected by human activities. Up to now, studies into food webs and the anthropogenic impact on their structure and functioning in ecosystems of Lithuanian rivers using the method of stable isotope analysis have not been been conducted.

As catchment areas of the above mentioned rivers are among the largest ones, the amount of the data obtained allows drawing statistically significant conclusions about the anthropogenic impact on changes in food webs and organic matter sources in rivers of

different ecological state. The data obtained are significant for environment protection, the study of climate change impact on riverine fish communities, sustainable development, protection of biodiversity, primary ecosystems and ecological processes in the Baltic region. In addition, findings of this study are supposed to deepen the knowledge of the impact physical and chemical factors have on river ecosystems. In the future, these findings could prove helpful in predicting productivity of riverine ecosystems, changes in fish communities and in ecological state of rivers. Also, the data obtained could be used in future stable isotope analysis-based studies into the impact of invasive species on ecosystems, into trophic levels of hydrobionts in river food webs, seasonality impact on the diet of hydrobionts, fish migrations, concentrated and diffused pollution, impact of human- and climate change-induced hydromorphological changes in rivers on food webs therein. The findings of this study could also be used for producing recommendations aimed at the upgrading and updating of complex studies into interactions between physical and chemical environmental factors and fish communities, monitoring programmes, river basin management plans and programmes of measures in Lithuania and other Baltic countries.

**Approbation of results**

Results of this study were published in 2 scientific publications and presented at 3 regional and international conferences.

**Structure of the dissertation**

The dissertation consists of the following chapters: Concepts and abbreviations, Introduction, Literature Review, Hydrographical and Hydrological Characterisation of Investigated Rivers, Material and methods, Results, Discussion, Conclusions, References and Annex. The scope of the dissertation is 176 pages, it contains 57 tables and 32 figures. References include 236 sources. The dissertation is written in Lithuanian with the summary in English.

**Literature Review**

This section presents a review of the studies, which were conducted in Lithuania and abroad, into the impact of hydrogeomorphology and chemical water quality on fish

parameters, food webs in river ecosystems, river typology and ecological state assessment.

## MATERIAL AND METHODS

**Analysis and assessment of fish indices.** During the study period (July – August 2010–2015) physical, chemical, and fish community data were repeatedly collected in eleven 250–300 m long river stretches in the Nevėžis River and in 6 stretches in the Žeimena River. All physicochemical samples used for the study were collected together with with fish samples. Water quality parameters and hydrological parameters were measured *in situ* in each study stretch. Fish sampling was performed using the backpack electric fishing equipment (CEN, 2003). Fish density and biomass of the population were calculated for a unit of area (100 $m^{-2}$ and g 100 $m^{-2}$). The caught fish were divided into groups by their species, with the number of individuals of each species (N), their lengths (L and l, cm) and weight (Q and q, g) determined (Thoresson 1993). The ecological state of a fish community was assessed employing the Lithuanian Fish Index (LFI) estimate method (Ministry of Environment of the Republic of Lithuania, 2007; Virbickas and Kesminas, 2007).

**Investigations of food webs and organic matter sources.** Fish and macroinvetrebrate surveys were carried out in the Nevėžis and Žeimena rivers, which are of the same 5$^{th}$ type (according to the catchment area and riverbed slope), but differ in some parameters of water, habitat quality and ecological state. The study was conducted in the river stretches located in the following parts of the Nevėžis River: the upper reaches (55°68'N, 24°45'E; 151–159 km of the river estuary), the middle reaches (55°45'N, 24°04'E; 84–76 km) and the lower reaches (55°17'N, 23°82'E; 36–30 km). In the Žeimena River, we performed studies in river stretches located in: the upper reaches (55°23'N, 25°97'E; 75–70 km), the middle reaches (55°10'N 25°94'E; 48–45 km) and the lower reaches (54°91'N, 25°64'E; 2–5 km) (Fig. 1). The impact of the riverbed morphology on foodwebs was evaluated in natural (55°68'N, 24°45'E; 151–159 km of the river estuary) and regulated (55°58'N, 24°62'E; 183–175 km) sections of the Nevėžis River upper reaches (Fig. 1). The criterion for distinguishing these sections was the riverbed morphology.

Benthic macroinvertebrates were sampled following standard methods for the monitoring of macroinvertebratesin flowing water (Ministry of the Environment of the Republic of Lithuania, 2010; Arbačiauskas, 2009). For the stable isotope analysis,12 invertebrate genera belonging to 5 classeswere selected: *Bivalvia* (*Pisidium* sp.); *Gastropoda* (*Bithynia* sp.); *Oligochaeta*; *Crustacea* (*Gammarus* sp., *Asellus* sp.); *Insecta* (*Baetis* sp., *Nemoura* sp., *Calopteryx* sp., *Aphelocheirus* sp., *Brachycentrus* sp., *Simulium* sp., *Cricotopus* sp.) and 6 fish species belonging to three families: *Cyprinidae* (*Rutilus rutilus, Leuciscus cephalus, Leuciscus leuciscus*, *Gobio gobio*); *Esocidae* (*Esox lucius*); *Percidae* (*Perca fluviatilis*). The stable isotope ($\delta^{13}C$ and $\delta^{15}N$) analysis of a total of 249 macroinvertebrate and 229 fish samples was performed for this study.

The stable isotope analysis was performed at the State Scientific Research Institute Center for Physical Sciences and Technology (FTMC). Ratios of carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) in the samples were determined using continuous-flow isotope mass spectrometry (Post, 2002; Fry, 2006). $\delta^{13}C$ and $\delta^{15}N$ values were used to estimate the trophic level (TL) of consumers and the index of allochthonous organic matter (AO) showing the source of the organic matter (allochthonous or autochthonous) in river ecosystems (Doucett et al., 2007; Solomon et al., 2009). Terrestrial detritus (e.g., leaves from riparian trees/shrubs; $\delta^{13}C_{base1}$) and benthic periphyton (macrophytes and algae) ($\delta^{13}C_{base2}$) were used as river reach-specific baselines. $\delta^{13}C$ and $\delta^{15}N$ values plotted in a bivariate fashion, and also referred to as a isotopic niche, describe the structure of food webs and their parameters such as food web overlap or interconnection of food chains within a food web (Layman et al., 2007a, 2011).

**Statistical methods.**The following statistical parametric and non-parametric methods were used for data analysis: Student‘s t-test, ANOVA and Tukey HSD test, multiple regressions, Pearson and Spearman correlations, cluster analysis and canonical correspondence analysis (CCA). Prior to analysis,all species data were log (1 + x) transformed. Statements of statistical significance refer to $p < 0.05$.

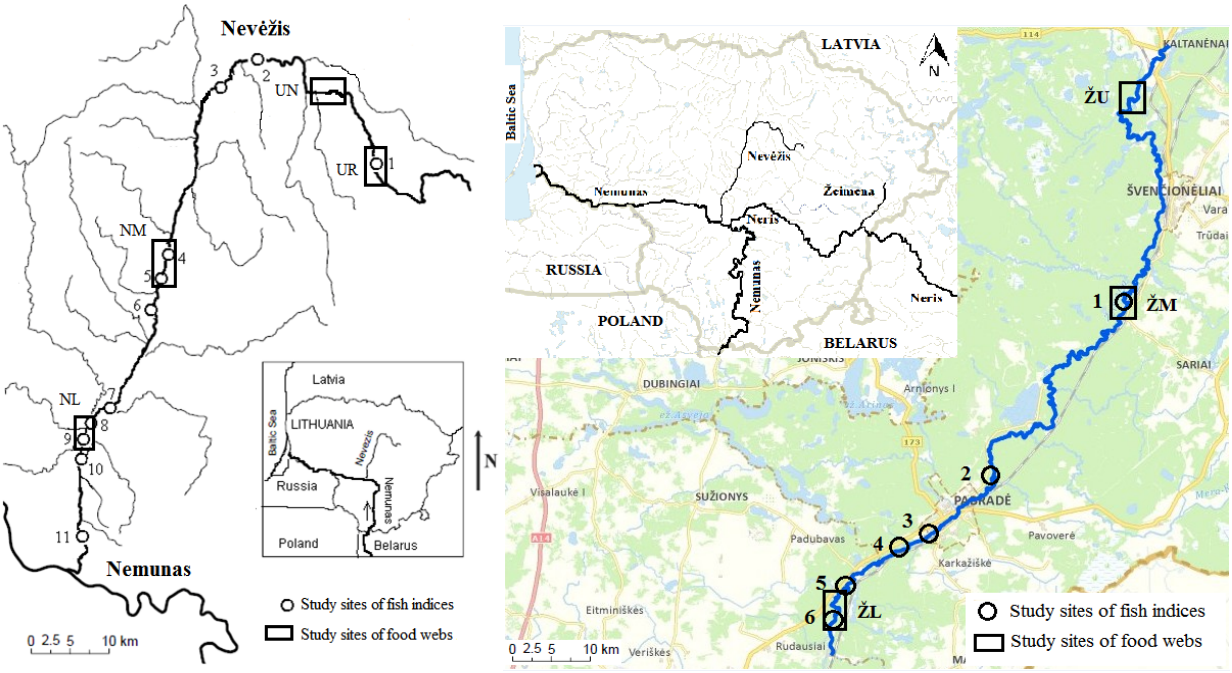


**Fig. 1** Location of study stations in the Nevėžis and Žeimena Rivers: UR - regulated stretch of the Nevėžis River upper reaches; UN - natural stretch of the Nevėžis River upper reaches; NM - middle reaches of the Nevėžis River; NL - lower reaches of the Nevėžis River; ŽU – upper reaches of Žeimena River; ŽM - middle reaches of the Žeimena River; ŽL - lower reaches of the Žeimena River.

## RESULTS AND DISCUSSION

**Ichthyofauna and ecological state of the Nevėžis River.** During the study period, 23 fish species were recorded in total. At the study sites, fish density varied from 16.6 to 60.7 ind. 100 $m^{-2}$ (38.6 ind. 100 $m^{-2}$ on average), and biomass from 467.2 to 1544.3 g 100 $m^{-2}$ (906.9 g 100 $m^{-2}$ on average). In the Nevėžis River fish community, dominant by abundance was roach (21.4%). Fish communities were found to be dominated by omnivorous and tolerant fishes. The ecological state of the investigated river stretches was assessed as either moderate or poor. The most important factor to affect LFI, abundance of intolerant, lithophilic, rheophilic and omnivorous fishes was substrate.

**Ichthyofauna and ecological state of the Žeimena River.** By $BOD_7$, total N and total P, the ecological state of the river at all study sites was found to be very good. A total of 19 fish species were recorded during the study period. At the study sites, fish

density varied from 31.6 to 56.6 ind. 100 $m^{-2}$ (on average, 47.4 ind. 100 $m^{-2}$), and biomass from 295.6 to 1991.3 g 100 $m^{-2}$ (on average, 858.1 g 100 $m^{-2}$ ). By abundance, the Žeimena River community was dominated by salmon (20.0%) and European bullhead (15.8%). Fish communities were dominated by lithophilic and rheophilic species. The ecological state of the Žeimena River at all study sites was assessed as good or very good. The study showed that LFI varied from 0.85 to 1.03. It was found to correlate positively with stream flow velocity.

**Stable isotopes of carbon and nitrogen in river ecosystems.** Investigations of stable isotopes in river ecosystems showed that the lowest $\delta^{13}C$ values were characteristic of macrophytes and algae (periphyton). The lowest $\delta^{13}C$ values among invertebrates were found in herbivorous gastropod molluscs (*Bithynia* sp.). Among fish, the lowest $\delta^{13}C$ values were recorded in small roach and, in some river locations, in small individuals of chub (*L. cephalus*) that do not avoid vegetation in their diet.The highest $\delta^{13}C$ values were recorded in terrestrial detritus. Among invertebrates, it was detritus-feeding stoneflies (*Nemoura* sp.), amphipods (*Gammarus* sp.) and water louse (*Asellus* sp.) that showed the highest $\delta^{13}C$ values. Among fish, the highest $\delta^{13}C$ values were found in larger individuals of dace (*L. leuciscus*) and chub (*L. cephalus*), which like feeding on terrestrial insects that fall into the water. Among samples of organic matter, the lowest $\delta^{15}N$ values were determined in detritus and the highest in periphyton. The lowest $\delta^{15}N$ values among invertebrate samples were found in typical primary consumer molluscs (*Bithynia* sp.) and freshwater crustaceans (*A. aquaticus)*, while the highest $\delta^{15}N$ values were recorded in predatory water bugs (*Aphelocheirus* sp.) and dragon-flies (*Calopteryx* sp.). Among fish samples, the lowest values of $\delta^{15}N$ were found in small-sized individuals of roach (*R. rutilus*) and chub (*L. cephalus)* and the highest $\delta^{15}N$ values in top predator fish species – perch (*P. fluviatilis*) individuals of larger size and pike (*E. lucius).* SI analysis revealed that $\delta^{13}C$ and $\delta^{15}N$ values in most genera of macrobenthic invertebrates and fishes significantly differ among different river sites. Thus, when assessing isotopic niche parameters of invertebrate and fish communities, hydrobionts were analysed at genus and species levels respectively. Values of $\delta^{13}C$ and $\delta^{15}N$ in most fish were found to depend on fish body length. Therefore, for the impact assessment of various factors on $\delta^{13}C$ and $\delta^{15}N$ values in fish and for the calculation of isotopic niche

parameters of a fish community, fish were divided into length groups. Significant differences in $\delta^{13}C$ and $\delta^{15}N$ values were also found among ecological groups of macrobenthic invertebrates and fish.

**Isotopic niche parameters of hydrobiont communities.** In members of the invertebrate community, the $\delta^{15}N$ range (NR), reflecting the average food chain length in the community, varied from 3.9 (in the middle reaches of the Žeimena River) to 1.9 (in the regulated stretch of the Nevėžis River upper reaches), while NR of fish was found to be the largest (3,9) in the lower reaches of the Žeimena River and the smallest (2.2) in the regulated stretch of the upper reaches and lower reaches of the Nevėžis River. The greater length of food chains in the Žeimena River was due to a bigger difference between the highest and lowest means of the measured $\delta^{15}N$ values. The range of the average $\delta^{13}C$ values (CR) in invertebrate communities varied from 3.7 (the upper reaches of the Žeimena River) to 2.1 (the regulated stretch in the Nevėžis River upper reaches). In fish communities, the largest range of $\delta^{13}C$ values (3.1) was recorded in the Žeimena upper reaches, and the smallest (1.9) in regulated stretches of the Nevėžis upper reaches. The decrease in the range of $\delta^{13}C$ values in the regulated stretch of the Nevėžis upper reaches could be explained by the decreased variety of primary production that is assimilated by hydrobiont communities. The total area (TA) of the isotopic space covering $\delta^{13}C$ ir $\delta^{15}N$ values of the invertebrate community members on the biplot ranged from 11.5 for the middle reaches of the Žeimena River to 3.6 for the regulated stretch in the Nevėžis upper reaches.

The total area of the isotopic space (TA) covering $\delta^{13}C$ and $\delta^{15}N$ values of the fish community on the biplot ranged from 5.4 for the middle reaches of the Žeimena River to 2.6 for regulated stretches in the Nevėžis upper reaches. Such a decrease in the isotopic space can be explained by a smaller $\delta^{13}C$ range (CR). Rather small CD and NND values of the invertebrate community in the regulated stretch of the Nevėžis River upper reaches are also related to the decrease in $\delta^{13}C$ range (CR) and in total area (TA). The largest mean distance of fish community members to the centroid (CD) was recorded throughout the Žeimena River and in natural stretches of the Nevėžis upper reaches (1.9–2.1), and the smallest one in the remaining parts of the Nevėžis River (1.1–1.4), which evinces a greater trophic variety in fish communities of the Žeimena River. The mean

nearest neighbour distance (NND) in both rivers was similar, slightly smaller only in the regulated stretch of the Nevėžis upper reaches (1.3). The standard deviation of the nearest neighour distance (SDNND) in both rivers varied slightly. It was found to be somewhat larger in the Žeimena River (0.8–0.9), implying a larger spatial dispersion of invertebrate community members within the common space. SDNND was somewhat smaller (0.5) in the regulated stretch of the Nevėžis upper reaches exhibiting a more even distribution of community members in the $\delta^{13}C$ and $\delta^{15}N$ biplot space (Table 1).

Table 1. Values of isotopic niche parameters of hydrobiont communities in the investigated rivers.

| **Study site** | **Isotopic niche parameters** | | | | | |
|---|---|---|---|---|---|---|
| | **NR** | **CR** | **TA** | **CD** | **NND** | **SDNND** |
| **Macroinvertebrates communities** | | | | | | |
| **Žeimena** | | | | | | |
| Upper reaches | 3.7 | 3.7 | 6.0 | 2.2 | 1.6 | 0.8 |
| Middle reaches | 3.9 | 3.0 | 11.5 | 2.3 | 1.8 | 0.9 |
| Lower reaches | 3.2 | 2.8 | 7.4 | 2.2 | 1.6 | 0.9 |
| **Nevėžis** | | | | | | |
| Upper reaches (natural stretch) | 3.4 | 3.5 | 4.8 | 2.2 | 1.7 | 0.9 |
| Upper reaches (regulated stretch) | 1.9 | 2.1 | 3.6 | 1.1 | 1.3 | 0.5 |
| Middle reaches | 2.3 | 2.8 | 4.4 | 1.9 | 1.6 | 0.8 |
| Lower reaches | 2.1 | 2.5 | 3.7 | 1.9 | 1.4 | 0.6 |
| **Fish communities** | | | | | | |
| **Žeimena** | | | | | | |
| Upper reaches | 3.8 | 3.1 | 4.2 | 2.0 | 1.4 | 0.7 |
| Middle reaches | 3.9 | 2.5 | 5.4 | 1.9 | 1.9 | 0.7 |
| Lower reaches | 4.0 | 2.8 | 4.3 | 2.1 | 1.6 | 0.8 |
| **Nevėžis** | | | | | | |
| Upper reaches (natural stretch) | 3.4 | 2.8 | 4.1 | 1.9 | 1.7 | 1.2 |
| Upper reaches (regulated stretch) | 2.2 | 1.9 | 2.6 | 1.1 | 1.3 | 0.8 |
| Middle reaches | 2.8 | 2.4 | 3.1 | 1.4 | 1.9 | 0.9 |
| Lower reaches | 2.2 | 2.4 | 3.2 | 1.3 | 1.5 | 0.7 |

Parameters: the range of the average $\delta^{15}N$ values in community members (NR), the range of the average $\delta^{13}C$ values in community members (CR), The total area of the isotopic space covering $\delta^{13}C$ and $\delta^{15}N$ values of community members on the biplot (TA), mean distance of community members to the centroid (CD), the mean nearest neighbour distance (NND) and the standard deviation of the nearest neighour distance (SDNND).

Results of the performed canonical correspondence analysis (CCA) showed that environmental factors affect isotopic niche parameters of invertebrate communities. CCA was also used for the impact assessment of environmental factors on isotopic niche parameters of fish communities. Relations of the first axis with environmental variables

revealed the anthropogenic impact on the river food web. The second axis demonstrated the impact of the river gradient on isotopic niche parameters of invertebrate and fish communities.

**Trophic levels of macrozoobenthic genera and sources of organic matter in their diet.** Significant differences ($p < 0.05$) were revealed among different invertebrate genera in the trophic level they occupy and allochtonous organic matter proportion in their diet. This fact shows that genus is a statistically significant factor in determining trophic levels of macrozoobenthos and the proportion of allochtonous organic matter in their diet. Among the invertebrates studied, *Calopteryx* sp. and *Aphelocheirus* sp. were found to occupy the highest trophic levels, while *Brachycentrus* sp., *Simulium* sp. and *Bithynia* sp. the lowest**.** Allochthonous organic matter dominated the diet of *Gammarus* sp. and *Nemoura* sp., constituting the least proportion in that of *Bithynia* sp. The impact of study site-related factors on trophic levels and organic matter proportion in the diet of Žeimena River macrozoobenthos was not significant ($p > 0.05$). This could be explained by the fact that hydrological conditions throughout the Žeimena River are quite similar. Meanwhile, in the Nevėžis River, where ecological conditions are varying, the cumulative impact was found to significantly ($p < 0.05$) affect trophic levels of aquatic invertebrates and the proportion of organic matter in their food. The lowest trophic levels and the least proportion of organic matter in the diet of macrozoobenthos were recorded in the lower reaches, and the highest in the upper reaches of the river.

**Trophic levels of macrozoobenthos ecological groups and sources of organic matter in their diet.** The analysis of invertebrate trophic levels and the proportion of allochthonous organic matter in their food ($p < 0.05$) revealed significant differences among ecological groups of invertebrates. Predators were found to occupy the highest and scrapers the lowest trophic levels among the investigated invertebrates. Allochthonous matter constituted the largest proportion in the diet of shredders, and the smallest in that of scrapers. The impact of study site-related factors on trophic levels of Žeimena River macrozoobenthos ecological groups and the proportion of organic matter in their diet was insignificant ($p > 0.05$) (Fig. 2, 3). This can be explained by the fact that hydrological conditions do not vary much throughout the Žeimena River. Meanwhile, in the Nevėžis River, due to varying ecological conditions, the cumulative impact on

trophic levels and the proportion of organic matter in invertebrate food was found to be statistically significant ($p < 0.05$). The lowest trophic levels and the smallest proportion of allochthonous organic matter were recorded in the lower reaches, while the largest in the upper reaches of the river (Fig. 2, 3).

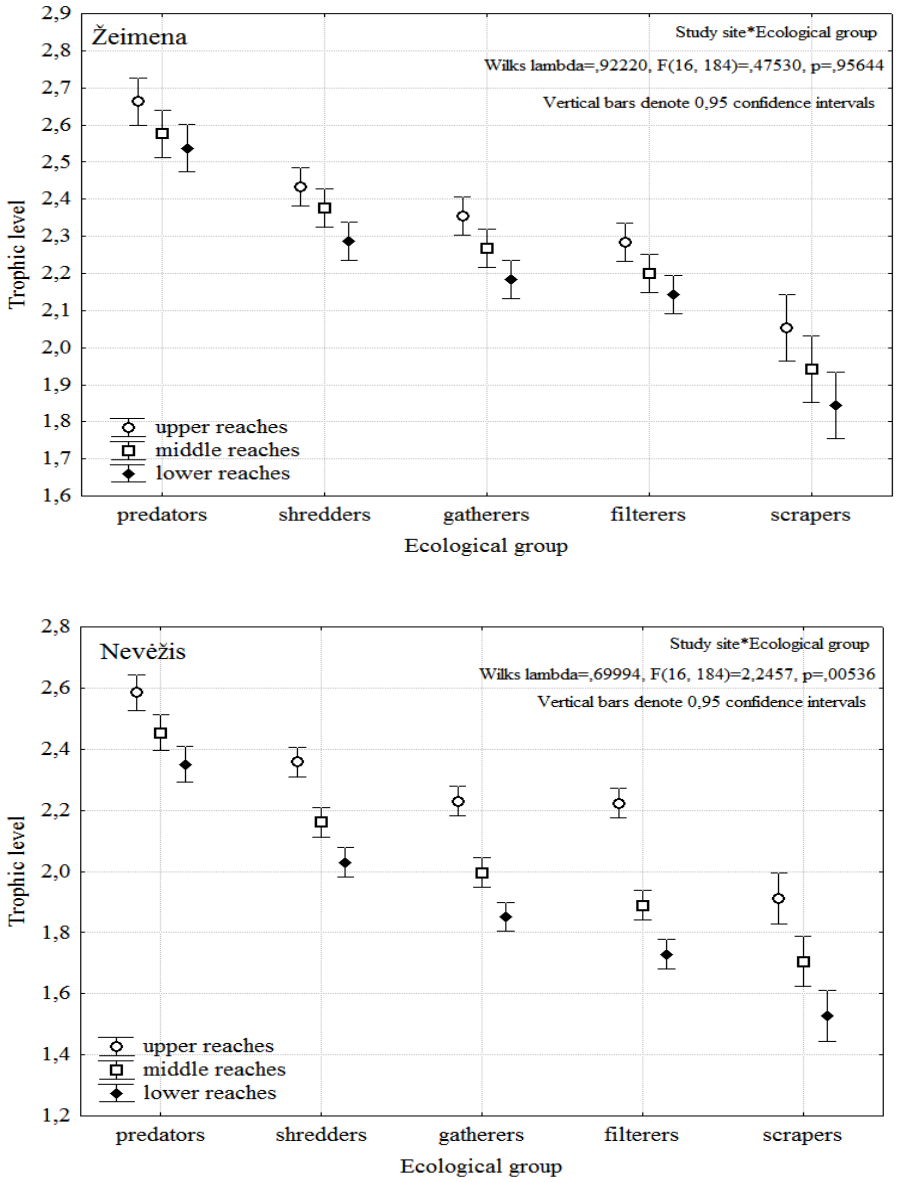


Fig. 2. Impact of study site-related factors on trophic levels of Žeimena River macrozoobenthos ecological groups.

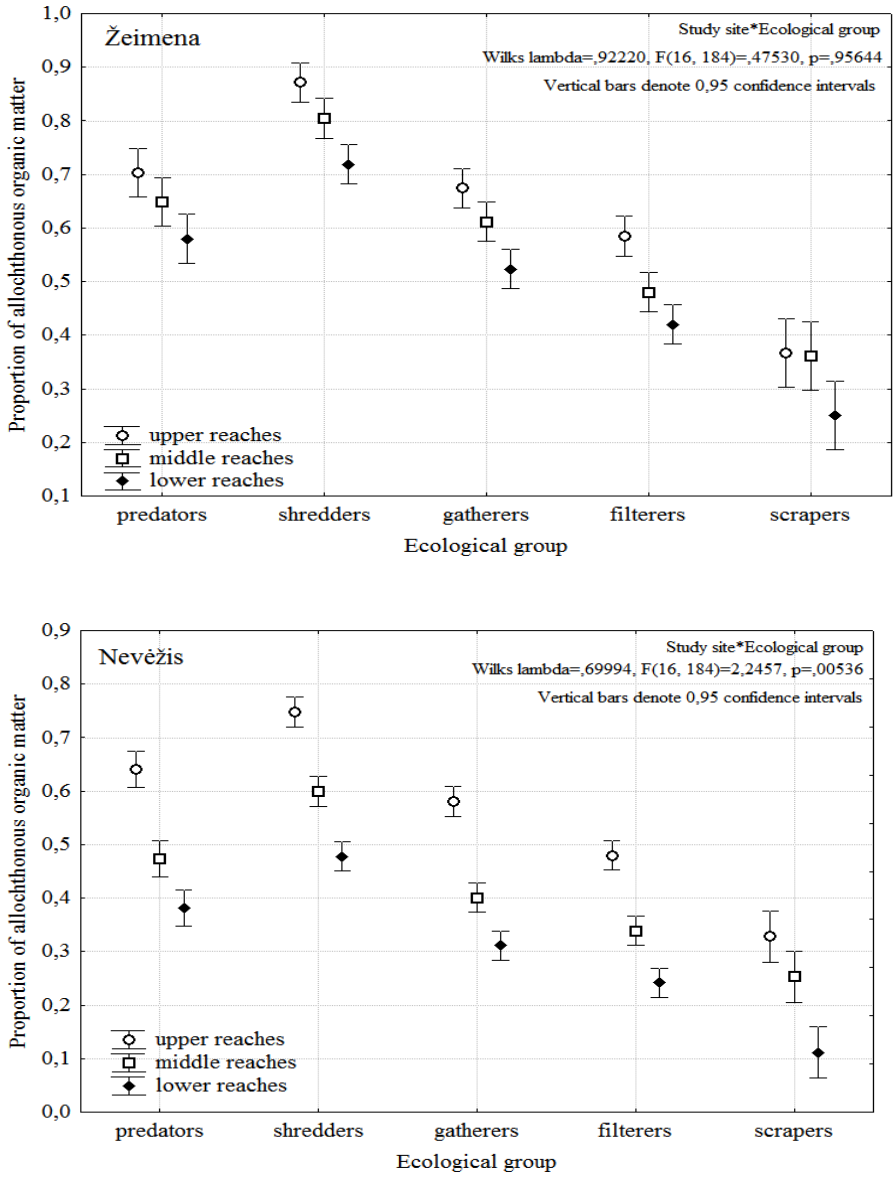


Fig. 3. Impact of study site-related factors on organic matter proportion in the diet of Žeimena River macrozoobenthos ecological groups.

**Spatial impact on trophic levels of macrozoobenthos and sources of organic matter in their diet.** As a factor, the part of the riverbed was found to have a significant impact ($F = 25.44$; $p < 0.05$; $df = 5$) on ML and AO indices of macrozoobenthos. Average trophic levels of invertebrates were found to be the lowest in the Nevėžis lower reaches (1.92±0.1), and the highest in the upper reaches of the Žeimena River (2.38±0.1) (Fig. 4). The lowest index averages of allochthonous organic matter were recorded in the Nevėžis lower reaches (0.33±0.1), and the highest in the Žeimena upper reaches (0.68±0.1) (Fig. 5).

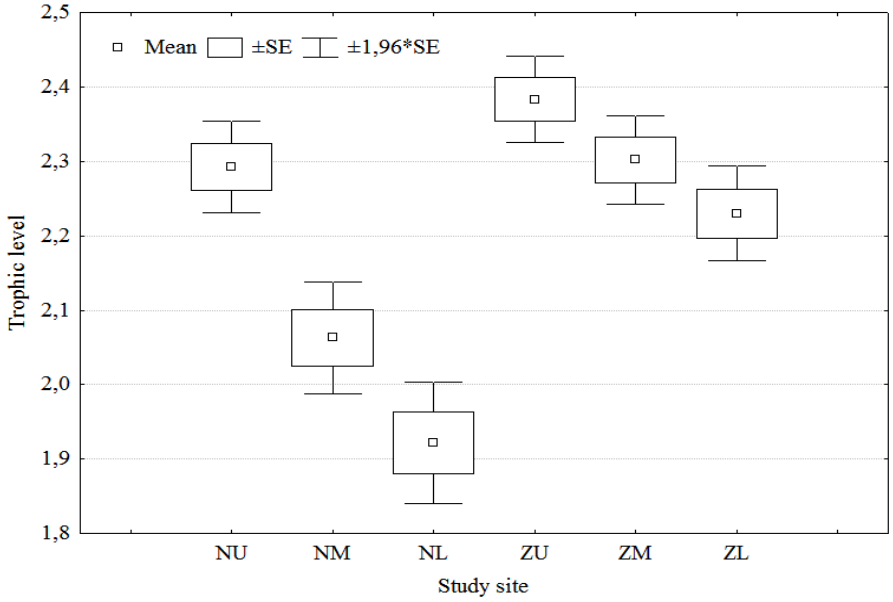


Fig. 4. Trophic levels of invertebrates in the Žeimena and Nevėžis Rivers (NU – upper reaches of the Nevėžis River; NM – middle reaches of the Nevėžis River; NL – lower reaches of the Nevėžis River; ZU – upper reaches of Žeimena River; ZM – middle reaches of the Žeimena River; ZL – lower reaches of the Žeimena River).

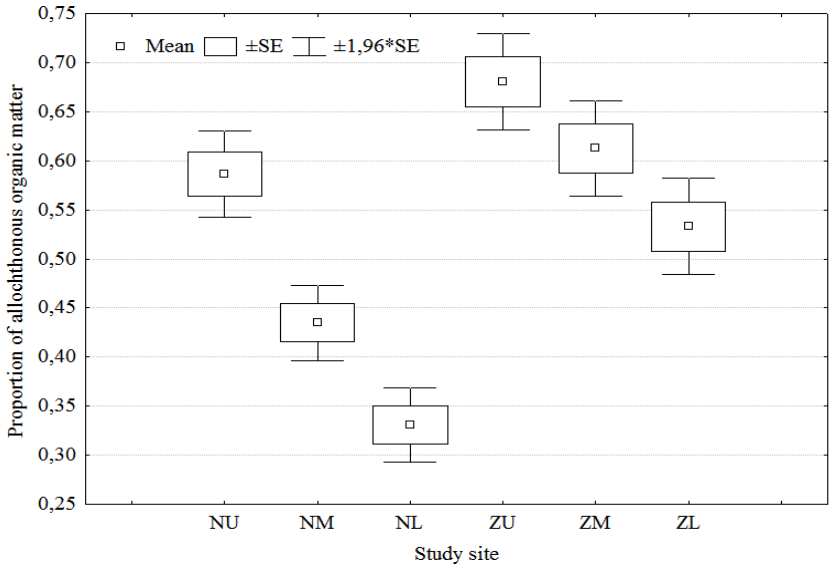


Fig. 5. Proportion of allochthonous organic matter in invertebrates diet in the Žeimena and Nevėžis Rivers (NU – upper reaches of the Nevėžis River; NM – middle reaches of the Nevėžis River; NL – lower reaches of the Nevėžis River; ZU – upper reaches of Žeimena River; ZM – middle reaches of the Žeimena River; ZL – lower reaches of the Žeimena River).

**Trophic levels of fish species.** The analysis of trophic levels and proportion of allochthonous organic matter in fish diet revealed significant differences ($p < 0.05$) among different species. Among the species studied in these rivers, *E. lucius* was found to occupy the highest trophic level and *R. rutilus* the lowest. The highest proportion of allochthonous organic matter was determined in the diet of *L. leuciscus* and *L. cephalus,* and the lowest in that of *R. rutilus* and *G. gobio.* The impact of study site-related factors on trophic levels of Žeimena River fish and organic matter proportion in their diet was not significant ($p > 0.05$), which was due to similar hydrological conditions throughout the river. On the contrary, in the Nevėžis River, where ecological conditions are varying, the cumulative impact on trophic levels of fish and proportion of organic matter in their diet was found to be significant ($p < 0.05$). The lowest trophic levels and the smallest proportion of allochthonous organic matter were documented in the lower reaches, and the highest in the upper reaches.

**Trophic levels of ecological fish groups and allochthonous organic matter in their diet.** The study of fish trophic levels revealed statistically significant ($p < 0.05$) differences among ecological fish groups at all river sites. We did not find any statistically significant differences in allochthonous organic matter indices between benthivorous and piscivorous fishes. Among the investigated ecological fish groups, piscivorous fish were found to occupy the highest trophic levels, while benthivorous the lowest. As a factor, the part of the Žeimena riverbed was not found to affect ($p > 0.05$) ML indices of piscivorous and benthivorous fish. However, it had a significant ($p < 0.05$) impact on indices of the allochthonous organic matter. Meanwhile, the part of the Nevėžis riverbed, as a factor, made a significant ($p < 0.05$) impact on ML and AO indices of ecological groups of benthivorous fish (Fig. 6, 7).

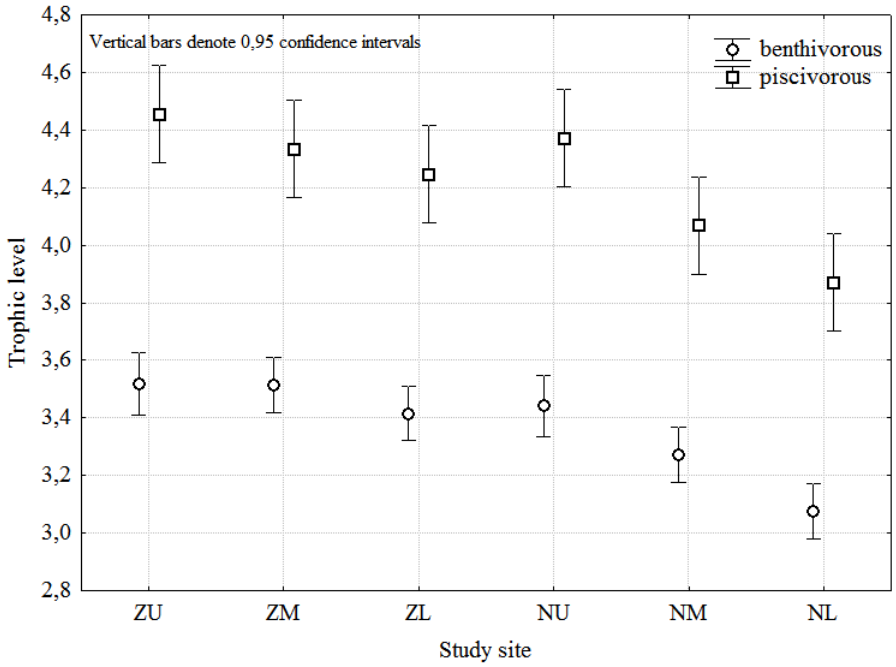


Fig. 6. Trophic levels of ecological fish groups in the Žeimena and Nevėžis Rivers (NU – upper reaches of the Nevėžis River; NM – middle reaches of the Nevėžis River; NL – lower reaches of the Nevėžis River; ZU – upper reaches of Žeimena River; ZM – middle reaches of the Žeimena River; ZL – lower reaches of the Žeimena River).

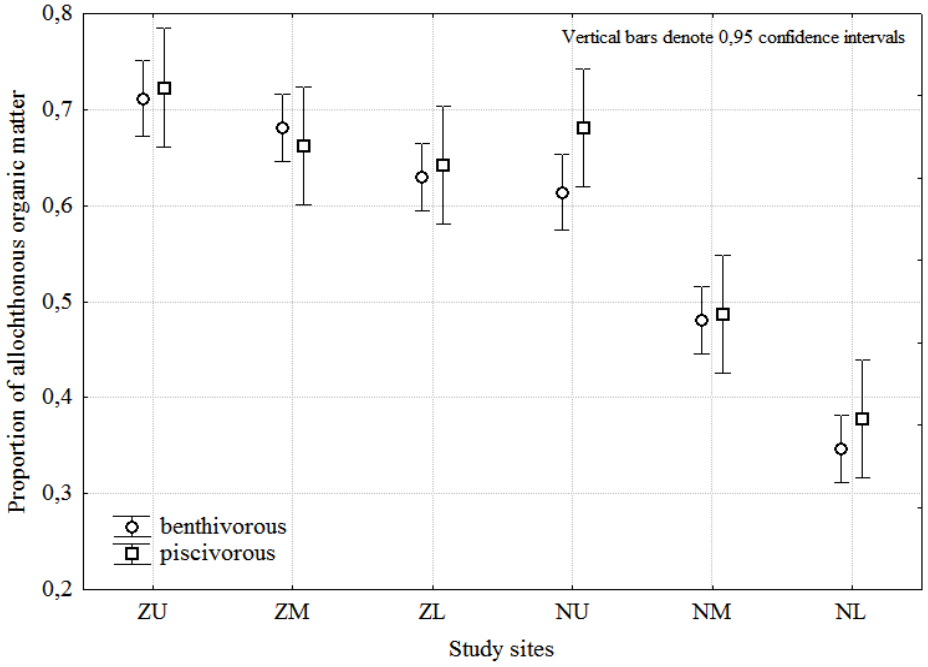


Fig. 7. Proportion of allochthonous organic matter in ecological fish groups in the Žeimena and Nevėžis Rivers (NU – upper reaches of the Nevėžis River; NM – middle reaches of the Nevėžis River; NL – lower reaches of the Nevėžis River; ZU – upper

reaches of Žeimena River; ZM – middle reaches of the Žeimena River; ZL – lower reaches of the Žeimena River).

**Spatial impact on trophic levels and source of organic matter.** As a factor, the part of the riverbed was found to exert a significant ($p < 0.05$) impact on fish ML indices. Average trophic levels of fish were found to be the lowest (3.26±0.1) in the lower reachs of the Nevėžis River, and the highest (3.78±0.1) in the upper reaches of the Žeimena River (Fig.10). As a factor, the part of the riverbed proved to have a significant ($p < 0.05$) impact on fish AO indices. The lowest index averages of the allochthonous organic matter were determined in the Nevėžis lower reaches (0.35±0.1), and the highest in the upper reaches of the Žeimena River (0.71±0.1) (Fig. 11).

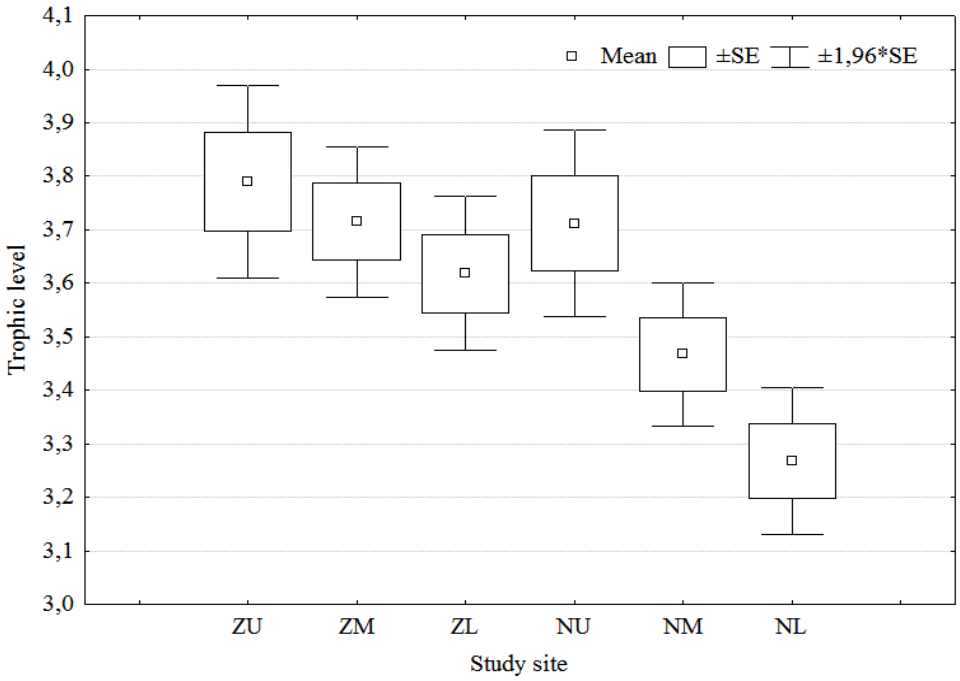


Fig. 10. Trophic levels of fish in the Žeimena and Nevėžis Rivers (NU – upper reaches of the Nevėžis River; NM – middle reaches of the Nevėžis River; NL – lower reaches of the Nevėžis River; ZU – upper reaches of Žeimena River; ZM – middle reaches of the Žeimena River; ZL – lower reaches of the Žeimena River).

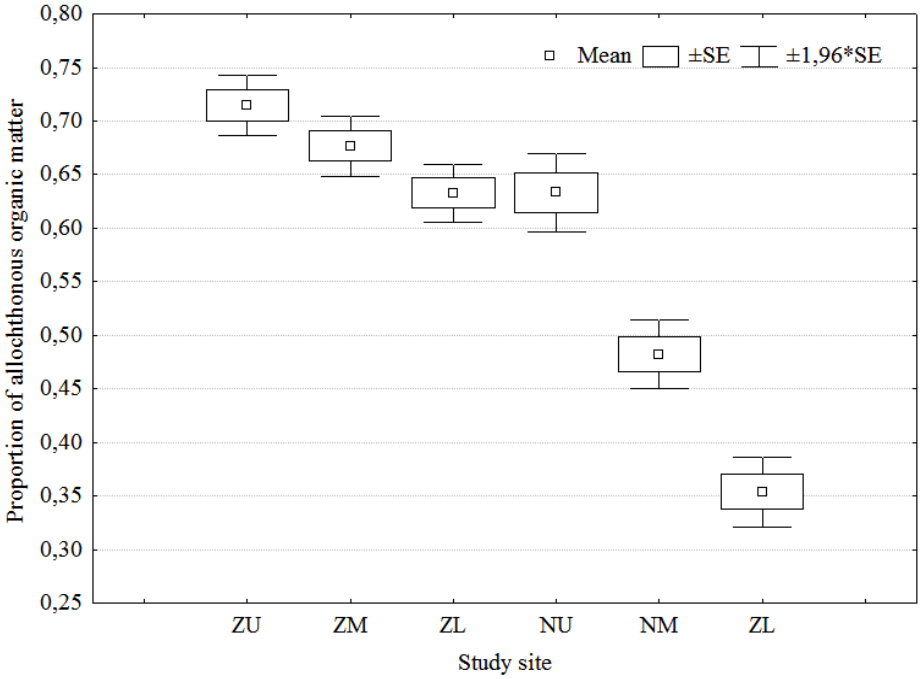


Fig. 11. Proportion of allochthonous organic matter in ecological fish groups in the Žeimena and Nevėžis Rivers (NU – upper reaches of the Nevėžis River; NM – middle reaches of the Nevėžis River; NL – lower reaches of the Nevėžis River; ZU – upper reaches of Žeimena River; ZM – middle reaches of the Žeimena River; ZL – lower reaches of the Žeimena River).

**Impact of environmental factors on food chains and organic matter proportion**. Distance from the confluence, watershed area, macrophyte coverage, canopy cover, channel width, mean depth, flow velocity, bottom substrate, discharge, suspended solids, $BOD_7$, total N and total P were the most important factors for the food chains of macroinvertebrates and fish and organic matter proportion in their diet.

**Influence of the riverbed morphology on river foodwebs.** Invertebrates collected from natural and regulated stretches of the Nevėžis upper reaches showed significantly different trophic levels and averages of allochthonous organic matter proportion. In a natural river stretch, invertebrate trophic levels were found to be significantly ($p < 0.05$) higher both overall (natural: 2.28 ± 0.1; regulated: 2.13 ± 0.1) and for each genus individually. The proportion of the allochthonous organic matter in the diet of invertebrates was significantly ($p < 0.05$) higher both overall (natural: 0.57 ± 0.0; regulated: 0.45 ± 0.0) and for each genus individually. Among the invertebrates studied,

*Calopteryx* sp. and *Aphelocheirus* sp. were found to occupy the highest trophic levels, and *Bithynia* sp.the lowest. The highest proportion of allochthonous organic matter was determined in the diet of shredders *Gammarus* sp. and *Asellus* sp. feeding on detritusand the lowest inthat of the scraper *Bithynia* sp. feeding on periphyton.

Algal carbon isotope ratios at all study sites in the Nevėžis River were found to be more depleted (mean $\delta^{13}C = -34.2 \pm 0.1$) than those of terrestrial (riparian) sources (mean $\delta^{13}C = -29.0 \pm 0.2$). However, there was no statistically significant difference observed in isotope ratios of terrestrial detritus ($p > 0.05$) collected from natural and regulated sites. The mean $\delta^{15}N$ values of periphyton ($\delta^{15}N = 9.31 \pm 1.22‰$) were higher than those of terrestrial detritus at all sites ($\delta^{15}N = 6.3 \pm 0.17‰$; $t = -5.99$, $p < 0.001$, $df = 10$) (Fig. 12). Neither $\delta^{13}C$ nor $\delta^{15}N$ of periphytonsignificantly differed between natural and regulated reaches. Likewise, neither $\delta^{13}C$ nor $\delta^{15}N$ of terrestrial detritus differed between natural and regulated stretches.

Averages of $\delta^{15}N$ isotope values in benthivorous fishes from the regulated river stretch were found to be significantly higher (15.90±1.34 ‰ in the regulated stretch; 13.75±1.55 ‰ in the natural one; $p < 0.001$). The nitrogen isotope ratio increase is related to the eutrophication and pollution. Benthivorous fish collected from natural and regulated sites exhibited significantly different mean (±SD) nitrogen isotope ratios ($\delta^{15}N$ for regulated $15.90 \pm 1.34‰$ and $13.75 \pm 1.55‰$ for natural sites; $t = -3.18$, $p < 0.001$). As for carbon isotope ratios in fish from regulated and natural stretches, the difference was also significant ($\delta^{13}C$ for regulated $-33.01 \pm 0.71‰$ and $-31.82 \pm 1.15‰$ for natural sites, $p < 0.001$). As for piscivorous fish, the difference in $\delta^{15}N$ values was significant between natural ($14.17 \pm 1.74‰$) and regulated ($15.68 \pm 1.10‰$) sites ($t = -2.20$, $p < 0.05$, $df = 16$). The decrease in $\delta^{13}C$ values was also significant: from $-30.02 \pm 0.83‰$ in natural, to $-32.06 \pm 0.46‰$ in regulated river stretches ($t = 6.24$, $p < 0.001$, $df = 16$). Piscivorous fish were consistently found enriched in $\delta^{13}C$ compared with benthivorous fish ($p < 0.001$).

All fishes at natural sites were more $^{13}C$-enriched indicating their feeding on terrestrial (riparian) food sources. The mean $\delta^{13}C$values of fish at natural sites (–30.02±0.8 ‰) were more similar to those of detritus (–29.0 ± 0.2) than to those of algae

(–34.2±0.1). Fish at regulated sites show depletion in $\delta^{13}C$ (–32.06±0.4 ‰), suggesting that the basal resources for aquatic invertebratesare not of allochthonous, but of autochthonous origin.

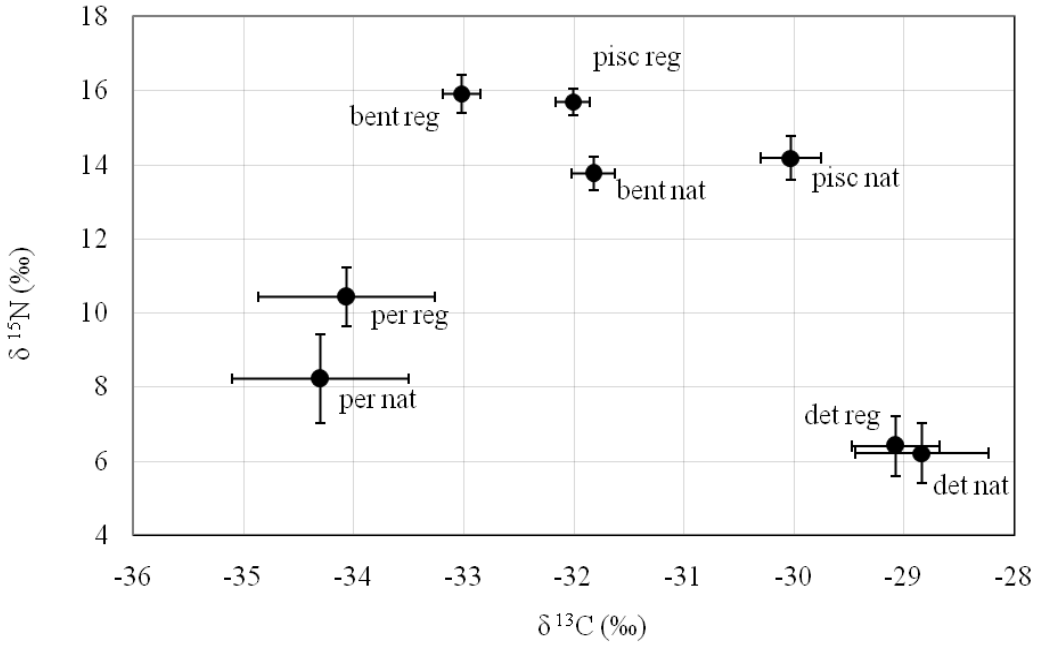


Fig. 12. Mean (±SE) carbon and nitrogen isotope ratios for benthivorous (bent) and piscivorous (pisc) fish, perphyton (per) and detritus (det) collected from natural (nat) and regulated (reg) stretches of the Nevėžis River.

## CONCLUSIONS

1. Fish community structure in the rivers studied was found to depend on ecological conditions therein.The ecological state of the Žeimena River was assessed as good or very good (LFI = 0.85–1.03), whereas that of the Nevėžis River as moderate (LFI = 0.41–0.39).
2. Trophic levels and the proportion of organic matter in hydrobionts diet were found to be statistically significantly dependent on their function in the food web. Among invertebrates, the largest proportion of allochthonous organic matter was recorded in the diet of shredders and the smallest in that of scrapers.Among fish, the largest proportion of allochthonous organic matter was recorded in the food of water column feeders and the smallest in that of bottom feeders.
3. There were no differences found in trophic levels and organic matter proportion in the diet of hydrobionts in the Žeimena River, whereas in the Nevežis River, due to variations in hydrological conditions along the river gradient, these parameters were found to be statistically significantly different, with the lowest values recorded in the lower reaches, and the highest – in the upper reaches.
4. The study showed that the proportion of allochthonous organic matterin the diet of hydrobionts from natural Nevėžis River stretches is statistically significantly higher than in that from regulated stretches. The higher $\delta^{13}C$ values estimated in hydrobionts from natural river stretches indicate that their food sources are from riparian ecosystems. The lower $\delta^{13}C$ values in hydrobionts from regulated river stretches suggest that their basic food resources are of the autochthonous origin.
5. Trophic levels of hydrobionts in natural Nevėžis River stretches are statistically significantly higher than in regulated stretches. Due to increased eutrophication and anthropogenic pollution, the average isotope $\delta^{15}N$ values calculated for hydrobionts from regulated stretches of the Nevėžis River were found to be statistically significantly higher than those from natural stretches.
6. The results obtained show that trophic levels of hydrobionts, the proportion of organic matter in their diet and parameters of river food webs depend on the river stretch morphology and the cumulative effect of environmental factors determining the general ecological state of rivers.

## LIST OF PUBLICATIONS ON THE DISSERTATION TOPIC

### Articles

**Čivas L.,** Kesminas V., Sullivan S.M.P. 2016. Influences of hydrogeomorphology and chemical water quality on fish assemblages in the Nevėžis River, Lithuania: implications for river basin management plans in the Baltics. *Environmental Monitoring and Assessment* **188** (109): 1–16.

**Čivas L.,** Kesminas V., Barisevičiūtė R. 2016. Impact of the Riverbed Morphology on the Source of Organic Material and the Trophic Structure of Fish Community along the Upper Reaches of the Nevėžis River, Lithuania. *Acta Ichthyologica et Piscatoria* **46** (4): 303–312.

### Conference reports

**Čivas L**. Fish distribution and ecological state of the Nevėžis River. Conference of Young Scientists "Biofuture: perspectives of natural and life sciences", Lithuanian Academy of Sciences, Vilnius, 2014.

**Čivas L**. Fish stable istope $\delta^{13}C$ ir $\delta^{15}N$ analysis in natural and regulated stretches of the Nevėžis River. Conference of Young Scientists "Biofuture: perspectives of natural and life sciences", Lithuanian Academy of Sciences, Vilnius, 2015.

Kesminas V., Virbickas T., Leliūna E. and **Čivas L.** Status and restoration of salmon (Salmo salar L.) stocks in the Nemunas River Basin, Lithuania. XV European Congress of Ichthyology. Porto (Portugal), September 7–11, 2015

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