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Diversity of Fish and Zoobenthic Communities in Lowland River Related to the Factors of Hydromorphological, River Continuum and Pollution Disturbances

Janusz Golski University of Life Science in Poznań, Poland https://orcid.org/0000-0002-2326-5111

Krzysztof Dajewski^{*} Chief Inspectorate of Environmental Protection, Poland

Maria Urbańska University of Life Science in Poznań, Poland

Wojciech Andrzejewski University of Life Science in Poznań, Poland

Sławomir Runowski University of Life Science in Poznań, Poland

*corresponding author's e-mail: kdajewski@ymail.com

Abstract: The response of zoobenthos and ichthyofauna to different levels of habitat degradation was estimated on a small lowland river. The level of fragmentation, the hydromorphological modifications of the watercourse bed, and the water quality of different river stretches were assessed as proxies of the degree of anthropogenic transformation of habitats. The degree of fauna similarity between the study sites, as well as the relationships between habitat quality and biotic indexes were estimated. A strong response of both assemblages to changes in environmental conditions was demonstrated, however, reaction to individual pressures differs. This confirms that these organisms are excellent, universal bioindicators and both groups should be used together. The key role of a well developed riparian zone in shaping the diversity of freshwater biota has also been demonstrated.

Keywords: lowland stream, anthropopressure, fish fauna, benthic macroinvertebrates, biodiversity



1. Introduction

Surface waters in Poland are still subject to strong anthropogenic pressures despite measures to reduce the impacts required by the implementation of the European Directive have been undertaken. Running waters are subject to hydromorphometric modifications that profoundly alter their natural course and hydrological regime, and to the impact of pollutants that alter water quality. These main drivers of anthropogenic alteration lead to a loss of habitat heterogeneity and to a consequent impoverishment of biocoenoses (Allan 1998, Golski et al. 2010, Mueller et al. 2020, Pytka et al. 2013). According to the concept of river continuum, the longitudinal variation of the characteristics of the river ecosystem is the result of a gradient of physical conditions (width and depth, velocity and volume of flow, temperature, bottom substrate) and of resources along which the biota and ecosystem processes are gradually adjusted. According to the concept, the effects of processes in the head course of the river affect the dynamics of processes occurring in the down section (Allan 1998, Branco et al. 2014, Vannote et al. 1980). Even the smallest hydro-technical building or other interference in the river channel disrupts the continuity of the river system. Currently, most rivers in Europe suffer to a greater or lesser extent from river continuum disturbances (Mazurkiewicz-Boroń & Starmach 2009, Prus et al. 2016, Przybylski et al. 2020). In addition to the direct modifications of the river channel, the use of the surrounding land also has an impact on the characteristics of the river ecosystem, for example by modifying the type of sedimentation and the load of material of terrestrial origin that enters the river. Commonly carried out maintenance works - consisting of profiling and strengthening the banks, straightening and deepening of the channels, removing the bottom substrate and macrophytes – lead to a loss of heterogeneity of the micro-habitats and, consequently, to drastic changes in the structure of the assemblages (Golski et al. 2010, Kalny et al. 2017, Przybylski et al. 2020). Habitat fragmentation is also a major driver of diversity loss. As an example, river sections within larger cities were an insurmountable barrier for many species (Czerniawska-Kusza 2001, Penczak et al. 2010, Przybylski et al. 2020).

Until the end of the 1980s, flowing water pollution was the most important factor limiting the abundance and composition of biotic assemblages. After the political transformation, and especially after the enlargement of the European Union, the approach to environmental protection in Central and Eastern Europe has changed significantly. The quality of water began to gradually improve, which enabled the reconstruction of biocenoses, although still not enough to bring most of the running waters to a good ecological state, according to the requirements of the Water Framework Directive (Kruk et al. 2016, Marszał et al. 2014). Currently, phosphorus and nitrogen pollution continues to be one of the major problems (Pytka et al. 2013).

Macroinvertebrates and fish are particularly sensitive to river fragmentation, profiling and pollution (Bis & Mikulec 2013, Branco et al. 2014, Pietraszewski et al. 2008, Prus et al. 2016). Fish, due to their high mobility, are particularly associated with the disruption of the watercourse, and their species composition reflects the effects of anthropogenic disturbances over many years (Branco et al. 2014, Prus et al. 2016, Rechulicz & Płaska 2016). Unlike fish, the species richness of benthic macroinvertebrates depends more closely on the heterogeneity of river microhabitats, and therefore more effectively reflects changes in local conditions (Bis & Mikulec 2013, Czerniawska-Kusza 2001). The presence, persistence and composition of both biotic assemblages depends on natural morphological characters that are key functional elements for river ecosystems. These include stony-gravel reefs that spawn rheophilic fish (Prus et al. 2016), as well as ponding water areas that are essential sites for young fish development (Brylińska 2000). The presence of shelters against strong currents and predation (Prus et al. 2018, Wolter 2010), as well as of natural barriers, including debris, may favor fish and zoobenthos development (Kałuża & Radecki-Pawlik 2014, Wyżga et al. 2012).

In spite of the wide use of biotic responses to anthropogenic stressors for the assessment and monitoring of ecosystem integrity, the causal relationship between environmental and biotic characteristics is still largely to be clarified. The aim of the study was to supplement the knowledge about changes in the species structure of ichthyofauna and zoobenthos of a small lowland river as a function of the level of habitat degradation. To this aim we assessed how the main sources of directional variability – fragmentation, hydromorphological transformations and pollution – affect the diversity of two assemblages.

The following research hypothesis was formulated: The studied communities show a different response to pressures. Fish better represent large, while macrozoobenthos local scale of impact.

To represent a wide degree of anthropogenic impact we identified the Lutynia and its tributaries Żybura and Lubianka (West Poland), as an optimal experimental site. These rivers experienced strong anthropopressure during the past Kołaczkowski & Kniat (1959), data from the Voivodeship Inspectorate for Environmental Protection- VIEP, while have been gradually improving in more recent times, but comprehensive hydrobiological studies are still lacking.

2. Study area

Lutynia flows through the Wielkopolskie Voivodship and its basin is an area characterized by the occurrence of small water reservoirs of anthropogenic origin (RZGW 2015). Land cover is dominated by a 76% of agricultural, while only a 17% is made by semi-natural areas (Corine Land Cover 2012). Water covers 0.08% of the Lutynia basin.

Lutynia is a third-order stream (Strahler 1957) through its left side of the Warta with a length of 63 km and basin of 606 km². According to the abiotic typology of rivers proposed by Błachuta et al. (2010), watercourse should be classified according to the height and size of the basin as a small lowland river. There are 19 damming devices along the entire course of the river, the watercourse river channel is subjected to regulatory works for a considerable length, while the quality of water can be affected by sewage from three cities and area pollution of agricultural origin (VIEP).

Żybura and Lubianka are fourth-order streams, and permanent tributaries of Lutynia with a length of 9 and 21 km, respectively. These are lowland streams whose catchment area does not exceed 100 km². Two damming dams were located on each of the watercourses, the quality of water may be affected by pollution from the agricultural catchment (VIEP).

The location of watercourses and research sections along with the types of pressure is shown in Figure 1 and Table 1.



Fig. 1. Location of study sites in the Lutynia river system

Research section SdD		E P	Expecte Pressure	d s	Depth	Width	tom strate	
		В	НМ	Р	[MIN.M.	Bot Subs		
L1	51.861575 17.680681	+	+++	++	$\frac{18-70}{34}$	$\frac{265-400}{375}$	M/S	
L2	51.894653 17.629475	++	++	+++	$\frac{4-30}{20}$	<u>210-315</u> 275	S/M/G	
L3	51.952111 17.596211	++	+++	+++	<u>9-115</u> 57	<u>290-460</u> 400	S/M/ST	
L4	51.974561 17.555461	-	+	+	<u>5-140</u> 55	<u>315-670</u> 550	S/G/ST/M	
L5	51.998467 17.542314	-	+	+	<u>10-125</u> 48	<u>500-990</u> 850	S/G/ST/M	
L6	52.037967 17.551286	++	+++	+++	<u>30-85</u> 52	<u>650-810</u> 720	S/M	
L7	52.111183 17.576344	+++	++	++	<u>10-96</u> 25	<u>400-515</u> 475	S/M	
L8	52.124175 17.506128	++	++	++	<u>22-150</u> 74	<u>370-580</u> 475	S/M	
ŻB	51.951389 17.595303	+	++	++	$\begin{array}{c c} \hline 7-94 \\ \hline 18 \\ \hline 156 \\ \hline \end{array}$		S/M	
LB	52.033225 17.670222	++	++	+++	$\frac{5-70}{42}$	<u>110-190</u> 160	S/M	

Table 1. Types of pressure and GPS research section

Explanations: B – barriers, HM – hydromorphology, P – pollutions, (+) - strength of anthropopressure, S – sand, G – gravel, ST –stones, M – mud. Abiotic typology (according to Błachuta et al. 2010): loess or clay lowland stream (L1, L3, ŻB, LB), sandy lowland stream (L2), gravel lowland stream (L4, L5), sandy-clay lowland river (L6, L7, L8)

3. Material and Methods

The research was carried out in 2015-2016 on 10 river stretches. These were defined by dividing the course of the river into sections of 2-3 km and identifying in each a stretch of 200 representative of the local conditions as a reference area for the study. One 150 m long stretch was tested on the tributaries.

The level of impact of the analyzed pressures was expressed on a 5-point scale, where class I corresponds to the lowest level, while class V corresponds to the highest. The degree of disturbance of watercourse continuity was estimated based on the number of barriers per section. This number ranged from 0 (class I) to 4 and more (class V). Transformations of the river channel morphology were characterized by the method of scoring selected parameters: depth and flow variation, the presence of shelters and natural elements of the river channel (riffle-pool sequences, meanders), the structure of the bottom substrate (Table 2).

Parameter	Component	Points
	Imperceptible	1
	Two types of flow	2
Flow	Three types	3
Flow Shelters Morphometry of river channel (natural elements) Coefficient of variation for depth (Depth CV)	Four types	4
	Over four types	5
	Absence	1
Shelters	One type of shelter	2
	Two types	3
	Three types	4
	Four and more shelters	5
Mamphamatry of vision above al	Absence	0
(natural elements)	Riffle-pool sequence	1
(natural elements)	Meander	2
	0-15	1
Coefficient of variation	16-30	2
for depth (Depth CV)	31-45	3
for depth (Depth C V)	46-60	4
	ComponentImperceptibleTwo types of flowThree typesOver four typesOver four typesAbsenceOne type of shelterTwo typesThree typesFour and more sheltersAbsenceRiffle-pool sequenceMeander0-1516-3031-4546-60> 60SlimeClaySandGravel > 20%< 5041-5026-4016-25< 15	5
	Slime	1
	Clay	2
Bottom substrate	Sand	3
	Gravel > 20%	4
	Gravel and stones $> 20\%$	5
	> 50	1
	41-50	2
W/D	26-40	3
Interprioritient of variation (natural elements) Coefficient of variation for depth (Depth CV) Bottom substrate W/D	16-25	4
	< 15	5

 Table 2. Scoring assessment of hydromorphological parameters of the watercourse

Explanations: W/D – ratio of average width to average watercourse depth (source: Dajewski 2016, Golski et al. 2010, 2013, 2015, Graczyk 2014)

The higher the sum of points on a given research section, the smaller the hydromorphological transformations (Table 3). A maximum of 35 points can be awarded if there are at least 3 meanders and 4 riffle-pool sequences (a total of max. 10 points). All hydromorphological surveys were made once a year.

Class	Total points
Ι	≥28
II	21-27
III	14-20
IV	7-13
V	≤ 6

 Table 3. Final classification of evaluation of waterway morphology transformations

Source: Dajewski 2016, Golski et al. 2010, 2013, 2015, Graczyk 2014

The degree of watercourse pollution was determined using selected physicochemical indicators (Table 4). Temperature, oxygen content, conductivity and reaction were measured in the field with a YSI 556MPS multiparameter device. Water samples were collected at each site every month during summer (June-September) and transported to the laboratory for chemical and BOD5 analyses. The obtained results were assigned to one of five water quality classes according to modified Regulation of the Minister of the Environment of February 11, 2004. The use of a five-point scale, which is not currently applied to physicochemical parameters, allowed for a more precise differentiation of the stands in terms of their contamination.

Quality of water in diaster	Luit	Limit values in class I-V								
Quality of water indicator	Unit	Ι	II	III	IV	V				
Water temperature	°C	≤15	≤ 18	≤ 20	≤23	>23				
Oxygen dissolved	$mgO_2l^{\text{-}1}$	≥ 8	≥6	≥5	≥ 3	< 3				
pH reaction	pН	6.5-8.5	6.0-8.5	6.0-9.0	5.5-9.0	<5.5>9.0				
Conductivity	μS cm ⁻¹	≤ 300	≤ 500	≤ 800	≤ 1000	> 1500				
Substance dissolved	mg l ⁻¹	\leq 300	≤ 500	≤ 800	≤ 1200	> 1200				
BOD ₅	mg O ₂ l ⁻¹	< 2	< 3	< 6	< 12	> 12				

Table 4. Limit values of indicators in surface water quality classes modified in terms of fish requirements

Source: Regulation of the Minister of the Environment, modified data

The degree of anthropogenic transformation was determined by scoring the naturalness of the river channel, coastal zone and river valley zone based on: the degree of river channel adjustment, the presence of barriers, the development of the river basin, the shape of the coastal zone, the presence of vegetation, according to Ilnicki & Lewandowski (1997) parameters.

Benthic macroinvertebrates were collected twice in Spring, during the period of the highest taxonomic diversity. Tubular (L1, ZB) and Surber (other sites) samplers were used. On each site 15 tubular or 3 Surber samplers were taken. The organisms were marked to the level of genus, and their density was expressed on 1 m² of bottom surface. The fish were caught twice, in Autumn, by the electro-fishing to ford up a watercourse over a distance of 150-200 meters. An IUP12 type backpack generator set was used. The collected fish species were ranked according to ecological reproductive groups, according to the division proposed by Balon (1990). The density was calculated per 100 m^2 of the bottom surface. After the measurements were completed, all fish were released into the river at the place of catch. In order to assess biodiversity, the Shannon-Wiener species diversity index was selected. Biocenotic indices of dominance in abundance and stability of occurrence were also used. For benthic organisms, the ASPT_B (Average Score Per Taxa), EPT (Ephemeroptera, Plecoptera, Trichoptera) indices and the share of stenotype taxa (S) were also calculated. For ichthyofauna, the $ASPT_F$ (Average Score Per Taxa) indices and the ratio of eurytopic to rheophilic species (E/R) were calculated. Indexes used in national monitoring were also calculated when assessing the ecological status of rivers - the European Fish Index (Adamczyk et al. 2013) and the Polish Multi-Metrics Index MMI PL based on the benthic macroinvertebrates assemblages (Bis & Mikulec 2013). Both indicators are based on a probabilistic model, referring the current state of fauna to the reference state. Thus, they reflect the degree of change resulting from anthropopressure. The calculations were made in the XLSSTAT 2016 and SAS Enterprise Guide 4.3 programs. Relationships between environmental parameters and assemblage diversity were estimated using Spearman rank correlations. The paper contains only statistically significant correlations, where "R" is the correlation coefficient, while "p" the significance of the correlated variables. Species similarity between assemblages at individual survey sites was depicted using dendrograms, performing cluster analysis using the complete linkage method based on the Jaccard formula (Czachorowski 2004).

4. Results

4.1. Environmental conditions

The largest diversity of habitats and the lowest degree of anthropopression was found in the middle -L4, L5 and downstream current Lutynia, on section L8

(Table 5). These sites were characterized by a significant diversity in the structure of the river channel – the coefficient of depth variation in the flow exceeded 60%. High morph-dynamic activity of the watercourse was found here, including the meandering the river channel and the occurrence of numerous riffle-pool sequences. On sections L4 and L5 no damming devices were noted, while on the lowest section L8 there are two gabion buildings, and the river banks are partially profiled.

According to the ecomorphological valorization of watercourses, sections L4, L5 and L8 were placed in II and III category of the degree of naturalness, respectively, which means that they are slightly transformed by humans (Table 5). They belong to the semi-natural section.

	L1	L2	L3	L4	L5	L6	L7	L8	ŻB	LB
Barriers	II	III	III	Ι	Ι	III	IV	III	III	II
Hydromorphological parameters	III	III	IV	II	II	IV	III	III	III	III
Physicochemical parameters	III	IV	IV	II	II	IV	IV	III	III	IV
Anthropogenic modification	IV	IV	IV	II	II	IV	IV	III	IV	IV

Table 5. Anthropopression and environmental conditions in the studied rivers

The river valleys of these sections are relatively intact and well preserved, and the shore zone is dominated by forests and rush vegetation, with many species of grass and ruderal flora. In terms of river channel hydromorphology, the best-looking forest sections are L4 and L5 (II class), which are characterized by a significant depth variation and the presence of shelters. The best thermal-oxygen conditions were also observed on these sections. More pressure and less hydromorphological diversity were found in the headwater of Lutynia – L1, L2, L3, as well as in downriver – L6, L7 and tributaries – ŻB, LB (Table 5). The aforementioned group of sections was classified into the IV category of naturalness. These are anthropogenically modified river stretches, which are characterized by low landscape attractiveness and low depth variation – up to 48% for ŻB. Relatively narrow river valleys are dominated by agricultural areas, and the width of the coastal zone does not exceed 10 meters. The hydromorphological parameters of the river channel were between class III and IV, and the final low assessment of physicochemical indicators was primarily due to low oxygen content.

4.2. Species structure of zoobenthos

There were 24 families of benthic macroinvertebrate in the Lutynia River system. Chironomidae (Diptera), Sphaeriidae (Bivalvia) and Tubificidae (Oligochaeta) had the highest stability of occurrence as well as the highest values of the dominance index in abundance (Table 6). The highest density (2274 sp./m^2) was recorded at section L8. In turn, the largest number of families (12) took place at L4 section.

	Inc	lex				Resea	arch se	ction l	D [%]			
Family	D [%]	C [%]	L1	L2	L3	L4	LS	L6	L7	L.8	ŻB	LB
Bithynidae	0.5	20	-	-	1	-	-	-	-	-	6	-
Lymnaeidae	1.0	40	11	-	-	-	-	-	-	1	4	2
Planorbidae	1.0	40	4	-	-	-	-	-	4	-	4	1
Sphaeriidae ¹	14	70	16	19	2	-	-	-	16	1	38	74
Unionidae	0.5	10	-	1	-	-	-	-	-	1	1	-
Lumbriculidae ¹	3.0	40	21	23	2	-	-	-	8	-	-	-
Naididae	2.5	20	-	-	-	1	1	-	-	-	-	-
Tubificidae ¹	17	70	-	-	38	9	19	33	60	20	19	-
Erpobdellidae	1.5	40	5	-	3	1	-	-	-	-	7	-
Asellidae ¹	2.0	60	6	8	1	-	-	18	7	-	-	7
Gammaridae	14	50	5	1	-	12	17	-	-	62	1	10
Baetidae ²	1.5	30	-	1	-	5	6	3	-	-	1	-
Coenagrionidae ²	0.2	10	-	-	-	-	-	-	-	-	-	2
Mesoveliidae	0.2	10	-	3	-	-	-	-	-	-	I	-
Sialidae	0.5	10	15	I	-	-	-	-	-	-	I	-
Nepidae	0.3	20	-	I	-	-	-	-	-	1	1	-
Glossosomatidae ²	0.5	20	-	-	-	2	3	-	-	-	-	-
Hydropsychidae ²	1.0	30	-	-	1	29	7	11	-	-	-	-
Leptoceridae ²	1.5	20	-	-	-	1	-	-	-	-	-	-
Limnephilidae ²	0.5	10	-	5	-	4	-	-	5	-	-	-
Polycentropodi- dae ²	2.6	20	-	-	-	10	9	-	-	-	-	-
Gyrinidae	0.5	10	-	-	-	1	-	-	-	-	-	-
Chironomidae ¹	33	90	17	42	52	24	35	35	-	14	21	4
Limoniidae	0.7	20	-	-	-	1	3	-	-	-	-	-
Total 24 f	family		9	6	8	13	9	5	6	7	8	7
ASPT	Гв		3.4	4.0	3.5	5.4	5.6	3.8	3.7	4.3	4.0	4.1
ЕРТ			0.0	1.0	1.0	5.0	3.0	2.0	1.0	0.0	0.0	0.0
Н			0.9	0.6	0.5	0.8	0.7	0.6	0.6	0.5	0.8	0.4
S [%]			0.0	5.0	1.0	51	25	14	5.0	0.0	0.0	0.0
MMI/class			0.2 V	0.2 V	0.1 V	0.6 III	0.5 III	0.2 V	0.2 V	0.2 V	0.0 V	0.1 V
Density (ind.* m ⁻²)			452	861	1319	982	781	1727	500	2274	931	1501

Table 6. Benthic macroinvertebrates in the Lutynia River system

Explanations: D - Structure of dominance, C - stability of occurrence $ASPT_B - Average$ Score Per Taxa index, EPT- Ephemeroptera, Plecoptera, Trichoptera index, H - Shannon-Wiener index, S(%) - share of stenotype taxa, MMI - Multimetric Macroinvertebrate Index, **Bolded**¹ - eurytopic taxa, Bolded² - stenotypic taxa Oligochaeta (*Lumbriculus sp.*, *Tubifex sp.*), Chironomidae (*Chironomus plumosus*) and Sphaeriidae (*Pisidium sp.*) dominated most of the surveyed sites, only on sections L4 and L8 Hydropsychidae and Gammaridae, respectively (Table 6). The share of organisms from the EPT group (Ephemeroptera, Plecoptera, Trichoptera), with the exception of L4 and L5 sites, was limited to only one taxon at the site (L2, L3, L6) or none at all (L1, L7, ŻB, LB). The highest share of stenotype taxa, as well as the highest values of the ASPT_B index, were recorded on sections L4 and L5, the lowest on L1, L3, ŻB and LB.

Species diversity is different from other indicators. The highest diversity values were found on the anthropogenically modified section L1 as well as on the more natural section close L4. The MMI macroinvertebrate multimeter was the highest in sections L4 and L5 – class III (Table 6).

The largest taxonomic similarity is found in the L4 and L5 sites, i.e. the middle, forest course of Lutynia (Fig.2). The families of Beatidae (Ephemeroptera), Glossosomatidae, and Polycentropodidae (Trichoptera) were only found here. Other cluster groups are pairs L1 and LB, L2 and L7 as well as L3 and L6. The L1 and LB positions had 6 common taxa, including the Sphaeriidae, Asellidae and Gammaridae families. At two sites with the highest pressure (L3 and L6) 5 common taxa were recorded, including the dominant species *Chironomus plumosus* and *Tubifex tubifex*.



Fig. 2. Agglomerative hierarchical clustering by Jaccard coefficient for zoobenthos

Statistically significant correlations were noted between biological indexes and individual parameters describing the pressure on the river (Table 7). The ASPT_B index was highly significantly correlated with almost all parameters,

except temperature and conductivity. The strongest compounds are found in dissolved matter content (R = -0.891, p < 0.01), oxygen content (R = 0.867, p < 0.01) and bottom substrate (R = 0.812, p < 0.01). The EPT index was strongly correlated only with the number of shelters available (R = 0.926, p < 0.01 and the type of bottom substrate (R = 0.752, p < 0.02). Species diversity was significantly influenced by the five parameters tested, of which the conductivity was the most correlated (R = 0.818, p < 0.01) and oxygen content (R = 0.771, p < 0.01). Invertebrate multimeter which includes, among others, ASPT, EPT and Shannon-Wiener species diversity, significantly correlates with the availability of shelters (R = 0.871, p < 0.01), but also depth variations, flow and bottom substrate strongly influence this index.

	Barriers	Depth CV	Natural elements	Flow	Shelters	Bottom substrate	Temperature	Oxygen	Conductivity	Dissolved matter
ACDT	-0.789	0.768	0.752	0.766	0.792	0.812	-0.15	0.867	-0.085	-0.891
ASPT _B	0.007	0.009	0.012	0.009	0.006	0.004	0.681	0.001	0.815	0.0005
EDT	-0.397	0.261	0.283	0.342	0.826	0.752	-0.39	0.006	-0.215	-0.095
EPI	0.25	0.466	0.427	0.332	0.003	0.012	0.261	0.986	0.550	0.794
п	-0.630	0.062	0.265	0.276	0.046	0.067	-0.68	0.771	-0.818	-0.638
п	0.041	0.864	0.459	0.44	0.899	0.853	0.029	0.009	0.004	0.047
мл	-0.406	0.611	0.201	0.743	0.871	0.631	-0.15	0.207	-0.243	-0.480
IVIIVII	0.24	0.041	0.577	0.014	0.001	0.04	0.662	0.567	0.498	0.160

 Table 7. Correlations of biotic indexes with environmental conditions – zoobenthos

Explanations: **bold font** was used for statistically significant correlations (p < 0.05)

4.3. Species structure of fish

The ichthyofauna of Lutynia and its tributaries was represented by 19 species (Table 8). The gudgeon (*Gobio gobio*), which was found in all positions (C = 100%), was the numerically dominant species, while the share of other species did not exceed 10%. The highest species richness was recorded at L7 and L8 sites – 12 and 10 species of fish, respectively, while the highest density was found at L8 and L4 sites.

The most ecologically demanding lithophilic fish occurred almost exclusively at L4 and L5 sites in the middle course of the river. Single asp (*Aspius aspius*) fry were caught in the downstream section (L8). Protected species were represented by a spined loach (*Cobitis taenia*) and a bitterling (*Rhodeus sericeus amarus*), found in the lower part of Lutynia and the stone loach found in both main watercourse and tributaries. Alien species, including the Prussian carp (*Carassius gibelio*) and topmouth gudgeon (*Pseudorasbora parva*), appeared on heavily modified sections of Lutynia and Lubianka.

The structure of dominance in terms of abundance, $ASPT_F$ indexes, H species diversity, E/R ratio and EFI+ value are presented in Table 8. In seven positions, the dominant was a gudgeon or stickleback (*Gasterosteus aculeatus*). Only the L8 position was dominated by a dace – 40%, while the LB topmouth gudgeon – 50%. The positions in the lower reaches of the L7 and L8 were characterized by the largest species diversity – the Shannon-Wiener index exceeded 0.70. The smallest species diversity of ichthyofauna was found at L2 (D = 0.37) and L3 (H = 0.25). The ASPT_F index reached the highest values at L4 and L5 stations, the lowest on L6 sections and in the LB inflow. The E/R index looks similar. As far as EFI+ values are concerned, the index reached the highest values in positions L4, L8 and L5 – II class. In other positions, it adopted class III or IV, respectively.

The similarities in the species structure between the sites are presented by means of cluster analysis in Figure 3. Sites L1 and L3 as well as L2 and $\dot{Z}B$ had the same species structure (100%). The similarity between the two groups located in the upper reaches was 65%. The psammophilic species observed in these stretches – gobies, stone loaches and sticklebacks. L4 and L5 grouped in another cluster. They have 88% identical species composition. The middle course of Lutynia was characterized by the occurrence of lithophils, namely brown trout (*Salmo trutta* m. *fario*) and barbell (*Barbus barbus*) not found in other sites. Another cluster formed the lower reaches of Lutynia – L7, L8, with a 58% of similarity in species.

As already mentioned, the greatest species richness was observed here (Table 8). Common species for both sites are chub (*Leuciscus cephalus*), dace (*Leuciscus leuciscus*) and spined loach, while asp and bitterling were also caught at L8. The Lubianka inflet (LB) was characterized by a significantly different species composition compared to the other sites, and two out of four caught species were alien: the Prussian carp and topmouth gudgeon.

Table 8. Ichthyofauna of the Lutynia (L1-L8) and its tributaries Żybura (ŻB) and Lubianka (LB)

4 4 11 9
4 4
00
6.2 5
Leuciscus cephalus (L.)

Explanations: ASPT_F – Average Score Per Taxa index, S – share of stenotypic (lithophilic) species, H – Shannon-Wiener index, EFI(+) – European Fish Index, **Bolded – stenotypic species**, **Bolded – eurytopic species**, **Bolded – alien**, **invasive species**



Fig. 3. Agglomerative hierarchical clustering by Jaccard coefficient for ichthyofauna

The results of the Spearman correlation analysis between the indices describing the state of ichthyofauna and the environmental parameters describing the pressures are presented in Table 9. The ASPT_F index was significantly correlated with almost all environmental parameters reflecting the pressures. The analysis shows that the index has the greatest impact on the oxygen content (R = 0.906, p < 0.001), conductivity (R = -0.863, p < 0.01) and the presence of barriers (R = -0.839, p < 0.01). The correlations of the S index look similar, but the relationships are not that strong. Species diversity is significantly related to depth (R = 0.676, p < 0.05) and flow (R = 0.669, p < 0.05), number of shelters (R = 0.662, p < 0.05) and the type of bottom substrate (R = -0.674, p < 0.05). Attention is drawn to the negative, recent correlation, which shows that the high proportion of gravel in the bottom substrate negatively affects the species diversity. The most advanced EFI+ index significantly correlates with depth variation (R = 0.875, p < 0.01) and flow (R = 0.944, p < 0.001).

	Barriers	Depth CV	Flow	Shelters	Bottom substrate	Temperature	Oxygen	Conductivity	Dissolved matter
N spec	0.169	0.615	0.612	0.694	0.480	0.453	0.306	0.324	-0.073
1 1 . spec.	0.639	0.048	0.049	0.026	0.160	0.189	0.390	0.361	0.840
ASDT	-0.839	0.741	0.859	0.725	0.592	-0.735	0.906	-0.863	-0.802
ASIT	0.002	0.015	0.001	0.018	0.051	0.015	0.0003	0.001	0.005
E/D	0.703	-0.625	-0.659	-0.321	-0.749	0.732	-0.745	0.788	0.615
E/K	0.023	0.043	0.038	0.366	0.012	0.016	0.0135	0.007	0.048
TT	0.038	0.676	0.669	0.662	-0.674	0.409	-0.116	0.237	-0.262
п	0.917	0.032	0.034	0.037	0.032	0.241	0.749	0.508	0.464
EEL	-0.336	0.875	0.944	0.507	0.561	-0.151	0.467	-0.454	-0.551
EF1+	0.342	0.001	<0.001	0.135	0.091	0.676	0.174	0.186	0.098

Table 9. Correlations of biotic indexes with environmental conditions – ichthyofauna

Explanations: **bold font** was used for statistically significant correlations (p < 0.05)

5. Discussion

The results presented in the work testify to the strong reaction of the studied assemblages to anthropogenic watercourse transformations. The strong correlation between anthropopression (expressed by the indicator of naturalness) and the diversity of microhabitats, which in turn is reflected by the structure of zoobenthos and ichthyofauna. On the untransformed river stretches, the multi-structured shape of the river channel, the presence of boulders and limbs of trees, spawn optimal living conditions for fish and invertebrates as stated by relevant literature (Branco et al. 2014, Pander & Geist 2016, Thompson et al. 2017, Wolter et al. 2013). Most of the river stretches were dominated by eurytopic benthic taxa (Oligochaeta, Chironomidae, Sphaeriidae) while the most exigent taxa were rare or absent due to the synergistic impact of three pressures, out of which the most important appeared to be river channel modification and pollution. Confirming the causal relationship between environmental quality and macrobenthos composition, stenotypic taxa are present with significantly greater frequency and abundance in the two river sections with little or no impact that cross the forest. According to Liu et al. (2016) and Wolter et al. (2013) the dominance of Oligochaeta and Diptera along with the reduction or elimination of other taxa is evidence of watercourse degradation. As can be logically expected, this study also confirmed that macro-invertebrate diversity is correlated negatively with temperature and

positively with oxygen. The decisive role of thermal regime and oxygenation in shaping aquatic biocenoses is also emphasized by (Bis et al. 2013, Krepski et al. 2018, Łaszewski et al. 2016). Since the presence shore vegetation and the covering the river channel with tree crowns is a determinant factor that influences temperature of the watercourses (Broadmeadow et. al. 2011, Kalny et al. 2017) the most natural river stretches crossing the forested areas also provided the most favorable conditions for macro-invertebrate assemblages. This further confirms the important role played by the riparian areas that provide both a mitigation of temperature variation and a source of organic matter, from large woody debris which is a component of habitat diversification, to the smallest organic particulate that is an important food supply for aquatic invertebrates (Thompson et al. 2017).

The ichthyofauna was dominated by the gudgeon, and in the mostchanged sections almost exclusively this species was found, with only a small contribution of the stickelback and stone loach. This corresponds to the results of Witkowski et al. (2007) who noted the dominance of the gudgeon in all sub-basins of the Oder, in particular those degraded anthropogenically. According to many authors, the occurrence of only a few psammophilic species is a phenomenon typical of small rivers, organically polluted (Marszał et al. 2014, Mueller et al. 2020, Penczak et al. 1991, Prus et al. 2016).

Brown trout and barbell only occurred at two forest sites with minimal pressure. Both taxa belong to the group particularly susceptible to negative changes resulting from anthropopressure (Marszał et al. 2014, Przybylski et al. 2020) and their presence positively indicates the ecological status of the water-course (Admaczyk et al. 2013, Prus et al. 2016, Rechulicz & Płaska 2016). For-ested watercourse sections are least exposed to pressure, and the presence of the riparian zone has also a positive effect on ichthyofauna (Broadmeadow et al. 2011, Four et al. 2017, Prus et al. 2018).

The highest number of fish species was found in estuaries, of which only one (L8) has a relatively high degree of naturalness. The high number of species in the heavily impacted section L7 is not surprising, since mainly eurytopic or stagnophilic species, including one alien species, were present. The occurrence of <u>rheophiles</u>, such as chub, ide and dace, as well as a protected spined loach, can be explained by the location of the section a few kilometers from the mouth of the river (near Warta), from which, despite the presence of two gabion buildings, they migrate up Lutynia. The general tendency, repeatedly emphasized in the literature, confirms that along with the size of the watercourse and the distance from the sources, the quantity and diversity of species increases (Broadmeadow et al. 2011, Four et al. 2017, Prus et al. 2018). The significant correlation between species composition and environmental conditions shows that fish assemblages react very clearly to all kinds of pressure, that act contemporarily making difficult to disentangle the relative importance. Only species diversity appears to be strongly correlated only with hydromorphological transformations. According to the authors, the Sh-W index in some cases does not reflect the actual state of ichthyofauna. This is especially true for small gravel streams, in which several (2-4) species are often found, but they are fish with high environmental requirements. The above thesis can be confirmed by the example of sections L4 and L5.

Alien species – Prussian carp and topmouth gudgeon were caught in the sites under pressure, with the second species appearing in significant densities just on one sampling date. The presence of these taxa is associated with carp and grass carp farming fish in nearby ponds fed with river water and with transformations of watercourses favoring tolerant over exigent species. These species are a threat to native ichthyocoenoses, because they have broad ecological tolerance and can compete for microhabitats and food resources, and Prussian carp reduces the spawning efficiency of common crucian carp (*Carassius carassius*) through gynogenesis (Kirankaya & Ekmekci 2013, Jakubčinová et al. 2013, Simonović et al. 2017, Szumiec et al. 2006, Witkowski & Grabowska 2012).

Alien species that get into the fish farming ponds along with young fish, are then easily introduced into the connected rivers (Witkowski & Grabowska 2012, Záhorská et al. 2013). In addition, fish ponds negatively affect the ecological state of the receiving rivers through post-production water discharges (Four et al. 2017, Francová et al. 2019, Hlavac et al. 2014, Szumiec et al. 2006).

The ASPT index calculated for both biotic assemblages indicates a strong response to most of the tested parameters and reflects well all types of disturbances. Similar results for invertebrates can be found in the publication of Bis & Mikulec 2013, however, no attempt has been made to apply this indicator to ich-thyofauna so far.

Comparing the results of our own study with general, accessible data from Kołaczkowski & Kniat (1959) and archival results of physicochemical analyzes conducted by VIEP in Poznań, we can notice a progressive improvement of the ecological condition of the middle and down current of Lutynia. Reduction of impacting activities in the river surroundings and of pollution loads prompted a gradual recovery of habitat conditions, as well as a slow increase in the number of species.

6. Conclusions

- The watercourses studied, despite the observed improvement in water quality, are still subject to strong anthropopressure, which is manifested mainly by modifications of the river bed reducing the diversity of microhabitats, continuity disturbances limiting the possibility of organism migration and organic pollutants deteriorating oxygen conditions.
- The level of pressure on individual sections is varied, and this is reflected in the differences in the composition and structure of the groups studied. The sections least transformed in terms of continuity and hydromorphology are also characterized by the highest water quality. In these sections, an increase in species diversity and the presence of indicator species with higher requirements were noted.
- The presence of the wide, well developed riparian zone is one of the most important factors determining the high diversity in the river biocenoses.
- Alien fish species are found mainly in transformed environments. The presence of all three disturbances is conducive to the occurrence and even the dominance of alien species.
- Macroinvertebrates and fish assemblages are responding slightly differently to each single impact source. For macroinvertebrates, the value of biological indexes depends on maintaining continuity; diversification of flow, depth and bottom substrate; the presence of hiding places; oxygen content and dissolved organic matter. Fish respond to all types of pressure, but the diversity of microhabitats and the water quality expressed in oxygen are particularly important.
- Zoobenthos and fish are very good, universal bioindicators and both groups should be used together providing a complimentary view on large and small scale impacts.
- The thesis about a different reaction of communities to pressures has been confirmed, but it is difficult to link a specific group of organisms with the scale of the pressure impact.
- The analyses show that ASPT is the most reliable among the biological indices used, for both benthos and fish.

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