UK INVERTEBRATE SAMPLING AND ANALYSIS PROCEDURE FOR STAR PROJECT

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Introduction

This manual is based on the Environment Agency's standard sampling and analysis manual (BT001) and describes the methods used by regulatory authorities in England, Wales, Scotland, Northern Ireland and Isle of Man for collecting and analysing samples of invertebrates to assess the quality of rivers. It is based on methods developed and required for RIVPACS. Other systems and methods used by the Environment Agency also rely on these methods.

All activities must be subjected to an assessment of risk and appropriate measures taken to minimise the risks of injury and ill health. You must comply with your organisation's health and safety management procedures. Anyone using the procedures described in this document for the STAR project who has any health and safety concerns relating to these tasks should contact the STAR management team.

1 The surveyor and analyst

Anyone using this method must have had *practical* training in the use of all the equipment that they use for it. This includes both safety and sampling equipment. Every field worker must have attended a course on river safety, so that they are capable of undertaking risk assessments on-site. All field workers must have experience of using their life-jacket in water whilst wearing the clothing (including waders) that they use when sampling or surveying. Knowledge of waterborne diseases is essential. Users must follow local codes for working in the field (in UK this means following the country code).

Anyone using this method to analyse biological samples should have received training and must be competent in sorting and identifying the macro-invertebrate taxa. All users must know their laboratory's emergency procedures and the safe systems of work used in their laboratory.

Field workers must understand the accident procedures enforced by their employers. The potential dangers at every site should be recorded in a site manual. Detailed risk assessments must be recorded for every site using an appropriate checklist.

Staff collecting biological samples must be physically fit, particularly if they work alone. Field workers should have current immunisation for diseases as appropriate in the country in which they are working. In UK, this includes polio and tetanus and field workers are asked to consider hepatitis-a immunisation. Field workers should seek advice from their employer's health advisor or doctor.

2 Health and safety in the outdoor working environment

Being near rivers, streams or any other water environment, either for work or recreation, is potentially dangerous.

Every effort must be made to minimise risks in the field by:

- Avoiding steep or unstable banks unless suitably equipped
- Avoiding rivers in spate: apart from the increased danger, it is impossible to collect

representative biological samples from streams that are in spate

- Always testing the depth and stability of the river-bed with a pole or pond-net handle before proceeding when the river-bed is not visible, and taking special care when sampling under these conditions

- Working in pairs (which is strongly recommended when collecting invertebrate samples), particularly in remote places

- Watching out for hazards, especially in urban rivers, such as broken glass, sharp metal, decomposing waste, and potential sources of attack, including other people and animals

- Washing hands before eating or drinking during field work

- Wearing the right clothes for the job and weather conditions

- Always wearing a life jacket when considered appropriate in risk assessment, not just in deep rivers or upstream from weirs or deep pools, and using a safety harness where necessary; follow the advice on risk assessment given on river safety courses

- Carrying a basic first-aid kit and knowing how to use it

- Letting someone know where you are going and when you will return and reporting to them when you return and at appropriate intervals; this is especially important for surveyors working alone

- Survey supervisors ensuring that the location of each field worker is known every working day

- Following the agreed system of emergency action in case a field worker does not report-in or sign-off at the end of the day

- Ensuring that COSHH regulations, local safety codes, and any safe-systems of work procedures are understood and complied with

- Ensuring that all equipment and vehicles are in safe working order before setting-off, and during field work.

Treat both formalin fixative and industrial methylated spirit (IMS) preservative with care. Both are harmful. All containers and storage facilities must be labelled with hazard and poison signs. In addition, those used for IMS must be marked with flammable liquid signs. Fume cupboards or extractor hoods must be used when using formalin or IMS in the laboratory.

It is essential that every laboratory using IMS and formalin has a solvent and a formaldehyde neutraliser and spillage clean-up kit. Formaldehyde exposure meters should be used when new procedures are used, and periodically thereafter to ensure that the equipment and working practices remain effective.

Keep laboratory work surfaces clean and passages and fire exits clear. Do not consume food or drink in the laboratory. When analysing samples, wash your hands before leaving the laboratory. Samples that have not been fixed or preserved pose particular health risks, even when they do not come from obviously polluted sites.

3 Selecting sites

Some places on river are unsuitable for collecting biological samples for RIVPACS. If the site is unsuitable, the results from RIVPACS and other analyses may be misleading. Biological samples and water samples do not have to be collected from the same place to be comparable. The easiest place for collecting water samples for chemical analysis (for example, fords and bridges) is often unsuitable for biological sampling for RIVPACS.

The physical and chemical features within each site must be relatively homogeneous. Sites should not be extended to include all types of physical conditions, for instance both shallow gravel riffles and deep soft-bottomed pools. This is because RIVPACS predicts the biota according to average environmental conditions at the site. If, for example, both shallow riffles and deep pools were included, RIVPACS would predict the fauna likely to be found at a site predominantly of intermediate depth. This is may be very different to either the pool or the riffle communities present at the site. Too diverse conditions in the sampling area may cause 'over-sampling' of the biota (see below).

If the watercourse is divided into sub-channels, it will be difficult to estimate the size of the channel and for RIVPACS to associate this with the sample. Avoid such places if you can. If they are unavoidable, locate the site on the largest natural channel: the fauna should be typical of a larger river (based on distance from source) and small channel dimensions may confuse RIVPACS. Width and depth measurements should reflect the channel being sampled. If all the channels are the same size, only sample from one. See also the notes on measuring stream width (Section 6.1) and depth (Section 6.2) if there is temporary braiding because of low flow.

Sites used to evaluate the ecological quality of a reach containing many types of physical characteristics should include only the most prevalent features, not all of them. For RIVPACS, sites do not have to be restricted to riffles. However, for assessing water quality, riffles should be used in preference to other habitats, because their faunas are easier to sample and provide the best assessment of water quality. Sites must not be located in isolated biotopes (e.g. riffles when these are not common in the reach) because isolation causes faunas to be less diverse (Begon *et al.*, 1996: Chapter 23) and RIVPACS is likely to predict a more diverse fauna than the site actually supports.

Avoid obvious disturbances, particularly upstream from the site, (unless the aim of the survey is to evaluate the impact of those disturbances). This includes places near dams, bridges, fords, weirs or livestock drinking areas, and immediately downstream from confluences or discharges where the waters are not fully mixed. Sites should be as natural as possible because RIVPACS predicts and classifies the faunas of natural or semi-natural sites. Differences between the observed fauna and RIVPACS' predictions will reflect the impact of any such disturbances. These could be confused with the impact of whatever is being investigated, such as water quality. Areas that are canalised, or where there is

regular dredging or weed cutting should be avoided, for the same reason. Where these disturbances are unavoidable, such as in cities, the site must be representative of the reach as a whole, and the disturbances must be recorded so that they can be taken into account when the data is analysed.

Safety is very important. Risk assessments must be undertaken at every site. A record of the risk assessment must be kept for sites used periodically which should take account of conditions throughout the year and should record steps necessary at the site to reduce risks (for example, double manning and use of additional safety equipment). This is in addition to assessments of risk undertaken by biologists at the time of sampling (see Section 7.1). If an alternative site that meets the survey requirements but poses less risk, the alternative site must be used in preference. Places where it is necessary to climb slippery or steep banks, or to cross deep mud or peat should be avoided. Sites that are easily accessible also reduce time and costs.

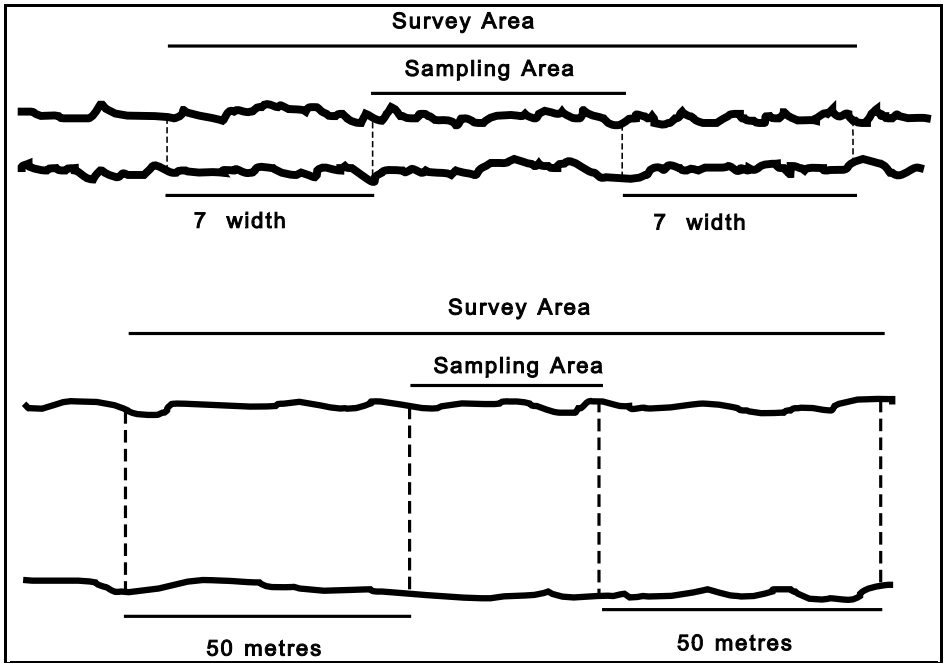


Figure 1 The survey area and the sampling area

Canals and rivers that cannot be traced to a point source, or which follow an upstream direction to the sea (i.e. are pumped or are below sea level at any time), are not accommodated by RIVPACS. RIVPACS seems to predict BMWP indices of the expected magnitude in many of these. However, EQIs derived from them should be treated with caution because of the non-conformity with the RIVPACS ideal.

RIVPACS is unsuitable for watercourses that are normally dry for part of the year, such as winterbournes. It is also unsuitable for in-stream ponds, lakes and reservoirs. Sites should be well away from their influence. Sites that are predominantly on bedrock are unsuitable.

RIVPACS III encompasses headwaters, unlike RIVPACS II.

The criteria for locating sites have been illustrated in a training video (National Rivers Authority 1990).

At each site, you should define both the sampling area from which the samples are collected and a more extensive survey area. The boundaries of the sampling area must be recorded in a site manual. The boundaries of the survey area only need to be recorded if they are not obvious, for instance if the sampling area is not central in the survey area.

The sampling area covers the whole width of the stream wherever possible, but its length will depend on the width of the stream and the variability of its habitats. The sampling area must be a single area of river bed whose major habitat types can be sampled in the recommended sampling period. It must not be a collection of separate places along an extended length of river, for instance to include both riffles and pools in an attempt to increase the variety of animals captured. This would cause over-sampling and result in an apparent under-prediction by RIVPACS. The sampling area will usually be between five and twenty metres long. It will be longer in narrow streams than in wide rivers. Sample environmental data (i.e. water width, mean water depth and substratum composition) collected for RIVPACS relates to the average conditions in the whole sampling area. Parts of the sampling area that are inaccessible for sampling are still considered to be a part of it. The sampling area is therefore more than simply the precise location from which the invertebrate sample is collected.

The survey area extends either seven channel widths or 50 m either side of the sampling area, (see Figure1), whichever is the shorter (this depends on the width). The survey area must be similar to the sampling area that it encompasses. This will ensure that differences between samples from the same site caused by slight differences in the area that is sampled are minimised, and will enable the sampling area to be extended for conservation assessments or to allow replicate samples to be taken. Placing the sampling area within a survey area also ensures that the sampling area is homogenous, and not an isolated area distinct from its immediate surroundings, which may be vulnerable to damage.

The procedures described below provide a standard sampling procedure that can be used in a wide range of habitats, even if the site is unsuitable for RIVPACS itself.

4 Permission for access to sites

Although not always possible, make every effort to obtain permission for access to private land. Usually, you will only need to do this when a site is first established, but at some sites you will need to contact someone before every visit. Land or riparian owners who must be contacted before every visit should be recorded in the site manual. Unless indicated otherwise indicated, assume that riparian land is privately-owned.

If not obtained in advance, field workers should always attempt to obtain permission by asking people living or working near the site.

If you are working in the field without permission and are challenged by an owner or tenant, you should:

- Provide proof of identity (e.g. official identity cards)
- Apologise for not obtaining permission before and then request it
- Describe the work that you are doing
- Explain what the sampling involves and how long it will take
- Leave the site without fuss if the person becomes aggressive or upset
- Report the incident to the survey supervisor.

The guidelines above should be followed under normal circumstances. You must only use official powers of entry when absolutely necessary.

FIELD WORKERS SHOULD ALWAYS BE COURTEOUS AND HELPFUL TO LANDOWNERS AND OTHER MEMBERS OF THE PUBLIC, AND MUST ABIDE BY THE COUNTRY CODE.

5 Obtaining site registration data

Wherever possible, site registration data (map-based environmental data) used for RIVPACS should be the average of measurements made by two (or more) people.

5.1 Map Grid Reference

The Ordnance Survey National Grid Reference (NGR) for the site must be centred on the Sampling Area (see Section 3).

RIVPACS needs accurate six-figure (100 m) NGRs. It is recommended that eight-figure NGRs be recorded even though the last figures will be imprecise.

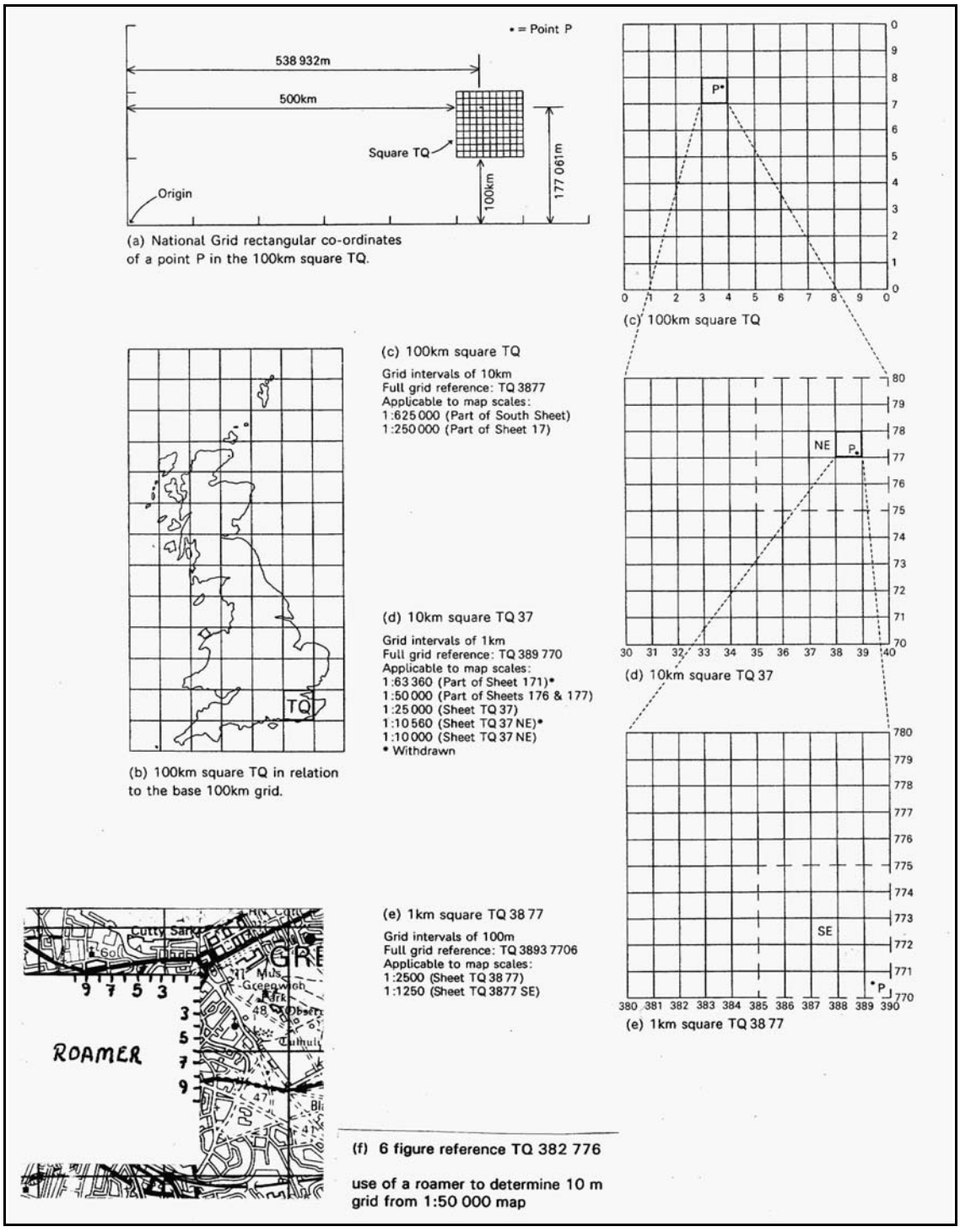


Figure 2 **Determining national grid references at various scales**, adapted from Harley (1975), reproduced with permission of Ordnance Survey.

An NGR for a particular point comprises two prefix letters indicating the 100 km grid square in which it falls. These are marked on the legend and the corners of 1:50 000 maps. The two letters are followed by six (six-figure NGRs) or eight figures (eight-figure NGRs) in two groups of 3 or 4 respectively, referring to the distance from the western and southern sides of the 100 km square (see Figure 2). The distance eastward (easting) is always given before the distance northwards (northing). Successive numerals of the eastings and northings represent successively smaller grids (see Figure 2 c to d). The eastings and northings for the grid square in which the point is marked on the map should be read directly from the values printed along each side of the map. The point can be located accurately to one-tenth of the grid interval with the aid of a map roamer.

Remember that when estimating eastings and northings, it is the position of the south-western corner of the relevant grid square that you should be estimating, not the position of the point. This is because the last figures of the eastings and northings are truncated, and not rounded up. Eastings and northings are always recorded to an equal number of figures, even though some of them may be zeros.

Six-figure NGRs can be determined from 1:50 000 scale printed or digitised Ordnance Survey maps.

Eight-figure NGRs can only be estimated approximately from 1:50 000 scale maps. Even at 1:25 000 or 1:10 000 scale, the last digit of eastings and northings will be imprecise. Eight-figure NGRs can be determined from larger scale (1: 2500 or 1:1250) published or digital maps, or by global positioning systems (GPS) with an accuracy of at least 10 m, with the receiver located in the sampling area.

Grid references obtained from printed or digital maps must be consistent and independent determinations obtained by at least two people. NGRs obtained by GPS and entered directly to databases electronically can be based on a single determination.

5.2 Altitude

The altitude (in metres above mean sea level) must be obtained from the Ordnance Survey 1:50 000 scale maps. The estimates should be to the nearest 5 m, except in mountainous areas where this is impossible because of the lack of contours on steep slopes, where estimates should be to the nearest 10 m.

5.3 Distance from source

This is the distance along the watercourse (in kilometres, to the nearest 0.1 km) between the site and its furthest source, regardless of whether that source is on a tributary known by a different name. The source is considered to be the beginning of the line marking the watercourse on the latest edition of the Ordnance Survey 1:50 000 scale map. Underground reaches must be treated as if they were marked on the map. Assume that underground reaches follow a straight line, unless the

watercourse obviously follows a valley. Similarly, reservoirs and other impoundments should be treated as part of the watercourse: measure the shortest distance within the water body (straight lines), ignoring any islands but not promontories, from inflow to outflow, see Figure 3.

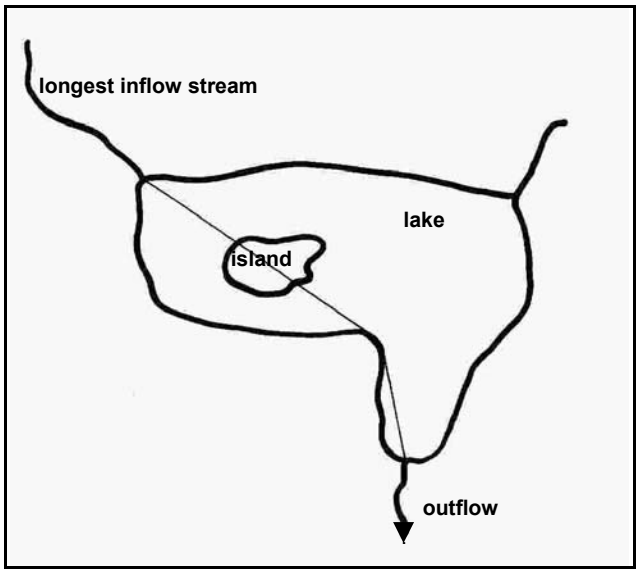


Figure 3 **Measuring distance from source across an in-stream lake or reservoir**. Measure the shortest distance within the water-body, i.e. along straight lines from inflow to outflow, ignoring islands, but not promontories. Always use the stream with the longest distance from source.

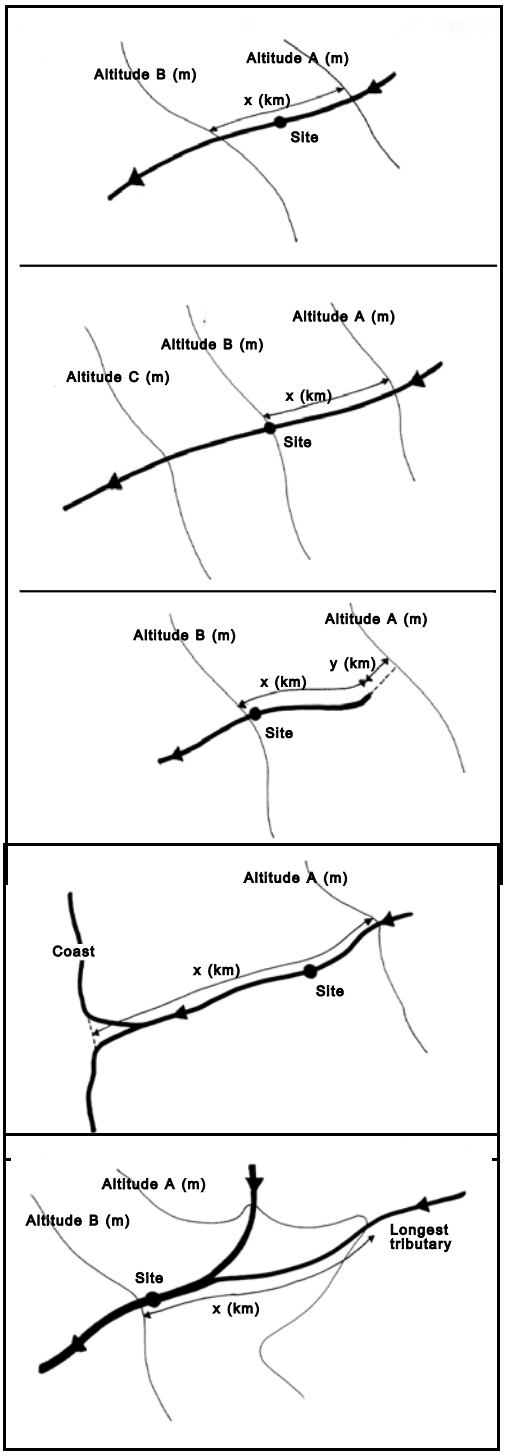
Measure the distance by following the line on a 1:50 000 map using an accurate curvimeter or a planimeter.

The NGR of the source should be recorded for future reference.

5.4 Slope

The slope at the site, in metres per kilometre, must be estimated to the nearest 0.1 $m.km^{-1}$ using the procedures explained in Figure 4. The slope must be determined from the latest edition of the Ordnance Survey 1:50 000 scale maps.

When measuring the slope, follow the watercourse that is used for determining the distance from source, i.e. the longest tributary, even if this is not the main river by name. Use an accurate curvimeter or planimeter.



Site is between contours

$$\text{Slope} = \frac{\text{A - B}}{\text{x}}$$

More than one site may lie between the same contour lines. They would both have the same slope.

Site situated on a contour

$$\text{Slope} = \frac{\text{A - B}}{\text{x}}$$

The distance between contours is measured between the contour intersected and the next contour upstream. The slope upstream from a site is more likely to affect it than the slope downstream.

Upstream limit is the source

$$\text{Slope} = \frac{\text{A - B}}{\text{x + y}}$$

x is the distance between the site and the source
y is the shortest distance between the source and the next highest contour.

Downstream limit is the coast

$$\text{Slope} = \frac{\text{A}}{\text{x}}$$

The altitude at the coast is zero. Distance x is measured from contour A to the theoretical line that extends the natural line of the coast across the estuary.

Site is downstream from a tributary

$$\text{Slope} = \frac{\text{A - B}}{\text{x}}$$

x is measured along the longest tributary marked on the 1 : 50 000 scale map, even if that tributary has a different name or a smaller discharge.

Figure 4 **Computing slope under a variety of circumstances**, adapted from Furse *et al*. (1986).

5.5 Discharge category

Estimates of the mean annual discharge at each site should be recorded using the categories in Table 1.

This information should be obtained using the Institute of Hydrology's Micro Low Flow system for predicting discharge, or by direct gauging. Micro Low Flows may give unrealistic estimates under certain conditions, so it is recommended that you seek the advice of hydrometric staff to obtain the most reliable estimate. Regional hydrometric staff should be able to provide estimates of the discharge at any location, but give them plenty of time to do this. This data will be more reliable than the discharge categories marked on previous Regional or national river quality maps (e.g. National Rivers Authority, 1994a).

Table 1 Discharge categories for RIVPACS

Discharge Category	mean annual discharge (cubic metres per second)
1	< 0.31
2	0.31 - 0.62
3	0.62 - 1.25
4	1.25 - 2.50
5	2.50 - 5.00
6	5.00 - 10.00
7	10.00 - 20.00
8	20.00 - 40.00
9	40.00 - 80.00
10	> 80.00

5.6 The precision of site registration data

The precision necessary for the site registration data will depend on the precision required of the predictions or EQIs derived from them.

IFE has investigated the precision necessary to make reliable predictions of BMWP-score system indices for deriving EQIs (Clarke *et al.*, 1994). They assumed that an error in EQI N-taxa of 0.02, and in EQI ASPT of 0.01 was acceptable (these represented 10% of a class interval in the NRA 5M biological quality grading scheme, described in Institute of Freshwater Ecology, 1991 and Clarke *et al.*, 1994, and summarised in National Rivers Authority, 1994a). The standard errors of site registration data necessary to achieve this at 90% and 95% of sites are shown in Table 2. For GQA surveys, site registration data should meet the criteria for 90% compliance. The precision required for other purposes has not been decided. The

values in Table 2 assume that the error is solely from the parameter in question: this is most unlikely in practice; errors from the imprecision of some parameters may be cancelled-out or added to imprecision in others. Errors should be reduced below the guideline values in the table wherever possible. In general, large errors are tolerable for most parameters except the discharge category, which must be accurate.

Table 2 **Estimates of the tolerable standard errors of the environmental site registration parameters at any site for RIVPACS** (based on Clarke *et al*. 1994). $\bar{y}$ = mean value of measurements for a site. Values equal or less than those in the table are tolerable.

Variable	Range of values of $\bar{y}$	90% site compliance EQI N-taxa	90% site compliance EQI ASPT	95% site compliance EQI N-taxa	95% site compliance EQI ASPT
Stream slope	0.2 - 1.0	40% of $\bar{y}$	40% of $\bar{y}$	30% of $\bar{y}$	35% of $\bar{y}$
(m km^{-1})	1 - 5	40% of $\bar{y}$	40% of $\bar{y}$	30% of $\bar{y}$	35% of $\bar{y}$
	5-75	40% of $\bar{y}$	40% of $\bar{y}$	25% of $\bar{y}$	30% of $\bar{y}$
Distance from	0.2 - 8.0	30% of $\bar{y}$	40% of $\bar{y}$	20% of $\bar{y}$	30% of $\bar{y}$
source (km)	8 - 40	40% of $\bar{y}$	40% of $\bar{y}$	20% of $\bar{y}$	30% of $\bar{y}$
	40 - 203	40% of $\bar{y}$	40% of $\bar{y}$	30% of $\bar{y}$	35% of $\bar{y}$
Discharge category	1 - 2	no error allowed			
	3 - 10	none	± 1 category	none	± 1 category

Remember that all environmental site registration data used for RIVPACS must be averages of at least two independent measurements. Cartographic measurements should be made by different people to prevent systematic errors (bias) caused by the way in which individuals make their measurements.

The standard error of the average (of two or more measurements) of a site registration parameter that is used for RIVPACS can be determined by:

$$SE \text{ of } \bar{y} = \sqrt{\frac{\sum_{i=1}^{n} (y_i - \bar{y})^2}{n(n-1)}}$$

where SE = standard error; n = number of values on which y,_ is based.

If the standard error is small compared to the average that is used for RIVPACS (i.e. smaller than the percentage given in Table 2), it is considered to be acceptable. If the standard error is larger, the average value should be based on more measurements. If the error is large, check that no mistakes have been made, for example, that the correct source or contours have been used.

For large sets of data, it is recommended that standard errors be screened on a spreadsheet or database.

Worked example

Suppose that two independent measurements of distance from source for a site were 50 km and 80 km.

The mean value used for RIVPACS would be:

(50 + 80) ÷ 2 = 65.

The standard error of this mean value is calculated according to the formula above:

$$SE = \sqrt{\frac{(50 - 65)^2 + (80 - 65)^2}{2\,(2 - 1)}}$$

$\Rightarrow$

$$SE = \sqrt{\frac{225 + 225}{2}}$$

$\Rightarrow$

$$SE = 15$$

The tolerable standard error for this distance from source (between 40 and 203 km in Table 2), assuming 90% site compliance, is 40% of y,_ or less, for both EQI ASPT and EQI N-taxa.

40 % of 65 = 26

The actual standard error in the example (15) was less than this, so the estimate of slope is acceptable.

In this example, common-sense suggests that the difference between the two measurements was larger than one would expect simply from

imprecision in measuring two distances on a map. The measurements in this example should be checked again, to make sure that the same watercourse was followed in both measurements, and that the same source was used. If they were not, a new measurement should be substituted for the erroneous one, and the mean distance re-calculated.

6 Obtaining sample environmental data

All measurements of site environmental parameters relate to the conditions in the whole sampling area, i.e. the full width of the watercourse along the whole length of the sampling area, even if parts of it are inaccessible for sampling.

RIVPACS requires information on the modal or average environmental conditions, even when RIVPACS is used to predict single or paired season faunas. These must be based on measurements taken throughout the year, not just in one season because this will give a biased estimate.

As a minimum, annual means should be based on measurements made in spring, summer and autumn. If the annual averages are based on more data, the measurements must be relatively evenly spaced throughout the year and not concentrated in one season. To prevent distortion by unusual conditions in any one year, it is recommended that sample environmental data be based on the mean of annual averages collected over five years. Unusually dry or wet years should be excluded. As a minimum, data collected in three seasons in one year is acceptable.

It is recommended that long-term data is used whenever available. When data for one season is exceptional, because of floods or drought, it is better to replace the actual measurements taken in such circumstances by estimates of what they would be under normal circumstances (these may be common-sense guesses, or better still, actual measurements from a normal year), before calculating the annual average. Do this if the sample environmental data is to be based on an average of less than five years' data: if it is to based on five years' data, base all the annual averages on actual measurements, excluding exceptional years. Remember that RIVPACS is unsuitable for watercourses that are dry at some time of the year under normal conditions: it will not be able to predict their natural fauna accurately.

Even if a good estimate of the average conditions is available already, the environmental data should be collected whenever macro-invertebrate samples are collected so that unusual conditions at the time of sampling can be recognised and taken into account when analysing the biological data.

The standard procedures described below must be used to collect these data to ensure its compatibility with RIVPACS.

When collecting environmental data, the objective should be to measure the modal conditions in the Sampling Area at the time of sampling. Avoid isolated features such as boulders or narrows, which would cause the measurements to be atypical of the Sampling Area as a whole. Choosing the best place to measure these is easier when the Survey Area is restricted to a relatively discrete range of physical conditions.

Some of the environmental measurements will have to be estimated if the stream is too deep or dangerous to wade across. The estimates must be actual values and not inequalities (such as >60 cm or 60+ cm).

To reduce systematic bias caused by the surveyor, it is recommended that, whenever practicable, either the sample environmental data is collected by more than one person on each visit and the average values recorded, or it is collected by a different person in each season (this would require different staff to visit each site in different seasons), see Clarke *et al.*, 1994.

6.1 Stream width

The stream width should reflect the predominant conditions in the sampling area.

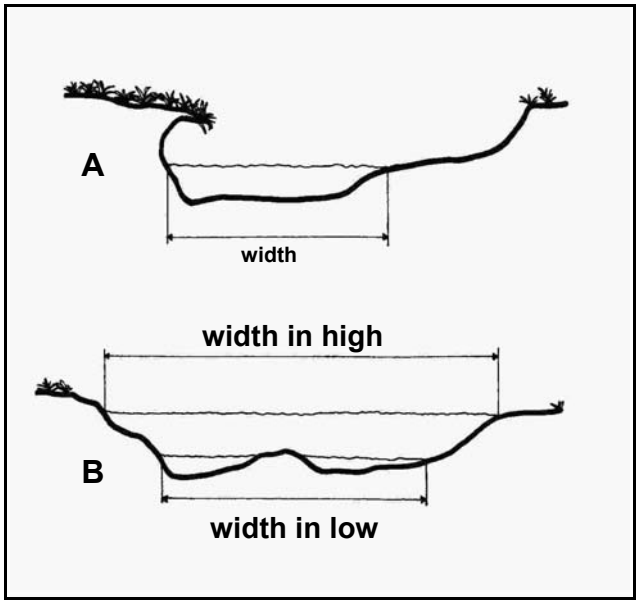


Figure 5 **Measuring stream width**: A, measure the width of the water, not the width of the channel, and measure water beneath overhanging banks; B, include gravel bars and other temporary islands when measuring width during low flows.

Measure the stream width at right-angles to the channel. Measure the width of the water surface, not the stream channel, and include water under overhanging banks; see Figure 5. If temporary islands form in the channel because of low flow, include them in the measurement. Choose a place to measure the width that gives an approximately modal value for the site. Alternatively, where the modal width is difficult to estimate and it is safe and easy to cross the river, an average of more than one measurement from the vicinity of the sampling area can be used, although this should not normally be necessary. Where both banks can be reached by wading, use either a metre rule, an accurately marked pond-net handle or river-crossing pole, or a tape measure. Metre rules may be attached to a stout length of wood to act as a river-crossing staff. On wide or deep rivers, either estimate the

stream width, making use of nearby bridges (although sites should not be in the immediate vicinity of bridges), or use a rangefinder that has been calibrated. Wherever possible, width should be measured rather than estimated. Guesses can be surprisingly inaccurate, even for narrow rivers. As a last resort, whilst standing near to the river bank, measure the distance to an object along the bank that appears to be as far away as the opposite bank. As a minimum, estimate widths of less than one metre to the nearest 10 cm; widths of between one and two metres to the nearest 20 cm; and widths between two and ten metres to the nearest 50 cm; and widths greater than ten metres to the nearest metre.

6.2 Depth

The depth should reflect the predominant conditions in the sampling area.

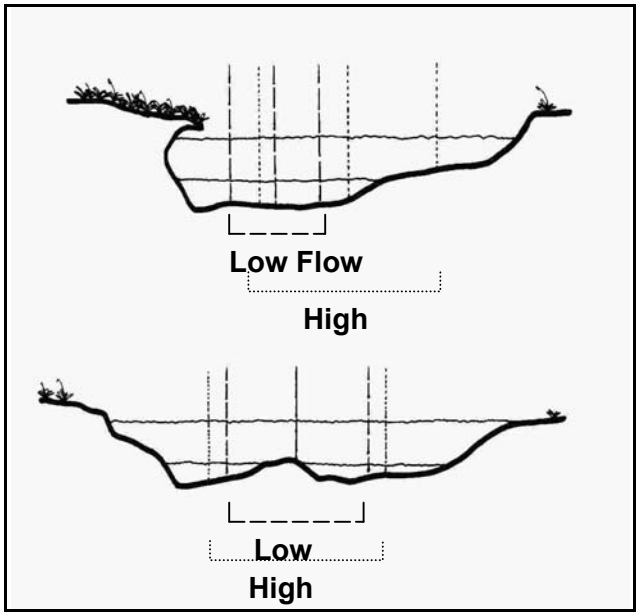


Figure 6 **Measuring stream depth**: A, position of depth measurements may vary with flow; B, the middle depth measurement may be zero in low flows if temporary islands or gravel bars form.

Depth should be based on the average of measurements from approximately a quarter, half, and three-quarter distance along a transect across the stream in the Sampling Area. Do not measure depth where it is atypical of the site, for instance over or close to boulders. In periods of low flow, the depth at a quarter and three quarters channel width will be measured closer to mid-channel than at other times. When a temporary island appears in mid-channel, the depth there will be zero, and should be recorded as such (see Figure 6). Where the stream is wadeable, record the depth to the nearest centimetre. Depth may also be measured against gradations marked accurately on a pond-net handle or river-crossing staff or against a metre rule with its narrow edge facing into the current. Although not mandatory, it is recommended that the individual measurements on which the average depth is

based be recorded, so that changes in the cross-sectional profile can be detected: for this, the depths must always be measured along the same transect.

Where the depth has to be estimated, record depths to 1 m to the nearest 10 cm, and greater depths to the nearest 50 cm. It is difficult to estimate depth in deep rivers. The predictive equations of RIVPACS are based on logarithmic values, so they are robust enough to withstand a reasonably wide range of error in the greater depth range.

6.3 Substratum characteristics

The composition of the stream bed must be assessed over the whole Sampling Area, i.e. the full width of river along the whole length sampled, even if some parts of it are inaccessible for sampling. Estimates should represent a bird's eye view. They should only include particles on the surface of the stream bed, including the equivalent superficial layers under macrophytes. A fine layer of silt or clay through which the shape of the underlying stones can be seen should be typed according to the underlying substratum, but if the shapes of the underlying stones are not distinct, the silt or clay should be recorded instead. Compacted clay should be recorded as clay, even when broken-up into gravel-sized fragments.

Record the percentage cover of the categories in Table 3, *ignoring areas of bedrock*. The sum of the four percentages must be 100%. The size categories can be marked on sample data sheets, clip-boards, or pond-net handles, for comparison in the field.

Table 3 Substratum particle size categories recorded for RIVPACS

Category	Longest axis (mm)	Description
silt/clay	<0.06	soft in texture and not abrasive to the hands when rubbed
sand	0.06 - 2	smaller than coffee granules, and unlike silt/clay, abrasive to the hands when rubbed
pebbles/gravel	2 - 64	coffee granule to half fist size
boulders/cobbles	>64	half-fist size or larger

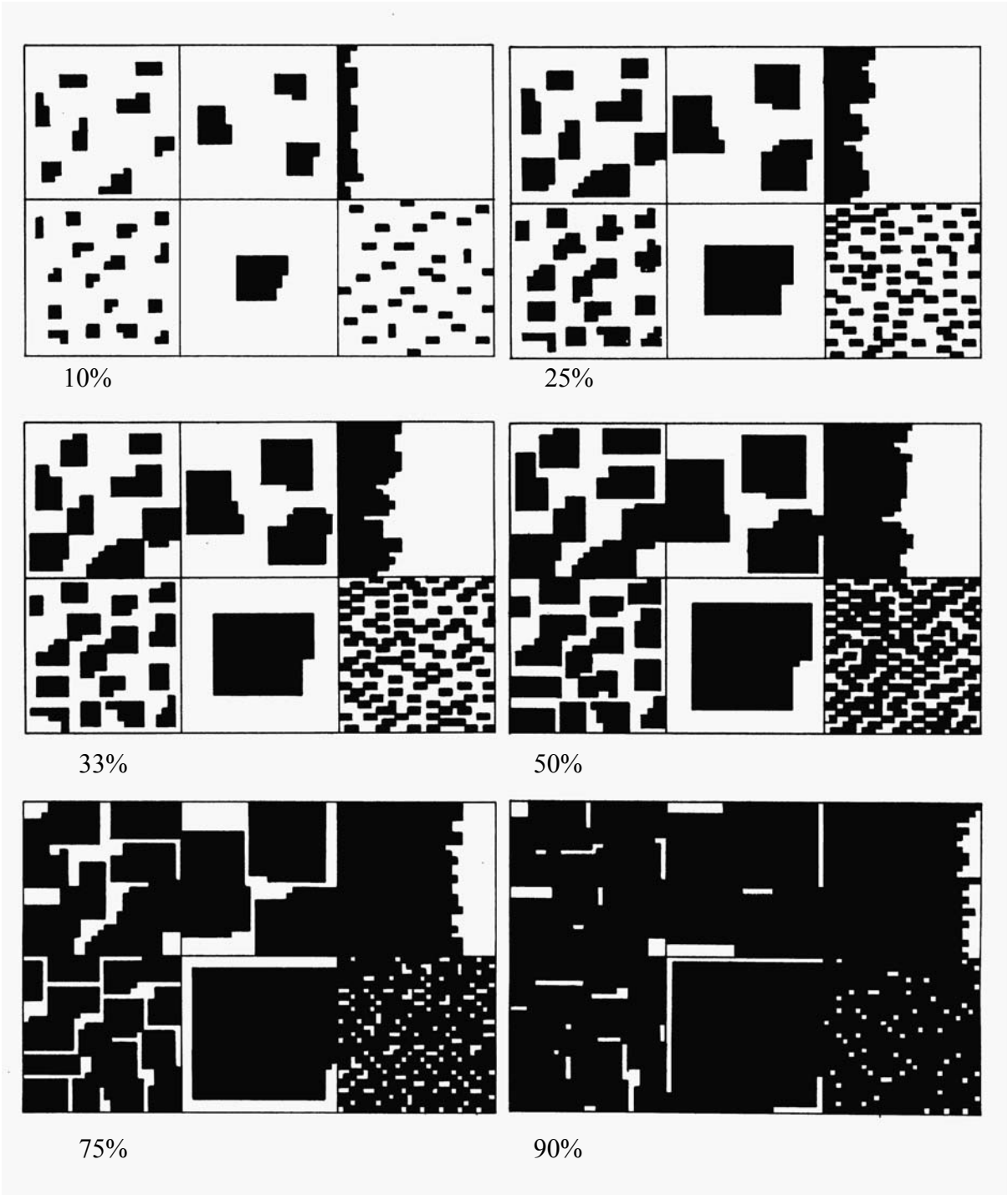


Figure 7 **An aid to determining percentage cover**. See text for guidance on using this figure; there is a slight imprecision in these diagrams owing to the thickness of the lines with which it was drawn, this can be gauged by comparing size of black squares in 10% with white squares in 90%.

Visual estimates of percentage cover are known to vary considerably between individuals. This variation can be reduced by experience. An individual can only gain this experience by comparing their estimates against a benchmark: only then will they know whether they tend make over- or under-estimates, and under what circumstances. Experience has shown that variability within teams can be reduced by all of them estimating percentage cover at the same site and comparing results. The only way to improve the accuracy (as opposed to the precision) of visual estimates of cover is to compare them against the results of measured surveys.

Figure 7 can be used to test variability and accuracy. To do this, cut-out individual squares for each percentage cover from a photocopy of the diagram. Mix the 36 squares and estimate the cover on each. Compare the estimates with the true value. Multiple copies of the top right hand square of each block can be jointed together an arranged to represent cover at the edge or in the middle of a stream channel. This may indicate whether you are prone to over or underestimation, but is not a substitute for field exercises. Copies of the figure may also be attached to the back of notebooks or clipboards and used as an aid in the field.

It is useful to record the percentage cover of bedrock, because this can affect the abundance of animals in the sample. It is not required for RIVPACS, and is therefore not mandatory for this procedure.

Walk along the river bank and make a preliminary note of the substratum after any surface-living animals have been collected. These initial evaluations will be particularly useful at silty sites, and will be necessary for apportioning the sampling effort (see Section 7.1). After the rest of the biological sample has been collected, walk over the whole Sampling Area before making the final estimates. It is difficult to judge the composition of the river bed in deep or turbid water. The substratum visible at the water's edge, the feel of the stream-bed under foot, the contents of the sampling net, previously recorded data, and local knowledge may all be used as guides.

Estimates of the composition of the substratum will be subjective. When there is more than one person on site, a consensus of opinion should be used.

6.4 Water geo-chemistry

The annual average alkalinity, total hardness, calcium concentration or electrical conductivity of the water is required for RIVPACS. Alkalinity is preferred, because RIVPACS was based on this. The others can be used as surrogates. If none of this information is available from other sources, such as a concurrent chemical survey, it must be measured specially. Conductivity can be measured with a hand-held meter. If a meter is not available, a water sample must be collected for laboratory analysis.

An absolute minimum of three relatively evenly spaced chemical measurements are necessary, but monthly values collected over a twelve month period provide a much more acceptable basis for calculating the annual average.

Make a note if a site's long-term alkalinity (or its surrogates) are affected markedly by human influences, e.g. acidic mine drainage. Under such conditions, RIVPACS should be run using an option that does not require alkalinity or its surrogates. Even if RIVPACS analysis is to be based on an option not requiring this parameter, alkalinity should still be measured if possible.

Objective guidance on deciding whether alkalinity is so affected that you should not use it for RIVPACS is difficult. Decisions can only be based on the experienced judgement of local staff who may wish to take account of long-term records for the site, alkalinity upstream of the apparent source of perturbation and the alkalinity of other local streams of comparable size and catchment geology.

At sites with very soft-water, it is recommended that alkalinity is measured by a method giving a limit of detection of 2 $mg.l^{-1}$. The analytical methods for alkalinity that are used generally by most Environment Agency laboratories have a reliable limit of detection of 10 $mg.l^{-1}$. In very soft water, where the lower limit is to be used, it will be necessary to limit the time between sample collection and analysis to a few hours to ensure that the sample does not deteriorate, and especial care will have to be taken to avoid contamination of the water samples. Elsewhere, a limit of detection of 10 $mg.l^{-1}$ will be adequate.

When alkalinity is based on analyses with a limit of detection of 10 $mg.l^{-1}$ at sites having alkalinity less than this, substitute half the face value (i.e. 5 $mg.l^{-1}$) for any “less-than” values when calculating the mean for RIVPACS. This is in accordance with the recommendations in Ellis *et al*. (1993).

Procedures for collecting water samples for laboratory analysis, and for using conductivity meters, are given in the Environment Agency sampling procedures manual (NRA, 1994b) which must be followed.

RIVPACS III or later versions do not use chloride concentration.

Remember that all the parameters mentioned in this section can be affected by waste water discharges. Depending on the aim of the survey, it is worth considering omitting alkalinity (or its substitutes) from RIVPACS analyses.

6.5 The precision of sample environmental data

The precision necessary for the sample environmental data will depend on the precision required of the predictions or EQIs derived from them.

IFE have investigated the precision necessary to make reliable predictions of BMWP indices for deriving EQIs (Clarke *et al.*, 1994; Furse *et al*., 1995). They assumed that an error in EQI N-taxa of 0.02, and in EQI ASPT of 0.01 was acceptable (these represented 10% of the NRA 5M class band). The standard errors of sample environmental data necessary to achieve this at 90% and 95% of sites are shown in Table 4. For GQA surveys, the data should meet the criteria for 90% compliance. The precision required for other purposes have not been decided.

Table 4 **Estimates of the tolerable standard errors of the average sample environmental parameters at any site for RIVPACS** (based on Clarke *et al*. 1994). $\bar{y}$ = mean of annual averages (which are based on the mean of measurements taken throughout the year) for a site; SE = standard error. Values equal or less than those in the table are tolerable.

Variable	Range of values of $\bar{y}$	90% site compliance EQI N-taxa	90% site compliance EQI ASPT	95% site compliance EQI N-taxa	95% site compliance EQI ASPT
Stream width (m)	0.3 - 2.0	30% of $\bar{y}$	30% of $\bar{y}$	20% of $\bar{y}$	20% of $\bar{y}$
	2 - 4	30% of $\bar{y}$	30% of $\bar{y}$	25% of $\bar{y}$	25% of $\bar{y}$
	4 - 20	40% of $\bar{y}$	40% of $\bar{y}$	25% of $\bar{y}$	30% of $\bar{y}$
	20 - 120	40% of $\bar{y}$	40% of $\bar{y}$	30% of $\bar{y}$	35% of $\bar{y}$
Stream depth (cm)	4 - 10	20% of $\bar{y}$	25% of $\bar{y}$	20% of $\bar{y}$	25% of $\bar{y}$
	10 - 20	35% of $\bar{y}$	40% of $\bar{y}$	30% of $\bar{y}$	35% of $\bar{y}$
	20 - 50	40% of $\bar{y}$	40% of $\bar{y}$	30% of $\bar{y}$	35% of $\bar{y}$
	50 - 120	40% of $\bar{y}$	40% of $\bar{y}$	30% of $\bar{y}$	35% of $\bar{y}$
Alkalinity	2 - 30	10% of $\bar{y}$	30% of $\bar{y}$	10% of $\bar{y}$	20% of $\bar{y}$
($mg.l^{-1}$ $CaCO_3$)	30 - 150	15% of $\bar{y}$	20% of $\bar{y}$	15% of $\bar{y}$	15% of $\bar{y}$
	150 - 250	7.5% of $\bar{y}$	7.5% of $\bar{y}$	5% of $\bar{y}$	5% of $\bar{y}$
	250 - 314	5% of $\bar{y}$	7.5% of $\bar{y}$	5% of $\bar{y}$	5% of $\bar{y}$
Mean substratum particle size (MSUB; φ units)	-7.75 - -6	SE = 2.5	SE = 2	SE = 1.5	SE = 1.5
	-6 - -3	SE = 2.5	SE = 2	SE = 1.5	SE = 1
	-3 - 3	SE = 2.5	SE = 1.5	SE = 1.5	SE = 1
	3 - 8	SE = 2.5	SE = 1.5	SE = 1.5	SE = 1

The influence of substratum composition at a site is only represented in RIVPACS predictions by the mean substratum size category (MSUB), (*not* the median size, as conventionally used in sediment analysis).

MSUB = (- 7.75 B - 3.25 P + 2.00 S + 8.00 C □ 100)

Where B = % boulders & cobbles; P = % pebbles & gravel; S = % sand; C = % silt & clay

Remember that, ideally, the sample environmental data should be based on the

mean of a number of annual averages. The standard errors in Table 4 refer to the variation of these annual averages and not to the variation of individual measurements within years.

The standard errors of sample environmental data derived from annual averages from more than one year can be determined using the formula given in Section 5.6. Note that the individual values (of y in the formula) will themselves be annual averages, and the number of values (n) will be the number of annual averages used to determine the (long-term) average used for RIVPACS.

If the standard errors of long term averages are greater than those in Table 4 (see worked example in Section 5.6), they must be re-calculated using more samples, i.e. the multiple-year averages should be based on annual averages from more years. The variation within years will be much greater in watercourses with large changes in flow throughout the year, for instance mountain streams flowing over granite. However, for RIVPACS, the most important variation is that between years. There may be greater variation in the estimates of annual average conditions between years in the types of stream mentioned above, because the same number of measurements will yield less precise estimates of the true mean.

Table 5 An approximate guide to changes in mean substratum particle size category (MSUB) arising from changes in a range of typical substrata

	particle size	example 1	example 2	example 3	example 4
boulders/cobbles	> 63 mm	80% → 60%	60% → 40%	0%	0%
pebbles/gravel	2 - 63 mm	20% → 40%	30% → 50%	80% → 50%	0% → 10%
sand	0.06 - 2 mm	0%	0%	10% → 40%	50% → 10%
silt/clay	< 0.06 mm	0%	10%	10%	50% → 80%
MSUB (φ units)		-6.85 → -5.95	-4.82 → -3.92	-1.60 → -0.03	5.00 → 6.28

It is best to estimate how many samples of the environmental data will be needed before the survey starts, based on information from previous years or from similar sites. The conditions in any season will not be representative of the year as a whole and data from one year will not always reflect the long-term range of conditions.

7 Collecting macro-invertebrate samples

7.1 General principle

The primary objective is to collect a **comparable** sample (comparable with RIVPACS predictions and with other samples collected by the procedures in this document), rather than examples of every taxon at the sampling site, or any other type of 'representative' sample. Comparability is ensured by strictly controlling the sampling procedure. Each invertebrate habitat in the Sampling Area must be sampled with an effort proportional to its cover. *Within the limitations imposed by this procedure*, as many different taxa in the Sampling Area as possible should be collected. On average, about 60% of families present will be collected in a single three minute kick sample, excluding the manual search (Furse *et al.,* 1981). Although the method is essentially qualitative, the standardisation is sufficient to justify the allocation of logarithmic categories of abundance to each BMWP taxon and for these to be used for comparisons between samples.

Immediately before collecting any biological sample, you must assess the risks of doing so at the site. If you are not confident that you can collect the whole sample safely using the equipment and assistance that you have with you, do not collect the sample. Use the risk assessment record for the site (if there is one) as a guide to prepare for sampling, but remember that conditions on rivers can change.

Avoid sampling during and immediately after spates. Samples collected in these conditions will not be comparable with samples collected at other times, or with RIVPACS and will not reflect the underlying environmental quality of the site accurately.

Wash the collecting net thoroughly before and after taking samples, and check that it is neither damaged nor contaminated with animals from previous samples. Wash the net again in clean tap water after every sampling run: this is most important after sampling from polluted waters. To avoid the risk of spreading the fungal spores of crayfish plague, boots, nets and other equipment must be disinfected or cleaned if it cannot be dried thoroughly when moving between sites where native crayfish *Austropotamobius pallipes*, or signal crayfish, *Pacifastacus leniusculus*, may exist. Follow the instructions in Appendix B.

1st Part: Manual Search
Seek and collect individual animals from the water surface.
Spend a total of one minute on the manual search split between Parts 1 and 3.

2nd Part: Main Sample
3 minute pond-net sample collected by a combination of kicking and sweeping, depending on the nature of the substratum, current and habitats, for benthos and free-swimming animals.
Sample from all habitats samples, in proportion to their cover.

3rd Part: Manual Search
Search and collect animals attached to submerged rocks, logs or vegetation.
Spend a total of one minute on the manual search split between Parts 1 and 3.

Figure 8 Summary of sampling procedure in shallow waters

Wherever possible, collect samples by sampling for three minutes with a pond-net and one minute of manual searching. The search is mandatory, although it will not always be fruitful. In deeper water where it is not possible to sample adequately with a pond-net, deep water methods compatible with RIVPACS may be used, but these are not included in the STAR project. (These are three to five trawls of a medium naturalist's dredge, or a transect across the river with an air-lift, together

with a one minute sweep of marginal areas with a pond-net, and a one minute manual search). A combination of kick-sampling and dredging is not permissible. The Sampling Area should not encompass such a wide range of features *along* the river that it includes both deep and shallow areas, see Section 3, though there may be this much variation *across* large rivers.

There is no limit to the size of the sample collected: only the sampling time or number of trawls controls the size of the sample. The only exception to this is that material collected by dredge can be reduced to about three litres after collection (see Section 7.4).

The same procedure should be used for every sample from the same site whenever possible, to improve the comparability between successive samples and so make any changes easier to interpret.

Whatever sampling method is used, material collected in the net must be removed periodically, to prevent the mesh becoming blocked and the sample being washed-out. As a minimum, this must be done after every minute of sampling with a pond-net, and more frequently if the net is filling rapidly or becoming blocked.

Wash fine sediment through the net more frequently than this, to minimise the amount retained in the sample. The dredge must be emptied after every trawl. Large stones and pieces of vegetation can be discarded, but before doing so, agitate them vigorously in the collecting net whilst it is half-submerged, to wash any animals back into it. Check that no animals are still attached before discarding this material

Retain only enough water to keep the sample damp. This reduces the amount of fixative or preservative that has to be added to it, and reduces damage and the activities of carnivores in samples that are to be transported or sorted live.

Do not retain fish, amphibians, and readily identified rare species in the samples, such as large specimens of the freshwater pearl mussel (*Margaritifera margaritifera*), medicinal leeches (*Hirudo medicinalis*) and crayfish, but (except for non-native crayfish) return them to the water with care and unharmed. Record their presence in the sample. If removed live for identification, return them only to the site where they were collected. Retain voucher specimens of these taxa for identification and confirmation only when necessary. Bear in mind that it is particularly important to identify rare taxa correctly, and to differentiate native from introduced crayfish, because of their high conservation value and need for protection, so voucher specimens may be important.

The sample data must relate only to specimens that are actually caught. With the exception of the three rare taxa mentioned above, all specimens must be retained in the sample for identification in the laboratory. Other taxa observed at the site but not forming part of the sample may be recorded in a note (this may be useful, for instance for conservation purposes), but they should not be included in the sample data itself.
Pond-net samples must not be collected from a boat, unless it is unsafe to do otherwise (this is the only acceptable caveat).

7.2 Manual searching

The manual search is similar, whatever methods are used to collect the main sample. Unlike the main sample in which animals are collected without seeing them, individual animals seen by the sampler are collected in the search and added to the main sample.

The search is in two parts that, together, last one minute although the time spent on each part may vary. The first part is to seek and collect animals living on the water surface, such as whirligig beetles, water crickets and pond skaters. This must be done before any other sampling, because these animals are easily disturbed and will either leave the Sampling Area or be much more difficult to find later. They are best caught with a pond-net. Most surface dwellers are very active and they should be secured in a tied bag or vial immediately after capture. Whilst searching for these animals, note the area covered by different habitats within the Sampling Area, so that you can apportion the sampling effort amongst them in the main sample.

The second part of the search is for animals from habitats that are not sampled effectively by the methods use to collect the main sample. Pick-off animals attached or clinging to the submerged stems of emergent plants, rocks, logs, or other solid objects, with forceps or a stiff paint brush. Examine rocks at several places across the river to cover the different biotopes and areas covered by different sized substrata. Always search for animals attached to floating-leafed plants. Inspect the under-surfaces of floating leaves as well as the upper surface and stems.

The whole search must last one minute. It is standardised by time alone, and not by searching a certain number of rocks or locations. This period only covers the time spent actually searching, and excludes the time spent moving around the site. A stopwatch or watch with second hand must be used to ensure that the cumulative time spent actively searching is one minute. At some sites, the search will be fruitless, either because no suitable or accessible places to search are found within the minute, or because no animals are found in the places that are searched. Even where the sampler suspects that nothing will be found, the search must be undertaken honestly, to maintain consistency.

7.3 Pond-net sampling

The pond-net can be used in different ways depending on the nature of the Survey Area. Different biotopes at the same site may be sampled by a combination of the methods described below. The total sampling time must be three minutes.

If a site comprises discrete habitats, apportion the sampling effort according to their cover in the Sampling Area. If a site appears to be homogeneous in character, continuous diagonal transects will suffice for most of the sample.

Always move upstream and diagonally across the stream a number of times whilst sampling, rather than straight upstream. This will ensure that a greater number of habitats are sampled, even if they are not apparent, and therefore a higher

proportion of the taxa present at the site are collected (see Woodiwiss, 1980).

The three minutes covers only the time spent actively sampling, and excludes the time spent emptying the net, or moving around the site. It is recommended that sampling is done in short bursts of 15-20 seconds. There will be 9 to 12 bursts in a three minute sample, which is worth remembering when apportioning the sampling effort to the different habitats. A stopwatch or watch with second hand must be used to ensure that the cumulative time spent actively sampling is precisely three minutes. If two people are on-site, it may be easier for one to time the sampling with the stopwatch so that the other can concentrate on collecting the sample. The sampler should call-out to the timekeeper when to start and stop the watch, and the timekeeper can remind the sampler when each sampling burst should end.

Pond-net samples collected with less effort, in an attempt to prevent denuding sites on very small watercourses, are not compatible with RIVPACS. A longitudinal extension of the site will be required in these streams.

In general, more material will be collected from lowland streams than from stony mountain streams.

7.3a Kick sampling from gravel or cobbles

When kick sampling, hold the net vertically with the frame at right-angles to the current, downstream from your feet, and resting firmly on the river bed; disturb the stream bed vigorously by kicking and rotating the heel of your boot to dislodge the substratum and the fauna within it to a depth of about 10 cm. Lifting and disturbing the substratum with your heel and toe by rotating your foot is particularly effective. There is no need to kick-up froth. 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 (see Figure 9). Hold the net further away where the substratum is finer or the current swifter, to prevent it clogging. Move large stones by hand if they cannot be shifted by foot, and sample the finer sediment that collects beneath them.

7.3b Sampling from soft sediments

Where the stream bed is soft silt or clay, kick sampling is ineffective because the net will become blocked rapidly. Instead, skim the bottom edge of the net gently through the top few centimetres of the substratum, which is where most of the animals will be found. Alternatively, stir-up the surface of the sediment by foot or with the back of the net, and pass the open net through the clouded water. Rinse the silt away through the net frequently, by agitating the net in the current or at the water surface.

Figure 9 **Kick sampling from a shallow, fast-flowing gravely stream**. The sampler is facing at right angles to the current and is moving diagonally to the right and towards the photographer, for safety. The sampler is dislodging the substratum with his left foot, holding the net close in the

plume of disturbed sediment, to catch the animals that are dislodged.

7.3c Sampling from boulders

It is not easy, and sometimes impossible, to take a kick sample amongst boulders. Most of the invertebrates will be in the finer deposits that accumulate under the boulders. To reach them, boulders may have to be moved by hand, though small ones may be prised away by foot. Move boulders away at right-angles to the current or upstream and away from feet, so that the net can be held downstream from the area disturbed. Sample the exposed river bed by kicking in the normal way.

It is impossible to sample effectively where large boulders dominate the stream-bed, particularly near waterfalls or where the gradient is steep. Replace these sites by ones that can be sampled effectively.

7.3d Sampling from vegetation

Sample from submerged and emergent vegetation and tree roots by pushing the net into them with a variety of forward, upward, and lateral movements. Dislodge animals from dense tangles of tree roots by kicking. Sample the sediment that accumulates beneath plants by kicking or skimming the surface of the sediment. Do not overlook water under overhanging banks, because invertebrates may be hiding there.

7.3e Sampling from still or slow-flowing water over gravel or cobbles

When sampling from still or slow-flowing water, a different procedure is necessary because there is no current is to carry dislodged animals into the net. Disturb the substratum with your feet and catch the dislodged animals by sweeping the net through the water immediately above the disturbed area. Use this technique wherever the current is weak, to supplement the methods described above.

7.3f Sampling from deep waters

In watercourses that are too deep for a conventional kick sample, a sweep sample may be taken from the marginal vegetation and the shoreline with a pond-net, using an extension handle if necessary. This is preferred to using a dredge or air-lift, both of which are less easily controlled, and inefficient on very soft or detrital stream beds. (Note that neither the dredge nor air-lift is included in the STAR project.)

All habitats must be sampled. If possible, use a combination of sweeping and kicking. Wherever practicable, collect the sample from both banks. Although each habitat should be sampled in proportion to its cover, this is unlikely to be possible in the main channel. Sample discrete habitats in proportion to their linear predominance along the river.

Some of the sample *must* come from the river bed in the main channel; limit this fraction to no more than 3 litres. If it is not possible to get material from any of the main channel with a long-handled pond net, a dredge or air-lift sample must be collected instead (these are not covered by the STAR project).

In fenland rivers, it is recommended that sites be chosen that support some emergent vegetation, this being the more natural state. Do not extend the Sampling Area to include stands of different species if this causes a gap in the Sampling Area. The Sampling Area must be a single discrete area, but remember that it covers both banks.

The dangers of falling into the water are greater with this type of sampling than any other. It is necessary to get close to the water's edge and to lean out into it, so there is a greater danger of falling into the river. Many slow-flowing deep watercourses are deepened for land drainage and therefore have steeply shelving but soft banks, so it is also difficult to get back onto the shore. Careful consideration should be given to double manning such sites following a risk assessment.

7.4 Dredge sampling

Sampling deep rivers is not included in the STAR project. This method is being revised because of health and safety risks.

7.5 Air-lift sampling

Sampling deep rivers is not included in the STAR project.

7.6 Removing samples from the collecting net

Rinse the sample, following the instructions in the preceding sections, to remove silt and clay, and to discard stones, wood, and large fragments of vegetation before removing the sample from the net.

The easiest way to remove a sample from a pond-net is to wash the catch into one corner of the net first, by dipping the net into the water and gently shaking it from the opposite corner as it is lifted out of the water. Then, by gradually everting the corner of the net, the bulk of the sample can be dropped into a labelled sample container, or polythene bag. Material clinging to the net can be shaken, or flicked-off from the other side of the net, into the container. Alternatively, it can be removed by dipping the everted net into some water in a tray, bucket, or wide-mouthed sample container, using the surface tension to dislodge any material clinging to the net. A combination of these processes may be used, and they should be repeated until all the animals have been removed. A plastic tea-strainer with a fine mesh is useful for decanting the collection from a tray or bucket into a polythene bag or sample container. Recalcitrant specimens may be picked-off the net by hand or with forceps. Always wash the collecting net thoroughly to prevent contaminating subsequent samples.

Drain the sample before putting it into a collecting jar or polythene bag. Do not add water to the sample.

Fill the sample containers to no more than about half-full with collected material. This will leave sufficient room for fixative or preservative, and an air space. Never cram material into a sample container, and never fill it completely: use an additional container instead. Every container must be labelled according to the instructions in Section 9.2.

7.7 Sampling for conservation assessment

This supplementary procedure is to collect additional material from 50 m either side of the sampling area to provide additional information for conservation assessment. It is not used for environmental quality assessment and is therefore not included in the STAR project.

8 Sampling season

Samples should be collected in the following seasons recognised by RIVPACS:

spring (March - May)
autumn (September - November).

RIVPACS also recognises samples collected in summer (June-August). However, for environmental assessment, samples are only collected in summer when it is not possible to guarantee that it will be possible to collect samples in sorting or autumn, for example in areas where spates are common in one of these seasons.

Samples from the same site collected in consecutive seasons must be separated by at least two months.

Sample environmental data (see Section 6) must be collected in all three seasons, regardless of how many seasons the invertebrate samples are collected in. This is because RIVPACS relates the macro-invertebrate communities present in any season or combination of season, to the modal or average environmental conditions at the site throughout the year.

RIVPACS will not predict the communities found outside these seasons accurately, because they were not represented in the data on which RIVPACS was based. If it is necessary to relate samples collected outside these seasons to predictions made by RIVPACS, samples collected in December and January should be considered as if they were collected in autumn, and samples collected in February as it they were collected in spring. Remember that RIVPACS cannot predict the invertebrate communities outside the three RIVPACS sampling seasons reliably. Any comparison of samples collected in winter with RIVPACS predictions will incorporate additional, un-quantified errors as a result. Never plan to collect samples for comparison with RIVPACS predictions in these months.

9 Transporting and storing samples

At present, not all Environment Agency biology laboratories can use formalin and some prefer not to analyse preserved samples. All its biology laboratories must be able to use fixative and preservative. Formalin is the only fixative that is known to be effective for most freshwater macro-invertebrate samples.

Samples that are to be sorted dead should be treated as soon as possible after capture. The ideal is to fix samples immediately after capture, and to transfer them to preservative if they are to be kept for more than a few months. If it is not possible to fix the samples in the field, the next best option is to do so immediately on return to the laboratory. In the mean time, they must be treated as live samples (see below). There is some risk of carnivores altering the sample with this option (see Hiley, 1995). Other options are further from the ideal, but may be necessary if laboratory facilities or local systems do not permit otherwise, for example adding alcohol preservative without using fixative beforehand. It is strongly recommended that fixative is added to samples that are to be kept for more than a couple of months or if they are to be retained for auditing, even though formalin fixative is less effective when added to samples that have been in alcohol.

Samples that are to be transported live must be kept in the minimum amount of liquid, and they must be kept cool during transport at between 1 and 3°C, in a cool-box or mobile fridge. This will reduce damage and the activity of carnivores, and prevent the condition of the sample from deteriorating rapidly. If the sample fills more than about 60% of the sample

container, transfer some of it to another correctly labelled container.

Samples that are to be sorted live must be put in a storage fridge at between 1 and 3°C immediately on return to the laboratory.

Not all couriers will carry samples in formalin or from polluted waters. If you intend to despatch samples by courier, you must check that they will accept them.

9.1 Sample containers

Usually one sample container will suffice, but two, or a larger container, may be necessary for large samples.

Some find it easier to transfer the sample from the collecting net into a polythene bag rather than directly into a pot. Polythene bags may be used for transporting live samples only and these must be drained of excess liquid. They must never be stacked without support.

Sample containers should be transported in an air-tight crate if they contain formalin. The minimum requirement for all other samples is that they are transported in a lidded crate, to protect them, and to reduce the movement of air around them. Cool boxes or refrigerators must be used with live samples.

9.2 Labelling samples

Labels should be written on the outside of all sample containers, including polythene bags, using a waterproof marker pen. Do this before the container gets wet, and give the ink time to dry to improve the ink's adhesion. Lids should not be labelled because they can become separated easily from the rest of the sample whilst it is being analysed in the laboratory. Waterproof paper labels marked in soft-leaded pencil or waterproof (alcohol resistant) ink must be added to each sample container as an additional precaution. Alcohol resistant labels are particularly important for vials because these are kept inside sample containers to which alcohol preservative may be added. Few inks are alcohol proof.

Labels must include the following:

Watercourse name
Site name
Site code
Date of sampling
Sampler's initials.

If a sample is contained in more than one container, add the following:

X of Y containers

where X is the individual number assigned to the container and Y is the total number of containers in which the sample is placed.

If only a portion of the material collected by dredge is retained you must indicate whether the sample container holds dredged material (and its proportion) or the sweep and search, and the vial of specimens found in the dredge material that was discarded must also be labelled fully.

When rare taxa (native crayfish, medicinal leeches and large pearl mussels, described in Section 7.1) are identified and returned to the river, record their presence and abundance on the labels placed in the sample containers, as well as on the sample data sheet.

When the sample has been sorted, the following must be added to identify samples that are ready for analytical quality control (AQC) inspection or auditing:

"SORTED"

and optionally:

date of sorting
sorter's initials.

When a sample has been re-analysed for AQC, the following must be added:

"AQC CHECKED"

and optionally:

date of AQC inspection
AQC analyst's initials.

Sorting and AQC inspection dates, and AQC inspectors' initials must be distinguished clearly from the sampling date and the sampler's initials.

Labelling can be washed-off the sample containers using a solvent such as acetone or IMS, although IMS is ineffective on alcohol-proof inks. Gloves and a fume cupboard or extractor must be used for this.

Samples in formalin are harmful, and samples in IMS are flammable and harmful. Every container must be labelled with the appropriate warning signs.

After sorting, vials containing identified animals should be labelled in pencil, either on labels already attached to the vial (after testing that they are not soaked- off by the alcohol preservative), or on a slip of waterproof paper placed in the vial. This helps to ensure that they are not misplaced during AQC inspection or auditing. It is helpful to include on the vial's label a list of the rare taxa not retained in the sample, or specimens that are too large to fit in it.

Information on the sample data sheets (and audit data sheets) must correspond to the information on the sample container and the vials.

Consignments of samples transported by courier, including those sent to the auditors, must be labelled in accordance with the Chemicals (Hazard Information and Packaging) Regulations 1993, and be accompanied by an appropriate Transport Emergency Card (TREM card).

9.3 Fixing samples

The procedures below are currently used in some laboratories that have the necessary facilities. The procedures have been tested fully and COSHH assessed at these laboratories. Other laboratories adopting these procedures will need to obtain the necessary facilities and undertake their own COSHH assessments.

Fix samples in 10% neutral buffered formalin (4% aqueous formaldehyde). It is best to do this immediately after they have been collected, to prevent carnivores, particularly beetles and Gammaridae, from eating other specimens. The fixative hardens insect and oligochaete cuticle, reducing the chances of specimens disintegrating during storage or handling (except for flatworms and the soft bodies of molluscs). The fixative will also kill most pathogens in the sample, which may be useful if it is from polluted water. It is far better to fix samples when they are fresh than when they have been preserved. If samples have been preserved, keep the samples in alcohol preservative and add the fixative to this, rather than replacing the alcohol with water.

Samples must remain in fixative at least overnight to ensure that it penetrates sufficiently. Samples may be stored in formalin until they are sorted. However, it is better if the fixative can be washed-out after a while and the samples stored in alcohol, which is a better preservative (see Section 9.4). Formalin can seriously affect the colour of specimens if used for long-term storage; it is also more pungent than IMS. If samples have been fixed in formalin, do not return them to fixative but preserve them in alcohol.

Fixative can be added in the field either in concentrated form from small bottles stored in each sample container, or in dilute form (approximately 10% formalin) from a large jerry can.

If concentrated fixative is used, put about 100 ml of neat buffered formalin (buffered 40% aqueous formaldehyde) inside a 150 ml Nalgene screw-capped bottle, using a fume cupboard or fume extractor in the laboratory. Place a pre-filled bottle of fixative in each sample pot. In the field, add sufficient fixative to the sample to result in a 10% formalin solution. Cap the bottle containing unused neat formalin securely, and put it back in the sample pot. Do this outside the vehicle in a well ventilated area. This ensures that neat formalin is always double-sealed, prevents large volumes of fixative from being carried in the same container, and limits the total volume carried to that needed for sampling.

If adding dilute formalin to samples from a jerry can, do this outside the vehicle in a well ventilated area.

Distribute the fixative evenly throughout the sample by gently tumbling it in its container after the lid has been firmly secured. You must leave some air in the container (and polythene bag if used) so that the mixing is thorough. You must do this in the open air when in the field (never in a closed vehicle), or in a fume cupboard or extractor in the laboratory. Check that the sample container's lid seals properly and that no liquid seeps out. If an adequate seal cannot be made, use another lid or transfer the sample to another container. Live specimens can be found in samples after a few weeks if the fixative has been mixed inadequately. If this happens, you must review your procedures to prevent it reoccurring.

Gloves are essential when using concentrated formalin, regardless of whether a barrier cream is used, and the use of barrier cream is recommended. Eye protection and a fume cupboard or fume extractor must be used when concentrated stock solutions are being decanted, and when washing samples containing formalin. Although contact with small quantities is not unduly harmful if rinsed-off immediately with plenty of water, it will sting any breaks in the skin. Individual laboratories should follow the guidelines given in this document, but must also establish their own detailed procedures that are tailored to their particular laboratory conditions and facilities. They must be fully assessed for COSHH by their Regional Safety Advisor.

9.4 Preserving samples

Samples should be transferred from fixative to preservative if they are to be kept for more than a few months. Preservative penetrates biological material better than formalin, and causes less discoloration. Samples should be preserved after sorting if they are to be retained.

Preserve samples in an aqueous solution of 70% industrial methylated spirit (IMS) and 5% glycerol. The alcohol may have to be replaced a number of times to ensure that there is an adequate concentration in the sample after it has penetrated any organic material, and been diluted by water that it displaces. It may be more convenient to add a more concentrated solution of IMS to reduce the number of times that it has to be replaced or to obviate this stage altogether. As a rough guide, add about 8 cm depth of 90% IMS to every 3 cm of sample in a parallel-sided container: add less if there are stones or sand in the sample. The glycerol will prevent the sample drying-out completely if the seal on the sample container is not completely air-tight, and the relatively volatile alcohol evaporates. Distribute the preservative evenly throughout the sample by gently tumbling it in its container with the lid firmly attached. It is important to leave some air in the container (or polythene bag if used) to do this effectively. An air space of about one fifth of the container's volume is sufficient. Dredge samples containing large amounts of silt need particular care to ensure that they are preserved effectively.

Store preserved samples at cool temperatures, away from room heaters or radiators. Store them in the dark to minimise the loss of colour.

10 Examining samples

All sorting and identification for RIVPACS analysis must be undertaken in the laboratory, not in the field, with the exception of material collected by dredge that is not retained in the sample, see Section 7.4. The whole sample must be sorted, even when few animals are found in some fractions of it.

Samples may be examined in the field, to check for the effects of pollution, in particular for the presence of dead animals. These examinations must not materially affect the sample that is returned to the laboratory for sorting and identification for RIVPACS. Only readily identified rare macro-invertebrate taxa may be removed from the sample, as specified in Section 7.1. These must be recorded on the sample data sheets.

Remember that even when they are washed thoroughly in accordance with Section 10.1, specimens may contain undiluted fixative or preservative. This can spurt out of soft bodied specimens when their skin is ruptured, so whenever possible, handle them under water, particularly during identification.

10.1 Washing samples prior to sorting

Fixative, preservative, and silt must be washed from the samples thoroughly with tap water before they are sorted. Fixative will also have to be rinsed from the samples if it is to be replaced by preservative.

Washing must be done in a fume cupboard or under a fume extractor and into water if the samples contain fixative or preservative. Even if they do not, the use of a fume cupboard or extractor is recommended, particularly if the samples are from polluted sites.

The 500 μm sieve is mandatory. Everything retained on it, or on larger aperture sieves, is considered to be part of the sample. It is recommended that a 250 μm sieve be placed below the 500 μm sieve to prevent coarse silt from blocking drains that are not fitted with a silt trap, but beware of water in this 250 μm sieve from blocking the washing process. It is helpful to use larger aperture sieves to split the sample into size fractions to aid sorting. A 4 or 8 mm sieve and a 1 mm sieve are recommended, and may be stacked above the 500 μm sieve during washing.

Always rinse the outside of the sample container first. Decant the liquid in the sample through the sieves and into running water in the sink. Add more water to the sample container, swirl it gently, and pour the liquid off into the sieves. Repeat this until the liquid runs clear. Do not throw stones or debris away. Do not swirl the sample in the sieve and do not use a high pressure spray because this can damage the specimens. Be gentle. Fixed or preserved samples must be rinsed thoroughly: this will take at least five minutes, and longer if the sample is large. Ideally, leave fixed or preserved samples totally immersed in water for a few minutes before the final rinse, particularly if plant material is present. Wash the sink thoroughly to remove any residual formalin or preservative from it. The sample itself is now ready for washing.

If the sample is more than about a litre, wash it in smaller parts to avoid damaging the animals.

If the sample contains a substantial amount of weed or filamentous algae, put the sample in a bucket of water. Let the weed float to the surface and then transfer it to a large bowl or other container of water. If the weed does not float (e.g. when preserved in alcohol), swish it in the upper layers of the bucket by hand to remove most of the animals before lifting it out. Subdivide the weed if there is a large amount of it and wash it in several parts. Wash the weed by teasing it out and letting trapped animals fall to the bottom. Allow the weed to float to the surface again, and put it in another container. The animals and other material that falls out of the weed should be drained on a 500 μm sieve or a stack of sieves. Wash the weed again, and repeat until no animals come out of it. Put the weed into a container to await sorting.

Wash other samples and material removed from weed by one of the procedures below.

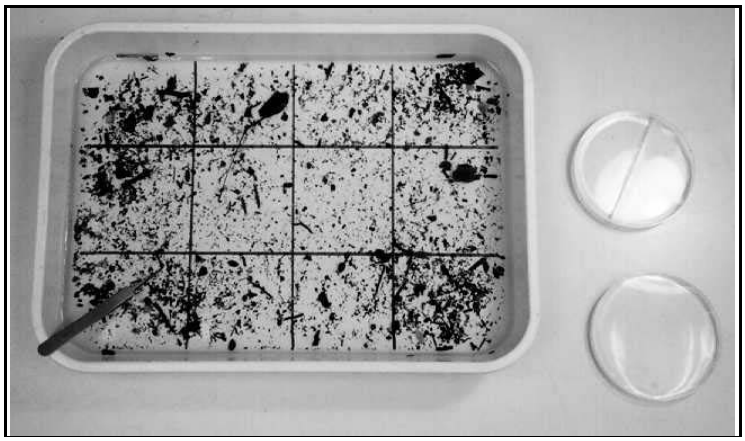
(a) Empty small amounts of sample into the coarsest sieve and wash it by dipping the sieve up and down in a large bucket, bowl or jug of water, in a puddling motion. Smaller objects will fall through the sieve and into this first container. Puddle the sieve until no more material passes through it. The material retained on the sieve should be back-washed into a second container for sorting later. Empty the finer material in the first container into the next finer sieve over a third container, and puddle it. Again, back-wash material on the sieve into the second container. Continue this process until the 500 μm sieve. This should be the last to be used, and anything that passes through it can be discarded. The second container can be replaced by a series of containers, one for each sieve, to separate the sample into size fractions to aid sorting. This is the least damaging method of washing, and also the most efficient, because the whole mesh is cleared every time that the sieve is dipped into the water (so long as not too much material is put in the sieve). This procedure is strongly recommended.

(b) Empty small amounts of the sample into a sieve, or stack of sieves, and rinse with water using a hose attached to the tap or a low pressure spray. Rinse the sample very gently and *never* use a high-pressure spray because this will damage the animals or force them through the mesh.

Never swirl the sample in a bucket or sieve, because this will grind the sample and damage or destroy delicate animals: gills, legs and tails will be lost. Separating animals from coarse silt or gravel by swirling it in a bucket of water and rapidly decanting the animals and debris into a 500 μm sieve whilst leaving the heavier stones in the bucket is not recommended because of this.

Always check that no animals are left in the sample container. Leeches, flatworms and molluscs in live samples often cling to the sides and lid of the container. Add fixed or preserved specimens to the rest of the sample for rinsing. If the sample is live, do not do this, but put the animals into a lidded Petri dish.

10.2 Sorting samples



The Environment Agency's biological laboratories sort samples either live, preserved, or both.

The need to standardise procedures, for RIVPACS and more generally throughout the Agency, has been recognised. This is the aim of the Biological Methods project of which this document is a part. For RIVPACS itself, the ideal is for samples to be sorted preserved, because little is known about the effects of sorting live versus preserved samples from different habitats (e.g. see Hiley, 1995), and because RIVPACS and the quality assurance procedures for the error module are based on preserved samples. The practice followed in a particular laboratory is not only determined by RIVPACS but also by past practice and the views, experience and priorities implemented by the biologists in charge. The Environment Agency's policy on the use of biology in aquatic systems (Environment Agency, 1997) recommends that a comparison of the effects of live and preserved procedures on the quality of sample examination should be carried out, and that following this exercise, a decision should be taken on future policy.

Live samples should be sorted and identified as soon as possible after capture, and must be sorted within 48 hours of collection. This includes any re-analysis of live samples for AQC. A storage temperature of between 1 and 3°C must be maintained during this period. Any live samples not processed within this time or not kept at this temperature must be discarded and new samples taken.

For dredge samples in which only a sub-sample of material collected in the dredge is retained, sort the dredge fraction separately from that of the sweep and search. This is because the abundances in the dredge fraction will have to be multiplied-up by proportion but not the material from the sweep and search.

Small trays are recommended for most purposes. Large trays may be needed when checking through stones and larger material. Some people prefer larger trays for all sorting.

A

B

Figure 10 **The amount of material recommended in tray for sorting a sample**. The amount shown in A is suitable for an experienced biologist, a smaller amount, shown in B, is recommended for inexperienced biologists.

It is much more effective to sort small portions of the sample at a time than sorting the whole sample in one go. Place a small amount of the sample into the sorting tray, see Figure 10. Do not completely cover the bottom of the tray, otherwise you will waste time moving material around to uncover animals that are hidden. A number of trays can be prepared at the same time, and stacked ready for sorting. This saves time in repeated visits to the sink, especially when small amounts of sample are put in each tray. However, you should move around frequently and not spend prolonged periods is the same position: periodic visits to the sink will help stretch your muscles (see Section 10.3). It is recommended that you keep the waterproof label from the sample container(s) in the tray whilst you sort the sample. Add sufficient water to completely cover the sample to reduce reflections: the water will also absorb any fixative or preservative remaining. Spread the sample evenly around the tray, it should not be necessary to move detritus around much. It is helpful if the sample has been split into size fractions so that you can concentrate on searching for animals of a particular size (when searching for small animals it is

easy to miss a large one). When using the range of sieves suggested in Section 10.1, it is usually easier to sort the middle size fraction first (retained on 1 mm mesh, and containing most taxa), then the largest (4 or 8 mm mesh), then the smallest (500 μm).

Good lighting is essential for sorting. The bench lamp should shine from the side and slightly forwards so that reflections from the surface of the water shine away from you, and should be quite close to the tray to prevent shadows. These precautions will maximise the contrast between specimens and the tray and detritus, and reduce the chances of you missing them. You may use a low power magnifier if this helps you (e.g. to overcome impairment caused by short-sightedness).

When sorting a live sample, first view the sample for up to five minutes and remove any obvious fast moving animals such as beetles, corixids or shrimps. Remove these as soon as you see them, and secure them in a lidded container so that they cannot walk or fly away.

Table 6 **Taxa most commonly missed during sorting samples for the BMWP-score system**. This data is from the audit of all such analyses by CEH for the Agency and SEPA during 1995, including primary analyses and AQC inspections; lists covering all BMWP-scoring taxa are provided in the annual reports of the results of AQC audits produced by CEH.

Taxon	n	% of all missed Taxa in 1995 audit
Hydrophilidae (incl. Hydraenidae)	68	5.97
Hydroptilidae	59	5.18
Sphaeriidae	52	4.57
Hydrobiidae (incl. Bithyniidae)	50	4.39
Planariidae (incl. Dugesiidae)	46	4.04
Caenidae	40	3.51
Elmidae	39	3.42
Leptoceridae	39	3.42
Psychomyiidae (incl. Ecnomidae)	39	3.42
Lymnaeidae	33	2.90
Simuliidae	32	2.81
Nemouridae	31	2.72
Limnephilidae	30	2.63
Planorbidae	29	2.55
Haliplidae	28	2.46

Sort through the sample systematically, using the grid marked on the sorting tray as an aid. Scan the tray for floating specimens. Large objects such as stones, sticks, and leaves may be removed from the tray. When sorting through coarse gravel or pebbles, look out for animals that sink rapidly, such as molluscs and cased caddis. 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. See Table 6 for most commonly missed taxa

in samples sorted for analysis to the level required for BMWP-score system; further information about sorting errors is given in Furse *et al.*, 1995. Care must be taken to ensure that similar looking specimens are examined in detail to ensure that examples of each taxon present are recognised. It is recommended that these be put into a Petri dish or similar container during sorting, so that they can be examined more closely for identification and enumeration. Cell culture trays are particularly useful for this, as they have eight to ten separate compartments and a lid. When identification is to genus or species, it is recommended that at least 50 specimens of each major taxonomic group be removed from the sample for identification. Taxa that can be differentiated, but need to be identified under the microscope should also be put into a Petri dish. Always pick-out material that you suspect may be a part of an invertebrate. If it turns out to be detritus you can always discard it later. Use past data as an aid. This is especially useful when unusual taxa, or a wider than normal range of taxa is likely to be found.

Examples of every taxon must be placed in a vial containing preservative, for quality assurance, see Section 11 and BT003.

After the main sort, move the tray or stir its contents with blunt forceps to displace the animals, turn the tray through 90 or 180°, and spend a few minutes searching for any missing taxa. In live samples, snails and flatworms will congregate around the edges of the tray.

After sorting, pour the contents of the tray into a fine sieve so that the sample can be saved for AQC or auditing. A 250 µm mesh sieve is recommended for this, but it must be no coarser than 500 µm. Large diameter sieves are particularly useful for this. Return the sample to the original sample container(s) and add fixative or preservative as appropriate. Never return a live-sorted sample to any watercourse other than the site from which it was collected. This is to prevent the spread of diseases and alien species.

There is no time limit for sorting a sample: this must depend on the sample and the experience of the sorter. Samples identified to family will usually take no more than two hours to sort, unless they come from sites which are very rich, or are particularly difficult to sort (such as peaty samples from fenland rivers). Dredge samples taking more than three hours to sort for BMWP analysis might be too large. These times do not include that spent on identification, which will depend on the expertise of the biologist. Identification to family (excluding chironomids and oligochaetes) can be completed in two hours for *most* samples, whereas identification to RIVPACS species level can take up to two days: one day for oligochaetes and larval chironomids which must be cleared and mounted on slides, and one day for other taxa.

If you break from sorting material whilst it is in a tray, cover it completely (for example with another tray) and put a lid over any Petri dishes to protect the sample and to reduce evaporation. Although not advisable, partly sorted preserved samples can be left overnight like this, but live samples should be returned to their sample container and put back in the refrigerator: if the Petri dish contains preservative, its contents should be put into a vial to prevent the preservative from evaporating.

Biologists and laboratories may develop their own techniques for sorting samples within the general guidelines described above.

10.3 Health implications of sorting samples in the laboratory

Unless the process of sorting biological samples is properly managed, there are a number of health risks. The main risk comes from the biologist sitting in one position for long periods. The following measures are recommended to minimise the risks. However, it is the responsibility of the laboratory manager to ensure that all tasks are undertaken by his staff have undergone a suitable risk assessment and that the assessment is recorded. Adequate control measures must be in place to ensure that the risks of harm are reduced as far as is reasonably practicable. The controls must be reviewed regularly, to ensure that appropriate use is made of up-to-date technology. It is also essential that appropriate maintenance routines be in place to ensure that the equipment remains serviceable.

Figure 11 **Reccomended posture for sorting samples**, the bench top is relatively high and the tray is close to the analyst so that they can maintain a straight back; the object just behind the sorting tray is a fume extractor vent.

The law requires that every laboratory undertakes a risk assessment of all the tasks that it undertakes. This should follow the procedures in the Agency's Risk

Assessment Manual. The assessment should take all relevant factors into consideration, including those listed below. It is recommended that risk assessments be undertaken specifically for major surveys to address any risks associated with substantially increased workloads.

Sit well back and upright in the chair when sorting, in order to maintain a straight back and place the chair close to the workbench.

The bench top should be relatively high, about level with your diaphragm, as shown in Figure 11. This is higher than the level needed for writing. If the work surface is too low, you will tend to bend your head forward to see into the tray and this can put strain on your neck. However, it should not be so high that it puts a strain on your shoulders. It should have a low reflectance surface and provide adequate space. If the height of the workbench is not suitable, a height adjustable pad should be provided that is sufficient to support the sorting tray. Adjust the height of the chair and use a footrest if necessary. The sorting tray should be close to you, and small trays will reduce the distance that you have to bend over to reach the far side. Shallower trays (up to 3 cm deep) can reduce aching wrists, though some people prefer slightly deeper trays that provide some wrist support (up to about 4 cm). Trays deeper than this are not recommended. Use the size and depth of tray that you feel most comfortable with. Your workstation should be adjusted to fit you, not *vice versa*. See Newman (1994) for further advice on workstations for sorting.

Sorting should be undertaken in a well and evenly lit area. Prevent glare, which can cause eye-strain, by positioning bench lamps so that they shine from the side and slightly forwards, causing any reflections to shine away from you. You may have to use a window blind to prevent glare from the sun at certain times of the day if you are working near a window. Conversely, the light source should not cause shadows over the work area.

The temperature of the laboratory should be in the region of 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 if necessary.

Sorting should not be undertaken at a rate that puts your health at unnecessary risk. It is recommended that you 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 can also help. There will be times when the workload is particularly large. At such times, you should be particularly vigilant in assessing your own and others health and safety risks. It is likely that managing the workload will be the aspect of control that requires particularly close monitoring. Managers should ensure that their staff take frequent breaks from sorting, as a matter of course. It is not sufficient to leave this solely to the discretion of staff. In all circumstances, it is recommended that the full breaks are taken, but in times of heavy workload this is especially important and a break from sorting of an hour should be taken in the middle of the day.

10.4 Identifying specimens

Identify all the aquatic macro-invertebrates in the sample, including caddis and Diptera pupae.

The terrestrial or aerial stages of aquatic animals, and wholly terrestrial animals, are not part of the sample. Specimens that were obviously dead when the sample was collected are not part of the sample either; this includes empty snail shells, caddis

cases, puparia and exuviae. These may be recorded in a note accompanying the sample but must not be recorded as part of the sample.

Fragments of damaged specimens can cause errors, particularly when abundances are estimated. Do not include taxa for which only the posterior end is found, even if that part of the specimen could have been healthy when the sample was collected and can be identified. If the thorax and abdomen of an insect is found, it will constitute a record, but if only an abdomen or only a head is found, it must not be included. If such a specimen is recorded on the sample data sheet it will be regarded as an error in the AQC and the audit.

It may be necessary to break mollusc shells and poke caddis cases to check for occupants. Cased caddis should be pushed out of their cases from the rear, because they are liable to break if pulled out from the front.

Identify specimens to 'BMWP' level according to the nomenclature given in Appendix C. If the analysis is to the level required for the BMWP-score system, it is recommended that non-scoring taxa be recorded in the taxonomic groups listed in Appendix C. This is to ensure the standardisation of records for 'other taxa'. Furthermore, it is recommended that abundance categories be also recorded for these taxa.

The taxonomic nomenclature in the latest version of the 'Maitland' coded check-list should be used (Biological Dictionary Determinand Working Group, 1989), with the exceptions noted in the two paragraphs below. Copies are available on disk and as computer listings in each Region, or can be obtained from Mike Furse at IFE. Where the nomenclature has been revised since this checklist was produced, the new name may be recorded in a supplementary note.

The taxonomic groups used in the BMWP-score system follow those given in the original Maitland coded checklist (Maitland, 1977), as does RIVPACS III at family level for BMWP-scoring taxa. Although it is obsolete, it must be used to derive a BMWP-score, ASPT and N-taxa. Thus, for example, the BMWP family Rhyacophilidae follows the definition given in Maitland and encompasses what are now considered to be two separate families: Glossosomatidae and Rhyacophilidae. Note that Chrysomelidae, Clambidae and Curculionidae are not used in the BMWP-score system by the Environment Agency or by RIVPACS III.

The health risks posed by prolonged periods of identification work, particularly using a microscope, must be recognised and action taken to reduce it. As with sorting, correct posture is important. A lower bench than is recommended for sorting, or raised chair and footrest, are necessary for microscopy. The microscopes' eyepiece(s) should be slightly lower your eyes when looking straight ahead, so that they fall naturally to the eyepiece when you bend your head down. The objectives should be about 10 cm in from the edge of the bench, though this will vary from person to person. As with sorting, it is important not to sit in the same position for prolonged periods. When identifying to species, you will probably move around sufficiently as you consult different keys and record results; when identifying specimens to family, alternating between periods of sorting and identification will help. You will have to take particular care when identifying large batches of similar

taxa, for example, when identifying large batches of chironomids or oligochaetes, because there will be less need to move around. In these circumstances, it may be necessary to rest from identification for about ten minutes in every hour.

The work station should be well lit.

Good illumination of the specimens under the microscope is essential, as is clear focusing. You must clean the eyepiece lenses frequently (at least twice a day if use is continual) and the bottom lens at least weekly, using only proper lens cleaning tissue. Zoom microscopes that loose focus when you alter the magnification may need to be serviced. All microscopes must be serviced regularly to ensure that the optics are in good order, properly aligned and focused. On binocular microscopes, you should use eye-cups if you find them more comfortable.

10.5 Enumerating abundances

Although abundances are not currently necessary for the BMWP-score system and environmental quality assessments based on it, they should always collected. They are used for the LIFE index and diagnosis of environmental pressures. They may be used for assessments for Water Framework Directive reporting.

Count only free-living individuals. Do not count individuals of a colony, buds of oligochaetes or coelenterates, or brooding young of leeches. Count each of these 'colonies' as one individual.

Record the abundance of each taxon on the logarithmic scale of abundance categories in Table 7. If another scale is used for any reason, it must be possible to convert the abundances to the scale in Table 7, as this is the scale used by LIFE index (Extence *et al.* 1999), Artificial Intelligence-based diagnostic systems (Walley *et al.* 2001), RIVPACS III, and revisions to BMWP score systems (Walley & Hawkes 1996, 1997).

If taxa are enumerated and recorded at species or mixed taxonomic level as abundance categories, the abundance categories for BMWP-scoring taxa (listed in Appendix C) must also be enumerated and recorded separately. Similarly, if the abundances of families are recorded, the abundance categories for BMWP-scoring taxa that comprise two or more families must be enumerated and recorded separately. The abundance categories of composite and higher taxa cannot be calculated from the abundance categories of their component taxa. Such calculations are only possible from numerical counts or estimates of abundance.

You must distinguish abundance records relating to the whole sample at family (or BMWP-scoring taxa) level from the abundances of undifferentiated specimens identified no further than family when other specimens have been identified to genus or species. When samples are identified and enumerated beyond BMWP, you must check that data used for BMWP indices includes the abundances for the whole sample at BMWP-scoring taxon level.

Although the whole sample must be sorted, the numbers of very abundant taxa in

large samples may be estimated by counting specimens in a portion of the sample (or of a size fraction of the sample) and calculating the total by proportions. Use the grid marked on the sorting tray to estimate the abundances of taxa in the trays. For this, the sample must be distributed evenly in the trays. It is recommended that counts be based on counting at least 50 specimens or at least a third to a fifth of the whole sample. If the sample has been split into size fractions for sorting, remember to estimate totals for each size fraction and sum it to get the total in the sample. Tally counters may be useful for counting taxa that are abundant.

There are a number of different approaches to estimating abundances, depending on the experience of the analyst and the procedure used to sort the sample.

A Select the proportion of the sample on which you intend to base counts on (e.g. ½ or ¼), having first had a brief look at the sample. Count the number of each taxon in this proportion of every tray. For proportions less than ¼, it is recommended that you select individual squares marked on the tray along a diagonal, as the edge of the tray can affect the distribution of the sample in it.

B You may wish to base abundances on counting different proportions from different size fractions of sample (e.g. all the coarse material, 1/4 of the medium size fraction, and 1/8 of the fine fraction).

C Based on a brief inspection of the sample, select different proportions of the sample on which to base the counts of abundant taxa (e.g. Chironomidae 1/10; *Gammarus* 1/4; *Baetis* 1/3; rest 1/2; very rare taxa should be based on the whole sample).

Options A and B are recommended for inexperienced staff, or when analysing samples to family. Option C, or a combination of B and C is more likely to be used by experienced staff, in particular when analysing samples to species.

When analysing samples to family or the level required for the BMWP-score system, easily identified taxa can be counted in the tray without removing them (for example Gammaridae (including Crangonyctidae) and Asellidae). Less easily identified taxa should be removed from the tray, particularly if they are to be identified to species. In this case, all specimens from the proportion of each tray to be counted are removed and placed in a vial, labelled with the proportion of the sample that they represent. These specimens should be enumerated when they are identified. Specimens removed from the rest of the tray or rare taxa whose abundance is not being estimated by proportion should be put into a separate vial to avoid the risk of their abundances being multiplied up by proportion accidentally.

If you are part way through a sample and realise that you need to count a larger proportion of the sample, record the abundance, proportion of the tray and number of trays, then start recording abundance in a larger proportion of each tray and the number of trays in which you record that proportion. If you are simply removing specimens to be counted when they are identified (to species), use separate vial when you alter the proportion, and record on both vials the number of trays (or proportion of the total sample) that they relate to.

Table 7 **Abundance categories.**

Category	Abundance
A	1 - 9
B	10 - 99
C	100 - 999
D	1000 - 9999
E	10000 +

The worked example below is complicated in order to demonstrate all the calculations that could be necessary.

Worked Example

In this example, the estimation of the abundance of Chironomidae is described. Remember that the aim is to base estimates on counts of about 50 specimens.

A quick look at this portion of the sample whilst it was being washed in the laboratory indicated that Chironomidae were abundant, therefore numbers were counted in ¼ of each tray of material.

To simplify this example, the samples are assumed to fill 8 trays. In reality, a large sample is likely to need between 50 and 100 trays when filled with the amount of material shown in Figure 10, and chironomids would be counted in only one square of each tray.

Number of Chironomidae in ¼ of each tray

tray 1	=	6
tray 2	=	7
tray 3	=	10
tray 4	=	8
tray 5	=	4
tray 6	=	12
tray 7	=	7
tray 8	=	5

Number of Chironomidae in ¼ of the sample

Σ	=	59

Number of Chironomidae in whole sample

	=	4 x 59
	=	236

Abundance category of Chironomidae in the sample = C

It is possible for an experienced biologist to estimate the abundance category of BMWP families and species by eye. Whilst sorting, a mental note is made of the

abundance of each taxon using the grid lines for guidance. Having sorted the whole sample, a value judgement as to the appropriate abundance category for each taxon/species can be made. For abundant taxa it may help to estimate the abundance in one or more of the grid squares and multiply to give an estimate for the whole tray.

Inexperienced biologists may need to make a written note of abundances in each grid or tray as they sort the sample.

Trainee biologists should have samples checked by an experienced biologist for accuracy of abundance estimates until they reach a satisfactory level of proficiency.

11 Quality assurance

The aim of quality assurance is to minimise and quantify errors. Full quality control and auditing would involve independent sampling, sorting and analysis. This is impractical because it would be too expensive and time-consuming. Instead, formal systems are limited to laboratory analyses, in particular sorting and identification.

This document, the training video (National Rivers Authority, 1990) and training workshops on sample collection serve for quality assurance for sample collection, the precision of which has been estimated in Furse *et al.*, 1995. If the procedures described in this manual (and others in this series, when appropriate) are followed precisely, errors from variations in sample collection and examination should be minimised.

Analytical Quality Control (AQC) is undertaken within laboratories to enable them to control the quality of their analyses, so that they can ensure that it meets the quality specified by the client, or required for the purposes of the sampling programme. The Environment Agency's standard AQC scheme for sorting and identification for samples collected and analysed by these procedures to the taxonomic level needed for the BMWP-score system involve the re-analysis of 1 in every 10 samples, and control charts based on cusum analysis to ensure that its quality target is met. The procedure is described in detail in BT003. It is most important that AQC inspection is of the highest quality. In practice, this means that it should only be undertaken by experienced biologists. It is also very important that sufficient time is allocated for the AQC inspection. For any AQC scheme to be effective, samples must be inspected as soon as possible after the primary analysis, and the AQC statistics must be kept up-to-date. Experience has shown that the Agency's standard AQC scheme works best when the reanalysis of a sample for AQC inspection is done within two weeks of its primary analysis. New or inexperienced biologists should not be treated in the same was as experienced biologists. Initially, all their samples should be checked by an experienced biologist, followed by a period in which a higher proportion of their samples are checked, until they are of an acceptable quality to join the main laboratory AQC scheme. All data that is archived or used operationally should be subject to AQC.

Auditing is undertaken to measure the final quality of the results of a survey, to check that the analyses comply with the quality specification, to provide error statistics for determining the significance of differences between samples, and to measure the quality of AQC inspection so that it can be taken into account in the AQC parameters. It should be commissioned by whoever commissions the work being audited, to ensure that their quality

specification has been met. The precision of an audit depends on the number of samples on which it is based. Therefore, for any survey, a fixed number of samples should be audited in contrast to a laboratory's AQC in which a proportion of samples should be inspected. The auditor must be independent of those whose work is being audited. Usually this is an independent organisation. The reanalysis of samples for auditing must be of a very high quality.

Since 1990, an independent audit has been undertaken by CEH for samples collected by this procedure and analysed to the taxonomic level required by the BMWP-score (principally to family). Details of this procedure are given in BT003. A similar scheme has also been devised for auditing samples analysed to species, although this is not as well developed as that for BMWP level. Similar schemes will have to be devised for samples analysed to other taxonomic levels, and for estimates of abundance.

It is recommended that the auditors inspect some samples to be analysed by outside contractors before they are sent to the contractor for analysis. It will not be possible to check whether the contractor loses specimens if they are inspected after they have been analysed by the contractor. Further advice is given in BT003.

Additional procedures have to be followed during sorting to accommodate quality assurance. For each sample, primary analysts must place two or three specimens, if available, of every invertebrate taxon being recorded for the survey in a small vial containing alcohol preservative. The specimens retained should include representatives of taxa that are not being recorded (for BMWP analysis these are families that are not included in the BMWP-score system), solely to help identify the causes of errors: errors involving these taxa do not contribute to the measurement of errors. The specimens should be good quality examples rather than the first encountered on sorting. Because they are prone to breaking-up, it is recommended that three specimens of each taxon of flatworms are included in the vial whenever possible. Pupae must be put in the vial if larvae of the same taxon are not present in the sample. Use more than one vial if necessary, but label all multiple vials as being vial number 'x' of 'n' vials. This may be necessary when many taxa are present, or when the identification of different taxonomic groups is to be confirmed by different experts. If a specimen is too large for a vial, such as large mussels, place a note to this effect in the vial. Vials should be stored in one of the sample containers containing the rest of the sample.

If the sample is inspected for AQC, the AQC analyst must not alter the contents of the primary analyst's vials in any way. They must use a separate labelled vial for any additional taxa that they find: its label should indicate that this vial contains taxa found by the AQC inspector but not the primary analyst.

Quality is primarily measured in terms of gains and losses. Gains are taxa found in the vial(s) or the sample by AQC analysts or auditors that were not recorded by the primary analysts. Losses are taxa that were recorded but which are not found by AQC analysts or auditors. Auditors also record gains and losses in the performance of the AQC inspectors. In the Environment Agency's standard AQC and audit for samples analysed to BMWP-scoring taxa, quality is measured in terms of gains only. This is because, at this taxonomic level, gains represent the majority of errors, as most errors are associated with sorting rather than identification. Identification errors are likely to be more common in analyses to species or genus, and losses will be more important. The quality target for analyses involving the BMWP-score system is an average of no more that two gains. The error module in RIVPACS III+ uses net gains (gains − losses).

Sample data must not be altered in the light of AQC or audit results, because this will invalidate the measure of analytical quality provided by the audit and may cause difficulties when analytical quality is taken into account in the comparison of data from different samples. However, it is helpful if quality assurance results can be taken into account for certain purposes, such as when investigating the distribution of individual taxa.

12 Archiving data

Sample data, in particular that used for environmental assessment, must not be corrected in the light of results from the AQC or audit. If corrected data is to be retained, it must be archived separately.

When samples are identified and enumerated beyond BMWP-scoring taxa, you must check that data in GQA datasets, or used for the abundance or full family modules of RIVPACS, includes the abundances for the whole sample at BMWP-scoring taxon level and not of the of component families of composite BMWP-taxa or the abundances of the few specimens which could not be identified further when the rest of the sample has.

Errors can be reduced if the staff entering the data have a biological background. Double entry of data can also be used to reduce errors: data being entered twice, each time by a different individual.

All data entered onto databases must be checked for transcription errors against the original data records. This is most easily done by two people; one reading out the original data records, the other checking against a listing from the database. A record of checking must be kept (see Section 13).

13 Sample traceability

A record of the status of all samples should be maintained. It should include items 1, 5, and 6 from the list below. The other records are optional.

1. Sample identification (i.e. watercourse name, site name, site code, sample code).
2. The reason for the sample (purpose code or survey number).
3. An indication of whether a water sample or meter reading for alkalinity or one of its surrogates is to be collected with the biological sample.
4. Date by which the sample must be analysed.
5. Date when the samples was collected, and the initials of the sampler.
6. Date when the sample was analysed and the initials of the analyst.
7. An indication that a record for the sample has been entered on the database.
8. Date when the sample record on the database was completed (except chemical information). This will enable samples with incomplete data to be identified easily.
9. Date when the chemical information was entered onto the database (e.g. alkalinity. if it is to be used), if appropriate.
10. Date when the record on database was checked for data-entry error.

The loss of samples or data should be recorded where appropriate.

A similar record should be kept for samples to be re-analysed for laboratory AQC, however it is most important that primary analysts do not know which samples are to be inspected until the inspection has been completed.

This record must be maintained continuously on either a computer or paper log.

14 References

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BT001
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Appendix A Long-handled pond-net

A standard FBA-pattern long-handled pond-net should be used. This has a frame with a straight lower edge of 20-25 cm, and straight, vertical sides of 19-22 cm. It should be fitted with a 1 mm mesh collecting net, as specified below. The net bag should be square with rounded corners, not conical. Nets and frames from different manufacturers vary in design (see Figure A.1), but their principal dimensions must not differ from those above.

Figure A.1 Different pond-net frames and collecting nets. Frames, from left to right: SM Davis; GB Nets (the example illustrated is larger than the standard pattern currently produced); original FBA. Collecting nets, from left to right: multi-filament polyester, 30 cm deep (not recommended for use with RIVPACS); multi-filament polyester, 50 cm deep, suitable for RIVPACS; shallow heavy-duty monofilament net **which must not be used for this procedure**, recognisable by canvas reinforcements over seams and its stiffness.

Polyester nets are available 30 cm deep and 50 cm deep. For this procedure, 50 cm nets should be used. They become blocked less easily because of their greater mesh surface so perform much better on soft sediments, or where there is a large amount of vegetation or detritus, and they are less prone to emptying in eddy currents. Monofilament nylon nets are usually about 25 cm deep or less, and must not be used for this procedure.

Pond-net handles should be about 1.5 m long. Longer handles may be used in deep waters, for example for collecting sweep samples from deep rivers, but they are not recommended for general use.

Periodically, check that the bottom-edge of the frame is not bent, because this reduces its sampling efficiency. Thin gauge aluminium frames are particularly prone to this type of damage.

The collecting net is a critical part of the sampling equipment because it determines the size of the animals caught. Collecting nets used for this procedure must be woven from multi-filament polyester with an oval shaped 0.8 □ 1.4 mm ± 0.2 mm aperture (loosely termed a 1 mm mesh), whatever type of sampler is used. Note that this is larger than the maximum specified in British

Standards Institute, 1994 and 1995.

Use only multi-filament polyester nets for this procedure. Do not use monofilament nylon nets. Although multi-filament polyester nets are manufactured to less precise tolerances and are more easily holed, they are easier to repair than monofilament nets. Because they are more flexible, they are much easier to empty.

Damaged nets must not be used. Always carry spare nets when sampling to replace damaged nets, together with tools for changing them (usually a small screwdriver and spanner). Nets are prone to damage, and require care and regular inspection. Repair small holes with rot-proof thread: discard nets with large tears or more than a few repairs. Dry the nets after each day's sampling: this is particularly important for nets bound with cotton thread.

Nets can be protected from accidental damage with stout covers whenever they are not being used.

Appendix B Crayfish Plague: Guidelines to Prevent Its Spread

Introduction

These guidelines describe measures the Environment Agency must take to prevent the spread of crayfish plague by its own activities.

Background

The introduction of the American signal crayfish, *Pacifastacus leniusculus*, into the UK has undoubtedly contributed to the decline in numbers of our only species of native crayfish, *Austropotamobius pallipes*. Apart from direct competition between the two species, the signal crayfish is a vector for the fungus *Aphanomyces astaci*, more commonly referred to as the crayfish plague fungus. This has wiped out populations of native crayfish in many rivers.

Incidents of crayfish plague were first recorded in the early 1980s, and staff at the Centre have confirmed thirteen outbreaks for Environment, Fisheries and Aquaculture Science (CEFAS. Twelve other cases that were not confirmed as plague have been suspected as causing crayfish kills. No outbreaks have been reported since 1993, but it is believed that signal crayfish can carry the plague indefinitely and it is highly likely that the plague is still present in Britain.

Technical advice

Transmission of fungal zoospores

A. astaci produces free-swimming zoospores that are specific to crayfish. These spores can be transmitted in water, mud and via damp equipment. An outbreak of plague in the midland lakes in Ireland wiped out about one million crayfish in 1987. In the absence of any exotic crayfish species, it is believed that the disease was introduced inadvertently on contaminated angling equipment. The risk of picking up spores is greatest at times of a plague outbreak, when the number of free-swimming zoospores is high.

Human activities are widely recognised as the main agent for transferring crayfish plague. In carrying out our duties such as biological sampling and fisheries surveys there is a chance that we could introduce *A. astaci* where it could infect populations of our native crayfish or infect other populations of 'clean' signal crayfish. It is believed that the spores of *A. astaci* have been introduced into water-bodies on fish scales during restocking operations. This has implications for fisheries staff, particularly when moving fish from a water-body known to contain signal crayfish or when moving fish into a water-body known to contain native crayfish.

An action plan for *A. pallipes* has been drawn up in the Biodiversity UK Steering Group Report, setting out action plan objectives and targets. Section 4.1 of this (page 157) states: “Attempt to maintain the present distribution of this species [*A. pallipes*] by limiting the spread of crayfish plague, limiting the spread of non-native species, and by maintaining appropriate habitat conditions.”

Preventing the spread of *A. astaci*

As the spores remain viable only when damp, complete drying of equipment that has been in contact with the water or sediments is an effective way of killing the spores. Washing mud off waders on site, preferably with a scrubbing brush, will also reduce the risk of transferring spores elsewhere.

Depending on the distribution of crayfish in an area, it may be possible to arrange sampling runs to sample sites containing signal crayfish at the end of the day. This would make the drying of equipment much easier (i.e. overnight) and reduce the need to carry disinfectant in the vehicle.

It may also be necessary to arrange biological sampling runs from upstream to downstream, contrary to normal sampling methods. The more usual downstream-to-up practice (to prevent sampling invertebrates which have been disturbed from upstream sites and drifted down) would be acceptable only if there was a reasonable distance between sites. This distance varies and depends on several factors, particularly flow velocity. If sites are considered too close together then sampling downstream-to-up is not acceptable. To increase flexibility, a second set of clean equipment could be used to eliminate the need to carry disinfectant in vehicles.

Spores can be killed by disinfectants. Iodophore-based compounds used by fisheries staff to prevent the transfer of fish diseases and parasites are effective. Disinfectants can be applied using a spray applicator although it may also be necessary to use a bowl to dip nets and equipment.

Recommendations

All equipment used at sites known to contain signal crayfish must be dried out thoroughly before it is used at other sites. All sites known to contain native crayfish should be sampled with clean and dry, or disinfected equipment.

All mud must be washed off waders and other equipment on site.

A second set of clean equipment could be carried in the vehicle and used when appropriate.

When necessary and preferably after drying, equipment should be decontaminated using an iodophore-type disinfectant at 100ppm available iodine for at least five minutes. If equipment cannot be dried then it must at least be cleaned thoroughly to avoid transfer.

Sampling programmes should be planned accordingly. Where possible, a site containing signal crayfish should be sampled last in the day.

If you suspect a plague outbreak

Crayfish suffering from the plague show abnormal behavioural signs, they are normally nocturnal but will tend to wander out in the daytime. They often walk in an upright manner, sometimes

described as 'on stilts' and in an uncoordinated 'trembly' way. In the latter stages the crayfish lie on their backs in a torpid state. To confirm the cause as *A. astaci*, specimens should be sent with minimal delay to an expert. (The contact details for the expert used by the Environment Agency are given in it procedures manual BT001.)

In addition, specimens can be sent and/or incidents of crayfish mortality reported to Dr David Rogers at Crayfish Consultants International Ltd. There is no charge for this service. Dr Rogers is collaborating with the University of Munich in a research project looking at all crayfish diseases and investigating the various strains of crayfish plague. The contact details are as follows:

Dr David Rogers
Crayfish Consultants International Ltd
9 The Moat
Castle Donnington
DERBY DE74 2PD
Tel. and fax 01332 850156

For both the above, specimens should be sent in a cool box with icepacks and kept damp, and sent by courier. It is essential to telephone in advance to request diagnosis.

Appendix C Taxonomic Groups Recorded for this Procedure

The taxonomic groups recorded for the BMWP-score system are listed below, together with other families used in full family RIVPACS analyses. Except for families of Oligochaeta and sub-families or tribes of Chironomidae (listed here in square brackets if included in RIVPACS or curly brackets if not included in RIVPACS), these other families are recorded by the Environment Agency during river quality assessment surveys. Although not used for classifications based on BMWP-score system, the other families recorded by the Environment Agency are used for other systems, or may be used in the future. Taxa used for the BMWP-score system are marked in the list by an asterisk or (if not included in RIVPACS) a plus sign. Where the families used by the BMWP-score system have since been sub-divided, the current families (according to Biological Determinand Dictionary Working Group, 1989) are given in parentheses alongside the original BMWP family name. Both families are also recorded separately (as needed by LIFE index). Where the nomenclature used in RIVPACS III differs from that given in Maitland (1977), the current version is given and the original follows it in square brackets.

Spongillidae
Hydridae
* Planariidae (incl. Dugesiidae)
* Dendrocoelidae
Chordodidae
Ectoprocta
* Neritidae
* Viviparidae
* Valvatidae
* Hydrobiidae (incl. Bithyniidae)
* Physidae
*Lymnaeidae
* Planorbidae
* Ancylidae (incl. Acroloxidae)
Margaritiferidae
* Unionidae
* Sphaeriidae
Dreissenidae
* Oligochaeta
{Aelosomatidae}
[Lumbriculidae]
[Haplotaxidae]
[Enchytraeidae]
[Naididae]
[Tubificidae]
[Lumbricidae]
{Dorydrilidae}
{Branchiobdellidae}
* Piscicolidae
* Glossiphoniidae
* Hirudinidae
* Erpobdellidae
Hydracarina
Argulidae
* Astacidae
* Asellidae
* Corophiidae
* Gammaridae (incl. Crangonyctidae & Niphargidae)
* Siphlonuridae
* Baetidae
* Heptageniidae
* Leptophlebiidae
* Potamanthidae
* Ephemeridae
*Ephemerellidae
* Caenidae
* Taeniopterygidae
*Nemouridae
* Leuctridae
* Capniidae
* Perlodidae
* Perlidae
* Chloroperlidae
* Platycnemididae
* Coenagriidae
+ Lestidae
* Calopterygidae [=Agriidae]
* Gomphidae
* Cordulegasteridae
* Aeshnidae
+ Corduliidae
* Libellulidae
* Mesoveliidae
* Hydrometridae
Veliidae
* Gerridae
* Nepidae
* Naucoridae
* Aphelocheiridae
* Notonectidae
+ Pleidae
* Corixidae
* Haliplidae

+ Hygrobiidae
* Dytiscidae (incl. Noteridae)
* Gyrinidae
* Hydrophilidae (incl. Hydraenidae)
* Scirtidae [=Helodidae]
*Dryopidae
* Elmidae [= Elminthidae]
*Sialidae
  Osmylidae
  Sisyridae
* Rhyacophilidae (incl. Glossosomatidae)
* Hydroptilidae
* Philopotamidae
* Psychomyiidae (incl. Ecnomidae)
* Polycentropodidae
* Hydropsychidae
* Phryganeidae
* Brachycentridae
* Lepidostomatidae
* Limnephilidae
* Goeridae
* Beraeidae
*Sericostomatidae
* Odontoceridae
* Molannidae
* Leptoceridae
  Pyralidae
* Tipulidae
  Psychodidae
  Ptychopteridae
  Dixidae
  Chaoboridae
  Culicidae
  Thaumaleidae
  Ceratopogonidae
* Simuliidae
* Chironomidae
    [Tanypodinae]
    [Diamesinae]
    [Prodiamesinae]
    [Orthocladiinae]
    [Chironomini]
    [Tanytarsini]
  Stratiomyidae
  Rhagionidae
  Tabanidae
  Empididae
  Dolichopodidae
  Syrphidae
  Sciomyzidae
  Ephydridae
  Muscidae