

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

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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 Ichthyol. Piscat.* 46 (4): 303–312.

Background. The catchment of the Nevėžis River, the sixth longest river in Lithuania, is among the most affected by agricultural activities. Although seriously disturbed by land reclamation and agricultural pollution, the Nevėžis River still has natural, anthropogenically unaffected stretches. Fish species were studied at two sites of the upper part of the river with different hydrological regime, exhibiting clear differences in the trophic status of fish. The combined effect of hydrogeomorphic and water-chemistry variability on the stable isotope composition of fish assemblages in Lithuania has not been investigated. The presently reported study was intended to explore the possible effects of the riverbed morphology and agricultural activities on the chemistry and stable isotope composition of the three main fish species.

Materials and methods. This study covered three species of freshwater fishes: the European perch, *Perca fluviatilis* Linnaeus, 1758; the roach, *Rutilus rutilus* (Linnaeus, 1758); and the northern pike, *Esox lucius* Linnaeus, 1758. The sampling was performed in both natural and regulated river stretches using backpack electrofishing equipment. Stable isotope composition of the samples was revealed by applying stable isotope analyses (SIA) with an elemental analyser connected to an isotope ratio mass spectrometer. To obtain more information about environmental variability and variation of stable isotopes $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in fish, the entire data matrix was analysed using Principal Component Analysis (PCA).

Results. In the study sites on the Nevėžis River, algal carbon isotope ratios were more ^{13}C -depleted than those from terrestrial sources. The fishes were clearly dependent on terrestrial carbon sources in natural locations. However, the fishes collected from regulated sites had carbon isotope signatures more similar to algal than to terrestrial sources. Mean values of $\delta^{15}\text{N}$ of the fishes from the natural sites were lower than those from the regulated ones.

Conclusion. The presently reported study revealed impact of abiotic habitat variables indicating the importance of physicochemical and morphological characteristics on $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotopic values variance of fish at natural and regulated river sites. Our findings would help to evaluate and predict changes on river food webs that occur due to hydromorphological alterations determined by human activity and climate change. The results could be useful for fisheries, i.e., impact of environmental factors on fish nutrition and growth.

Keywords: food web, hydrogeomorphology, organic matter, C and N stable isotopes, water chemistry, perch, roach, pike

INTRODUCTION

Many rivers and streams have been channelized for various purposes. Channelization reduces the heterogeneity of physical habitats in rivers and streams (Bis et al. 2000, Pliūraitė and Kesminas 2010) and is the most significant threat to the biodiversity and functioning of the river ecosystems worldwide (Rosenberg et al. 2000, Nakamura and Yamada 2005).

Stable isotopes are a useful tool for quantifying the energy and nutrient flow in ecosystems (Fry 2006, Jardine et al. 2006). When using stable isotopes in ecological research, the most common elements are carbon ratios ($^{13}\text{C} \div ^{12}\text{C}$), whose isotopic signatures reveal the source material, and nitrogen ratios ($^{15}\text{N} \div ^{14}\text{N}$), whose signatures reflect the trophic position of animals (Post 2002). Stable isotope analysis is a powerful analytical tool that complements classical studies because it provides

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data about the partitioning of nutrients to body tissues, revealing the source of the food that is assimilated rather than simply ingested (Trakimas et al. 2011).

Stable nitrogen isotope ratios in animals can serve as indicators of the trophic level because animals tend to be enriched in $\delta^{15}\text{N}$ by 3‰–4‰ relative to the dietary ^{15}N (Deniro and Epstein 1981, Minigawa and Wada 1984, Fry 1988). Elevated nitrogen isotope ratios in particular have been related to increasing eutrophication and pollution (Cabana and Rasmussen 1996, Costanzo et al. 2001, Savage and Elmgren 2004), whereas carbon isotope ratios (in littoral and pelagic environments) may depend more on the lake area (Post 2002) and/or the relative contributions of autochthonous and allochthonous organic matter (Grey et al. 2001).

For species that are unlikely to show appreciable dietary differences due to sex or habitat segregation, the approach could be particularly effective in revealing individual specialization, which has recently received more attention (Bolnick et al. 2003, Svanbäck and Persson 2004, Sánchez-Hernández et al. 2012, Pliūraitė et al. 2013, Pilinkovskij et al. 2014). The European perch, *Perca fluviatilis* Linnaeus, 1758; the roach, *Rutilus rutilus* (Linnaeus, 1758); and the northern pike, *Esox lucius* Linnaeus, 1758 are common fish species in many central European rivers including the Nevėžis River. In addition, these species typically become more abundant with progressive river eutrophication leading to intensified competition for resources (Syväranta and Jones 2008). Each species was studied in two stretches of the upper part of the Nevėžis River with different hydrological regime, i.e., in natural and regulated stretches, exhibiting clear differences in the trophic status of fish.

Despite the strong influence of human activities in the basin, the Nevėžis River still supports a diverse ichthyofauna, composed of a mixed cool- to warm-water assemblages (Žiliukas 1999, Čivas et al. 2016). However, the dual influence of hydrogeomorphic and water-chemistry variability on the stable isotope composition of fish assemblages has not been investigated. In Lithuania, the stable isotope analysis of aquatic ecosystems was carried out only in the Curonian Lagoon (Lesutienė et al. 2007, 2008) and in some lakes (Rakauskas et al. 2013, Rakauskas 2014) for the purpose of ascertaining the role of invasive crustaceans in food webs.

The presently reported study was intended to explore the possible effects of the riverbed morphology and agricultural activities on the chemistry and stable isotope composition of three main fish species: perch, *Perca fluviatilis*; roach, *Rutilus rutilus*; and pike, *Esox lucius*. The goals of this study were: to estimate the natural abundance of stable carbon ($^{13}\text{C} \div ^{12}\text{C}$) and nitrogen ($^{15}\text{N} \div ^{14}\text{N}$) isotopes in the main fish species representing different feeding guilds in the river; to assess and compare physicochemical characteristics in natural and regulated stretches of the river; to explore the influence of physical and chemical water parameters' variability on fish stable carbon and nitrogen isotopes; and to evaluate the relative contribution of basal energy sources, i.e., macroalgae and terrestrial detritus, to the nutrition of the main fish species.

MATERIALS AND METHODS

Study area. The study was conducted in July 2013 in the Nevėžis River in two sections: natural (55°68'N, 24°45'E; 151–159 km of the river course) and regulated (55°58'N, 24°62'E; 183–175 km; Fig. 1). The criterion for distinguishing the sections was their riverbed morphology. In total, the studied stretch was ~16 km long. The longitudinal distance between the investigated natural and regulated river stretches sites was 13 km of the river flow. To eliminate the impact of season and meteorology, samples of environmental variables, fish and primary sources were collected simultaneously, repeatedly three times for each site, during the same month, in July, when low stream flows facilitated effective sampling and large-scale seasonal fish movement was unlikely to occur.

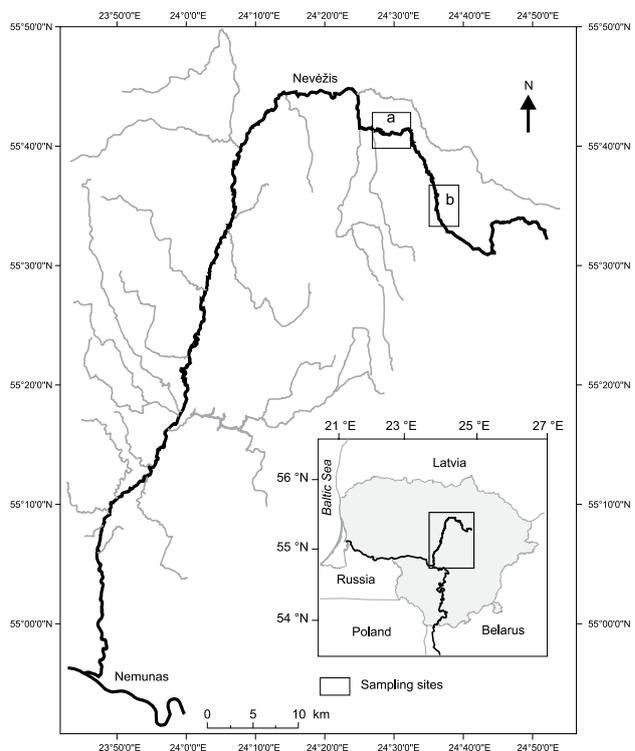


Fig. 1. Location of sampling stations on the Nevėžis River: a = natural stretch; b = regulated stretch

The Nevėžis River (the district of Panevėžys, central Lithuania) drains the catchment of 6141 km² and flows for 210 km before merging with the Nemunas River (Fig. 1). It is one of the longest rivers in Lithuania, the fourth largest (by basin area), and one of those with the lowest elevation (Kilkus and Stonevičius 2011). In the upper part of the river valley, the Nevėžis River flows in the northwest direction. Some upper sections of the river are channelized. Industrial operations have led to both the water withdrawal and the elevated levels of inorganic substances in the water (Paukštys et al. 2011). Agricultural areas account for ~45% of the catchment area, and ~90% of this land is drained (Kilkus and Stonevičius 2011). Riparian areas are largely deforested although remnant riparian bands are quite common. Although seriously affected by land reclamation and agricultural pollution,

the Nevėžis River still has natural, anthropogenically unaffected stretches. For the current research, we selected two stretches: natural and regulated. They are both located in the upper river reaches, between which there are no hydraulic barriers, with the exception of some 0.5 m high spillways for slowing down water.

Physicochemical characteristics. On each sampling site, 13 environmental variables were recorded (Table 1). All the environmental variables were measured or visually estimated on sampling sites along 12 evenly spaced transects using standardized procedures (Simonson et al. unpublished*). Biochemical oxygen demand (BOD_7) ($mg\ O_2 \cdot L^{-1}$), total nitrogen ($mg \cdot L^{-1}$), total phosphorus ($mg \cdot L^{-1}$) concentrations, and total suspended solids ($mg \cdot L^{-1}$) were determined according to APHA (Anonymous 1985). Water sampling as well as in situ measurements of physicochemical parameters was performed in the subsurface of the mid-channel.

Wet river width (m) and water depth (m) were measured. Flow rate was estimated at four-tenths of the water depth above the stream bed using a flow meter (type Swoffer 2100). Water discharge ($m^3 \cdot s^{-1}$), mean flow ($m^3 \cdot s^{-1}$), and the maximum and mean depths (m) were then calculated. The percentage of the riparian zone coverage (at the edge between the water channel and the banks) as well as that of its canopy over the water surface, aquatic vegetation, and substrate composition were estimated visually. The substratum class within each study location was classified and estimated visually (%) according to Platts et al. (1983) as silt (particle size < 0.6 mm), sand (0.6–2.0 mm), gravel (2.0–20.0 mm), pebble (20.0–60.0 mm), and stones (more than 60.0 mm). Characteristics of the bottom substrate were presented in scores by coding classes in order of coarseness: 1 = silt; 2 = sand; 3 = gravel; 4 = pebble; 5 = stones.

The channel width and BOD_7 values did not differ between natural and regulated sites (Table 1). Canopy cover, flow velocity, and discharge values were lower on regulated than on natural sites. Water depth was considerably lower on natural than on regulated sites. The substrates of regulated sites differed from those of natural sites. Regulated sites had sand-silt substrates, whereas natural ones had pebble substrates. The highest values of suspended solids and macrophyte coverage parameters were recorded on regulated sites. Total nitrogen and total phosphorus concentrations on regulated sites in the Nevėžis River were greater than on natural sites. Descriptive statistics for the variables analysed can be found in Table 1, showing an array of conditions throughout the study area.

Fish surveys. Fish sampling was performed in both natural and regulated river stretches using backpack electrofishing equipment (HANS Grassl GmbH IG 200/2, Germany). Fish were sampled across the study reach performing single-pass electric fishing by wading upstream against the current (Arbačiauskas et al. 2009). All habitats were sampled, including riffles, lotic flats, and some oxbows. We collected fish species that appeared to be the most abundant on the study sites. All individuals were measured and the total length (TL), standard length (SL) (mm), and weight (W) (g) of each fish were recorded (Thoresson 1996).

Perch, roach, and pike were divided into two length classes (see Table 2) as it is known that these fish undergo ontogenetic niche shifts (Hjelm et al. 2000, Horppila et al. 2000, Svanbäck and Eklov 2002, Hjelm et al. 2003). In this study, roach and perch individuals < 150 mm and pike individuals < 250 mm were regarded as immature and were assigned to “group I” while mature individuals to “group II” (Kesminas and Virbickas 2000, Virbickas and Kesminas 2007).

Table 1

Comparison of environmental variables' characteristics in natural and regulated stretches surveyed in the Nevėžis River

Physicochemical conditions	Riverbed morphology		<i>t</i> -test value	<i>P</i> value
	Natural	Regulated		
Distance from confluence [km]	154.1 ± 1.1	181.0 ± 1.9	12.71	< 0.001
Watershed area [km ²]	331.9 ± 48.9	179.1 ± 14.1	3.00	0.039
Macrophyte coverage [%]	35.0 ± 2.3	76.0 ± 3.8	8.91	< 0.001
Canopy cover [%]	30.0 ± 2.8	5.0 ± 1.7	7.42	0.002
Channel width [m]	15.0 ± 1.7	13 ± 1.2	0.96	0.391
Mean depth [m]	0.4 ± 0.1	1.0 ± 0.0	5.19	0.006
Flow velocity [$m \cdot s^{-1}$]	0.4 ± 0.1	0.1 ± 0.0	8.00	0.001
Discharge [$m^3 \cdot s^{-1}$]	6.3 ± 0.4	1.3 ± 0.1	12.63	< 0.001
Suspended solids [$mg \cdot L^{-1}$]	2.7 ± 0.2	6.1 ± 0.5	11.60	< 0.001
BOD_7 [$mg\ O_2 \cdot L^{-1}$]	1.5 ± 0.2	2.2 ± 0.2	2.46	0.069
Total N [$mg \cdot L^{-1}$]	3.5 ± 0.4	5.7 ± 0.5	3.35	0.028
Total P [$mg \cdot L^{-1}$]	0.07 ± 0.0	0.19 ± 0.0	5.64	0.004
Bottom substrate	stones – gravel	sand – silt	4.65	< 0.001

As data on the bottom substrate were non-parametric, Mann–Whitney U test was used for the comparison of mean values.

* Simonson T.D., Lyons J., Kanehl P.D. 1994 Guidelines for evaluating fish habitat in Wisconsin streams. General technical report, U.S. Department of Agriculture, Forest Service, North Central Forest Experiment Station, Minnesota, St. Paul.

Table 2

Fish species in respective length classes studied for stable isotope analyses

Functional feeding group	Species	Length class		TL range [mm]	N
		Class	TL [mm]		
Benthivorous	<i>Rutilus rutilus</i>	I	<150	50–147	18
		II	>150	167–260	18
Piscivorous	<i>Perca fluviatilis</i>	I	<150	90–143	18
	<i>Perca fluviatilis</i>	II	>150	175–202	15
	<i>Esox lucius</i>	I	<250	215–240	12
	<i>Esox lucius</i>	II	>250	285–340	9

TL = total length, N = number of replicates.

Stable isotope analysis. The major primary sources were collected by hand from submerged rocks or logs in all available habitats on a given site, including leaves from riparian trees, aquatic macrophytes, filamentous green algae (*Cladophora* sp.). For periphyton, acid-washed (10% HCl) filters were used to remove inorganic carbon. We rinsed macroalgae with deionized water and then removed invertebrates and particulate matter. All samples were placed into separate vials, then kept on ice in the field and subsequently frozen until prepared for stable isotope analysis. A small piece of fish white muscle tissue was dissected posteriorly to the dorsal fin and dried at 60°C for 48 h, homogenised (ground to fine powder) using an agate pestle and a mortar, and assigned to 3–6 analytical replicates (3–5 individuals of each taxonomic/size group per replicate), which were weighed in tin cups and combusted with an elemental analyser (FlashEA 1112) connected to an isotope ratio mass spectrometer (Thermo Finnigan Delta Plus Advantage). Carbon and nitrogen isotope data are reported as δX values (where X represents the heavier isotope ^{13}C or ^{15}N) or differences from the given standards, expressed in parts per thousand (‰) and are calculated according to the formula:

$$\delta X = (R_{\text{sample}} \times R_{\text{standard}}^{-1} - 1) \times 10^{-3}$$

where R_{sample} is the $^{13}\text{C} \div ^{12}\text{C}$ ratio or $^{15}\text{N} \div ^{14}\text{N}$ of sample, $R_{\text{standard}} = ^{13}\text{C} \div ^{12}\text{C}$ or $^{15}\text{N} \div ^{14}\text{N}$ of standard. The global references were atmospheric N_2 for the $^{15}\text{N} \div ^{14}\text{N}$ ratio, and Vienna Pee Dee belemnite for the $^{13}\text{C} \div ^{12}\text{C}$ ratio. Typical precision for a single analysis was $\pm 0.2\text{‰}$ for $\delta^{13}\text{C}$ and $\pm 0.3\text{‰}$ for $\delta^{15}\text{N}$.

Statistical analysis. Comparisons of the environmental variables, carbon and nitrogen isotope ratios between natural and channelized river sites were performed using a paired t -test. Variation in spatial fish $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotopic values with habitat and environmental variables on natural and regulated study sites in the Nevėžis River was summarized through the principal components analysis (PCA). To present PCA results, we calculated eigenvalues, loading factors (at level > 0.5) and projected them onto two first factors presented as a factors' biplot. The mean difference in stable isotope values among fish species was analysed using one-way ANOVA and Tukey's HSD test. Statements of statistical significance refer to P

< 0.05 . All species data were $\log(1 + x)$ transformed prior to analysis. Calculations were done with Statistica for Windows, Version 6.0 (StatSoft, Tulsa, Oklahoma, USA).

RESULTS

Fish surveys. The TL of the fish analysed ranged from 50 to 260 mm for roach, from 90 to 202 mm for perch, and from 215 to 340 mm for pike. Overall, individuals of each fish species were clearly divided into two distinct groups by their feeding mode and $\delta^{13}\text{C}$ – $\delta^{15}\text{N}$ signatures. To illustrate isotopic variation in size, perch, roach, and pike specimens were subsequently classified into length classes I and II (Table 2).

Fish individuals assigned to the first group exhibited depleted $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotopic signatures compared to those of the second group and included mostly benthivorous fish, except pike. The second group of fish had considerably enriched $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures and included some individuals of benthivorous roach, piscivorous perch and pike. There were differences in $\delta^{15}\text{N}$ values among size classes of perch and pike (t -test: $P < 0.05$), but no difference was recorded in $\delta^{15}\text{N}$ between different-sized roach. With respect to roach, there was no difference in $\delta^{13}\text{C}$ between different-sized fish (t -test: $P < 0.05$).

Benthivorous fish collected from natural and regulated sites had significantly different mean (\pm SD) nitrogen isotope ratios ($\delta^{15}\text{N}$ for regulated $15.90\text{‰} \pm 1.34\text{‰}$ and $13.75\text{‰} \pm 1.55\text{‰}$ for natural sites; $t = -3.18$, $P < 0.001$, $df = 18$). As for carbon isotope ratios, the difference was also significant ($\delta^{13}\text{C}$ for regulated $-33.01\text{‰} \pm 0.71\text{‰}$ and $-31.82\text{‰} \pm 1.15\text{‰}$ for natural sites; $t = 4.59$, $P < 0.001$, $df = 18$) (Fig. 2).

As for piscivorous fish, the difference in $\delta^{15}\text{N}$ values was significant between natural ($14.17\text{‰} \pm 1.74\text{‰}$) and regulated ($15.68\text{‰} \pm 1.10\text{‰}$) sites ($t = -2.20$, $P < 0.05$, $df = 16$). The decrease in $\delta^{13}\text{C}$ values was also significant: from $-30.02\text{‰} \pm 0.83\text{‰}$ on natural, to $-32.06\text{‰} \pm 0.46\text{‰}$ on regulated sites ($t = 6.24$, $P < 0.001$, $df = 16$). Piscivorous fish were consistently found enriched in $\delta^{13}\text{C}$ compared with benthivorous fish ($P < 0.001$). However, there was no statistically significant difference in $\delta^{15}\text{N}$ values found between benthivorous and piscivorous fishes ($P > 0.05$).

Algal carbon isotope ratios from all investigated sites in the Nevėžis River were more depleted (mean $\delta^{13}\text{C} = -34.2\text{‰} \pm 0.1\text{‰}$) than those from terrestrial (riparian) sources (mean $\delta^{13}\text{C} = -29.0\text{‰} \pm 0.2\text{‰}$). However, there was no statistically

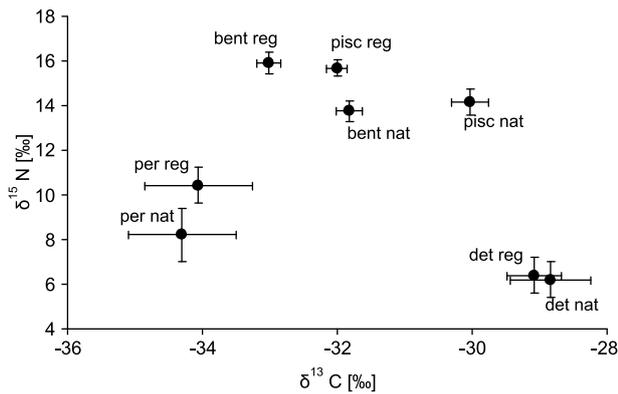


Fig. 2. Mean (\pm SE) carbon and nitrogen isotope ratios for benthivorous (bent) and piscivorous (pisc) fish, periphyton (per) and detritus (det) collected from natural (nat) and regulated (reg) stretches of the Nevėžis River

significant difference in isotope ratios of terrestrial detritus ($P > 0.05$) collected from natural and regulated sites. The mean $\delta^{15}\text{N}$ values of periphyton ($\delta^{15}\text{N} = 9.31\text{‰} \pm 1.22\text{‰}$) were higher than those of terrestrial detritus on all sites ($\delta^{15}\text{N} = 6.3\text{‰} \pm 0.17\text{‰}$; $t = -5.99$, $P < 0.001$, $df = 10$) (Fig. 2). Neither $\delta^{13}\text{C}$ ($t = -1.50$, $P = 0.205$, $df = 4$) nor $\delta^{15}\text{N}$ ($t = -4.15$, $P = 0.060$, $df = 4$) of periphyton significantly differed between natural and regulated reaches. Likewise, neither $\delta^{13}\text{C}$ ($t = -1.47$, $P = 0.194$, $df = 4$) nor $\delta^{15}\text{N}$ ($t = -3.61$, $P = 0.100$, $df = 4$) of terrestrial detritus were different between natural and regulated reaches.

Mean stable isotope values differed among fish species on both natural (one-way ANOVA: $\delta^{13}\text{C}$, $F_{5,13} = 43.72$, $P < 0.001$; $\delta^{15}\text{N}$, $F_{5,13} = 19.19$, $P < 0.001$) and regulated (one-way ANOVA: $\delta^{13}\text{C}$, $F_{5,13} = 20.39$, $P < 0.001$; $\delta^{15}\text{N}$, $F_{5,13} = 55.02$, $P < 0.001$) sites.

Mean $\delta^{13}\text{C}$ values of roach, perch, and pike from natural sites were higher than those from regulated sites, whereas mean values of $\delta^{15}\text{N}$ of the above mentioned fish from natural sites were lower than those from the regulated ones. $\delta^{13}\text{C}$ of fish on regulated sites was significantly lower than that of fishes on natural sites ($P < 0.05$) and generally close to that of algal carbon collected from the same site. Roach of group I were the most ^{13}C -depleted

(natural: mean = $-32.40\text{‰} \pm 0.2\text{‰}$, regulated: mean = $-33.58\text{‰} \pm 0.2\text{‰}$) of all other fish. Meanwhile, pike of group II had ^{13}C -enriched mean values (natural: $-29.06\text{‰} \pm 0.3\text{‰}$, regulated: $-31.73\text{‰} \pm 0.2\text{‰}$) (Table 3).

Predatory fish were ^{15}N -enriched in comparison with benthivorous fish species. The mean $\delta^{15}\text{N}$ value of perch belonging to group I (natural $-12.17\text{‰} \pm 0.2\text{‰}$, regulated $-13.67\text{‰} \pm 0.3\text{‰}$) was depleted compared with other fish. The most enriched in $\delta^{15}\text{N}$ of all other fishes were pike of group II on natural and roach of group II on regulated sites (Table 3). Pike was considered to be the top predator in food chains of the Nevėžis River.

All fish species on natural sites were more ^{13}C -enriched indicating their foraging for terrestrial (riparian) food sources. The mean $\delta^{13}\text{C}$ value of fish on natural sites was more similar to that of detritus than to that of algae. Fish on regulated sites show depletion in $\delta^{13}\text{C}$, suggesting that the basal resources for aquatic invertebrates (which we did not collect) are not derived from terrestrial detritus. In general, on regulated sites, all fish species, except for perch and pike of group II, exhibited the most ^{15}N -enriched values.

To verify the relations between fish $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotopic signatures and environmental variables (fifteen variables were tested in total), the principal component analysis (PCA) was performed in the first place. The PCA extracted eleven factors, of which the first two factors showed eigenvalues > 1 (Table 4). The first three extracted factors explained 89.8% of the variance. Macrophyte coverage, discharge, flow velocity, suspended solids, distance from the confluence, total P, canopy cover, total N, mean depth, BOD_7 , watershed area, bottom substrate, $\delta^{13}\text{C}$, and $\delta^{15}\text{N}$ were strongly related to factor 1 (eigenvalues > 0.5). PC 1 described the gradient from regulated sites with poor water quality to pristine natural sites with relatively good water quality. Regulated sites in agricultural landscape generally had the highest macrophyte coverage, suspended solids, the largest distance from the confluence, total P, total N, mean depth, and BOD_7 values. Natural site groups generally exhibited low macrophyte coverage, suspended solids, total P and total N values and high flow velocity values. PC 2 separated sites on the basis of river channel width, watershed area, bottom substrate and BOD_7 .

Table 3

Comparison of isotopic variation of different fish size groups from natural and regulated sites in the Nevėžis River. Paired t-tests were used to compare isotopic parameters between natural and regulated study sites,

Species	LC	FFG	$\delta^{13}\text{C}$ [‰]		P value (t-test)	$\delta^{15}\text{N}$ [‰]		P value (t-test)
			Natural	Regulated		Natural	Natural	
<i>Rutilus rutilus</i>	I	B	-32.40 ± 0.2	-33.58 ± 0.2	0.05	13.79 ± 0.5	16.54 ± 0.1	0.05
<i>Rutilus rutilus</i>	II	B	-31.72 ± 0.1	-32.72 ± 0.1	0.05	15.26 ± 0.1	17.28 ± 0.1	0.05
<i>Perca fluviatilis</i>	I	B	-31.13 ± 0.1	-32.55 ± 0.1	0.05	12.17 ± 0.2	13.67 ± 0.3	0.05
<i>Perca fluviatilis</i>	II	P	-30.18 ± 0.2	-31.73 ± 0.2	0.05	14.08 ± 0.4	15.39 ± 0.4	n.s.
<i>Esox lucius</i>	I	P	-30.83 ± 0.1	-32.51 ± 0.1	0.05	12.25 ± 0.1	14.65 ± 0.1	0.05
<i>Esox lucius</i>	II	P	-29.06 ± 0.3	-31.73 ± 0.2	0.05	16.17 ± 0.2	17.02 ± 0.2	n.s.

LC = length class (as defined in Table 1), FFG = Functional feeding group, B = benthivorous, P = piscivorous, n.s. = not significant.

Table 4
Loading values for the first two factors and 17 variables with eigenvalues extracted by the principal component analysis

Variable	Factor 1	Factor 2
$\delta^{13}\text{C}$ [‰]	0.64	-0.24
$\delta^{15}\text{N}$ [‰]	-0.58	-0.21
Mean depth [m]	-0.85	-0.49
Flow velocity [$\text{m} \cdot \text{s}^{-1}$]	0.96	-0.24
Discharge [$\text{m}^3 \cdot \text{s}^{-1}$]	0.97	-0.21
Suspended solids ($\text{mg} \cdot \text{L}^{-1}$)	-0.96	-0.18
BOD ₇ [$\text{mg O}_2 \cdot \text{L}^{-1}$]	-0.82	-0.53
Total N [$\text{mg} \cdot \text{L}^{-1}$]	-0.89	-0.39
Total P ($\text{mg} \cdot \text{L}^{-1}$)	-0.94	0.22
Macrophyte coverage [%]	-0.98	-0.13
Canopy cover [%]	0.94	-0.32
Channel width [m]	0.38	-0.90
Watershed area [km^2]	0.80	-0.54
Distance from confluence [km]	-0.96	0.19
Bottom substrate	0.80	0.53
Eigenvalue	10.91	2.55
Total variance [%]	72.76	17.03

Values in bold type are loading factors (at level > 0.5).

DISCUSSION

The majority of rivers and streams flow through open agricultural landscapes, due to which they are heavily affected by human activities, i.e., channelization, pollution (Kesminas and Virbickas 2000). The Nevėžis River, whose upper sections are channelized, is not an exception either (Kilkus and Stonevičius 2011).

In the presently reported study, we used physicochemical characteristics and stable isotope composition of fish tissues as well as multivariate analysis to assess the impact of anthropogenic activities along the upper reaches of the Nevėžis River. Environmental elements revealed that the ecological status of the river in regulated stretches was worse than in natural ones. Physicochemical characteristics and fish stable isotope composition clearly responded to changes in riparian morphology characteristics. Studies such as this one into European and more particularly into Baltic rivers are scarce (Sullivan et al. 2015, Čivas et al. 2016, Kautza and Sullivan unpublished*), whereas it is the first detailed research work revealing influence of the riverbed morphology on the source of organic material and the trophic structure of fish community along the Nevėžis River, using stable isotope analysis method.

Stable isotope ratios differed statistically among fish functional feeding groups, with predators having higher nitrogen isotope ratios than benthivorous fish within each river section. This result is consistent with trophic shifts of isotope ratios reported by other studies (McCutchan et al. 2003, Duda et al. 2011) and is likely to be a reflection of differences in food sources among these groups.

As many fish species undergo ontogenetic shifts in the feeding niche, fish size is another factor possibly

affecting fish $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (e.g., Grey et al. 2001, Post 2002). Persson and Hansson (1999) found that large perch were exclusively piscivorous and had, accordingly, significantly higher $\delta^{15}\text{N}$ values than roach or other size classes of perch. Differences between the diet of roach belonging to different size-classes and that of perch of smaller classes were too slight to be traced in the stable isotope content. Likewise, we also found that there were differences in $\delta^{15}\text{N}$ values among size-classes of perch and pike, but there was no difference in $\delta^{15}\text{N}$ between different-sized roach. There was a positive relation between fish length and $\delta^{15}\text{N}$ of fishes. Similar results have been found for other fish species and are often attributed to either an ontogenetic change in the diet or to differential metabolic fractionation of nitrogen with age (Beaudoin et al. 1999, Overman and Parrish 2001). The trophic shift of N was higher for carnivores and other consumers with high-protein diets (vertebrates, microbes, and animal-based prepared diets) than for consumers with plant or algal diets (including plant-based prepared diets), but not so significantly (McCutchan et al. 2003).

Top predators such as the northern pike ($\delta^{15}\text{N} = 12.5\text{‰}$), and the big European perch ($\delta^{15}\text{N} = 12.2\text{‰}$), were plotted at the top, while benthivorous roach ($\delta^{15}\text{N} = 10.1\text{‰}$), remained at the bottom of the isotopic niche of Drūkšiai Lake (Lithuania) (Rakauskas 2014).

We found that $\sim\delta^{15}\text{N}$ values differed by 2‰–4‰ between the two size groups of fish (carnivorous/benthivorous), which is consistent with findings by Camusso et al. (1999) who reported that this isotope shift indicates that these groups of animals are at different trophic levels. Similar isotope compositions in different fish species reflect either similar feeding strategies or that one or both species feed on a variety of sources incorporating a wide range of isotope compositions. The low $\delta^{15}\text{N}$ values in small roach and small perch suggested the presence of plants and zooplankton in their diet and similar feeding strategies; whereas the high values in pike (which is carnivorous) and large roach (which is omnivorous, feeding on algae, macrophyte detritus and insects) probably reflect different food sources that nonetheless integrate to give similar isotopic compositions (Camusso et al. 1999).

As expected before, algae and terrestrial detritus differed significantly in their $\delta^{13}\text{C}$ values in both investigated stretches of the river. However, they did not differ significantly in their isotope ratios between natural and regulated stretches. Terrestrially derived C3 organic matter has mean $\delta^{13}\text{C}$ between -20‰ and -32‰ (mean: -27‰) (Leng and Marshall 2004). The mean $\delta^{15}\text{N}$ value of terrestrial detritus in the Nevėžis River was -29.0‰. The mean $\delta^{15}\text{N}$ values of periphyton were higher than those of terrestrial detritus across all sites.

Algal carbon isotope ratios from all the investigated sites in the Nevėžis River were more ^{13}C -depleted (mean $\delta^{13}\text{C} = -34.2\text{‰} \pm 0.1\text{‰}$) than those of terrestrial (riparian) sources (mean $\delta^{13}\text{C} = -29.0\text{‰} \pm 0.2\text{‰}$) and were within the range of previously reported values (Fry 1984, Harrigan et al. 1989, Lee 1995, Boon et al. 1997, Bouillon

* Kautza A., Sullivan S.M.P. (In press.) The energetic contributions of aquatic primary producers to terrestrial food webs in a mid-size river system. Ecology.

et al. 2002). However, there was no statistically significant difference in isotope ratios of terrestrial detritus collected from natural and regulated sites. The mean $\delta^{15}\text{N}$ values of periphyton ($\delta^{15}\text{N} = 9.31\text{‰} \pm 1.22\text{‰}$) were higher than those of terrestrial detritus across all sites ($\delta^{15}\text{N} = 6.3\text{‰} \pm 0.17\text{‰}$; $t = -5.99$, $P < 0.001$, $df = 10$). This is consistent with the findings of Angradi (1994), who indicated that dual isotope plots reveal that lotic algae, riparian vegetation, and upland vegetation had distinct isotope signatures.

According to Kruk (2007) pike and roach did not respond to changes in the quality of the aquatic environment. Conversely, we found that $\delta^{15}\text{N}$ values of roach, perch, and pike followed an upward trend from natural to regulated sites. According to Andersson and Cabana (2005), $\delta^{15}\text{N}$ in aquatic organisms would increase in a predictable way, following the increasing amounts of agricultural N inputs. Comparable relations between $\delta^{15}\text{N}$ of indicator organisms and agricultural land use were observed in previous studies (Harrington et al. 1998, Hebert and Wassenaar 2001, Udy and Bunn 2001). Our results complement these studies as longitudinal trends within watersheds can help us to better understand how progressive changes in land use affect $\delta^{15}\text{N}$. Furthermore, the use of organisms of different trophic levels may help to distinguish those components of the food web that are affected by anthropogenic activities from those that avoid them through migration (Anderson and Cabana 2005).

All fish species on natural sites were more ^{13}C -enriched indicating their foraging for terrestrial (riparian) food sources. Similarly to our results, blacknose dace, *Rhinichthys atratulus* (Hermann, 1804); and brown trout, *Salmo trutta* Linnaeus, 1758, the only species collected on both forested and unforested sites, was more negative by 3‰ on the unforested site, implying a greater dependence on autochthonous carbon. Despite that, there is very little overall difference in absolute fish $\delta^{13}\text{C}$ between sites (Rosenfeld and Roff 1992). Fish from natural stretches would therefore appear to derive more of their carbon base from terrestrial sources than aquatic invertebrates from the same site. This suggests that terrestrial inputs in the form of infalling invertebrates (secondary producers) may contribute to vertebrate production in a forested stream as much as primary terrestrial carbon inputs (leaves, detritus) via the aquatic food chain do (Rosenfeld and Roff 1992).

Our findings from the field experiment are consistent with previous studies of middle-sized rivers that report enhanced algal and macrophyte growth with P supplementation, even in heavily shaded river sections (Taulbee et al. 2005, Hill and Fanta 2008). Similarly to previous studies (Briand and Cohen 1987, Schoener 1989, Vander Zanden and Fetzer 2007, Sullivan et al. 2015), we found fish $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotopic signatures to differ significantly among riverbed morphology types, with the PCA model explaining almost 90% of the variation.

In conclusion, our results showed that $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ of fish at different trophic levels reacted strongly to spatial patterns in the intensity of eutrophication associated with N and P inputs, agricultural land use and channelization

within and among watersheds. Furthermore, they reveal that within-site variation in $\delta^{15}\text{N}$ among functional feeding groups remained small compared with among-site variation, thus supporting the general use of $\delta^{15}\text{N}$ values of fish as landscape integrators of the impact of land use on aquatic ecosystems (Andersson and Cabana 2005).

Agricultural land use has caused marked changes in the stable isotope composition of perch, roach, and pike populations in the investigated Lithuanian river. Fish on regulated sites show depletion in $\delta^{13}\text{C}$, suggesting that the basal resources for aquatic invertebrates (which we did not collect) are not derived from terrestrial detritus. The overall $\delta^{13}\text{C}$ values of fish in channelized stretches are usually lower than those in natural ones and are generally close to those of algal carbon. Similarly to previous studies (Rosenfeld and Roff 1992, Angradi 1994, Coat et al. 2009, Sullivan et al. 2015) the fish assemblages of natural stretches were found to be more ^{13}C -enriched indicating their feeding on the terrestrial (riparian) food sources. Fish from the natural sites had isotopic labels much closer to terrestrial one than to aquatic carbon. Our results, together with those of the previous studies, suggest that isotopic variation of fish coupled with hydrological changes in middle-sized rivers is a common phenomenon and is associated with allochthonous organic input and autochthonous phytoplankton growth (Mariotti et al. 1991, Barth et al. 1998, Kendall et al. 2001).

ACKNOWLEDGMENTS

The authors would like to thank Dr. Gintautas Vaitonis for providing vector graphic figures. The authors are grateful to Laima Monkienė for additional editing of English language of the revised manuscript.

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Received: 8 March 2016

Accepted: 9 November 2016

Published electronically: 31 December 2016