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RESEARCH PAPER — OPEN ACCESS

Shaping of macroinvertebrate structures in a small fishless lowland stream exposed to anthropopressure, including the environmental conditions

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Abstract – In studies of abiotic and biotic factors influencing macroinvertebrate assemblages, there is always the problem of which factor – fish predation or environmental conditions – has the strongest impact on the invertebrates and whether the impact is positive or negative. The aim of our study was to determine the impact on the structures of macrozoobenthos in a small field watercourse exerted by abiotic conditions, with the concurrent lack of predators and varied intensity of anthropopressure. During the entire study period, the presence of 49 taxa of macroinvertebrates was recorded. The highest number of taxa and value of biodiversity was observed in the upper part of the watercourse, and subsequently decreased down the stream, reaching the lowest value at the sites located near the outlet. The tributaries significantly differed between each other in the number of taxa. In the tributary carrying water from wetland, a much higher number of taxa was noted than in the tributary carrying municipal water where the density achieved a significantly higher value of individuals than the remaining sites. The most limiting factors for the abundance of the investigated taxa were the oxygen concentration, nutrients and ammonia.

Keywords: Macroinvertebrates / small streams / environmental conditions / anthropopressure

Résumé – Variations des structures des macroinvertébrés dans un petit cours d'eau de plaine sans poissons exposé à la pression anthropique influant les conditions environnementales. Dans les études sur les facteurs abiotiques et biotiques qui influencent les assemblages de macroinvertébrés, il y a toujours le problème de savoir quel facteur – prédation des poissons ou conditions environnementales – a l'impact le plus fort sur les invertébrés et si l'impact est positif ou négatif. Le but de notre étude était de déterminer l'impact, sur les structures du macrozoobenthos dans un petit cours d'eau de plaine, des conditions abiotiques, avec absence concomitante de prédateurs, et l'intensité variable de la pression anthropique. Pendant toute la période d'étude, la présence de 49 taxons de macroinvertébrés a été observée. Le nombre le plus élevé de taxons et la valeur de la biodiversité la plus élevée ont été observés dans la partie supérieure du cours d'eau et ont ensuite diminué le long du cours d'eau, pour atteindre la valeur la plus faible aux sites situés près de l'exutoire. Les affluents différaient considérablement les uns des autres quant au nombre de taxons. Dans l'affluent transportant l'eau d'une zone humide, on a observé un nombre beaucoup plus élevé de taxons que dans l'affluent transportant de l'eau domestique, où la densité a atteint une valeur significativement plus élevée d'individus que dans les autres sites. Les facteurs les plus limitants de l'abondance des taxons étudiés étaient la concentration d'oxygène, les nutriments et l'ammoniac.

Mots-clés : Macroinvertébré / petits cours d'eau / conditions environnementales / pression anthropique

1 Introduction

Small field watercourses are a common component of the landscape of Central and Eastern Europe. These watercourses flow into larger rivers or lakes, often affecting the abiotic and biotic conditions in their recipient water bodies (Wohl, 2017). Small watercourses are a unique type of environment, completely different from that found in other parts of the catchment area (Wohl, 2017). They can constitute sites of permanent existence of resident fauna, or be a transitional shelter for migratory fauna (Williams *et al.*, 2003; Wohl, 2017). They can also constitute a refuge for fauna colonizing lower parts of the catchment area, currently often subject to

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maintenance activities. Small watercourses are a difficult research subject, as they are under strong influence of local factors disrupting their functioning, such as convective storms, fires or landslides (Wohl, 2017).

Depending on whether the investigated watercourse is a natural stream or a drainage ditch, its impact on the recipient water body can be different. Drainage ditches, due to their anthropogenic nature, are obviously a more frequent research subject than small streams (Verdonschot *et al.*, 2011; Leslie *et al.*, 2012; Verdonschot and Verdonschot, 2014; Whatley *et al.*, 2014; Leslie and Lamp, 2017). Small watercourses, natural and artificial, present significant differences. Simon and Travis (2011) reported that water in streams affected by drainage ditches, as well as drainage ditches themselves, have a higher biodiversity than natural streams, although no significant taxonomic differences between the two environments have been demonstrated. However, Williams *et al.* (2003) in drainage ditches recorded the lowest diversity of all investigated environments, including small streams. As can be noted, the evolution of benthic structures in small streams depends on the local environmental conditions.

Relatively many studies are also conducted in larger rivers (Brabender *et al.*, 2016; Rico *et al.*, 2016; Leitner *et al.*, 2017). Among studies of macroinvertebrates in small streams, the most common topic has been the impact of environmental factors on the benthic structure (Souto *et al.*, 2011; Zhang *et al.*, 2014; Kakouei *et al.*, 2017), as well the top-down and bottom-up relationships (Nyström *et al.*, 2003).

In small watercourses, in which predatory fish that effectively restrict the components of macrozoobenthos are present, it is difficult to determine the exact effect exerted on the investigated organisms by environmental conditions. For example, Choe *et al.* (2014) showed that in the examined regulated watercourse, predatory fish can be the main limiting factor for certain groups of invertebrates. On the other hand, Nicola *et al.* (2010) found that the structures of benthic macroinvertebrates are shaped primarily by water chemism, rather than by the presence of predatory fish. The aim of our study was to determine the impact on the structures of macrozoobenthos in a small field watercourse exerted by abiotic conditions, with the concurrent lack of fish and varied intensity of anthropopressure.

2 Material and methods

2.1 Area of study

The study was conducted in a small watercourse subjected to a strong anthropopressure (NW Poland, GPS coordinates: N 53° 13′ 15″, E 15° 45′ 50″). The length of the watercourse is approx. 1 km. The waters of the catchment area are wetlands on the right bank, and fields and meadows on the left bank. The investigated watercourse flows into Lake Grazyna (area around the Drawa National Park), through which the river Drawa flows. In order to conduct the study, six sites located in the main watercourse were established (following the direction of the water flow, within an average distance of approx. 0.2 km from each other) and one in each tributary (Fig. 1). The left tributary (LT) originated from the nearby wetland of approx. 20 m^2, while the right tributary (RT) was a wastewater

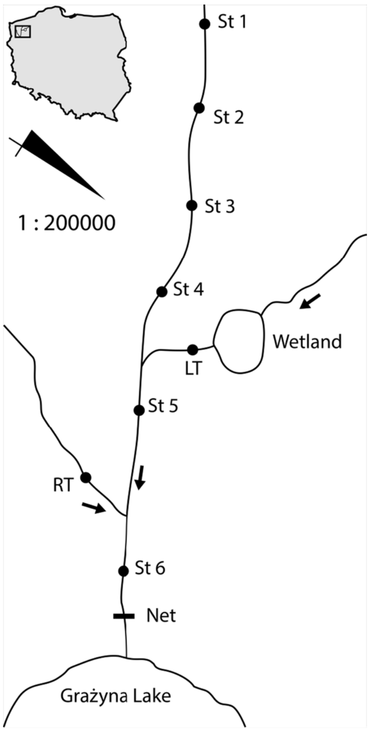

Fig. 1. Study area.

discharge. Below the mouth of the watercourse into the lake, a net with a mesh of 2.0 mm was installed, spanning from the bottom to 0.5 m above the water surface. The grid was cleaned every day. In April and July, an attempt of a catch was conducted in the watercourse using a generator (Hans Grassl ELT60 II, Germany). Fish were not found in either of the two months.

2.2 Sampling methods

Samples were taken from all sites, every month from March to October of 2009 and 2010. The samples were collected using a dragnet of 0.20 × 0.35 m, scraping off a fragment of the bottom of 1 m in length. Measurements of temperature, pH and conductivity of water, as well as oxygen concentration were conducted using a CX-401 versatile device by Elmetron (Poland). The content of $N–NO_2$, $N–NO_3$, $N–NH_3$ and N_{TOT}, PO_4 and P_{TOT} was measured using a DR-850 colorimeter manufactured by Hach Lange (USA). At each site in the watercourse, water current velocity was measured using an OTT electromagnetic water flow sensor (Germany). To calculate flow, the width and depth of the watercourse were additionally measured. The percentage of bottom coverage by macrophytes was determined visually.

2.3 Statistical analysis

Biodiversity of macroinvertebrates at the investigated sites was determined using the Shannon–Weaver index. The identified taxa were grouped into classes of dominance following Biesiadka and Kowalik (1980), where: Eudominants: abundance >10%; dominants: 5.01–10%, subdominants: 2.01–5%; recedents: <2%. In order to demonstrate taxonomic similarity between sites, the Jaccard index was used.

In order to determine statistically significant differences in the mean abundance of taxa, overall abundance, biodiversity and number of taxa, the Scheffe post-hoc test was used (Statistica 12).

In order to determine the influence of the abiotic and biotic variables of the stream on the abundance of macroinvertebrates, taxa that reached a frequency of 50% and higher, and simultaneously a dominance ratio of 2% and higher, were taken into account. To conduct calculations, The Canonical Correspondence Analysis (CCA) with the forward selection procedure of variables by permutation test (ANOVA) was used. Vegan 1.15.1 software was used to perform CCA analyses.

3 Results

3.1 Environmental factors

The largest change in the morphology of the watercourse was observed in width, which varied from less than 1 m (the source section) to over 3.5 m (the lower section). Both tributaries were morphologically similar. There were also differences in water temperature at the sampled sites. The left tributary, which receives warmer waters from the floodplain, affected the water temperature at the S5. However, this influence was smaller at the site 6. The right tributary carried water of similar temperature to the waters of the main watercourse. In the case of nutrients concentration, the largest load was observed on the right tributary, where the highest ammonia values ($1.64\,\mathrm{mg\,l^{-1}}$) were present. Moreover, the following values were noted: total nitrogen of $2.66\,\mathrm{mg\,l^{-1}}$, phosphate concentration of $2.77\,\mathrm{mg\,l^{-1}}$ and total phosphorus of $7.25\,\mathrm{mg\,l^{-1}}$. These values exceed several times the nutrients concentration observed in the main watercourse or on the left tributary. The detailed values of the abiotic and biotic parameters at the investigated sites are presented in Table 1.

3.2 Macroinvertebrates structures

During the entire study period, the presence of 49 taxa of macroinvertebrates was recorded (Tab. 2). There were significant differences in the number of taxa between the investigated sites. The highest number of taxa was observed at site 2, and subsequently decreased down the watercourse, reaching the lowest value at site 6 (Fig. 2, Tab. 3). The tributaries significantly differed between each other in the number of taxa. In the left tributary, a much higher number of taxa was noted than in the right tributary.

In the case of density, only on the right tributary achieved a significantly higher value of individuals per m^2 than the remaining sites (Fig. 3, Tab. 3).

There were some significant differences in the Shannon–Weaver biodiversity index between the investigated sites. Generally, the lowest biodiversity index was observed in the lower section of the stream (Fig. 4. Tab. 3). It increased from site 1 to site 3, where it reached the highest value, and then decreased to site 6. The lowest value was observed in the right tributary.

Among the recorded taxa were 3 eudominants (Chironomidae, Asellidae and Gammaridae), 2 dominants (Sphaeriidae and Lumbriculidae), one subdominant (Culicidae) and 2 recedents (Baetidae and Limnephilidae) (Tab. 4). The absolutely permanent taxa included 18 families, but only 10 of them were present at each site (Tab. 5). 6 taxa were permanent, 12 taxa were accessory and 13 taxa were recognized as accidental.

3.3 Shaping the macroinvertebrate structures in the stream

The Jaccard's coefficient divided the sampling sites into 3 taxonomically similar groups (Fig. 5): the first group including site 6 and in the right tributary; the second group including sites 2, 3, 4 and the left tributary, and the third group including sites 1 and 5. Apart from the third group, sampling sites of the other two groups were located near to each other.

The results of the statistical analysis ANOVA showed that the largest difference in the abundance of macroinvertebrates between the examined sites was noted for 14 families (Tab. 6): Sphaeriidae, Lymnaeidae, Lumbriculidae, Nematoda, Erpobdellidae, Gammaridae, Asellidae, Beatidae, Limnephilidae, Dytiscidae, Chironomidae, Culilicdae Ptychopteridae and Sciomyzidae ($p > 0.05$).

3.4 Influence of environmental conditions

The first axis of Canonical Corresponding Analysis (CCA) explained 20.9% of relationship between the occurrence of macroinvertebrates and environmental factors, while the second axis explained 9.7% (Fig. 6). There was a strong correlation of biogenic compounds (such as total phosphorus and phosphates content, total nitrogen and ammonia content) with the first axis. An equally strong correlation, but opposite to that of nutrients, was observed for the oxygen concentration. Moreover, the amount of chlorophyll a was strongly correlated with the second axis. In the case of macroinvertebrates, the CCA divided the organisms into 4 groups. The 1st group one gathered ubiquitous organisms, such as: Culicidae, Ptychopteridae, Nematoda and Chironomidae. These group of organisms showed very small correlation with the first axis. The 2nd group with Nemouridae, Leptoceridae, Gyrinidae and Gammaridae, was strongly correlated with the second axis, similarly to chlorophyll a. In the 3rd group there was one taxon: Crambidae, which was also correlated with the first axis, similarly to the 1st group, but with the opposite vector, similarly to oxygen concentration. The 4th group included organisms without any clear trends: Sciomyzidae, Psychodidae, Lumbriculidae, Dytiscidae, Baetidae, Simuliidae, Asellidae, Ceratopogonidae, Limnephilidae, Glossiphoenidae, Lymnaeidae, Sphaeriidae and Erpobdellidae. CCA divided the sites in three groups (Fig. 7). The first group were the sites located in the first section of the watercourse (S1–S4). The second group were the site located in the left tributary and the sites below them. To the last group one site was assigned—the right tributary—with the lowest oxygen concentration and the highest nutrients concentration.

Table 1. Mean value and SD of abiotic and biotic factors observed on the sampling sites. O_2–oxygen dissolved, Cond – conductivity, NO_3–nitrates, NO_2–nitrites, NH_3–ammonia, N_{TOT} – total amount of nitrogen, PO_4–phosphates, P_{TOT} – total amount of phosphorus, Chla – chlorophyll a, Bottom – type of bottom: S – sandy, M – muddy, Veget – bottom cover by macrophytes, Macrophytes: most common macrophytes on site (ST – number of site, LR – Left Tributary, RT – Right Tributary).

	S1		S2		S3		S4		S5		S6		LT		RT	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Width (m)	0.85	0.20	0.56	0.22	0.70	0.27	0.95	0.10	1.63	0.34	3.56	0.55	0.40	0.15	0.50	0.14
Depth (m)	0.20	0.06	0.23	0.13	0.24	0.17	0.29	0.16	0.34	0.07	0.32	0.11	0.11	0.05	0.14	0.09
Veloc ($cm\,s^{-1}$)	0.05	0.04	0.06	0.03	0.05	0.03	0.04	0.02	0.03	0.01	0.03	0.01	0.01	0.01	0.03	0.02
Disch ($cm^3\,s^{-1}$)	0.01	0.02	0.01	0.02	0.02	0.03	0.01	0.02	0.02	0.02	0.03	0.03	<0.01	0.00	<0.01	0.00
Temp (°C)	11.29	4.25	12.78	4.62	14.06	5.25	14.41	5.44	15.59	6.73	14.81	6.03	17.38	7.22	13.94	4.70
O_2 ($mg\,l^{-1}$)	10.24	1.97	10.77	2.29	8.79	3.17	10.51	2.36	8.48	1.94	6.91	2.74	7.25	1.27	2.87	1.13
pH	7.71	0.32	7.50	0.63	7.74	0.39	7.62	0.29	7.49	0.28	7.47	0.26	7.39	0.26	7.11	0.36
Cond ($\mu S\,cm^{-1}$)	964	181	1031	208	1039	223	1056	224	1073	235	1023	226	1052	372	931	147
NO_3 ($mg\,l^{-1}$)	0.89	0.43	1.00	0.69	1.05	0.66	0.90	0.58	0.89	0.43	0.81	0.31	0.77	0.24	0.62	0.23
NO_2 ($mg\,l^{-1}$)	0.01	0.00	0.01	0.00	0.01	0.00	0.01	0.00	0.01	0.00	0.01	0.01	0.01	0.01	0.01	0.01
NH_3 ($mg\,l^{-1}$)	0.04	0.05	0.04	0.03	0.04	0.04	0.06	0.03	0.05	0.02	0.43	0.57	0.05	0.03	1.64	1.40
N_{TOT} ($mg\,l^{-1}$)	1.29	0.69	1.36	0.93	1.41	0.96	1.18	0.69	1.13	0.49	1.64	0.81	1.03	0.24	2.66	1.82
PO_4 ($mg\,l^{-1}$)	1.66	1.56	1.49	1.27	1.32	0.88	0.99	0.76	1.18	0.81	1.68	1.02	1.40	1.02	2.77	1.24
P_{TOT} ($mg\,l^{-1}$)	4.41	4.60	4.34	4.21	3.40	2.69	2.63	2.02	3.03	2.03	4.62	3.05	3.48	2.83	7.25	3.56
Chla ($\mu g\,l^{-1}$)	25.48	4.92	28.76	6.40	28.70	6.33	36.09	5.94	63.01	17.13	73.29	23.65	69.33	15.34	46.19	9.04
Bottom %	100 % S		95% S, 5% M		70% S, 30% M		40% S, 60% M		50% S, 50% M		95% M, 5% S		100% S		90% M, 10%S	
Veget (%)	48	38.12	94	10.94	85	25.79	73	35.54	48	29.89	39	25.23	87	22.51	62	27.57

Macrophytes *Nasturtium officinale Nasturcium officinale,* Carex sp., *Glyceria maxima* Carex sp., *Phragmites australis, Acorus calamus, Glyceria maxima, Typha latifolia Phragmites australis, Acorus calamus, Glyceria maxima, Typha latifolia Phragmites australis, Acorus calamus, Glyceria maxima, Typha latifolia, Callitriche palustris, Acorus calamus, Glyceria maxima, Typha latifolia Veronica beccabunga, Glyceria maxima, Phragmites australis Glyceria maxima, Phragmites australis*

Table 2. List of taxa identified on sites.

Bivalvia	**Collembola**	**Heteroptera**	**Diptera**	**Lepidoptera**
Sphaeriidae	Isotomidae	Corixidae	Ceratopogonidae	Crambidae
Gastropoa	**Crustacea**	Gerridae	Chironomidae	
Bithyniidae	Gammaridae	Nepidae	Culicidae	
Lymnaeidae	Asellidae	Notonectidae	Dolichopodidae	
Planorbidae	**Insecta**	**Trichoptera**	Empididae	
Valvatidae	**Ephemeroptera**	Hydropsychidae	Ephydridae	
Oligochaeta	Baetidae	Limnephilidae	Limoniidae	
Lumbriculidae	Leptophlebiidae	Polycentropodidae	Muscidae	
Naididae	**Plecoptera**	**Coleoptera**	Psychodidae	
Nematoda	Nemouridae	Donaciidae	Ptychopteridae	
Hirudinea	**Odonata**	Dytiscidae	Sciomyzidae	
Erpobdellidae	Aeschnidae	Gyrinidae	Simuliidae	
Glossiphoniidae	Calopterygidae	Haliplidae	Stratiomyidae	
Hirudinidae	Libellulidae	Hydrophilidae	Tabanidae	
		Scirtidae	Tipulidae	

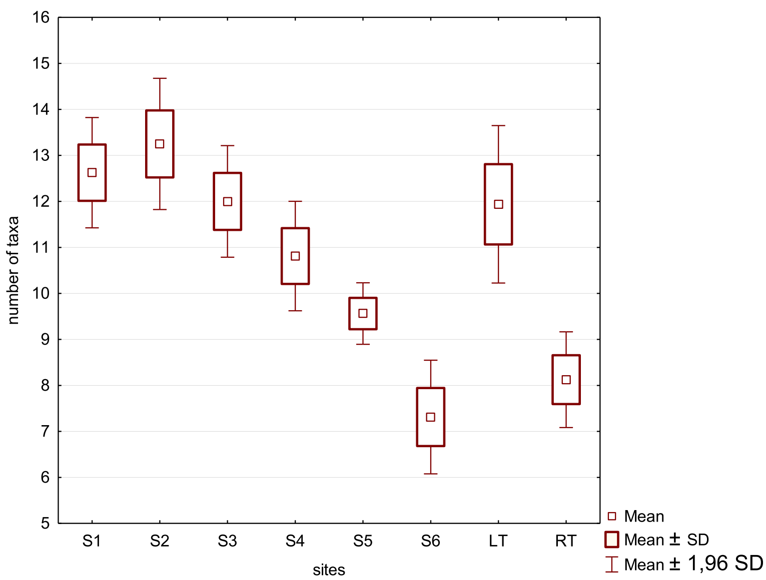

Fig. 2. Mean + SD values of number of taxa founded on examined sites (ST – number of site, LR – Left Tributary, RT – Right Tributary).

4 Discussion

At the investigated sites, high diversity of the physico-chemical and biotic properties was observed. This was probably primarily due to the terrain type and the area through which the watercourse flowed, as well as the intensity of anthropopressure.

Considering all studies by Williams *et al.* (2003), in which they proved that ditches and streams are characterized by the smallest number of macroinvertebrate taxa, we made an assumption that in such a small, regulated and partially polluted stream we would find a low number of macro-invertebrate taxa. However, the results were much higher than we had expected. This relatively high diversity of macro-invertebrates can be explained mainly by the lack of predatory fish, the large amount of biogenic compounds and the relatively large number of samples taken.

In studies of abiotic and biotic factors influencing macroinvertebrate assemblages, there is always the problem of which factor—fish predation or environmental conditions—has the strongest impact on the invertebrates and whether the impact is positive or negative. Multiple authors provide different answers, and in the opinion of many of them, it mostly depends on the type of reservoir. For example, Nicola *et al.* (2010) claimed that in streams, benthic communities are most affected by water chemistry, and less by fish predation.

Table 3. Statistically significant differences in number of taxa, density and biodiversity between the sampling sites. The symbol>indicates the index is significantly higher on examined sites of column than the sites of the row. > means $0.05>p\geq 0.01$; >> means $0.01>p\geq 0.001$; >>> means $p<0.001$ (ST – number of site, LR – Left Tributary, RT – Right Tributary).

	Sites	Scheffe post-hoc test					
		S1	S3	S4	S5	S6	RT
Number of taxa	S1					>>>	>>
	S2				>	>>>	>>>
	S3					>>>	>
	S4					>	
	LT					>>>	>
Density	RT	>	>>	>	>	>	
Biodiversity	S1					>>>	>>>
	S2				>	>>>	>>>
	S3				>>	>>>	>>>
	S4				>	>>>	>>>
	S5						>
	LT				>	>>>	>>>

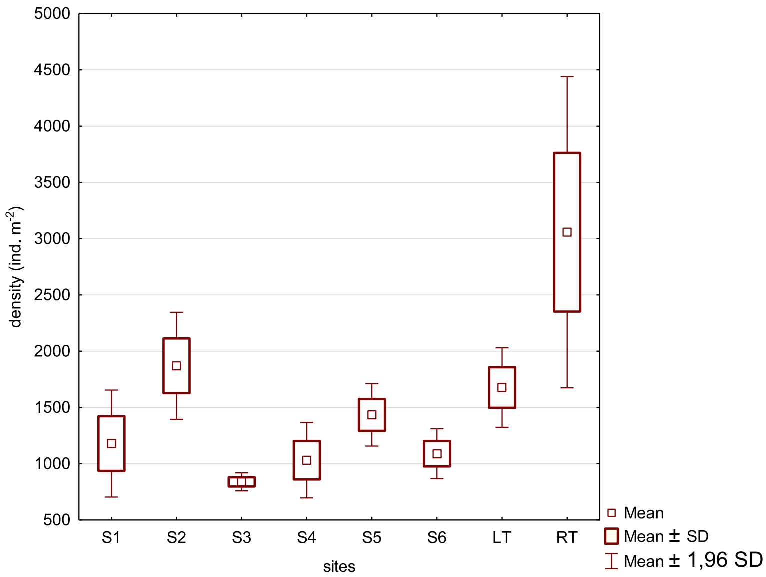

Fig. 3. Mean + SD values of total individuals per m^2 on the examined sites (ST – number of site, LR – Left Tributary, RT – Right Tributary).

On the other hand, Williams and Taylor (2003) found a significant influence of fish predations on the density and taxonomic structure of invertebrates in isolated pools of an intermittent stream. Moreover, Bonneau and Scarnecchia (2015) found that *Cyprinus carpio* can cause impoverishment of the macroinvertebrate taxa richness in streams. In our earlier paper regarding food selectivity of juvenile trout in small streams (Domagała *et al.*, 2015), we found that the investigated fish preyed on selected groups of invertebrates, not necessarily those most abundant. All the above facts allow a conclusion that more accurate biotic and abiotic factors for benthos can be obtained, when these effects are examined separately.

In the investigated fishless stream, a large number of taxa was considered as absolutely permanent, yet most of them had a low abundance—even <1% of all organisms. Accordingly, it seems that with the absence of fish, invertebrates can spread unhindered. However, while the absence of fish allows a potential spread of macroinvertebrates, there are some environmental factors that limit the abundance of macroinvertebrate taxa. Among the factors taking for consideration in this study, it seems that the most limiting for the abundance of the investigated taxa were those that reached extreme values at the sites.

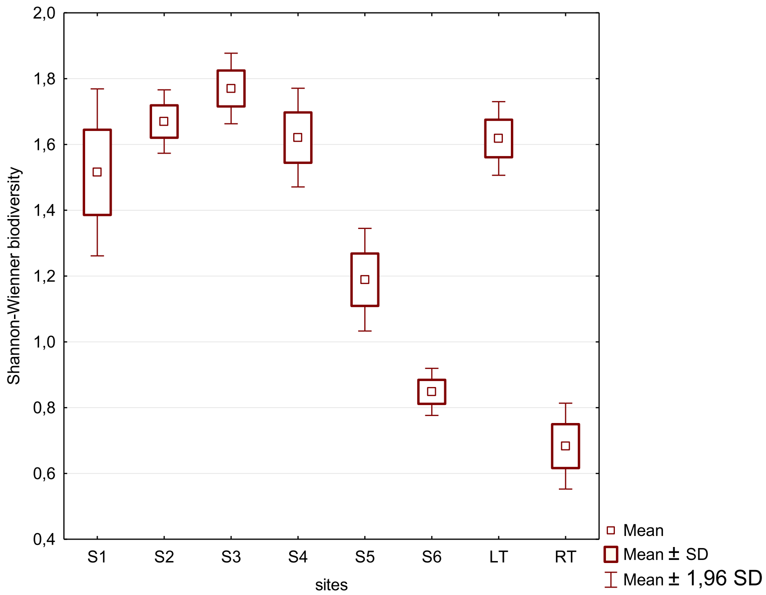

Fig. 4. Mean + SD values of Shannon-Wienner biodiversity index on the examined sites (ST – number of site, LR – Left Tributary, RT – Right Tributary).

Table 4. Dominant class of macroinvertebrates on sampling sites.

Class	Eudominants			Dominants		Subdominants	Recedents		
Taxa	Chironomidae	Asellidae	Gammaridae	Sphaeriidae	Lumbriculidae	Culicidae	Beatidae	Limnephilidae	Other
% of dominance	37.61	22.37	12.75	8.87	7.31	2.68	1.75	1.27	5.39

Table 5. Frequency of taxa which were present in at least half of the sites.

	Sphaeriidae	Lumbriculidae	Nematoda	Glossiphoniidae	Asellidae	Baetidae	Limnephilidae	Dytiscidae	Ceratopogonidae	Chironomidae	Erpobdellidae
Frequency	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	87.5

	Leptophlebiidae	Psychodidae	Lymnaeidae	Gammaridae	Nemouridae	Culicidae	Gyrinidae	Sciomyzidae	Simuliidae	Crambidae
Frequency	87.5	87.5	66.7	66.7	66.7	66.7	62.5	62.5	62.5	50.0

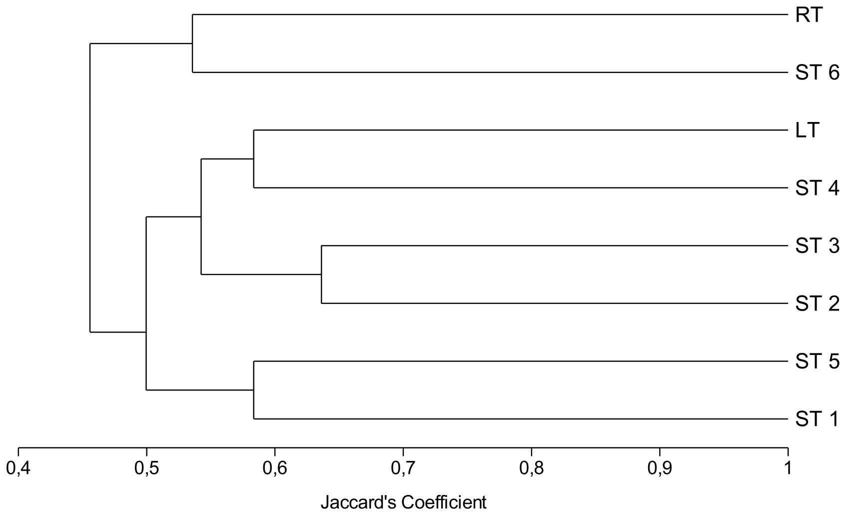

Fig. 5. The Jaccard's Coeffiicient shows taxonomic similarity of the examined sites (ST – number of site, LR – Left Tributary, RT – Right Tributary).

Table 6. Statistically significant differences in the mean abundance of taxa between the sampling sites. The symbol>indicates the mean abundance of taxa is significantly higher on examined sites of column than the sites of the row. > means $0.05 > p \geq 0{,}01$; >> means $0.01 > p \geq 0.001$; >>> means $p < 0.001$ (ST – number of site, LR – Left Tributary, RT – Right Tributary).

Taxa	Sites	Scheffe post-hoc test							
		S1	S2	S3	S4	S5	S6	LT	RT
Sphaeriidae	S2	>>>		>>	>>>	>>>	>>>		>>>
	S3						>		>>
	LT	>>>		>>>	>>>	>>>	>>>		>>>
Lymnaeidae	S1								>
Lumbriculidae	S6	>							>>
Nematoda	RT	>	>	>	>			>	
Erpobdellidae	S1					>	>		
Gammaridae	S1			>	>>	>>>	>>>	>>>	>>>
	S2			>	>	>	>	>	>
Asellidae	S5	>					>		
Baetidae	LT	>	>	>	>				>
Limnephilidae	LT						>		>
Dytiscidae	LT	>	>	>	>	>	>		>
Chironomidae	RT	>>>	>>>	>>>	>>>	>>>	>	>>>	
Culicidae	RT		>	>		>	>	>	
Ptychopteridae	RT	>>>				>>>			
Sciomyzidae	S5						>		
	LT						>		

The use of CCA allowed us to determine that such factors were the oxygen concentration, nutrients and ammonia. In our study, we observed the lowest oxygen concentration and the highest amount of nutrients and ammonia in the right tributary, which supplied waste water. This tributary had the strongest influence on macroinvertebrate structures in the main watercourse, and was followed by the left tributary in which only water temperature was slightly higher than at the remaining sites. Despite the difference in water temperature between the left tributary and at the sites above it, and the fact that the watercourses came from various sources, there was no significant difference in the number of taxa, density and biodiversity between the left tributary and the sites situated above its outlet. Following the above statements and CCA results, the oxygen concentration and biogenic matter were the most important chemical factors shaping the macroinvertebrate communities in a fishless, regulated stream. It seems that physical and morphological factors, because of their homogeneity, were less important. Oxygen, as the main biogenic element, is often mentioned as an important determinant of

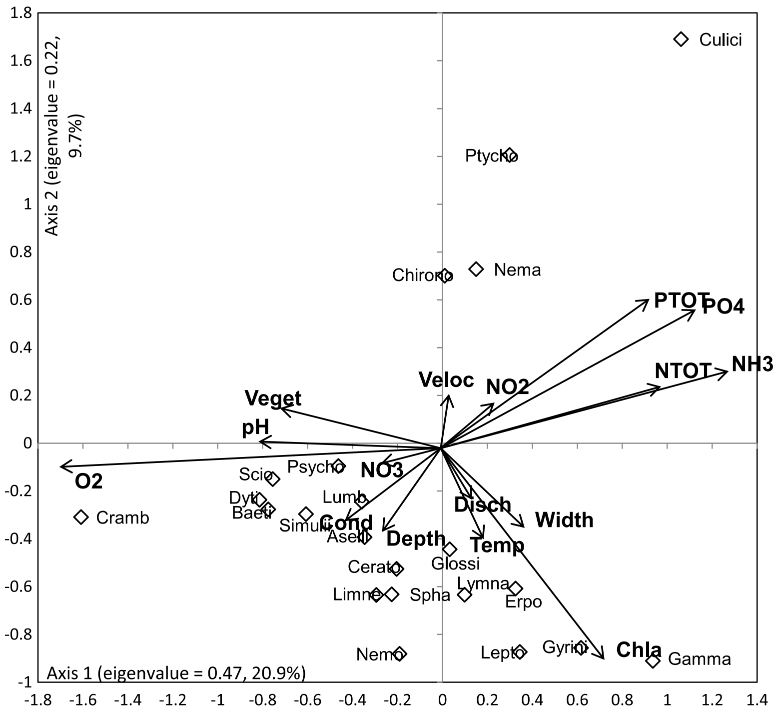

Fig. 6. The Canonical Corresponding Analysis (CCA) – the macroinvertebrates ordination. Spha – Sphaeriidae, Lymna – Lymnaeidae, Lumb – Lumbriculidae, Nema – Nematoda, Erpo – Erpobdellidae, Glossi – Glossiphoniidae, Gamma – Gammaridae, Aselli – Asellidae, Baeti – Beatidae, Lepto – Leptophlebiidae, Nemo – Nemouridae, Cramb – Crambidae, Limne – Limnephilidae, Dyti – Dytiscidae, Gyrini – Gyrinidae, Cerato – Ceratopogonidae, Chirono – Chironomidae, Culici – Culicidae, Psycho – Psychodidae, Ptycho – Ptychopteridae, Scio – Sciomyzidae, Simulii – Simuliidae, O_2–oxygen dissolved, Cond – conductivity, NO_3–nitrates, NO_2–nitrites, NH_3–ammonia, N_{TOT} – total amount of nitrogen, PO_4–phosphates, P_{TOT} – total amount of phosphorus, Chla – chlorophyll a, Veloc – velocity, Disch – discharge, Temp – water temperature, Cond – conductivity, Veget – % bottom covered by macrophytes.

invertebrate presence (Souto *et al.*, 2011, Wynne and Linnane, 2009, Zhang *et al.*, 2014). Based on the CCA results, the most favourable oxygen conditions during the whole period of study were observed at the first four sites of the watercourse, where the biodiversity reached the highest values.

In the investigated watercourse, a small scale of the River Continuum Concept (RCC) (Vannote *et al.*, 1980) is reflected, even though it was developed for large scale areas. The model assumes that as the watercourse flows, accumulation of biogenic compounds and decrease in the biodiversity of benthic macroinvertebrates occur. The last section of the watercourse and the right tributary carrying wastewater presented the lowest values of biodiversity and number of taxa. Moreover, in the right tributary, the highest values of density were observed, caused by a high abundance of ubiquitous taxa, such as Chironomidae or Nematoda, and larvae breathing atmospheric oxygen (Culicidae and Ptychopteridae) that were also abundant below the mouth. Both the tributaries and changes in morphology affected variations in the structures of macroinvertebrates in the small watercourse.

Of course, there are other factors, such as the exact bottom structure, type and amount of macrophytes (Traversetti *et al.*, 2015), or amount of heavy metals (Malaj *et al.*, 2012), that could shed a bit more light on the shaping of benthic structures in this type of streams. For example, Graça *et al.* (2015) in their experiment showed that the differences in the benthos from sites with a less grainy bottom and sites with a more grainy bottom are visible only in the taxa composition and not in the quantity of taxa and abundance. This observation is also reflected in this study. We observed that the sites located in the upper section of the watercourse (ST 1, ST 2, ST 3 and ST 4) differed primarily in the type of bottom and the structure of macrophytes, while no significant differences were demonstrated in the number of taxa biodiversity and density of the benthos at these sites.

Thus, small watercourses, due to their size and the uniqueness of local conditions, as well as variability of environmental conditions often dependent on human-made transformation (Wohl, 2017), are a difficult research subject. Based on the results of this study, it can be concluded that aquatic macroinvertebrates in small fishless watercourses can spread in an uninterrupted manner. Physico-chemical conditions limit mainly the abundance of organisms. Among the abiotic parameters taken into account, the oxygen concentration in water and biogenic elements affected the most the abundance of benthic structures. Wastewater supplied by one tributary affected the benthic structures below its mouth in the recipient more strongly than water from field wetlands supplied by another tributary.

Despite the attempt to describe the topic in a comprehensive manner, there are still unanswered questions, such as what is the impact of the vegetation on the benthic structure in small fishless watercourses, or what is the impact of the graininess of the bottom on the occurrence of aquatic invertebrates. It is therefore necessary to conduct further studies of the evolution of macrozoobenthic structures in small watercourses if the

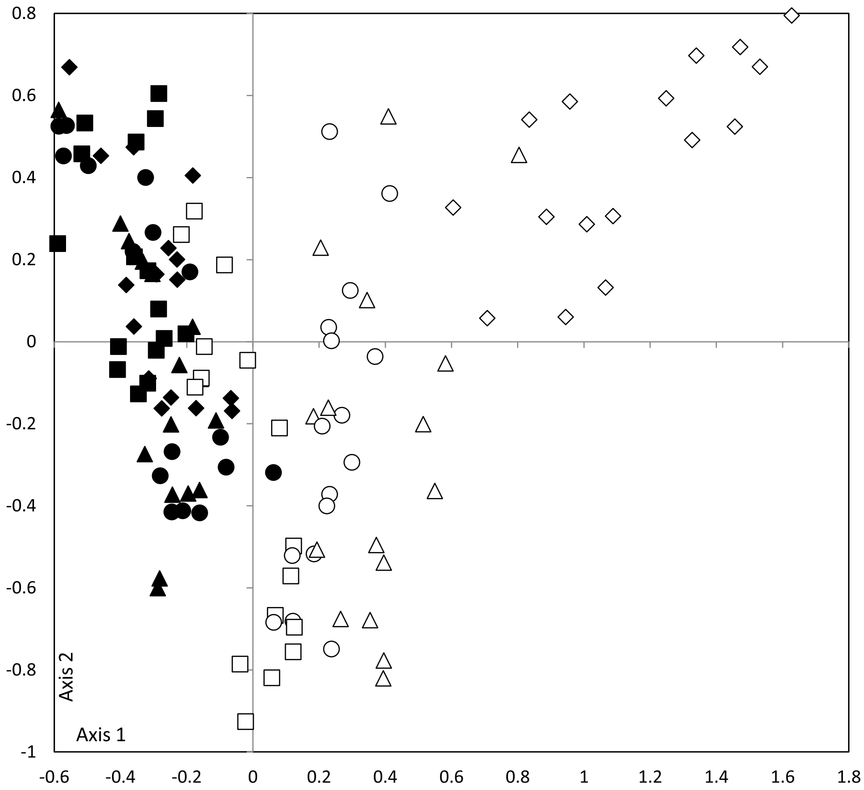

Fig. 7. The Canonical Corresponding Analysis (CCA) – the sites ordination. ■ – Site 1, ● Site – 2, ◆ – Site 3, ▲ – Site 4, ○ – Site 5, Δ – Site 6, □ – Left Tributary, ◇ – Right Tributary.

ecological dependencies in this type of environment are to be thoroughly understood.

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