

Freshwater macro-invertebrate sampling in rivers

Operational instruction 018_08

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What's this document about?

Describes how to collect and sample river macro-invertebrates. It is the first in a series of documents related to macro-invertebrate monitoring in rivers. It replaces the manual known as BT001 (Murray-Bligh, 1999).

Who does this apply to?

This document applies to all staff planning surveys, creating sampling sites or taking samples of river macro-invertebrates.

This document is for staff at level 2 capability of the data and information management in the environmental monitoring and appraisal technical development framework.

Contact for queries and feedback

- [Helpdesk services](#)
- Anonymous feedback for this document can be given [here](#)

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Introduction

Standard method

This method is the standard for the Environment Agency, the Scottish Environment Protection Agency and Northern Ireland Environment Agency.

Training and competency

To carry out the procedures in this manual you must be signed off as capable to sample invertebrates under the [technical coaching scheme](#) or be under supervision.

To operate an airlift sampler you must have done the compressed gas course (T535).

Health and safety

When you use the methods described here, you must follow all the relevant health and safety instructions. The important ones are listed in [Related documents](#).

In some habitats, especially in industrial or urban areas, take care when sampling (manual search and emptying the collecting net) to avoid sharp objects that may have been washed into the net or lie on the substrate.

You must wear correct Personal Protective Equipment (PPE), such as cut resistant gloves.

Biosecurity

Biosecurity is the measures we must take to reduce the risk of spreading diseases, parasites and invasive non-native plants and animals. As Environment Agency staff move between water bodies more frequently than other people, we need to adopt good practice to ensure that we do not aid the movement of invasive non-native species, diseases and parasites.

Monitoring staff must always take a precautionary approach. Where you or your equipment is in contact with the water, you must always follow [Check, Clean Dry](#).

Good planning is the best way to reduce the risk of spreading invasive non-native species. It may be appropriate to put in extra measures at vulnerable locations such as using separate equipment. Staff should not carry out monitoring activity where the biosecurity risk is high and appropriate measures are not in place.

Equipment

Essential

Your essential equipment includes:

- a pond net, long handled pond net or airlift sampler;
 - sample containers (you may need two for large samples);
 - a metre rule or pond net handle/river crossing staff marked in 5 cm intervals;
 - a watch or stop watch, that is water resistant;
 - cut resistant gloves;
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Extras for airlift sampling

If you are airlift sampling, you will also need:

- air cylinders;
 - boat
 - spare collecting nets.
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Net

The collecting net is critical for all methods described in this document. It determines the size of the animals caught. All methods use nets of the same fabric and mesh size.

Type of net required

Follow the guidelines below to ensure that you use the right type of net:

- collecting nets must be woven from multi-filament polyester with an oval shaped 1.4 mm ± 0.2 mm aperture;
- do not use monofilament nylon nets because they are less flexible and harder to repair;
- use a square net bag with rounded corners - not conical.

Caring for nets

Follow the guidelines below to care for your net:

- do not use damaged nets;
 - carry spare nets and tools to change them, a small screwdriver and a spanner;
 - inspect the nets regularly and 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, especially nets that are bound with cotton;
 - protect nets with stout covers when they are not being used..
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Container

Your containers can be pots, buckets or polythene bags.

They must be capable of being securely sealed and be strong enough to handle knocks in the field or damage from sharp items, such as twigs and stones, in the sample.

Only use polythene bags for transporting live samples if they are placed in a rigid container.

For further information about containers refer to [95_06 Fixing and preserving freshwater macro-invertebrate samples](#) on the use of preservatives.

When to sample

For RIVPACS

Take samples for use with RIVPACS and RICT predictions in:

- Spring (March, April and May);
- Summer (June, July and August);
- Autumn (September, October and November).

Note: Winter samples are not adequately represented in the RIVPACS reference collection. They can be compared with Autumn (December, January) or Spring (February) predictions, but this is not recommended. Sampling for some drivers occurs within set periods within these seasons,

for examples drought monitoring is in April and May and September and **October** only.

You must sample in the time frame set by the sampling calendar set on RouteLIMS.

Consider purpose

Follow any specific instructions related to the reason that you are doing the survey.

Example: WFD classification samples must be taken in Spring and Autumn.

For a new site, you must make at least three visits in one year to collect environmental parameters.

Not during and after spates

Avoid sampling during and soon after spates.

Reason: As well as the health and safety risks, samples will not be comparable with those collected at other times (or with predictive models) and will not accurately reflect the underlying ecological quality of the site. This is because some macro-invertebrates may have been displaced from their normal habitat.

Where to sample

Sampling site

Sampling sites cover a 'sampling area' and a larger 'survey area'. The whole site should be broadly similar in its physical characteristics.

Physical characteristics of the sampling site should be as natural as possible, similar throughout the section and representative of the river stretch. Predictions from RIVPACS or other tools are based on the fauna expected under natural conditions.

You can sample watercourses that are normally dry for part of the year, such as winterbournes, using the standard methods. However these types of river are not well represented in RIVPACS and you should treat any predictions for these sites with caution.

Sampling area

This is where you actually collect the sample.

The sampling area must cover the whole width of the stream, if it is accessible.

Its length will depend on the width of the stream and the variability of its habitats. It will usually be between 5 and 20 metres long and will be longer in narrow streams than in wide rivers.

It must be a single area of river bed, where the major habitat types can be sampled within a 3 minute period.

Any environmental measurements you take must reflect the nature of the sampling area. Where the survey area has a variety of physical characteristics, the sampling site should possess the most prevalent features, not necessarily all of them.

What to avoid

The sampling area must not be a collection of separate sampling points within an extended length of river, for instance do not attempt to include both riffles and pools in an attempt to increase the variety of animals

captured. This would cause over-sampling and an overly rich sample resulting in a higher classification representing better quality than exists and mean depth, width and substrate would not be representative.

Survey area

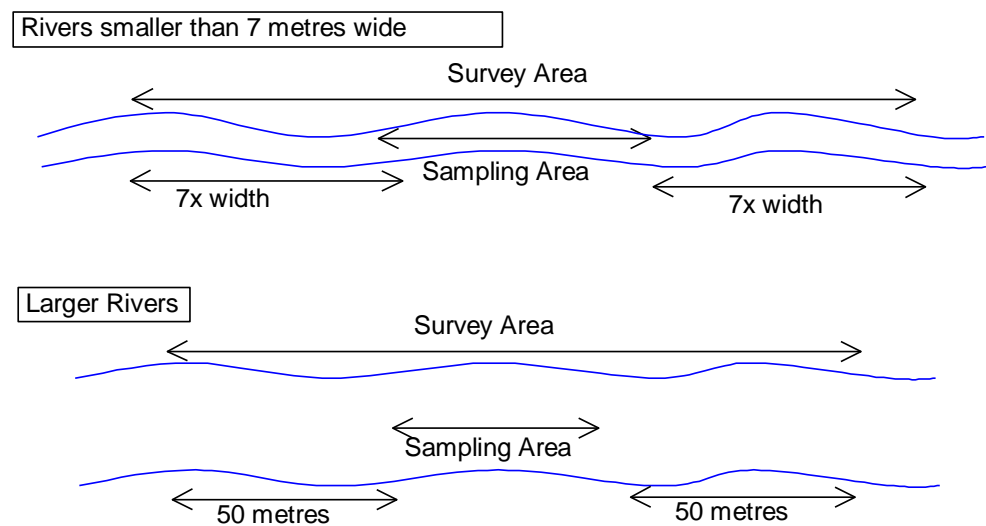
The survey area must be seven channel widths, either side of the sampling area up to a maximum of 50 metres. The [diagram](#) below, illustrates this.

Having a sampling area within a larger but similar survey area reduces differences between samples taken on different occasions that are not from the exact location sampled previously.

Surveys that need more intensive sampling, such as those for conservation purposes or those requiring replicate samples, can also use this larger area.

Diagram of sampling site, survey area and sampling area

A sampling site showing the location of the survey area and the sampling area.



Sites to avoid

Aim to avoid sites that non representative of the waterbody for example sites that are:

- close to artificial influences, such as dams, bridges, fords, weirs or livestock watering areas; If this is not possible, the site must represent the reach as a whole. Record any artificial influences on the field data form and take them into account in data analysis.
- immediately downstream of confluences or discharges where waters are not fully mixed;
- too close to structures associated with in-stream lakes and reservoirs;
- on stretches subject to dredging or regular weed removal;
- in isolated habitats, such as riffles that are uncommon in the rest of the reach. Isolated habitats have less diverse invertebrate assemblages (see Begon et al., 1996: Chapter 23), that RIVPACS does not recognize causing over prediction and inaccurately poor status classification.
- on braided or divided channels. If the site cannot be located elsewhere, sample from the largest natural channel.
- predominantly on bedrock, as it is difficult to sample the invertebrate fauna and the fauna will be sparse.

Exception: If the survey is to examine the impact/communities of the features described.

Choosing a sampling method

First visit

On the first visit to a site, follow the instructions in the [flow diagram](#) below to choose the appropriate sampling method. If in doubt use the deep-water method. This first assessment must be done under normal flow conditions, as once the sampling method for the site is established it must always be used at this site to ensure consistency. The method must be detailed in the site file and in MSIS additional comments so that the same task type is consistently entered on Routelims.

Selecting the correct method

At sampling sites where the average depth across the channel is 80cm or less you must use the standard 3-minute kick sample with 1-minute manual search.

In rivers where the average water depth across the channel exceeds 80cm, the conventional kick sample technique becomes ineffective. In these situations, you must select the appropriate deep river sampling method using [the flow diagram](#).

There are two allowable deep river sampling techniques:

- Sweep sampling using a long-handled pond net
- Airlift sampling from a boat

Important! Sampling using a dredge of any type is no longer permitted.

All methods include an additional 1-minute search. Do not combine any of the three distinct sampling techniques (kick sample with pond net, sweep samples with long handled pond net, airlift sample) for the main sample within a single sample. Pond nets must be used for the search and the sweep that accompanies deep-water samples.

The choice of sampling techniques for deep rivers (over 80cm) is determined solely by the average width. The table below describes the decision process summarised in the [flow diagram](#) in more detail.

Step	Action
1	Measure the depth of the river. If the depth, averaged across 3 measurements taken at $\frac{1}{4}$, $\frac{1}{2}$ and $\frac{3}{4}$ river width, is less than or equal to 80cm, a reasonable proportion (at least half) of the sampling area is deemed to be wadeable and a standard pond net kick sample is appropriate.
2	If the average river depth is more than 80cm, kick sampling is no longer suitable, you must use one of the two deep-water methods. The deep-water method is determined by the width of the river at the sample site. Determine the river width using a range finder.
3	If the average width of the river is 15m or less, the river is deemed to be deep but narrow and a sweep sample using a long-handled pond net is appropriate.
4	If the river is more than 15m wide, the river is deemed to be deep and wide, and an airlift from a boat must be used to collect a sample. This is the only way to represent properly the benthic

	fauna of all the habitats in rivers that are both deep and wide.
5	This approach, called the 80:15 rule, must be the sole basis for determining the choice of sampling method.
6	The average width and depth measurements can be spot measurements taken on the day of sampling, but it is better to use long-term averages to determine the choice of sampling method used at the same site on repeated visits. Base your choice on the deepest and widest measurements taken during periods of normal flow, to ensure that the same method can always be used.
7	You must read the 426_05 Generic Risk Assessment on working in or near water and should generally take two people for invertebrate sampling.

Exception to 80:15 rule

If your site is shallower than 80cm but has deep soft silt substrate making it unsafe to enter the channel and kick sample the sweep sample with a long handle pond net method should be used. This is for health and safety reasons and the ONLY reason you can choose a method outside of the 80:15 rule.

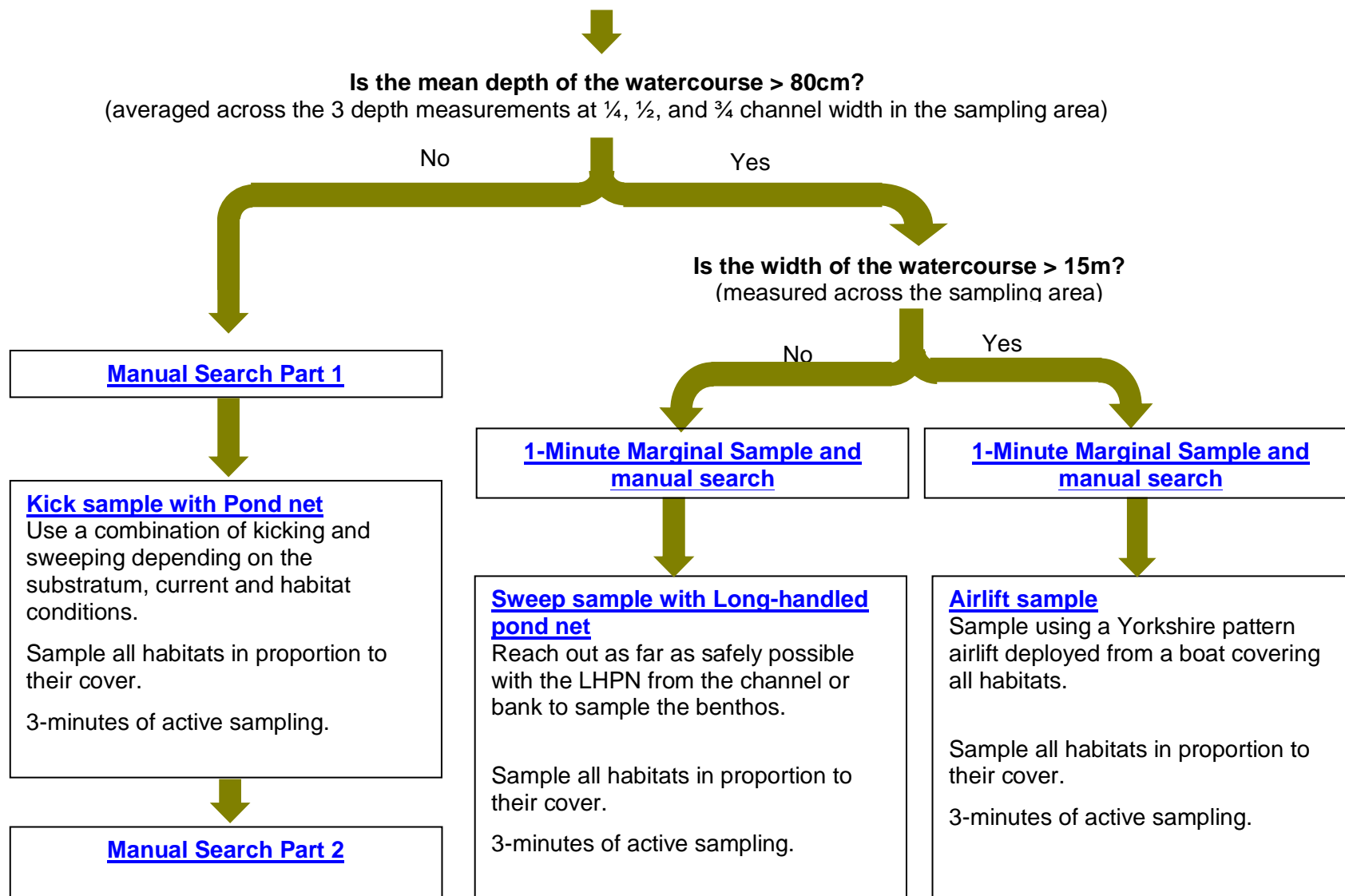
Recording the method

Record the sampling method on BIOSYS. See [Store data on BIOSYS](#) for more information.

Important! Once the method for a particular site has been determined, you must continue to use that same method for collecting all samples from that site. For example if you visit a site which is normally sampled using kick sample methods and flows are slightly elevated making average depths greater than 80cm, do not switch sampling methods to sweep sampling with along handled pond net or airlift. You must revisit and sample the site when the flows have dropped below 80cm.

Samples collected using the 3 techniques are not directly comparable.

How to select a sample method



Site base data

Base data

This is the physical information about a site and is needed for all new sites.

It is needed by RIVPACS and other classification tools to generate predictions. All site registration data used for RIVPACS should be an average of at least two independent measurements.

Collect grid reference, altitude, [distance from source](#), [slope](#) and [discharge category](#) in the office. Do this after the first visit to the site when you have confirmed that it is suitable to sample.

Collect and record this data for all sites sampled with standard methods. How to collect and calculate this data is covered in the [BIOSYS user guide](#).

Store the data on BIOSYS.

Sample environmental parameters

Recording data

Record the data electronically on your PDA.

If conducting a survey without a PDA, for example during incident response you may use the [paper survey form](#). However all data will need to be manually entered onto BioSys if this is used.

Data at new sites

Plan for and collect sufficient environmental data at new sites. To generate predictions in RIVPACS, you need annual average environmental parameter values, even if the prediction is for a single season's fauna.

Base annual average environmental parameters on a minimum of three measurements taken in each of spring, summer and autumn. If the annual averages are based on more data, take the measurements evenly throughout the year.

Mandatory parameters

For all samples taken using the standard methods, you must record width, depth, and substrate composition. These are needed to generate a RICT prediction and classification.

Supporting parameters

In addition to the mandatory parameters, there are many supporting parameters that are useful for interpreting your invertebrate data. They must be collected in the field alongside standard samples. Record all data on BIOSYS via the PDA.

Collect environmental parameters for the whole sampling area. That is, the full width of the watercourse along the whole length of the sampling area, even if parts are inaccessible for sampling. Do this before you take your sample.

Many supporting parameters are subjective categories. Compare them with colleagues to ensure consistency. Only record them as 'not present' if you have specifically looked for and not found them.

Example: 'Sewage fungus under stones' should be left blank or entered as 'not recorded' if a search has not been made.

NB: Some drivers have particular site information required. For example, all invertebrate sites sampled for drought purposes must have site photos taken every time the site is sampled.

Chemical parameters and RIVPACS

RIVPACS requires annual average alkalinity to make its predictions. An absolute minimum of three, relatively evenly spaced alkalinity measurements at each site are required. We recommend monthly values collected over a 12-month period for calculating the annual average. Store raw measurements on WIMS.

Make a note in the BIOSYS site comments if a site's long-term alkalinity is affected markedly by human influences, such as acidic mine drainage.

Alkalinity is preferred because it is used in the RIVPACS reference database. If alkalinity is not available you can use total hardness, calcium concentration or electrical conductivity as a surrogate.

Mandatory parameters

Stream width

The table below describes how to record the stream width.

Step	Action
1	<p>Choose a point that reflects the predominant conditions in the sampling area.</p> <p>Measure the width of the water surface (not the stream channel) at right angles to the channel.</p> <p>Include water under overhanging banks and any temporary islands that have formed in the channel because of low flow.</p> <p>Use a metre rule, marked pond net handle, river-crossing pole or tape measure.</p>
2	<p>For wide or deep rivers that cannot be waded, use a calibrated rangefinder or estimate the stream width making use of nearby bridges.</p>
3	<p>Try not to estimate. However, if you have to, estimate widths as follows:</p> <ul style="list-style-type: none">• <1 metre to the nearest 10 cm;• 1 - 2 metres to the nearest 20 cm;• 2 - 10 metres to the nearest 50 cm;• >10 metres to the nearest metre.

4	<p>Use the definition of widths below.</p>
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Stream depth

The table below describes how to record the stream depth.

Step	Action
1	<p>Reflect the predominant conditions in the sampling area.</p> <p>Average measurements from a quarter, half and three-quarter distances, across the stream transect, within the sampling area (where the width is the water surface).</p>
2	<p>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.</p> <p>If there is a temporary island at a measuring point, the depth there will be zero. Record it as this.</p>
3	<p>Use a marked pond net handle or a metre rule.</p> <p>When using a rule, ensure that the narrow edge is facing the current to avoid distortion.</p> <p>In deep rivers when airlifting a sample, mark the anchor rope and airlift rope with 10cm intervals to aid depth measurements.</p>
4	<p>If the stream is wadeable, record the depth to the nearest centimetre.</p> <p>If the depth cannot be directly measured, estimate it as follows:</p> <ul style="list-style-type: none"> ● <1 metre to the nearest 10 cm; ● >1 metre to the nearest 50 cm.
5	<p>It is difficult to estimate depth in deep rivers. The predictive equations in RIVPACS are based on logarithmic values. Therefore, they are robust enough to withstand a reasonably wide range of error in the greater depth range. If using an airlift you can measure the depth using a line and sinker from the boat.</p>

Substrate composition

The table below describes how to record the substrate composition.

Step	Action
1	<p>Assess the composition of the stream bed over the whole sampling area. That is, the full width of the river along the whole length sampled, even if parts are inaccessible.</p> <p>Your estimates should represent a bird's eye view. Only include particles on the surface of the stream bed, including the equivalent superficial layers under macrophytes.</p>

	In deep rivers when airlifting an underwater camera (on an extendable pole) linked to a tablet in the field to video the substrate is very helpful for making the assessment.
2	If you can see the shape of underlying stones through a fine layer of silt or clay, record the underlying substrate. Record the silt as 'overlying silt' in BIOSYS. If the shapes of the underlying stones are not distinct, record the silt or clay.
3	Record compacted clay as clay, even when broken up into gravel-sized fragments.
4	Record bedrock as part of the substrate percentage cover. It is useful to record the percentage cover of bedrock because this can affect the abundance of animals in the sample.
Note	RIVPACS calculations are based on an adjusted substrate cover, excluding the bedrock and adding up to 100% cover. The Biosys to RICT data extractor will make this adjustment for you.
6	The categories can be recorded as their individual components within BIOSYS. Examples: silt and clay separately The components are automatically combined by the Biosys to RICT data extractor.

Substrate size categories

The table below lists the substratum particle size categories recorded for RIVPACS.

Category	Width	Description
Clay	<0.06	Sticky and cohesive.
Silt	<0.06	Soft in texture and not abrasive to the hands when rubbed. Not cohesive or sticky
Sand	0.06 - 2	Smaller than instant coffee granules and, unlike silt/clay, abrasive to the hands when rubbed.
Gravel	2 - 16	Instant coffee granule to broad bean
Pebble	16-64	Broad bean to half fist size.
Cobbles	64-256	Half fist to head size
Boulders	>256	Head size and larger

Percentage cover

The table below describes how to record the percentage cover.

Step	Action
1	Walk along the river bank after collecting surface living animals and make a preliminary note of the substratum. Initial evaluations are very useful at silty sites and help decide the appropriate level of sampling effort in different habitats.
2	After sampling, walk over the whole sampling area to make final estimates.

3	<p>It is difficult to judge the composition of the river bed in deep or turbid water.</p> <p>Use 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 to help.</p>
4	<p>Percentage cover estimates are subjective but can be improved by experience and by comparison with a colleague's estimates.</p> <p>Use the exercise in the Ecological Sampling CD to test your estimates to see if you tend to over or underestimate.</p>
5	<p>The aid to determining percentage cover may be useful in the field.</p>

Supporting parameters

Supporting environmental data

The table below sets out the details of these fields

Detritus - the amount of leaf litter and woody debris at the sampling site	<p>None: None present</p> <p>Local: Occasional patches - < 30% of area</p> <p>Widespread: 30 - 60% of area</p> <p>Extensive: > 60% of area</p>
Sewage litter - the amount of sewage litter at the sampling site	<p>None: None present</p> <p>Local: Occasional patches - < 30% of area</p> <p>Widespread: 30 - 60% of area</p> <p>Gross: > 60% of area.</p>
Bed stability - the 'kickability' or ease of sampling at a site - used to assess potential effects of the substrate on sampling efficiency	<p>Solid: Substrate is not disturbed by kick sampling</p> <p>Stable: Substrate difficult to disturb by kick sampling</p> <p>Unstable: Substrate easy to disturb by kick sampling</p> <p>Loose: Substrate solid but shifts underfoot</p> <p>Soft: Substrate gives underfoot and difficult to walk in</p> <p>Dangerously soft: Sampler sinks to below ankle depth in substrate</p>
Odour - any smells at the site	<p>None: No discernible odour</p> <p>Slight: Odour detectable within the channel</p> <p>Strong: Odour obvious within the channel or noticeable away from it</p>
Turbidity - water turbidity at the site	<p>Clear: Water not visibly turbid</p> <p>Slight: Visible turbidity but no significant effect on light penetration</p> <p>Moderate: Significant effect on light penetration</p> <p>High: Visibility limited to 10cm depth or less</p>
Shade - overall shade at the sample site (note these categories are not the same as those used for macrophyte and diatom physical information)	<p>None: Channel is unshaded</p> <p>Light: < 25%</p> <p>Moderate: 25 - 50%</p> <p>Heavy: > 50%.</p>

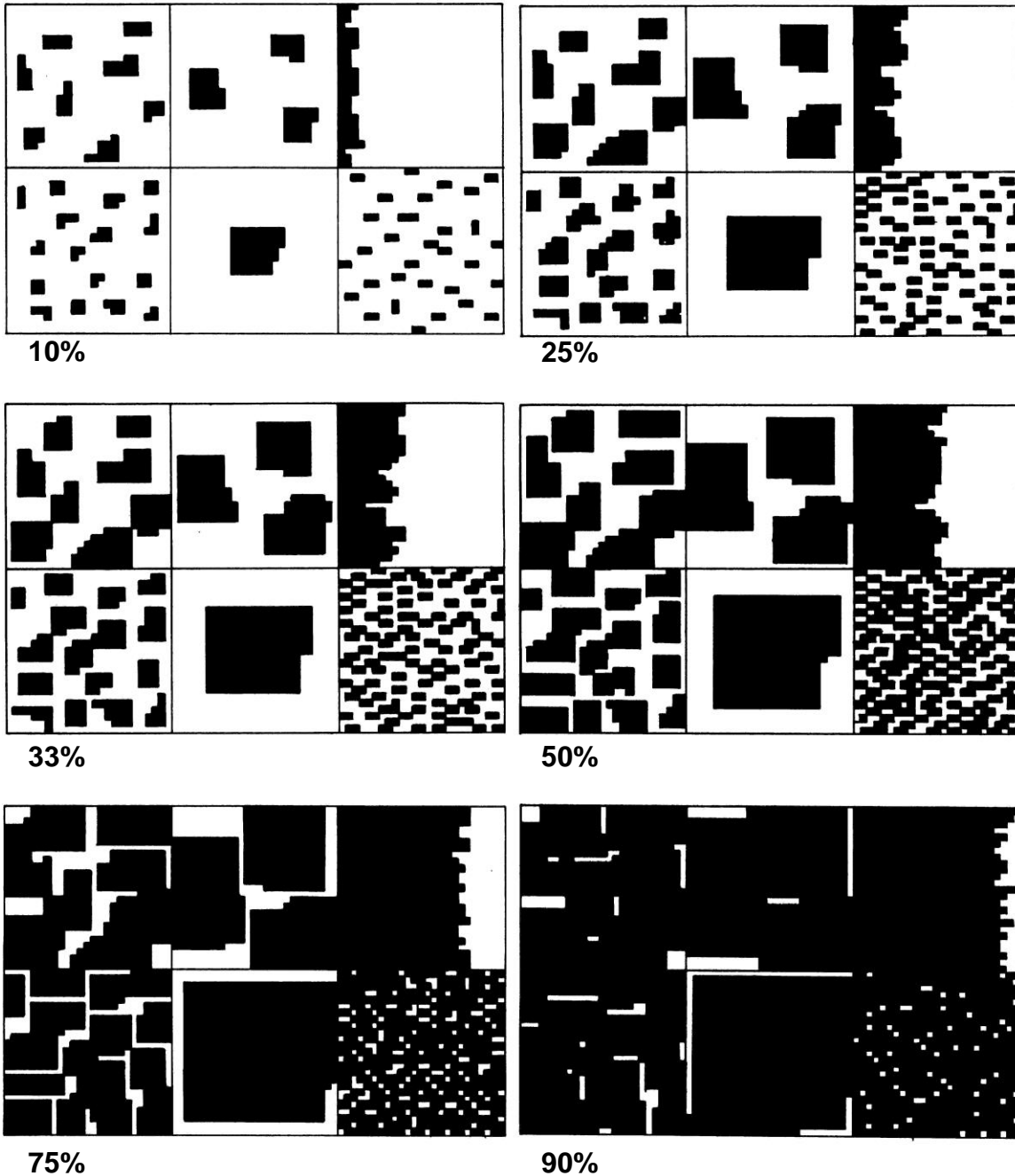
<p>Flow status - the flow status of the watercourse on your visit. The measurement should be based on relative characteristics such as inundation of terrestrial vegetation for high flow and stranding of normally submerged features for low flow</p>	<p>Dry: No water No flow: Still water/unconnected pools Low: Flow present but below normal level Normal: Normal flow rate High: Faster than normal rates Spate: Flood or near flood conditions, watercourse near bankfull.</p>	
<p>Dredging, weed cut, saline, rubbish, oil film, oil deposit - indicate whether different pressures at the site may have influenced the sample.</p>	<p>Tickboxes</p>	
<p>Habitat - presence of particular habitats at the sample site. Most use River Habitat Survey definitions. More than one feature can be recorded in a sample</p>	<p>Torrent Riffle Pool Run Glide Slack Ditch Waterfall Cascade Rapid Ponded reach</p>	<p>Marginal dead water Exposed bedrock Mature island Unvegetated mid bar Vegetated mid bar Unvegetated Side bar Vegetated side bar Silt deposit Sand deposit Trickle</p>
<p>The following categories can be recorded as a cover category or percentage cover and/or a thickness value. Estimates of percentage cover should be made to the nearest 10%.</p>		
<p>Overlying silt - the extent and thickness of silt overlaying the substrate at the site. This overlying silt should be excluded from the substrate percentages</p>		
<p>Sewage fungus above stones – Visible on darker stones as a whitish bloom.</p>		
<p>Ochre - the extent and thickness of ochre at the site</p>		
<p>Filamentous algae -the extent and thickness of filamentous algae at the site. Filamentous algae refers to any algal species that is forming macroscopic chains or strands and is not confined to a film on the surface of the substrate.</p>		
<p>Non-filamentous algae - the extent and thickness of non-filamentous algae at the site. Usually referring to a diatom or other algal film on the surface of the substrate.</p>		
<p>Bank Structure – vegetation characteristics of the river bank. Primary refers to dominant features within the sampling area; secondary refers to less common features within the sampling area</p>	<p>Predominantly Bare Ground: Uniform : (1 dominant vegetation type) Simple: (2-3 dominant vegetation types) Complex: (>4 dominant vegetation types)</p>	
<p>Land Use – Land use within 50m of bank top. Primary refers to predominant land use within the sampling area; secondary refers to less common uses within the sampling area.</p>		

<p>Broadleaf wood: Woodland containing predominantly deciduous broadleaved trees. Natural or plantation.</p> <p>Coniferous wood: Woodland containing predominantly conifers. Natural or plantation.</p> <p>Open water: Any body of standing water, natural or artificial.</p> <p>Suburban/urban development: Domestic buildings, roads etc.</p> <p>Rock and scree: outcropping rock, dunes, mountain scree</p> <p>Orchard: horticultural crop of fruit trees and managed to produce fruit crops (includes hop fields and vineyards)</p> <p>Wetland: Bog, marsh and fen</p> <p>Moorland/Heath: typically heather present</p>	<p>Parkland/Garden: parks/golf courses, public amenity spaces, private gardens etc.</p> <p>Scrub: scrub and woody shrubs (gorse, brambles, blackthorn, hawthorn etc.)</p> <p>Tall herbs/ Rank vegetation: vegetation at least waist high, dominated by herbs (not grasses or reeds)</p> <p>Tilled land: Agricultural land with crops grown on regularly ploughed soil. Includes allotments.</p> <p>Improved pasture: pasture/grassland which has been reseeded or artificially fertilised</p> <p>Rough pasture: pasture/grassland not reseeded or fertilised. Includes hay meadows</p> <p>Industrial: Industrial buildings, roads etc.</p> <p>Farm buildings: Farm buildings other than dwellings</p> <p>Road/Railway: railway lines, large roads.</p>
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An aid determining percentage cover

Percentage cover aid

You can use this figure to test the variability and accuracy of a surveyor's estimates of percentage cover. 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 and arranged to represent cover at the edge of a stream channel. This can show if you are prone to over or underestimation, but is not a substitute for field exercises.



General principles

Planning a survey

The following points must be considered.

- Frequent sampling can effect macro-invertebrate communities, especially in smaller streams. Avoid repeat sampling of a site in short time periods.
 - Follow the [biosecurity](#) principle set out in the introduction and [998_08 Biosecurity - for field and monitoring work](#).
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What to sample

For all methods, sample each invertebrate habitat in the sampling area with an effort proportional to its cover.

Example: If the substrate is 50% silt and 50% gravel, you must allocate half the sampling time to each type. For a kick sample, this would be 90 seconds on the silt and 90 seconds on the gravel.

About 60% of the invertebrate families present in the sampling area will be collected by a single 3-minute kick sample. The manual search will add more, depending on the habitats present (Furse et al., 1981).

Remember to include areas under overhanging banks in your habitat assessment and sampling, as this is a good habitat for invertebrates that is often overlooked.

For all methods

For all sample methods, you must also carry out a [one-minute manual](#) search. For deep-water sites this typically involves using a pond net to sweep the marginal areas and shallows close to the banks.

The marginal sample comprises 1-minute of active sampling and should seek to represent the fauna of the margins and surface which are poorly represented by long-handled pond net or airlift samples alone. It can incorporate elements of the manual search, for example to capture surface dwelling animals or those attached to solid substrates, but it is 1-minute of active sampling rather than 1-minute of searching for individual animals.

Keeping your net clear

For all methods it is important to keep the net clear by removing material from the net periodically to prevent the mesh becoming blocked.

Do this at least after every minute of sampling with a pond net. Do it more often if the net is filling rapidly or blocking.

At silty sites, wash fine sediment through the net frequently to prevent blocking the mesh and reduce the amount of sediment retained in the sample.

You can discard large stones and pieces of vegetation, but agitate them vigorously first in the collecting net and carefully check them for attached animals.

What you MUST not keep

Do not retain:

- fish;
- amphibians;
- the freshwater pearl mussel (*Margaritifera margaritifera*);
- the medicinal leech (*Hirudo medicinalis*);
- the native crayfish (*Austropotamobius pallipes*).

Return these species to the water with care.

Record their presence on the container sample label and in the “sampler’s comments” section on your PDA. The A&R staff will then add them to the taxon list (and remove from the comments field) in BIOSYS.

If you retain any rare taxa live to confirm identification, only return them to the site where you collected them. Retain voucher specimens of these rare or protected taxa for identification and confirmation when necessary.

What to collect and keep

With the exception of the protected taxa mentioned above, keep all invertebrate specimens in the sample for identification in the laboratory.

! Important: In all samples, including those that will only be identified to family level, all crayfish must be identified and recorded at species level.

If you find identifiable, non-native invertebrate species (such as signal crayfish, *Pacifastacus leniusculus*) in the sample, do not return them to the site. Record them as part of the sample, kill them humanely and dispose of them appropriately.

Record any non-native species from active sampling in the sampling area (whatever taxonomic group) as part of the analysis on [BIOSYS](#).

Species of interest outside sample area

You may also record in the sample comment field, other taxa which you see outside the sampling area. This information may be useful to your local biodiversity team. Do not add this data to the BIOSYS analysis.

Examples: Stands of Japanese knotweed (*Fallopia japonica*) or sightings of water voles (*Arvicola terrestris*).

Record non-native fish species and keep a specimen or photograph. Report the record to the National Fisheries Technical Team at the Brampton Laboratory.

Note: Invasive species must be recorded in the sample comment with IS and protected species with PS in front of the species name. Further instructions on how and where to record invasive species found outside of the survey area is given in [OI 302_09](#).

Sampling Methods

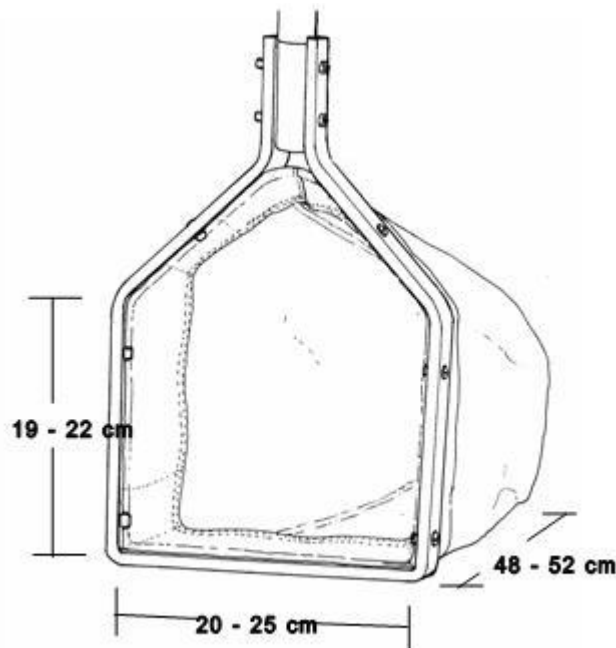
Kick Sample with pond net

Selecting a net Use a standard pond net for kick and sweep sampling.

Nets and frames vary slightly between manufacturers but their basic features must not differ from those described below:

- the frame must have a straight lower edge of 20 - 25 cm and straight, vertical sides of 19 - 22 cm;
 - regularly check that the bottom edge of the frame is not bent, because this reduces its sampling efficiency;
 - thin gauge aluminium frames are prone to this type of damage.
 - use nets 50 cm deep;
 - They are less easily blocked because of their greater mesh surface.
 - the pond net handle should be about 1.5 metres long.
-

Net dimensions



General principles of method

The pond net can be used in different ways, depending on the nature of the sampling area. Ensure you follow the principles below:

- Total sampling time is 3 minutes. The 3 minutes only covers the time spent actively sampling. It excludes time spent rinsing and emptying the net or moving around the site.
- Ideally, sample in short bursts of 15 - 20 seconds, allowing 9 to 12 bursts in a 3-minute sample. Remember this when apportioning sampling effort to different habitats.
- If a site includes discrete habitats, apportion your sampling effort according to their proportion in the sampling area. If a site appears uniform in character, use continuous diagonal transects.
- Always move upstream and diagonally across the stream a number of times while sampling. Do not move straight upstream. This ensures a greater number of habitats are sampled, even if they you do not notice them, and, therefore, collect a higher proportion of the taxa present at the site (see Woodiwiss, 1980).
- Use a stopwatch to ensure that the cumulative time spent actively sampling is precisely 3-minutes.

Important! All 3-minute pond net samples need an additional 1-minute [marginal sweep/search](#) to be undertaken as part of this sampling procedure.

In gravel or cobbles

The table and photo below describe how to collect a kick-sample from gravel or cobble river beds.

Step	Action
1	Hold the net vertically with the frame at right-angles to the current, downstream from your feet and resting on the stream bed.
2	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 a froth!
3	<p>Hold the net:</p> <ul style="list-style-type: none"> • 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. <p>See the photo below of someone doing this.</p> <p>Note:</p> <ul style="list-style-type: none"> • Hold the net further away when the substratum is finer or the current swifter, to prevent it clogging. • Move large stones by hand if they cannot be shifted by foot. Sample any finer sediment collected beneath them.

Photo of kick sampling

Kick sampling from a shallow, fast flowing stream.

The sampler is facing at right angles to the current and is moving diagonally to the right and towards the photographer. He is dislodging the substratum with his left foot and holding the net close in the plume of disturbed sediment to catch the animals that are dislodged.



In soft sediments

Where the stream bed is soft silt or clay, kick sampling is ineffective because the net will become blocked rapidly. The table below describes how to sample from soft sediments.

Step	Action
1	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.
2	Alternatively, stir up the surface of the sediment by foot or with the back of the net. Pass the open net through the clouded water.
3	Rinse the silt away through the net frequently, by agitating the net in the current or at the water surface.

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 with your foot.

Important Waders with steel toe caps must always be worn when sampling in areas dominated by boulders.

The table below describes how to do this.

Step	Action
1	Move boulders away at right-angles to the current, or upstream and away from your feet, so that the net can be held downstream from the area disturbed.
2	Sample the exposed river bed by kicking in the normal way.
3	Where the whole survey area is dominated by large boulders, particularly near waterfalls or where the gradient is steep, it may be impossible to sample effectively. Replace the site by one more amenable to sampling

From vegetation

Different types of vegetation will require slightly different techniques to dislodge the animals. The table below describes what to do.

Step	Action
1	The best general technique is to push the net into plants with a variety of forward, upward and lateral movements.
2	Dislodge animals from dense tangles of tree roots by kicking.
3	Sample the sediment that accumulates beneath plants by kicking or skimming the surface of the sediment.

Slow flowing water over gravel or cobbles

When sampling from still or slowly flowing water, a different procedure is necessary because there is no current to carry dislodged animals into the net. Use this technique wherever the current is weak, to supplement the methods described above. The table below describes what to do.

Step	Action
1	Disturb the substratum with your feet.
2	Catch the dislodged animals by sweeping the net through the water immediately above the disturbed area. Be careful to keep the net moving or organisms already trapped may float out.

Sweep sample using a Long-handled pond net

What to use

Use a long-handled pond net with an overall length including the net frame of 4 metres. Longer nets must not be used as they present health and safety risks to the user.

The long-handled pond net is essentially the same design of net as the

FBA-pattern pond net for kick and sweep sampling, except that it has a much longer handle in three separate screw together sections.

The frame must have a straight lower edge of 20 - 25cm and straight, vertical sides of 19 - 22cm. Regularly check that the bottom edge of the frame is not bent, because this reduces its sampling efficiency. Thin gauge aluminium frames are prone to this type of damage. Use nets 50 cm deep; they block less easily because of their greater mesh surface.

The pond net handle including frame should be graduated so that it can be used to measure river depth.

Important! All long handled pond net samples need an additional 1-minute [marginal sweep/search](#) to be undertaken as part of this sampling procedure.

Staffing levels

Long-handed pond net sampling must always be carried out by at least two people.

Preparing the long-handed pond net

Attach the three sections of the long-handed pond net together tightly to prevent them working loose during sampling.

A long handled pond net



Sweep sampling with Standard long-handed pond net sweep

A standard sweep with long-handed pond net involves two different sampling strategies:

- 3-minutes of active long-handed pond net sampling in the main channel;
- [1-minute of active marginal sampling](#).

3-minute sample portion

The table below describes the procedure for the 3-minutes of active long-handed pond net sampling from the main channel.

The 3-minute long-handed pond net component and the 1-minute marginal component are pooled to form the complete long-handed pond net sample.

Step	Action
1	A long-handed pond net sample should be taken by standing on the river bank. This avoids the need to wade into water with a mean depth greater than 80cm deep.
2	The long-handed pond net should be pushed out into the channel with the net opening facing down, and then drawn back to the operator while applying down force to sweep the net through the

	top of the benthic substrate on the return stroke.
3	Stream beds in narrow deep rivers tend to be composed of medium sized to fine substrates. These can usually be dislodged relatively easily using a long handled pond net.
4	At the end of the sampling stroke, the net should be rotated so that the opening faces upwards and then pushed outwards again towards the mid channel with the net lifted up, so that it is close to the water surface. The net should then be rotated to face downwards and pushed down again for the next sampling stroke.
5	Each sampling stroke across the streambed must sample a new area of previously un-sampled river bed to avoid repeatedly sampling the same area. This is easiest to achieve by moving upstream at least one or two steps between each sampling stroke.
6	Take care not to lose any of the sample by moving the net through the water column with it facing downwards when not actually sampling.
7	The aim is to include as many separate sweeps of the river bed as possible in the 3 minutes. This should simulate the effect of taking a kick sample in shallower water, where a large number of separate 'kicks' are performed.
8	The long-handled pond net sweeps should sample all of the habitats discernible in the river channel in proportion to their occurrence.
9	The 3 minutes of active sampling includes both the sampling strokes, and the return strokes where the net is pushed out towards the mid channel again for the next sampling stroke. If you need to reposition yourself on a different area of river bank, or empty the net, the stopwatch should be stopped as you are no longer actively sampling.
10	Empty the net bag when it becomes too heavy to move easily through the water, or at least after every minute of sampling.
11	Try to avoid lifting the fully assembled long-handled pond net from the far end of the extension handles, especially when laden with a sample. This places considerable stress on the handles and your arms and back. When recovering samples from the river, it is better to feed the handles back through the hands until the net is close to your body so that the weight is evenly distributed

Airlift sampling

What to use

You must use a Yorkshire pattern airlift sampler or similar from a boat if you need to take samples in wide deep rivers for use with RIVPACS/RICT.

You can purchase an airlift through the National Instrumentation Framework contract. There is a small annual servicing cost associated with this item of kit. Please contact [Matt Loewenthal](#) in the National Instrumentation Service.

Alternatively you may be able to borrow airlift equipment from a neighbouring Area. Contact [Helpdesk services](#) if you want to make contact with owners of this equipment.

Important! All airlift samples need an additional 1-minute [marginal sweep/search](#) to be undertaken as part of this sampling procedure.

Yorkshire pattern airlift sampler

The photos below show a Yorkshire airlift sampler. It includes the following:

- a 1.4 metre long, 10cm diameter plastic pipe, with a 67.5 degree bend, a net collar at the top and a weight at the bottom;
- guide ropes, to move the airlift over the river bed and to retrieve it;
- a collecting net fixed to the collar at the top of the pipe with a quick release Jubilee clip;
- the end of the net is closed by tying a knot in it or using an industrial clip (like a freezer bag clip) so that it can be emptied without removing it from the pipe;
- an air supply cylinder, typically a standard 232 bar scuba diving cylinder fitted with a DIN (screw) connection and hose reel. Inlet pressure of 232 bar (3365 psi), regulated at the outlet to 7 bar (0 - 100 psi), with a separate on/off lever/tap;
- an air pressure control box which allows the flow of air to the airlift tube to be controlled;
- an air hose to supply air from the regulator to the base of the airlift pipe;
- a tool kit with a screwdriver, pliers, wrenches, Allen keys, and spares;
- three spare collecting nets;
- a gas cylinder carrier.

Further technical details of the Yorkshire pattern airlift sampler are in [A specification for the Yorkshire pattern airlift sampler](#).

Photos of sampler



Compressed air

We recommend using two 12 litre air cylinders, giving you at least three, but up to 10, samples with each cylinder. You can conserve air by regulating both the pressure and volume regulators.

! Important If you use compressed gases, you must store and transport them in a way that complies with health and safety requirements. Follow guidance in [Transport of dangerous goods by road](#). Staff that operate compressed gas systems must complete [T535 Compressed Gas Safety Training](#).

Standard airlift sample

A standard airlift sample involves two different sampling strategies:

- 3-minutes of active airlift sampling from the main channel;
- [1-minute of active marginal sampling](#).

The 3-minute airlift sample is usually done first, followed by the 1-minute sample from the river margins.

The airlift sample can be either a single transect across the river (if the river is very wide) or a number of smaller transects, covering the range of habitats at the site in proportion to their occurrence. If the stream bed and its associated habitats cannot be seen from the boat, preventing you from sampling different habitats in proportion to their cover, you should try to cover as many different areas of river as is possible within the sampling area.

Use at least two people for the airlift, one to control the air supply and boat, another to control the sampler itself.

Each airlift sample takes an absolute minimum of 3-minutes to collect, but often takes longer due to the need to bring the boat around to re-position for another sampling transect. The amount of time spent actively airlift sampling must be 3 minutes. This must be measured using a stopwatch. The time spent both sampling and bouncing the airlift between sampling patches is included within the active sampling period. Time spent re-positioning the boat must not be included and the stopwatch must be stopped whilst this is being done.

Both the 3-minute airlift component and the 1-minute marginal component are pooled to form the complete airlift sample.

Choosing a site

The amount of material lifted by the sampler depends on the nature of the river bed.

Airlifts work best on gravel or stony river beds. Airlifts can raise large items such as half bricks. On sandy or silty river beds, the airlift may bury itself in the sediment. This can clog the net bag and impede the airflow. You may need to bounce it gently on the river bed to prevent it digging in too deeply.

If the net becomes full of fine material you must swap it with a clean empty net head and continue sampling.

As with pond net and long-handled pond net sampling, airlift sampling becomes less efficient on large boulders. If the airlift fails to recover any material for a period of sampling, for whatever reason, you should add additional time to the 3 minutes to ensure that a full three minutes of effective active sampling has been achieved.

!Important: Airlift sampling must be carried out using a boat so that the whole stream bed can be sampled. Sites under bridges are unsuitable. Airlifting should not be attempted by throwing the airlift from the bank.

The table below describes how to deploy the airlift and collect the sample.

Before you start sampling

Step	Action
1	Check the air pressure to ensure the cylinder is full enough to complete the sample. Check that the on/off lever on the air supply panel is 'off'.

2	Turn the air 'on' at the cylinder. Check the pressure gauge attached to the cylinder regulator	
	If the reading is...	then...
	200 and 240 bar (2900 – 3480 psi)	the cylinder is full.
	less than 35 bar (510 psi)	the cylinder is nearing empty, and should not be used.

Operating airlifts

The airlift is usually deployed fully submerged, see [figure](#) below. There is no need to alter the length of the riser pipe to match the depth of the river.

Follow local safe system of work and manufacturer's instructions for your design of airlift.

The table below describes what to do.

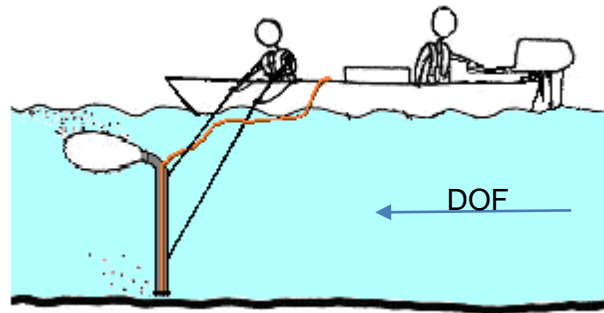
Step	Action
1	Either standing in the boat (if the river flow is slow), or kneeling (if the river flow is fast), lower the airlift into the water.
2	Use the control ropes to maintain the tube between vertical and 40 degrees. Do not allow it to lie horizontally on the bed.
3	Turn the air supply on for two to five seconds without the net attached to clear contaminant specimens from the system. If functioning properly, air bubbles will surface in a cloud of silt. The airlift should now be almost vertical, buoyed up by the air in the upper part of the pipe.
4	Reattach the net and turn the air supply on for two to five seconds. If functioning properly, air bubbles should surface in a cloud of silt. The airlift should now be almost vertical, buoyed up by the air in the collecting net and the upper part of the pipe.
5	Pull in any excess rope. Check that the lower end is on the river bed by the feel of the rope.
6	Use one of the following methods to collect the sample: <ul style="list-style-type: none"> On loose riverbed substrates, leave the air flowing and move the airlift continuously across the river bed; On more compacted substrates sample in a series of short bursts in different locations by turning the air supply on and off and bouncing the airlift to help disturb the river bed. <p>Whichever method you use, you must aim to sample the habitats present in proportion to their cover.</p>
7	It is important to keep the ropes and the airline away from the boat's propeller. In slow to moderate flow conditions, the boat must be moved diagonally upstream in reverse. Airlift sampling is carried out with the airlift and ropes over the bow downstream (see figure and photo).
8	In faster rivers, if the helmsman tries to reverse upstream, the speed of the water makes it difficult to keep the airlift vertical. In these situations, the boat must be moved upstream of the sampling site with the airlift out of the water. The engine power should then be reduced so that the boat floats downstream through the site with the river flow. During this time, the airlift can be deployed and a series of sampling bounces can be obtained.

	Several such manoeuvres will usually be needed to complete 3-minutes of active sampling.
9	<p>Airlift sampling requires good communication between the sampler and the helmsman to coordinate their actions. The airlift operator will standing and care should be taken by the skipper not to move the boat suddenly.</p> <p>After the first 30 seconds stop and check the sample in the net. If it is very small you can turn up the air, if you are getting large object (cobble or bricks) you should turn the air down.</p> <p>Once the sample is collected, the operators should inform the skipper who will give the all clear to lift the equipment back into the boat. Once all the equipment is back in the boat, the operators should inform the skipper.</p>
10	Make sure that the airlift is in contact with the stream bed and not suspended from the boat when sampling. A cloud of disturbed sediment appears in the water when the airlift is working correctly.
11	<p>Recover the airlift bottom end first, by pulling it upwards with the bottom rope.</p> <p>This will wash the material into the collecting net, see figure below.</p>
12	<p>Check sample for fish and other species such as large mussels and <i>Gomphus</i>) that should not be put in the pot. Identify these in the field, take a photo of them, and put them back. Write them down in pencil on a piece of paper and add the paper to the pot.</p> <p>Empty the sample into a large pot. Net should be removed and rinsed clean. Whilst the net is off check inside the bend of the airlift for contamination. The joints can get plants caught up on them which if not removed would contaminate the next sample and pose a biosecurity risk if not removed. See section below about removing the sample from the net.</p>

Photos of sampling



Figure of how to use



Boatwork

The table, photo and figure above describes how to deploy the airlift from a boat.

You must follow all the procedures relating to the use of equipment from small boats.

You must refer to the guidance on the [Safe Management of Boatwork](#) and follow the procedures in [06_10 generic risk assessment boatwork](#).

Manual search

1- minute manual search

All three sampling methods (pond net, long handled pond net and airlift) need a manual search, which is in two parts:

- part one: surface dwelling animals;
- and part two: attached animals.

The two parts together must last for one minute. The time on each part of the search varies, depending on the habitat being sampled. The minute covers only the time you spend actually searching. It does not include time spent moving around the site.

Use a stopwatch or watch with a second hand to ensure that the cumulative time spent actively searching is one minute. The search is mandatory, even if you suspect nothing will be found. You may find nothing, either because:

- no suitable or accessible places to search are found within the minute;
- no animals are found in the places that are searched.

Surface/marginal search

This method is used with the deep-water sampling methods as well as the 3-minute pond net technique. This sweep is designed to collect free-swimming animals and animals from shoreline vegetation and other habitats not sampled by the airlift or long handled pond net.

The table below describes how to carry out the first part of the search, for surface dwelling animals. Where it is safe to do so, this part of the search should cover both banks.

Note: This search collects animals which would swim away during active sampling so you must do it before the sampling area has been disturbed.

Step	Action
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1	Make a visual search and sweep for animals on the water surface and margins, such as whirligig beetles and pond skaters.
2	Collect the animals in a pond net. They are usually very active, so secure them in a tied bag or vial immediately after capture.
3	While you are undertaking this search, make a note of the area covered by different habitats within the sampling area. This will help you to apportion the sampling effort between them in the main sample.

Attached animals search

This is only used in shallow waters in association with the 3-minute pond net technique. The table below describes how to carry out the second part of the search two search, for attached animals.

Step	Action
1	You are looking for animals that are not freed from the substrate during normal sampling. Examples: Caddis pupae and larvae, limpets, leeches. Search for animals attached to logs, stones, solid objects, vegetation, floating leafed plants (check the surfaces underneath the substrates as well as the upper surface) and stems.
2	Search in habitats that are not sampled effectively by your chosen sampling method.
3	Use forceps or a stiff paint brush to pick off animals.
4	Examine stones and rocks of various sizes at several places across the river to cover the different habitats sampled.

Transferring sample to container

Removing sample from the pond net

The table below describes how to remove samples from the collecting net for 3 minute pond net sample.

Step	Action
1	Before you remove the sample from the net: <ul style="list-style-type: none"> • rinse it to remove silt and clay; • discard stones, wood, and large fragments of vegetation, following the instructions in section Keeping your net clear; • gently shake or swing the net to remove as much water as possible from the sample material.
2	The easiest way to remove a sample from the pond net is to wash the catch into one corner of the net so it forms into a ball. Drop the bulk of the sample into a labelled sample container.
3	Material clinging to the net can be shaken or flicked off, from the outside of the net, into the container. Alternatively, remove it by turning the net inside out and dipping it into some water in a tray, bucket, or wide-mouthed sample container, using the surface tension to dislodge any material clinging

	to the net.
4	<p>Use a combination of these methods and repeat them until all the animals have been removed.</p> <p>A plastic tea strainer with a fine mesh is useful for decanting the collection from a tray or bucket into the sample container.</p> <p>You can pick recalcitrant specimens off the net by hand or with forceps.</p>
5	<p>Do not fill sample containers more than about two thirds full with collected material. Leave sufficient room for fixative or preservative and an air space.</p> <p>You must never cram material into a sample container or fill it completely. Use an additional container, if necessary.</p>
6	<p>Only retain enough water to keep the sample damp.</p> <p>This reduces the amount of fixative or preservative needed, reduces mechanical damage and inhibits the activities of carnivores in the sample. Put any large carnivores observed (especially from slower flowing sites) into a separate plastic vial if possible.</p>
7	<p>Wash the collecting net thoroughly at each site, before and after sampling.</p> <p>Carefully inspect it for damage and for any animals left from the previous sample.</p>

Removing the sample from the Air lift net

The table below describes how to remove material from an airlift.

Step	Action
1	<p>The procedure for removing material from an airlift collecting net is similar to that described above.</p> <p>Rinse out most of the silt and remove larger material, as described for the pond net above.</p> <p>Untie the knot at the end of the net. Empty the contents into another container or standard net so that any remaining silt, stones, vegetation and other fragments can be washed and discarded.</p>
2	<p>Follow the procedure for removing material from the pond net described above</p> <p>Drain the sample before putting it into a suitable container. Do not add water to the sample.</p>
3	<p>While the net is detached clean the airlift tube and remove any material caught in the joints to prevent contaminating the next site and sample.</p>

Labelling and transportation

Labelling

Follow the steps below for labelling

Step	Action
1	Label the outside of all sample containers, including polythene bags, using a water and alcohol proof marker pen. Do this before the container gets wet. Give the ink time to dry to improve its adhesion. Do not label lids because they can become separated from the rest of the sample.
2	Place a waterproof paper label in each sample container as an additional precaution, marked in soft-leaded pencil or water and alcohol proof ink. Labels must include the following: <ul style="list-style-type: none"> • watercourse name; • site name; • BIOSYS site ID; • date of sampling; • sampler's name or initials; • if necessary, the type of preservative and any warning notices required; • if a sample is contained in more than one container, add the following: X of Y containers; • which portions of an airlift sample are in the container, including the proportion of material retained if appropriate.
3	If you have identified rare taxa (native crayfish, medicinal leeches or pearl mussels, as described in What you must not keep) and returned them to the river, you must record their presence and abundance on the labels placed in the sample containers, as well as on the PDA.
4	Leave space on the labels so that analysts can record the sorting and analytical quality control (AQC) details.
5	Ensure information on the PDA corresponds with the information on the sample container.

Transporting samples

Before any sample is fixed or preserved, treat it as a live sample to minimise damage from decomposition and the actions of carnivores (see Hiley, 1995).

Keep samples cool during transport. You may need a cool box or mobile fridge for surveys on warm days. Stow the cool box or fridge and the sample containers safely in the vehicle (see the [guidance on driving](#)).

Put samples for live sorting in a fridge as soon as you return to the laboratory. Make sure that analysts are aware of new samples.

Return samples that will be preserved to the laboratory and preserve or fix them, ideally no more than 10 hours after collection.

Related documents

General

- [95_06 Fixing and preserving freshwater macro-invertebrate samples](#);
- [206_06 Data entry to the BIOSYS biological database](#).
- [18_07 Technical reference material: lake macro-invertebrate sampling and analysis](#)
- [227_06 Ecological Appraisal attendance at a pollution incident](#)

- [998_08 Biosecurity - for field and monitoring work](#)
 - [318_10 Hydroecological validation using macroinvertebrate data](#)
 - [302_09 Finding and recording invasive non-native \(alien\) species during routine ecological field monitoring](#)
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River Sampling

- [118_05 Quality Assurance \(AQC, Audit and Ring Test\) programme for freshwater macro-invertebrate riverine sample analysis](#)
 - [024_08 Freshwater macro-invertebrate analysis of riverine samples;](#)
 - [776_15 Hydroecological **Monitoring** for Flow Pressure Assessment](#)
 - Bass J. A. B., Wright J. F., Clarke R. T., Gunn R. J. M. & Davy-Bowker J. (2000) *Assessment of sampling methods for macroinvertebrates (RIVPACS) in deep watercourses*. Environment Agency R&D Technical Report E134.
 - Davy-Bowker J., Jones J.I. & Murphy, J.F. (2014) *Standardisation of RIVPACS for deep rivers: Phase I - deriving a standard approach to deep river sampling*. Environment Agency, Bristol.
<http://www.fba.org.uk/river-invertebrate-classification-tool-riect-and-rivpacs>
 - Jones J.I. & Davy-Bowker J. (2014) *Standardisation of RIVPACS for deep rivers: Phase I - review of techniques for sampling benthic macro-invertebrates in deep rivers*. Environment Agency, Bristol.
<http://www.fba.org.uk/river-invertebrate-classification-tool-riect-and-rivpacs>
 - Neale M.W., Kneebone N.T., Bass J.A.B., Blackburn J.H., Clarke R.T., Corbin T.A., Davy-Bowker J., Gunn R.J.M., Furse M.T. & Jones J.I. (2006) *Assessment of the Effectiveness and Suitability of Available Techniques for Sampling Invertebrates in Deep Rivers*. North South Shared Aquatic Resource (NS Share) Final Report T1(A5.8) – 1.1.
-

Lake sampling

- [18_07 Taking and analysing lake macro-invertebrate samples](#)
 - [A guide to monitoring ecological quality of ponds and canals using Predictive System for Multi-metrics \(PSYM\)](#), from the Ponds Conservation Website.
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Health and safety

- Generic Risk Assessment for taking freshwater macro-invertebrate samples with an airlift
- [65_07 Safe storage of Industrial Methylated Spirit \(IMS\) for preserving ecological samples](#)
- [52_05 Ecology laboratory safety](#)
- [07_01 Generic Risk Assessment for Fieldwork in Rural Locations](#)
- [242_06 Generic Risk Assessment for Ecology Laboratory Safety](#)
- [09_10 Generic Risk Assessment: Transporting samples](#)
- [56_04 Risk Assessment - Fish kill response](#)
- [428_05 Generic Risk Assessment: Ecological Sample Collection from Freshwaters](#)
- [62_05 Generic Risk Assessment - Towing of trailers](#)
- [426_05 Generic Risk Assessment – Working in or near water](#)

- [05_10 Generic risk assessment - Working in proximity of Avian Influenza](#)
 - [017_08 Generic risk assessment - Collecting ecological evidence at an environmental incident](#)
 - [725_06 Fieldwork](#)
 - [06_10 Boatwork](#)
 - [425_05 Driving on Agency Business](#)
 - [Transport of dangerous goods by road](#)
 - [Take care with oxygen.](#)
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References

Begon, M., J. L. Harper & C.R. Townsend (1996) *Ecology : individuals, populations and communities*. (3rd edn.) Oxford: Blackwell Scientific Publications

Clarke, R. T., M. T. Furse & J. F. Wright (1994) *Testing and further development of RIVPACS Phase II: aspects of robustness*. Interim NRA R&D Report 243/7/Y. Bristol: Environment Agency

Clarke, R. T., Cox, R., Furse, Wright, J. F., and Moss, D. (1997) *RIVPACS III+ User Manual. R&D Technical Report E26*. Bristol: Environment Agency

Furse, M. T., J. F. Wright, P. D. Armitage & D. Moss (1981) An appraisal of pond net samples for biological monitoring of lotic macro-invertebrates. *Water Research* **15**, 671-689

Hiley, A. (1995) *Predation in Freshwater Macro-invertebrate Samples - Project Summary*. Newcastle: NRA North East Region, Northumbria Area

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

Glossary

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

Term	Description or definition
80:15 rule	Method for determining the sampling method for deep rivers based on 80cm depth and 15m width.
airlift	a sampler in which material from the stream bed and the animals associated with it are driven up a pipe and into a collecting net by the release of compressed air near to the base of the pipe
alkalinity, total alkalinity	free or excess base, measured by titration at pH 4.5. Quantified as the equivalent concentration of calcium carbonate. One of the parameters for the sample environmental data for RIVPACS.
AQC	Analytical quality control. Procedures to control errors in laboratory analyses within specified limits. Formal AQC procedures, covering sorting and identification are described in the Operational Instruction for analytical quality control and external audit of freshwater macro-invertebrate analysis to the level required by the BMWP and LIFE system.
audit	an independent measurement of the quality of the laboratory analysis of samples or the quality of the AQC inspection
bar	a standard unit of pressure, equivalent to 1 x 10 ⁵ pascals. 1 bar = 14.504 psi
bias	error introduced when the observed measurements of a surveyor are consistently different to the actual value. Note: This sampling definition of bias is not the same as the term used in the audit of invertebrate analyses. That definition is very specific. Refer to the Operational Instruction for analytical quality control and external audit of freshwater macro-invertebrate analysis.
biotic index	A scale for showing the quality of an environment by indicating the types of organisms present in it.
BMWP score	Biological Monitoring Working Party score. A biotic index of ecological quality, based on numerical values assigned to each BMWP scoring taxon which represent their tolerance to organic pollution. The BMWP score of a site is the sum of the values of each taxon in a sample collected from it. It is, therefore, based on both the tolerance of the taxa to organic pollution and the taxonomic richness.
calcium	the concentration of calcium ions in water is a surrogate for alkalinity, one of the sample environmental data parameters for RIVPACS
CAMS	Catchment Abstraction Management Strategy. A process designed to: <ul style="list-style-type: none"> • inform the public on water resources and licensing practice; • provide a consistent approach to local water resources management; • help to balance the needs of water users and the environment; • involve the public in managing the water resources in their area.
conductivity	electrical conductivity is a surrogate for alkalinity as one of the sample environmental data parameters for RIVPACS
corixid	an insect of the family Corixidae.
detritus	finely divided and partially decomposed particles of organic matter.

discharge	the volume of water flowing in a watercourse per unit time.
discharge category	based on the average discharge in cubic metres per second (Cumecs) - a site registration data parameter for RIVPACS
easting	the distance eastwards in a grid reference. Eastings are given before northings in an NGR.
EQR	Environmental Quality Ratio. A biotic index expressed as a proportion of what it is expected to be present under WFD reference conditions, predicted by RIVPACS in RICT.
electrical conductivity	see conductivity
environmental quality	a general term encompassing water quality, ecological quality and physical (habitat) quality
FBA	Freshwater Biological Association. An independent association of freshwater biologists conducting research into all aspects of freshwater science.
fixative	Maintains cell and tissue constituents in as life-like a state as possible and allow them to undergo further preparative procedures without change.
Formalin	Solution of formaldehyde in water used to preserve and fix macro-invertebrate samples.
GIS	Geographical information system. Computer database(s) linked to a geographical display (a map) allowing visual display and analysis of spatially related information.
GPS	Global positioning system. A system for determining a position on Earth, based on a receiver that can accurately determine its position relative to an array of satellites. GPS is widely used for navigation and surveying. Differential GPS have a static unit in addition to the portable unit and provide more accurate and precise readings.
Long handled pond net	A standard pond net with an extra long handle, usually in three sections that screw together.
habitat	the type of environment in which an organism lives, defined in biotic and abiotic terms
hardness, total hardness	the concentration of carbonate and bicarbonate ions in water. Quantified as the equivalent concentration of calcium carbonate - a surrogate for alkalinity as one of the sample environmental data parameters for RIVPACS.
HEV	Hydroecological Validation (HEV) tool for water resources purposes. See 318 10 Hydroecological validation using macroinvertebrate data
IMS	Industrial Methylated Spirit – used to preserve macro-invertebrate samples. Also known as Industrial Denatured Alcohol (IDA).
kick sample	a biological sample taken by kicking the substratum to disturb it and collecting the organisms that are dislodged with a pond net. It is the most widely used qualitative method for collecting macro-invertebrates from streams and shallow rivers.
LIFE	Lotic-invertebrate Index for Flow Evaluation. A macro-invertebrate biotic index that compares the ecological sensitivity of a community to low water velocity and the changing flow character of the reach. LIFE (F) uses family level data and LIFE (S) uses species level.
macro-invertebrate	an invertebrate animal large enough to be seen without magnification. Often defined as an animal retained on a 500 µm aperture sieve, but for this procedure it is an animal captured by a net of approximately 1 mm mesh.

monofilament	material made of single, continuous strands of artificial fibre. c.f. multi-filament.
MSUB	Mean substratum particle size. The mean size of particles covering the stream bed. MSUB is calculated within RIVPACS, based on estimates of the percentage contribution of different sized particles.
multi-filament	material made of woven strands of fibre c.f. monofilament.
NGR	Ordnance Survey national grid reference
northing	the distance northwards in a grid reference. Eastings are given before northings in an NGR.
ochre	a rust-coloured, flocculent deposit caused by the oxidation of dissolved iron and sometimes other metals. The oxidation is often mediated by bacteria. Ochre usually indicates pollution by acidic or metalliferous drainage.
pool	a part of a watercourse that is distinctly deeper than the rest of the watercourse and which, as a result of the reduced current, usually has a silty stream-bed, often covered in detritus Pools alternate with shallower riffles along many small rivers.
pond net	a hand held sampler comprising a square framed collecting net on the end of a pole the length of a broom handle. Also known as a hand net.
PDA	Personal digital assistant
PPE	Personal Protective Equipment such as waders, gloves and lifejacket.
precision	the closeness of repeated measurements of the same item. c.f. accuracy, which is the closeness of a measured or computed value to its true value. Bias affects accuracy but does not affect precision.
preservative	a substance that protects biological material from decomposition. Formalin or IMS are used to preserve freshwater macro-invertebrate samples. c.f. fixative. Some preservatives are also fixatives.
PSI	Index derived from proportion of sediment-sensitive Invertebrates (PSI) for sediment assessment.
psi	Pounds per square inch. A non-standard unit of pressure – see also bar. 1 psi = 0.069 bar.
qualitative (samples or sampling)	<p>samples or sampling methods optimised to provide information about the range of different organisms rather than their abundance (for which quantitative samples are required).</p> <p>Qualitative samples tend to be less precise, but more extensive than quantitative samples. Qualitative samples that are sufficiently standardised to provide comparable estimates of the abundance (number) of taxa and the abundances of individual taxa, including samples collected by the procedures described in this document for RIVPACS, are known as semi-quantitative samples.</p> <p>Semi-quantitative samples can only provide estimates of relative abundance because the samples do not relate to a particular area or volume of habitat.</p>
quantitative (samples or sampling)	<p>samples or sampling methods optimised to provide information about the abundance of organisms. c.f. qualitative samples or sampling.</p> <p>Quantitative samples cover a small and precisely measured line, area or volume of habitat, or a point.</p>
RAM	Resource Assessment Methodology. Provides a consistent technical approach to water resource assessment and management within the Catchment Abstraction Management Strategy (CAMS) process. It sets out the principles and process of resource assessment and management for the formulation of a CAMS. It provides guidance on the various 'tools' and alternative approaches which can be used to carry out each stage of the

	process and defines standard output formats.
rangefinder	a device for measuring distances, normally using optics
region	an Environment Agency administrative division based on river catchment boundaries for the purposes of managing the water environment
RICT	River Invertebrate Classification Tool. Software that implements RIVPACS and determines WFD status.
right bank (of a watercourse)	the bank on the right hand side, when facing downstream
riffle	a shallow area of fast, turbulent water with reasonably well sorted gravel beds. Many small rivers have alternating riffles and deeper pools along their length.
River Habitat Survey	River Habitat Survey (RHS) is a method for assessing the physical character and quality of river habitats.
RIVPACS	River Invertebrate Prediction and Classification System. A computer system used to predict the macro-invertebrate fauna and biotic indices which could be expected at a site if it were unstressed.
roamer	a simple instrument comprising two linear scales at right angles, or a grid, to help estimate grid references within a grid square on a map
sample environmental data	environmental data collected in the field at the same time as a macro-invertebrate sample. Specifically that collected for RIVPACS, which comprises stream width, depth, percentage cover of boulders, gravel, sand and silt on the river bed, surface current velocity category (a surrogate for discharge category which is a site registration data parameter) and alkalinity or a surrogate (calcium concentration, total hardness, conductivity).
sampling area	the area at a sampling site from which the samples are actually collected. c.f. survey area.
sampling run	the sites to be visited and the route to be taken in a sampling excursion
site registration data	information defining a site and environmental data that can be obtained from maps. It comprises watercourse name, site location name, site code, NGR, altitude, slope, distance from source and discharge category.
SE	Standard error. The standard deviation of a statistic, usually a mean if none other is specified.
semi-quantitative samples	see quantitative samples
substratum, pl. substrata (also substrate, pl. substrates)	the material comprising the stream bed
survey area	a length of watercourse encompassing the sampling area and extending either seven stream widths or 50 metres either side of it. It should have the same physical characteristics (and therefore the same macro-invertebrate habitats) as the sampling area.
sweep sample	a component of all samples collected principally by long handled pond net or airlift, in addition to the 1-minute search. Sweep samples are collected by pond net to capture free swimming animals and those in vegetation and other habitats not sampled adequately by the long handled pond net, airlift or the manual search. Sweep samples are of 1-minute duration.
taxon, pl. taxa	a type of organism, irrespective of the taxonomic level at which it is defined
total alkalinity	see alkalinity

total hardness	see hardness
velocity category	based on the median or modal surface current velocity of the main flow channel in centimetres per second. A sample environmental data parameter for RIVPACS, which is used as a surrogate for discharge category (a site registration data parameter) when this is unavailable.
WFD	Water Framework Directive. EU legislation that shifts the emphasis of water quality assessment from chemical to ecological measures.
water quality	The physical and chemical nature of water

**FRESHWATER
INVERTEBRATE SAMPLING SHEET**



**ENVIRONMENT
AGENCY**

<u>RIVER</u>	<u>DATE</u>	<u>TIME</u>	<u>BIOSYS SITE ID</u>		
<u>SITE NAME</u>		<u>NGR</u>	<u>COLLECTOR</u>		
Sample ID	Analysis ID		<u>SAMPLE METHOD</u> 3 MIN NET ↑ 1 MIN NET AIRLIFT LONG HANDLED PONDNET BOX SURBER OTHER		
SITE DIAGRAM and ECOLOGICAL COMMENTS (MACROPHYTE ID, MOSS ID, HABITAT, TREES, INVASIVES, ODOUR TYPE.....)			<u>SAMPLE REASON AND COMMENT</u> (ROUTINE, LOW FLOW, ECN, UWWTD, MINEWATER...)		
			<table border="1" style="width:100%; border-collapse: collapse;"> <tr> <td style="width:50%;"><u>Invasive species</u> Himalayan balsam Japanese Knotweed Giant Hogweed Rhododendron other</td> <td style="width:50%;"><u>Protected species (returned to river)</u> Medicinal leech Freshwater pearl mussel Native crayfish</td> </tr> </table>	<u>Invasive species</u> Himalayan balsam Japanese Knotweed Giant Hogweed Rhododendron other	<u>Protected species (returned to river)</u> Medicinal leech Freshwater pearl mussel Native crayfish
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<u>ALKANITY.</u> (mg/l)					

<u>AV. WIDTH (m)</u>	<u>SUBSTRATE</u>	<u>DETRITUS</u>	<u>SEWAGE LITTER</u>	<u>TURBIDITY</u>	<u>GENERAL</u>
	BEDROCK _____	NONE ↑	NONE ↑	CLEAR	Y N
<u>AV. DEPTH (cm)</u>	BOULDERS -----	LOCAL ↑	LOCAL ↑	SLIGHT	RUBBISH ↑
	COBBLES _____	WIDESPREAD	LOCAL ↑	MOD	OIL FILM
	PEBBLES -----	GROSS	WIDESPREAD	HIGH	OIL DEPOSIT ↑ ↑
<u>FLOW</u>	GRAVEL _____	<u>SHADE</u>	<u>ODOUR</u>	<u>BED STABILITY</u>	<u>INFLUENCES</u>
DRY	SAND _____	NONE	NONE	SOLID	DREDGING
PONDED	SILT -----	LIGHT	SLIGHT	STABLE	WEEDING
LOW	CLAY _____	MOD	STRONG	UNSTABLE	SALINE
NORMAL		HEAVY		LOOSE	OTHER
HIGH					
SPATE ↑					

<u>HABITAT</u>		<u>% cover</u>	<u>Trace</u>	<u>Thin</u>	<u>Thick</u>	<u>Massive</u>
TORRENT	Sewage fungus above stones					
RIFFLE	Ochre					
POOL	Filamentous algae					
RUN	Non filamentous algae					
GLIDE	Macrophyte					
SLACK	Moss					
DITCH	Overlay silt					
WATERFALL						
CASCADE	<u>LANDUSE</u>	<u>PRM</u>	<u>SEC</u>	<u>PRM</u>	<u>SEC</u>	<u>BANK STRUCTURE</u>
RAPID	BROADLEAF WOOD					PREDOMINANTLY
PONDED REACH	CONIFEROUS WOOD			TALL HERB/RANK		BARE GROUND
MARG DEAD WATER	OPEN WATER			TILLED LAND		
EXPOSED BEDROCK	SUBURBAN			IMPROVED PASTURE		
MATURE ISLAND	URBAN			ROUGH PASTURE		UNIFORM
UNVEG MID BAR	ROCK & SCREE			INDUSTRIAL		
VEG MID BAR	ORCHARD			FARM BUILDINGS		SIMPLE
UNVEG SIDE BAR	WETLAND			ROAD/RAILWAY		
VEG SIDE BAR	MOORLAND HEATH					COMPLEX
SILT DEPOSIT	SCRUB					
TRICKLE						