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Gabriela STASTNA[1], David STRANSKY[1], Ivana KABELKOVA[1] and Roman BABKO[2]

SIMPLIFIED SAMPLING OF BENTHIC MACROINVERTEBRATES FROM SMALL STREAMS

UPROSZCZONE POBIERANIE PRÓBEK MAKROFAUNY BEZKRĘGOWEJ Z NIEWIELKICH STRUMIENI

Abstract: European Union legislation requires achievement of good ecological status of European streams and rivers. Because of that, the ecological status of all streams has to be assessed and evaluated. If there is a status identified less than good the remedial measures must be applied. For effective remediation it is necessary to find the cause of worse than good status classification. In urbanized areas is a number of urban drainage outlets with consequences in a necessity of very detailed biomonitoring. Due to time and money demands of such detailed monitoring, it is not possible to make standardized macroinvertebrates field sampling and processing to evaluate the ecological status. Therefore, admissible simplification of field sampling of macroinvertebrates compared to the standard AQEM method was searched for. Both the effect of the subjectivity of multihabitat sampling and the effect of the reduction of the number of sampling points and their uncertainties were studied with help of field experimental work and probabilistic Monte Carlo simulations. It was proved, that a substantial reduction of the number of sampling units (from 20 sampling units requested by European protocol AQEM) is possible only for ASPT and saprobity index (7 sampling units is sufficient) and diversity (9 sampling units is sufficient). A certain reduction (to 14 sampling units) is also possible for the number of individuals, % EPT and IBI index but no reduction can be applied in case of number of taxa and BMWP, where already the replicate 20 unit samples were biased by an unacceptable uncertainty.

Keywords: benthos, macroinvertebrates, sampling method, screening, uncertainties

Introduction

European Union legislation - Water Framework Directive 2000/60/EC [1] requires achievement of good ecological status of European streams and rivers. For this reason, the ecological status of all streams has to be assessed. The assessment of ecological status is based on several indicators, benthic macroinvetebrates being one of them. However, the realization of extensive macroinvertebrates monitoring programs requires a lot of effort and time. Thus, it is not always possible to evaluate the ecological status by the detailed macroinvertebrates field sampling and processing [2-4]. For this reason, studies searching for a simplification of this procedure are necessary and urgent [5].

The biomonitoring process contains several steps, which can be simplified to save time, and hence money: field sampling, laboratory processing and taxonomical identification. Most studies of possible simplification have focused on the laboratory processing by subsampling when only a part of the sample is processed. However, the minimum number of individuals that has to be taken into account to obtain a valid result varies widely between 100 and 700 [6, 7]. Little attention has been paid to the possibilities of the field sampling simplification. Only Vlek et al [3] studied the effect of the sample size

[1] Faculty of Civil Engineering, Czech Technical University in Prague, Thakurova 7, 166 29 Prague, Czech Republic, phone +420 22435 5412, email: gabriela.stastna@fsv.cvut.cz
[2] Schmalhausen Institute of Zoology of National Academy of Sciences of Ukraine, B. Khmelnitsky 15, 01601 Kiev, Ukraine, email: rbabko@ukr.net
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on individual benthic metrics and final bioassessment of the streams. Metrics dependent on the absolute abundance were more sensitive to the sample size than those dependent on the relative abundance. Also the effect of the variability of multihabitat samples has not been investigated systematically. Lorenz and Clarke [4] studied the similarity of replicate samples on one sampling site and found out that it varied 83-100%.

Comparing various countries, field sampling protocols differ substantially. Some methods require multihabitat sampling when the sampling site is covered by sampling units distributed relatively to the microhabitats occurrence. Other methods doesn't use a certain number of sampling points but the sampling is performed for a certain time, *e.g.* 3 minutes AQEM [8], PERLA [9]. In 2006 the comparison and intercalibration of the national methods within EU with the AQEM one was performed in the STAR project [10]. This project found that some methods were compatible with AQEM and their variance was < 10% (*e.g.* Czech method PERLA) [9].

Thus, in our study on admissible simplification of field sampling of macroinvertebrates of standard AQEM method was searched for including both the effect of the subjectivity of the multihabitat sampling and the effect of the reduction of the number of sampling points and their uncertainties.

The requirements on the uncertainties imposed by the simplification of the sampling method are that the simplified sampling must be able to reveal stream disturbances caused by discharges from the urban drainage system. A site is considered to be significantly disturbed by urban drainage if the difference in total abundance from reference conditions (usually a site upstream of the disturbance source) is higher than 30% or the difference in number of taxons greater than 20% or 3 or more species with at least mean abundance or one highly abundant species do not overlap [11]. For other metrics the uncertainty of about 15% reflects the natural variability [12]. Thus, the relative error of the simplified sampling method compared to the standard method must be lower than these values.

Materials and methods

Seven streams of different morphological quality were sampled. Three of the streams were in urban areas affected by combined sewer overflows, four were natural streams. The stream morphological status was assessed according to the Swiss methodology [12].

In order to find the multihabitat sampling subjectivity the AQEM procedure (reach length 20 m at least, multihabitat sampling, 9 seconds per unit) was applied with the exception of the number of units. Contrary to 20 units required by AQEM, 34 to 41 units were sampled in individual streams. It allows both to determine the uncertainty of the subjective selection of 20 sampling units in the AQEM method and to study the possibility of a further simplification in a screening method.

Organisms from each sampling unit were collected, kept separately and preserved in 90% ethanol. In the laboratory all organisms were identified to the lowest practicable level (usually species) except Oligochaeta, Nematoda, Turbellaria, Acarina, Chironomidae, Ceratopogonidae, Psychodidae which were not identified any further.

In order to identify uncertainties introduced by the reduction of sampling units, Monte Carlo method was used. In each simulation (randomization) a desired number of sampling units x was randomly chosen and benthic metrics were calculated. The randomization was

repeated y times (y = 500). It resulted in y possible values of each benthic metrics, which were statistically analyzed to obtain probability density functions (PDF) (log-normal distribution was considered).

The process described was applied to x values ranging from 8 to 20 in order to find a relation between the number of sampled units and the uncertainty of individual metrics. The values of metrics from the original sample (*i.e.* from all sampled units) served as a reference.

In order to ensure that selected sampling units are not similar (as a biologist selects different representative habitats) criteria restricting the selection were implemented. A maximum difference of 40% from the original sample was set for all criteria (*e.g.* in case 30% of sampling units in the original sample had flow velocity of 1 m/s, then 18-42% of units had to be in the same category in the reduced sample). To allow a comparison of this restricted selection with a non-restricted one, a second run of simulations was done for the random selection without restrictions.

Eight metrics were evaluated: number of individuals, number of taxa, metrics describing general degradation: % of EPT taxa and diversity, and metrics characterizing organic pollution: Si (Saprobity Index), ASPT (the Average Score per Taxon) and BMWP (Biological Monitoring Working Party) [7]. Also B-IBI (Benthic Index for Biological Integrity) describing general and morphological degradation was calculated [13].

Results and discussion

In our study on admissible simplification of field sampling of macroinvertebrates of standard AQEM method was searched for. Both the effect of the subjectivity of multihabitat sampling and the effect of the reduction of the number of sampling points and their uncertainties were studied with help of field experimental work and probabilistic Monte Carlo simulations.

Simulations showed that the restriction criteria applied on the sampling units selection had only a minor influence on results as they caused less than a 3.5% decrease of uncertainty (less than 2% in most cases). Therefore, the results of random selections are presented.

Figure 1 shows two typical patterns of behavior of benthic metrics: 1. nearly no effect of the systematic error, and 2. a pronounced effect of the systematic error (*i.e.* a systematic under- or overestimation of the metrics due to the insufficient number of sampled units). Number of individuals, Saprobic Index, diversity and ASPT belong to the first group as they are not affected by a systematic error larger than 2%. Percentage of EPT taxa is systematically overestimated by up to 4% and IBI shows no systematic error until 15 sampling units (if the number of sampling units is further reduced, the systematic underestimation increases). Number of taxa and BMWP are representatives of the second group as they are highly affected by the systematic error caused by the increased probability of missing rare species when number of sampling points is reduced.

The full line in Figure 1 represents the mean value of metrics in unrestricted simulations, dotted line mean value of metrics in restricted simulation, dashed lines mean random error as 90% uncertainty of unrestricted simulations. Relative uncertainty of 0% corresponds to the reference value of metrics (*i.e.* from all sampled units).

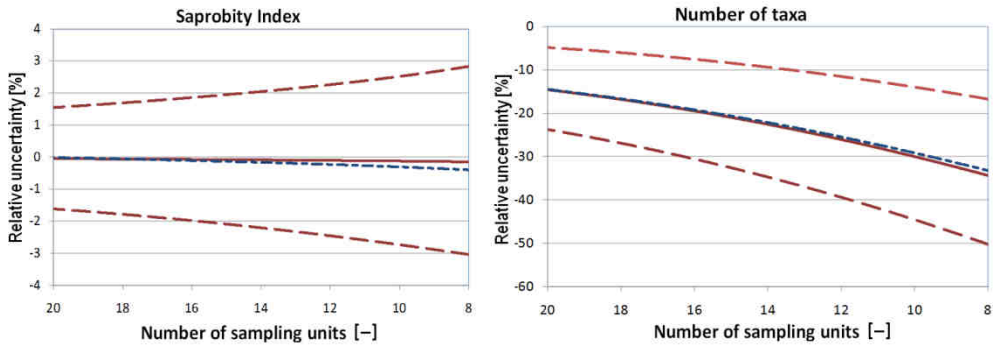

Fig. 1. Course of metrics uncertainties in relation to the decrease of the number of sampled units for one stream sampled

The minimum number of sampling units needed to meet the criteria for the relative uncertainty of individual metrics can be derived from the courses of uncertainties of the individual metrics (Table 1).

Table 1

Number of sampling units needed to meet criteria for relative uncertainty

Metrics	Tritrubecky	Cerveny	Obecnice	Sazavka	Hloucela	Rokytka	Botic	Mean value	Standard deviation	90% conf. interval
Individuals	13	20	11	12	13	16	10	13.6	3.2	19
Taxa	20	20	20	20	20	20	20	20.0	0.0	20
%EPT	6	16	6	8	11	20	20	12.4	5.8	~20
Saprobity index	6	11	6	6	6	6	6	6.7	1.7	10
ASPT	6	6	6	6	6	8	6	6.3	0.7	8
BMWP	20	20	20	20	20	20	20	20.0	0.0	20
Diversity	6	20	6	6	9	7	6	8.6	4.8	17
IBI	7	20	6	6	6	20	19	12.0	6.7	~20

Conclusions

No sampling simplification is admissible for the number of taxa and BMWP, where already the replicate 20 units samples were biased by an unacceptable uncertainty. On the other hand, only 7 sampling points are in average necessary for ASPT and Si and 9 points for the diversity. 14 sampling points should be in average sufficient for the number of individuals, % EPT and IBI. However, standard deviations of the necessary number of sampling units are quite high for some metrics (especially IBI, % EPT and diversity).

Benthic metrics studied exhibited a different dependency on the samples size as also confirmed by [3, 7, 10]. A further reduction of the number of sampling units is possible for metrics, for which the calculated relative uncertainty based on the 20 units sample is lower than the requested uncertainty. Thus, a substantial reduction of the number of sampling

units is possible only for ASPT and saprobity index, which are very robust. A certain reduction is also possible for diversity, number of individuals, % EPT and IBI index. However, in case of the number of taxa and BMWP the average uncertainties are higher than the requested ones even for replicate 20 units samples no reduction can be applied.

As a conclusion, no screening method of the field sampling reducing the number of sampling units from the original 20 unites used in the AQEM method can be recommended.

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UPROSZCZONE POBIERANIE PRÓBEK MAKROFAUNY BEZKRĘGOWEJ Z NIEWIELKICH STRUMIENI

Abstrakt: Normy legislacyjne Unii Europejskiej (Ramowa Dyrektywa Wodna 2000/60/WE) wymagają osiągnięcia dobrego statusu ekologicznego rzek i strumieni na obszarze państw członkowskich. Z tego też powodu status ekologiczny rzek i strumieni krajów Unii powinien być określony i oceniony. W przypadku gdy status ten zostanie oceniony jako mniej niż dobry, powinny zostać podjęte odpowiednie środki zaradcze. Dla wdrożenia efektywnych działań polepszających stan ekologiczny strumieni należy najpierw zidentyfikować powody, dla

których jest on niezadowalający. Na terenie obszarów zurbanizowanych zlokalizowanych jest zwykle wiele wylotów z systemów odprowadzających wody opadowe, które powinny być poddane odpowiednim procedurom biomonitoringu. Ze względu na znaczne nakłady finansowe i czasochłonność nie jest możliwe prowadzenie standardowych procedur pobierania i analizy próbek makrofauny bezkręgowej dla każdego z tak licznych punktów wraz z wymaganą oceną statusu ekologicznego. Stąd też poszukiwane są sposoby dopuszczalnego uproszczenia metod pobierania próbek makrobezkręgowców w odniesieniu do standardowych metod AQEM. W ramach przeprowadzonych badań, za pomocą terenowych prac eksperymentalnych oraz symulacji z wykorzystaniem metody Monte Carlo, analizowano efekty subiektywnego próbkowania siedlisk wielogatunkowych oraz zmniejszenia liczby punktów pobierania próbek powiązane z niepewnością próbkowania. Dowiedziono, że znaczne zmniejszenie liczby próbek (z 20 wymaganych w protokole AQEM) jest możliwe tylko dla indeksu ASPT i indeksu saprobowości (wynosi 7 próbek) oraz indeksu różnorodności (wystarczająca liczba 9 próbek). Zauważalne zmniejszenie liczby (do poziomu 14 próbek) jest możliwe również dla ilości osobników oraz % EPT i indeksu IBI. Jednakże niemożliwe jest zmniejszenie liczby próbek w celu prawidłowego określenia liczby taksonów oraz wartości BMWP, gdzie już w przypadku liczby powtórzeń na poziomie 20 próbek wynik obarczony jest wysokim poziomem niepewności.

Słowa kluczowe: bentos, makrobezkręgowce, testy przesiewowe, niepewność pomiarów