ELSEVIER

Limnologica 34, 349–365 (2004)
http://www.elsevier.de/limno

LIMNOLOGICA

Assessing streams in Germany with benthic invertebrates: development of a practical standardised protocol for macroinvertebrate sampling and sorting

Peter Haase[1,*], Susanne Lohse[1], Steffen Pauls[1], Karin Schindehütte[1], Andrea Sundermann[1], Peter Rolauffs[2], Daniel Hering[2]

[1] Senckenberg Research Institute and Natural History Museum, Department of Limnology and Conservation, Biebergemünd, Germany
[2] University of Duisburg-Essen, Department of Hydrobiology, Essen, Germany

Received: March 26, 2004 · Accepted: September 13, 2004

Abstract

In the past, no single standardised method for sampling and sorting benthic macroinvertebrates has been implemented in Germany. Therefore, we tested the suitability of two common sorting protocols, RIVPACS and AQEM/STAR, by taking samples with each protocol at 44 sampling sites. Our results reveal that different methods deliver slightly different assessment results. Moreover these two methods differ in costs. Although the AQEM/STAR protocol takes longer than the RIVPACS protocol, we favoured the AQEM/STAR protocol because of its higher level of standardisation. In order to limit costs to an acceptable level, a modification of the AQEM/STAR protocol (MAS method) is developed. This method is highly standardised, gives stable assessment results and is relatively inexpensive (€ 224.00 for processing of an average sample). A detailed protocol of the newly developed method is given.

Key words: Stream assessment – methods – macroinvertebrate sampling – benthos – multihabitat sampling

Introduction

The assessment of running watercourses in accordance with the EU Water Framework Directive (EU-WFD) is based much more strongly on biological criteria than in previously implemented systems (e.g. LAWA 2001). Besides fishes and aquatic flora, benthic macroinvertebrates have become an integral component. Concerning benthic macroinvertebrates, previous experience has shown that data precision and quality often are closely linked to the methodology applied and expertise of the person sampling (e.g. CARTER & RESH 2001; HERING et al. 2001; ROSENBERG & RESH 1993). There are manifold methods for taking a sample from running waters (e.g. area-based or time-based methods) (BARBOUR et al. 1999; BIRK & HERING 2002; ENVIRONMENT AGENCY 1997; ISO 7828; ROSENBERG & RESH 1993). The advantages and drawbacks of various sampling methods have been tested in a number of studies (CLARKE 2000; CLARKE et al. 2002; CAO et al. 2003) and are relatively well-known. The determination of the organisms collected within a sample is highly dependent on specialist knowledge: a taxonomic group for which a person is a specialist may be more reliably determined in more tax-

***Corresponding author:** Peter Haase, Senckenberg Research Institute and Natural History Museum, Department of Limnology and Conservation, D-63599 Biebergemünd, Germany; e-mail: Peter.Haase@senckenberg.de

onomic detail than groups with which the analyst is not as familiar.

All in all, different sampling and analysis methods can lead to very different taxa lists at a single site. For biological stream assessment, heterogeneous taxa lists are of little use, as they may not only reveal differences in ecological quality, and are prone to variability in data acquisition. To implement the EU-WFD, it is of utmost importance to attain and use comparable and standardised data sets, since the newly developed assessment tools are designed and calibrated to detect differences in ecological quality and cannot compensate for differences brought forth by different methods. Thus, several European countries already have highly standardised sampling and sample treatment procedures (Nordic countries: FRIBERG & JOHNSON 1995; UK: ENVIRONMENT AGENCY 1997).

In order to obtain comparable macroinvertebrate data for stream assessment it is necessary to ...

- standardise the sampling procedure,
- standardise the proceeding sample treatment (subsampling, sorting),
- and standardise the sample analysis (determination of taxa, data analyses, interpretation of results) (not subject matter of this article).

With such highly standardised procedures, data also become verifiable. Thus a vital and to date very variable aspect in stream assessment becomes more transparent, reproducible and can be subjected to quality control measures. Since stream rehabilitation measures will be based on the assessment results, questions concerning quality control will become increasingly important during the implementation process of the EU-WFD.

From this vantage point we developed a standardised method for sampling and treatment of macroinvertebrate samples from streams, which meets the needs of water managers in Germany. We did this based on a comparative study where method precision was considered the primary factor. Other aspects such as required time and related costs were also taken into consideration.

In this study we compared the relatively new AQEM/STAR protocol (STAR CONSORTIUM 2003), a highly standardised, area based multi-habitat sampling method and the RIVPACS protocol (WRIGHT et al. 1984; ENVIRONMENT AGENCY 1997), a standardised, time-related sampling approach, which is widely used in Great Britain. The main questions to be addressed were how these methods compared concerning the assessment results and whether the assessment methods are robust enough to cope with different sampling methods.

Based on the comparison of the two established methods, we developed and tested a modified protocol of the AQEM/STAR method. The robustness of this **M**odified **A**QEM/**S**TAR method (MAS method) is tested.

Material and Methods

Protocols compared

Short summaries of the protocols compared in this study are given in the following. For full protocols of these methods, please see the citations given.

- **RIVPACS** (WRIGHT et al. 1984; STAR CONSORTIUM 2003)

The RIVPACS protocol was developed and widely used in Great Britain. In the meantime it has also been adapted for use in several other countries (Australia: AusRivAS, SMITH et al. 1999; Canada: BAILEY et al. 1998, REYNOLDSON et al. 2001; New Zealand: JOY & DEATH 2003). The protocol followed for this comparison is given in STAR CONSORTIUM (2003). The protocol is based on sampling in a stream reach. Each invertebrate habitat at the site is sampled with an effort proportional to its cover at the sampling site. The sample is taken in three steps: 1st a manual search is done for collecting organisms on the water surface; 2nd a three minute pond-net sample is taken by means of kick-sampling and sweeping; 3rd animals on submerged rocks, logs or plants are collected manually. For steps 1 and 3 a total time effort of one minute sampling time should be spent. We thus have a sample based on 4 minutes sampling time. The sorting procedure is laboratory based and incorporates a subsampling procedure. When sorting a sample, the operator selects a fraction of the sample (1/2, 1/4, ...), which is sorted completely without magnification into a "fraction vial". From the unsorted rest of the sample individuals of taxa, which have not been picked in the sorted fraction, are picked and put into a separate "extras vial". All individuals found in either a "fraction vial" or the "extras vial" are determined. The result is a taxa list giving the number of individuals extrapolated to whole sample.

- **AQEM/STAR** (STAR CONSORTIUM 2003)

The AQEM/STAR method was developed in the course of two successive EU-funded projects, the aim of both being to develop and standardise macroinvertebrate assessment systems (HERING et al. 2004b). Sampling is based on distributing 20 sampling units in habitats proportional to their cover at the sampling site. Habitat cover is estimated in 5% steps and only those habitats which cover more than 5% area at the sampling site are sampled. Each sampling unit (25 × 25 cm) is sampled by means of kick sampling and manual searching using a hand net (25 × 25 cm frame; 500 μm mesh).

The AQEM/STAR sorting protocol is done completely in the laboratory and requires that a defined subsample is taken prior to sorting. The subsample corresponds to 1/6 of the sample and at least 700 individuals sorted. If 1/6 of the sample contains < 700 individuals, the sub-

sample is increased until ≥700 organisms are sorted. All individuals of the subsample are picked and counted without magnification and then determined. The result is a taxa list giving the number of individuals extrapolated to the whole sample.

- **Modified AQEM/STAR protocol (MAS)**

The AQEM/STAR protocol is highly standardised but also extremely time consuming. Over 80% of the time required to complete an AQEM/STAR protocol lies in the follow-up sample treatment, especially in the sorting and determination times (c.f. Table 1). Therefore we focused the modification of the AQEM/STAR protocol on these time consuming activities, and drew up the following hypothesis:

"The validity of the assessment system increases with rising taxonomic level, at best the species level, because this level bears the most detailed and differentiated information. Thus omitting those individuals which are usually only determinable at the family or genus level at best – this is often the case for juvenile stages – should only have minor influence on the assessment result."

Based on this hypothesis we modified the original AQEM/STAR protocol in the following manner: after subsampling, an AQEM/STAR sample was separated into a coarse (> 2 mm) and a fine (≤2 mm) fraction by pouring it through cascade of sieves. The underlying assumption is that most of the juvenile individuals that are only determinable at lower taxonomic levels are found in the fine fraction, while most of the older, larger stages remain in the coarse fraction. Thus most of the species information should remain in the coarse fraction, and losing the fine fraction should not greatly influence the assessment result.

Comparative data

- **RIVPACS, AQEM/STAR**

Parallel samples with the AQEM/STAR protocol and the RIVPACS protocol were taken from 44 sampling sites from three different stream types. The stream types studied were type 5 ("Small siliceous cobble-bottom streams") and type 5.1 ("Small siliceous sandstone streams") in lower mountainous areas of Central Europe and type 15 ("Mid-sized to large sand-bottom streams") in the Central Lowlands (stream type numbers according to POTTGIESSER & SOMMERHÄUSER 2004). At these 44 sampling sites, samples were taken with both protocols, giving us a total data set of 88 samples. These data were collected within the EU funded project STAR[1]. For each stream type, the same person collected the samples from both protocols.

- **MAS, AQEM/STAR**

To test the above stated hypothesis and the robustness of assessment results of our MAS approach, we separated 70 AQEM/STAR protocol subsamples into the coarse and fine fraction before sorting and determination.

The 70 samples for the comparison covered six different stream types from both lowland (two types) and highland (four types) regions in Germany. The size spectrum covered both small streams and mid-sized rivers for both lowland and highland regions. These samples were collected within a LAWA funded project[2].

For all data sets a multimetric index to evaluate ecological stream quality (MMI, BÖHMER et al. 2004) was calculated using the stream type relevant core metrics (HERING et al. 2004a). The taxa lists resulting from all protocols were also compared regarding number of taxa and number of individuals determined.

Differences between core metrics and MMI values for two protocols were tested for significance using Wilcoxon-Test. In order to determine whether there are any significant differences between more than two protocols the Friedman ANOVA by ranks was used. Both tests compare variables that were measured in dependent samples. In the case of getting a significant result from the Friedmann ANOVA the Wilcoxon-Test was calculated in order to show which of the protocols differ from each other. To do this, the significance level had to be adjusted according to ENGEL (1997). The Spearman R was calculated in order to find out whether two variables were correlated. All statistical analyses were done with the STATISTICA 6.1 software package (STATSOFT 2002).

Time sheets were kept for the sampling and laboratory treatment. Time was recorded separately for subsampling, sorting and determination of the actually picked individuals. Based on these time sheets, the personnel costs for each sorting method were calculated, taking into consideration that different tasks should be performed by differently qualified persons. Three categories of personnel were differentiated: student assistants for simple tasks (€ 15/hour); technical staff for tasks requiring training (€ 30/hour); scientists for tasks requiring specialist skills (€ 50/hour).

To compare time effort between the RIVPACS and AQEM/STAR protocols, we had to use a smaller data set (N = 16), because the sampling, subsampling, sorting and determination times were only recorded for 16 samples. To compare the MAS protocol to AQEM/STAR, time effort was recorded for all 70 samples.

[1] Standardisation of River Classifications: Framework method for calibrating different biological survey results against ecological quality classifications to be developed for the Water Framework Directive. Contract No: EVK1-CT 2001-00089.

[2] Länderarbeitsgemeinschaft Wasser (LAWA) Contract Nr. O 4.02.

Results

RIVPACS, AQEM/STAR

• **Assessment results**

The sampling done in the STAR project aimed at covering sites from all stages of stream degradation, ranging from high to bad ecological quality. Thus, both protocols show a wide range of assessment results. In small siliceous cobble-bottom streams, small siliceous sandstone streams and in mid-sized to large sand-bottom streams these ranged from high to poor, high to bad and from good to bad respectively (Fig. 2).

The mean difference in MMI values, which can range from 0 to 1, between the two methods at all 44 sampling sites is 0.01 ± 0.08. Deviation between methods ranges from –0.2 to +0.16. In 19 of 44 cases (43%), the differences in MMI results between the AQEM/STAR protocol and the RIVPACS protocol lead to different classifications of streams by one ecological quality class (Fig. 2). There is no discernible tendency (Wilcoxon p = 0.48) in either method to favour lower or higher MMI values compared to the other method. The correlation between MMI values for the two protocols is high (R = 0.92, p < 0.01) (Fig. 1).

The differences in single core metrics for each stream type are shown in Fig. 3.

• **Time and effort for each protocol**

On average an AQEM/STAR protocol subsample consisted of a quarter of the whole sample (Table 1). An AQEM/STAR protocol subsample requires an average of 12.6 hours to complete. It returns an average of 912 individuals and recovers a mean of 52 taxa. Projected on the whole sample this leads to an average of 3968 individuals collected in the field (Table 2). An average RIVPACS protocol sample is completed in approximately half the time (6.5 hours), while recovering 45 taxa from 512 sorted individuals. Projected on the whole sample this gives an average of 2843 individuals per RIVPACS protocol sample (Table 2).

The RIVPACS protocol is thus much faster than the AQEM/STAR protocol. It recovers 72% of the individuals and 88% of the taxa found using the AQEM/STAR protocol.

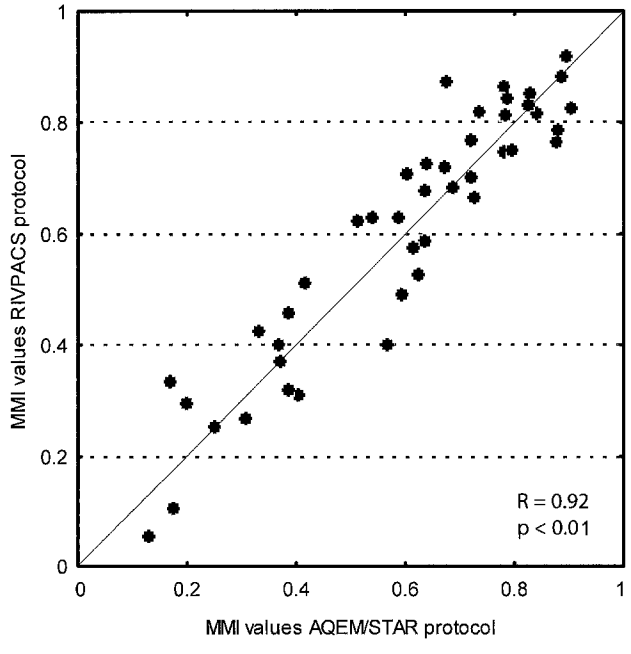

Fig. 1. Correlation results between the MMI values obtained from the AQEM/STAR protocol and RIVPACS protocol.

MAS, AQEM/STAR

• **Assessment results**

The mean difference in MMI values between the AQEM/STAR protocol and MAS is –0.01 ± 0.04 (Fig. 4). The correlation between MMI values for the two protocols is very high (R = 0.97, p < 0.01) (Fig. 5). Compared with the result from the AQEM/STAR protocol, MAS achieves the same ecological quality class in 61 of 70 cases (87%). The mean difference in MMI values for the AQEM/STAR fine fraction to the AQEM/STAR protocol is 0.08 ± 0.07 (Fig. 4). Here the correlation between MMI values for the two protocols is somewhat lower (R = 0.90, p < 0.01) (Fig. 5). For the AQEM/STAR fine fraction the same ecological quality class is obtained in 51 of 70 cases (73%). The calculation of the Friedman ANOVA by ranks determined significant differences between the three protocols (Friedman ANOVA p < 0.01). All protocols differ significantly from each other (Wilcoxon-Test: all comparisons p < 0.01). The results remain significant after correcting the

Table 1. Average time required to complete a sample following the AQEM/STAR protocol or RIVPACS protocol.

	AQEM/STAR protocol [h]*	RIVPACS protocol [h]
Sampling (in the field):		
sampling	0.75 (*N* = 123)	0.5 (*N* = 16)
Sample treatment (in the lab):		
subsampling	0.5 (*N* = 123)	–**
sorting	5.7 (*N* = 123)	3.4 (*N* = 16)**
taxa determination	4.9 (*N* = 70)	2.1 (*N* = 16)
data entry	0.75 (*N* = 70)	0.5 (*N* = 16)
Total time	**12.6**	**6.5**

*Additional data used; ** Subsampling occurs while sorting, thus the time can only be given for the combined step.

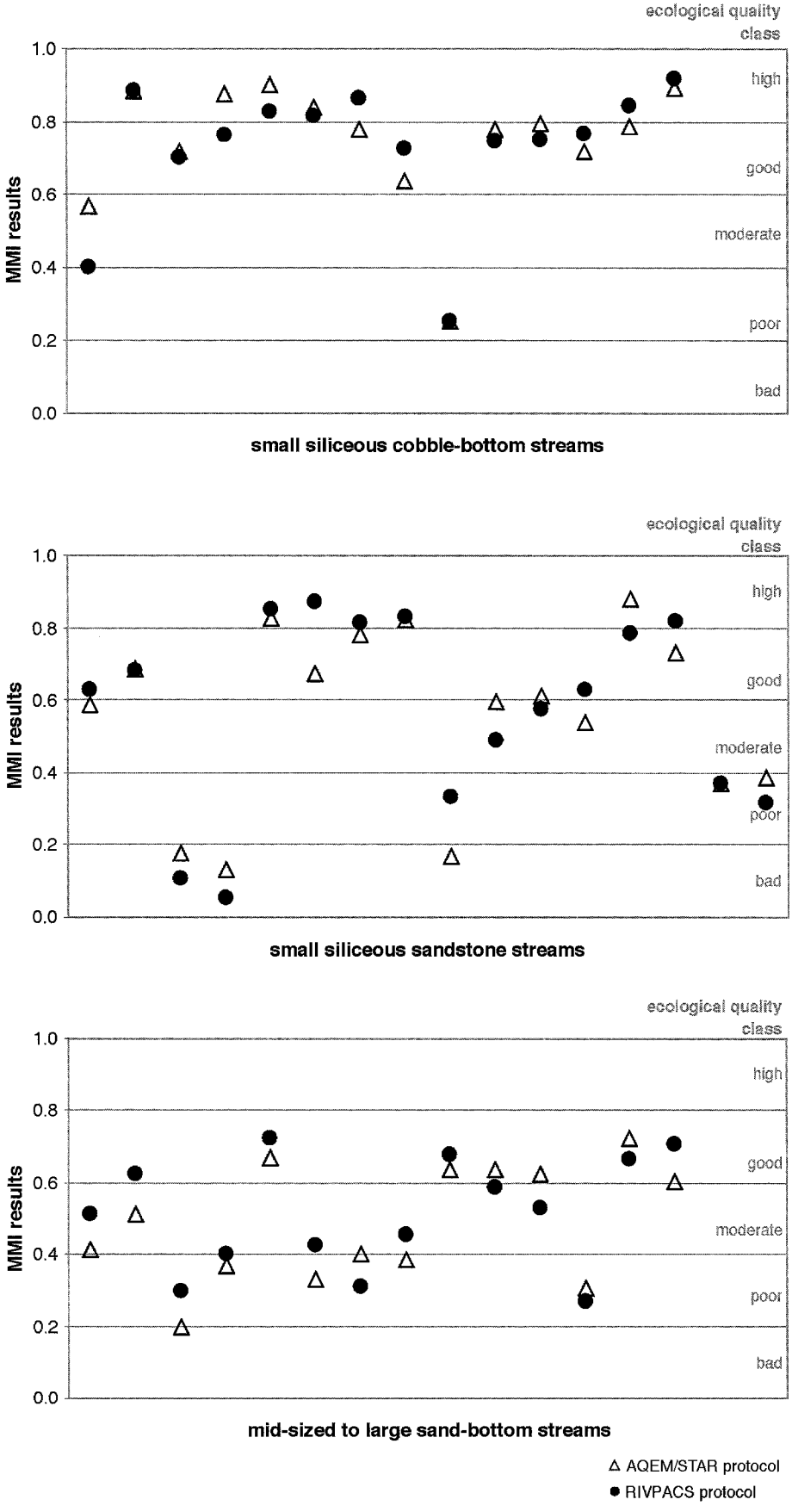

Fig. 2. Comparison of ecological quality, given as MMI results, between the AQEM/STAR protocol and the RIVPACS protocol. Shown are the results from small siliceous cobble-bottom streams in lower-mountainous areas (top), small siliceous sandstone streams (middle) and mid-sized sand-bottom streams in the lowlands (bottom). The resulting ecological quality class based on the MMI is inserted on the right.

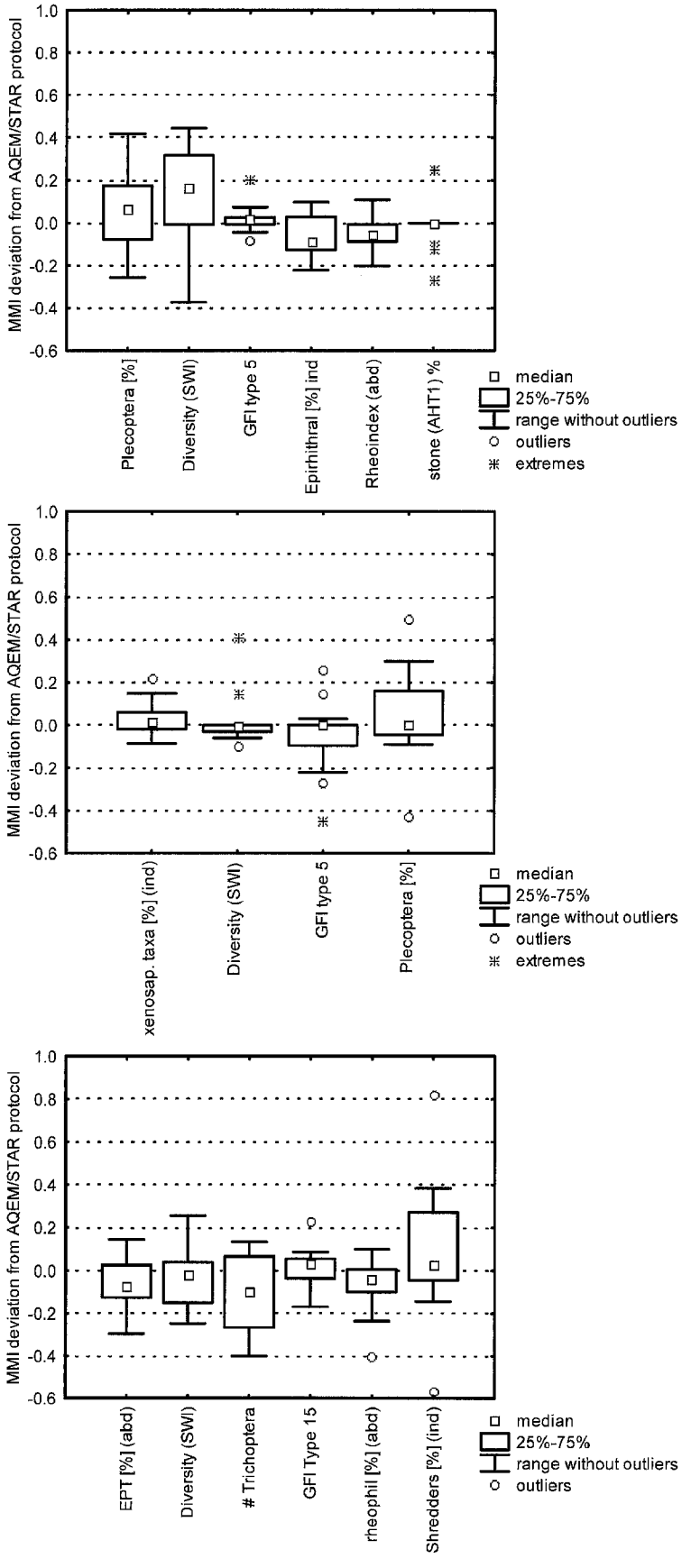

Fig. 3. Mean deviation of the core metric results from the RIVPACS protocol to the AQEM/STAR results. The results are shown separately for each stream type. The stream types shown are top: type 5 ("small siliceous cobble-bottom streams") (top) and type 5.1 ("small siliceous sandstone streams") (middle) in lower mountainous areas of Central Europe and type 15 ("mid-sized to large sand-bottom streams") in the Central Lowlands (bottom).
Description of "Core Metrics": Epirhithral [%] ind = % individuals with preference Epirhithral (upper trout region); EPT [%] (abd) = % individuals Ephemeroptera, Plecoptera, Trichoptera based on abundance classes; Plecoptera [%] = % Plecoptera individuals; rheophil [%] (abd) = % rheophile individuals based on abundance class; GFI type 5 = German Fauna Index type 5; GFI type 15 = German Fauna Index type 15; # Trichoptera = number of Trichoptera taxa; Shredders [%] (ind) = % shredders (individuals); Rheoindex (abd) = Rheoindex according to Banning (individuals) (BANNING 1998); stone (AHT1) % = stone dwelling taxa "AHT1" [%]; Diversity (SWI) = Diversity (Shannon-Wiener-Index); xenosap. Taxa [%] (ind) = proportion of xenosaprobic taxa [%] (individuals).

level of significance according to ENGEL (1997).

- **Time and effort for each protocol**

Sorting and determination effort and resulting number of taxa and individuals for AQEM/STAR, the AQEM/STAR fine and MAS are summarised in Table 3. The data in Table 3 show that ...

- Sorting and determining either the fine or the coarse fraction reduces the time required to sort the AQEM/STAR protocol by a half.
- The time required to sort and determine the MAS is somewhat lower than that required for the fine fraction. This corresponds to the slightly lower number of individuals in the coarse fraction.
- On average the MAS recovers 81% of the taxa from the AQEM/STAR protocol, the AQEM/STAR fine fraction recovers 77%.

Table 4 gives an overview of the total time effort required to sample, treat and analyse a subsample following the AQEM/STAR protocol, using only the AQEM/STAR fine fraction or the AQEM/STAR coarse fraction.

Table 5 gives a financial and time effort comparison of the RIVPACS, AQEM/STAR and MAS protocols. Further costs, like travel

Table 2. Average number of individuals and taxa from AQEM/STAR and RIVPACS samples.

$N = 44$ for each protocol	AQEM/STAR-protocol (individuals picked from subsample)	AQEM/STAR-protocol (projected on whole sample*)	RIVPACS-protocol (individuals picked while sorting)	RIVPACS-protocol (projected on whole sample**)
Number of individuals	912	3968	512	2843
Number of taxa	51	–	45	–

* whole AQEM/STAR protocol sample covers 1.25 m² area; ** whole RIVPACS protocol sample refers to 3 minutes kick sampling plus 1 minute free collecting time.

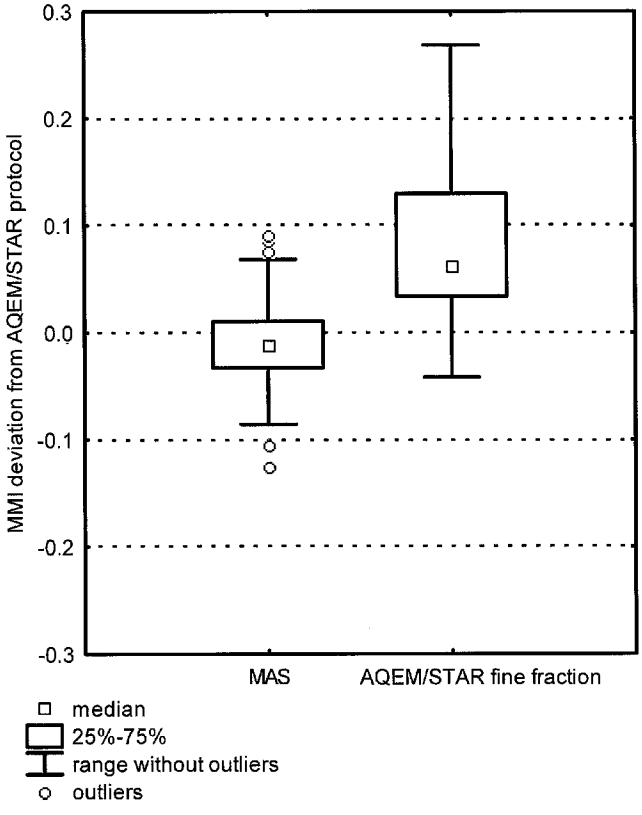

Fig. 4. Box-Whisker plots showing the deviation of MMI results obtained by either MAS or AQEM/STAR fine fraction from the AQEM/STAR protocol results. Outliers are defined by lying 1.5 times outside the 25th and 75th percentile range.

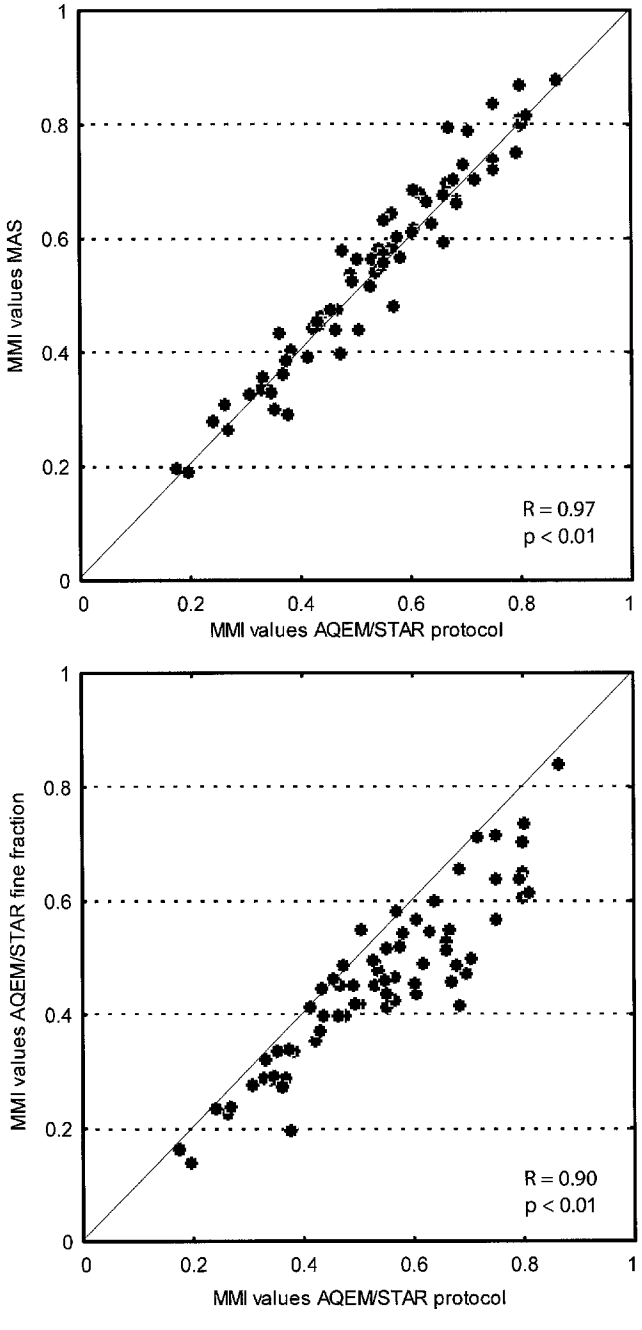

Fig. 5. Correlation results between the MMI values obtained from the AQEM/STAR protocol and either the MAS (above) and AQEM/STAR fine fraction (below).

Table 3. Sorting effort and results for three compared fractions AQEM/STAR, AQEM/STAR fine fraction and AQEM/STAR coarse fraction.

	AQEM/STAR fine fraction (≤2 mm only)	AQEM/STAR coarse fraction (>2 mm)	AQEM/STAR (fine & coarse fractions)**
Sorting [h] ($N = 123$)	3.1	2.6	5.7
Taxa determination [h] ($N = 70$)	2.4	2.5	4.9
Number of individuals ($N = 70$)*	597	469	1066
Number of taxa ($N = 70$)	40	42	52
Relative number of taxa [%] ($N = 70$)	77	81	100

* Actually picked from the subsample fraction (not projected on whole sample); ** Results calculated from the values from the AQEM/STAR fine fraction and the AQEM/STAR coarse fraction.

Table 4. Average time required to complete a sample following three protocols AQEM/STAR, AQEM/STAR fine fraction and the AQEM/STAR coarse fraction.

	AQEM/STAR fine fraction (≤2 mm only)	AQEM/STAR coarse fraction (>2 mm)	AQEM/STAR (fine & coarse fractions)**
Sampling (in the field)			
sampling [h] ($N = 123$)	0.75*	0.75*	0.75*
Sample treatment (in the lab)			
subsampling [h] ($N = 123$)	0.5*	0.5*	0.5*
sorting [h] ($N = 123$)	3.1	2.6	5.7
taxa determination [h] ($N = 70$)	2.4	2.5	4.9
data entry [h] ($N = 70$)	0.5	0.5	0.75
Total time [h]	**7.25**	**6.85**	**12.6**

* Values for sampling and subsampling are identical, because they occur before separation into coarse and fine fractions; ** Results of an AQEM/STAR protocol subsample, calculated from the values from the AQEM/STAR fine fraction and the AQEM/STAR coarse fraction.

to sampling site, sampling materials, taxes, are the same for all protocols and were thus omitted from the table as they are irrelevant for a direct comparison of the protocols. The cost for a sample following the AQEM/STAR protocol comes to € 398.00. A RIVPACS protocol comes to € 247.00, a sample with the MAS protocol is slightly less expensive and costs € 224.00.

Discussion

In terms of individuals sorted and determined the AQEM/STAR protocol delivers almost double the number obtained using the RIVPACS sample. In terms of taxa number these differences are smaller, although AQEM/STAR delivers more taxa than RIVPACS. Comparing the assessment results obtained with the two methods shows that the two protocols do not differ significantly and show slight differences in mean deviation. On the other hand, the standard deviation is 0.08 and the spread of deviation is relatively high (0.36). The same is true for single metrics (cf. Fig. 3). Based on this, the differences in classifications shown in our data (43% different classifications) probably are overestimated, and fewer differences would be observed for a larger data set. Nonetheless, our data support the notion, that although differences may be small, different sampling schemes can lead to different assessments (c.f. Carter & Resh 2001).

The AQEM/STAR method required much more time and money than the RIVPACS protocol, while concerning assessment results, both methods seem applicable for implementation processes in regard to the EU-WFD. The AQEM/STAR protocol seems too expensive and time consuming to be readily applicable in water management practice.

For reasons we will discuss below, we think that the high level of standardisation of the AQEM/STAR

method validates a modification aimed at keeping this level of standardisation, while cutting costs dramatically. The modification we tested, which we name MAS, divides the subsample used in the AQEM/STAR protocol into a coarse and a fine fraction and limits further treatment to the coarse fraction. With this we nearly halve the time required to finish a sample (Table 3). Furthermore, MAS recovers an average of 81% of the taxa from the complete subsample. Concerning assessment results, MAS differs only slightly from AQEM/STAR in (1) scoring of ecological quality classes, (2) mean difference of MMI scores (mean deviation = –0.01) and (3) spreading of MMI scores, which is also limited (standard deviation ± 0.04). This slight difference is significant. This means that MAS minimally, but systematically underestimated MMI values in comparison to AQEM/STAR. This slight systematic error can easily be accounted for by minor adjustments of class boundaries.

These results are supported by a more detailed analysis of omitting the fine fraction (HAASE et al. 2004). This implies that the individuals and taxa, which are lost by omitting the fine fraction are of subordinate importance for the assessment.

We have shown that RIVPACS and AQEM/STAR applied by the same operator led to comparable assessment results at stream sites. While AQEM/STAR is much more time consuming and costly, our suggested modification of this protocol (MAS) delivers the same robust assessment results. These results support our hypothesis, that omitting the fine fraction has little detrimental effect on the assessment result. We thus suggest that the MAS presents a practical and stable alternative to the AQEM/STAR protocol. We now discuss advantages and disadvantages of the two protocols, MAS and RIVPACS.

The RIVPACS and the MAS methods are comparable in time effort, costs and also deliver comparable results concerning number of individuals and taxa. There are, however, important differences in sampling and sorting procedures between the two systems, which we discuss separately below.

Sampling

A major requirement of a sampling method is its reproducibility. Accurate results should be obtained in both quantitative and qualitative aspects. For qualitative aspects, the relative consideration of different microhabitats according to their presence at a sampling site is important. A stable sample volume is important from a quantitative point of view.

The MAS method fulfils both of these requirements:

- The multi-habitat sampling approach with 20 sampling units standardises the sampling of different microhabitats at each sampling site (qualitative aspects).
- Each sampling unit is a defined area (25 × 25 cm), which standardises the sample volume (quantitative aspects).

The comparability of results from multi-habitat macroinvertebrate samples is highly dependent on the method used to define the cover of habitats and how, when and whether or not they should be sampled. This decision is prone to operator bias in almost any approach, and is difficult to standardise (CARTER & RESH 2001). Both the RIVPACS and the MAS protocol are subject to these difficulties. The RIVPACS sampling method does not define where exactly (at which habitats) the samples should be taken, which is also true for other methods (e.g. BRAUKMANN 2000). In the MAS protocol we consider the 5% classes and the proportional distribution of sampling units at a site to be a practical and clearly outlined approach to habitat estimation. This approach is transparent and leads to comparable sampling unit distribution within and among sites.

Time-limited sampling, as in RIVPACS, Quality Rating System (MCGARRIGLE et al. 1992) or PERLA (KOKES 2001) may be more prone to volume biases, than an area-based approach depending on substrate (c.f. STAR CONSORTIUM 2003).

Table 5. Average time and costs for RIVPACS, MAS and AQEM/STAR protocols.

	RIVPACS			MAS			AQEM/STAR		
	Time [h]	Person*	Costs [€]	Time [h]	Person*	Costs [€]	Time [h]	Person*	Costs [€]
Sampling	0.5	Sci	25	0.75	Sci	37.50	0.75	Sci	37.50
Subsampling	–	–	–	0.5	Stu	7.50	0.5	Stu	7.50
Sorting	3.4	Tech	102	2.6	Stu	39	5.7	Stu	85.50
Determination	2.1	Sci	105	2.5	Sci	125	4.9	Sci	245
Data entry	0.5	Tech	15	0.5	Tech	15	0.75	Tech	22.5
Total time	**6.5**		**247**	**6.85**		**224**	**12.6**		**398**

* Stu (Student assistant) = 15 €/h; Tech (technical staff) = 30 €/h; Sci (Scientist) = 50 €/h.

Sorting

Looking at the sorting protocols of both methods we can clearly recognise the following differences:

- When following the RIVPACS protocol, the person sorting must decide which fraction of the sample should be sorted (1/4, 1/2 ...). This brings a fair amount of variability into the procedure. After sorting the chosen subsample fraction, the worker must scan the rest of the material in the sorting tray and identify taxa that have not yet been picked. Thus the person sorting must have sufficient taxonomic knowledge and experience to be able to identify and sort taxa that have not yet been found in the fraction sampled. For this type of sorting, skilled technical staff are required (CLARKE 2000). The MAS sorting on the other hand can also be done by student assistance or other less-skilled workers (Table 5) and can nevertheless be subject to quality control.
- It is thus also necessary to train the "RIVPACS"-personnel more proficiently, which in turn bears higher costs.
- Within the RIVPACS sorting method, differences in taxonomic expertise of the person sorting can lead to different sorting results (CLARKE 2000).

Conclusions

We have shown that there are only minimal differences in assessment results between AQEM/STAR, MAS and RIVPACS. However, the AQEM/STAR approach is both very costly and time consuming. RIVPACS is a little less time-consuming than MAS, on the other hand, the MAS protocol costs slightly less. Based on the points of protocol variability we discussed here, we feel that MAS may be less affected by operator bias than RIVPACS sampling and sorting. In our opinion, the MAS method offers a highly standardised and practical sampling and sorting protocol for macroinvertebrate investigations in running water as required by the EU-WFD. A detailed description of all aspects of the method (sampling, subsampling, sorting etc.) is given in the following section of this paper.

The Modified AQEM/STAR (MAS) sampling and sorting protocol

The MAS method is based on sampling microhabitats according to their representation at the sampling site (multi-habitat-sampling). First, all microhabitats are recorded in 5% intervals. Every 5%-microhabitat is sampled as a "sampling unit" over an area of 25 × 25 cm. A complete sample comprises 20 "sampling units" (1.25 m^2 total sample area), which are pooled for further treatment and analysis. Each "sampling unit" is basically sampled using a kick sampling method (BARBOUR et al. 1999).

Using a careful washing method, the total sample is separated into an organic and a mineral fraction. The mineral substrate is discarded in the field. From the organic fraction a subsample is taken in the lab, which is then divided into a coarse fraction (>2 mm) and a fine fraction (≤2 mm). The coarse fraction consists of at least 1/6 of the whole sample and 350+ individuals. It is sorted by removing all organisms. The organisms are separated according to their order. In Germany, samples are then determined according to the "Operational Taxalist for Running Water in Germany" (HAASE & SUNDERMANN 2004).

The MAS sampling method is applicable in both wadable and non-wadable streams and rivers. Necessary minor adjustments of the method in non-wadable situations are described at the end of the protocol in section C.3.

A. Selecting the sampling period

In Germany, the sampling season depends on catchment size of the water body, based on a general agreement within water authorities on the best-suited sampling season. Thereby most suitable sampling time for larger streams and rivers (catchment area >100 km^2) is early summer (June, or if need be July), for smaller water courses (catchment area <100 km^2) March or April. In exceptions samples from small streams can already be taken in mid-February. In any case a sample should never be taken during or directly after a flood or a no-flow dry period. Ideally a sample should be taken during a time when stream flow is somewhat lower than the annual mean.

B. Selecting a sampling site

The sampling site should be representative of a longer reach of the watercourse. The reach should be more or less homogeneous over a stretch of several hundred metres. If riffles and pools alternate in this reach, both should be represented in the sample. The length of the sampling site should extend between 20 and 50 m for small streams (<100 km^2 catchment area) and 50–100 m for mid-sized and large rivers (100–10,000 km^2 catchment area)

C. Taking a sample

• C.1. Necessary equipment

To take a sample one requires a hand net with a long pole. The net should be attached to a square metal frame

(25 × 25 cm). The 70 cm deep bag or cone shaped net is made of a standard 500 μm mesh size screen. For the further sampling steps and sample treatment more accessories are required. Thus, in the field you will need the following: hand net; site protocol (Fig. 6); two or three 10-litre pails; two large sorting trays (white, approx. 30 × 50 cm); several sample containers (approx. 1 to 2 l; preferably with wide opening); a small sieve (500 μm); a funnel for filling the sample containers; two or three small sorting containers for pre-sorted individuals; preprinted labels for all sample containers; tweezers; a soft brush for scrubbing organisms from rocks or coarse woody debris; long gloves; about 2 l Ethanol (96%) per sample; cooler with ice packs; hand disinfectant (if necessary); life jacket (if necessary); safety line (if necessary).

• C.2. Estimating microhabitat composition and distributing sampling units

The first step in taking a sample requires an estimation of microhabitat distribution. Microhabitat composition and distribution are estimated from the bank wherever possible in order not to disturb the substrates. Based on the microhabitat list given in the site protocol the coverage of all microhabitats with at least 5% cover is recorded to the nearest 5% interval in the column *"Coverage (5% intervals)"* in the site protocol. In the site protocol example (Fig. 6), these are 55% mesolithal, 25% psammal, 15% CPOM, 5% akal. The sum of microhabitat coverage must add up to 100%.

If mineral substrates are covered by organic substrates (e.g. CPOM or macrophytes), the organic cover is recorded. If a microhabitat is present but covers less than 5% area of the sampling site, it is marked on the site protocol with an "X" in the column *"Coverage (5% intervals)"*. The column *"Comments"* in the site protocol should be used to note particularities of a sampling unit, like a very high amount of organic material in sandy substrates or a high amount of sand in mesolithal areas. If the substrate is very heterogeneous (e.g. a mix of mesolithal, akal and psammal) then the dominant substrate should be recorded.

Based on the estimation of coverage (site protocol) the number of sampling units to be taken from each microhabitat is determined. For every 5% cover of a microhabitat one sampling unit is taken, thus a total of 20 sampling units are collected. If, for example, the substrate estimation was 55% mesolithal, 25% psammal, 15% CPOM, and 5% akal (compare Figs. 6 and 7), the sampling units would be distributed as follows: 11× mesolithal, 5× psammal, 3× CPOM, and 1× akal. The number of sampling units is recorded in the column "number of sampling units" on the site protocol.

When distributing sampling units dependent on substrate and microhabitat composition in the stream the following guidelines should be considered:

- Sampling units placed in substrates with a high degree of coverage should include areas along shore and in the stream channel, more or less in the form of a transect.
- Two or three sampling units should be taken directly along the stream margin.
- If a common substrate occurs in both riffles and pools, the sampling units of this substrate should be distributed according to the relative abundance of the substrate in each of these sections.
- Habitats or substrates with less than 5% cover are not considered during the sampling.
- If substrates cannot be recorded from the bank, one may enter the stream at single entry points. The sample units should then however be taken away from these "disturbed" areas.

When sampling macrophytes within a watercourse, the following should be considered:

- Generally macrophyte samples should be taken from the anchoring region of the plants in larger stands of macrophytes (e.g. *Callitriche* sp., *Elodea* sp., *Myriophyllum* sp. or *Potamogeton* sp.).
- If macrophytes grow in single thin but long floating stands (e.g. *Callitriche* sp. or *Ranunculus* sp.), where the anchoring region is relatively small (< 20%) compared with the total projected coverage of the stand, the samples may also be taken outside the anchoring region.
- If only a single sampling unit is taken from macrophytes, it is important that this sample represents the dominant growth form of macrophytes within the sampling reach.
- If several sampling units are taken from macrophytes, they should be distributed with the aim of covering the diversity of species and growth forms present at the site.

• C.3. Modifications of recording substrate coverage for non-wadable streams and rivers

Water courses are considered non-wadable, when most stretches cannot be passed. In these cases the substrate recording is done only in the wadable areas, usually close to shore, where sampling will be undertaken subsequently.

If only some small regions of a stream or river are wadable (e.g. riffle sections) the number of sampling units in the middle of the stream is limited to retain the comparability of data with non-wadable streams. Thus a maximum of 5 sampling units should be taken from these wadable sections, the other 15 sampling units should be taken close to shore.

MAS macroinvertebrate sampling ("Multi-Habitat-Sampling")
Field protocol to determine sampling units

Sampling site	Date	Person sampling
Windach at Hirschberg	*March 20, 2003*	*Sundermann*

Information in 5%-intervals, microhabitats with coverage <5% are marked with an "x"

MINERAL SUBSTRATES	Coverage (5% intervals)	Nr. subsampling units	Comments
Hygropetric zones water layer on mineral substrates			
Megalithal (> 40 cm) upper sides of large boulders and blocks; bedrock			
Macrolithal (> 20 cm - 40 cm) coarse blocks, head-sized cobbles, with variable %-age of smaller fractions			
Mesolithal (> 6 cm - 20 cm) fist to hand-sized rocks, with variable %-age of smaller fractions	*55*	*11*	
Microlithal (> 2 cm - 6 cm) coarse gravel – egg-sized rocks with variable %-age of smaller fractions			
Akal (> 0.2 cm - 2 cm) fine and mid-sized gravel	*5*	*1*	
Psammal / Psammopelal (> 6 µm - 2 mm) sand and/or (mineral) mud	*25*	*5*	
Argyllal (< 6 µm) silt, loam, clay (inorganic)			
Technolithal 1 (artificial substrate) fixation structures of rocks or rock piles			
Technolithal 2 (artificial substrate) fixation structures without seams or interstices (plaster or concrete)			
ORGANIC SUBSTRATES			
Algae filamentous algae, algal tufts			
Submerged macrophytes macrophytes, incl. moss and Characeae			
Emergent macrophytes e.g. *Typha, Carex, Phragmites*			
Living parts of terrestrial plants fine roots, floating riparian vegetation	*X*		
Xylal (wood) tree trunks, dead wood, branches, roots	*15*	*3*	
CPOM deposits of coarse organic matter (e.g. fallen leaves)			
Sewage bacteria, -fungi and sapropel waste water induced „Aufwuchs" (e.g. *Sphaerotilus*) and/or organic mud			
Debris organic and inorganic material deposited in the splash zone area (e.g. mollusc shells deposited by wave motion)			
Sum	**100%**	**20**	

Fig. 6. Example of a filled-in site protocol for the MAS protocol. The microhabitat distribution corresponds to that shown in Fig. 7.

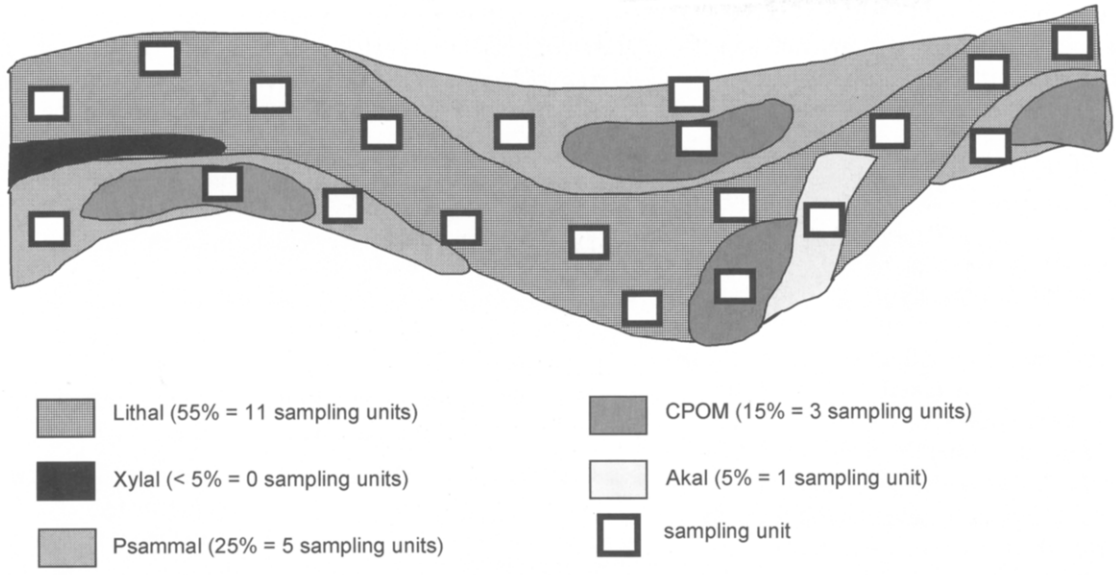

Fig. 7. Example of substrate composition and corresponding distribution of sampling units in a stream. The substrate "xylal" is not considered in the sampling units distribution and thus not sampled (from HERING et al. 2003, altered).

• C.4. Sampling procedure

Sampling always takes place against the direction of flow starting with the sampling unit furthest downstream. Each sampling unit is sampled over an area of 25 × 25 cm (frame size of hand net). The hand net is held vertically to the substrate, which is disturbed with the foot in front (upstream) of the hand net. With this method fine and medium sized substrates are disturbed to a depth of 2 to 5 cm. In order to catch dislodged or drifting organisms, the disturbed area should be close to the hand net frame. In shallow water, larger stones are brushed by hand or with a brush. After taking three to five sampling units, the hand net is emptied into a 10-litre pail filled with 2 to 3 l of water. The sample volume should not exceed more than half a pail. If need be, the sample is distributed into several pails. If a large stone rolls into the hand net while sampling any organisms clinging to it should washed from the stone into the net. The stone may then be removed before the next sampling unit is taken.

Sampling macrophytes:
Generally there are two situations that a worker will encounter when sampling macrophytes. These are either...

- long floating stands (e.g. *Callitriche* sp., *Elodea* sp., *Myriophyllum* sp. or *Potamogeton* sp.) or
- macrophytes in deeper, lentic reaches (e.g. *Sparganium emersum, Sagittaria sagittifolia* or *Nuphar lutea*).

Sampling floating stands:
When samples are taken in the anchoring region of macrophyte stands, the underlying substrate also becomes subject to the macrophyte sampling. The sampling is done in the way described above. The hand net is placed vertically below the macrophyte stand and the substrate and macrophytes are disturbed by hand or by foot in an area of 25 × 25 cm. If the sample is taken outside the anchoring region of macrophytes (e.g. in very long, thin stands of floating vegetation as described above), both the floating macrophytes and the underlying substrate are sampled within the sampling unit.

Sampling macrophytes in deeper water:
In deeper, or lentic, reaches, macrophytes can stand vertically extending in depth to over 1m. For each sampling unit an area of 25 × 25 cm is projected onto the substrate surface. Starting from the water surface the hand net is slipped over the macrophyte stand toward the stream bottom. While doing this one should attempt to keep the hand net against the direction of flow, so organisms dislodged from the plants will drift into the net. Once the stream bottom is reached, the macrophytes are completely dislodged from the substrate and put into the hand net. The substrate in the anchoring region is also part of this sampling unit and is sampled in the same way as other benthic substrates. Large volumes of macrophytes are taken from the hand net and placed in a pail with water.

Particularities while sampling:
If the current is too slow to ensure dislodged organisms drift into the hand net, the upper 2 to 5 cm of the substrate are collected completely into the hand net.

In streams with high levels of unstable substrate, e.g. alpine streams or highly mobile sandy streams, the number of sampling units is allotted in microhabitat parity as described above. The exact location of each sampling unit for each substrate type should then however be positioned in more stable regions of this particular substrate type. In some cases the regions with little substrate stability host so few organisms, that taking a larger number of sampling units from these areas, would lead to extremely low numbers of individuals.

• C.5. Sample treatment in the field

After the 20th sampling unit is taken, the entire sample material is unified in a container, e.g. 10-litre pail, unless so much material was taken, that the material is divided among several pails. If this is the case, each pail, with the exception of pails filled only with macrophytes, must be treated in the way described in the following.

First, large stones or pieces of wood are removed and searched for organisms. These are picked off using tweezers and placed back into the hand net. The substrate can then be discarded. After this, most samples still contain a large amount of mineral substrates. Using a simple washing method, these can be removed to simplify the proceeding sorting process. This wash is done as follows:

Fill the pail containing the substrate about 3/4 with water. The water and material are slowly set into rotation by hand. The resuspended material (usually primarily organic material) is then poured back into the hand net, while the mineral substrate remains in the pail. While the material is poured back into the net, the end of the net should be lying in the stream. The pail is then refilled with water and the wash repeated until only mineral substrate remains in the pail. This substrate is then put into a sorting tray and searched for remaining organisms (e.g. case bearing Trichoptera, molluscs, clinging leeches or Turbellaria). These organisms are then put into the hand net with the remaining organic sample. The mineral substrate is now free of organisms and is discarded in the field. If the total sample was originally divided among several pails, this washing step is repeated for each pail. The organic sample material is pooled in the hand net.

If macrophytes were separated in a separate pail, these are rinsed in the pail with water and then searched for clinging or sessile organisms. Pupal stages, e.g. of Simuliidae, do not need to be removed since they are of no importance for further sample treatment. Once the plants have been searched and organisms removed they can be discarded. Organisms washed or picked from the plants are placed into the hand net with the rest of the sample, which is now complete.

Picking "pre-sorted" organisms:

The sample material is removed from the hand net and placed in a sorting tray. Organisms clinging to the net are removed and placed in the sorting tray. Slightly cover the sample material with water. The material is searched and single organisms (pre-sorted organisms) removed from the sample according to the following guidelines:

- taxa which are protected and must not be killed (e.g. *Astacus astacus, Margaritifera margaritifera, Unio crassus* in Germany),
- taxa which can no longer be determined when fixed in Ethanol (e.g. Turbellaria),
- sensitive taxa which are no longer readily determinable after being exposed to mechanical force (e.g. Ephemeroptera),
- taxa, which after a preliminary sighting only occur once or twice in the sample.

The taxa from the first group are determined in the field according to the requirements (in Germany according to HAASE & SUNDERMANN 2004) and then released in the field. The taxa and number of individuals are marked in the field "Comments" on the site protocol.

One or two individuals from each separated taxon from group 2 are determined in the field according to requirements (in Germany according to HAASE & SUNDERMANN 2004) and these are then placed in a vial filled with 70% Ethanol, which is specially marked with "pre-sorted individuals". The total abundance of these taxa is then estimated and marked in the field "Comments" on the site protocol.

Taxa from groups three and four are picked and placed into the "pre-sorted" vial. However, the number of animals pre-sorted from groups 2 to 4 must not exceed 30 individuals. The maximum number of 30 individuals seems low, but in practice this number has proved fully sufficient. Picking the pre-sorted organisms should not take longer than ten minutes.

Be careful to search and clean the hand net well before proceeding to avoid losing organisms from the sample and more importantly to avoid contamination of any proceeding samples or other water bodies.

• C.6. Conserving and storing the samples

The water from the sorting tray is drained over a small sieve (500 μm). The sample material is then transferred from the sorting tray into a sample container using a funnel. The container and funnel should have relatively wide openings to facilitate the transfer. Remaining material can be washed from the sorting tray into the sample container using 96% Ethanol. The sample volume should not exceed 3/4 of the container volume. The whole sample is preserved in the container with 96% Ethanol. Fill the sample container at least half full with 96% Ethanol, preferably so Ethanol covers the whole sample. Usually a sample will easily fit into one or two 1-litre sample containers.

Sample labelling should contain at least the following information: Name of the stream and sampling site, date,

name of the person who took the sample and sample code (optional).

To ensure that preserved samples do not begin to rot, the samples should be stored cool, e.g. in a cooler in the field or the fridge in the lab. 12 to 24 hours after initial preservation, the liquid in the sample container should be drained carefully over a 500μm mesh or sieve and replaced with 96% Ethanol. Any organisms on the sieve are returned to the sample. The sample is stored in a cool place again. After another 24 to 48 hours, the Ethanol is drained and replaced with 70% Ethanol. After this the sample can be stored for longer periods of time at room temperature before further treatment. It is always important that Ethanol reaches all parts of the sample. It is therefore advisable to carefully shake and upend the closed sample container directly after each filling with Ethanol.

D. Laboratory Treatment: Subsampling and sorting

To further reduce the sample volume, a defined subsample is taken.

• D.1. Technical requirements

To take the defined subsample, a subsampling grid/sorting tray combination is required. The outer tray is a "normal" sorting tray, while the inner grid is a modified subsampling sieve with an inner area of 30 × 36 cm and a grid mesh bottom (500 μm). The mesh is divided into 30 equal units (6 × 6 cm each) (Fig. 8). The lines extend up the sides of the grid, where along the upper edge the grids are numbered 1 through 5 and 1 through 6 respectively. Thus each unit is given a unique coordinate code. This apparatus allows taking a defined subsample.

Besides the subsampling grid/sorting tray combination you will need the following material in the lab: a "cookie cutter" (6 × 6 cm) and the fitting shovel to remove a subsample unit; about 15 sorting vials (approx. 25 ml); two or three small sorting trays (about 12 × 20 cm); tweezers; ethanol (70%); a hand counter; a pair of dice; printed labels; a sorting sieve with a 2 mm grid and a fitting collector with closable outlet.

• D.2. Taking a subsample

If a sample was divided into several sample containers, the total sample is first poured over the subsampling grid and rinsed under running water (Fig. 8). If larger pieces of material (leaves, small twigs) are still in the sample, they are washed under running water over the grid to remove any organisms. Once leaves or twigs are clean they are discarded. This whole rinsing procedure should only take a few minutes.

After rinsing, the subsampling grid with the sample is placed inside the outer sorting tray, which is filled with water. The sample material is carefully and evenly distributed across the grid. It is important that the corners are also filled evenly with material. Once the material is distributed, the sieve is removed from the outer sorting tray, so the water can run off. With the help of the dice, 5 of the 30 units (1/6th of the whole sample) from which the subsample is to be taken are randomly selected. If for example a "2" and a "4" are tossed with the dice, the first unit taken has the coordinates 2 (from the short side) and 4 (from the long side of the grid). The other four units are selected in the same way.

With the help of the "cookie cutter" and the shovel each subsample unit is removed from the grid and transferred into a sorting tray. Plant material, which extends beyond the edge of the unit, should be cut with a pair of scissors before the cookie cutter is placed on the unit and the material removed with the shovel. This makes the removal more accurate and much easier. If an animal lies directly on the edge of two units, it is counted into the unit where the larger part of its body lies.

After the five subsample units have been removed from the grid, the remaining sample material is left undisturbed on the subsampling grid, until the taken subsample has been analysed in terms of individuals within. This is necessary, because a MAS subsample must fulfil both of the following criteria:

- The subsample must correspond to at least 1/6th of the whole sample, and
- it must contain at least 350 individuals.

If this number of individuals is not reached with 1/6th of the sample additional units must be randomly selected from the subsampling grid, sorted completely and counted. The process is repeated until at least 350 individuals are sorted from the whole subsample.

While the subsample units are sorted and individuals therein counted it is important that the remaining sample in the subsampling grid does not dry out. This can be avoided by covering the grid with plastic wrap or aluminium foil, or by carefully squirting water on the sample. Only when the subsampling is finished and 350+ in-

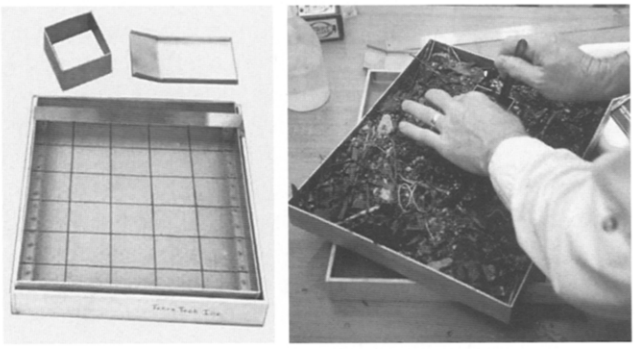

Fig. 8. Subsampling grid: left, the grid, the "cookie cutter" and the subsampling shovel; right, the same in action.

dividuals are sorted can the remaining sample be discarded.

- **D.3. Separating the coarse fraction from the subsample**

After the subsample is taken, the material is placed in a sieve with a 2 mm grid. The sieve itself is placed in a fitting collector with an outlet. Water is poured into the cascade of sieves until the 2 mm sieve is filled with water. During this procedure the substrate is automatically resuspended in the sieve. Once the sieve is filled the spout of the collector is opened and the water can run off. The separated material (≤2 mm), which passed into the collecting tank, is discarded. This rinsing is repeated another four times. The material that remains in the 2mm sieve afterwards is considered the MAS subsample which is sorted and analysed.

- **D.4. Sorting**

The MAS subsample is now transferred into a sorting tray, in small portions using a teaspoons or tablespoon (depending on the size of the sorting tray). The material is slightly covered with water and distributed evenly. To make for comparable sorting and to facilitate recognition of small and dark organisms it is important that no more than half of the sorting tray bottom is actually covered with material. This is especially important in samples with a lot of organic matter.

All organisms are removed from the sample and sorted by taxonomic order into sorting vials filled with 70% Ethanol. While sorting the total number of sorted individuals is monitored continually. This is most easily done using a hand counter while sorting. The following material is not counted: adult stages of insects (except Coleoptera), exuviae, empty mollusc shells or individuals where the taxonomic order is no longer recognisable due to mechanical damage. Cases of caddis flies are only counted when they clearly host larvae. With the exception of the readily recognisable Blephariceridae (Diptera), pupae are not counted.

If the number of individuals sorted from the subsample exceeds 350 at the end of sorting a subsampling unit, the sorting process is finished. If the number of individuals is <350 after a subsampling unit is completely sorted, one other subsample unit is randomly selected, removed from the subsampling grid, the coarse fraction (> 2 mm) separated and sorted completely. This process is repeated until at least 350 individuals have been sorted from the MAS subsample. It is important that every started subsample unit must be completely sorted, even if the number exceeds 350 individuals early in the sorting of that subsampling unit!

The sorted MAS subsample containing 350+ individuals is used for further analysis.

E. Taxa determination

The determination of the organisms sorted from the MAS subsample and the pre-sorted individuals follows set guidelines. In Germany, the guidelines and recommended determination literature are given in HAASE & SUNDERMANN (2004). Some taxonomic groups may reach very high numbers of individuals in samples, e.g. Simuliidae, Gammaridae. At the same time, when following the determination guidelines, these groups may only be represented by few taxa. If this is the case, determination can be simplified with the following procedure:

At least 50 individuals are randomly picked and determined. The remainder of the animals are only counted and projected proportionally onto the determined taxa. For Chironomidae the same procedure may be used, with a minimum of 100 individuals determined.

F. Taxa list generation and data analysis

For further analysis the number of individuals determined is projected onto a whole sample, thus 1.25 m^2. In this way abundance data remain comparable. If for example the subsample part was 1/6 (5 of 30 subsampling units sorted), the number of individuals for each taxon is multiplied by 6. Only after the subsample data have been projected on a whole sample (1.25 m^2) are the pre-sorted individuals and those individuals that were determined alive in the field added to the total taxa list!

Acknowledgements

This study was supported by Länderarbeitsgemeinschaft Wasser (Project number: LAWA O4.02) and by the European Union under the 6th Framework Programme (STAR project; contract number: EVK1-CT-2001-00089).

References

BAILEY, R. C., KENNEDY, M. G., DERVISH, M. Z. & TAYLOR, R. M. (1998): Biological assessment of freshwater ecosystems using a reference condition approach: comparing predicted and actual benthic invertebrates communities in Yukon streams. Freshwater Biology **39**: 765–774.

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, 2nd edition. EPA 841-B-99-002. U.S. Environmental Protection Agency; Office of Water; Washington, D.C.

BANNING, M. (1998): Auswirkungen des Aufstaus größerer Flüsse auf das Makrozoobenthos dargestellt am Beispiel der Donau. Essener ökologische Schriften, Band 9. Westarp-Wiss., Hohenwarsleben.

BIRK, S. & HERING, D. (2002): Waterview web-database – a comprehensive review of European assessment methods for rivers. FBA news no. 20, winter 2002. Freshwater Biological Association, Ambleside: 4.

BÖHMER, J., RAWER-JOST, C., ZENKER, A., MEIER, C., FELD, C. K., BISS, R. & HERING, D. (2004): Assessing streams in Germany with benthic invertebrates: Development of a multimetric invertebrate based assessment system. Limnologica **34**: 416–432.

BRAUKMANN, U. (2000): Hydrochemische und biologische Merkmale regionaler Bachtypen in Baden-Württemberg. Oberirdische Gewässer, Gewässerökologie **56**: 1–501.

CAO, Y., HAWKINS, C. P. & VINSON, M. R. (2003): Measuring and controlling data quality in biological assemblage surveys with special reference to stream benthic macroinvertebrates. Freshwater Biology **48**: 1898–1911.

CARTER, J. L. & RESH, V. H. (2001) After site selection and before data analysis: sampling, sorting, and laboratory procedures used in stream benthic macroinvertebrate monitoring programs by USA state agencies, J. N. Am. Benthol. Soc. **20**: 658–682.

CLARKE, R. T. (2000): Uncertainty in estimates of river quality based on RIVPACS. In: J. F. WRIGHT, D. W. SUTCLIFFE & M. T. FURSE (eds.), Assessing the biological quality of fresh waters: RIVPACS and other techniques, pp. 39–54. Freshwater Biological Association, Ambleside.

CLARKE, R. T., FURSE, M. T., GUNN, R. J. M., WINDER, J. M. & WRIGHT, J. F. (2002): Sampling variation in macroinvertebrate data and implications for river quality indices. Freshwater Biology **47**: 1735–1751.

ENGEL, J. (1997): Signifikante Schule der schlichten Statistik. Filander Verlag, Fürth.

ENVIRONMENT AGENCY (1997): Procedures for collecting and analysing macro-invertebrate samples. Quality Management Systems for Environmental Monitoring: Biological Techniques, BT001. Environment Agency, Bristol.

FRIBERG, N. & JOHNSON, R. K. (1995): Biological monitoring of streams – methods used in the Nordic countries based on macroinvertebrates. TemaNord **1995**: 640, 58 pp.

HAASE, P. & SUNDERMANN, A. (2004): Standardisierung der Erfassungs- und Auswertungsmethoden von Makrozoobenthosuntersuchungen in Fließgewässern. Unveröffentlichtes Gutachten der Länderarbeitsgemeinschaft Wasser (LAWA).

HAASE, P., PAULS, S., SUNDERMANN, A. & ZENKER, A. (2004): Testing different sorting techniques in macroinvertebrate samples from running waters. Limnologica **34**: 366–378.

HERING, D., HAASE, P., MOOG, O. & SOMMERHÄUSER, M. (2001): Vorschlag zur Standardisierung fließgewässerökologischer Untersuchungen in der Bundesrepublik Deutschland – Probennahme und Taxaliste. DGL Tagungsbericht 2000 (Magdeburg): 164–168.

HERING, D., BUFFAGNI, A., MOOG, O., SANDIN, L., SOMMERHÄUSER, M., STUBAUER, I., FELD, C. K., JOHNSON, R., PINTO, P., SKOULIKIDIS, N., VERDONSCHOT, P. F. M. & ZAHRÁDKOVÁ, S. (2003): The development of a system to assess the ecological quality of streams based on macroinvertebrates – design of the sampling programme within the AQEM project. Internat. Rev. Hydrobiol. **88**: 345–361.

HERING, D., MEIER, C., RAWER-JOST, C., BISS, R., FELD, C. K., ZENKER, A., SUNDERMANN, A., LOHSE, S. & BÖHMER, J. (2004a): Assessing streams in Germany with benthic invertebrates: selection of candidate metrics. Limnologica **34**: 398–415.

HERING, D., MOOG, O., SANDIN, L. & VERDONSCHOT, P. F. M. (2004b): Overview and application of the AQEM assessment system. Hydrobiologia (The Hague) **516**: 1–20.

ISO 7828 (1985): Water quality – methods of biological sampling – guidance on handnet sapling of aquatic benthic macro-invertebrates. Ref. No. ISO 7828–1985 (E).

JOY, M. K. & DEATH, R. G. (2003): Biological assessment of rivers in the Manawatu-Wanganui region of New Zealand using a predicative macroinvertebrate model. New Zealand Journal of Marine and Freshwater Research **37**: 367–379.

KOKES, J. (2001): Prediction Models for River Ecosystems (Predikcní modely rícních ekosystému). Report of the grant No. 510/7/99 of the Council of the Government of the Czech Republic for Research and Development, T.G.M. Water Research Institute Prague (in Czech).

LAWA (Länderarbeitsgemeinschaft Wasser) (2001): Gewässerstrukturgütekartierung in der Bundesrepublik Deutschland – Verfahren für kleine und mittelgroße Fließgewässer. LAWA Publikationen „Oberirdische Gewässer und Küstengewässer", ISBN 3-88961-233-4-227-x.

MCGARRIGLE, M. L., LUCEY, J. & CLABBY, K. C. (1992): Biological assessment of river water quality in Ireland. In: NEWMAN, P. J., PIAVAUX, M. A. & SWEETING, R. A. (eds.), River water quality. Ecological assessment and control, pp. 371–385. Commission of the European Community, Brussels.

POTTGIESSER, T. & SOMMERHÄUSER, M. (2004): Die Fließgewässertypologie Deutschlands: System der Gewässertypen und Steckbriefe zu den Referenzbedingungen. In: STEINBERG, C., CALMANO, W., WILKEN, R.-D. & KLAPPER, H. (eds.), Handbuch Angewandte Limnologie. 19. Erg.Lfg. 7/04. VIII-2.1: 1–16 + Anhang. ecomed Verlagsgesellschaft Landsberg.

REYNOLDSON, T. B., ROSENBERG, D. M. & RESH, V. H. (2001): Comparison of models predicting invertebrate assemblages for biomonitoring in the Fraser River catchment, British Columbia. Canadian Journal of Fisheries and Aquatic Sciences **58**: 1395–1410.

ROSENBERG, D. M. & RESH, V. H. (1993): Introduction to freshwater biomonitoring and benthic macroinvertebrates. In: ROSENBERG, D. M. & RESH, V. H. (eds.), Freshwater biomonitoring and benthic macroinvertebrates. Chapman and Hall, New York.

SMITH, M. J., KAY, W. R., EDWARD, D. H. D., PAPAS, P. J., RICHARDSON, K. S., SIMPSON, J. C., PINDER, A. M., CALE, D. J., HORWITZ, P. H. J., DAVIS, J. A., YUNG, F. H., NORRIS, R. H. & HALSE, S. A. (1999): AusRivAS: using macroinvertebrates to assess ecological condition of rivers in Western Australia. Freshwater Biology **41**: 269–282.

STAR CONSORTIUM (2003): The AQEM sampling method to be applied in STAR. Unpublished report, available from http://www.eu-star.at/tops/top_protocols.htm

STATSOFT, Inc. (2002) STATISTICA für Windows [Software-System für Datenanalyse] Version 6. www.statsoft.com.

WRIGHT, J. F., MOSS, D., ARMITAGE, P. D. & FURSE, M. T. (1984): A preliminary classification of running-water sites in Great Britain based on macroinvertebrate species and the prediction of community type using environmental data. Freshwater Biol. **14**: 221–256.