Hydrobiologia (2006) 566:543–555

M.T. Furse, D. Hering, K. Brabec, A. Buffagni, L. Sandin & P.F.M. Verdonschot (eds), The Ecological Status of European Rivers: Evaluation and Intercalibration of Assessment Methods
DOI 10.1007/s10750-006-0073-8

Influence of seasonal variation on bioassessment of streams using macroinvertebrates

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Key words: bioassessment, macroinvertebrates, seasonal variation, Slovakia, stream

Abstract

The EU Water Framework Directive requires assessment of the ecological quality of running waters using macroinvertebrates. One of the problems of obtaining representative samples of organisms from streams is the choice of sampling date, as the scores obtained from macroinvertebrate indices vary naturally between seasons, confounding the detection of anthropogenic environmental change. We investigated this problem in a 4th order calcareous stream in the western Carpathian Mountains of central Europe, the Stupavský potok brook. We divided our 100 m study site into two stretches and took two replicate samples every other month alternately from each stretch for a period of 1 year, sampling in the months of February, April, June, August, October and December. Multivariate analysis of the macroinvertebrate communities (PCA) clearly separated the samples into three groups: (1) April samples (2) June and August samples (3) October, December and February samples. Metric scores were classified into two groups those that were stable with respect to sampling month, and those that varied. Of the metrics whose values increase with amount of allochthonous organic material (ALPHA_MESO, hyporhithral, littoral, PASF, GSI new, DSI, CSI), the highest scores occurred in February, April, October and December, while for metrics whose values decrease with content of organic material (DSII, DIS, GFI D05, PORI, RETI, hypocrenal, metarhithral, RP, AKA, LITHAL, SHRED, HAI) the highest values occurred in February, April, June and December. We conclude that sampling twice a year, in early spring and late autumn, is appropriate for this type of metarhithral mountain stream. Sampling in summer is less reliable due to strong seasonal influences on many of the metrics examined while sampling in winter is inappropriate for logistical reasons.

Introduction

With the implementation of the Water Framework Directive (WFD) every EU member state is obligated to assess the effects of human activities on the ecological quality of all water bodies (European Commission, 2000). Assessment of the ecological state of surface waters based on selected groups of living organisms as required by the Water Framework Directive (WFD) poses the problem of obtaining samples representative of the stream community. In collecting macroinvertebrate samples temporal and spatial changes in the community composition are two of the most important aspects that should be taken into account when collecting representative samples.

Temporal distributions of freshwater communities, both on the bottom and in the water column, are known to be influenced by the life histories of the various species (Hynes, 1972; Williams, 1981a). Ormerod (1987) showed that the most precise categorisation of assemblage type required a sampling strategy that combines both habitat and seasonal data. While many physical factors that have been shown to affect faunal assemblages are known to change seasonally (e.g., hydrological regime, water chemistry, light levels and temperature), lotic assemblages of invertebrates vary both seasonally and with spatial position within the stream (Matthews & Bao, 1991; Cowell et al., 2004). Setting a suitable time period for sampling a given habitat type is therefore a complex problem.

The establishment of reliable biomonitoring programmes is central to the effective implementation of the WFD for surface waters. Water managers prefer cost efficient methods, e.g. sampling in most cases only once a year for the purpose of surveillance monitoring. In contrast, studies aiming to assess conservation value normally require more than one sampling occasion within a given year to obtain adequate site evaluations (Furse et al., 1984). The choices made related to sampling strategies are always a trade off between biological reliability and economic considerations. When cost do not allow to take more than one sample a year at a site for the purpose of surveillance monitoring a higher level of standardisation and between site comparability could be reached if samples from the same area were collected in the same time period, thereby minimising variability in the observed communities due to natural seasonal differences. In many European countries there is an agreement about the period most suited for sampling macroinvertebrates, however in most cases scientific background to these agreements is lacking.

The aim of this study was therefore (1) to examine the variation in macroinvertebrate community composition between months (2) to assess the effects of natural seasonal community variation on metric values, and (3) to determine whether a preferred sampling period(s) could be identified for mountainous streams in Slovakia. A similar study in lowland streams (Heelsumse beek) was performed in the Netherlands (Vlek, in press). In combination these two studies combined make it possible to evaluate the influence of seasonal changes in macroinvertebrate community composition on metrics used for bioassessment purposes across two widely differing European stream types.

Materials and methods

Study site and data collection

Samples were collected from the Stupavský potok brook (N 48° 15′ 09.1″ E 17° 06′ 44.4″), a small, calcareous, 4th order stream in the Carpathian Mountains of central Europe (Fig. 1). The long-term discharge of Stupavský potok brook is characteristic of highland snowmelt streams (Šimo & Zaťko, 1980), with the highest discharges occurring at the beginning of spring (March and April; Fig. 2). It should be noted that the discharge during the study period was to some extent atypical, being generally lower than the long-term average and lacking a peak in the usual snow-melt period (gradual spring snow melt; Fig. 2).

The study site was a relatively uniform 100 m section of the stream (average width 5.1 m: average depth 0.16 m). This 100 m section was divided into two 50 m stretches. Two (replicate) samples were taken every other month in the last week of the month (April, June, August, October, December* and February, actually sampled 8th January), alternately from the two stretches (stretch 1 in April, stretch 2 in June etc.). Prior to sampling, habitat coverage was estimated for the complete 100 m section (AQEM consortium, 2002). For each habitat an area of 25×25 cm was sampled by kick-sampling using a 500 μm hand-net. Each habitat with a coverage of more than 5% was sampled separately. The area sampled per habitat was the same on all sampling occasions and the same operator collected all of the sub-samples. The samples were preserved in 4% formaldehyde prior to transportation to the laboratory for processing. In the laboratory the samples collected from the different habitats were sieved using 1000 and 500 μm sieves, and fully sorted under a stereomicroscope. Sorting was performed by a group of three people. The same specialist preformed all identifications of each major organism group. Macroinvertebrates were identified to the lowest taxonomic level possible (species level for almost all groups).

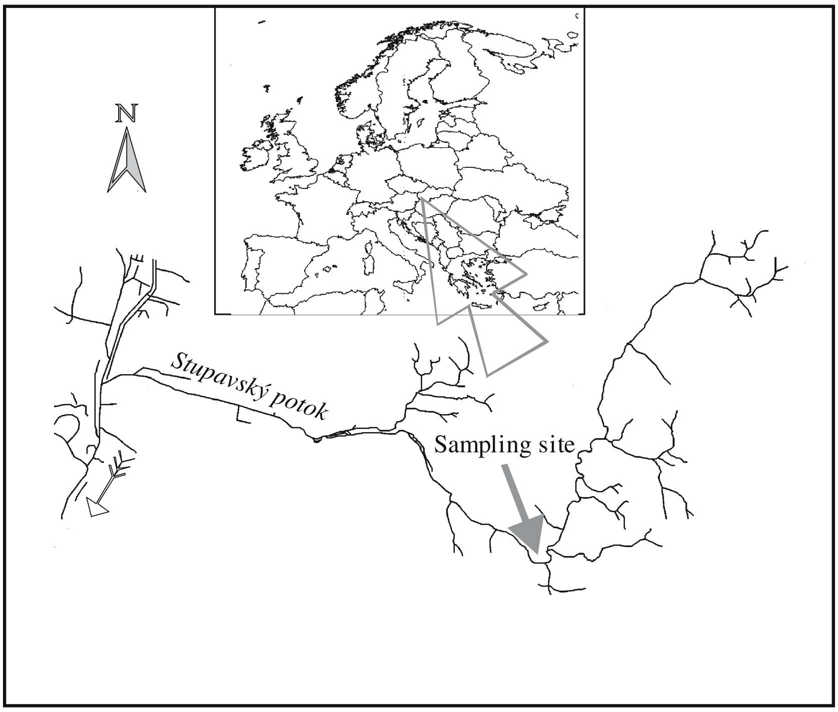

Figure 1. The catchment area of the Stupavský potok brook with sampling site.

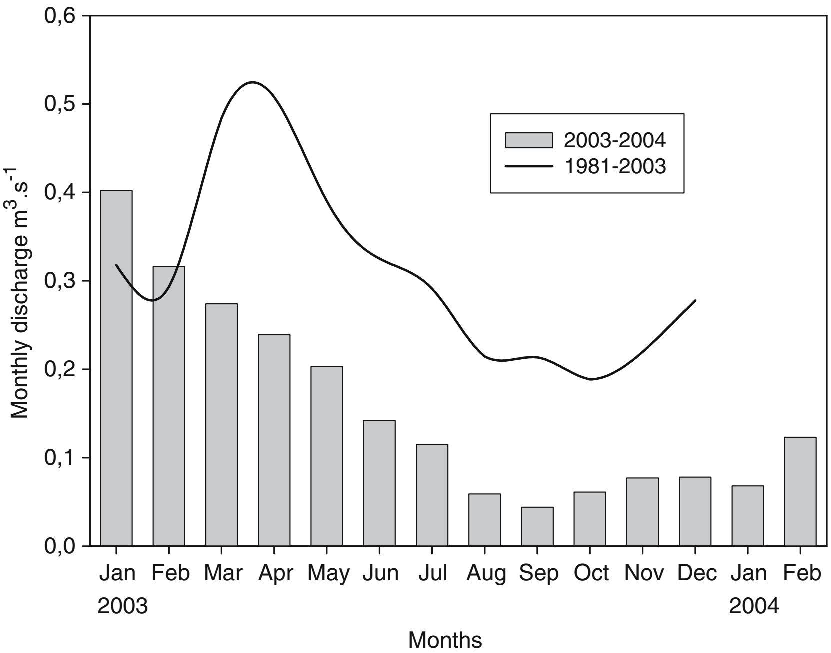

Figure 2. Average monthly discharge of the Stupavský potok brook based on a 23-year long-term average (1981–2003) and individual monthly averages between the months of January 2003 and February 2004.

Data analysis

Prior to analysis, samples from the different habitats were pooled together to form two composite samples. The number of individuals per taxon were standardised to a total sample area of 1.25 m^2 for each composite sample based on habitat coverage and sampled area (abundance* 1.25/area sampled). A Principal Components Analysis (PCA) using CANOCO 4.5 (ter Braak & Smilauer, 2002) was performed to examine variation in macroinvertebrate community composition between months. Species data were $\log_2 (x+1)$ transformed before analysis.

The effects of natural seasonal variation in community composition on metric values were assessed using a list of metrics commonly used in Europe (supplementary material).[1] The metrics were selected from an extensive list given by Hering et al. (2004). In addition to these metrics the number of taxa and the number of individuals for each major macroinvertebrate group (e.g. Diptera, Ephemeroptera, Plecoptera) was also evaluated. Some groups were only present at low abundances and in just a few samples. These groups were therefore excluded from our analyses because of the difficulties of finding appropriate transformations to normalise the data and the problems of having many zero values (Metzling et al., 2003). Metric values were calculated with the software ASTERICS version 1.0 (AQEM/STAR Ecological RIver Classification System; http://www.aqem.de) for all composite samples, except for the Slovak Saprobic index which is not included in the software. Slovak Saprobic index values were obtained from Šporka (2003). The coefficient of variation (CV = SD/mean), a measure of variability, was calculated for the different metrics. One-way analysis of variance (ANOVA) was used to identify significant differences between months ($\alpha = 0.05$) by SigmaStat 3.1 for Windows software.

Assumptions for normality and homogeneity of variance could not be tested in a reliable way due to the low number of samples. For this reason it might have been more appropriate to perform a non-parametric test. However, a non-parametric test would never be able to detect significant differences between protocols based on two replicates. Therefore it was decided to use the ANOVA and to transform metric values based on experiences in other studies. Abundance metrics were $\ln(x+1)$ transformed (Supplementary Material type 1). Taxa counts were not transformed and proportions were transformed $\ln(x+1)–\ln(y+1)$ (Supplementary Material type 2), where x = the number of individual taxa and y = the number of total taxa (Kerans et al., 1992). Biotic index data (e.g. Saprobic Index, BMWP, ASPT) were not transformed (Norris & Georges, 1993). Metrics like XENO (%), SHRED (%) and littoral (%) are not simple proportional metrics. The values for these metrics also depend on the strength with which a species prefers a certain category (AQEM consortium, 2002). The decision was made not to transform values of these metrics, since no information could be found to describe a suitable transformation. Acronym, metric description and type of transformation are given in Supplementary Material.

Results

Taxa analysis

In total 218 taxa were collected during this study. Each replicate contained on average 42% of the total number of taxa, and the total number of taxa occurring in both replicates from any 1 month varied between 56% and 70%. In macroinvertebrate community of the Stupavský potok brook the highest of number of taxa reached Diptera and Trichoptera (Fig. 3). Samples from different months did not exhibit major differences in the number of taxa per organism groups (Fig. 3, Table 1). Similarly, there was no significant difference in the total number of taxa between months ($p = 0.185$). There was also no significant difference in the total number of individuals between months ($p = 0.062$), although, the percentage of individuals for some of the major organism groups did vary significantly between months (Fig. 4, Supplementary Material).

During most months (except February and April) the Crustacea formed the largest proportion of the community (varying between 25 and 57%), followed by the Diptera (varying between 15 and 38%). In February however, the Diptera

[1] Electronic supplementary material is available for this article at <http://dx.doi.org/ 10.1007/s10750-006-0073-8> and accessible for authorised users.

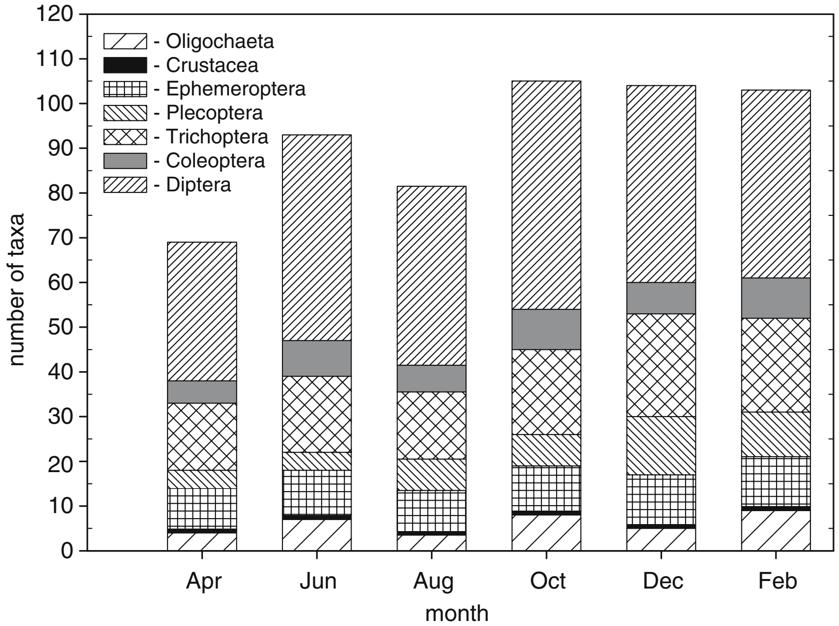

Figure 3. Between month variation in the number of taxa in the Stupavský potok brook based on the sum of both replicates. Only those groups that formed more than 5% of the total abundance are shown.

represented the largest part of the community, while Crustacea numbers were far lower and conversely represented the smallest proportion of the community (Fig. 4).

Multivariate analysis clearly divided the samples into three groups: (1) April samples (2) June and August samples (3) October, December and February samples (Fig. 5).

Dominant taxa that were found in high abundance more than 5% in at least 1 month are compiled in Table 2. *Gammarus fossarum* and species of the family Simuliidae predominated in the summer months. *Rhithrogena semicolorata* dominated in early spring, as did the caddisflies *Agapetus* sp., *Hydropsyche instabilis* and midges of the genus *Micropsectra*. Midges also formed a large proportion of the macroinvertebrates assemblage in October and December and *Hydraena gracilis* dominated in February.

Metric analysis

About 31 out of 76 metrics showed significant ($p < 0.05$) differences between months (Table 1). Between which months significant differences occurred depended on the metric. Metrics showing significant differences between individual months were classified into three groups – (a) those with values increasing with anthropogenic stress (e.g. organic pollution, general degradation, acidification) (b) those with values decreasing with anthropogenic stress and (c) those showing no direct relation to degradation (Hering et al., 2004) or being based on insufficient knowledge:

group a

Metrics that increase values with degradation – ALPHA_MESO, hyporhithral, littoral, PASF, GSI new, CSI. Five out of six metrics reached their lowest values in April and one in February.

group b

Metrics that decrease values with degradation – DSII, DIS, GFI D05, PORI, RETI, hypocrenal, RP, AKA, LITHAL, SHRED, HAI, EPHE, PLEC%, PLEC taxa, PLEC, TRIC. Five out of sixteen metrics reached their highest values in April, 3 out of 16 in August and October, 2 out of 16 in February, June and December.

group c

Metrics with unidentified or insignificant relationships with degradation: GRA + SCRA, metarhithral, DSI, COL taxa, RHYTI, CRUS,

Table 1. Months between which metrics values differed significantly ($p < 0.05$) in the Stupavský potok brook, based on the Least Significant Difference (LSD, $\alpha = 0.05$) and months when metrics reached minimal and maximal value

Acronym	p	Significant differences between	Min value	Max value
ALPHA-MESO (%)	0.003	Apr–other	Apr	Aug
GFI D03	0.045	None		
GFI D05	<0.001	Apr–other	Jun	Apr
		Dec–other (except Feb)		
		Feb–Jun		
GSI new	0.018	Apr–Feb/Jun/Oct	Apr	Feb
DSI	<0.001	Jun–other (except Aug)	Oct	Aug
		Aug–Feb/Oct/Dec		
		Apr–Feb/Oct		
		Dec–Feb		
CSI	0.013	Feb–Apr/Aug	Apr	Feb
		Apr–Oct		
MTS	0.049	None		
HAI	0.001	Feb–Jun/Oct/Dec	Jun, Oct	Feb, Aug
		Aug–Jun/Oct/Dec		
DSII	<0.001	Feb–Jun/Aug	Aug	Feb
		Apr–Jun/Aug		
		Dec–Jun/Aug		
		Oct–Jun/Aug		
DIS	<0.001	Dec–Jun/Aug	Aug	Feb
		Feb–Jun/Aug		
		Oct–Jun/Aug		
		Apr–Jun/Aug		
EVENNESS	<0.001	Dec–Jun/Aug	Aug	Dec
		Apr–Jun/Aug		
		Feb–Jun/Aug		
		Oct–Jun/Aug		
RP (%)	0.004	Aug–Feb/Oct	Feb	Jun
		Jun–Feb		
		Dec–Feb		
AKA (%)	0.034	Jun–Apr	Apr	Jun
LITHAL (%)	0.026	Apr–Feb/Oct	Feb	Apr
Hypocrenal (%)	0.011	Jun–Feb/Dec	Feb	Jun
Littoral (%)	0.014	Apr–Jun/Aug/October	Apr	Jun
Metarhithral (%)	0.01	Apr–other	Feb	Apr
Hyporhithral (%)	0.018	Aug–Apr/Feb	Apr	Dec
SHRED (%)	0.008	Aug–Febr/April	Feb	Aug
		Jun–Feb		
PASF (%)	0.006	Aug–other (except Dec)	Feb	Aug
GRA + SCRA (%)	0.001	Apr–other	Aug	Apr
RETI	0.044	Apr–Feb	Feb	Apr
EPT taxa	0.05	None		
PLEC (%)	0.021	Dec–Apr/Jun	Feb	Apr
CRUS	0.006	Apr–other (except Feb)	Apr	Oct
EPHE	0.022	Oct–Jun/Aug	Aug	Oct

Continued on p. 549

Table 1. (Continued)

Acronym	*p*	Significant differences between	Min value	Max value
PLEC	0.018	Oct–Aug	Aug	Oct
PLEC taxa	0.03	Dec–Jun/Aug	Jun	Dec
TRIC	0.009	Oct–others (except April)	Jun	Oct
COL	0.005	Oct–Apr/Aug/Dec Feb–Apr/Aug	Apr	Feb
COL taxa	0.032	Feb–Aug	Apr	Feb
DIP	0.02	Apr–Feb/Oct	Apr	Feb
PORI	0.012	Apr–Jun/Aug	Aug	Apr
RHYTI	0.032	Apr–Oct	Oct	Apr

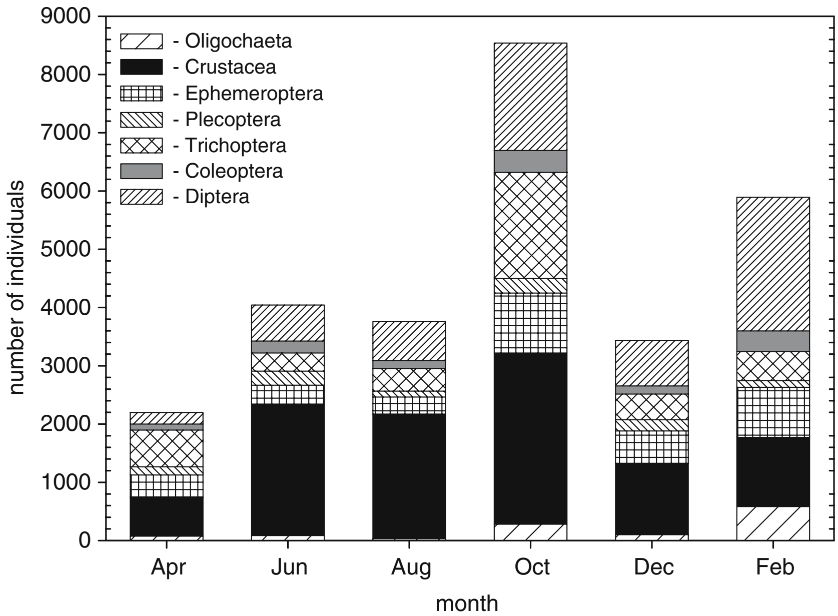

Figure 4. Between month variation in the number of individuals in the Stupavský potok brook, based on the average of both replicates. Only those groups that formed more than 5% of the total abundance are shown.

COL, DIP, Evenness. Among them, three metrics showed highest values in February and April and one in August, October and December, respectively. Four metrics reached the lowest values in April, two metrics in August and October and one in February.

Metrics that reached their maximum values in summer (group a) and differed significantly in value between summer and the other months were associated with poor water quality caused by low discharges (high CSI, PASF %, littoral %). Values of metrics indicating impairment of water quality in summer samples (June, August) are also influenced by summer emergence and the consequent absence of larval stages. The effects of summer emergence were also evident in the low values of the diversity (DIS, DSII) and evenness and low abundance values for certain taxonomic groups e.g., Plecoptera (Table 1). Percentage of dominant feeding types shows differences in individual months during the year (Fig. 6).

The coefficient of variation (CV) of significant metrics varied from 4.2 to 90.6% during the year

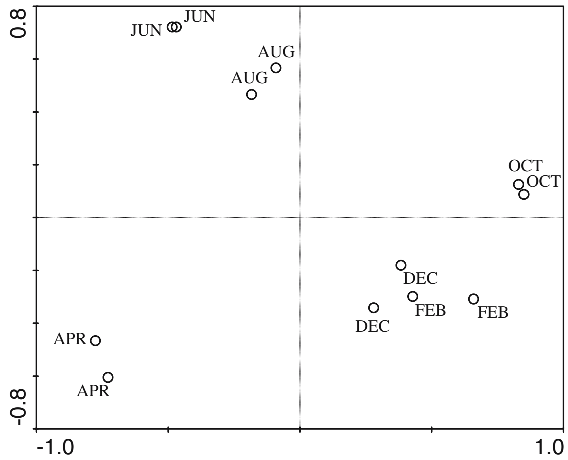

Figure 5. The first two axes of a PCA ordination of Stupavský potok brook macroinvertebrate samples from different seasons.

(Table 3). CV of the most of qualitative metrics does not exceed 20%. However, the highest CV values (above 40%) were found for the quantitative metrics that were based mainly on the abundance of a particular taxonomic group.

Discussion

It is a well-established fact that many insect species have life cycles that are seasonal, and that this results in fluctuations in the numbers of certain groups of macroinvertebrates occurring in samples taken from the streambed at different times of the year (Hynes, 1972). Our analyses show how the community as a whole is affected by macroinvertebrate seasonality and how individual bioassessment metrics can differ significantly between months as a consequence. We found that the majority of metrics exhibiting significant differences between months were quantitative metrics. So, when using quantitative metrics in assessment it is important to recognise that the season in which samples are taken can and often will have a strong influence on the results obtained. In terms of individual metrics, differences between months strongly depend on the metric under evaluation. This makes it difficult to give a general recommendation for a preferred sampling month or season. One option (although not a very practical one) might be to select a preferred season for each individual metric. For metrics directly related to the number of taxa or the number of individuals, the preferred sampling period might be the month in which their values are typically at their highest. In the Stupavský potok brook, the highest numbers of individuals of most major taxonomic groups were found at the end of October. Hynes (1972) showed that autumn is a period of egg hatching, and for many species it is a period of increasing or often of maximum, numbers, including many small individuals. Similarly, in lowland headwater streams of the Alafia River, Cowell et al. (2004) also found the highest abundances in autumn.

On the other hand, EPT metric values did not markedly differ between seasons because in any single month a reasonably representative selection of the three groups that make up this index was always present. Sprules (1947) similarly showed that while the number and diversity of Plecoptera decreases with increasing average summer temperature, the number and diversity of Ephemeroptera and Trichoptera increase, thereby

Table 2. Taxa with abundances more than 5% in one month. Percentage of individuals based on the average of both replicates

Months	Number of individuals (%)						
	Gammarus fossarum	*Rhithrogena semicolorata*	*Agapetus* sp.	*Hydropsyche instabilis*	Simuliidae Gen. sp.	*Micropsectra* sp.	*Hydraena gracilis*
Feb	20	7	1	3	1	23	8
Apr	31	3	16	7	1	0	0
Jun	56	1	1	3	3	3	0
Aug	57	0	7	1	12	2	0
Oct	35	0	12	5	1	9	0
Dec	36	6	1	5	4	7	0

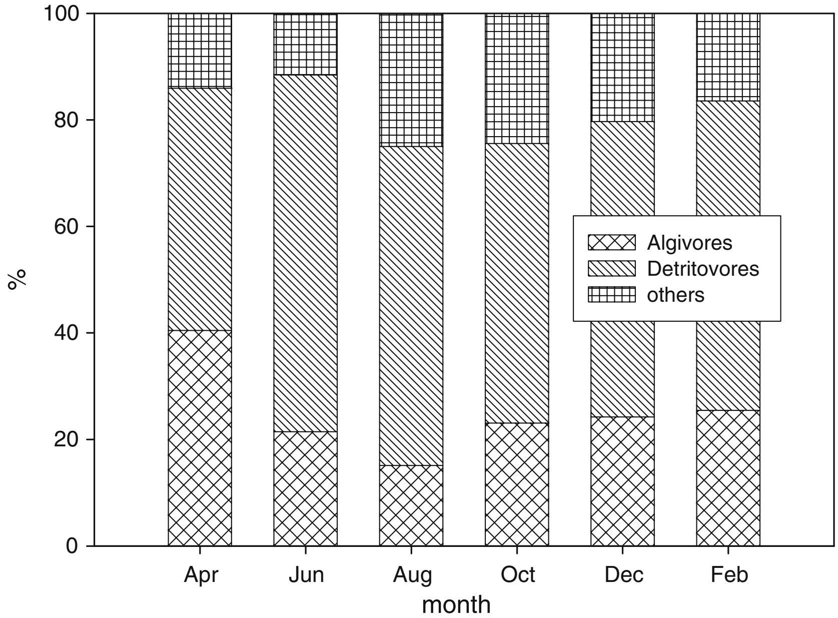

Figure 6. Between month variations in invertebrate food guilds of in the Stupavský potok brook. Percentage of functional feeding groups based on the average of both replicates. Only dominant food guilds are shown.

Table 3. The coefficient of variation (CV) of significant metrics for samples from the Stupavský potok

Metric	CV	Metric	CV	Metric	CV
GSI new	4.2	EPT-taxa	20.4	GRA + SCRA (%)	34.6
RHYTI	7.7	LITHAL (%)	22.8	PLEC	40.1
HAI	9.1	GFI D03	23.3	PLEC (%)	40.7
DSII	12.0	ALPHA-MESO 9%)	23.5	PLEC taxa	45.7
EVENNESS	13.2	RP (%)	23.7	CRUS	48.4
RETI	13.5	Littoral (%)	24.7	COL	52.8
DIS	14.2	Hypocrenal (%)	26.3	EPHE	54.2
MTS	15.4	Metarhithral (%)	26.8	PASF (%)	63.3
GFI D05	16.2	COL taxa	27.9	TRI	81.4
DSI	16.9	PORI	29.5	DIP	90.6
Hyporhithral (%)	17.8	SHRED (%)	29.8	–	–
AKA (%)	20.2	CSI	31.7	–	–

avoiding strong seasonal differences of EPT index scores. This effect has also been observed in the lowland stream Heelsumse beek in the Netherlands (Vlek, in press).

By examining the whole community using multivariate analyses we identified three distinct seasonal assemblages from spring (April), summer (June and August), and autumn and winter (October, December, and February). Individual metric results also indicated that macroinvertebrate community composition in the Stupavský potok brook in April differed from all other months. ALPHA-MESO (%) values were significantly lower in April than in all other months. The low values of ALPHA-MESO (%) in April indicate low amounts of allochthonous organic material. The significantly low CSI values can also be related to organic pollution. The low CSI values and the high values of RETI, GFI, PLEC (%), PORI in April suggest that the water quality of the

Stupavský potok is better in April than in all other months.

With increasing temperature in summer oxygen levels decrease and therefore saprobity increases. Under extreme conditions these changes become readily apparent, as shown by Coimbra et al. (1996) in their investigation of macroinvertebrate community in a temporary stream in Portugal. On the basis of multivariate analysis they classified macroinvertebrate communities into three groups according to environmental variables related to seasons and anthropogenic influences. Morais et al. (2004) studied the robustness of metrics under different hydrological conditions in temporary streams. Seasonal changes over the study period followed the general temporal pattern observed in other Mediterranean streams, with taxa sensitive to organic pollution being present under high discharge and more tolerant taxa under low discharge. The same pattern could be observed in the Stupavský potok brook. In summer due to low discharge the fauna consisted mostly of eurytopic species e.g., *Simulium* sp.

Several other studies have also shown that eurytopic species of the family Simuliidae are dominant in streams of the Small Carpathians Mts. in summer (Halgoš & Jedlička, 1974; Illéšová & Halgoš, 2003). Dahl et al. (2004) stated "However, though a summer sampling window may result in a better detection of oxygen stress, the summer emergence by aquatic insects often precludes the use of this season in bioassessment programmes in Sweden." Nijboer & Schmidt-Kloiber (2004) found that taxa indicating oligosaprobic conditions were taxa with small distribution ranges living in close proximity to stones and gravel (i.e., lithal). In the Stupavský potok brook, colonisation of the lithal substrate was at its greatest in April.

Many studies have shown that seasonal abundance of food may strongly influence the life cycles of the stream community (Ross, 1963; Neel, 1968; Williams & Hynes, 1973; Cummins, 1977; Moore 1977; Townsend & Hildrew, 1979; Williams 1981b). Based on the evaluation of energy flow, Krno (1996) distinguished two significantly different time periods within a year in terms of abiotic factors and food availability:

Cold season – High discharge, periphyton biomass and production of scrapers

Warm season – High temperature, biomass FPOM and production of filterers and collectors.

In the Stupavský potok brook similar relationships between abiotic factors, food resources, and the composition of trophic groups were found.

The highest values of the metrics GRA + SCRA % were found in April when discharge was highest. Representation of feeding types during the year in Stupavský potok brook shows a strong dominance of algophagous forms in spring and, on the contrary, dominance by detritophagous taxa during other parts of year. Similarly, Krno & Hullová (1988) found the largest proportion of this trophic group in the metarithral stretch of the Vydrica stream in the Carpathians in spring, when periphyton (representing an important food resource in this system) develops under the influence of increasing illumination. Krno (1996) also recorded the highest percentage of PASF % in summer when water temperatures were highest. These studies support the view that temperature is a key abiotic factor influencing macrozoobenthos structure (Sprules, 1947, Williams & Hynes, 1974). High temperatures result in high microbial activity and subsequently low oxygen concentrations (Dahl et al., 2004). The metrics reaching significantly higher values in August and June in relation to other months are typically regarded as indicators of poor water quality caused by reduced discharges and high temperatures (CSI, PASF %, hypocrenal % and littoral %).

In this study we have shown that seasonal changes in macroinvertebrate community composition have marked effects on many biotic indices. The life cycles of stream invertebrates, and the seasonal changes in community composition reflect on metric values, are caused primarily by the seasonal dynamics of variables such as temperature, light regime and the supply of nutrients and allochthonous organic material (Clifford, 1978; Bunn, 1986; Krno & Hullová, 1988; Doledec, 1989; Krno 1996). Spring is characterised by an increase in temperature, discharge, light and nutrient supply which results in an increase in primary production and abundance of algophagous invertebrates. This situation is accompanied by a stronger representation of lithophiles and rheophils, and the rapid development of spring forms of macrozoobenthos and emergence of water insects. In spring the metabolism of Small

Carpathian streams has been shown to be predominantly autotrophic (Krno & Hullová, 1988; Rodrigez & Derka, 2003). In the Stupavský potok brook this was confirmed by the highest values of the metric LITHAL % in April and the dominance of algophagous invertebrates (GRA + SCRA%). The progression to summer is characterised by relatively stable and high temperatures, reduced discharge and reduced illumination due to shadowing, and the concurrent development of summer forms of the macrozoobenthos. Signatures of these changes are readily apparent in the metrics littoral, hypocrenal and hyporhithral, which all peak in summer. In autumn and winter, a marked decrease in temperature, lower illumination, and (in contrast to earlier months of the year) a strong supply of allochthonous organic material result in the development of detritophagous invertebrates. Development of detritophagous invertebrates can however be slower than the onset of the preceding seasonal changes in the macroinvertebrate community and in winter it can be strongly inhibited or even stopped. During the winter, the metabolism of Small Carpathian streams has been shown to predominantly heterotrophic (Krno & Hullová, 1988; Rodriguez & Derka, 2003). The strong development detritophagous Crustacea, Plecoptera, Ephemeroptera, and Coleoptera in our study confirms these findings.

The question of determining an appropriate number of sampling occasions during the year is important. From an economic perspective there is a desire to minimise the frequency of sampling while biological studies tend to indicate the reverse. Several studies (e.g., Ormerod, 1987) have demonstrated the benefit of combining datasets from at least two seasons so that taxa rarely recorded in one season are gained from the additional season. Similarly, Furse et al. (1984) showed that combined season data enabled better categorisation and prediction of macroinvertebrate communities than single season data. They advocated sampling in three seasons wherever feasible to allow the characteristic annual pattern of change in the fauna of a site to be incorporated into the analyses. The advantage of taking more than one sample a year was also evident from this study. The complementary value of a late autumn or winter sample to a spring sample was obvious. The autumn and winter community consisted of many species that were uncommon in spring yet were found in high abundances in the later part of the year. It should be noted, however, that mid winter sampling is not suitable for purely logistic reasons (e.g., problems reaching and entering streams and sampling in ice and snow). Futhermore sampling three times a year can be very time-consuming, particularly if identifications are to be taken to species level.

Since seasonal changes are a natural phenomenon it is not possible to give advice on the time period most suited for sampling. For metrics that show high seasonal variation the best solution would be to always sample during the same month or to take into account seasonal variation in setting class boundaries for assessment purposes.

Many of the metrics evaluated in this study depend on indicator values. In many cases indicator values for these taxa were unknown and the influence of taxa with indicator values (and high abundance) and the sensitivity of the metrics to seasonal variation will be overestimated. Increasing the knowledge of autecology will help to reduce this problem. For metrics where the optimal sampling period is not directly related to the highest metric value, the best solution would be to sample in a comparable month or months or to take into account seasonal variation in setting class boundaries.

In this study only the effects of seasonal variation in macroinvertebrate community composition on metric values were evaluated. When selecting metrics for the development of a biological assessment system apart from variability and differences in values between months it is most important to know whether metrics are (highly) correlated to anthropogenic stress.

Acknowledgements

This study was carried out within the STAR project, a research project under the 5th Framework programme of the European Union (EVK1-CT-2001-00089) and grant 1/292/04 from the Slovak Grant Agency for Sciences. We would like to thank John Davy-Bowker for linguistic revision of the manuscript. We would like to thank Tomáš Derka, Daniela Illéšová and Zuzana Pastuchová for their efforts in collecting the data on which this study was based.

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