The AQEM sampling method to be applied in STAR

Document history

1.) base: AQEM CONSORTIUM (2002)[1].(chapters 7 and 8)

2.) adaptation to the requirements of the STAR project (20/4/02)

- the distribution of sampling units is described more detailed;
- the sampling of selected microhabitats is described more detailed;

3.) further refinement after the sampling workshop in La Bresse (11/5/02)

- a revised form for estimating the microhabitat cover is included (more or less coherent to the original version used in AQEM)
- the allocation of sampling units is described in a different way (preparation, point 3)
- the sampling of selected microhabitats is described more detailed
- a subsampling procedure is described more detailed
- several of the short subchapters under "sample processing" have been deleted or included in the chapter "subsampling and sorting"
- several minor additions have been included
- terminology has been changed: pools → lentic sites; riffles → lotic sites; replicates → sampling units
- the annex has been changed to a short description of sampling unit allocation, to be used in the field
- lentic and lotic sampling units are added to one final sample containg all the 20 sampling units
- the fine and coarse fraction is subsampled together
- sorting of the complete samples can only be done on "dead" organisms in the laboratory
- defining the length of the sampling site has been slightly altered
- the use of low power magnification when sorting the sample is allowed if necessary

[1] AQEM CONSORTIUM (2002). Manual for the application of the AQEM system. A comprehensive method to assess European streams using benthic macroinvertebrates, developed for the purpose of the Water Framework Directive. Version 1.0, February 2002.

SAMPLING

This chapter describes the AQEM procedures for collecting and examining macroinvertebrate samples to be applied in the STAR project. The methods are based on the Rapid Bioassessment Protocols (BARBOUR et al. 1999), the procedures of the Environment Agency (ENVIRONMENT AGENCY 1999a), the Austrian Guidelines "Saprobiology" (MOOG et al. 1999) and ISO 7828. These guidelines have been tested and adapted by the AQEM partners to provide standardised procedures for collecting and analysing macroinvertebrate samples within the AQEM stream assessment procedures and be further adapted to meet the requirements of the STAR project.
The description does not aim at, nor is it able of competing with or replacing the references cited above. The information given here focuses on the application of the AQEM approach to guarantee a standardised procedure.

Sampling season

In general no samples should be taken

- during or shortly after floods
- during or shortly after droughts
- during any other man-induced or natural disturbances
- if unnatural turbidity prevents a proper sampling of the stream bottom.

The preferred sampling seasons for each individual stream type are given in the AQEM manual.

Sampling gear

Either a hand-net/shovel sampler or a Surber sampler must be used.

Hand-Net/Shovel Sampler

- Shape of the frame: D-Frame (shaped as a "D") or rectangular. A frame in front of the hand net of 625 cm^2 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.

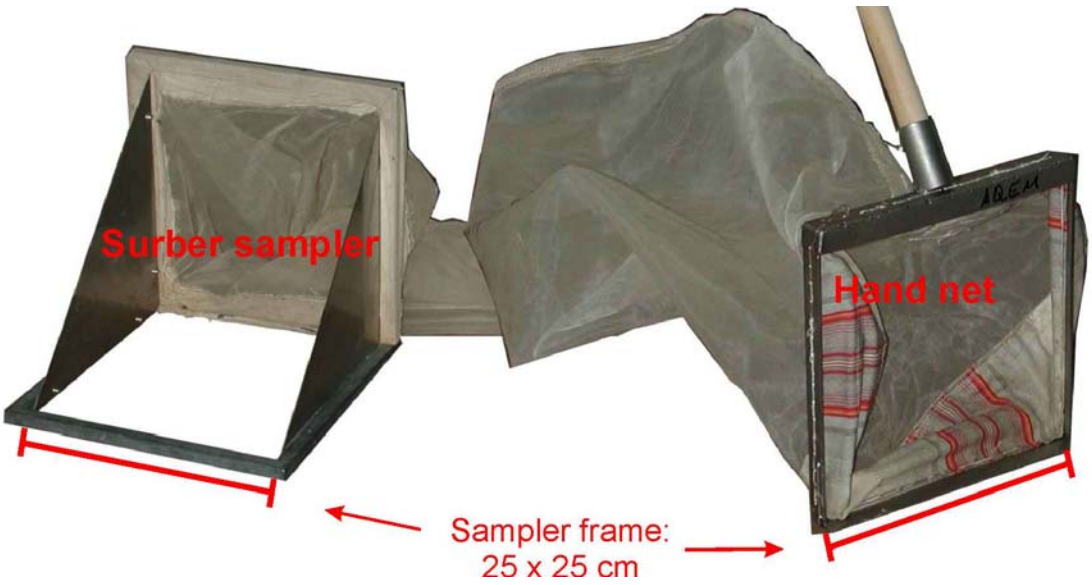

FIGURE 1: Surber sampler and hand-net.

Surber Sampler

For sampling stony substrates an open Surber Sampler, with lateral metal boundaries or no-box frame can be used in place of a hand-net. Dimensions of the sampler are 0.25 m x 0.25 m. The sampler is horizontally placed on the substrate to delineate a 0.0625 m^2 area. The net attaches to the vertical brace of the frame and captures the dislodged organisms from the sampling area. The sampling frame is also surrounded by a box frame with 500 µm meshes. The use of the open Surber sampler (without a net frame) is usually restricted to shallow, fast-flowing habitats; in standing or lentic zones a hand-net should be used instead .

Field sampling procedures

The AQEM method is based on BARBOUR et al. (1999) and focuses on a multi-habitat scheme designed for sampling major habitats proportionally according to their presence within a sampling reach.

A sample consists of 20 “sampling units” taken from all microhabitat types at the sampling site with a share of at least 5% coverage. A “sampling unit” is a stationary sampling accomplished by positioning the net and disturbing the substrate for a distance that equals the square of the frame width upstream of the net (0.25 x 0.25 m). The 20 “sampling units” must be distributed according to the share of microhabitats. For example, if the habitat in the sampling reach is 50% psammal (sand), then 10 “sampling units” must be taken there. The categories of microhabitat composition are to be taken from the site protocol (parameters 23 and 24). This procedure results in sampling of approximately 1.25 m^2 stream bottom area.

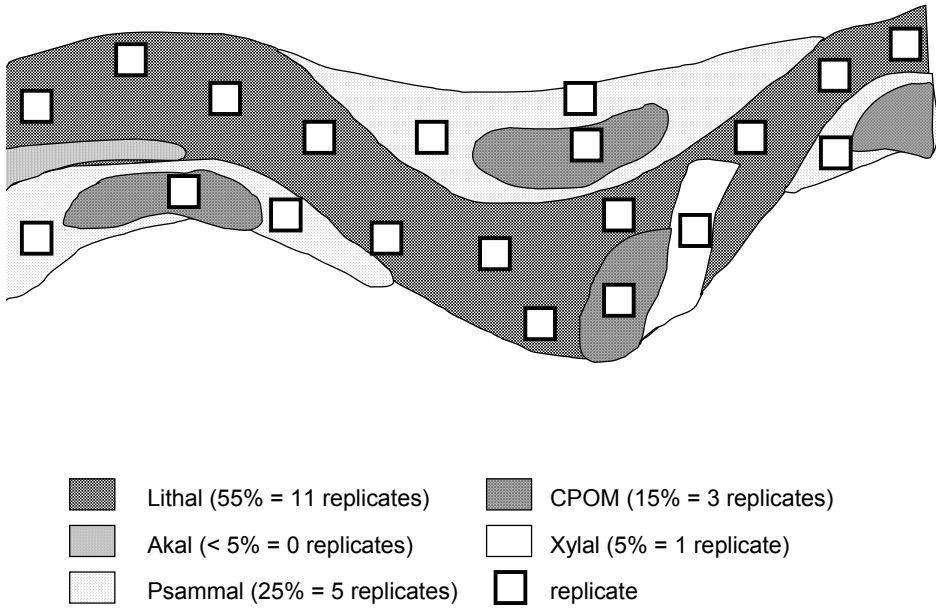

FIGURE 2: Example of sampling unit position in a theoretical sampling site according to the „multi habitat sampling“ method applied in AQEM.

More specifically, the “multi-habitat sampling“ procedure is performed in the following steps:

Preparation

1. **Select an appropriate sampling site** (compare chapter 5 AQEM manual). The lenght of the sampling site depends on the stream width and the variability of the habitats. As a general rule it should 20 to 50 metres in length in small (1-100 km^2) streams and 50-100 metres in length in medium (100-1000 km^2) streams. The sampling site must cover the whole width of the stream and be representative for a minimum survey area of 500 meters stream length or 100 x average stream width, whichever is longer.

2. Before sampling, the **site protocol** should be completed (compare Chapter 6 AQEM manual). However, the sampling area should not be disturbed by physical contact, if at all possible. Therefore, after sampling, this information should be reviewed for accuracy and completeness (step 12).

3. 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, the presence of other microhabitats (<5% cover) is only indicated (see form in the Annex).

   If you have problems to estimate microhabitat composition from the bank, it is allowed to enter the stream (one access point per 20 metre stream length). However, the area accessed must not be sampled. Since especially the proportion of the rare habitats around 5% coverage is difficult to estimate, the estimation of microhabitat cover might be refined during sampling.

The estimation of microhabitat composition comprises the following steps:

- Estimation of the cover of mineral substrates; the sum of the coverage of the individual mineral microhabitats must be 100% (1st column of the table on the next page, upper part).
- Estimation of the cover of biotic microhabitats (seen as an additional layer); the sum of the coverage of the individual biotic microhabitats is variable (0 to 100%) (1st column, lower part).
- For step (3.) (distribution of sample replicates) mineral and biotic microhabitats together are regarded as just one layer. The sum of the cover of ***all*** microhabitats (mineral and biotic) must be 100% (2nd column).
- Based on estimation of microhabitat coverage (3.) the **number of sampling units** in the individual habitats is determined and indicated in the site protocol (3rd column). For example, if a sampling site consists of 50% mesolithal (pebbles and stones), 25% psammal (sand) and 25% CPOM, then 10 sampling units should be taken in the mesolithal, 5 sampling units in the psammal and 5 sampling units in the CPOM. Every microhabitat covering at least 5% of the stream bed must be sampled.
- For the mineral substrates it must be indicated, whether they are artificial (e.g. 'technolithal' = riprap) (4th column).

When allocating your sampling units you should take into consideration that microhabitats may be unequally distributed between:

- Lentic and lotic sites; the distribution of sampling units in an individual microhabitat should, therefore, reflect its proportion in lentic and lotic zones.
- The sampling units in habitats with a very high proportional coverage (e.g. mesolithal in lotic sites) should be distributed between the stream margin and central zones of the stream bed, e.g. in form of a transect.
- The distribution of sampling units in organic microhabitats should consider, which mineral substrates they are covering. E.g., if submerged macrophytes are equally distributed on macrolithal and akal, half of the macrophyte sampling units should be taken in macrophytes growing on macrolithal, and the other half in macrophytes growing on akal.

Sampling

4. **Sampling** starts at the downstream end of the reach and proceeds upstream.
5. When **sampling the "sampling units"** use the hand-net either as a kick net, or for "jabbing", "dipping" or "sweeping". When kick-sampling, hold the net vertically with the frame at right angles to the current, downstream from your feet, and disturb the stream bed vigorously by kicking or rotating the heel of your boot to dislodge the substratum and the fauna within a depth of at least 10-15 cm. Disturb the substrate in the 0.25 x 0.25 m area upstream of the net. 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 drop before entering the net. Move cob-

bles and large stones by hand, sweep or brush the surfaces to dislodge clingers and sessile organisms. It is recommended to deposit wood and cobbles in a plastic bucket for a later inspection to remove adhering animals by hand-picking with forceps. To dislodge the animals from the interstices of the sediments, the substrate should be disturbed with a screwdriver or similar device. The surface of soft sediments and fine or organic microhabitats should be sampled by pushing the hand-net gently through the uppermost 2-5 cm of the substratum. In shallow waters with a strong current an open Surber sampler can be used instead of a hand-net. To sample with an open Surber sampler in slow-flowing areas the sediment within the Surber frame can be disturbed using the hands, in the normal fashion, and then a current created by pushing water through the net with the hands to trap the animals. It is possible to use different devices for different microhabitats, as long as the same area is sampled.

Some further comments on sampling selected microhabitats:

- Stones with noticeable algae cover can also be considered as part of mineral substrate. Algae that grow in tufts or bigger fractions are considered as organic habitat.
- Megalithal (boulders): It is recommended to sample different positions (frontal, sideways) for different sampling units.
- Xylal (woody debris): Avoid relatively new deadfall that lacks microbial conditioning. Washing the samples into a bucket is effective. Alternatively: take the woody debris out, spray on a net, lay down in sun and pick the animals. Sweeping followed by vigorous shaking is effective for roots.
- CPOM (leaf litter): wash carefully in field, avoid taking a large amount of leaves to the lab.
- Macrophytes should be searched for pupae and taken to the lab for further examinations, because espacially Simuliidae can not be washed off in the field. The sampling of an adequate area of the macrophyte stand is recommended instead of taking some sweeps with the hand net. For this purpose, macrophytes covering an area of 25 x 25 cm must be cut and transferred into the net. The sediments below the macrophytes must be sampled, too. This procedure is to be applied for both, floating and emergent macrophytes.
- Microlithal/Akal: Care should be taken to minimise the amount of sand in the sample.
- Deep parts of a river which can not be sampled by moving cobbles and large stones by hand, must be sampled by kicking the substrate. For this purpose, hold the net vertically with the frame at right angles to the current, downstream from your feet, and disturb the stream bed vigorously by kicking or rotating the heel of your boot to dislodge the substratum and the fauna within a depth of at least 10-15 cm.

6. **Rinsing**: After 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. If clogging occurs, 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.

FIGURE 3: Using a hand-net for jabbing and sweeping.

FIGURE 4: Kick-sampling.

Follow-up treatment

7. **Removal of large material and sorting**: Large wood and stones can be removed after being rinsed and inspected for clinging or sessile organisms. Any organisms found have to be placed into the sample container. Generally, it is recommended not to spend time inspecting small debris in the field; however, large and fragile organisms (e.g. Ephemeroptera) or species that cannot be preserved (e.g. Tricladida, Oligochaeta) should could be picked out of the sample in the field (a maximum of 30 representative organisms in total). These organisms should be stored in a small separate container containing only organisms but no substrate. They must be kept separately for auditing purposes.
8. **Removal of large organisms**: Large and rare organisms, which can easily be determined in the field (such as large mussels), should be removed from the sample and be placed back in the stream.
9. **Sieving**: [deleted; see new desription in Chapter “sample processing”]
10. **Storing**: Transfer the sample from the net to sample container(s) and preserve with formalin (4% final concentration) or in enough 95% ethanol to cover the sample immediately after collection. This form of fixation is important to prevent carnivores, particularly stoneflies (Setipalpia), beetles (Adephaga), caddis larvae (e.g. Rhyacophilidae), Sialidae and certain Gammaridae, from eating 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 close tightly. The samples should be stored cool. The whole sample (lentic and lotic parts) should be lumped together and treated as one sample.

11. **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 (optional)
    - date of sampling
    - investigators name (optional)

    The outside of the container should include the same information and the words "preservative: formalin 4%, 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, etc.). If rare taxa (e.g. crayfish, large mussels) have been identified in the field and returned to the river (step 9), record their presence and abundance on the label placed in the sample containers as well as on 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.
12. **Refine the site protocol**, particularly the share of microhabitats, after sampling has been completed. Having sampled the various microhabitats and walked the reach helps ensure a more accurate assessment. Note the sampling gear used, and comment on conditions of the sampling, e.g. high flows, treacherous rocks, difficult access to stream, or anything that would indicate adverse sampling conditions.

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

Safety

Fieldwork always holds a 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 regarded when selecting a sampling site.

- Never take samples alone. When taking samples always be accompanied by at least one other person that can help you.
- The attending person should have clear sight of the sampling person at all times.

- Do not take samples when the conditions at a sampling site may be dangerous. In particular you should
    - avoid sampling rivers in flood conditions
    - avoid sampling during severely cold conditions
    - avoid steep or unstable banks
    - check depth and stability of the river bottom
    - watch out for hazards (broken glass, sharp metals etc.).
    - Wear a life jacket when sampling either in deep rivers, upstream from weirs or deep lentic sites, in streams with strong current, or during extremely cold conditions with bottom ice. 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 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 sampler)
- mobile phone

Quality control in the field

- Sample labels must be completed properly, including the sample identification code, date, stream name, sampling location, and collector's name, 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 in 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 cray-fish plagues.

SAMPLE PROCESSING

Subsampling and sorting

Rationale: Subsampling

- reduces the effort required for sorting and identification
- provides an unbiased representation of a large sample
- provides a more accurate estimate of time expenditure
- thus, it reduces costs for the process of macro-invertebrate samples

The following subsampling procedure is based on CATON (1991).

Subsampling devices

The subsampling device comprises (see. figures 5 and 6):

- the (inner) gridded pan with 6 x 5 grids, each 6 x 6 cm, and a 500 µm mesh bottom
- the outer tray (watertight) to facilitate homogenization of the sample
- “cookie cutter” (6 x 6 cm)
- small shovel or a teaspoon to remove the material from the grids
- pair of dices or a random number table

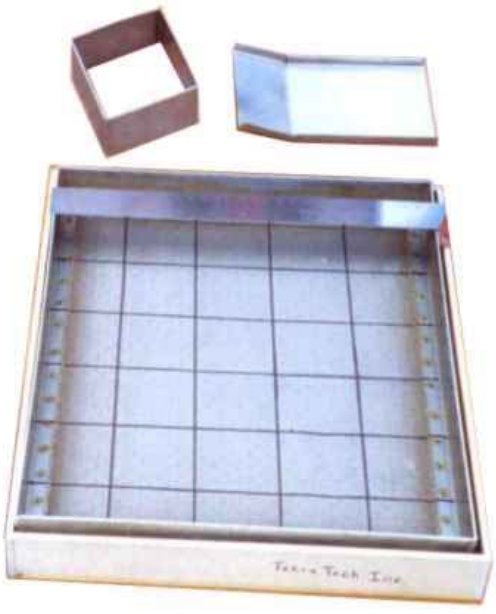

FIGURE 5: Subsampling gear: pan, grid and devices to remove debris from selected grids.

FIGURE 6: Subsampling procedure.

The following steps of subsampling are to be processed in the lab under controlled conditions:

1. If the sample was stored in more than one container, the contents of all containers for a given sample are combined.
2. The preservative is carefully decanted from the preserved sample through a 500 µm sieve. Inspect the sieve for any organisms and retrieve attached organisms.
3. Thouroughly rinse the sample over a 500 µm sieve to remove preservative and fine sediment. It is particularly recommended to follow this step if the samples are preserved with Formalin. Avoid direct contact with formalin! Do not use too much pressure on the water in order not to force the specimens through the sieve mesh.
4. Transfer the washed material into the gridded pan (inner pan) and homogenise the material. Therefore, place the gridded pan into the outer tray and add a sufficient amount of water to facilitate the procedure. Spread the sample material evenly throughout the gridded pan. Move the material also into the corner. Do not remove large or rare species from the pan.
5. If macrophytes are included into the sample they have to be treated the following way: If no sessile animals are attached the macrophytes have to be rinsed in a bucket by vigorously shaking and need to be controlled for remaining specimen afterwards. If few sessile organisms are attached, they should be removed by hand and treated as above. If many sessile organisms are attached, the macrophytes have to be sub- sampled in the pan.
6. Lift the gridded pan out of the outer tray to drain. Pour the water off the tray, but leave just enough water to keep the bottom of the gridded pan moist when it is returned to the tray.
7. Use dices or a random number table to select a grid to process.

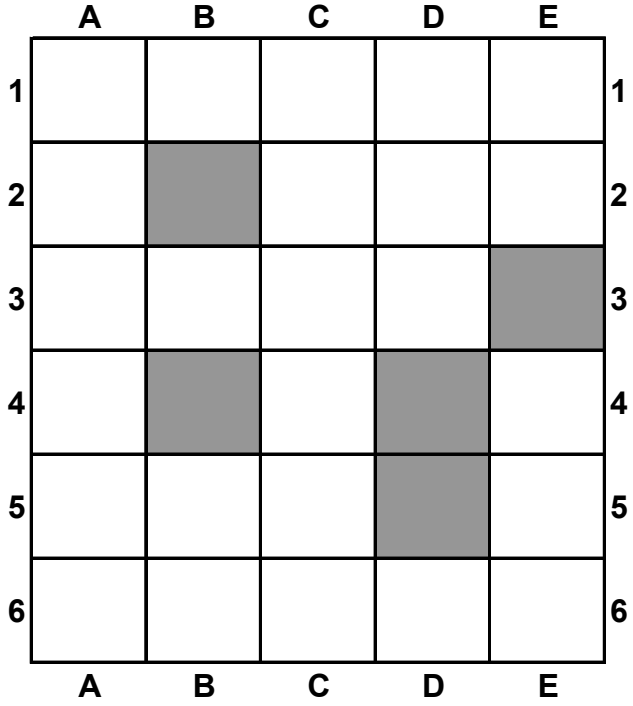

Figure 7: Subsample pan with 5-grids to be subsampled at least (= 1/6th of the whole sample) Grids to be processed: B2, B4, D4, D5 and E3.

8. Remove all the material from that grid with a scoop or teaspoon, and place the removed material into a separate white tray. Repeat this process until 5 grids have been processed (5 grids represent a 6^{th} of the total sample material).
9. It might be necessary to cut the material along the outside of the "cookie cutter" with scissors to push the cookie cutter carefully onto the bottom of the gridded pan. Inspect the bottom for any remaining organisms.
10. If there appear to be a number of organisms that equal or exceed the targeted number (700 ind.)[2], then subsampling is finished. Sort out the whole 5-grid composite sample. Place the sorted organisms in vials with sufficient preservative, and label every vial and the sorted material accurately for further processing. The animals sorted should be separated into systematic units.
11. If the 5-grid composite subsample contains less than 700 organisms, then randomly select and process one additional grid. Pick out the specimens from the material. Repeat this process until the targeted number of 700 organisms is achieved. Remember: Each grid once begun has to be sorted out completely, even if the 700^{th} organism is counted halfway.
12. While sorting out the subsampled material, prevent the remainder material in the gridded pan from desiccation. This can be done by covering it with aluminium foil. It is recommended to periodically moisten the sample with water from a spray bottle if necessary.
13. Avoid disturbance of the subsampling device in order not to redistribute the sampling material and containing organisms between two subsampling operations.

[2] For certain river types, with very low densities of organisms, the targeted number can be reduced. The expert panel must be consulted on forehand.

Sorting rules

Some rules for the removal of organisms:

- An organism belongs to the grid containing its head.
- If the head is difficult to locate, the organism is considered to be in the grid containing most of its body.
- If the head of an organism lies on the line between two grids, all organisms on the top of a grid and those on the right side of a grid belong to that grid.
- The animals sorted in the lab should be separated into systematic units.

Do not count

- empty snail or mussel shells, empty cases of caddis
- exuviae and fragments such as legs, antennae, gills or wings,
- for Oligochaeta try to remove and count only whole specimens or fragments that include the head.

Note the total number of processed grids and the number of organisms contained in the grids in the laboratory bench sheet.

Storing and labelling

Store separately:

1. The organims sorted from the subsample (≥ 700 organisms), separated into systematic units. Labels contain: sample number, date of sampling, name of the person, who has taken the sample, date of sorting, number of grids sorted, “subsample”, name of the person sorting the sample, systematic unit.
2. Stones, sand and debris from the sorted subsample. Labels contain: sample number, date of sampling, name of the person, who has taken the sample, date of sorting, number of grids sorted, “subsample”, name of the person sorting the sample.
3. The 30 organisms sorted in the field. Labels contain: sample number, date of sampling, name of the person, who has taken the sample, “fraction sorted in the field”.
4. The remaining fraction, which has not been sorted (sand, debris and organisms). Labels contain: sample number, date of sampling, name of the person, who has taken the sample, date of sorting, number of grids sorted, name of the person sorting the sample.

Since some partners aim to sort the complete sample, fraction 4. can be sorted in a separate step. In this case, fraction 4. is split into:

5. All organisms sorted from the remaining fraction (without those grids, from which 700 organisms have been sorted). Labels contain: sample number, date of sampling, name of the person, who has taken the sample, date of

sorting,"remaining fraction", name of the person sorting the sample, systematic unit.

6. Stones, sand and debris from the sorted "remaining fraction". Labels contain: sample number, date of sampling, name of the person, who has taken the sample, date of sorting, number of grids sorted, "remaining fraction", name of the person sorting the sample.

Since it has not been decided about the process of auditing, all 4 or 5 fractions have to be kept and stored.

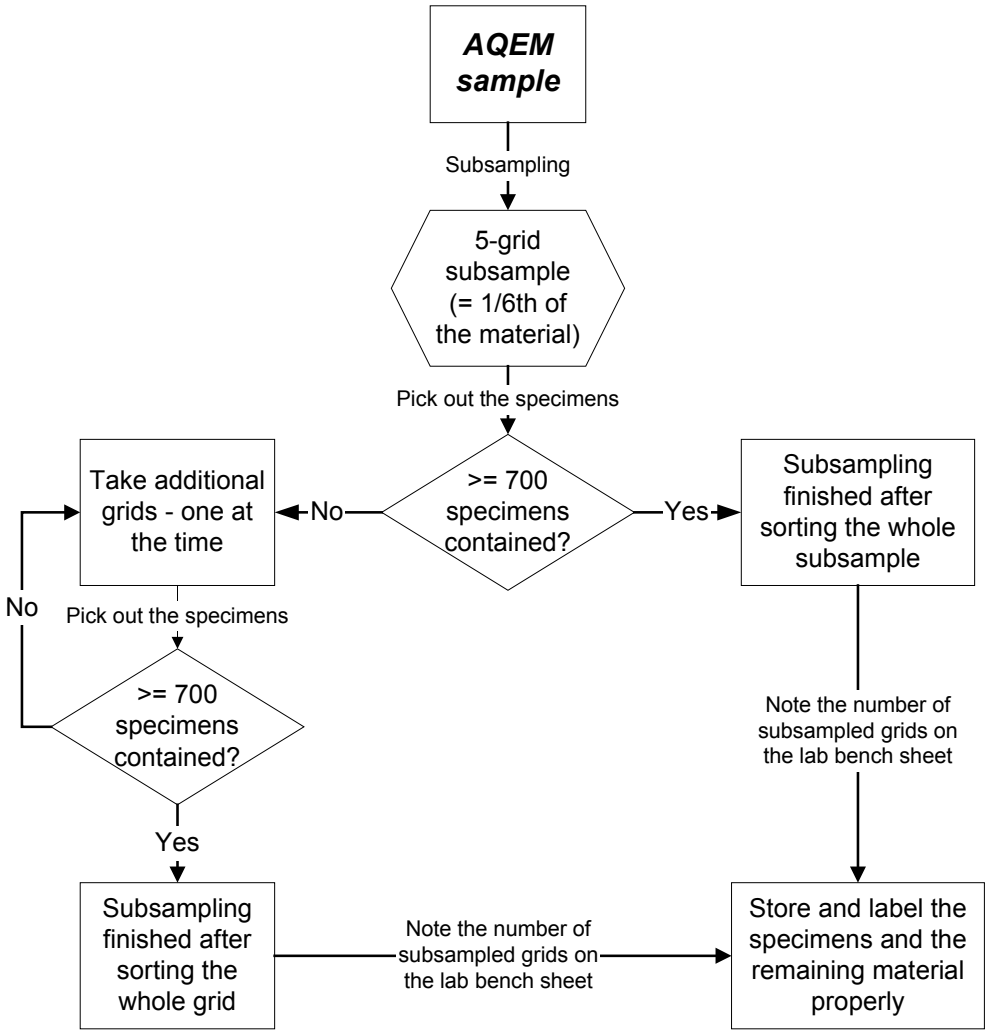

Figure 8: Flowchart of the subsampling procedure. The example is based on the original subsampler after (Caton 1991) with 30 grids, each 6 x 6 cm. A minimum amount of 1/6th of the material has to be subsampled, containing a minimum number of 700 specimens.

Quality assurance and quality control in the lab

(partly based on: ENVIRONMENT AGENCY *1999a)*

The aims of quality assurance and control in the lab are to minimise errors in the treatment of biological samples and thus secure the validity of the biological assessment results. One must distinguish between the general improvement of the treatment of the samples in the laboratory (as a part of "quality assurance") and the quality control by an auditor. This chapter does not cover any aspects of auditing.

Important elements of quality assurance in the lab are:

Treatment of the samples during the process of sieving and sorting

In order to minimise damage to specimens in the process of sieving, e.g. loss of gills, legs and tails

- rinse very gently and never use a high-pressure spray when you separate specimens from substratum e.g. by means of a hose attached to the tap
- never swirl the sample violently in a bucket or sieve
- decant water very carefully.
- If necessary use low power magnification when sorting the sample, the important thing is that *all* organisms in the subsample is picked out of the sample

When picking out the specimens from the sieved samples a soft pair of tweezers should be used in order to minimise damage to the animals.

Identification level and taxonomic nomenclature

To apply the assessment system correctly, the required minimal level of identification must be achieved for all specimens (for the different levels see AQEM manual). The nomenclature and the taxa list to be used are provided by the digital version of the AQEM data base (available from www.aqem.de).

Identification

The correct identification of the specimens according to the level and nomenclature required is crucial for the correct application of the system. Use only state-of-the-art-determination literature as specified in the AQEM manual. All aquatic macroinvertebrates in the sample, including caddis and Dipteran pupae, have to be identified to the given taxonomic level. Terrestrial or aerial stages of aquatic animals, empty mollusc shells, exuviae, empty puparia, empty caddis cases and eggs are not part of the samples. Fragments of damaged specimens can cause errors particularly in the calculation of abundances. In case of fragments use only head *and* thorax or thorax *and* abdomen parts, not single heads, single abdomens, legs or other smaller parts.

Use suitable and regularly serviced binoculars and microscopes for identification (minimum magnification 100 x). The work area should be well illuminated; especially

the lighting of the animals under the binoculars should be good. Cover the specimens with enough liquid to avoid reflections of light.
Sometimes it may be necessary to break mollusc shells and poke caddis cases to check for occupants.

Fitness of the lab personal staff

Sorting usually is a hard and very time-consuming work. Samples, which are easy to sort may be finished in two hours, laborious samples may consume more time. The work area should always be well lit and health implications should be considered during the whole process of sample treatment in the lab. A good physical condition during the process of treating the samples contributes to a good result in the process of biological evaluation. Short, regular breaks from the sorting every hour are highly recommended. If the tray is left for longer breaks, the sample should be covered completely in order to reduce evaporation.

Controlling methods

AQEM is not suggesting a detailed controlling or auditing system. However, particularly for larger monitoring programmes, a controlling and auditing system is highly recommended.
A very well-suited system for controlling sorting errors has been described by ENVIRONMENT AGENCY (1999b). Auditing needs to be undertaken for a set number of samples by a person or institution independent of those whose work is being audited.