DE GRUYTER

Biologia **72**/4: 431—444, 2017
Section Zoology
DOI: 10.1515/biolog-2017-0049

Bioassessment of streams based on macroinvertebrates – can sampling of some substrate types be excluded?

Daniela Kalaninová[1], Iľja Krno[2], Ladislav Hamerlík[3], Eva Bulánková[2], Pavel Beracko[2] & Ferdinand Šporka[1]

[1]*Institute of Zoology, Slovak Academy of Sciences, Dúbravská cesta 9, SK-84506 Bratislava, Slovakia; e-mail: daniela.kalaninova@savba.sk, ferdinand.sporka@savba.sk*
[2]*Department of Ecology, Faculty of Natural Sciences, Comenius University Bratislava, Mlynská dolina B-2, SK-84215 Bratislava, Slovakia*
[3]*Faculty of Natural Sciences, Matej Bel University, Tajovského 40, SK-97401 Banská Bystrica, Slovakia*

Abstract: Attempting to help reduce the costs of bioassessment of aquatic habitats, the aim of this study was to estimate how particular substrate types influence the ecological quality evaluation based on a multihabitat scheme proposed by the AQEM/STAR consortium. Samples of macroinvertebrates were taken from the Stupavský potok brook, a small, 4th order calcareous stream in the Small Carpathians in Slovakia (Central Europe). Eight most suitable metrics for small Slovakian streams forming the Slovak multimetric index on seven substrate types were tested and compared with the multihabitat sample. The Saprobic Index (SI) and Index of Biocenotic Region (IBR) showed considerably worse (higher) values in the psammal and the best (lowest) values on coarse mineral substrates (lithal, akal). Similarly, values of the metrics Oligo (%), BMWP Score, Rheoindex, Rhithron Typie Index (%) and EPT reached their worst (lowest) values on psammal and the best (highest) values on coarse mineral substrates. Psammal sample showed the worst ecological quality expressed by the lowest EQR (Ecological Quality Ratio) value, most significantly differing from the multihabitat sample (Multiple Comparisons of Means: Dunnett Contrasts: –8.25, $P < 0.01$). We conclude that substrate types considerably influence selected metrics. Because of a relatively substantial proportion of psammal in some small Slovakian streams and its marginal influence on the overall ecological quality of the site, we suggest conduct further research addressing the effectiveness of its usage in the water management.

Key words: ecological status; metrics; microhabitats; psammal; benthic invertebrates; small stream

Abbreviations: SI – Saprobic Index (Zelinka and Marvan), Oligo (%) – proportion of individuals with a preference for oligo-saprobic conditions (scored taxa = 100%), BMWP – Biological Monitoring Working Party, RTI – Rhithron Typie Index, IBCR – Index of Biocoenotic Region, Aka+Lit+Psa (%) – proportion of individuals with a preference for gravel, lithal and sand (scored taxa = 100%), EPT – number of Ephemeroptera, Plecoptera and Trichoptera taxa, Rheoindex – Banning, with abundance classes. Term "scored taxa = 100%" means that only taxa for which autecological information was available were included in the metric calculation.

Introduction

Widely used methods for the ecological quality assessment have constantly been revised for improvement reasons (e.g., Buffagni et al. 2001; Springe et al. 2006; Vandewalle et al. 2010). One of these methods is a multihabitat method for sampling, processing and evaluation of aquatic macroinvertebrates, proposed by the AQEM consortium for the purpose of implementing the EU WFD (European Commission 2000) (AQEM Consortium 2002). According to this method, 20 samples of the macroinvertebrates are taken from all dominant microhabitats present (with coverage at least 5%), but later they are pooled into one sample. This proposed method meets the water managers' need to reduce the costs of obtaining the primary data required for assessment of the ecological status of waters. On the other hand, it makes it impossible to obtain data on community structure in individual microhabitat types. This information is particularly useful for answering questions about the effects of different types of impact at a range of different scales (Buffagni et al. 2004). Several studies dealt with differences in benthic fauna between riffles (transport units) and pools (depositional units) (e.g., Logan & Brooker 1983; Brown & Brussock 1991; Buffagni et al. 2004; Brabec et al. 2004), the others dealt with these differences at the microhabitat level, mostly limited to individual habitats, primarily organic

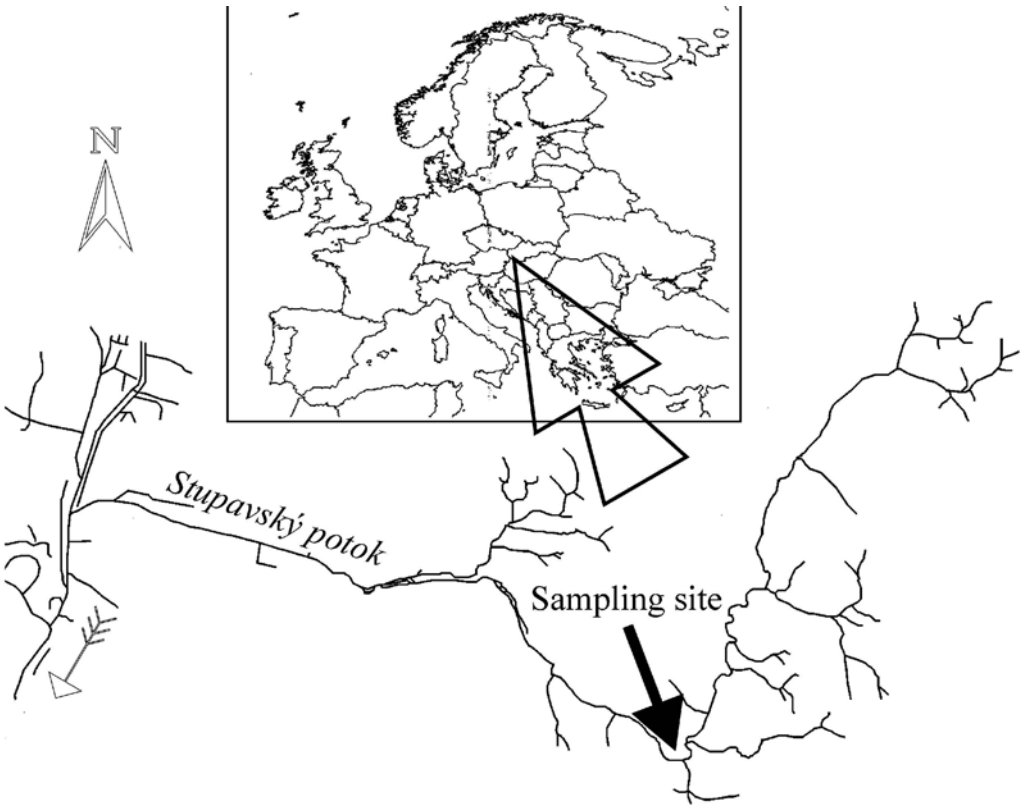

Fig. 1. Catchment area of the Stupavský potok brook in Slovakia and the location of the sampling site.

substrates. The importance of woody debris in various types of streams has been widely studied in Europe (O'Connor 1992; Hering et al. 2000; Hoffmann 2000; Hoffmann & Hering 2000; Mutz 2000; Warmke & Hering 2000; Godfrey 2003) as well as in America (Gregory 2005). The importance of submerged roots and wood as a site for the development and emergence of insects was described by Derka et al. (2001), Gavlasová & Derka (2004) and Hoffmann (2000). Macroinvertebrate species associated with macrophytes in running waters were studied by Dudley (1988), Harrod (1964), Korsu (2004), Lindegaard & Thorup (1975), and in sand by Jensen & Madsen (1989). McElhone & Davies (1983), Williams (1980), Jowett & Richardson (1990), Jowett et al. (1991), Wood (1998) and others focused on the influence of mineral substrates on the micro-distribution of macroinvertebrates. Information on substrate preferences were revised and extended in Schröder et al. (2013). However, there is still little knowledge about influence of different substratum types as key elements in restoration processes (Verdonschot et al. 2016) on the ecological quality.

The final ecological status, in terms of the scheme proposed by the AQEM/STAR consortium is the result of a multihabitat sample consisting of individual substrates in various proportions. Therefore, in this study we analysed the influence of different substrates in the final multihabitat sample on eight metrics [SI; Oligo (%), BMWP, Rheoindex, RTI; Aka+Lit+Psa (%), EPT taxa and IBR – explained in detail in Material and methods]. Moreover, they were also chosen for calculation of a multimetric index for small streams (catchment area (10–100 km^2) in 200–500 m a.s.l. in Slovakia (Šporka et al. 2009).

Our main goal was to statistically evaluate and compare the influence of the habitat type on the selected metrics and the overall ecological quality.

Specific aims were to: (1) Find out species composition of individual substrates as a base for calculation of selected metrics; (2) Calculate the metrics and the total EQR (representing ecological quality) for each substrate and the multihabitat sample and statistically evaluate the significance of differences of each substrate values from the multihabitat sample.

Material and methods

Study site and data collection

Samples were collected from the Stupavský potok brook (48°15′09.1″ N, 17°06′44.4″ E), a small, 4th order, calcareous stream in the Carpathians in Slovakia (Central Europe) (Fig. 1). The sampling site was located at 290 m a.s.l. According to the Slovak water typology, the Stupavský potok brook is a small stream with a drainage area of 29 km^2. The discharge fluctuated between 0.4 m^3 s^{-1} (January 2003) and 0.05 m^3 s^{-1} (September 2003). For more details see Šporka et al. (2006).

The study site was a relatively uniform 100 m section of the stream (average width 5.1 m; average depth 0.16 m) divided into two 50 m stretches. Two (replicate) samples were taken in the last week of these months: June, August and October 2003 and April 2004. Prior to the first sampling occasion, microhabitat coverage was estimated for the

Table 1. Characteristics of microhabitats sampled.

Substrate type	Origin	Size of grains (cm)	Coverage (%)	Number of replicates taken
Psammal/psammopelal	mineral	> 0.006–0.2	20	4
Akal	mineral	> 0.2–2	10	2
Microlithal	mineral	> 2–6	10	2
Mesolithal	mineral	> 6–20	40	8
Macrolithal	mineral	> 20–40	10	2
Moss (submersed)	biotic		5	1
Roots (submersed)	biotic		5	1

complete 100 m section (AQEM consortium 2002). Characteristics of microhabitats from which material was collected are given in Table 1.

One replicate represents an area of 25 × 25 cm, kick-sampled with a 500 μm hand-net. Samples from the different habitats were stored separately in buckets. The area sampled per habitat was the same on all sampling occasions and processed by the same operator. The samples were preserved in formaldehyde at a final 4% concentration. In the laboratory the samples collected from the different habitats were rinsed and fully sorted under a stereomicroscope. The same specialist performed all the identifications of each major organism group. Macroinvertebrates were identified to the lowest possible taxonomic level (species level for almost all groups).

Data analysis

The number of individuals per taxon was standardized to a total sampled area of 1.25 m^2. The reference (multihabitat) sample was also standardised to a sampled area of 1.25 m^2. Multihabitat samples were obtained by merging the microhabitats according to the number of sample replicate described above, which was based on the % coverage of the individual microhabitats. Overall we had a set of eight samples from individual microhabitats and eight multihabitat samples. Prior to analysis, samples consisting of more than one sample replicate were transformed in order to apply the rank species abundance model. The Zipf-Mandelbrot model $[A_i = A_1(i+\beta)^{-\gamma}]$ (Frontier 1985) was used to fit the observed species abundance and then to estimate species abundance in one sample replicate of each type of substratum (A_i – the abundance of the species on microhabitat at rank i. A_1 – adjusted abundance of the most inhabited type of microhabitat of species. γ – constant representing the average probability of the occurrence of a species. β – ecologically it can be considered as the potential diversity of the environment). This procedure was carried out with GenStat 12.1. (Payne et al. 2009).

Metrics suitable for assessment of small Slovakian streams were chosen for the analysis (Šporka et al. 2009). Moreover, these metrics are commonly used in EU countries for stream assessment: **SI; Oligo (%)** (metrics indicating organic pollution), **RTI**; **IBR; Aka+Lit+Psa (%)** (metrics indicating organic pollution and degradation of stream morphology), **Rheoindex** (metrics indicating degradation of stream morphology), **EPT taxa and BMWP** (metrics indicating organic pollution, degradation of stream morphology and general degradation). For detailed information about individual metrics see AQEM Consortium (2002).

In addition we also analysed the species composition of the individual microhabitats as well as the multihabitat samples. Average values of abundances for all seasons were used in this analysis.

Metrics with increasing predicted response to increasing perturbation are SI and IBR. Metrics with decreasing predicted response to increasing perturbation are Oligo (%), BMWP, RTI, Aka+Lit+Psa (%), EPT and Rheoindex. Term 'best values' of metrics used in text refer to lowest values for SI and IBR, and to highest values for Oligo (%), BMWP, RTI, Aka+Lit+Psa (%), EPT and Rheoindex; term 'worst values' of metrics thus means the opposite.

Based on these metrics, we calculated the EQR value for each substrate and the multihabitat sample. Ecological Quality Ratio is the ration between the value of the observed biological parameter for a given surface water body and the expected value under reference conditions. The ration is expressed as a numerical value between 0 and 1, with high ecological status represented by values close to one and bad ecological status by values close to zero (Anonymous 2003).

Statistical analysis

Linear regression was used to relate the number of taxa to number of microhabitats to reveal how the taxa number change with increasing number of microhabitats. Microhabitats were arranged from fine – psammal to coarsest – megalithal microhabitas, followed by roots and moss.

Cluster analysis (complete linkage, Jaccard similarity coefficient for qualitative samples, Bray-Curtis for quantitative samples) was used to analyse the similarity of individual microhabitats in terms of their macroinvertebrate communities. Prior to statistical analysis, abundance data were $\log 10(x+1)$ transformed. For individual microhabitats an average abundance from all sampling occasions was used in analysis.

Spearman Rank Order Correlation was used to assess how the metric values, based on individual microhabitats, correspond to the metric values based on the multihabitat sample, i.e., how the sample from a single microhabitat differs from a complex sample.

Multiple Comparisons of Means: Dunnett Contrasts were used to assess how the EQR values, based on individual metrics, correspond to the EQR value based on the multihabitat sample, i.e., how the ecological quality value differs from the ecological quality value of the multihabitat sample.

Results

Taxonomic composition

A total of 246 taxa of macroinvertebrates were recorded in the Stupavský potok brook. In the cold season (October – April) 222 taxa, in the warm season (June – August) 143 taxa were identified (Appendix 1).

The lowest numbers of taxa were detected in moss (132), macrolithal (135) and roots (139). In contrast,

Table 2. Total numbers of taxa in individual taxonomic groups in the microhabitats of the Stupavský potok brook and proportion (%) of taxa contributing to the total numbers in the multihabitat sample created from all samples. Total number of taxa was calculated as sum of taxa number at all sampling occasions.

	Psammal	%	Akal	%	Microlithal	%	Mesolithal	%	Macrolithal	%	Roots	%	Moss	%	Multihabitat
Turbellaria	1	100	1	100	1	100	1	100	1	100	1	100	1	100	1
Amphipoda	1	100	1	100	1	100	1	100	1	100	1	100	1	100	1
Oligochaeta	12	63	9	47	8	42	8	42	8	42	7	37	8	42	19
Ephemeroptera	12	71	13	76	14	82	16	94	14	82	15	88	12	71	17
Plecoptera	13	65	13	65	15	75	17	85	15	75	13	65	13	65	20
Coleoptera	4	27	5	33	8	53	12	80	11	73	12	80	11	73	15
Heteroptera	0	0	0	0	0	0	1	100	0	0	0	0	0	0	1
Trichoptera	22	56	33	85	21	54	27	69	29	74	23	59	22	56	39
Chironomidae	49	64	44	57	41	53	42	55	30	39	42	55	40	52	77
Simuliidae	6	60	8	80	9	90	9	90	9	90	9	90	9	90	10
other Diptera	27	59	23	50	19	41	17	37	17	37	16	35	15	33	46
Total taxa	147		150		137		151		135		139		132		246

mesolithal and akal habitats belonged to those with the highest numbers of taxa (151 and 150).

Oligochaeta, Chironomidae and other Diptera had the highest numbers of taxa in psammal. Taxa richness of Ephemeroptera, Plecoptera and Coleoptera was highest in the mesolithal, while Trichoptera richness was highest in the akal. The lowest diversity of these groups was detected in psammal, moss, akal, roots and microlithal (Trichoptera) (Table 2).

The highest numbers of taxa from all microhabitats were recorded in the mesolithal and akal, the lowest in macrolithal, however, the differences were not substantial. Individual microhabitats hosted 50–60% of overall taxa number. Multihabitat samples were characterized by the highest number of taxa, which was more than one third times higher in comparison with all the microhabitats (Table 2). The number of taxa increased with every other microhabitat involved (regression coefficient $R = 0.973$, $P < 0.001$) which means that every microhabitat contributes to the overall taxa number by unique species (Fig. 2).

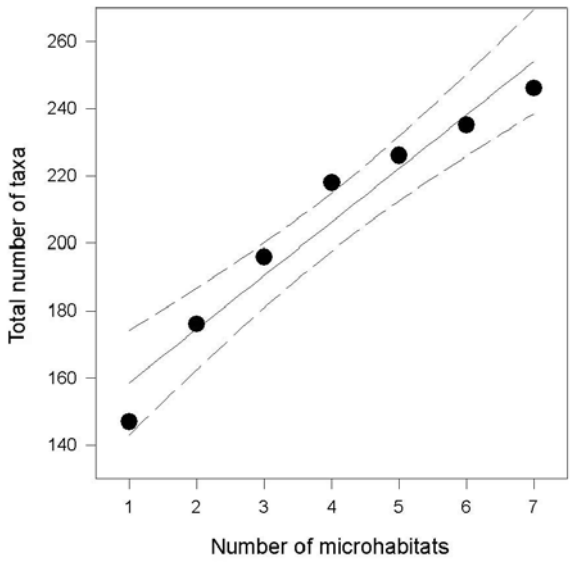

Fig. 2. Linear regression (Y = 142.714 + (15.893 * number of microhabitas) between the numbers of microhabitats and numbers of taxa in the Stupavský potok brook (solid – regression line, dash line – 5%, 95% confidence intervals). $R = 0.973$, $P < 0.001$. Microhabitats were added as follows: psammal, akal, microlithal mesolithal, macrolithal, roots and moss. On the first axis next microhabitat is added to the pool of previous microhabitats, starting with single microhabitat (psammal).

Cluster analysis of microhabitats

According to the quantitative data – abundances of macroinvertebrate taxa – on individual microhabitats, several clusters can be distinguished. Coarse mineral substrates (meso- macrolithal) and the multihabitat sample were clustered together on a level of 90% similarity. Mesolithal was the most similar habitat to the multihabitat sample and together they created a separate cluster with 96% similarity. Fine mineral substrates (akal and microlithal) showed greater dissimilarity with the lithal and multihabitat sample and were grouped together as identical. Psammal – sand showed very low similarity with other mineral substrates. On the other hand, organic substrates – roots and moss clearly separated from the others (Fig. 3A).

Analysis of the qualitative samples eliminated the effect of taxa abundance and emphasized the effect of presence and absence of individual taxa. On this level the dissimilarity of individual groups increased compared with the quantitative data. Similarity of coarse mineral substrates is still apparent (similarity more than 80%), and the same applies to organic substrates (75%). Multihabitat sample which cumulates the number of taxa from all substrates was separated from other substrates and showed only low similarity (Fig. 3B).

Metrics indicating organic pollution – SI, Oligo (%)

Comparison of metrics indicating organic pollution [SI, Oligo (%)] from individual microhabitats with values from the multihabitat sample (Figs 4A, B) showed that the psammal was characterised by the greatest variation and the worst values together with roots. Similar values to those in the multihabitat sample were detected in moss, the best values were detected in akal and all types of lithal.

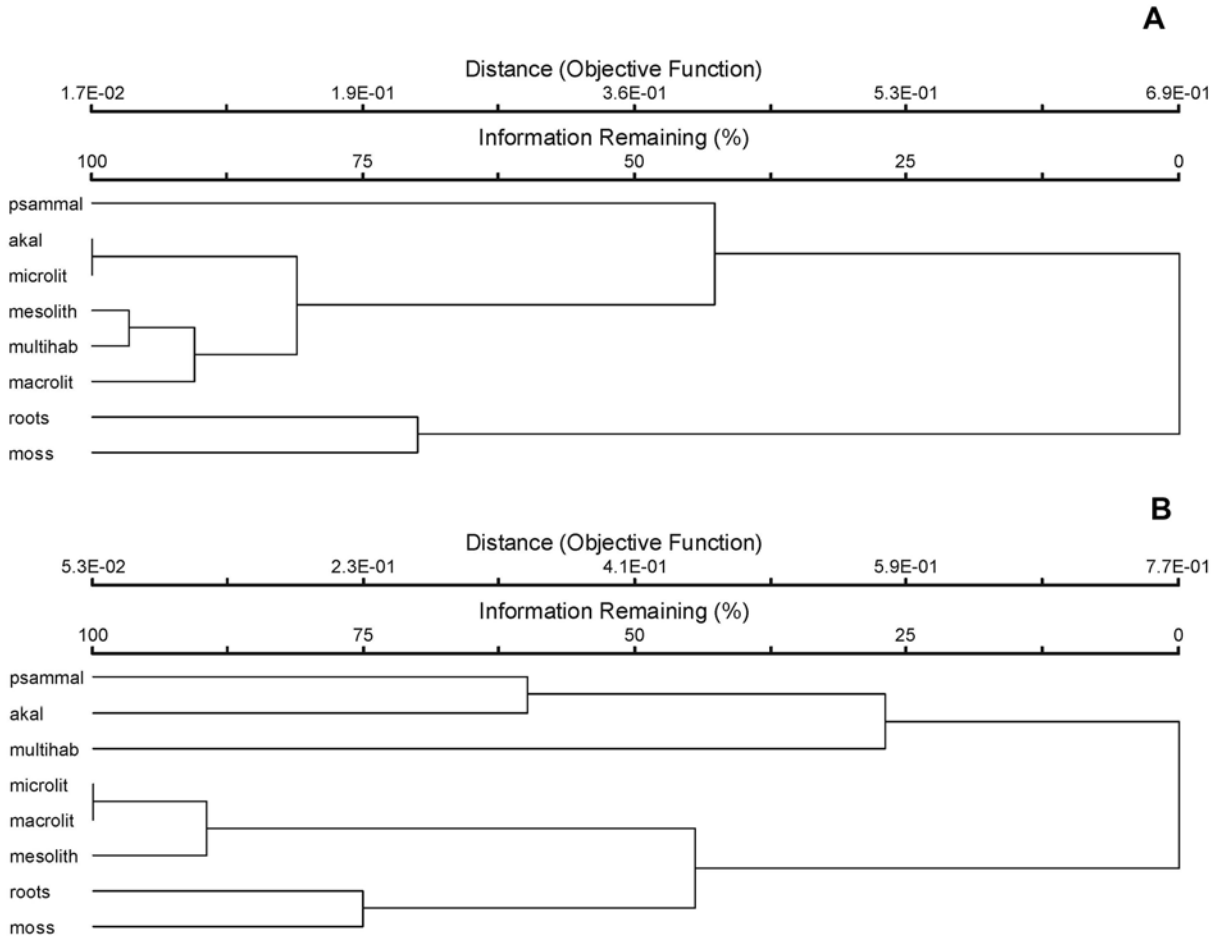

Fig. 3. Dendrograms of (A) quantitative samples (Bray -Curtis coefficient, complete linkage) for different microhabitats in the Stupavský potok brook and (B) qualitative samples (Jaccard coefficient, complete linkage). For individual microhabitats an average abundance from all sampling occasions was used in analysis.

Table 3. Correlation coefficients (R) and significance levels of metric values tested in individual microhabitats versus the multihabitat substrate of the Stupavský potok brook.

	SI	Oligo (%)	BMWP	RTI	Rheoindex	IBR	AkLiPs	EPT
Psammal	ns	–0.810**	ns	ns	-0.905**	ns	ns	ns
Akal	ns	ns	0.743*	ns	ns	ns	ns	ns
Microlithal	ns	ns	0.693*	ns	ns	ns	ns	0.750*
Mesolithal	ns	0.905**	0.778*	ns	ns	ns	ns	0.790*
Macrolithal	ns	0.857**	ns	ns	0.761*	ns	ns	0.963**
Roots	ns	ns	0.825**	ns	ns	ns	ns	0.884**
Moss	ns	0.952**	ns	ns	ns	ns	ns	0.927**

Explanations: * $P < 0.05$, ** $P < 0.001$, ns – not significant.

Correlation analysis was performed in order to examine to what extent the metric values from individual microhabitats were correlated with the multihabitat sample. Analysis of metrics showed a negative relationship of Oligo (%) in the psammal microhabitat and a positive relationship in the mesolithal, macrolithal and moss habitats on the one hand and the multihabitat sample on the other hand. For SI no significant relationship was detected (Table 3).

Metrics indicating organic pollution and degradation of stream morphology – RTI, IBR, Aka+Lit+Psa (%)
The worst values of RTI and IBR were detected in the psammal microhabitat, whereas the worst values of Aka+Lit+Psa (%) were in the roots. All these metrics showed better values in the lithal microhabitats compared with the multihabitat sample (Figs 4E, F, H). There were no significant correlations of metrics between microhabitat and multihabitat samples.

Metrics indicating organic pollution, degradation of stream morphology and general degradation – BMWP, EPT taxa
These metrics indirectly reflect biodiversity to some extent; their values in the multihabitat samples are the accumulation from all habitats. Both metrics reached the

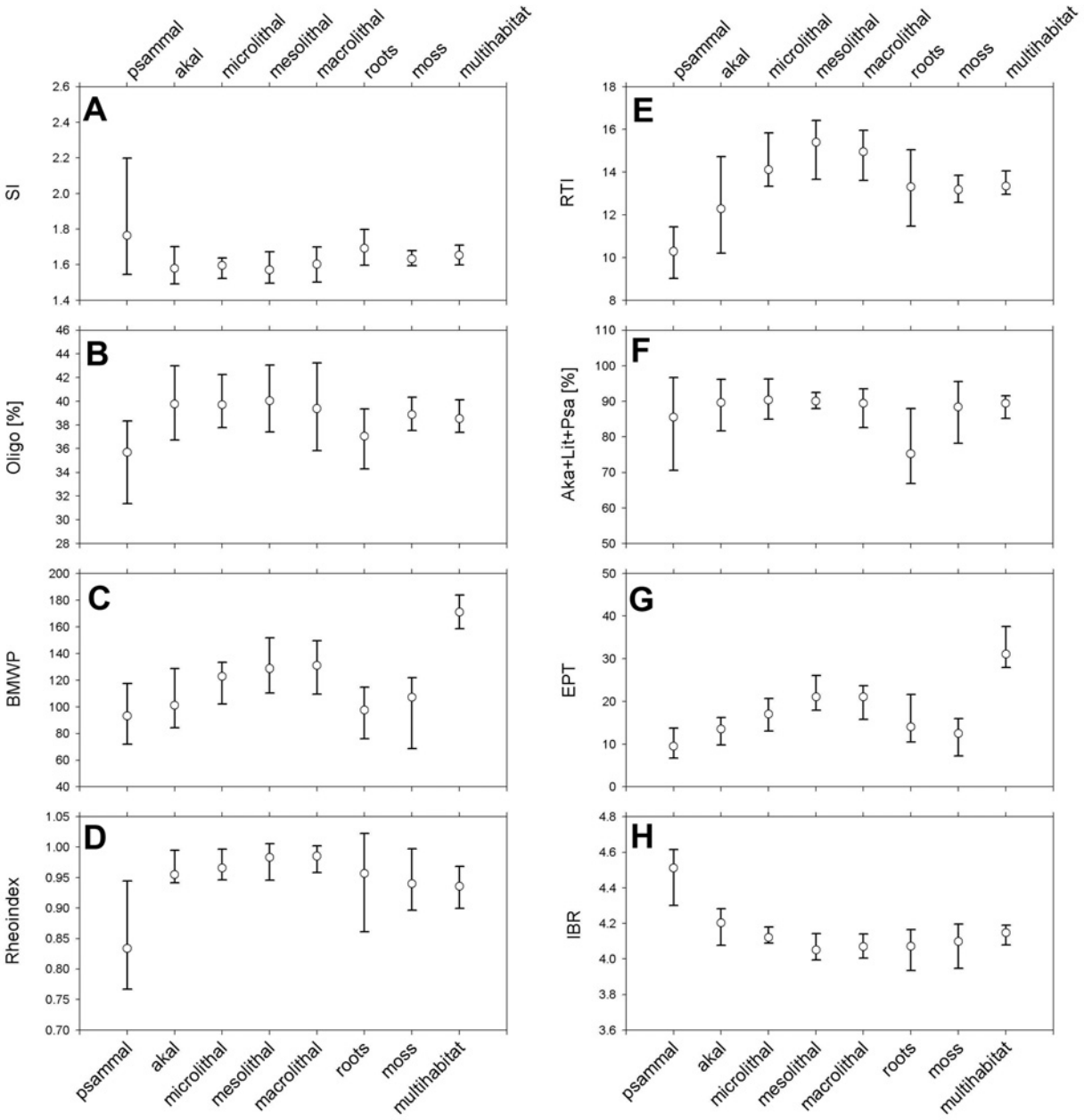

Fig. 4. Values of eight selected metrics measured in the individual microhabitats of the Stupavský potok brook and in the multihabitat sample (circle – median, whiskers – standard deviation). Set of eight samples for every microhabitat was used for calculation.

best values in multihabitat samples (Figs 4C, G). From the microhabitats surveyed, meso- and macrolithal were characterized by the best values of both these metrics; while the psammal had the worst values for both metrics.

BMWP of akal, micro – mesolithal and roots showed a positive relationship to multihabitat samples. EPT values from multihabitat samples correlated positively with all types of lithal, as well as with roots and moss (Table 3).

Metrics indicating degradation of stream morphology

Rheoindex

The worst values were observed in the psammal, while the rest of the microhabitats were characterized by better values, together with the multihabitat sample (Fig. 4D). Multihabitat samples correlated significantly with macrolithal (positively) and psammal (negatively) (Table 3).

EQR

The worst ecological quality expressed by the lowest EQR value as well as the largest significant divergence from the multihabitat sample value was observed in psammal sample (–8.25, $P < 0.01$). Moss, roots and akal also differed significantly (–4.21, –4.04, –3.33, respectively, $P < 0.01$). Mineral substrates – microlithal, mesolithal and macrolithal were most similar to the multihabitat sample (no significant difference was confirmed) (Fig. 5, Table 4). Considering the borders of the AQEM Consortium (2002) ecological classes (0–0.20 = bad, 0.21–0.40 = poor, 0.41–0.60 = average,

Table 4. Pair comparison of substrate samples vs. multihabitat (AQEM) sample in terms of the EQR value.

Substrate	Psammal	Akal	Microlithal	Mesolithal	Macrolithal	Moss	Roots
EQR	**–8.25****	**–3.33****	–0.99^{ns}	0.35^{ns}	–0.06^{ns}	**–4.21****	**–4.04****

Explanations: Multiple Comparisons of Means: Dunnett Contrasts. t- statistic; ** $P < 0.01$, * $P < 0.05$, ^{ns} – non significant.

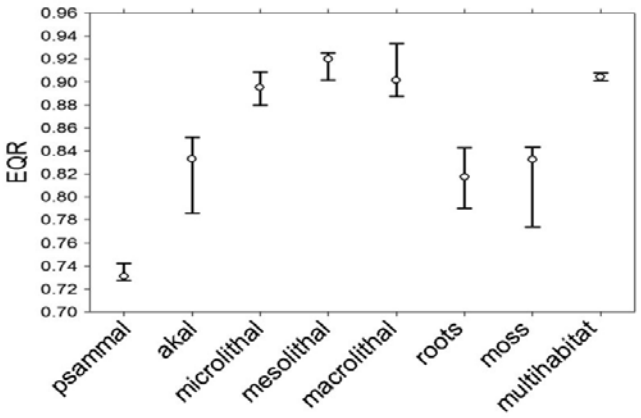

Fig 5. The EQR values calculated in the individual microhabitats of the Stupavský potok brook and in the multihabitat sample (circle – median, whiskers – standard deviation). Set of eight samples for every microhabitat was used for calculation.

0.61–0.80 = good, 0.81–1 = very good), all the microhabitats showed very good ecological status, apart from psammal.

Discussion

The surveyed stretch of the Stupavský potok brook included a number of different microhabitat types, which allowed us to test the degree of influence of different substrates in a final assessment on the ecological status of this stream section.

Mineral substrates, specifically mesolithal and psammal, were the most frequent types of microhabitat, covering 40% and 20% of the total area, respectively. In contrast, roots and mosses (biotic microhabitats) were the most sparsely represented habitats, with each covering only 5% of the area.

While submersed roots occurred by the river banks in pools and riffles, mosses only occurred in riffles, due to their growing on boulders normally located in riffles. In spite of their low coverage, submersed roots and mosses belonged to the most important microhabitats of the Stupavský potok brook because of their association with the highest mean abundances of macroinvertebrates. This was confirmed by the cluster analysis of the quantitative data whereas, according to the qualitative data, the taxonomical structure of the communities in mosses is similar to macrolithal. Mosses mainly overgrow the macrolithal and therefore it was not possible to distinguish the assemblages of these two substrates. The reason for the high percentage of macroinvertebrate taxa, e.g., many Trichoptera species (Skuja 2010), inhabiting stream bryophytes is probably its large total surface area. These extremely important habitats for stream invertebrates (Korsu 2004), functioning as shelters and source of food can be regarded as key habitat elements for a relatively large number of (specialized) species in restoration processes as well (e.g., Louhi et al. 2011).

As expected, the highest total number of taxa we found in a complex multihabitat sample, as it included all taxa from all microhabitat types sampled. Different substrate types thus contributed by different assemblages of more common species inhabiting a wide range of habitats (Kubošová et al. 2010) and species with specific habitat requirements (Dallas 2007). Confirmed by a highly significant linear regression between the tested variables, the number of macroinvertebrate taxa increased with the number of microhabitats tested, which is an obvious phenomenon in natural or near natural streams.

This fact was reflected also by cluster analysis of qualitative data. The multihabitat sample was relatively dissimilar to the individual substrates, fine substrates (psammal, akal) were separated from coarse (lithal) and organic substrates (roots, moss).

Cluster analysis of the quantitative dataset emphasised the distinctiveness of organic substrates, and also psammal, from coarser mineral substrates by its lower abundances and different taxonomic composition. It seems that the multihabitat sample is strongly influenced by the substrate type with the highest coverage (mesolithal in this case), which makes more difficult to evaluate the similarity of the multihabitat sample to the individual substrates. However, comparison of similarity among different microhabitat types was possible and resembled that of the qualitative data analysis.

The worst values of both metrics indicating organic pollution [SI and Oligo (%)] were found in the case of psammal habitats. This suggests that macroinvertebrate fauna dwelling in this habitat type is the most tolerant of organic pollution. These results highlight the need for care when using biotic indices on sandy sites (Wood 1998). Sandy substrates occur primarily in pools, and the data from pools could have influence on the ecological water quality based on metrics values. While, in the case of the Oligo (%) metric, a correlation between multihabitat samples and single substrate types was found in four different sediments, in the case of SI there was no significant correlation at all. This difference could be caused by the fact, that oligosaprobic taxa prefer more rheophilic environments (for which coarse substrates and mosses are typical) with a higher oxygen content. Also, among the metrics indicating organic pollution and degradation of stream morphology [RTI, IBR, Aka+Lit+Psa (%), BMWP and EPT], the worst values were obtained from psammal.

The low values of the Aka+Lit+Psa (%) metric observed in the case of roots are not surprising, taking into consideration that this metric represents the sum of taxa dwelling on akal, lithal and psammal. In contrast, high values of Aka+Lit+Psa (%) were found in moss; tufts of mosses function as natural traps of fine sediment and are thus colonised by species preferring this kind of substrate. It also supports the fact, that species living in mosses have a requirement for high flow rates similarly to lithal dwelling species.

Values of BMWP as well as EPT, also indicating general degradation of streams, were significantly higher in the multihabitat sample compared to the single microhabitat samples. This is not surprising because both these metrics are to certain level measures of biodiversity and their value increases cumulatively with habitat type variability and thus comparison of the multihabitat and single habitat samples is quite problematic. The same could be said about the whole taxa number, which could be explained by the variation of taxa in different substrates. Brabec et al. (2004) analysed a subset of 16 samples with equal proportions of riffles and pools and found that both number and percentage of EPT taxa were higher in riffle habitats. Study at unaffected sites had shown that EPT taxa richness was more stable and more predictable than total taxa richness (Lenat & Penrose 1996). We found that EPT taxa had a more regular distribution in all microhabitats, while Oligochaeta, Chironomidae and other Diptera had a closer affinity to psammal (Table 2). Our results thus confirmed the predictions of Barbour et al. (1999): Case studies focused on microhabitat scale patterns would be needed for a better understanding of the interrelationships between natural patterns and the effects of impairment. It is not clear how well some changes in these measurements of ecological traits are related to changes in water or habitat quality.

Metrics of meso- and macrolithal habitats have higher explanatory values compared with the multihabitat sample, which represents an average of different microhabitats and which may reflect the structure of the stream bottom instead of the water quality. This fact has already been pointed out by Gregory et al. (1991), who proposed a correction factor to be applied to ecological condition metrics that adjusts for the presence or absence of the riffle habitat in Piedmont streams. Similarly, other authors assessing differences between pools and riffles without distinguishing their microhabitats (Logan & Brooker 1983; Brown & Brussock 1991; Buffagni et al. 2004; Brabec et al. 2004) pointed out that ratios of samples taken from riffles and pools could influence the final evaluation of the ecological status of a stream. Our study not only confirmed but also specified these findings in terms of individual microhabitats. We found out that lithal (consecutively mesolihal, macrolithal and microlithal) showed the highest ecological quality. This result is consistent with findings of Jähnig & Lorenz (2008) and Verdonschot et al. (2016) which pointed out a great significance of cobbles with coarse organic matter especially in restoration processes. On the contrary, seven out of eight metrics had their worst values in the psammal (with the exception of Aka+Lit+Psa, having its worst value in roots). Not surprisingly, psammal community indicated significantly the lowest values of EQR and thus the worst ecological status. These findings raise an important question about re-evaluation of usage of individual substrate types for bioassessment purposes.

Conclusion and recommendation for bioassessment of streams

Our study confirmed that the character of the substrate type significantly influences macroinvertebrate communities and ecological quality. We showed that mesolithal had the best ecological status expressed by the EQR value, whereas psammal had the worst and differed most from other substrates (including the multihabitat sample) in species composition as well as in metrics values. However, despite its relatively low richness and abundance compared to less dynamic substrates, pure psammal harbours a distinct community of macroinvertebrates (Yamamuro & Lamberti 2007), and thus contributes to the biodiversity increase and subsequently to more realistic assessment of the ecological status. Therefore we suggest to reduce only samples of psammal with organic matter (which covered most of sandy substrates sampled in the current study). Moreover, as our research was conducted in a submontane stream and some species show different substrate preferences in mountain and lowland streams (Schröder et al. 2013), we suggest to reduce psammal sampling from submontane streams only. This time and costs saving measure would help water managers mainly in those cases in which restoration processes would be applied based on the underestimated ecological status derived from metrics from psammal. In order to specify the psammal sampling reduction and to increase efficiency of water management, serving thus to a better water resources protection, it would be fruitful to pursue further research regarding significance of microhabitats in bioassessment in more detail.

Acknowledgements

This study was carried out within the STAR project, a research project under the 5th Framework programme of the European Union (EVK-1-CT-2001-00089), and by the Slovak Scientific Grant Agency (VEGA, project No. 1/0119/16). We would like to thank Tomáš Derka, Daniela Illéšová, Zuzana Čiamporová-Zaťovičová and Zuzana Pastuchová for identification of samples.

References

Anonymous 2003. Monitoring under the Water Framework Directive. Common Implementation Strategy for the Water Framework Directive (2000/60/EC) Guidance Document No. 7,

Produced by Working Group 2.7 – Monitoring, Office for Official Publications of the European Communities, Luxemburg, 153 pp. ISBN: 92-894-5127-0

AQEM Consortium 2002. Manual for the Application of the AQEM System. A Comprehensive Method to Assess European Streams Using Benthic Macroinvertebrates, Developed for the Purpose of the Water Framework Directive. Version 1.0, February 2002, 202 pp.

Barbour M.T., Gerritsen J., Snyder B.D. & Stribling J.B. 1999. Rapid bioassessment protocols for use in streams and wadeable rivers: Periphyton, benthic macroinvertebrates and fish. Second Ed. EPA 841-B-99-002. U.S. Environmental Protection Agency, Office of Water, Washington, D.C., 339 pp.

Brabec K., Zahrádková S., Němejcová D., Pařil P., Kokeš J. & Jarkovský J. 2004. Assessment of organic pollution effect considering differences between lotic and lentic stream habitats. Hydrobiologia **516:** 331–346. DOI: 10.1023/B:HYDR.00000 25274.47757.85

Brown A.V. & Brussock P.P. 1991. Comparisons of benthic invertebrates between riffles and pools. Hydrobiologia **220:** 99–108. DOI: 10.1007/BF00006542

Buffagni A., Erba S., Cazzola M. & Kemp J.L. 2004. The AQEM multimetric system for the southern Italian Apennines: assessing the impact of water quality and habitat degradation on pool macroinvertebrates in Mediterranean rivers. Hydrobiologia **516:** 313–329. DOI: 10.1023/B:HYDR.0000025273. 15958.6a

Buffagni A., Kemp J.L., Erba S., Belfiore C., Hering D. & Moog O. 2001. A Europe-wide system for assessing the quality of rivers using macroinvertebrates: the AQEM Project and its importance for southern Europe (with special emphasis on Italy). J. Limnol. **60 (suppl. 1):** 39–48. DOI: 10.4081/jlimnol.2001.s1.39

Dallas H.F. 2007. The influence of biotope availability on macroinvertebrate assemblages in South African rivers: implications for aquatic bioassessment. Freshwater Biol. **52 (2):** 370–380. DOI: 10.1111/j.1365-2427.2006.01684.x

Derka T., Kováčová J. & Bulánková E. 2001. Substrate importance for selected macrozoobenthic communities in Rudava river. Folia Faunistica Slovaca **6:** 59–68.

Dudley T. 1988. The role of plant complexity and epiphyton in colonizaton of macrophytes by stream insects. Verh. Int. Verein. Limnol. **23:** 1153–1158.

European Commision 2000, Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 Establishing a Framework for Community Action in the Field of Water Policy, Official Journal of the European Communities. L237, **Vol. 43:** 1–71, European Commission, Brussels.

Frontier S. 1985. Diversity and structure in aquatic ecosystems, pp. 253–312. In: Barnes H., Barnes M. (eds), Oceanogr. Mar. Biol. Annu. Rev. Vol. **23,** Aberdeen University Press, Aberdeen, 590 pp. ISBN: 9781482267259

Gavlasová S. & Derka T. 2004. Význam submerzných koreňov pobrežných drevín pre spoločenstvá makrozoobentosu rieky Jelešňa [The importance of submerged roots of riparian trees for the macroinvertebrate communities of the Jelešňa River]. Acta Facultatis Ecologiae, Fakulta ekológie a environmentalistiky Technickej univerzity Zvolen **12:** 65–72.

Godfrey P.J. 2003. A review of the invertebrate interest of coarse woody debris in England. English Nature Research Reports, Number 513. Northminster House, Peterborough, UK, 51 pp.

Gregory M.B. 2005. Microhabitat preferences by aquatic invertebrates influence bioassessment metrics in Piedmont streams of Georgia and Alabama. In: Hatcher K.J. (ed.), Proceedings of the 2005 Georgia Water Resources Conference, April 25–27, 2005, University of Georgia. https://water.usgs.gov/nawqa/urban/pdf/GWRC05_Gregory.pdf (accessed 01.12. 2016)

Gregory S.V., Swanson F.J., McKee W.A. & Cummins K.W. 1991. An ecosystem perspective of riparian zones. Bioscience **41:** 540–551.

Harrod J.J. 1964. The distribution of invertebrates on submersed aquatic plants in a chalk stream. J. Anim. Ecology **33:** 335–348. DOI: 10.2307/2634

Hering D., Kail J., Eckert S., Gerhard M., Meyer E.I., Mutz M., Reich M. & Weiß I. 2000. Coarse woody debris quantity and distribution in Central European streams. Int. Rev. Hydrobiol. **85:** 5–23. DOI: 10.1002/(SICI)1522-2632(200003)85: 1<5::AID-IROH5>3.0.CO;2-X

Hoffmann A. 2000. The association of the stream caddisfly *Lasiocephala basalis* (Kol) (Trichoptera: Lepidostomatidae) with wood. Int. Rev. Hydrobiol. **85:** 79–93. DOI: 10.1002/(SICI)1522-2632(200003)85:1< 79::AID-IROH79> 3.0.CO;2-U

Hoffmann A. & Hering D. 2000. Wood-associated macroinvertebrate fauna in Central European streams. Int. Rev. Hydrobiol. **85:** 25–48. DOI: 10.1002/(SICI)1522-2632(200003)85:1 <25::AID-IROH25>3.0.CO;2-R

Jähnig S.C. & Lorenz A.W. 2008. Substrate-specific macroinvertebrate diversity patterns following stream restoration. Aquat. Sci. **70:** 292–303. DOI: 10.1007/s00027-008-8042-0

Jensen K. & Madsen T. 1989. Invertebrate graze submersed rooted macrophytes in lowland streams. Oikos **55:** 421–423.

Jowett I.G. & Richardson J. 1990. Microhabitat preferences of benthic invertebrates in a New Zealand river and the development of in-stream flow-habitat models for *Deleatidium* spp., New Zeal. J. Mar. Fresh. **24:** 19–30. DOI: 10.1080/00288330.1990.9516399

Jowett I.G., Richardson J., Biggs B.J.F., Hickey C.W. & Quinn J.M. 1991. Microhabitat preferences of benthic invertebrates and the development of generalised *Deleatidium* spp. habitat suitability curves, applied to 4 New-Zealand rivers, New Zeal. J. Mar. Fresh. **25:** 187–199. DOI: 10.1080/00288330.1991.9516470

Korsu K. 2004. Response of benthic invertebrates to disturbance from stream restoration: the importance of bryophytes. Hydrobiologia **523:** 37–45. DOI: 10.1023/B:HYDR.0000033086. 09499.86

Kubosova K., Brabec K., Jarkovsky J. & Syrovatka, V. 2010. Selection of indicative taxa for river habitats: a case study on benthic macroinvertebrates using indicator species analysis and the random forest methods. Hydrobiologia **651:** 101–114. DOI: 10.1007/s10750-010-0280-1

Lenat D.R. & Penrose D.P. 1996. History of the EPT taxa richness metric. Bull. N. Am. Benthol. Soc. **13:** 305–306.

Lindegaard C. & Thorup J. 1975. The invertebrate fauna of the moss carpet in the Danish spring Ravnkilde and its seasonal, vertical, and horizontal distribution. Arch. Hydrobiol. **75:** 109–139.

Logan P. & Brooker M.P. 1983. The macroinvertebrate faunas of riffles and pools. Wat. Res. **17:** 263–270. DOI: 10.1016/0043-1354(83)90179-3

Louhi P., Mykrä H., Paavola R., Huusko A., Vehanen T., Mäki-Petäys A. & Muotka T. 2011. Twenty years of stream restoration in Finland: little response by benthic macroinvertebrate communities. Ecol. Appl. **21:** 1950–1961. DOI: 10.1890/10-0591.1

McElhone M.C. & Davies R.W. 1983. The influence of rock surface area on the microdistribution and sampling of attached riffle dwelling Trichoptera in Hartley Creek, Alberta. Can. J. Zool. **61:** 2300–2304. DOI: 10.1139/z83-302

Mutz M. 2000. Influence of woody debris on flow patterns and channel morphology in a low energy, sand-bed stream reach. Int. Rev. Hydrobiol. **85:** 107–121. DOI: 10.1002/(SICI)1522-2632(200003)85:1<107::AID-IROH107>3.0.CO;2-L

O'Connor N.A. 1992. Quantification of submerged wood in lowland Australian stream system. Freshwater Biol. **27:** 387–395. DOI: 10.1111/j.1365-2427.1992.tb00548.x

Payne R., Murray D., Harding S., Baird D. & Soutar D. 2009. GenStat for Windows, 12th Edition Introduction. VSN International Ltd: Hemel Hempstead, UK, 256 pp.

Schröder M., Kiesel J., Schattmann A., Jahnige S.C., Lorenz A.W., Kramm S., Keizer-Vlek H., Rolauffs P., Graf W., Leitner P. & Hering D. 2013. Substratum associations of benthic invertebrates in lowland and mountain streams. Ecol. Indic. **30:** 178–189. DOI: 10.1016/j.ecolind.2013.02.012

Skuja A. 2011. Microhabitat preference of caddisfly (Trichoptera) communities in a mediumsized lowland stream in Latvia**.** Zoosymposia **5:** 425–433. DOI: 10.11646/zoosymposia.5.1.36

Springe G., Sandin L., Briede A. & Skuja A. 2006. Biological quality metrics: their variability and appropriate scale for assessing streams. Hydrobiologia **566 (1):** 153–172. DOI: 10.1007/s10750-006-0099-y

Šporka F., Pastuchová Z., Hamerlík L., Dobiašová M. & Beracko P. 2009. Assessment of running waters (Slovakia) using benthic macroinvertebrates derivation of ecological quality classes with respect to altitudinal gradients. Biologia **64:** 1196–1205. DOI: 10.2478/s11756-009-0201-9

Šporka F., Vlek H.E., Bulánková E. & Krno I. 2006. Influence of seasonal variation on bioassessment of streams using macroinvertebrates, pp. 543–555. DOI: 10.1007/978-1-4020-5493-8_36. In: Furse M.T., Hering D.K., Vrabec K., Buffagni A., Sandin L. & Verdonschot P.F.M. (eds), The Ecological Status of European Rivers: Evaluation and Intercalibration of Assessment Methods, Hydrobiologia **566** Series title: Developments in Hydrobiology **Vol. 188,** Springer Netherlands, 555 pp. ISBN: 978-1-4020-5160-9

Vandewalle M., de Bello F., Berg M.P., Bolger T., Dolédec S., Dubs F., Feld C.K., Harrington R., Harrison P.A., Lavorel S., da Silva P.M,. Moretti M., Niemelä J., Santos P., Sattler T., Sousa J.P., Sykes M.T., Vanbergen A.J. & Woodcock B.A. 2010. Functional traits as indicators of biodiversity response to land use changes across ecosystems and organisms. Biodivers. Conserv. **19:** 2921–2947. DOI: 10.1007/s10531-010-9798-9

Verdonschot R.C.M., Kail J., McKie B.G. & Verdonschot P.F.M. 2016. The role of benthic microhabitats in determining the effects of hydromorphological river restoration on macroinvertebrates. Hydrobiologia **769:** 55–66. DOI: 10.1007/s10750-015-2575-8

Warmke S. & Hering D. 2000. Composition, microdistribution and food of the macroinvertebrate fauna inhabiting wood in low-order mountain streams in Central Europe. Int. Rev. Hydrobiol. **85:** 67–78. DOI: 10.1002/(SICI)1522-2632(200003)85:1<67::AID-IROH67>3.0.CO;2-7

Williams D.D. 1980. Some relationships between stream benthos and substrate heterogenity. Limnol. Oceanogr. **25:** 166–172. DOI: 10.4319/lo.1980.25.1.0166

Wood P. 1998. Reach- scale mesohabitat variations in a small chalk stream under low flow conditions, pp. 31–38. In: Bretschko G. & Helešic J. (eds), Advances in River Bottom Ecology, Backhuys Publishers, Leiden, the Netherlands, 344 pp. ISBN: 9073348870

Yamamuro A.M. & Lamberti G.A. 2007. Influence of organic matter on invertebrate colonization of sand substrata in a northern Michigan stream. J. North Am. Benthol. Soc. **26:** 244–252. DOI: 10.1899/0887-3593(2007)26[244:IOOMOI]2.0.CO;2

Received July 1, 2016
Accepted March 21, 2017

Appendix 1. Taxa list. Mean density ± SD of macroinvertebrates in 1.25 m^2 of all substrates (28 samples) from cold (October – April) and from warm (June – August) seasons in the Stupavský potok brook in 2003 and 2004 (+ density less than 1 specimens).

Taxon	October – April		June – August	
	Mean	± SD	Mean	± SD
Turbellaria				
Dugesia gonocephala (Duges, 1830)	46	± 81.45	20	± 28.48
Amphipoda				
Gammarus fossarum Koch, 1836	3422	± 3095.46	2535	± 1428.03
Oligochaeta				
Chaetogaster diastrophus (Gruithuisen, 1828)	1	± 1.89	0	0.00
Nais alpina Sperber, 1948	9	± 27.68	1	± 1.31
Nais barbata Müller 1774	1	± 0.95	0	0.00
Nais communis Piguet, 1906	3	13.62	1	± 0.95
Nais elinguis O. F. Müller, 1773	+	0.00	0	0.00
Nais stolci Hrabě, 1981	27	± 124.36	19	± 50.47
Pristinella sp.	1	± 1.89	0	0.00
Limnodrilus hoffmeisteri Claparède, 1862	1	± 5.67	1	± 0.95
Tubifex tubifex (O. F. Müller, 1774)	88	± 252.76	6	± 15.36
Stylodrilus heringianus Claparède, 1862	59	± 103.21	33	± 53.27
Trichodrilus tatrensis Hrabě, 1937	9	± 13.70	3	± 6.01
Haplotaxis gordioides (Hartmann, 1821)	1	± 4.16	1	± 1.89
Fridericia sp.	1	± 0.95	0	0.00
Cernosvitoviella sp.	+	0.00	1	± 0.95
Enchytraeidae g. sp.	3	± 6.45	0	0.00
Eiseniella tetraedra (Savigny, 1826)	1	± 4.22	1	± 0.95
Lumbricidae g. sp.	1	± 0.95	1	± 0.95
Ephemeroptera				
Baetis alpinus Pictet, 1843–1845	29	± 75.82	19	± 47.76
Baetis rhodani Pictet, 1843–1845	73	± 126.41	180	± 239.42
Baetis spp. juv.	1	± 3.78	1	± 5.67
Baetis (Nigrobaetis) muticus (L., 1758)	36	± 67.80	6	± 23.60
Centroptilum luteolum (Müller, 1776)	8	± 22.13	0	± 1.89
Electrogena samalorum (Landa & Soldán, 1982)	+	0.00	0	0.00
Electrogena ujhelyii (Sowa, 1981)	1	± 7.56	0	0.00
Electrogena spp. juv.	1	± 2.62	1	± 2.62
Epeorus assimilis Eaton, 1885	72	± 152.45	15	± 41.70
Rhithrogena carpatoalpina Klonowska, Olechowska, Sartori & Weichselbaumer, 1987	9	± 24.35	2	± 5.15
Rhithrogena iridina (Kolenati, 1859)	0	0.00	4	± 15.19
Rhithrogena semicolorata (Curtis, 1834)	23	± 32.98	10	± 22.87
Rhithrogena semicolorata gr. juv.	450	± 670.44	28	± 35.39
Habroleptoides confusa Sartori et Jacob, 1986	18	± 27.90	14	± 21.79
Ephemera danica Müller, 1764	3	± 9.66	2	± 5.31
Ephemerella mucronata (Bengtsson, 1909)	150	± 237.99	0	0.00
Serratella ignita (Poda, 1761)	2	± 7.74	85	± 226.68
Plecoptera				
Brachyptera seticornis (Klapálek, 1902)	+	0.00	0	0.00
Nemoura cambrica (Stephens, 1835)	+	0.00	0	0.00
Nemoura flexuosa Aubert, 1949	3	± 10.49	0	0.00
Nemoura marginata Pictet, 1836	1	± 1.89	0	0.00
Nemoura spp. juv.	21	± 33.72	6	± 11.89
Protonemura intricata (Ris, 1902)	67	± 237.14	0	0.00
Protonemura nitida (Pictet, 1835)	151	± 523.33	179	± 226.11
Protonemura praecox (Morton, 1894)	31	± 60.28	15	± 34.95
Protonemura spp. juv.	0	0.00	4	± 11.36
Leuctra hippopus Kempny, 1899	82	± 112.49	0	0.00
Leuctra prima Kempny, 1894	2	± 5.69	1	± 0.95
Leuctra spp, juv.	0	0.00	11	± 13.01
Capnia bifrons (Newman, 1839)	+	0.00	0	0.00
Isoperla oxylepis (Despax, 1936)	2	± 5.50	1	± 2.62
Isoperla tripartita Illies, 1954	2	± 5.28	0	0.00
Isoperla spp. juv.	18	± 28.53	3	± 5.35
Perlodes microcephalus (Pictet, 1833)	1	± 4.16	1	± 4.16
Siphonoperla taurica (Pictet, 1841)	2	± 5.68	0	0.00
Siphonoperla torrentium (Pictet, 1841)	+	0.00	0	0.00
Coleoptera				
Limnius sp., larvae	346	± 369.73	167	± 201.33
Hydraena gracilis Germar, 1824	83	± 80.31	26	± 36.36
Esolus parallelepipedus (Müller, 1806)	+	0.00	0	0.00
Elmis sp., larvae	23	± 56.56	5	± 10.27
Hydraena egoni Jach, 1986	12	± 26.14	5	± 8.04

Appendix 1. (continued)

Taxon	October – April		June – August	
	Mean	± SD	Mean	± SD
Limnius perrisi (Dufour, 1843)	10	± 15.69	6	± 10.89
Hydraena melas Dalla Torre, 1877	1	0.00	0	0.00
Hydraena riparia Kugelann, 1794	+	0.00	1	± 0.95
Hydraena pygmaea Waterhouse, 1833	2	± 4.76	2	± 7.72
Elmis aenea (Müller, 1806)	3	± 8.44	1	± 4.48
Elmis maugetii Latreille, 1798	1	± 2.62	1	± 1.89
Limnius volckmari (Panzer, 1793)	4	± 6.75	1	± 2.62
Platambus maculatus (L., 1758)	1	± 1.89	0	0.00
Elodes sp., larvae	8	± 21.15	0	0.00
Pomatinus substriatus (Müller, 1806)	1	± 0.95	0	0.00
Heteroptera				
Microvelia spp.	0	0.00	1	± 0.95
Trichoptera				
Rhyacophila fasciata Hagen, 1859	2	± 5.35	5	± 9.33
Rhyacophila obliterata McLachlan, 1863	1	0.00	3	± 8.44
Rhyacophila tristis Pictet, 1835	55	± 53.42	25	± 31.09
Rhyacophila vulgaris Pictet, 1834	7	± 16.52	0	0.00
Rhyacophila (s. str.) sp. juv.	12	± 27.10	6	± 10.28
Agapetus fuscipes Curtis, 1834	1	± 0.95	0	0.00
Agapetus ochripes Curtis, 1834	82	± 129.45	27	± 49.82
Glossosoma boltoni Curtis, 1834	1	± 1.89	0	0.00
Glossosoma conformis Neboiss, 1963	2	± 6.87	0	0.00
Glossosoma sp. juv.	2	± 10.50	0	0.00
Agapetinae g. sp. juv.	540	± 962.71	149	± 253.39
Hydroptilidae g. sp. juv.	1	± 1.89	+	0.00
Philopotamus montanus (Donovan, 1813)	6	± 12.99	3	± 13.61
Philopotamidae g. sp. juv.	0	0.00	1	± 2.62
Hydropsyche exocellata Dufour, 1841	0	0.00	1	± 1.89
Hydropsyche instabilis (Curtis, 1834)	18	± 27.97	70	± 132.70
Hydropsyche saxonica McLachlan, 1884	1	0.00	0	0.00
Hydropsyche sp. juv.	287	± 303.87	43	± 114.23
Plectrocnemia cf. *brevis* juv. McLachlan, 1871	1	± 1.89	0	0.00
Plectrocnemia conspersa (Curtis, 1834)	1	± 1.89	0	0.00
Lype reducta (Hagen, 1868)	1	± 0.95	1	± 1.89
Tinodes rostocki McLachlan, 1878	32	± 63.75	1	± 1.89
Tinodes sp. juv.	20	± 38.75	6	± 13.08
Psychomyiidae g. sp. juv.	1	± 0.95	1	± 4.48
Annitella obscurata (McLachlan, 1876)	1	± 7.56	0	0.00
Drusus annulatus (Stephens, 1837)	1	± 7.56	0	0.00
Drusus sp. juv.	1	± 0.95	0	0.00
Halesus digitatus (Schrank, 1781)	1	± 3.78	1	± 1.89
Chaetopteryx villosa (F, 1798) / *fusca* Brauer, 1857	0	0.00	9	± 26.69
Potamophylax latipennis (Curtis, 1834) / *luctuosus* (Piller & Mitterpacher, 1783)	7	± 19.97	2	± 7.87
Potamophilax rotundipennis (Brauer, 1857)	1	± 3.78	0	0.00
Potamophylax sp. juv.	0	0.00	1	± 1.89
Limnephilidae g. sp. juv.	60	± 168.24	2	± 5.97
Silo sp. juv.	1	± 2.08	1	± 0.95
Goeridae g. sp. juv.	5	± 10.09	1	± 1.89
Sericostoma personatum (Spencer, 1826) / *schneiderii* (Kolenati, 1848)	48	± 57.58	28	± 37.63
Sericostomatidae g. sp. juv.	23	± 42.38	0	0.00
Odontocerum albicorne (Scopoli, 1763)	65	± 88.11	11	± 25.19
Chironomidae				
Apsectrotanypus trifascipennis (Zetterstedt, 1838)	24	± 84.61	2	± 6.14
Conchapelopia sp.	46	± 162.60	6	± 11.23
Macropelopia sp.	1	± 0.95	1	± 2.62
Procladius (*Holotanypus*) sp.	1	± 7.56	1	± 1.89
Tanypodinae indet.	0	0.00	1	± 0.95
Thienemannimyia sp.	24	± 46.56	2	± 4.99
Diamesa cf. *tonsa*	19	± 64.63	0	0.00
Diamesa sp.	0	0.00	5	11.70
Zavrelimyia sp.	1	± 3.78	0	0.00
Potthastia longimana (Kieffer, 1922)	12	± 43.21	1	± 1.89
Odontomesa fulva (Kieffer, 1919)	5	± 12.98	5	± 13.81
Prodiamesa olivacea (Meigen, 1818)	17	± 60.68	14	± 47.51
Brillia longifurca Kieffer, 1921	0	0.00	1	± 2.62
Brillia modesta (Meigen, 1830)	25	± 61.25	18	± 40.76

Appendix 1. (continued)

Taxon	October – April		June – August	
	Mean	± SD	Mean	± SD
Corynoneura lobata Edwards, 1924	157	± 539.96	6	± 13.45
Corynoneura scutellata gr.	1	± 0.95	0	0.00
Cricotopus tremulus gr.	1	± 1.89	1	± 1.89
Cricotopus trifascia gr.	1	± 3.78	0	0.00
Cricotopus sp.	0	0.00	1	± 4.16
Chaetocladius piger gr.	10	± 52.92	0	0.00
Chaetocladius dentiforceps gr.	0	0.00	0	0.00
Chaetocladius vitellinus gr.	1	± 3.78	0	0.00
Epoicocladius flavens (Kieffer, 1924)	1	± 3.86	0	0.00
Eukiefferiella brevicalcar gr.	76	± 230.73	1	± 0.95
Eukiefferiella cf. *brehmi* gr.	+	0.00	0	0.00
Eukiefferiella clypeata (Thienemann, 1919)/ *pseudomontana* Goetghebuer, 1935	1	± 1.89	0	0.00
Eukiefferiella cf. *coerulescens* (Kieffer, 1926)	0	0.00	1	1.89
Eukiefferiella devonica (Edwards, 1929)/ *ilkleyensis* (Edwards, 1929)	14	± 71.81	0	0.00
Eukiefferiella cf. *fuldensis*	1	± 1.89	0	0.00
Eukiefferiella rectangularis gr./*brehmi* gr.	3	± 10.49	0	0.00
Eukiefferiella sp.	103	± 498.56	0	0.00
Heleniella ornaticollis (Edwards, 1929)	9	± 18.85	1	± 3.23
Limnophyes sp.	0	0.00	1	± 2.62
Krenosmittia camptophleps (Edwards, 1929)	1	± 2.24	0	0.00
Krenosmittia sp.	+	0.00	0	0.00
Metriocnemus fuscipes gr.	+	0.00	0	0.00
Metriocnemus hygropetricus gr.	1	± 3.78	0	0.00
Metriocnemus /Thienemannia sp.	0	0.00	0	0.00
Orthocladius obumbratus Langton & Cranston, 1991	7	± 22.58	0	0.00
Orthocladius obumbratus/oblidens (Walker, 1856)	+	0.00	0	0.00
Orthocladius sp.	2	± 7.58	0	0.00
Orthocladius rivicola Kieffer, 1911	139	± 394.23	0	0.00
Orthocladius rubicundus (Meigen, 1818)	8	± 24.84	1	± 1.89
Parakiefferiella cf. *triquetra*	0	0.00	1	0.95
Parakiefferiella sp.	1	± 1.89	0	0.00
Parametriocnemus stylatus (Kieffer, 1924)	15	± 37.60	24	± 49.95
Paraphaenocladius sp.	0	0.00	0	0.00
Paratrichocladius rufiventris (Meigen, 1830)	48	± 205.65	+	0.00
Paratrissocladius excerptus (Walker, 1856)	5	± 18.36	13	± 39.61
Rheocricotopus effusus (Walker, 1856)	+	0.00	0	0.00
Rheocricotopus fuscipes (Kieffer, 1909)	147	± 440.25	13	± 34.97
Rheosmittia spinicornis (Brundin, 1956)	12	± 31.14	0	0.00
Rheosmittia sp.	1	0.00	0	0.00
Symposiocladius lignicola (Kieffer, 1915)	4	± 9.94	4	± 13.60
Synorthocladius semivirens (Kieffer, 1909)	4	± 15.21	1	± 1.89
Thienemannia cf. *fulvofasciata* (Kieffer, 1921)	0	0.00	1	± 0.95
Thienemanniella vittata (Edwards, 1924)/ *clavicornis* (Kieffer, 1911)	33	± 101.25	6	± 17.09
Tvetenia calvescens (Edwards, 1929)	59	± 149.73	31	± 55.30
Tvetenia discoloripes (Gortghebuer, 1936)	54	± 156.59	53	± 64.34
Microtendipes pedellus gr.	+	0.00	0	0.00
Paracladopelma sp.	1	± 3.76	1	± 3.86
Phaenopsectra sp.	3	± 8.83	+	0.00
Endochironomus sp.	1	± 0.95	0	0.00
Polypedilum breviantennatum gr.	16	± 41.08	4	± 17.98
Polypedilum convictum gr.	33	± 126.51	1	± 3.06
Polypedilum pedestre gr.	23	± 78.31	35	± 64.79
Polypedilum scalaenum gr. (cf. pullum)	10	± 38.26	0	0.00
Polypedilum scalaenum gr.	0	0.00	0	0.00
Polypedilum sp.	1	± 3.76	0	0.00
Cladotanytarsus vanderwulpi gr.	0	0.00	1	± 5.91
Micropsectra cf. a*ristata* Pinder, 1976	0	0.00	3	± 9.28
Micropsectra cf. *Junci* (Meigen, 1818)	1	± 1.89	0	0.00
Micropsectra sp.	320	± 893.39	59	± 114.44
Rheotanytarsus sp.	1	± 3.78	0	0.00
Stempellinella sp.	14	± 46.64	2	± 7.60
Tanytarsus sp.	2	± 4.80	3	± 7.60
Chironomidae, pupae	+	0.00	0	0.00
Simuliidae				
Prosimulium tomosvaryi (Enderlein, 1921)	70	± 157.86	0	0.00

Appendix 1. (continued)

Taxon	October – April		June – August	
	Mean	± SD	Mean	± SD
Simulium costatum (Friederichs, 1920)	6	± 15.93	10	± 16.44
Simulium cryophilum (Rubtsov, 1959)	7	± 22.77	10	± 13.51
Simulium vernum (Macquart, 1838)	93	± 422.24	28	± 73.24
Simulium argyreatum Meigen, 1838	40	± 75.31	227	± 624.79
Simulium monticola Friederichs, 1920	1	± 5.25	3	± 13.34
Simulium ornatum Meigen, 1818	18	± 35.13	68	± 105.12
Simulium trifasciatum Curtis, 1839	0	0.00	1	± 2.62
Simulium variegatum Meigen, 1818	2	± 5.48	1	± 3.86
Simulium sp.	11	± 45.86	10	± 18.38
other Diptera				
Tipula fulvipennis De Geer, 1776	1	± 1.89	0	0.00
Dicranota gr. *robusta*	22	± 28.23	24	± 30.29
Pedicia (C.) straminea (Meigen, 1838)	1	± 1.89	0	0.00
Limoniidae	+	0.00	0	0.00
Ellipteroides (P.) alboscutellatus (von Roser, 1840)	1	± 3.23	4	± 9.46
Eloeophila maculata (Meigen, 1804)	5	± 7.69	1	± 2.74
Eloeophila mundata (Loew, 1871)	4	± 9.01	2	± 7.74
Eloeophila submarmorata (Verral, 1887)	1	± 0.95	1	± 1.31
Eloeophila spp. juv.	1	± 5.91	0	0.00
Molophilus sp.	1	± 1.89	0	0.00
Hexatoma vittata (Meigen, 1830)	5	± 9.95	2	± 4.18
Scleroprocta spp.	8	± 22.62	2	± 7.99
Paradelphomyia sp.	1	± 0.95	0	0.00
Gonomyia lucidula De Meijere, 1920	1	0.00	0	0.00
Idiocera punctata (Edwards, 1938)	1	0.00	0	0.00
Pilaria spp.	1	± 1.89	0	0.00
Rhypholophus haemorrhoidalis (Zetterstedt, 1838)	1	± 1.89	0	0.00
Erioptera vicina (Tonnoir, 1920)	1	± 1.89	0	0.00
Dixa puberula Loew, 1849	1	± 5.67	2	± 6.86
Dixa maculata-Gr.	1	± 1.89	+	0.00
Dixa nubilipennis Curtis, 1832	1	0.00	0	0.00
Bazarella subneglecta (Tonnoir, 1922)	4	± 12.27	1	± 2.74
Berdeniella unispinosa (Tonnoir, 1919)	1	± 5.67	0	0.00
Psychoda gemina (Eaton, 1904)	1	0.00	0	0.00
Satchelliella (Pneumia) stammeri Jung, 1956)	6	± 8.93	1	± 4.22
Pericoma spp.	1	± 4.16	0	0.00
Tonnoiriella pulchra (Eaton, 1893)	0	0.00	1	± 1.89
Sycorax spp.	0	0.00	1	± 1.89
Ptychoptera spp.	1	± 3.93	1	± 0.95
Bezzia spp.	8	± 17.66	1	± 3.15
Liponeura cinerascens minor Bischoff, 1922	3	± 11.82	0	0.00
Liponeura vimmeri Mannheims, 1954	48	± 99.42	0	0.00
Liponeura sp. juv.	8	± 16.53	0	0.00
Ibisia marginata (F., 1781)	29	± 40.68	7	± 11.56
Atherix ibis (F., 1798)	1	± 3.78	0	0.00
Oxycera meigenii Staeger, 1844	1	± 1.89	1	± 0.95
Oxycera pardalina Meigen, 1822	2	± 7.58	0	0.00
Oxycera pygmaea (Fallén, 1817)	+	0.00	0	0.00
Empididae	+	0.00	0	0.00
Clinocera spp.	1	± 1.31	0	0.00
Chelifera spp.	20	± 48.45	4	± 9.13
Hemerodromia spp.	1	0.95	0	0.00
Wiedemannia spp.	13	± 41.33	1	± 2.24
Chrysops caecutiens (L., 1758)	6	± 29.29	1	± 1.89
Chrysops spp.	2	± 12.28	1	± 1.89
Sciomyzidae	+	0.00	0	0.00
Liancalus virens (Scopoli, 1763)	1	± 1.89	0	0.00
Density	8699		4557	
Number of taxa	222		143	