Freshwater macro-invertebrate analysis of riverine samples

Operational instruction 024_08 **Issued 28 January 2014**

What's this document about?

Describes how to analyse samples of river macro-invertebrates. It is the fourth in a series of documents related to macro-invertebrate monitoring in rivers.

Who does this apply to?

- Environmental Monitoring (Analysis and Reporting) teams
- Any staff or contractors analysing invertebrate samples

Contact for queries and feedback

- **For further information please contact** Helpdesk Services
- **Anonymous feedback for this document can be given** here

Contents

Introduction

Mixed-Taxon Level Identification

This document outlines the process for analysing macro-invertebrate samples. From March 2014 all macro-invertebrate samples collected using a 3 minute kick sample or airlift method must be analysed to mixed taxon level.

Training and competency

This document is for staff at capability level three of the data and information management section of the Environmental Monitoring Technical Development Framework**.**

The Environmental Monitoring Technical Development Framework identifies the skills you need and the activities you must undertake to become fully capable in this activity. To carry out the procedures in this manual you must demonstrate you are competent. If not, you may only carry out these procedures under supervision.

For detailed training and competency requirements refer to 118_05 Quality Assurance (AQC, Audit and Ring Test) programme for freshwater macro-invertebrate riverine samples

The basic quality criteria for Primary Analysts sorting macro-invertebrate samples are:

- completion and pass of Modules 1 and 2 of the basic training course T309 Freshwater macro-invertebrate species ID;
- no more than an average of two gains, losses or omissions over the preceding 20 samples;
- takes part in all QA schemes: AQC, Ring Test and Reverse Ring Test.

Equipment

Analytical sieves

Analytical sieves are required to properly wash samples prior to analysis. You must use as a minimum a mesh size of 500µm.

It is useful to have a series of larger mesh size sieves to split the sample into size fractions for sorting and to help when washing your sample. You must make sure that you have a 500µm sieve at the bottom if you follow this method.

You can also use a 250µm mesh sieve to prevent coarse silt from blocking sinks where a silt trap is not fitted. Do not analyse the sample fraction from this sieve size.

If your sieve has a small gap between the top of the mesh and the sieve wall, fill the gap with plastic sealant. This prevents specimens becoming trapped.

Sorting tray

This must be white and have a completely flat bottom surface.

Mark the tray with a grid of thin lines to divide the tray into 12 or 16 equal sized areas. This helps to estimate abundances and sort methodically. Apply the lines with an indelible marker pen. Pale or mid-blue lines are better than black because dark coloured animals remain clearly visible on them.

Smaller trays (about 35 by 25 cm) are recommended for general use. They

focus your attention better. They are more comfortable to use because you do not have to lean so far over them, improving your posture.

Larger trays (about 45 by 35 cm) are useful for sorting stones and larger fragments of debris.

Forceps, needles and tweezers

Forceps are required for picking and manipulating specimens.

You will need:

Item	Points to note
Fine forceps	Made of steel with sharp-points. You can repair or sharpen the tips using fine abrasive paper or a grindstone.
Soft forceps	Made of spring steel for delicate organisms. Soft forceps are required for handling delicate animals, such as mayflies.
Mounted needles	These are useful for removing small body parts required for identification of some species. Keep them sharp by using fine abrasive paper or a grindstone.

Specimen containers

You will need:

Item	Points to note
Sample vials	Must have leak proof lids. Choose a size suitable for the numbers and sizes of taxa found in your samples.
Petri dishes/cell culture trays	Disposable dishes, preferably with divisions and lids, capable of holding invertebrate specimens in a small amount of liquid. Used for keeping aside specimens requiring further identification while you are sorting.

Microscopes

You will need:

Item	Points to note
Low power stereoscopic microscope	Good quality optics and good lighting are essential. A wide field of view and zoom facilities are very useful. Cold source fibre-optic lighting is best, because it will not heat the specimens. The additional facility to back light specimens can also be extremely useful.
High power microscope	For most purposes, when identifying species of macro-invertebrates, medium cost models with moderate fields of view but good optical quality are adequate.

Laboratory workspace

Each person sorting samples in a laboratory requires a bright and evenly lit area. There should be no glare on the surface of trays. Windows may, therefore, require blinds. The work bench must also have a non-reflective surface and be at a height that allows comfortable sorting without straining the neck forward. This is usually higher than the level needed for writing. If necessary, a box or stand can be used to raise the height of the tray when sorting. There must be enough space for the sorting tray, identification keys, notepads, microscope and Petri dishes.

See Newman (1994) for further advice on workstations for sorting.

The temperature of the laboratory should be around 20°C because the work is sedentary. You should take account of any drafts created by, for example, fume cupboards. Noise is unlikely to be a problem in laboratories but it must be assessed and controlled.

Adjustable chairs in the laboratory

Chairs must be height adjustable, as microscope and tray work require different positions. The seat back must also be adjustable in both height and tilt.

In addition, the chair must be stable, comfortable and allow easy freedom of movement. It should incorporate a foot-rest or an adequate foot-rest should be available when required, particularly if the seat is too high for the feet to rest on the ground comfortably.

Other equipment in the laboratory

You will need:

Item	Points to note
Adjustable bench lamp	Needs to be capable of directing light on to the sorting tray but away from the analyst. Incandescent bulbs may be replaced by florescent bulbs which give a cool white light, which some biologists find preferable.
Tally counters	We recommend a bank of at least four counters to help estimate abundances.
Pipettes	Small volume PVC pipettes are useful when analysing samples, for instance transferring excess preservative from vials, and when making temporary slide mounts, when identifying specimens.
Microscope mounted camera	Relatively cheap eyepiece mounted camera systems can be very useful for taking pictured of specimens to get identification confirmation from Area Macro-invertebrate specialists or analysts in other laboratories.

Solution for preserving specimens

95_06 Fixing and preserving freshwater macro-invertebrate samples provides details of preservatives and fixatives for samples.

Use an aqueous solution of 70% industrial methylated spirit (IMS) with 5% glycerol to store specimens for audit or reference collections.

Identification keys

A comprehensive library of identification keys, guides and taxonomic papers is essential for every biological laboratory.

The keys should cover the whole freshwater invertebrate fauna, including identification guides to terrestrial taxa that are sometimes found in freshwater samples or that could be confused with aquatic species. This especially includes terrestrial species of groups with aquatic members, such as snails and beetles.

Refer to Communities of Practice for a detailed list of available keys.

Storing and washing samples

Storing live samples

The table below describes how to store live samples.

Step	Action
1	Sort and identify live samples as soon as possible after capture, ideally within 48 hours of collection.
2	Store them in a fridge at a temperature between 1°C and 3°C.
3	Keep samples for a maximum of five days. Example: Collect samples on a Monday, then sort from Tuesday to Friday. ***! Important*** This time includes any re-analysis of live samples for AQC.
4	If samples cannot be refrigerated or sorted within the required 5 days, preserve the sample as soon as possible.
5	Discard any live samples not processed within five days or not kept between 1°C and 3°C. Take new samples.

Washing live samples before sorting

The table below describes how to wash live samples before you sort them. If you are sorting a preserved sample refer to 95_06 Fixing and preserving freshwater macro-invertebrate samples for details.

Note: You can use larger mesh size sieves to split the sample into size fractions. Refer to 95_06 Fixing and preserving freshwater macro-invertebrate samples for details.

Step	Action
1	Decant the sample (or portion of sample) into a 500µm sieve, or in the sieve stack.
2	Analyse everything retained in the 500µm sieve mesh size upwards. If a 250µm sieve is used under the 500µm sieve, do not analyse this portion.
3	Wash only a small portion of a live sample at any one time, keeping the remaining portion in the fridge
4	Rinse the sample container thoroughly into the sieves.

	Check that no animals remain clinging to the edges.
5	Using low pressure water, rinse the sample through the sieve (or sieve stack) until any silt in the sample has been washed through. Do not swirl the sample in the sieve or use high pressure spray. This can damage the invertebrates and make them difficult to identify.
6	For very silty samples, you should employ the 'puddling technique' Place the sieve in a bowl or deep tray filled with water. Agitate the sieve up and down to suspend and rinse the silt through the sieve. The 95_06 Fixing and preserving freshwater macro-invertebrate samples on the use of preservatives contains more details on this method.
7	To wash samples with large amounts of weed or leaves in the main sample put the sample (or large sieve fraction) into a bucket or bowl of water. Gently agitate the leaves and weed to remove most of the attached invertebrates before they are sorted in their own tray. Check the leaves and weed to ensure no macro-invertebrates remain attached and then return the detritus to the sample pot. Keep the remaining water and sample and pass it through the 500µm sieve.
8	Do not throw leaves, stones or other debris away. Even if you have checked them for invertebrates, they form part of the auditable sample and may be hiding taxa that you have missed.
9	Transfer an appropriate amount of the sample into the tray for analysis.
10	Begin analysing your sample. Once finished, check each sieve for attached animals, especially round the rim.

Analysing samples

Overview

The analysis process

This chapter describes the analysis process, including preparing to analyse samples and how to correctly carry it out.

Analysis Rules

Preserved samples

Remember that, even when samples are washed thoroughly in accordance with 95_06 Fixing and preserving freshwater macro-invertebrate samples, invertebrate specimens may still contain undiluted fixative or preservative. This can leak out of soft bodied specimens when their skin is ruptured, so whenever possible, handle specimens under water, particularly during identification.

Analysis must be done in laboratory

All sorting and identification of samples collected using the standard methodology must be undertaken in the laboratory, not in the field.

The whole sample must be sorted, even if few animals are found in some fractions.

The exceptions to these rules are:

- a field search of discarded material, when sub-sampling the contents of a dredge;
- samples related to pollution incidents; These may be examined in the field to check for the effects of pollution and, in particular, for the presence of dead animals. The guidance in 227_06 Ecological Appraisal attendance at a pollution incident describes the specific techniques and procedures to use when investigating pollution incidents;
- when you need to give an immediate, interim assessment of a standard sample. You may examine it in the field but you must not alter it in any way. Example: Any detritus you remove in the field sort must be retained in the sample container so it can later be properly sorted in the laboratory.

Sorting times

Time limits

The time taken to sort a sample will depend on the nature of the sample and the experience of the analysts.

! Important Time limits must not be imposed.

Time estimates

Analysis of a sample to mixed-taxon level will usually take no more than 5.8 hours. It may take longer if it comes from a site which has a diverse community or is particularly difficult to sort i.e. contains a lot of vegetation and/or detritus.

These time estimates include time spent on identification, which will depend on the taxa found and the expertise of the analyst.

Species and mixed-taxon level analysis

Taxonomic level

Identification of all macro-invertebrate samples collected using a 3 minute kick or airlift method must be carried out to mixed-taxon level.

The section 'Identification levels for mixed-taxon work', provides some guidelines on the taxonomic resolution required for different invertebrate groups.

You must never identify a specimen beyond the taxonomic level to which you can confidently assign it to. Do not be tempted to guess at what a specimen may be. If you cannot identify a specimen to the level required, leave it at the taxonomic level you are confident with.

!important. If you do not fully identify a specimen because you do not have the knowledge to do so, you must seek advice from a specialist or other experienced analysts. This will develop your identification knowledge to enable you fully identify this taxonomic group in the future

Setting up your workspace

Caring for your health

Follow the guidelines below to ensure that you work safely and comfortably:

- adjust your workstation to fit you, rather than you fitting it;
- sit well back and upright in the chair when sorting.
  Maintain a straight back and place the chair close to the workbench;
- position your bench lamp so that it is shining from the side and slightly forwards, directing reflections away from you and minimising shadows. This should prevent eye strain and maximise the contrast between specimens and the tray and detritus, making them easier to see;
- move around frequently so you do not spend prolonged periods in the same position. Periodic visits to the sink to refill your tray will help stretch your muscles;
- do not sort at a rate, or so continuously, that it puts your health at risk.
- You must break from sorting for about 10 minutes in every hour. You may achieve this by putting smaller amounts of material in the tray and increasing the number of visits that you make to the sink (this may also improve the accuracy of your sorting). Alternating between sorting and identification will also help.

Using microscopes

Health risks

You must recognise the health risks posed by prolonged periods of identification, particularly when using a microscope.

Take actions to reduce the health risks, using the guidelines in this section. See 242_06 Generic risk assessment for ecology laboratory safety.

Where you sit

As with sorting, correct posture is important.

If your microscope is where you sort samples, you must have either:

- a dedicated microscope bench with a lower work surface;
- or an adjustable height chair (your chair must have a foot-rest).

Positioning the microscope

The microscope eyepieces should be slightly below the level of your eyes when your head is upright so that they fall naturally to the eyepieces when you tilt your head forward.

The eyepieces should be about 10 cm in from the edge of the bench, though this will vary from person to person and the design of the microscope.

It is essential that specimens are properly illuminated and that you correctly focus the microscope.

You must clean frequently, using proper lens cleaning tissue:

- the eyepiece lenses at least twice a day, if use is continual;
- the objective lens at least weekly.

On binocular microscopes, use eye-cups if you find them more comfortable.

All microscopes must be serviced regularly to ensure that the optics are in good order, properly aligned and focused.

Taking breaks

As with sorting, it is important not to sit in the same position for prolonged periods. When identifying taxa, you will probably move around sufficiently as you consult different keys and record results. When identifying large batches of similar and demanding taxa, you should take breaks for about 10 minutes in every hour.

Workload

Taking breaks

When your team's workload is particularly high, you should be vigilant in assessing your own and other's health and safety risks and ensure you take sufficient breaks.

Team Leader's responsibility

It is the responsibility of the Team Leader to organise resources and ensure that all analysts take frequent breaks from sorting.

Setting up the tray

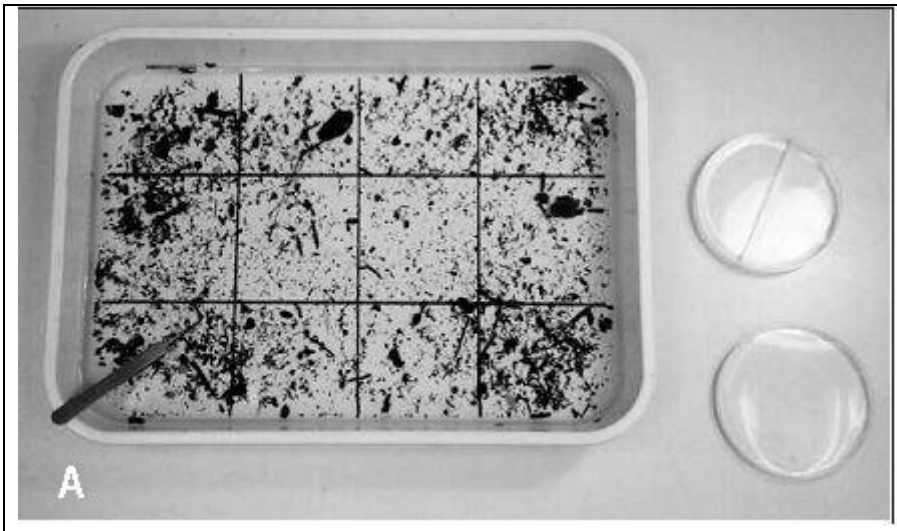
Actions

The table below describes how to set up the tray.

Step	Action
1	Sort samples in small portions, not all at once. This is not only more effective but it is also quicker.
2	Place a small amount of the sample into the sorting tray. See Figure 1, below, for guidance on the correct amount. Place smaller amounts of material in the tray when the sample material is fine.
3	Do not completely cover the bottom of the tray or you will waste time moving material around to uncover animals that are hidden.
4	Completely cover the sample material with water to reduce reflections.
5	Spread the sample evenly around the tray. You should not need to move detritus around very much.
6	Keep the waterproof label from the sample container(s) in the tray while you sort the sample.

Figure 1

The photos below show the amount of material recommended in a tray. The amount shown in A is suitable for an experienced biologist. A smaller amount, shown in B, is recommended for inexperienced biologists.



Picking out macro-invertebrates

Sorting the animals

The table below describes how to sort the animals from the sample.

Step	Action
1	Sort through the sample systematically, using the grid as an aid.
2	Scan the tray for floating specimens. Remove large objects, such as stones, sticks and leaves, from the tray after carefully inspecting them for attached organisms.
3	When sorting a tray from a live sample • View the whole tray first and remove any fast moving animals, such as beetles, corixids or shrimps, as soon as you see them. • Secure them in a lidded container so that they cannot walk or fly away.
4	When sorting through coarse gravel or pebbles • Look out for animals which sink rapidly, such as molluscs and cased caddis as they may become buried. • Take particular care with weedy fractions to look for large animals that are camouflaged, such as dragonflies and phryganeid caddis-flies, and for small hydroptilid caddis-flies in their cases.
6	Take care to examine in detail similar looking specimens, in case they include more than one taxon. Put them into a Petri dish, so that you can examine them later for identification and enumeration.
7	You must ensure you pick out representatives of all the different taxa types present in the sample from each family. To ensure you achieve this in samples with very abundant families, ensure you pick out at least 50 individuals from each family. This should ensure you get a representative of each taxa present in the sample

8	Put into a Petri dish the taxa that can be differentiated, but need to be identified under the microscope.
9	Always pick out material that you suspect may be a part of an invertebrate and inspect it closely, under a microscope if need be.
10	Use past data as an aid. This is especially useful when unusual taxa, or a wider than normal range of taxa, are likely to be found.
11	After you have sorted the whole tray, move the tray or stir its contents with blunt forceps. Turn it through 90 or 180 degrees and spend a few moments searching for any missed taxa. In live samples, snails and flatworms will congregate around the edges and corners of the tray.

After initial sorting

The table below describes what to do after the initial sort.

Step	Action
1	If you break from sorting material while it is in a tray, cover it completely (for example with another tray). Put a lid over any Petri dishes to protect the contents and reduce evaporation. Although not recommended, you can leave partly sorted preserved samples overnight like this. But return live samples to their sample container and put them back in the refrigerator. If the Petri dish contains preservative, its contents should be put into a vial to prevent the preservative from evaporating.
2	After sorting, pour the contents of each tray into a fine sieve so that the entire sample can be retained in case it is selected for AQC. We recommend a 250µm mesh sieve for this, but it must be no coarser than 500µm and a large diameter sieve is best.
3	When the sample has been sorted: **If it is…** / **Then…** an AQC sample — re-preserve the sample and store. Not an AQC sample, selected for reverse ring test — The AQC vial must be kept, but the sample can be discarded. not an AQC sample — the sample and vial can be discarded. Refer to 118_05 Quality Assurance (AQC, Audit and Ring Test) Programme for freshwater macro-invertebrate riverine samples to determine if a sample is needed for AQC or audit and 95_06 Fixing and preserving freshwater macro-invertebrate samples.

Identifying specimens

Actions

The table below describes how to identify specimens and what to exclude from the sample.

Step	Action
1	Place examples of every taxon in a vial containing preservative. Follow the procedures described in the 118_05 Quality Assurance (AQC, Audit and Ring Test) Programme for freshwater macro-invertebrate riverine samples for analytical quality control and external audit of freshwater macro-invertebrate analysis. Make sure you pick out and place a representative of each taxa in the vial, as it may be selected for AQC or Reserve Ring test. ***! Important*** Identify all the scoring aquatic macro-invertebrates in the sample to the required taxonomic level.
2	Identify all 3 minute kick and sweep samples to mixed-taxon level. However you must never identify a specimen beyond the level at which you are confident, even if this means leaving a specimen at family level. Identification levels for mixed-taxon work, provides a full list of required level of taxonomic identification for each invertebrate family.
3	Invertebrates that do not need identifying include • terrestrial or aerial life stages of aquatic animals and wholly terrestrial animals; • specimens that were obviously dead when the sample was collected. This includes empty snail shells, caddis cases, pupal cases and exuviae. You may record these in the sample comments but not as part of the analysis; • a head, or thorax or abdomen when found in isolation; ***! Important*** You must count any individual where two of the above body segments are found. • rear ends of animals alone, for taxa other than insects; • nymphs whose wings are fully unfolded. ***!Important*** Fragments of damaged specimens can cause errors, particularly when abundances are estimated.
4	It may be necessary to break mollusc shells and poke caddis cases to check for occupants. Push cased caddis out of their cases from the rear, using a blunt needle, because they are liable to break if pulled out from the front.

Recording abundances

How the count is used

All taxa identified must have a recorded abundance. This will usually be an estimated count.

This count is used by BIOSYS to calculate the national log abundance category (see Table 2 below) for each taxon and, importantly, allows counts to be aggregated in mixed level samples for indices such as WHPT

You must not provide absolute counts of taxa as the sampling method is not fully quantitative and so it introduces artificial levels of precision into the data.

Table 2

Abundance categories.

Step	Action
A	1 - 9
B	10 - 99
C	100 - 999
D	1,000 - 9,999
E	10,000 +

Recording the count

The table below describes how to record the estimated count.

Step	Action
1	Record estimated counts to one significant figure for each taxon - 1, 2, 3, 10, 20, 30, 100, 200, 300 and so on).
2	Make the estimate by eye or by using one of the methods described in Estimating abundances, below.
3	Abundances must relate to: • for mixed taxon level - the abundance of individuals identified to that taxonomic level; • for a family - the abundance of individuals which could not be identified further than family only. Do not include the count of individuals further identified to genus or species level.

Estimating abundances

Actions

The table below describes how to estimate the abundances of different taxa.

Step	Action
1	When recording specimens which are easily identified, count specimens in the tray without removing them.
2	Where an invertebrate family occurs in low numbers (<50 individuals) you should remove all representatives of the family from the tray and identify each specimen to species level (where required) These specimens should be enumerated as they are

	identified.
3	Where an invertebrate taxa occurs in high numbers (>50 individuals) then you should apply a stratified count approach. Distribute the sample evenly across the tray. Estimate the abundance of each taxa in two or more of the grid squares and multiply this up to give an estimate for the whole tray and sample.
4	Where high abundance family occur in a sample, you should select the first 50 individuals found from a family and then identify the different taxa present within the sample from this family. Using the ratio of occurrence of the different taxa calculated from these 50 specimens and the estimate of abundance for the whole family (from the method above) you can calculate an estimated abundance for each taxon in the sample. ***!Important*** You should continue to identify specimens of each family as you work through the sample to ensure you have identified all species of that family present.

Glossary

Terms defined

The table below lists a number of terms and abbreviations. Words or phrases in italics are defined elsewhere in the glossary.

Term	Description/Definition
AQC	Analytical Quality Control. Procedures to control errors in laboratory analyses, within specified limits.
Audit	An independent measurement of the quality of the laboratory analysis of samples or the quality of the AQC inspection.
Audit sample	a sample selected for auditing.
Biotic index	A scale for showing the quality of an environment by indicating the types of organisms present in it.
Family	A main grouping in the classification of living organisms, consisting of related genera. Family names are distinguished by the suffix -idae.
Fixative	Maintains cell and tissue constituents in as life-like a state as possible and allow them to undergo further preparative procedures without change.
Formalin	Solution of formaldehyde in water used to preserve and fix macro-invertebrate samples.
Genus, pl. genera	A main grouping in the classification of living organisms, consisting of related species. The next to smallest main group, the smallest being species. Related genera are grouped into families.
Identification	Determining the identity of the taxa in a sample.
IMS	Industrial methylated spirit - ethanol with 5% methanol.

	It is a good preservative for biological samples, but a poor fixative.
Mixed-Taxon Level	Analysing the taxa found within the sample to a lower taxonomic level than family (normally species). There are differing levels of taxonomic resolution depending on which group the animal belongs to.
Macro-invertebrate	An invertebrate animal large enough to be seen without magnification. Often defined as an animal retained on a 500µm mesh sieve. For this procedure, it is an animal captured by a net of approximately 1mm mesh.
Preservative	A substance that protects biological material from decomposition. Formalin or IMS are used to preserve freshwater macro-invertebrate samples. c.f. fixative - some preservatives are also fixatives.
Primary analysis	The main analysis of the sample, that is the sorting and identification, which produces the data for the sample. The primary analysis is usually the first analysis of the sample, but not always. For instance, if the AQC indicates that the original primary analysis was of unacceptable quality, the samples may undergo another primary analysis.
Qualitative samples or sampling	Samples or sampling methods optimised to provide information about the range of different organisms rather than their abundance (for which quantitative samples are required). Qualitative samples tend to be less precise but more extensive than quantitative samples. Qualitative samples that are sufficiently standardised to provide comparable estimates of the abundance (number) of taxa and the abundances of individual taxa, including samples collected by the procedures described in this document for RIVPACS, are known as semi-quantitative samples. Semi-quantitative samples can only provide estimates of relative abundance because the samples do not relate to a particular area or volume of habitat.
Quantitative samples or sampling	Samples or sampling methods optimised to provide information about the abundance of organisms. c.f. qualitative samples or sampling. Quantitative samples cover a small and precisely measured line, area or volume of habitat or a point.
Sorting	Searching for macro-invertebrate taxa amongst other material in a sample, and where necessary, removing representatives of each for identification. This procedure includes placing representatives of each identified taxon into a vial for quality assurance. Estimating the abundances of each taxon. In this procedure, estimating the logarithmic abundance

	categories of each BMWP/WHPT scoring taxon is also considered to be a part of sorting.
Taxon, pl. taxa,	A type of organism, irrespective of the taxonomic level at which it is defined.
Taxonomic level	The precision with which an organism is defined. It may be species, genus, or family, etc.

Appendix

Identification levels for mixed-taxon work

Note All samples require mixed level analysis. The recommended level of taxonomic identification is listed below:

Taxonomic Group				Identification
Phylum	**Class**	**Order/subclass**	**Family**	
Platyhelminthes	Turbellaria	Tricladida		**All species apart from species couplets, Polycelis nigra/tenuis and Dugesia lugubris/polychroa**
Nematoda				**Phylum**
Nematomorpha				**Phylum**
Annelida	Oligochaeta			**Class**
	Hirudinea			**Species**
Mollusca	Mollusca			**Species (except for Pisidium sp)**
Arthropoda	Crustacea	Malacostraca		**All adults to species and larvae to species or genus where keys allow.**
	Insecta	Ephemeroptera		
		Plecoptera		
		Odonata		
		Hemiptera		
		Coleoptera		
		Megaloptera		
		Trichoptera		
		Diptera		**Family except those specified below and the cranefly genera/species Tipula, Dicranota, Pedicia, Pilaria, Antocha vitripennis, Eloeophila,**

				Hexatoma.
			Chironomidae	**Tribe/Subfamily**
			Simuliidae	**Species**
			Dixidae	**Species**
			Thaumaleidae	**Species**
			Ptychopteridae	**Species**
			Stratiomyidae	**Species**
			Athericidae	**Species**
			Rhagionidae	**Species**
			Tabanidae	**Species**

References

See also

Furse, M. T., R. T. Clarke, J. M. Winder, K. L. Symes, J. H. Blackburn, N. J. Grieve and R. J. M. Gunn (1995) Biological assessment methods: Package 1 - The variability of data used for assessing the biological condition of rivers. NRA R&D Note 412. Bristol: National Rivers Authority

Maitland, P. S. (1977) A Coded checklist of animals occurring in freshwater in the British Isles. Edinburgh: Institute of Terrestrial Ecology

Murray-Bligh, J. A. D. (1999) Procedure for collecting and analysing macro-invertebrate samples. Quality Management Systems for Environmental Biology: Biological Techniques, BT001 Version 2.0. Bristol: Environment Agency

Newman, B. M. (1994) Workstation assessments at Environmental Appraisal Unit (South East), St Mellons and Environmental Appraisal Unit (South West), Llanelli, National Rivers Authority, Welsh Region. Report for NRA Welsh Region. Report No. 94672. Redditch: Risk Services Division, Sedgwick UK Limited

General links

- 206_06 BIOSYS data entry guide - data quality
- 95_06 Fixing and preserving freshwater macro-invertebrate samples.
- 227_06 Ecological appraisal attendance at a pollution incident

River sampling links

- 118_05 Quality Assurance (AQC, Audit and Ring Test) Programme for freshwater macro-invertebrate riverine samples
- 018_08 Freshwater macro-invertebrate sampling in rivers
- 018_01_SD01 Site forms for freshwater macro-invertebrate sampling

Health and safety links

- 52_05 Ecology laboratory safety
- 242_06 Generic risk assessment for ecology laboratory safety
- 09_01 Generic risk assessment: Transportation of samples
- 116_04 Safe system of work: macro-invertebrate collection with a dredge