



# **WATER QUALITY – GUIDANCE ON PRO-RATA MULTI-HABITAT-SAMPLING OF BENTHIC INVERTEBRATES FROM WADEABLE RIVERS IN THE HKH-REGION**

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Please check if our understanding corresponds to yours.

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## 1. Foreword

This is a working document that has been prepared by the AQEM, STAR and ASSESS-HKH consortium ([www.aqem.de](http://www.aqem.de), [www.eu-star.at](http://www.eu-star.at), [www.assess-hkh.at](http://www.assess-hkh.at)). The procedure described was tested at 478 sites across 8 European countries within the AQEM project. The exercise was repeated at 264 sites within the framework of the STAR project and about 800 sites in Austria within the national monitoring network. Altogether more than 20 institutions from 13 countries have actively tested and used the Multi-Habitat Sampling techniques.

For benthic macro-invertebrates, most assessment methods used **in the past** have focused only on the impact of **organic pollution**. Assessment methods developed for the implementation of the European Water Framework Directive (**WFD**) go a step further and aim at **detecting** the impact of **various stressors** (stream habitat degradation, effects of hydropower generation, toxicity, acidification, eutrophication) on the aquatic biota.

Sampling effort for detecting organic pollution was directed at sampling a **large number** of indicator species (e.g. sufficient for calculating a Saprobic Index) **independent** of their distribution/density in the field. Sampling for species richness may therefore bias many assessment features and will not reflect the actual conditions.

The sampling effort for a multipurpose evaluation methodology requires more precisely defined sampling protocols and sampling performance, as many approaches (e.g. the Multimetric Indices) depend on a pro-rata estimate of species richness and composition at a site. To ensure a comprehensible procedure, this does not require the sampling of rare or unique habitats with a share below 5%.

## 2. Scope

The recommended procedures focus on the pro-rata Multi-Habitat Sampling (MHS) of benthic macro-invertebrates in wadeable rivers and streams. The term "*pro-rata*" reflects the intention to sample adequate proportions of riverine habitats with a minimum occurrence of 5% of the total habitat.

The Multi-Habitat Sampling does not replace other techniques. But, among other applications, the pro-rata Multi-Habitat Sampling technique is a fundamental requisite of multimetric assessment approaches that evaluate the ecological status of running waters.

The MHS methodology is based on the Rapid Bioassessment Protocols (Barbour et al. 1999), the procedures of the ENVIRONMENT AGENCY of England and Wales (Murray-Bligh 1999), the Austrian Guidelines for the Assessment of the Saprobiological Water Quality of Rivers and Streams (MOOG et al. 1999), ISO 7828, the AQEM sampling manual (2002), the AQEM & STAR site protocol (2002), the German methodology as described in [www.fliessgewaesserbewertung.de](http://www.fliessgewaesserbewertung.de), and the Austrian Standards M 6232 and M 6119-2.

### 3. Normative References

This method incorporates by dated or undated reference, provisions from other publications. These normative references are cited at the appropriate places in the text and the publications are listed in the references.

### 4. Terms and Definition

For the purpose of this multi-habitat sampling method, the following terms and definitions should be applied. A common understanding of these terms is crucial for the sampling procedure. The terms are given in scientific (Latin or Greek) names to avoid any misunderstanding. The scientific background for the selection of habitats is based on the principle that each habitat is colonized by a habitat-specific benthic assemblage as described e.g. by Braukmann 1987 and the Austrian Standards M 6232 (1997).

#### **Akal**

Fine to medium-sized **gravel**; grain-diameter > 0.2 cm to 2 cm

#### **Argyllal**

Silt, loam, **clay**

#### **CPOM**

Deposits of **C**oarse **P**articulate **O**rganic **M**atter as e.g. fallen leaves

#### **Debris**

Organic and inorganic matter deposited within the splash zone area by wave-motion and changing water levels, e.g., mussels shells

#### **Emergent macrophytes**

Emergent (parts of) macrophytes (e.g. *Typha*, *Carex* and *Phragmites* species)

#### **FPOM**

Deposits of **F**ine **P**articulate **O**rganic **M**atter < 1 mm of diameter

#### **Hygropetric sites**

Thin water layer on solid (rocky) substrates

#### **Living parts of terrestrial plants**

Fine roots, floating riparian vegetation

#### **Macro-algae**

Three-dimensional filamentous algae, algal tufts

#### **Macrolithal**

**Coarse cobbles**, gravel and sand; grain-diameter > 20 cm to 40 cm

#### **Megalithal**

Upper sizes of large cobbles, **boulders** and blocks, bedrock; grain-diameter > 40 cm

#### **Mesolithal**

Fist to hand-sized **cobbles** with a variable percentage of gravel and sand; grain-diameter > 6 cm to 20 cm

**Micro-algae**

Two-dimensional algal cover, e.g., diatoms on a stone

**Microlithal**

**Coarse gravel** (size of a pigeon egg to child's fist) with variable percentages of medium to fine gravel; grain- diameter > 2 cm to 6 cm

**Pelal**

**Mud** and sludge; grain-diameter < 0.06 mm

**Psammal**

**Sand**; grain-diameter 0.06 mm to 2 mm

**Psammopelal**

Mixture of **sand and mud**

**Sewage bacteria and -fungi**

e.g. *Sphaerotilus*, *Leptomitus*, sulfur bacteria (e.g. *Beggiatoa*, *Thiothrix*), sludge

**Submerged macrophytes**

Totally immersed macrophytes, including water mosses, water ferns and Characeae

**Technolithal**

Artificial substrates, e.g. rip-rap, stone plastering with/without interstices, concrete with/without seams

**Xylal**

Tree trunks (**dead wood**), branches, roots

## 5. Description of the sampling approach

The method focuses on a multi-habitat scheme designed for sampling major habitats in proportion to their presence within a sampling reach. A sample consists of 20 "sampling units" taken from all habitat types at the sampling site, each with a share of at least 5 % coverage.

A "sampling unit" is a sample performed by positioning the net and disturbing the substrate in a quadratic area that equals the frame-size upstream of the net. Sediments must be disturbed to an adequate depth that ensures capture of all species present depending on substrate diameter, compactness and 'shape' (organic substrata). E.g. sediments should be disturbed to a depth of approximately 5-10 cm (finer substrates: psammal, pelal, FPOM), 10-15 cm (intermediate sized substrates: akal, microlithal, CPOM) or 15-20 cm (larger substrates: macrolithal; living parts of terrestrial plants).

A distribution of 20 sampling units proportional to the share of habitats means: if the total habitat in the sampling area consists of 50 % psammal (sand), then 10 "sampling units" will be taken from this substrate. The categories of habitat composition follow the descriptions in section 4 (terms and definitions).

Following the AQEM procedure with a square net of 25 x 25 cm, the sampling procedure equals a sampled area of approximately 1.25 m<sup>2</sup> of the stream bottom.

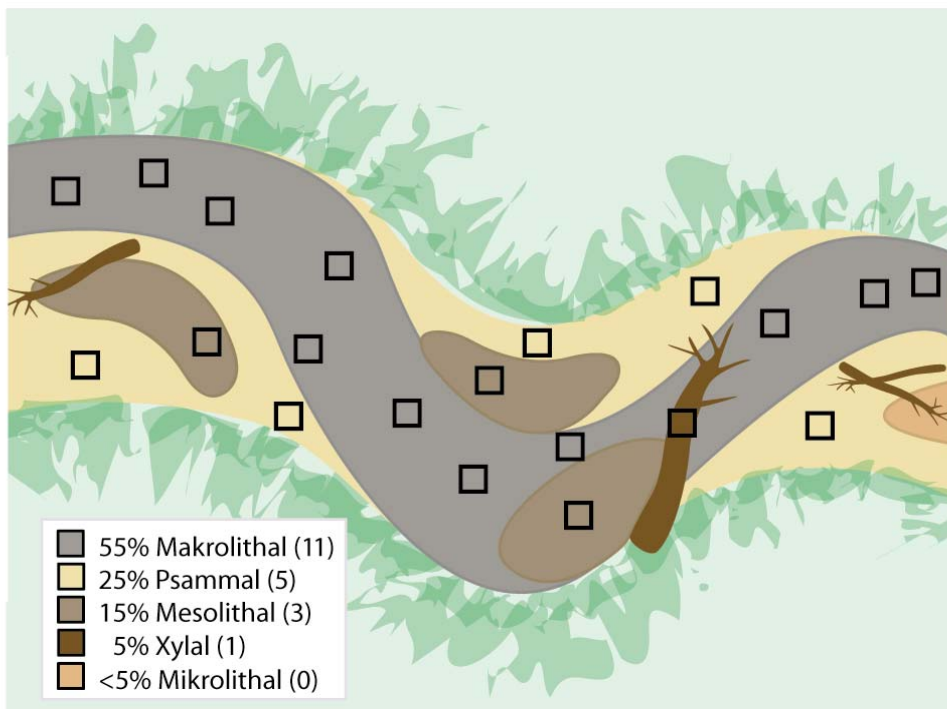


Figure 1: Example of sampling unit position in a theoretical sampling site according to the "multi habitat sampling" method (AQEM sampling manual 2002).

Figure 1 gives an example of sampling units' position in a river section that consists of five (major) habitats.

## 6. Sampling gear

The proposed sampling gear to be applied in wadeable rivers is the **AQEM/STAR net sampler**:

- Shape of the frame: rectangular. A frame in front of the hand net of 625 cm<sup>2</sup> area is recommended to enable the sampling of a distinct area.
- Dimensions of the frame: 0.25 m width by >0.25 m height. The frame attaches to a long handle, similar to a broom stick.
- Shape of the net: cone or bag shaped for capturing organisms. Mesh size of the net: standard mesh size of 500 µm nytex screen.



Figure 2: AQEM/STAR net sampler, with frame in front



Figure 3: The net is held vertically with the frame at right angles to the current, downstream from your feet

### Closed sampling nets (Box type-Samples):

In shallow waters with a strong current an enclosed piece of equipment (box type sampler with an appropriate sampling area) can be used instead of a hand-net. This is helpful in defining the sampling area and preventing too many animals drifting into the net from upstream areas.



Figure 4: Hess-Sampler



Figure 5: Box-type Sampler

Smaller grain sizes (gravel, sand) and soft sediments at sites that are not accessible by foot can be sampled with a **grab sampler** (e.g. Van Veen grab).

### Van Veen grab:

The Van Veen grab is used to take soft sediment samples from the river bottom. The operation of the grab is quite simple. At the surface the jaws are pushed open and kept in that position by a hook. To keep the hook in the right position the Van Veen grab should be sunk at a steady, not too high, pace. Both jaws are fitted with lids to allow air to escape during sinking. As soon as the jaws touch the bottom, the hook loosens its grip, so that, when hoisting the rope again the jaws will shut tight because of the leverage by the rods. The grab is opened and emptied over a plastic bucket.

The content of the grab is about 7 liters and covers a surface of 625 cm<sup>2</sup>.



Figure 6: Van Veen grab, top view, open

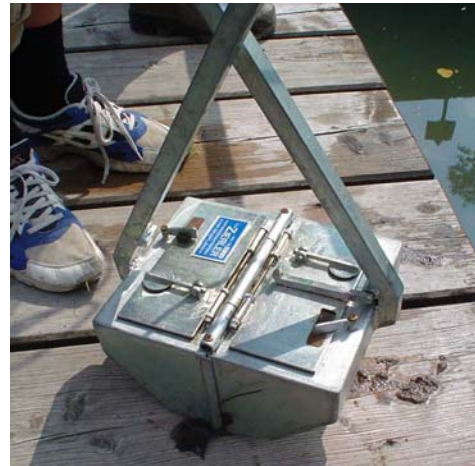


Figure 7: Van Veen grab, lateral view, closed

## ***Field sampling procedures***

### General remarks

In general, for the purpose of this study **no** sample should be taken

- during or shortly after floods; a recovery period of four to six weeks after a spate must be considered.
- during or shortly after droughts (completely dry river sections), (see above)
- during any other natural or man-induced disturbances, e.g. if unnatural turbidity prevents a proper estimation of the habitat composition or sampling of the stream bed.

In detail, the "multi-habitat sampling" procedure is performed in the following steps:

### Estimation of habitat composition and allocation of sampling units

1. Before sampling, the site protocol - especially the estimation of the coverage of habitats - must be completed. Whenever possible, the sampling area should not be disturbed by physical contact. If the estimation of the coverage of habitats needs to be corrected, e.g. due to hardly visible parts of the river bottom, this can be done during the sampling procedure. After sampling, the estimated coverage of substrates should be reviewed for accuracy and completeness. To estimate the coverage of habitats in naturally turbid rivers an (extendable) pole can be used.
2. Based on the habitats listed in section 4 or table 1, the coverage of all habitats in the river channel (including margins) with at least 5% cover is recorded to the nearest 5% interval. The presence of other habitats (< 5% cover) is indicated in the protocol by a cross. To ease the habitat estimation the sampling reach can be divided into smaller (e.g. 20-25 m) river segments.
3. The estimation of habitat composition can be done with the help of table 1 and consists of the following steps:
  - 3.1 Estimation of the cover of mineral habitats: the sum of the coverage of the individual mineral habitat must be 100 % (column1, upper part in table 1 (see appendix)).
  - 3.2 Estimation of the cover of biotic habitats (seen as an additional layer on the mineral substrates, e.g. macrophytes, macro-algae, woody material, roots, or CPOM): the sum of the coverage of the individual biotic habitats is variable (0 to 100 %) (column 1, lower part in table 1 (see appendix)).
  - 3.3 Habitats with a cover less than 5 % are not sampled but indicated by a cross (column 1, table 1 (see appendix)).

4. To define the number and the allocation of sampling units, table 1 (see appendix) has to be completed as follows:
5. To allocate the sampling units, the mineral (see 3.1) and the biotic (see 3.2) habitats are considered as one layer. Therefore the biotic habitat estimation is combined with the mineral substrate estimation. That means, samples taken from biotic habitats include the underlying (subjacent) mineral substrates. The sum of the cover of all habitats (mineral and biotic) must sum up to 100 % (= 20 x 5 %). %. (If the conditions allow, the estimation of the cover of mineral and biotic habitats can be done in one step: the sum of the coverage of the individual mineral and biotic habitats (top view) must be 100 %. This procedure may be helpful if a high proportion of biotic habitats is present in the river and consequently problems in assessing the % of the underlying mineral substrates arise).
6. The allocation of the 20 sampling units follows the combined (mineral and biotic) habitat estimation (5 % coverage equals 1 sampling unit) using table 2 (see appendix).
  - a) Copy the share (percentage) of mineral habitats (5% steps) from table 1 to row "mineral habitats" in table 2.
  - b) Copy the share (percentage) of biotic habitats (5% steps) from table 1 to column "biotic habitats" in table 2.
  - c) If bare mineral habitats exist exclusively, enter the percentage of these habitats in the corresponding cell of the row "bare mineral substrate" (column "%").
  - d) If mineral and biotic habitats are combined (e.g. FPOM on psammal or macro-algae on mesolitoral) the percentages have to be entered in the corresponding cells of the column "%".
  - e) If biotic habitats cannot be allocated properly to an underlying mineral habitat (bottom or margins, e.g. roots or macrophytes), then enter their percentage of cover in the column "not allocable". In that case this number of sampling units has to be subtracted from the nearest adjacent mineral habitat. If this procedure leads to a non-representative result, subtract the number of sampling units from the most frequent mineral habitat.
  - f) As a last step the final number of sampling units has to be entered in the different columns "SU" converting the percentages of the "%"-columns in sampling units (5 % coverage equals 1 sampling unit).

Example: If the overall cover of mesolitoral is 40 % (8 sampling units) and macro-algae are attached up to 10 % on mesolitoral 6 sampling units (SU) have to be taken at bare mesolitoral, 2 sampling units (SU) are to be taken from mesolitoral with attached macro-algae.
7. The final allocation of sampling units of a given habitat has to represent the overall structure of a sampling site. Note: sampling units should also be adequately distributed between: bed and banks; lentic and lotic areas as well as riffles and pools. It is particularly important that samples of biotic microhabitats should consider the

distribution of the underlying mineral substrates. For example, if CPOM occurs on psammal and akal, the sample units should be distributed proportionally. The column "comments" in table 1 (see appendix) can be used to indicate the proportion of lentic or lotic areas within the substrate coverage and the near-margin or in-stream situation, respectively.

8. Artificial substrates (e.g. techno-lithal = rip-rap, boulders below weirs) must be indicated in column "man-made" (table 1 (see appendix)).
9. Note again: habitats with a share of less than 5% are not sampled.

#### General recommendations for sampling

The sampling site (river reach) should be representative for the river-segment (in the sense of Frissell et al. 1986) and the purpose of the study. Therefore, the sampled stretch of about 100 m of length should reflect the typical situation (at least two riffle-pools sections) along a section of one to several kilometres. The length of the investigated river reach should increase with the size (stream order) of the river.

**Sampling** starts at the downstream end of the stretch and proceeds upstream. The river section to be sampled should not be disturbed by physical contact (don't step into the river upstream the sampling area).

When **sampling the "sampling units"** with the hand-net a controlled sampling by hand is the preferred method (see details in section 7). The sampling area per SU covers a quadratic area in the front of the opening of the net (25 x 25 cm). If kick-sampling is necessary (e.g. in deep sections), hold the net vertically with the frame at a right angle to the current, downstream from your feet (625 cm<sup>2</sup> sampling area), and disturb the stream bed vigorously by kicking or rotating the heel of your boot to dislodge the substratum and the fauna.

After at least every three sampling units (or more frequently if necessary) rinse the collected material by running clean stream water through the net two to three times. Transfer the material into a large (white) tray or a bucket. If clogging occurs in the hand-net, which may interfere with obtaining an appropriate sample, discard the material in the net and redo the sampling unit in the same habitat type but at a different location.

The **final** multi-habitat **sample** consists of **20 pooled sampling units**.

## **7. Detailed description of sampling performances**

(The examples refer to a square net with an opening of 25 X 25 cm)

### Megalithal (bedrock and boulders)

The sampling strategies in boulders depend on area and number of sampling units allocated. As only the surface can be sampled lifting is not applicable. Large stones are sampled by brushing and scraping the surface, then sweeping the animals into the net.

Individual samples of boulder substrates should be allocated to different positions (frontal, sideways) for different sampling units. If only one sampling unit in boulders is allocated, three positions (frontal and sideways/right and left) can be put together for this single sampling unit. However, the sampling location should be done with respect to algal cover respectively.



*Figure 8: Megalithal (> 40 cm)*

### Macrolithal & Mesolithal (coarse cobbles, cobbles and stones)

Sampling starts by gently sweeping the surface within the targeted area by hand to dislocate surface-dwelling animals and sweeping them into the net. Move cobbles and large stones by hand, sweep, brush or scratch the surfaces to dislodge clinging and sessile organisms. It is recommended that cobbles and bigger stones are put into a bucket to dislocate attached animals by hand-picking and controlled sweeping.

The remaining substrate is disturbed in the 0.25 x 0.25 m area upstream of the net. To dislodge the animals from the interstices of the sediments, the substrate should be disturbed with a screwdriver or similar device up to a depth of about 15 to 20 cm. A frame of 625 cm<sup>2</sup> placed on the substrate surface in front of the hand net is recommended for more precise sampling.

In shallow waters with a strong current an enclosed piece of equipment a box type sampler (with an appropriate sampling area) can be used instead of a hand-net. This is helpful in defining the sampling area and preventing too many animals drifting into the net from upstream areas. In lentic areas the sediment within the sampling area can be disturbed using the hands; also by using the hands, water can be pushed through the net to trap the animals. It is possible to use different devices for different microhabitats, as long as the same area is sampled.



*Figure 9: Macrolithal (>20 cm to 40 cm)*



*Figure 10: Mesolithal (> 6 cm to 20 cm)*



*Figure 11: Stone, put into a bucket to dislocate attached animals by hand-picking and controlled sweeping*

#### Micro lithal and smaller mineral substrates

The substrate is disturbed in the 0.25 x 0.25 m area upstream of the net. To dislodge the animals from the interstices of the sediments, the substrate should be disturbed with a screwdriver or similar tool up to a depth of about 15 to 20 cm. Hold the net close enough for the invertebrates to flow into the net with the current, but far enough away for most of the sand and gravel to sink in front of the net. Care should be taken to minimise the quantity of sand in the samples.

Areas of soft soil are sampled by 'bumping' the net along the surface of the substrate rather than dragging the net through soft substrates. Alternatively, kick the area to dislodge sediment and organisms into the water column and sweep the net through the suspended cloud of sediment to capture the dislodged animals. This reduces the amount of sediment and debris in the sample.

In shallow waters with a strong current an enclosed apparatus (box type sampler) can be used instead of a hand-net.

In lentic areas the sediment within the sampling area can be disturbed using the hands, in the normal fashion, and then a current can be created by pushing water through the net with the hands to trap the animals. Again it is possible to use different devices for different microhabitats, as long as the same area is sampled.



Figure 12: Microlithal (>2 cm to 6 cm)



Figure 13: Akal (> 0,2 cm to 2 cm)



Figure 14: Psammal (2 µm to 2mm)



Figure 15: Argyllal (< 2 µm)



Figure 16: The substrate should be disturbed with a screwdriver or similar tool up to a depth of about 15 to 20 cm

### Fine sediments in deep water bodies

For sampling soft substrates in large water bodies the use of grab samplers is recommended (e.g. Van Veen type with lids in the jaws). The grab can be operated from wadeable areas at banks and margins, from rubber rafts or transversal structures like (foot)bridges, weirs.



*Figure 17: Van Veen grab, lowered down from a footbridge*



*Figure 18: Van Veen grab, operated from a wadeable area near the bank*

## Technolithal

For sampling artificial substrates as rip-rap, correspond to the sampling performance described for mega- to mesolithal. Techno-megalithal as stone plastering with or without interstices or concrete, which has none or only very few substrate coverage is sampled by hand-net. Therefore the 0.25 x 0.25 m area upstream of the net is brushed off by a small brush. In shallow waters a current can be created by pushing water through the net with the hands to trap the animals.



*Figure 19: Techno-lithal (rip rap)*



*Figure 20: Techno-lithal (stone plastering)*



*Figure 21: Techno-megalithal, brushing off the surface of the plastering*

### Xylal (woody debris)

Avoid relatively new 'deadfall' that lacks microbial conditioning. Washing the samples into a net is the most effective mechanism. Alternatively: take the woody debris out, spray onto a net and pick the animals off using forceps.



*Figure 22: Xylal (wood)*



*Figure 23: Washing the wood carefully into the hand-net*

### Roots

Sweeping an area of 625 cm<sup>2</sup> followed by vigorous shaking can be effective.



*Figure 24: Roots*

### CPOM (leaf litter)

Wash carefully in the field and avoid taking large amounts of leaves back to the laboratory.



Figure 25: CPOM, Coarse Particulate Organic Matter

### Macrophytes

A quantitative macrophyte sample comprises the macrophytes attached to an area of 25 x 25 cm of the river bottom and includes the sediment surface layer. All parts of the macrophytes, i.e. roots, stems, and leaves are removed and transferred into the sampler that is located so that the current floats animals into the net. The macrophyte material is then rinsed thoroughly in a bucket (half filled with water) to separate the invertebrates from the plants. This procedure is repeated until no more invertebrate specimens are rinsed off. The rinsed macrophyte material needs to be taken to the laboratory for further examination, because clinging organisms such as *Simuliidae* and some chironomid tubes (e.g. *Rheotanytarsus*) cannot be completely removed from the plants in the field. This rinsed macrophyte material can be reduced if necessary. As a rule of thumb it will be sufficient to treat one quarter of this material in the lab (don't forget to multiply the number of this part of organisms by the appropriate split factor).



*Figure 26: Submerged macrophytes*



*Figure 27: Submerged macrophytes and filamentous algae*



*Figure 28: Emergent macrophytes*



*Figure 29: The macrophyte material is rinsed thoroughly in a bucket (half filled with water) to separate the invertebrates from the plants.*

## **8. Follow-up treatment in the field**

**Removal of large material and field sorting:** Branches, sticks and stones can be removed after being rinsed and inspected for burrowing, clinging or sessile organisms. Any organisms found should be placed into the sample container. Generally, it is recommended not to spend time inspecting small debris in the field; however, larger and fragile organisms (e.g. Ephemeroptera) or species of groups that cannot be preserved (e.g. Tricladida, Oligochaeta) should be sorted initially in the field (maximum 50 representative organisms). For field sorting the use of a white tray (with a size of about 300 x 150 x 50 mm) is recommended. These organisms should then be stored in a small separate containers without substrate.

**Removal of large organisms:** Large and rare organisms, which can easily be determined to the species level in the field (such as large mussels), should be recorded in the field protocol, removed from the sample and returned alive to the stream. If the benthic invertebrate biomass of a sample is to be determined these organisms need to be weighted before putting them back to the river.

**Storing:** Transfer the sample from the net to sample container(s) and preserve with formalin (4% final concentration of formaldehyde) or in sufficient 95% ethanol to cover the sample completely, immediately after collection. This form of fixation is important to prevent carnivores, particularly stoneflies (Setipalpia [=Systellognatha]), beetles (Adephaga), caddis larvae (e.g. *Rhyacophilidae*), *Sialidae* and certain *Gammaridae* from feeding on other organisms. The final ethanol concentration should be around 70 %. When using ethanol, water in the sample should be decanted before adding the fixation liquid. Forceps may be needed to remove organisms from the dip net. The sample container should be closed tightly. The samples should be stored cool.

**Labelling:** Place a label (written in pencil, printed on a laser printer or photocopied) indicating the following information inside the sample container: project (optional); stream name; site name; site code; date of sampling; sampling gear, fraction; investigator's name (optional).

The outside of the container should include the same information and the words "preservative: formalin 10 %", or "95 % ethanol", respectively. If more than one container is needed for a sample, each container should be labelled with all the information on the sample and should be numbered (e.g. 1 of 2, 2 of 2). If rare taxa (e.g. crayfish, large mussels) have been identified in the field and returned to the river, record their presence and abundance (when appropriate biomass) on the label placed in the sample-containers as well as in the sample-protocol. If possible, label and place the container with the rare and fragile organisms into the main sample-container and note its existence in the site-protocol.

**Refine the site-protocol,** particularly the share of microhabitats, after sampling has been completed. Having sampled the various microhabitats walking the reach helps ensure a more

accurate assessment. Note the sampling-gear used, and comment on conditions during sampling, e.g. high flows, treacherous rocks, difficult access to stream, or anything that could indicate an influence on the sample composition.

For health and safety reasons, not all laboratories can use formalin although it is known to be the most effective fixative for freshwater macro-invertebrate samples (ENVIRONMENT AGENCY 1999). If a laboratory cannot use formalin and the sample has been preserved with 95 % ethanol, it should be re-preserved in the lab. The sample can then be kept for several months before analysis.

## **9. Quality control in the field**

Sample labels must be completed properly (including at least stream name; site name; date of sampling; sampling gear; fraction) and placed inside the sample container. The outside of the container should be labelled with the same information. If chain-of-custody forms are required, they must include the same information as the sample- container labels.

After sampling has been completed at a site, all nets, pans, etc. that have come into contact with the sample should be rinsed thoroughly, examined carefully, and picked free of organisms or debris. Any additional organisms found should be placed into the sample containers. The equipment should be examined again prior to its use at the next sampling site.

The equipment should also be sterilised before taking new samples, e.g. by dipping it in alcohol or letting it dry for a number of hours. This is particularly necessary in areas affected by crayfish plague or notifiable fish diseases.

## **10. Safety**

Fieldwork always has the potential for personal injury from equipment-operation and exposure to environmental hazards. Every effort should be made to minimise risks in the field. Besides the scientific aspects, criteria for safe sampling should also be considered when selecting a sampling site.

Never take samples alone. When taking samples always ensure the presence of at least one other person.

The accompanying person should have a clear view of the sampler at all times.

Do not take samples when the conditions at a sampling site are dangerous. In particular you should: avoid sampling rivers in flood conditions; avoid sampling during extreme cold conditions; avoid steep or unstable banks; check depth and stability of the river bottom; watch out for man-made hazards (broken glass, sharp metals etc.).

Wear a life jacket when sampling either in deep rivers, upstream from weirs or deep pools, in streams with strong current. Have a bundled safety line stationed downstream that can be tossed out by the partner in the event the person sampling falls and is carried downstream by the current.

Wear appropriate clothing and use rubber gloves.

#### Precaution measures

Do not forget a country-specific first-aid kit and learn how to use it before setting off.

Prepare a list of telephone numbers of the nearest doctors and/or hospitals.

If direct communication is not possible follow an agreed system of emergency action in case a field worker does not report in or sign-off at the end of the day.


#### Safety equipment

Thigh or chest waders; elbow or shoulder length gloves preferably with elastic arm bands; life jackets (certified); safety goggles - for use with kits; rope; spare set of clothes inclusive a towel (one set for each sampling person); mobile phone.

## 11 References

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**Appendix-table 1**

Site name	date	sample code	investigator	
	1	2	3	4
<b>MINERAL HABITATS</b> 5% steps; <u>indicate microhabitats &lt;5% with 'X', indicate artificial microhabitats with 'X' in column 'man-made'</u>	% of coverage - 5% steps	SU (number of sampling units)	Comments	'man-made'
Hygropetric Sites water layer on solid substrates				<input type="checkbox"/>
Megalithal >40 cm large cobbles, bolders and blocks, bedrock				<input type="checkbox"/>
Macrolithal >20 cm to 40 cm coarse blocks, head-sized cobbles (with variable percentages of cobbles, gravel and sand)				<input type="checkbox"/>
Mesolithal >6 cm to 20 cm fist to hand-sized cobbles (with variable percentages of gravel and sand)				<input type="checkbox"/>
Microlithal >2 cm to 6 cm coarse gravel (size of a pigeon egg to child's fist) (with variable percentages of medium to fine gravel)				<input type="checkbox"/>
Akal >0.2 cm to 2 cm fine to medium-sized gravel				<input type="checkbox"/>
Psammal >6 µm to 2 mm sand				<input type="checkbox"/>
Psammopelal mixture of sand with mud				<input type="checkbox"/>
Pelal <6 µm mud (including organic mud and sludge)				<input type="checkbox"/>
Argyllal silt, loam, clay (inorganic)				<input type="checkbox"/>
sum=	<b>100 %</b>			
<b>BIOTIC HABITATS</b> 5% steps; <u>indicate microhabitats &lt;5% with 'X', indicate artificial microhabitats with 'X' in column 'man-made'</u>	<u>only biotic habitats</u>			
Micro-algae diatoms and other algae				<input type="checkbox"/>
Macro-algae filamentous algae, algal tufts				<input type="checkbox"/>
Submerged macrophytes macrophytes, including moss and Characeae				<input type="checkbox"/>
Emergent macrophytes e.g. <i>Thypha</i> , <i>Carex</i> , <i>Phragmites</i>				<input type="checkbox"/>
Living parts of terrestrial plants fine roots, floating riparian vegetation				<input type="checkbox"/>
Xylal (wood) tree trunks (dead wood), branches, roots				<input type="checkbox"/>
CPOM deposits of coarse particulate organic matter, as e.g. fallen leaves				<input type="checkbox"/>
FPOM deposits of fine particulate organic matter, detritus				<input type="checkbox"/>
Debris organic and inorganic matter deposited within the splash zone area by wave motion and changing water levels, e.g. mussel shells, snail shells				<input type="checkbox"/>
Sewage bacteria and -fungi e.g. <i>Sphaerotilus</i> , <i>Leptomitus</i> , sulfur bacteria (e.g. <i>Beggiatoa</i> , <i>Thiothrix</i> ), sludge				<input type="checkbox"/>
sum =	<b>variable</b>			

