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# Water quality — Guidance on pro-rata Multi-Habitat sampling of benthic macro- invertebrates from wadeable rivers

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## Water quality - Guidance on pro-rata Multi-Habitat sampling of benthic macro-invertebrates from wadeable rivers

Qualité de l'eau - Lignes directrices pour l'échantillonnage des macroinvertébrés benthiques en cours d'eau peu profonds au prorata des surfaces de recouvrement des habitats présents

Wasserbeschaffenheit - Anleitung für die pro-rata Multi-Habitat-Probenahme benthischer Makroinvertebraten in Flüssen geringer Tiefe (watbar)

This European Standard was approved by CEN on 16 March 2012.

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## Foreword

This document (EN 16150:2012) has been prepared by Technical Committee CEN/TC 230 "Water analysis", the secretariat of which is held by DIN.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by October 2012, and conflicting national standards shall be withdrawn at the latest by October 2012.

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## 1 Scope

This European Standard gives guidance on procedures for 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 reference to their percentage occurrence.

The pro-rata MHS technique does not replace other techniques, but is rather, alongside other applications, a fundamental requisite of some multi-metric assessment approaches used to evaluate the ecological status of running waters. The method described in this document is one of the possible techniques among the existing pro-rata MHS techniques.

The MHS methodology is based on Rapid Bioassessment Protocols [1], the procedures of the Environment Agency for England and Wales [2], the Austrian Guidelines for the Assessment of the Saprobiological Water Quality of Rivers and Streams [3], the AQEM sampling manual [4], the AQEM & STAR site protocol [5], EN 27828, the Austrian Standards M 6232 and M 6119-2 [6], [7], the German Standard DIN 38410-1 [8] and the French Standard XP T90-333 [9].

This European Standard also describes in a detailed manner how to sample different habitats that might be suitable for sampling approaches other than Multi-Habitat-Sampling.

## 2 Normative references

The following documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

EN 27828, *Water quality — Methods of biological sampling — Guidance on handnet sampling of aquatic benthic macro-invertebrates (ISO 7828)*

## 3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

- 3.1**  
**akal**  
fine to medium-sized gravel; grain diameter > 0,2 cm to 2 cm
- 3.2**  
**argyllal**  
silt, loam, clay
- 3.3**  
**debris**  
organic and inorganic matter deposited within the splash zone area by wave-motion and changing water levels
- 3.4**  
**hygropetric sites**  
thin water layer on solid (rocky) substrates
- 3.5**  
**investigation site**  
specific area of an investigated river reach for sampling benthic organisms

**3.6**

**macro-algae**

strands of filamentous algae and algal tufts

**3.7**

**macrolithal**

coarse blocks, cobbles, gravel and sand; grain diameter > 20 cm to 40 cm

**3.8**

**megalithal**

upper sizes of large cobbles, boulders, blocks and bedrock; grain diameter > 40 cm

**3.9**

**mesolithal**

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

**3.10**

**micro-algae**

algal films

**3.11**

**microlithal**

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

**3.12**

**pelal**

mud and sludge; grain diameter < 0,06 mm

**3.13**

**psammal**

sand; grain diameter 0,06 mm to 2 mm

**3.14**

**psammopelal**

sand and mud

**3.15**

**sampling unit**

benthic sample of a specific habitat

Note 1 to entry: One Multi-Habitat sample usually consists of a fixed number of sampling units.

**3.16**

**sewage bacteria and fungi**

filaments, tufts or coverage of bacteria and fungi, to be seen with the naked eye

EXAMPLE Sphaerotilus, Leptomitius, Beggiatoa, Thiothrix.

**3.17**

**xylal**

tree trunks (dead wood), branches, roots

**3.18**

**technolithal**

solid material (usually stones) or geotextiles inserted into a river for the purposes of river engineering

## 4 Description of the sampling approach

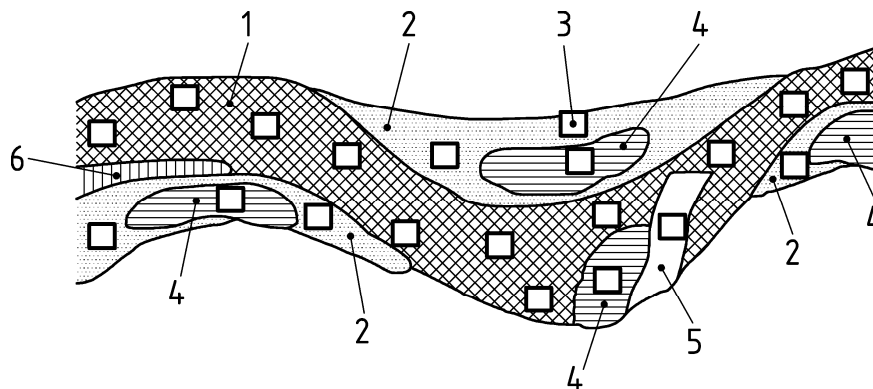
The method focuses on a multi-habitat approach, designed for sampling major habitats in proportion to their presence within a sampling reach. A sample consists of a number of sampling units (SU) taken from habitat types in relation to their spatial percentage cover. The AQEM and STAR projects [5] identified 20 "sampling units" taken from all habitat types at an investigation site, each with a share of at least 5 % spatial coverage, to be the optimum approach for ecological status assessment of wadeable rivers. Where habitat diversity is very low or taxa diversity within habitats is low and using 20 units would be an excessive repetitive sampling burden, fewer than 20 "sampling units" may be required to gain a good assessment of the ecological status. Where fewer than 20 units are used, the minimum spatial coverage should be adjusted accordingly: for example, 10 sampling units would have a minimum of 10 % spatial coverage. Throughout the rest of this document, the example of 20 "sampling units" is used to describe the approach; users should adjust numbers of unit accordingly in cases of low habitat or taxa diversity.

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 (EN 27828). Sediments are disturbed to an adequate depth that ensures capture of all species present depending on substrate diameter, compactness and 'shape' (organic substrata). For example, sediments should be disturbed to a depth of approximately

- 5 cm to 10 cm for finer substrates (psammal, pelal, fine particulate organic matter (FPOM)),
- 10 cm to 15 cm for intermediate sized substrates (akal, microlithal, coarse particulate organic matter (CPOM)), or
- 15 cm to 20 cm for 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" are withdrawn from this substrate. The categories of habitat composition follow the descriptions given in Clause 3.

If a square net of 25 cm × 25 cm is used, the sampling procedure equals a sampled area of approximately 1,25 m<sup>2</sup> of the stream bed (Figure 1).



### Key

- 1 lithal (55 % = 11 replicates)
- 2 psammal (25 % = 5 replicates)
- 3 replicate
- 4 CPOM (15 % = 3 replicates)
- 5 xylal (5 % = 1 replicate)
- 6 akal (< 5 % = 0 replicates)

**Figure 1 — Example of sampling unit position in a theoretical investigation site according to the "Multi-Habitat-Sampling" method [1]**



## 5 Field sampling procedures

### 5.1 Factors limiting effective Multi-Habitat-Sampling (MHS)

The effective use of the MHS procedure may be impaired under the following conditions which should be avoided and from which samples should not be taken (if possible):

- during or shortly after floods; consider allowing a recovery period of four to six weeks after a spate;
- during or shortly after droughts (completely dry river sections);
- 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.

The Multi-Habitat-Sampling procedure includes several steps which are described in 5.2 and 5.3.

### 5.2 Estimation of habitat composition

Before initiating sampling, complete the sampling protocol (see Annex A for an example), especially the estimation of the percentage coverage of the different habitats. 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 low visibility of parts of the river bed, this can be done during the sampling procedure. After sampling, the estimated coverage of substrates should be reviewed for accuracy and completeness.

With 20 sampling units (for example) and based on the habitats listed in Clause 4 or Table A.1, the coverage of all habitats in the river channel (including margins) with at least 5 % cover is recorded to the nearest 5 %. The presence of other habitats (< 5 % cover) is indicated by a cross.

NOTE It is advisable to divide the sampling reach into 20 m to 25 m segments, thus easing the habitat estimation.

Estimate habitat composition by referring to a suitable pro forma (for example Table A.1) and take into account the following steps:

- a) Estimation of the cover of mineral habitats: the sum of the coverage of the individual mineral habitat should be 100 % (column 1, upper part in Table A.1).
- b) 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 %).
- c) Depending on the objectives of the sampling, do not sample habitats with a cover of less than 5 % but rather indicate these with a cross.

### 5.3 Allocation of sampling units

Complete a survey specific pro forma (for example, Sampling Protocol II, Table A.2) in order to define the number and the allocation of sampling units (see Annex A).

To allocate the sampling units, consider the mineral (3.1) and the biotic (3.2) habitats as one layer, thus combining the biotic habitat estimation with the mineral substrate estimation. This ensures that samples taken from biotic habitats include the underlying (subjacent) mineral substrates. The sum of the cover of all habitats (mineral and biotic) should be 100 %. If the conditions allow, estimate the cover of mineral and biotic habitats in one step. This procedure may be helpful if a high proportion of biotic habitats are present in the river and if consequently problems in assessing the percentage of the underlying mineral substrates arise.

The allocation of the 20 sampling units (for example) follows the combined (mineral and biotic) habitat estimation (5 % coverage equals 1 sampling unit) using a suitable pro forma (see Table A.2).

- a) Copy the portion (percentage) of mineral habitats (5 % steps) from Table A.1 to row "mineral habitats" in Table A.2.
- b) Copy the portion (percentage) of biotic habitats (5 % steps) from Table A.1 to column "biotic habitats" in Table A.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 "%") in Table A.2.
- d) If mineral and biotic habitats are combined (e.g. FPOM on psammal or macro-algae on mesolithal), enter the percentages in the corresponding cells of the column "%" in Table A.2.
- e) If biotic habitats cannot be properly allocated to an underlying mineral habitat (bottom or margins, e.g. roots or macrophytes), enter their percentage of cover in the column "not allocable" in Table A.2. In such a case, subtract this number of sampling units 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, enter the final number of sampling units in the different columns "SU", converting the percentages of the "%"-columns into sampling units (5 % coverage equals 1 sampling unit).

**EXAMPLE** If the overall cover of mesolithal is 40 % (8 sampling units) and macro-algae are attached to 10 % of the mesolithal, take 6 sampling units from the bare mesolithal, and 2 sampling units from mesolithal with attached macro-algae.

The final allocation of sampling units of a given habitat represents the overall structure of an investigation site.

Sampling units should also be adequately distributed between bed and banks and lentic and lotic sites as well as riffles and pools. It is particularly important that samples from biotic layers reflect the distribution of the underlying mineral substrates. The column "comments" in the Table A.1 can be used, for example, to indicate the proportion of lentic or lotic areas within the substrate coverage and the near-margin or in-stream situation, respectively.

Indicate man made substrates (e.g. technolithal = rip-rap) in the column "man-made" (see Annex A).

## **6 Detailed description of sampling procedures**

### **6.1 General recommendations for sampling**

The investigation site should be representative of the river section and be suited to the purpose of the study.

Sampling starts at the downstream end of the reach and proceeds upstream.

When sampling the "sampling units", use the hand-net either as a kick-net, or for "jabbing", "dipping" or "sweeping", or a combination of these. Controlled sampling by hand is the preferred method (see details in Clause 7). 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 of the sampler's feet (sampling area), and disturb the stream bed vigorously by kicking or rotating the heel of the boot to dislodge the substratum and the fauna.

After every three sampling units (or more frequently if necessary), rinse the collected material by running clean water through the net two to three times as clogging may prevent the collection of a representative sample. In such cases, discard the material in the net and choose another sampling unit in the same habitat type but at a different location. The final Multi-Habitat sample consists of the pooled 20 sampling units. If the scope of the study is based on different sampling designs, the sampling units can be treated separately or pooled according to other characteristics (e.g. riffles-pools, habitat types, etc.).

## 6.2 Megalithal (bedrock and boulders)

The sampling strategies for boulders depends 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 and then sweeping the animals into the net.

Individual samples of boulder substrates should be allocated to different positions (front, side, etc.) for different sampling units. If only one sampling unit in boulders is allocated, three positions (front, right side and left side) may be combined for this single sampling unit. However, the sampling should also consider the extent of algal cover on the boulders.

## 6.3 Macrolithal and mesolithal (cobbles, stones, pebbles)

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

The remaining substrate is disturbed in the 0,25 m × 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 cm 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 (such as a Hess- or Surber sampler with an appropriate sampling area) may 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 may be disturbed by hand and water may be forced through the net to trap the animals. It is possible to use different devices for different microhabitats, as long as the same required area is sampled.

## 6.4 Microlithal and smaller mineral substrates

Disturb the substrate in the 0,25 m × 0,25 m area upstream of the net. To dislodge the animals from the interstices of the sediments, disturb the substrate with a screwdriver or similar tool up to a depth of about 5 cm to 15 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. Take care to minimise the quantity of sand in the samples.

Sample areas of un-vegetated or soft substrate 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 then 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 (such as a Hess- or Surber-Sampler) may be used instead of a hand-net.

In lentic areas, disturb the sediment within the sampling area by hand in the normal fashion; then create a current 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 required area is sampled.

## 6.5 Xylal (woody debris)

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

## 6.6 Roots

Sweeping followed by vigorous shaking can be effective.

## 6.7 Coarse Particulate Organic Matter (CPOM/leaf litter)

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

## 6.8 Macrophytes (emergent and submerged)

Macrophytes should be taken to the laboratory for further examinations because organisms such as *Simuliidae* and some chironomid tubes (e.g. *Rheotanytarsus*) cannot be washed off completely in the field. The quantitative sampling of an adequate area of the macrophyte stand with adequate portions of roots, stems and leaves is recommended instead of taking some sweeps with the hand net.

## 6.9 Technolithal

A variety of techniques may be required depending on the nature of the man-made substrate.

# 7 Sample treatment

## 7.1 Removal of large material and sorting

Branches, sticks and stones may be removed after being rinsed and inspected for clinging or sessile organisms. Any organisms found should be placed into the sample container. It is not recommended to spend time inspecting small debris in the field. However, larger and more 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). These organisms should then be stored in small separate containers with no substrate.

## 7.2 Removal of large organisms

Large and rare organisms which are easily identifiable on-site (such as large mussels or crayfish) should be recorded in the field, removed from the sample and returned to the stream.

## 7.3 Storage

Immediately after collection, transfer the sample from the net to the sample container(s) and preserve with formalin (4 % final concentration of formaldehyde) or in sufficient ethanol (95 %) to cover the sample completely. The final ethanol concentration should be about 70 %. When using ethanol, water in the sample should be decanted before adding the fixation liquid. Fixation is important to prevent carnivores, particularly stoneflies (*Setipalpia* [= *Systellognatha*]), beetles (*Adephaga*), caddis larvae (e.g. *Rhyacophilidae*), *Sialidae* and certain *Gammaridae* from eating other organisms. Forceps may be needed to remove organisms from the dip net. Tightly close the sample container and store the samples in cooled conditions.

## 7.4 Labelling

Place a label (written in pencil, printed on a laser printer or photocopied) inside the sample container indicating the following information: project (optional); stream name; site name and location; site code (optional); date of sampling; habitat, riffle or pool section; sampling gear, fraction; sampler'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, label with all the necessary information and number each container (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 on the label placed

in the sample containers as well as in the sample pro forma. If possible, label and place the container with the rare and fragile organisms into the main sample container and note their existence in the sampling protocol.

For health and safety reasons, formalin is often not used. However, it is known to be the most effective fixative for fresh water macro-invertebrate samples [2]. If a laboratory cannot use formalin and the sample has been preserved with 95 % ethanol, it should be re-preserved in the laboratory in fresh alcohol. The sample can then be stored for several months before analysis.

### **7.5 Refining the site-protocol**

Refine the sampling protocol, particularly the portion of microhabitats after the sampling has been completed. After having sampled the various microhabitats, walking the reach helps to ensure a more accurate assessment. Note the sampling gear used and comment on the conditions during sampling, e.g. high flows, treacherous rocks, difficult access to stream, or anything else that could indicate an influence on the sample composition.

## Annex A (informative)

### Sampling protocols

Table A.1 — Sampling protocol 1<sup>1)</sup>

Site name	Date		Investigator	
	1	2	3	
<b>MINERAL HABITATS</b> 5% steps; indicate microhabitats < 5 % with 'X', indicate artificial microhabitats with 'X' in column 'man-made'	% coverage - 5 % steps		Comments	'man-made'
<b>Hygropetric Sites</b> water layer on solid substrates				<input type="checkbox"/>
<b>Megalithal</b> > 40 cm large cobbles, boulders and blocks, bedrock				<input type="checkbox"/>
<b>Macrolithal</b> > 20 cm to 40 cm coarse blocks, head-sized cobbles (with variable percentages of cobbles, gravel and sand)				<input type="checkbox"/>
<b>Mesolithal</b> > 6 cm to 20 cm fist to hand-sized cobbles (with variable percentages of gravel and sand)				<input type="checkbox"/>
<b>Microlithal</b> > 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"/>
<b>Akal</b> > 0,2 cm to 2 cm fine to medium-sized gravel				<input type="checkbox"/>
<b>Psammal</b> > 6 µm to 2 mm sand				<input type="checkbox"/>
<b>Psammopelal</b> mixture of sand with mud				<input type="checkbox"/>
<b>Pelal</b> < 6 µm mud (including organic mud and sludge)				<input type="checkbox"/>
<b>Argyllal</b> silt, loam, clay (inorganic)				<input type="checkbox"/>
<b>Sum</b>	<b>100 %</b>			
<b>BIOTIC HABITATS</b>				
5 % steps; indicate microhabitats < 5 % with 'X', indicate artificial microhabitats with 'X' in column 'man-made'	<u>only biotic</u> habitats			
<b>Micro-algae</b> diatoms and other algae				<input type="checkbox"/>
<b>Macro-algae</b> filamentous algae, algal tufts				<input type="checkbox"/>
<b>Submerged macrophytes</b> macrophytes, including moss and <i>Characeae</i>				<input type="checkbox"/>
<b>Emergent macrophytes</b> e. g. <i>Thypha</i> , <i>Carex</i> , <i>Phragmites</i>				<input type="checkbox"/>
<b>Living parts of terrestrial plants</b> fine roots, floating riparian vegetation				<input type="checkbox"/>
<b>Xylal (wood)</b> tree trunks (dead wood), branches, roots				<input type="checkbox"/>
<b>CPOM</b> deposits of coarse particulate organic matter, as e. g. fallen leaves				<input type="checkbox"/>
<b>FPOM</b> deposits of fine particulate organic matter				<input type="checkbox"/>
<b>Debris</b> 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"/>
<b>Sewage bacteria and -fungi</b> e. g. <i>Sphaerotilus</i> , <i>Leptomitus</i> , sulfur bacteria (e. g. <i>Beggiatoa</i> , <i>Thiothrix</i> ), sludge				<input type="checkbox"/>
<b>Sum</b>	variable			

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Table A.2 — Sampling protocol II<sup>2)</sup>

		MINERAL HABITATS – percent coverage (sum: 100 %)																					
		hygropetric sites		megalithal > 40 cm		macrolithal > 20 cm to 40 cm		mesolithal > 6 cm to 20 cm		microlithal > 2 cm to 6 cm		akal > 0,2 cm to 2 cm		psammal > 6 µm to 2 mm		psammopelal		pelal < 6 µm		Argyllal < 6 µm		not allocable	
		%	SU	%	SU	%	SU	%	SU	%	SU	%	SU	%	SU	%	SU	%	SU	%	SU	%	SU
BIOTIC HABITATS - percent coverage (sum variable)	bare mineral substrate																						
	micro-algae																						
	macro-algae																						
	submerged macrophytes																						
	emergent macrophytes																						
	living parts of terrestrial plants																						
	xylal																						
	CPOM																						
	FPOM																						
	debris																						
sewage, bacteria and -fungi																							

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