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Iga Lewin*, Krzysztof Szoszkiewicz, Szymon Jusik, Agnieszka E. Ławniczak

Influence of selected environmental factors on macroinvertebrates in mountain streams

Abstract: The objectives of our survey were: to analyze the structure of macroinvetebrate communities in mountain streams in national parks and Biosphere Reserves (Poland, the Slovak Republic), to determine the environmental factors that influence the structure of macroinvertebrate communities and to assess the stream habitats including the bank and channel features, any modifications, land use and channel vegetation. Our results showed that in addition to the conductivity, the altitude, stream gradient and the values of the HQA index that reflected more natural features in the channel and river corridor were most important. The River Habitat Survey (RHS) method reflects not only the morphology but also the relationships between habitat features and the structure of macroinvertebrate communities and it provides a more holistic approach to assessing the health condition of stream ecosystems. Headwater streams support unique macroinvertebrate taxa that are found nowhere else in a catchment and may also constitute refuges for in-stream biota. Some of the least water pollution-tolerant macroinvertebrate taxa were recorded.

Keywords: Macroinvertebrates, Mountain stream, Biosphere Reserve, National park, Environmental variables, RDA analysis, River Habitat Survey

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***Corresponding authors: Iga Lewin:** The University of Silesia, Faculty of Biology and Environmental Protection, Department of Hydrobiology, 9 Bankowa Street, 40-007 Katowice, Poland, E-mail: iga.lewin@us.edu.pl

Krzysztof Szoszkiewicz, Szymon Jusik, Agnieszka E. Ławniczak: Poznań University of Life Sciences, Faculty of Land Reclamation and Environmental Engineering, Department of Ecology and Environmental Protection, 94C Piątkowska Street, 60-649 Poznań, Poland

1 Introduction

Headwater streams are especially vulnerable to anthropogenic disturbances. Changes in land and water use adjacent to or upstream of headwater sites often modify the water and habitat quality in these streams. Forested buffer zones protect headwater streams by promoting broad, shallow streams with a greater total area of aquatic habitats, and thus increase their diversity by protecting headwaters from point and diffuse sources of pollution and from any increase in the temperature of the water, thus forming a closed canopy as well as slower erosion from flooding [1]. The riparian vegetation provides the energy for the in-stream food web by allochthonously derived inputs of leaf litter. Macroinvertebrates (primarily aquatic insects) are the dominant organisms in temperate streams and rivers, including headwater streams and their adjoining wetlands and fill important trophic roles in stream ecosystems. For example, *Plecoptera* and *Trichoptera*, which are detritivores, algivores, and predators, fulfil important intermediate pathways of nutrient cycling and food web support. *Ephemeroptera*, *Plecoptera* and *Trichoptera* are significant components of headwater habitats and are widely used as indicators of the condition of streams. *Ephemeroptera*, *Plecoptera* and *Trichoptera* (EPT) are universally combined into the convenient and ecologically relevant, the %EPT index [2].

The habitat assessment method, *i.e.*, the River Habitat Survey (RHS), reflects the physical structure of streams, modifications of their channel, habitat and land use and any anthropogenic disturbances in a stream's corridor and the adjacent area. The RHS supports the survey of biological elements (*e.g.*, macroinvertebrates, macrophytes) that are most important in the assessment of the ecological status of streams in the light of the European Union Water Framework Directive (EU WFD) that was signed by Member States [3]. The RHS provides a practical way of characterising the physical structure of streams and combines the basic principles, approaches and terminology of fluvial morphology, freshwater ecology and nature conservation. The RHS has also been used to investigate the relationships between habitat features and associated biota, including benthic macroinvertebrates [4]. For example, the RHS was a useful tool in assessing the influence of the hydromorphology on caddisfly assemblages in high mountain streams [5]. The characteristic responses of macroinvertebrate communities to anthropogenic disturbances in a stream's corridor and the adjacent area are a reduction in the number of macroinvertebrate taxa and diversity, alterations of the community composition and feeding guilds. Many taxa of macroinvertebrates are eliminated or reduced, whereas a few taxa flourish under human-modified conditions. Therefore, their relatively high density is recorded in theses streams [6].

Even if the human disturbances and land use are limited in headwater streams such as commercial forestry operations (logging), tourisms (hiking trails, shelters, huts), cultivation, livestock grazing above the tree line etc., for example, in national parks or reserves, any changes in freshwater habitats may influence the structure of macroinvertebrate communities. Headwater streams may support unique species or communities of plants and animals that are found nowhere else in a catchment and may also constitute refuges for in-stream biota [7]. Thus, it is necessary to limit the human impact on headwater streams, including those in national parks or reserves. National parks, especially those located within mountain areas, are undoubtedly the most important since these protected natural spaces can be considered to be natural islands for the conservation of biodiversity [8].

The objectives of our survey were to analyze the structure of macroinvetebrate communities in mid- and high-altitude mountain streams in national parks and Biosphere Reserves (Poland, the Slovak Republic), to determine the environmental factors that are the most predictive parameters that influence the structure of macroinvertebrate communities and to assess the stream habitats including the bank and channel features, any modifications, land use and channel vegetation. We hypothesize that both the physical and chemical parameters of the water reflecting the differences in underlying geology, the presence of macrophytes and harsh environmental conditions are most important in explaining the gradient in distribution of macroinvertebrates in mountain streams.

2 Experimental Procedures

2.1 Study area

The study was carried out from 2007 to 2010 (June, July) in the Carpathians, Ecoregion no 10, which was established by the EU Water Framework Directive (EU WFD) [3]. Sampling sites were situated within national parks or reserves, *i.e.*, the Babia Góra National Park (BGNP) (one of the first Biosphere Reserves in the world, IUCN category II), the Gorce National Park (GNP), the Pieniny National Park (PNP), the Tatra National Park (TNP) (IUCN category II), the Tatranský Národný Park (TANAP) in the Slovak Republic, and the nature reserve "Wisła" (NRW) (the Silesian Beskids). In the Tatra Mts., the sampling sites were situated on the territory of the Tatras UNESCO Biosphere Reserve, which covers two national parks, *i.e.*, the TNP in Poland and the TANAP in the Slovak Republic. Approximately 70% of the Tatras UNESCO Biosphere Reserve is forested. There is a small amount of cultivation above 800 m a.s.l. but no livestock grazing above the tree line. Some characteristic mammals inhabit the Tatras UNESCO Biosphere Reserve, *e.g.*: chamois *Rupicarpa rupicarpa*, marmot *Marmota marmota*, brown bear *Ursus arctos*, lynx *Lynx lynx* or wolves *Canis lupus* [9].

A total of 39 sampling sites, which were situated mainly in the headwaters of mountain streams at mid- (200-800 m a.s.l.) and high altitudes (> 800 m a.s.l.) and with different geology (flysch, siliceous, calcareous) according to the EU WFD [3], were selected (Figure 1, Table 1).

2.2 Methods

2.2.1 Samples of water and benthic macroinvertebrates

Samples of water were collected from each sampling site immediately before the sampling of macroinvertebrates. Analyses of the physical and chemical parameters of the water, *i.e.*, conductivity, total dissolved solids, temperature, and pH were measured in the field using a portable HI 9811-5 pH/EC/TDS/°C meter HI 9811-5 (Hanna Instruments) and dissolved oxygen with a CO-401 oxygen meter (Elmetron). Analyses of ammonium, nitrite, nitrate, phosphate, iron concentrations in the water were carried out using colorimetric methods and alkalinity, the total hardness, calcium and chloride concentrations were carried out using titrimetric methods (meters and reagents from Hanna Instruments or Merck). Organic matter content in the bottom sediments was determined using the loss on ignition (LOI) method, which measures weight loss in the bottom sediment samples after burning at 550°C according to PN-88/B-04481 [10]. The grain size composition of the bottom sediments was determined by using both the sieve and aerometric methods. The particle size classification was characterised according to the Wentworth scale [11].

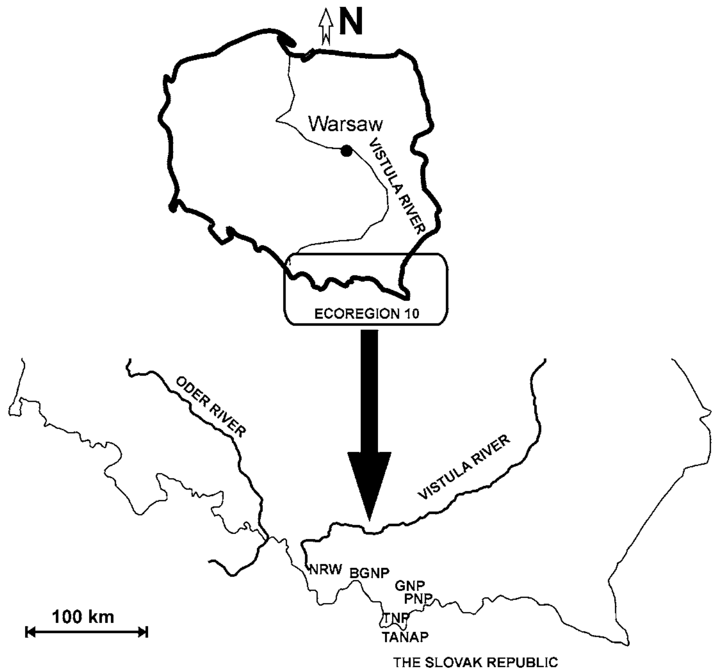

Figure 1:. Location of the study area. Abbreviations: BGNP - the Babia Góra National Park; GNP - the Gorce National Park; PNP - the Pieniny National Park; TNP - the Tatra National Park; TANAP - the Tatranský Národný Park; NRW - Nature reserve "Wisła"

Table 1: Geology and characteristic features of the mountain streams within the study area (national parks and reserves).

Criteria	**The Babia Góra National Park (BGNP) (Biosphere Reserve)**	**The Gorce National Park (GNP)**	**The Pieniny National Park (PNP)**	**The Tatra National Park, Poland (TNP), the Tatranský Národný Park, the Slovak Republic (TANAP) (the Tatras UNESCO Biosphere Reserve)**	**Nature reserve „Wisła" (NRW)**
Location/region	Outer Western Carpathians: the Babia Góra Massif	Outer Western Carpathians: the Gorce Mts.	Central Western Carpathians: the Pieniny Mts.	Central Western Carpathians: the Eastern (High) Tatra Mts.[a], the Western Tatra Mts.[b]	Outer Western Carpathians: the Silesian Beskids
Area (ha)	3,393	7,029	2,372	21,197 (TNP) 73,800 (TANAP)	17.61
Geology	flysch	flysch	calcareous	siliceous[a] calcalreous[b]	flysch
Number of sampling sites	8	13	3	12	3
Altitude of sampling sites (m a.s.l.)	705-1,217 (mid-altitude, high-altitude)	522-1,010 (mid-altitude, high-altitude)	498-674 (mid-altitude)	669-1,729[a] 925[b] (mid-altitude, high-altitude[a], high-altitude[b])	766-1,014 (mid-altitude, high-altitude)
Stream gradient (‰)	80.0-371.0	28.1-245.2	105.0-116.2	9.6-550.8[a] 68.5[b]	137.2-506.0
Size of streams	small, medium	small, medium	small, medium	small[a], medium[a,b]	small
Streams	Górny Płaj, Szumiąca Woda, Marków Potok, Jaworzynka, Dejakowy, Śpiowy	Kamienica, Koninka, Poręba, Łopuszanka, Głębieniec, Oberówka, Głęboki, Ustępny, Konina, Zapalac	Limbargowy, Pieniński, Straszny	Roztoka and its tributaries[a], Białka[a], Strążyski[b] (TNP), Biela voda[a], Bela[a], Zadná Tichá[a] (TANAP)	The headwater streams of the Vistula (Biała Wisełka and two tributaries)

The samples of benthic macroinvertebrates were collected using a hand net with a square frame (25 cm × 25 cm = sampling surface of 625 cm^2) and a mesh size below 500 µm according to the methodology of [12-14]. A total of 20 replicates (subsamples) was taken from all of the major habitat types in the reach (sampling surface of 1.25 m^2) using a hand net at each sampling site. The hand net was held vertically to the current of stream. The substratum was disturbed in the 0.25 × 0.25 m area upstream of the hand net and within a depth of 10 cm. In addition, benthic macroinvertebrates were collected from the softer bottom sediments among boulders and blocks using a core sampler (diameter 5 cm, sampling surface of 19.6 cm^2) within a depth of 10 cm. Twenty samples were collected using a core sampler at each sampling site. All of the collected material was preserved in 75% ethanol in the field and then brought back to the laboratory in plastic containers. The samples were sieved with a 0.23 mm mesh net and then sorted under a stereoscopic microscope in the laboratory. The benthic macroinvertebrates were counted and identified to the family level; *Heptageniidae* to the genus, *Hirudinea* and *Mollusca* to the species level [15-20].

The following metrics were calculated [12]:

1. The total number of macroinvertebrate taxa (S).

2. Density (D).
The density of benthic macroinvertebrates was estimated as the number of individuals per square metre.

3. The Average Score per Taxon (ASPT).
The ASPT is the value of BMWP (the Biological Monitoring Working Party) divided by the number of BMWP families that are present in the taxa list.

4. The index of %EPT (%EPT = sum of all individuals of *Ephemeroptera*, *Plecoptera*, and *Trichoptera* divided by the sum of all of the macroinvertebrates collected × 100).

The water and benthic macroinvertebrate surveys were supported by both a hydromorphological study and an assessment of the macrophytes (hydromorphological and biological elements) at the same sampling sites.

2.2.2 Hydromorphological and macrophyte surveys

The hydromorphological study was carried out according to the River Habitat Survey (RHS) methods as adapted to Polish conditions [21,22]. The RHS methodology includes an evaluation of the bank and channel features and modifications at 10 equally spaced spot-checks along a 500 m length of streams (rivers). The physical features (*e.g.*, flow type, substrate type, channel/bank modifications), land use and channel vegetation types were recorded at each spot-check. As a result, two indices were calculated *i.e.*, the Habitat Modification Score (HMS) and the Habitat Quality Assessment (HQA). Low values of the HMS indicate a limited artificial modification of watercourses, whereas higher values indicate a high degree of habitat modification. High values of the HQA indicate an extensive presence of a number of natural river features [9, 23]. The macrophyte surveys were carried out according to the Macrophyte Methods for Rivers as adapted to Polish conditions [24]. The total cover (%) of the stream beds by aquatic macrophytes was estimated. The Macrophyte Index for Rivers (MIR) was calculated based on the results of the survey.

2.2.3 Statistical analysis

Canonical ordination analyses to relate the biological data (the taxonomic composition of benthic macroinvertebrates) to the environmental variables were carried out using CANOCO for Windows version 4.5 [25]. The following environmental variables were included in the analysis: the physical and chemical parameters of the water; the total cover of the stream bed by macrophytes; the channel substrate; the values of the HMS, HQA, MIR indices; the organic matter content in bottom sediments; altitude, and the stream gradient. The appropriate type of analysis (redundancy analysis) was chosen in order to analyse the biological data using DCA (Detrended Correspondence Analysis) and the length of the gradient. Preliminary DCA on the biological data revealed that the gradient length was less than 3 SD (the standard deviation), thus indicating that the biological data exhibited linear responses to the underlying environmental variables, which justified the use of linear multivariate methods. Therefore, a linear direct ordination RDA with a forward selection was used to reduce of the large set of environmental variables. Taxa that occurred at fewer than 10% of the sampling sites were excluded from the statistical analyses following a preliminary exploration of their influence in an initial DCA analysis. The statistical significance of the relationship between the biological data and the physical and environmental variables was evaluated using the Monte Carlo permutation test (499 permutations). Both the biological and environmental data were log-transformed [25].

3 Results

3.1 The values of the environmental variables

Data summarizing the altitude of the sampling sites and gradients of the streams are given in Table 1. The altitude of the sampling sites ranged from 498 m a.s.l. (a calcareous stream, the PNP) to 1,729 m a. s. l. (a siliceous small stream, *i.e.*, a tributary of the Roztoka, the TNP) whereas the stream gradients ranged from 9.6 to 550.8‰. The physical and chemical parameters of the waters, the organic matter content in bottom sediments as well as the results of the hydromorphological and macrophyte surveys are given in Table 2. The lowest conductivity values, the total dissolved solids, pH, hardness, concentrations of nutrients or

Table 2: The values (ranges) of the environmental variables obtained for the sampling sites in the mountain streams within the study area (national parks and reserves).

Environmental variables	The Babia Góra National Park (BGNP) (Biosphere Reserve)	The Gorce National Park (GNP)	The Pieniny National Park(PNP)	The Tatra National Park, Poland (TNP), the Tatranský Národný Park, the Slovak Republic (TANAP) (the Tatras UNESCO Biosphere Reserve)	Nature reserve „Wisła" (NRW)
Temperature (°C)	6.4-14.5	10.5-15.6	14.7-17.6	6.2-14.1[a] 8.8[b]	12.0-14.2
Conductivity ($\mu S\ cm^{-1}$)	60-390	160-350	430-500	8-80[a] 270[b]	70-130
Total dissolved solids ($mg\ L^{-1}$)	30-190	80-170	210-250	3-40[a] 130[b]	30-60
Dissolved oxygen ($mg\ O_2\ L^{-1}$)	6.4-10.0	6.3-10.0	6.1-7.5	6.2-9.3[a] 6.6[b]	5.3-12.5
pH	7.6-8.1	7.4-8.4	7.7-8.0	5.3-7.1[a] 8.2[b]	6.8-7.2
Ammonium ($mg\ NH_4^+\ L^{-1}$)	0.049-1.408	0.024-0.182	0.049-0.133	0.0-0.024[a] 0.097[b]	0.012-0.097
Nitrites ($mg\ NO_2^-\ L^{-1}$)	0.0-0.197	0.033-0.132	0.007-0.218	0.0-0.033[a] 0.033[b]	0.033-0.099
Nitrates ($mg\ NO_3^-\ L^{-1}$)	0.44-8.86	0.44-7.97	5.32-10.63	0.0-1.33[a] 3.5[b]	3.10-5.76
Phosphates ($mg\ PO_4^{3-}\ L^{-1}$)	0.01-0.38	0.01-0.25	0.07-0.08	0.01-0.10[a] 0.07[b]	0.04-0.06
Hardness ($mg\ CaCO_3\ L^{-1}$)	48-100	56-210	310-335	2-68[a] 275[b]	18-67
Alkalinity ($mg\ CaCO_3\ L^{-1}$)	25-110	60-120	195-205	5-20[a] 140[b]	20-40
Chlorides ($mg\ Cl^-\ L^{-1}$)	4-10	4-10	4-6	1-4[a] 6[b]	4-6
Calcium ($mg\ Ca\ L^{-1}$)	10-44	26-42	62-78	2-18[a] 30[b]	6-12
Iron ($mg\ Fe\ L^{-1}$)	0.0-0.07	0.01-0.13	0.03-0.09	0.02-0.15[a] 0.05[b]	0.03-0.06
Organic matter (%)	3.19-11.05	1.59-5.07	0.54-18.38	1.05-16.81[a] 2.11[b]	2.22-4.34
Total cover (%)	0.80-6.35	0.20-15.05	0.10-2.35	0.20-46.40[a] 15.60[b]	6.05-22.40
MIR index	57.7-85.3	46.7-84.5	35.5-83.8	61.7-95.5[a] 82.8[b]	83-88.6
HQA index	41-76	50-68	67-69	39-60[a] 56[b]	60-75
HMS index	0-4	0-6	0-2	0-8[a] 3[b]	0-1

alkalinity were measured at the sampling sites in the siliceous streams located in the Tatras UNESCO Biosphere Reserve (Table 2). Water conductivity, total dissolved solids, concentration of nitrates and calcium, hardness and alkalinity were considerably higher at sampling sites in the calcareous streams of the PNP. A wider range of the organic matter content in the bottom sediments was recorded in the calcareous streams in the PNP.

The maximum value of the MIR index was recorded in the high-altitude siliceous stream in the TNP (tributary of the Roztoka) (Table 2). Lower values of the HQA index were obtained at a few sampling sites in both the high-altitude flysch and siliceous streams that are situated above 1,200 m a.s.l. in the Biosphere Reserves: 41-42 in the BGNP (the Górny Płaj, the Szumiąca Woda and the Dejakowy), 45-49 in the TANAP (the Biela voda), and 39-41 in the TNP (the Roztoka and its tributaries).

3.2 The structure of macroinvertebrate communities and the values of the metrics

In total, 53 macroinvertebrate taxa were recorded at the sampling sites. Among them some taxa that were most sensitive to water pollution according to the BMWP(PL) methodology [26] were recorded, *e.g.*, *Odontoceridae*, *Beraeidae*, *Glossosomatidae*, *Goeridae* or *Blephariceridae*. The total number of taxa (S) was low in the flysch stream at 1,208 m a.s.l. in the BGNP (the Górny Płaj) and siliceous stream at 1,729 m a.s.l. in the TNP (Table 3). Six to 11 taxa occurred on the sites located above 1,500 m a.s.l. *Chironomidae* and *Oligochaeta* were recorded at most of the sampling sites followed by *Limnephilidae*, *Baetidae*, *Nemouridae*, *Rhithrogena* and *Simuliidae*. Only one *Hirudinea* species, *i.e.*, *Erpobdella octoculata* (Linnaeus, 1758) was observed at sampling sites in the siliceous streams in the TANAP (*e.g.*, the Bela). *Bythinella* sp. was recorded in the flysch streams, *e.g.*, in the BGNP (the Dejakowy and the Marków Potok) and in the GNP (the Konina, the Koninka and the Głęboki); in the siliceous stream (the Bela) and calcareous stream (the Strążyski), which are located within the Tatras UNESCO Biosphere Reserve, but were mainly found in the calcareous streams of the PNP. The density (D) of macroinvertebrates was lowest in a small flysch stream in the GNP. The maximum density was recorded in the siliceous stream of the TANAP (the Bela) (Table 3). The density of some taxa was highest in the high-altitude siliceous stream and concurrently at a relatively small stream gradient of 12.4‰, *i.e.* in the Bela, the TANAP. For example, the density of *Perlodidae* ranged up to 70 individuals m^{-2}, *Capniidae* up to 125 individuals m^{-2}, whereas *Gammaridae* ranged up to 1,437 individuals m^{-2}. The maximum density of some taxa, *e.g.*, *Sericostomatidae* (92 individuals m^{-2}), *Elmidae* (110 individuals m^{-2}) and *Bythinella* sp. (153 individuals m^{-2}) were recorded in the calcareous streams in the PNP. Dragonfly larvae, *Cordulegasteridae,* were only recorded at two sampling sites with a density of up to 8 individuals m^{-2} (the Oberówka, the Straszny).

The maximum value of the ASPT index was calculated for the macroinvertebrates in the siliceous stream situated in the TANAP (the Zadná Tichá). The value of the %EPT index ranged from 1.81% in the siliceous stream located above 1,200 m a.s.l. (the Zadná Tichá, the TANAP) to 67.35% in the mid-altitude flysch stream (the Głębieniec,

Table 3: The values (ranges) of the metrics (macroinvertebrates) calculated for the sampling sites in the mountain streams within the study area (national parks and reserves).

Index	The Babia Góra National Park (BGNP) (Biosphere Reserve)	The Gorce National Park (GNP)	The Pieniny National Park (PNP)	The Tatra National Park, Poland (TNP), the Tatranský Národný Park, the Slovak Republic (TANAP) (the Tatras UNESCO Biosphere Reserve)	Nature reserve „Wisła" (NRW)
The total number of taxa (S)	5-21	11-24	14-23	6-28[a] 23[b]	12-18
Density (D) (individuals m^{-2})	267-903	111-1,322	401-1,673	314-5,224[a] 2,478[b]	457-652
ASPT	5.23-6.10	5.35-6.83	5.14-5.86	5.08-6.85[a] 5.65[b]	4.50-6.06
%EPT	15.39-45.69	10.83-67.35	7.47-20.67	1.81-39.61[a] 17.56[b]	8.90-53.61

the GPN). Relatively low values of the %EPT index were calculated for the sampling sites in the siliceous streams located within the Tatras UNESCO Biosphere Reserve above 1,200 m a.s.l. (*e.g.*, the Biela voda, the Roztoka and its tributaries) (Table 3).

3.3 Redundancy analysis (RDA)

RDA analysis based on the biological data and environmental variables showed that the first two axes explain 17.7% of the variance in the biological data and 78.8% of the variance in the biological data and environment relations. The altitude, stream gradient, conductivity and values of the HQA index were the parameters most associated (statistically significant according to the forward selection results) with the distribution of benthic macroinvertebrate taxa.

Some patterns of the distribution of macroinvertebrates were found. *Ecdyonurus*, *Bythinella*, *Elmidae*, *Perlidae*, *Hydropsychidae*, *Beraeidae* and *Leptophlebiidae* were the taxa positively correlated with higher values of conductivity whereas some taxa within *Trichoptera* (*Odontoceridae*, *Beraeidae*), *Plecoptera* (*Leuctridae*, *Nemouridae*) and *Coleoptera* or *Diptera* were positively correlated with the higher values of the HQA index (Figure 2). Most macroinvertebrate taxa within Ephemeroptera, Trichoptera as well as *Gammaridae* and *Radix balthica* (Linnaeus, 1758) were negatively correlated with the stream gradient. *Capniidae* and *Perlodidae* were positively associated with the altitude (Figure 2). The relation between the taxonomic composition of the benthic macroinvertebrate taxa and the environmental variables was statistically significant (the Monte Carlo test of significance of first canonical axis: F-ratio = 4.142, P-value = 0.002, test of significance of all canonical axes: F-ratio = 2.46, P-value = 0.002).

Figure 3 shows the samples in relation to the selected environmental variables. The samples of the Tatras UNESCO Biosphere Reserve (siliceous streams at a high altitude) are associated with the altitude and lower values of conductivity. The samples of the GNP (mid- and high-altitude flysch streams) are associated with higher values of conductivity and the HQA index. The samples of the PNP (mid-altitude calcareous streams) are associated with

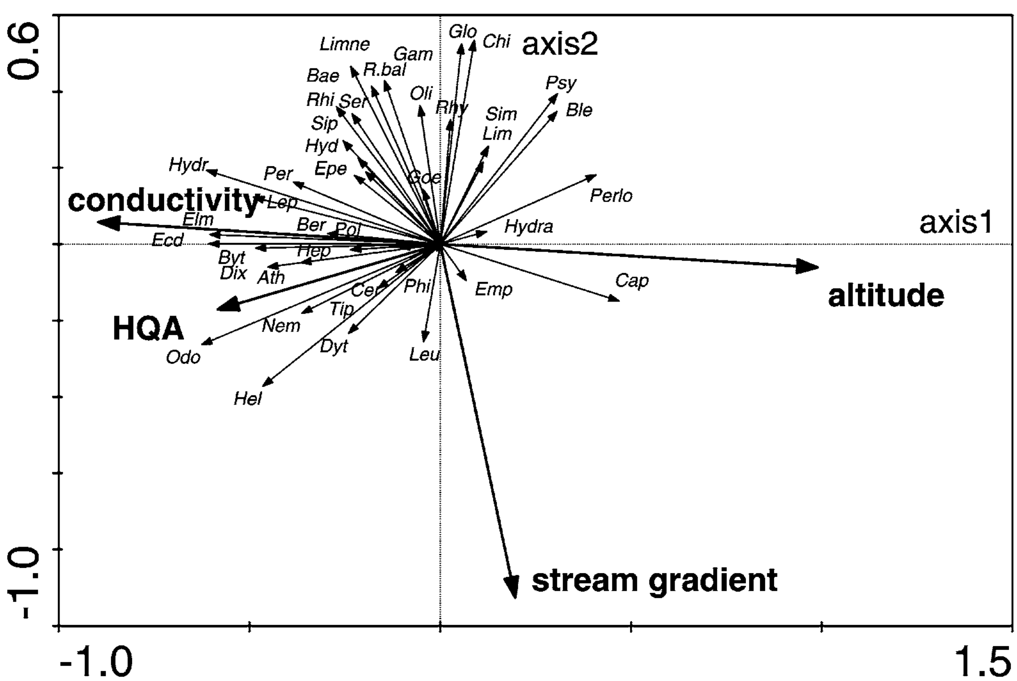

Figure 2: Ordination diagram (biplot) based on a redundancy analysis (RDA) of benthic macroinvertebrates and selected environmental variables. The long arrows representing some environmental variables emphasise their significant impact on the distribution of benthic macroinvertebrates. Abbreviations: *Ath Athericidae, Bae Baetidae, Ber Beraeidae, Ble Blephariceridae, Byt Bythinella, Cap Capniidae, Cer Ceratopogonidae, Chi Chironomidae, Dix Dixidae, Dyt Dytiscidae, Ecd Ecdyonurus, Elm Elmidae, Emp Empididae, Epe Epeorus, Gam Gammaridae, Glo Glossosomatidae, Goe Goeridae, Hel Helodidae, Hep Heptagenia, Hydra Hydracarina, Hyd Hydraenidae, Hydr Hydropsychidae, Lep Leptophlebiidae, Leu Leuctridae, Limne Limnephilidae, Lim Limoniidae, Nem Nemouridae, Odo Odontoceridae, Oli Oligochaeta, Per Perlidae, Perlo Perlodidae, Phi Philopotamidae, Pol Polycentropodidae, Psy Psychodidae, R.bal Radix balthica, Rhi Rhithrogena, Rhy Rhyacophilidae, Ser Sericostomatidae, Sim Simuliidae, Sip Siphlonuridae, Tip Tipulidae*

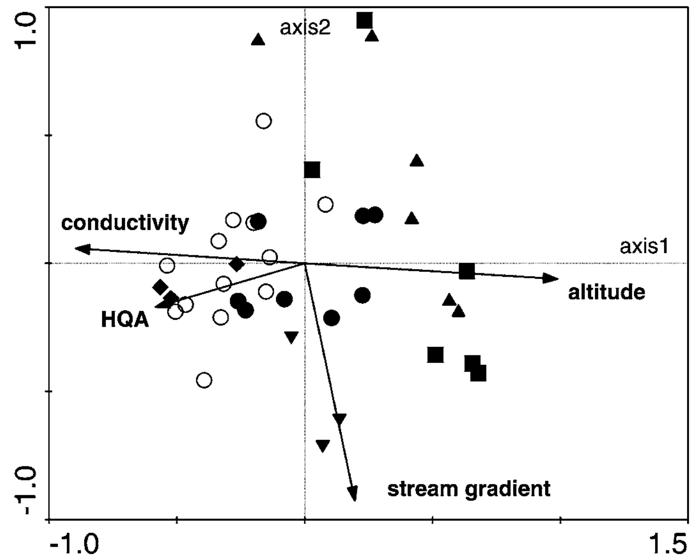

Figure 3: Ordination diagram (biplot) based on a redundancy analysis (RDA) of the samples in relation to the selected environmental variables. Legend: ● BGNP; ○ GNP; ♦ PNP; ■ TNP; ▲ TANAP; ▼ NRW

higher values of the HQA index and conductivity, whereas samples of the BGNP (Biosphere Reserve) (mid- and high-altitude flysch streams) are associated with the HQA index and altitude.

4 Discussion

4.1 The values of the HQA and HMS indices that reflect the natural features in the stream corridor and channel modifications

The habitat assessment method, *i.e.*, the RHS, which includes the calculation of the HQA and the HMS indices, reflects the physical structure of streams, any modifications of their channel, habitat and land use. The HQA index is a broad indication of overall habitat diversity that is provided by the natural features in the channel and river corridor [9]. Higher values of the HQA index reflect more natural features including eroding cliffs, waterfalls, in-channel vegetation, distribution of bankside trees and the extent of near-natural land use adjacent to the streams. Our results revealed a maximum value of the HQA index up to 76. The values of the HQA index above 47 are typical for the reference conditions of mountain streams [27]. In contrast, the present survey showed lower values of the HQA index which were obtained at a few sampling sites in the high-altitude flysch and siliceous streams that are situated above 1,200 m a.s.l. in the Biosphere Reserves (the BGNP and the Tatras UNESCO Biosphere Reserve) (minimum HQA values 41 and 39, respectively). Lower values of the HQA index are typical for streams that are situated in the alpine and subalpine zones. In comparison, Raven et al. [9] obtained minimum HQA values of 53 in the TANAP and 51 in the TNP (the Roztoka) for the high-altitude siliceous streams. According to [28], the values of the HQA index are positively correlated with tree cover. They showed a value of the HQA index of 61 for siliceous streams at high-altitude in the TANAP that were not affected by windstorms and 42 for those that were most affected by windstorms. In windstorm-damaged areas, streams that are situated on deforested valley slopes were more affected by erosion. In the Biosphere Reserves *i.e.*, the BGNP and the Tatras UNESCO Biosphere Reserve, there are distinct vegetation zones that are dependent on the habitat and climatic conditions. Snow cover on the highest peaks can last for 200 days a year and some snowfields are present throughout the year. The pine dwarf (*Pinus mugo* Turra) occurs above the tree line between about 1,500–1,800 m a.s.l. in the Tatras UNESCO Biosphere Reserve and from about 1,200 m a.s.l. in the BGNP. The value of the HQA index also includes the distribution of bankside forests and the extent of near-natural land-use adjacent to the streams, whereas in the alpine zone, the *Pinus mugo* is predominant rather than forests and the landscape is largely rock and scree. Thus, in our survey, lower values of the HQA index of streams that are situated above the tree line are not the result of their degradation but this phenomenon may be explained by the natural hydromorphological make-up and the harsh conditions.

In contrast to the HQA index, higher values of the HMS index reflect more artificial modifications to the river channel morphology, *e.g.*, culverts, weirs, re-profiling and reinforcement of banks. Our results found low values of the HMS index, that are characteristic of untransformed mountain streams or those that had been transformed to only a small degree [27]. Channel modification, which is reflected by the values of the HMS index, is generally greater in lowland rivers compared to higher altitudes and the values of HQA index increase with the altitude and gradient up to the tree lines because the HQA system is strongly influenced by the extent of trees and associated features [4]. In comparison [29] showed wider ranges of the HQA values, *i.e.*, 20-73 in high-altitude siliceous streams in a national park. This phenomenon was explained by the alignment and resectioning of banks, which caused the loss of natural water course sinuosity, the simplification of the substrate as well as the loss of bank vegetation.

4.2 Macroinvertebrate communities in mountain streams in relation to selected environmental factors

Environmental harshness including altitude, stream gradient or geological features is considered to be an important factor influencing the structure of macroinvertebrate communities in mountain streams [8,30-36]. Altitude is related to changes in the percentage of riparian cover and aquatic vegetation, which in turn, determine allochthonous inputs of energy into streams [37,38]. The character of mountain river beds is a function of these factors. The present survey revealed (RDA analysis) that the altitude, stream gradient, conductivity and values of the HQA index were the parameters most associated (statistically significant) with the distribution of benthic macroinvertebrate taxa. The results of [31-33] showed a similar pattern: the altitude, stream gradient, conductivity and also geology were among the major environmental variables.

The physical and chemical parameters of the water in unpolluted mountain streams are usually the function of geology: the water of calcareous or flysch streams are characterised by higher values of conductivity, alkalinity, hardness or pH in comparison with siliceous streams. For example, [39] found that the ephemeropteran species were positively correlated with conductivity in the mid-altitude calcareous streams of the PNP. Our results confirm their findings because some of the ephemeropteran taxa were associated with higher values of conductivity not only in the calcareous streams of the PNP but also in the flysch and siliceous streams. According to [40], the differences in the structure of the macroinvertebrate communities between mountain streams paralleled the differences in the chemistry of the stream water, which in turn, reflected the differences in the underlying geology. Mid- and high-altitude granite (siliceous) mountain streams supported fewer macroinvertebrate taxa than the others, *e.g.*, schist [40]. It was proved [41] that typical ephemeropteran species (*e.g.*: within the genera *Epeorus, Rhithrogena* and the family *Leptophlebiidae*) disappeared from the headwater mountain streams when pH dropped below 6.3. For comparison, our results revealed very low values of pH (minimum 5.3) in the high-altitude siliceous streams of the TNP. Thus, the relatively low values of the %EPT index that were recorded at these sampling sites of the TNP may be explained by the sensitivity of Ephemeroptera to low pH.

According to [30], an increase in the diversity of mayflies towards lower altitudes in siliceous mountain streams seems to be the rule; the diversity of mayflies increased with a decrease in altitude and stream gradient (the Bela River catchment, the TANAP). Our results (RDA analysis) confirmed the survey of [30] because the distribution of the mayfly taxa was negatively correlated with the stream gradient (*Rhithrogena*, *Epeorus*, *Baetidae* and *Siphlonuridae*). This pattern concerned not only the siliceous streams but also the flysch and calcareous streams. Our result is consistent with the survey of [42,43] who showed a negative association between the distribution of *Chironomidae* or *Perlidae* and the altitude as well as the stream gradient in mountain streams. Natural stressors such altitude and stream gradient also affected the composition of macroinvertebrate communities at the family level in mid- and high-altitude mountain streams [8,35,44].

According to [36], macroinvertebrate respiration rate decreased considerably less then the oxygen supply in streams at an altitude gradient from 400 m a.s.l. Therefore, the relatively large “gap” between the respiration rate and oxygen demand starts to occur from about 500 m a.s.l. As a result, macroinvertebrates may be living close to or under conditions of oxygen deficiency in high-altitude streams. Oxygen deficiency in the water can lead macroinvertebrates to adapt to such conditions or to the exclusion of non-adapted organisms. What is more, the oxygen saturation in the water depends on altitude [36]. The EPT taxa made up about 70% of the total invertebrate fauna in unpolluted, lower-altitude streams with about 100% oxygen saturation. In contrast, EPT taxa disappeared from the invertebrate fauna at an average minimum oxygen saturation of about 50%. The proportion of EPT taxa to macroinvertebrate fauna decreases with a decreasing oxygen saturation especially in high-altitude unpolluted streams. The EPT taxa was not recorded in streams with oxygen saturations under 80% relative to atmospheric pressure because EPT taxa of high-altitude streams are less resistant to a decrease in oxygen saturation than the macroinvertebrates of low-altitude streams [36]. Thus, in our survey, the negative correlation between altitude and the distribution of macroinvertebrates in mountain streams as well as lower values of the %EPT index that reflected the occurrence of EPT taxa in high-altitude, may be explained by their physiological make up.

The nature of a river bed, in addition to the physical and chemical parameters of the water affect the occurrence and development of aquatic biota in mountain river catchments [45]. Trees and the elements that accompany them, affect the spatial variability of mountain river habitats followed by natural morphological features (eroding cliffs, large bedrocks) and the hydromorphological condition as assessed by the RHS methodology. Our survey confirmed the results of [45] because both the nature of the river bed, which was reflected by the values of the HQA and HMS indices, as well as the physical parameter determined the structure of macroinvertebrate communities. According to [46], human land use and land cover within the Carpathian catchments are reflected in the instream macroinvertebrate distribution. *Ephemeroptera*, *Plecoptera*, *Trichoptera* and *Amphipoda* are numerous in less human-affected forested catchments in the Carpathian mountain streams. In comparison, we observed that macroinvertebrate taxa, *e.g.*, *Trichoptera* (*Odontoceridae*, *Beraeidae*), *Plecoptera* (*Leuctridae*, *Nemouridae*), *Coleoptera* or *Diptera* were positively correlated with the values of the HQA index, which reflects the extensive presence of a number of natural river features in the channel and river corridor and indicates the smallest degree of modification and human land use. Among them *Odontoceridae* and *Beraeidae* were the taxa that were most sensitive to water pollution according to the BMW(PL) methodology [26].

4.3 Endangered taxa in mountain streams of national parks

The spring snail genus *Bythinella* Moquin-Tandon 1856 comprises species that inhabit springs and subterranean water courses from a low altitude up to 1,465 m a.s.l. from Europe to western Asia and northern Africa [47,48]. Delimitation of species based on the morphological and anatomical features is difficult and may be controversial, and therefore, genetic data and molecular studies are also essential [48-50]. Our survey found that *Bythinella* was one of the taxa positively correlated with higher values of conductivity. *Bythinella* occurred in the flysch or calcareous streams with higher pH, alkalinity, hardness or a higher concentration of calcium, mainly in streams of the GNP, the BGNP and in the TNP (Western Tatra Mts., the Strążyski). However, the highest density of *Bythinella* was recorded in the mid-altitude calcareous streams of the PNP. The genus *Bythinella* is a typical example of a group for which the taxonomy can only be unravelled by an integrative approach that combines morphology, anatomy, biogeography and genetics [51]. Five species within the genus *Bythinella* have been identified in Poland [52,53]. Some of these are probably western Carpathian endemics that are known only from Poland [53]. According to the European Red List of Non-marine Molluscs [51], they are listed as Critically Endangered (CR), an Endangered (EN) or a Vulnerable (VU). Benke *et al.* [50,54] identified several biodiversity hotspots of the genus *Bythinella* based on genetic data *i.e.*, areas that contain an exceptional concentration of biodiversity as measured by species richness and species endemism. The Western Carpathians, which includes such localities in the Biosphere Reserves such as the Western Tatra Mts. (the Strążyski) of the TNP or the BGNP, is one hotspots of the genus *Bythinella*. According to [54], spring organisms such as *Bythinella* reflect a unique evolutionary history that is distinct from lentic and lotic taxa even though the genus *Bythinella* shows particularly high levels of uncertainty in the delimitation of species.

5 Conclusions

The macroinvertebrate communities in mid- and high-altitude mountain streams in national parks and Biosphere Reserves are influenced by several environmental factors acting together. Both the nature of river and the physical parameter of the water affected the structure of macroinvertebrate communities. In addition to the conductivity, the altitude, stream gradient and the values of the HQA index were most important (statistically significant).

The highest value of the HQA index, which reflects more natural features including eroding cliffs, waterfalls, in-channel vegetation, distribution of bank-side trees and the extent of near-natural land use adjacent to the streams, was recorded for a headwater stream located within the Babia Góra National Park (BGNP), which is one of the Biosphere Reserves. Lower values of the HQA index which were obtained for the high-altitude headwater streams (1,500-1,800 m a.s.l.) of the Tatras UNESCO Biosphere Reserve are the result of natural hydromorphological and extreme harsh environmental conditions (rock, scree, pine dwarf above the tree line, snow cover, snowfields) but are not the result of their degradation.

The novel finding of our survey showed that the River Habitat Survey (RHS) method reflects not only the morphology but also the relationships between habitat features and the structure of macroinvertebrate communities. The RHS supports the survey of macroinvertebrates (*i.e.*, the biological element in the light of the European Union Water Framework Directive) because it provides a more holistic approach to assessing the health condition of stream ecosystems as well as evaluating trends and environmental influences including anthropogenic disturbances and the degradation of streams. Therefore, the RHS should be recommended in future surveys.

The headwater streams of the national parks and the Biosphere Reserves support unique macroinvertebrate taxa that are found nowhere else in a catchment or that are highly restricted to the smallest first- and second-order streams and may constitute refuges for in-stream biota, *e.g.*, for *Bythinella*. However, the identification of molluscs to the species level requires further molecular surveys. Some of the least water pollution-tolerant macroinvertebrate taxa were also recorded (*e.g.*, *Odontoceridae*, *Glossosomatidae*, *Beraeidae*, *Goeridae* and *Blephariceridae*).

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