Comparison of the effectiveness of kick and sweep hand net and Surber net sampling techniques used for collecting aquatic macroinvertebrate samples

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Abstract: The objective of this work is to analyze the effectiveness of two widely used methods for collecting aquatic macroinvertebrate samples: the semiquantitative kick and sweep (K&S) and quantitative Surber net (SN) techniques. Based on our data, the methods were fully comparable as regards analysis of the macroinvertebrate metrics most often used in ecological status assessment (sensitivity/tolerance parameters), while K&S was found to be more successful in the evaluation of biodiversity. Thus, both methods could be used for routine monitoring of the status of water bodies, according to the recommendation of the EU Water Framework Directive, while for research, K&S is more advanced. K&S is also more effective timewise for material collecting. SN sampling is a quantitative method and could thus be used in studies of aquatic ecosystem productivity.

Key words: aquatic macroinvertebrates; kick and sweep technique; Surber net technique; sampling efficiency

INTRODUCTION

Aquatic macroinvertebrates are among the most frequently used biological quality elements in the assessment of the ecological status of water bodies [1,2] according to the requirements of the EU Water Framework Directive [3]. Selecting an appropriate sampling technique is a prerequisite for effective research and a reliable monitoring of the status of aquatic ecosystems. Sampling success significantly influences the overall results of a study, since it affects the number of species identified by the investigation, the proportion of different species groups per sample or target location [4–6], as well as proportions of indicator organisms. Thus, our capacity to detect species richness of a target water body or to discover some rare species depends not only on the sampling design, but also on the resulting indices which are used to assess water status and that significantly rely on the choice of effective sampling techniques. Limitations in the resources for monitoring and research (both financial and expert) have made the need for an effective methodology for collecting biological samples all the more important. The effectiveness of macroinvertebrate sampling and standardization of methodology has been extensively studied [7-12], but the issue remains open, especially in respect to some water types, such as large fluvial systems [13]. Sampling technique standardization is also important for studies on the relationship of biota and environmental factors, including analyses of the influence of single and multiple stressors on aquatic macroinvertebrates assemblages [14].

In order to contribute to the process of selection of appropriate sampling technique, we compared two widely used techniques of collecting macroinvertebrate samples in a wadeable hilly and mountainous stream: the semiquantitative kick and sweep technique (K&S) and the quantitative Surber net (SN) method [15].

MATERIALS AND METHODS

Sample collection

The material used in this study was collected in period 2005-2012 in different hilly and mountainous watercourses in Serbia. A total of 40 sites on 17 watercourses was sampled using two sampling techniques in parallel: the semiquantitative K&S technique using a standard hand net with mesh size of 500 μm, and a quantitative sampling using a SN with the same mesh size and 25x25 cm frame. The time needed for sample collection was measured using a stopwatch for 100 sampling occasions (50 for K&S and 50 for SN sampling). The K&S sampling technique was used in the shore region up to a 1.5-m water depth following the respective standard [16] and multihabitat procedure. The same sampling effort was made on each sampling occasion. About 100 m of the watercourse was taken into consideration for data collecting (visual assessment of dominant bottom substrate, evaluation of mean depth and width of the stream, assessment of shadow coverage, etc.) and sampling. Multihabitat sampling involves the assessment of available habitats within a sampling stretch and collection of material from at least 5% of accessible habitats [17].

Quantitative sampling with SN was done along the same sampling stretch as in the case of K&S. Each sampling occasion involved five subsamples, thereby providing a sample of 5 replicates with a surface area of 3.125 cm^2 (0.3125 m^2). Subsamples were collected from dominant substrate types in order to provide a representative sample for the stretch.

The visual classification of bottom substrate by particle size was performed using the following scale: 1) fine substrate (silt-clay and very fine sand; grains imperceptible by eye; <0.125 mm), 2) fine sand (grains perceptible by eye; 0.125-0.5 mm), 3) coarse sand (0.5-2 mm), 4) gravel (2-16 mm), 5) pebble (16-34 mm), 6) cobble (64-256 mm), and 7) boulder (>256 mm) [18].

Data analysis

The initial dataset comprised 400 samples, of which 230 were collected by the K&S technique and 170 by the SN method. To reduce any error that may be caused by analyzing data from different watercourse types, only samples collected from sites with a domination of coarse bottom type – classes 5-7 based on visual bottom substrate assessment, were included in the analyses. In such a way, the dataset covered the type group of hilly and mountainous small- to medium-sized streams with a domination of hard bottom substrate – types 3-5 according to Serbian typology of running waters. Thus, in the second step of analyses, 243 samples were included (133 collected by K&S and 110 by SN).

In the next step, out of 243 samples, 93 were selected (55 by K&S and 38 by SN) by the elimination of sites exposed to moderate to high anthropogenic pressure, and thus involved only the data from sites that were pre-assessed as possessing a good and better ecological status. This step was done to minimize the influence of stress factors on output results. Pre-assessment of ecological status (as identified in the EU Water Framework Directive [3]) was done based on previous studies [19], using the criteria described in Table 1.

For comparison of sampling techniques, the following biological metrics were used: 1) relative abundance parameters (total abundance of the community, abundance of principal macroinvertebrate taxa groups, all expressed as number of individuals per sample); 2) diversity parameters (total number of species, genera and families per sample, number

Table 1. Criteria for the indicative assessment of site quality (pre-assessed ecological status).

Reference or "near natural" site	No settlements or agricultural surfaces detected upstream of the site, or the influence is minor. Hydromorphological degradation is not detected in sampling stretch or upstream. Biological communities are not affected by human activities.	1 – high ecological status
Site under the insignificant influence	Only small settlements and extensive agriculture present upstream of the site. Hydromorphological degradation within sampling stretch or upstream is local. The biological communities are not adversely affected by human activities.	2 – good ecological status
Site under moderate influence and worse	The influence of human activities could be detected within the sample stretch or upstream; thus the influence on biological communities is evident.	3 – moderate ecological status and worse

Table 2. Tested metrics and results of MW-U-Test.

	U	Z	p-level	Z	p-level
Total Number of ind./sample	5433.50	**3.44967**	0.00056	3.44974	0.00056
Total Number of Taxa	5889.50	**2.61361**	**0.00896**	**2.61583**	**0.00890**
Saprobic Index (Zelinka & Marvan)	6936.00	0.694885	0.487128	0.694890	0.487125
% of xenosaprobic taxa	7031.50	0.519788	0.603211	0.526394	0.598615
% of oligosaprobic taxa	7109.50	0.376778	0.706339	0.376801	0.706322
% of beta-mesosaprobic taxa	7254.50	-0.110925	0.911676	-0.110925	0.911676
% of alpha-mesosaprobic taxa	7187.00	0.234684	0.814454	0.234685	0.814453
% of polysaprobic taxa	7045.50	-0.494120	0.621222	-0.541611	0.588087
BMWP Score	6379.50	1.71521	0.086308	1.71536	0.086280
Average score per Taxon	7137.00	0.32636	0.744154	0.32640	0.744125
Simpson Diversity Index	7163.50	0.27777	0.781189	0.27778	0.781184
Shannon Weaver Diversity Index	6679.50	1.16517	0.243951	1.16517	0.243950
Evenness index	6641.50	-1.23484	0.216890	-1.23487	0.216881
No. of sensitive taxa	6555.00	1.39344	0.163489	1.40414	0.160278
% of grazers and scrapers	6522.00	1.45394	0.145964	1.45396	0.145957
% of shredders	6109.00	**-2.21116**	**0.027025**	**-2.21921**	**0.026473**
% of gatherers and collectors	6490.50	1.51170	0.130612	1.51170	0.130612
% of filtrators	6292.50	1.87472	0.060832	1.87510	0.060779
No. of taxa Turbellaria	7295.00	0.03667	0.970749	0.05703	0.954524
No. of taxa Gastropoda	6817.00	-0.91307	0.361208	-1.07114	0.284107
No. of taxa Bivalvia	6935.00	0.69672	0.485980	1.46294	0.143485
No. of taxa Oligochaeta	7113.00	-0.37036	0.711114	-0.38473	0.700440
No. of taxa Hirudinea	7257.50	-0.10542	0.916039	-0.15631	0.875788
No. of taxa Crustacea	6247.50	1.95723	0.050322	**2.24106**	0.025023
No. of taxa Ephemeroptera	5275.50	**3.73936**	**0.000185**	**3.75931**	**0.000170**
No. of taxa Odonata	6609.50	1.29351	0.195835	1.89813	0.057680
No. of taxa Plecoptera	6663.50	-1.19450	0.232281	-1.26036	0.207541
No. of taxa Trichoptera	6910.00	-0.74255	0.457752	-0.75156	0.452315
No. of taxa Coleoptera	6485.00	1.52178	0.128065	1.64457	0.100060
No. of taxa Diptera	5686.00	**2.98672**	**0.002820**	**3.01517**	**0.002569**
No. of EPT taxa	6541.00	1.41910	0.155869	1.42129	0.155232
No. ind. – Turbellaria	7189.50	0.230100	0.818014	0.356524	0.721448
No. ind. – Gastropoda	6935.50	-0.695801	0.486554	-0.809359	0.418309
No. ind. – Bivalvia	6931.00	0.704052	0.481401	1.477452	0.139556
No. ind. – Oligochaeta	7297.50	0.032086	0.974404	0.032983	0.973689
No. ind. – Hirudinea	7257.50	-0.105424	0.916039	-0.155718	0.876256
No. ind. – Crustacea	6334.00	1.798633	0.072078	**2.016751**	**0.043722**
No. ind. – Ephemeroptera	5306.50	**3.682522**	**0.000231**	**3.689109**	**0.000225**
No. ind. – Odonata	6601.00	1.309097	0.190503	1.916056	0.055359
No. ind. – Plecoptera	7178.50	-0.250269	0.802380	-0.261547	0.793671
No. ind. – Trichoptera	7246.00	-0.126509	0.899329	-0.127121	0.898844
No. ind. – Coleoptera	6352.50	1.764714	0.077613	1.892118	0.058476
No. ind. – Diptera	5626.50	**3.095812**	**0.001963**	**3.098037**	**0.001948**
Number of Families	6077.50	**2.268918**	**0.023274**	2.272.694	0.023045
Number of Genera	6248.00	1.956311	0.050429	1.958927	0.050122

of species in principal macroinvertebrate taxa groups, number of Ephemeroptera, Plecoptera and Trichoptera taxa – EPT Index, Shannon Diversity Index [20]); 3) functional traits (percentage share of functional feeding groups – concept introduced by Cummins & Klug [21], and participation of taxa with defined saprobic preference); 4) number of sensitive taxa, as well as widely used indices, or tolerance/intolerance

Table 3. Number of species per macroinvertebrates taxa group.

Taxa group	No. of species
Turbellaria	7
Nematoda	1
Gastropoda	28
Bivalvia	17
Polychaeta	1
Oligochaeta	53
Hirudinea	10
Crustacea	18
Ephemeroptera	64
Odonata	17
Plecoptera	39
Heteroptera	10
Megaloptera	2
Trichoptera	92
Coleoptera	37
Diptera	82

Fig. 1. Box plots of the most important trait that reflects the differences between the effectiveness of the two sampling methods (1 – K&S sampling; 2 – SN sampling (five replicates)) widely used for collection of macroinvertebrate samples. Left side – number of individual metrics; right side – other diversity metrics that showed statistically significant difference (for p<0.05).
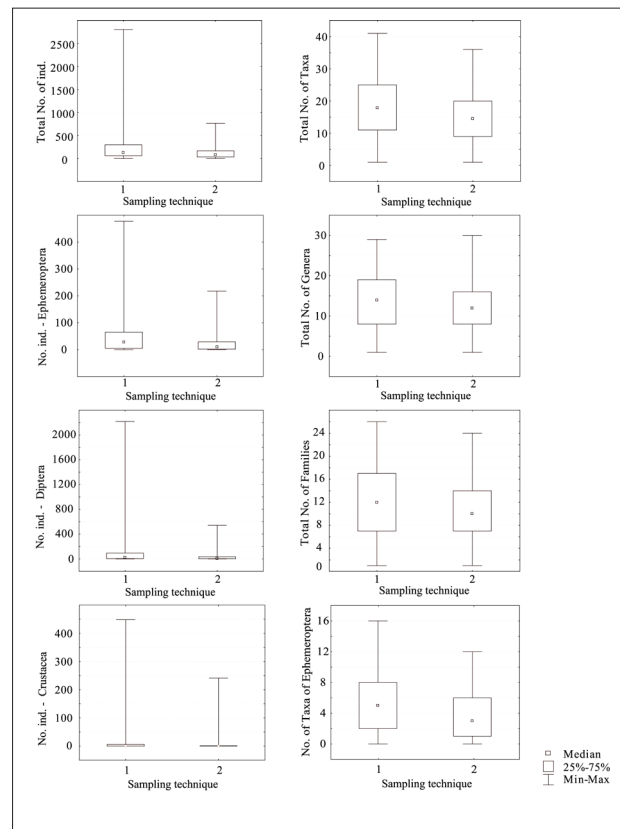
measures – saprobic index [22], biological monitoring working party (BMWP) score and average score per taxon (ASPT) [23]. The complete list of tested parameters is given in Table 2.

All mentioned parameters were calculated using the ASTERICS Software Version 4.0.4. For the assessment of statistical differences between results obtained by the two sampling techniques, the nonparametric Mann-Whitney U test (MW-U-Test) was used. FLORA Statistical software [24] was used for the data processing.

RESULTS

Of the material collected, 478 species of aquatic macroinvertebrates were identified in the investigated hilly and mountainous watercourses. Insects were the most diversified with 343 species belonging to 272 genera and 120 families. Trichoptera, Diptera and Ephemeroptera were found to be the principal components of macroinvertebrate communities with 92, 82 and 64 species, respectively. The number of species per macroinvertebrate taxa-groups is presented in Table 3.

Among identified species, organisms that indicate oligo- and beta-mesosaprobic conditions prevailed (35.39%), while alpha- and polysaprobic indicators were represented with 11.25%. For more than 50% of organisms, there were no data on saprobic prefer-

ence. In respect to feeding preference, scrapers/grazers, collector-gatherers and predators were almost equally represented in the communities, with 21.39, 23.27 and 23.26% of the total number of detected species, respectively.

All together 45 metrics out of numerous calculations provided by the ASTERICS Software Version 4.0.4 were used for comparison of effectiveness of the two sampling approaches. Based on the MW-U-test results (Table 2), the following metrics showed statistically significant difference (p<0.05) when the two sampling techniques were analyzed: total number of individuals, number of individuals of Crustacea, Ephemeroptera and Diptera, total number of taxa, number of Crustacea, Ephemeroptera and Diptera species, as well as number of families (Fig. 1). In addition, the share of shredders identified by the two

Table 4. Mean collection time for two tested sampling methods.

Method	Mean time needed for sampling	No. of measurements
K&S sampling	14±5	50
SN sampling (five replicates)	32±9	50

sampling techniques was significantly different. The other metrics, including the widely used tolerance/intolerance measures (saprobic index) [22], BMWP score and ASPT [23] did not show differences between the sets of samples. After the reduction of the dataset, when only samples collected from sites that have been pre-assessed as to having high or good status (93 samples; 55 collected by K&S and 38 by SN), we obtained similar results using the MW-U-test for comparison of the effectiveness, with the same set of metrics showing statistically significant difference, as well as number of individuals and number of Coleoptera taxa.

The results of time effectiveness are presented in Table 4. The time needed for the collection of data on the sampling sites (bottom substrate, stream width and depth, the level of hydromorphological degradation, etc.) was not taken into consideration, but only the sampling collection, reduction of sample volume (by elimination of coarse debris), sample packing and fixation. As can be seen from the measurements, SN sampling was much more time-consuming in comparison to the K&S technique.

DISCUSSION

The effectiveness of the K&S sampling method is very often underestimated. One of the major shortcomings of this approach is that it is often considered as qualitative [8], whereas the technique also allows for a semiquantitative approach (in defined time interval, or applying "the same sampling effort"), thus providing the data that are comparable along spatial and temporal gradients. Additionally, the sampling and processing of material collected by K&S are less time consuming in compare to other procedures, e.g. the Polyp grab [4], airlift sampling [25] or a detailed AQEM procedure [17]. Our data showed that K&S semiquantitative sampling in more effective in comparison to SN sampling as regards general taxa richness and taxa richness within the principal com-

ponents of the benthic communities in the type of watercourse covered by the study – small- to medium-sized streams with predominantly coarse bottom substrate. On the other hand, the metrics widely used for status assessment across Europe [1] belonging to the group of sensitivity/tolerance metrics, did not show significant differences in the resulting values based on the material collected by the two different sampling techniques. In that K&S was more effective in detecting the composition of the macroinvertebrate fauna, and that the tested sampling techniques were found to be of the same efficiency in respect to the mentioned metrics, indicates that both techniques are applicable in the routine monitoring of ecological status, but K&S is a better solution for investigative studies aimed at collecting information on taxa richness. Based on the data presented, the two methods are comparable in respect to sensitivity/tolerance metrics – e.g. saprobic index [22], BMWP and ASPT [23]. Similar results were obtained by comparing K&S with U-net sampling devices [26], where the methods were found to be similar in the values of benthic metrics and community composition. According to Brua et al. [26], U-shape net sampling provided slightly better data on diversity and thus the authors recommended this technique for biodiversity studies, despite the more time needed to complete sampling. It should be emphasized that K&S is much more efficient timewise than SN sampling, which is reflected in its economic effectiveness.

The advantage of the SN method is that it provides quantitative data, which is important in when dealing with the productivity of aquatic ecosystems, or if the aim of the research is to assess food availability for benthivorous fish, for example.

The selection of the most appropriate method to sample aquatic macroinvertebrates always depends on the particular goals, and there are several unanswered questions in this respect. Our study tried to answer a specific question regarding two widely used sampling methods for collecting appropriate faunistic information in small hilly water courses around Serbia.

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