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Lake benthic macroinvertebrates I: improving sampling methodology

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Authors:
M.T. O'Hare, A. Tree, M.W. Neale, K. Irvine, I.D. Gunn, J.I. Jones, & R.T. Clarke

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Research Contractor:
CEH Dorset
Winfrith Technology Centre
Winfrith Newburgh
DORCHESTER
Dorset DT2 8ZD
Tel : 01305 213500 **Fax :** 01305 213600

Environment Agency's Project Manager:
Ben McFarland, Exeter Office

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Executive summary

In order to fulfil the requirements of the Water Framework Directive on using biological indicators in assessing water quality and classifying water bodies, the existing Environment Agency method for collecting littoral invertebrates and the SEPA (Scottish Environmental Protection Agency) method for sampling profundal invertebrates have been reviewed and revised. Fundamentally, the existing methods were deemed suitable for producing data that can be used in the classification of standing waters and to produce necessary diagnostic tools and predictive models. Nevertheless, some revision was necessary, and the meso-habitats sampled using the littoral method have now been clearly defined and limited to two major types – hard substrate and vegetated areas dominated by submerged macrophytes. The objective of this modification was to reduce noise in the dataset and potentially improve model predictions. Minor alterations were also made to the profundal method, and both revised methods are included in Annex I.

Additional recommendations address sample site parameters, levels of taxonomic identification and sample times. The suggested list of parameters incorporates parameters required by the Water Framework Directive and includes both parameters which are not influenced by humans (for use in the classification of sites), and variables which are influenced by humans (for inclusion in predictive tools). In terms of taxonomic level, species-level identification has been proposed, in order to facilitate the production of the best possible tools. Finally, given the constraints on resources, a single sample per season per method has been advised.

The need to quantify uncertainty remains, however, and additional data collected specifically for that purpose are required. An experimental design to determine variability at a range of scales, and hence quantify sources of uncertainty, has been proposed for acidified water bodies. The proposed work would assess the variation between repeat samples, operatives, sites within each lake, and lakes suffering high or low impact of acidification. The influence of this variability on uncertainty should be determined at each level.

Contents

1 Introduction

This study was commissioned in response to the requirement of the Water Framework Directive (WFD) that standing water quality should be assessed using biological elements, specifically benthic invertebrates. This report – represents the first of three the project and brings phase 1 to completion. The project looks at the use of littoral and profundal benthic invertebrates in the classification of standing waters, and discusses the development of tools for estimating water quality on the basis of benthic invertebrates.

- Phase 1 – reviews the methods for sampling invertebrates and evaluates the suitability of the existing EA/SEPA methods for producing data for developing new predictive/evaluation tools;
- Phase 2 – looks at the processing of samples and quantification of levels of uncertainty associated with existing methods;
- Phase 3 – provides advice to EA/SEPA and those tasked with the development of tools.

Established sampling methods were already in place within EA and SEPA at the start of the project. This report examines these methods to assess whether they are sufficient to provide adequate data for classifying standing waters and developing predictive tools. The following chapters examine, current EA/SEPA sampling methods, current research on sampling strategy, the environmental parameters that should be included for robust tool development, and the taxonomic resolution most suitable for water classification and tool development.

Since chapter 1 integrates the recommendations of the other work packages by suggesting alterations in the existing littoral and profundal methods. For this reason there is a degree of overlap between the first and subsequent chapters.

2 Current EA/SEPA sampling methods

2.1 Task

To review existing EA/SEPA methods for sampling lake littoral and profundal benthic macroinvertebrates, in consultation with those experienced in sampling work, and to determine what, if any, modifications are necessary in order to generate data suitable for developing new biological tools . The recommended sampling method must be cost-effective, consistent with existing or developing European Committee for Standardisation (CEN) standards and compliant with existing and known future health and safety standards. The recommended methodology must also comprise techniques that introduce minimal sampling uncertainty.

2.2 Introduction

The evaluation criteria[1] for invertebrate sampling methods were that a) they must comply with CEN standards and existing and known future health and safety requirements, b) that they must be cost effective and c) that they should introduce minimal uncertainty. In addition, any revised methods should be based on the existing methods, while any data collected using such revised methods must be compatible with existing data (both those collected in previous years and, if possible, with those collected in the Republic of Ireland). Finally, it was essential that the data produced by the sampling methods should be capable of generating tools for assessing water quality.

2.3 Existing EA/SEPA sampling methods

Existing protocols for sampling invertebrate benthic communities in lakes involve two key methods – one for littoral communities, the other for profundal communities.

2.3.1 Littoral method

This method comprises a kick sample and a search – in this case a 3-minute kick sample together with a 1-minute search for animals, with three replicates taken. This semi-quantitative technique closely follows the technique used by EA and SEPA staff for sampling rivers (Murray-Bligh & Ferguson 1999).

2.3.2 Profundal method

This method is based on a technique designed by SEPA, where 10 replicate grab samples are taken from a single site to represent a water body. The method is quantitative because the surface area of the bed sampled is defined. However, as a result of the low densities and patchy distribution of benthic invertebrates found in oligotrophic systems, in practice the replicates must often pooled to gain a clear picture of the profundal invertebrate assemblage in such systems. Quantitative comparisons between lakes where replicates have been pooled and lakes where they have not are inappropriate, as the error cannot be quantified for pooled results. Thus, the advantage of a quantitative method can effectively be lost in datasets from oligotrophic water bodies.

[1] As detailed in the invitation to tender.

2.3.3 Health and safety, practicality and cost-effectiveness

The reviews of health and safety, practicability and cost effectiveness issues were based on three main sources: our own extensive field work experience, the existing EA/SEPA safety protocols for river invertebrate sampling (BT001) and the NERC's internal circulars on health and safety legislation. The latter was particularly significant in terms of identifying any up-and-coming legislation of relevance. The review identified a number of basic problems with the existing littoral method:

- health and safety considerations associated with the use of the long-handled pond net from small boats (because of the awkward movement required to take samples);
- unsuitability for use at some sites on cost-effective grounds due to the collection of large amounts of material, especially fine sediment.

The existing profundal method has been used in very deep lakes – 200m at their deepest point – without lifting equipment, and in cases staff were required to pay out and haul up the equivalent of 4 km of line. Again, a number of issues were identified:

- this was a slow and trying process and, unless personnel were trained and especially fit, could potentially lead to back injuries;
- profundal sampling does require the use of boats, but this need not be a dangerous activity if staff are suitably trained and high quality equipment is provided.
- due to low invertebrate densities common to oligotrophic water bodies, a large number of replicates were necessary when sampling profundal invertebrates in such sites. However,the same number of samples produced excess material when used in eutrophic water bodies, necessitating the use of sub-sampling.

2.3.4 CEN compliance and compatibility with existing data

It was not possible to review the compliance of the existing methods with CEN standard methods as the committee has yet to produce either a littoral or profundal standard sampling method for benthic invertebrates.

The littoral sampling methods used by SEPA, the EA, in Northern Ireland (Neale, submitted) and the EPA in the Republic of Ireland are all based on kick samples. They differ in duration from 3 minutes (EA; Neale, submitted) to 12 seconds (White 2000) and, to a degree, in the habitats sampled. The number of species collected increases towards an asymptote with increased sample time. This may have implications for determining reference conditions and classification of water bodies (Halse *et al.* 2002; Neale, submitted). It is currently not possible, therefore, to make any new methods compatible with all variants. The sampling period was left unchanged to allow integration of new and old data from within Great Britain. An experimental test for optimising sampling time has been proposed in Chapter 3, 'Sampling Strategy'.

2.3.5 Uncertainty

There are a number of possible sources of uncertainty in the invertebrate sampling methods. As yet, we cannot be certain of:

- the number of samples needed to represent a water body
- the number of samples necessary to detect change in a water body
- the error due to inter-operator variability

- the error introduced during sorting or the error introduced during the identification of animals.

While some of these sources of error have been quantified for river invertebrate sampling (Clarke *et al.,* 2002), they have not been quantified for profundal or littoral invertebrate sampling. To evaluate these aspects, an additional sampling regime has been proposed which would allow uncertainty to be quantified (see details in Annex II and Chapter 3). As a practical solution, all staff should be trained in the use of any new methods to help reduce uncertainty associated with inter-operator variability.

The profundal method is based on the assumption that a single sampling location can provide a representative sample of a lake but suggested splitting the sampling effort between different basins if present. This would reduce the number of samples per basin, making it difficult to compare these water bodies with other water bodies where a single location was sampled, hence increasing uncertainty.

2.3.6 Tool development requirements

Tool development requires the collection of data that will allow the use of both multimetric and multivariate analysis, the predominant approaches in ecological tool development (Norris & Hawkins, 2000), as well as artificial intelligence techniques. To be suitable for use in a tool development, a statistical sample for a water body must be both representative of that water body and sensitive to adverse change. Ideally, it should also have low levels of noise.

Representativeness

A water body may be represented by a 'single statistical sampling unit' or by a number of 'statistical sampling units' taken at one or more sites. These samples are then combined to represent the water body[2]. Where the samples are taken is therefore as important as how they are taken and the number taken. Chapter 3, 'Sampling Strategy', looks at the number of samples required to represent a water body, and the implications of taking a sample at a single site within a water body. The aim of the strategy was, in part, to test if a single location is representative of the littoral zone as a whole (Hurlbert 1984). The discussion is directed toward littoral zone invertebrates but applies equally to profundal invertebrates. When sampling oligotrophic systems using the profundal method, it is possible that so few animals are collected in an individual replicate that, statistically, the data generated are not useful for describing the community composition.

Reducing noise

Noisy data make modelling difficult because variability in data cannot be attributed to known drivers, either anthropogenic or natural. Noise can also be increased by a known factor, for example by sampling different meso-habitats in different water bodies. Lake littoral zones are heterogeneous with many meso-habitats and there is plenty of potential for noise to be introduced by this mechanism. As it stands, the existing EA/SEPA method for littoral sampling is likely to generate noisy data since it provides little direction of where samples should be taken. Indeed it implies that all meso-habitats within a site should be sampled during the kick sample, in proportion to their percentage occurrence. In addition, animals are collected in a 1-minute search and pooled with the kick sample.

Noise can be reduced by limiting the range of habitats sampled within a water body, as discussed in greater detail in Chapter 3. In addition, the existing method does not have a

[2] Ecologists often use 'sample' as a synonym for 'statistical sampling unit' which differs from the use of 'sample' by statisticians, i.e. a collection of sampling units (Jongman *et al* 1987)

well-focused list of variables to be recorded, which could explain variation in the data. Chapter 4 provides details of which variables should be recorded for both profundal and littoral methods.

Taxonomic resolution

Species level data are the best for tool development (see detailed discussion in Chapter 5) but has practical implications for the field methods. For example, to identify samples to species level requires samples to be properly preserved. The proposed methods advocate the use of formaldehyde as a fixative – the best means of preserving invertebrate samples for species level identification work. During discussions with Environment Agency/SEPA staff, however, it became clear that the use of formaldehyde was not common practice, with IMS being used as a substitute. There is an associated degradation in material and loss of data quality.

Detecting change in trophic status

The two impacts of most concern to the agencies are eutrophication and acidification. Our basic criterion, therefore, was to ensure that taxa sensitive to eutrophication and acidification would be sampled effectively.

As a lake becomes more eutrophic, all forms of primary production increase initially, but eventually phytoplankton dominate (Moss 1998). This increased production leads to changes in the invertebrate community, while related increase in detrital inputs into the littoral and profundal zones results in an increased representation of detritivores. The tendency for the hypolimnion to deoxygenate increases, and there is an associated shift in the profundal invertebrate assemblage to taxa which are tolerant of anoxic conditions.

In shallow lakes, there may be a shift from macrophyte dominance to phytoplankton dominance driven by biotic interactions rather than nutrient enrichment. The shift is controlled indirectly by fish predation on the invertebrates that graze epiphytic algae (Jones & Sayer, 2003). The epiphytic algae control the success of macrophytes by direct competition. If fish predators suppress grazer numbers sufficiently, epiphytic algae reduce macrophyte growth and allow dense growths of phytoplankton to develop.

Littoral fauna

Evidence from a study of Danish lakes showed that the distribution of littoral taxa in lakes reflected the trophic status of those lakes (Brodersen *et al.* 1998).

Data from a study of 37 lakes obtained using the existing EA/SEPA littoral sampling method were used to evaluate the potential for the method to provide data for the developing tools that are sensitive to acidification and eutrophication. To test the effectiveness of the method for collecting taxa that are sensitive to organic pollution and eutrophication the taxa collected from these 37 lakes were compared to those used to calculate BMWPscores for rivers. Almost the entire range of BMWP species scores were encountered and 55% of BMWP scoring taxa were recorded (Table 2.1).

Table 2.1 The number of BMWP scoring taxa collected in 2003 by the Environment Agency using the existing littoral sampling methodology at 37 standing waters

BMWP_SCORE	Number of riverine scoring taxa nationally	Lake littoral invertebrate scoring taxa	% of scoring taxa found in lake survey
1	1	0	0
2	1	1	100
3	10	9	90
4	3	1	33
5	21	11	52
6	9	2	22
7	5	4	80
8	10	4	40
10	22	13	59

Profundal fauna

Profundal fauna, especially chironomidae and oligochaetes, have long been used to determine the trophic status of water bodies (eg Thienemann, 1909, 1915, 1931; Naumann, 1931; Saether, 1979). And while no profundal data were made available to the current project, since the existing EA/SEPA method uses standard profundal sampling equipment, it is anticipated that both chironomids and oligochaetes are normally collected.

Detecting change in acidification

Existing work suggests that littoral invertebrates can be used to detect changes in acidification, while macroinvertebrates are used in routine monitoring of many acidified British and Scandinavian lakes (Fjellheim & Raddum, 1990; Wiederholm & Johnson, 1997; Monteith & Evans 2000). Molluscs, crustaceans and leeches have all been shown to be sensitive to pH (Okland & Okland 1986; Schindler 1988).

For example, in a study of 1500 Norwegian lakes, crustacean and mollusc species were present in lakes of pH greater than 6.3, but less than half the species were present in lakes of pH 5.8, while none were present below pH 4.5 (Okland & Okland 1986). A possible explanation for the loss of crayfishfrom acidic waters is the loss of calcium from their exoskeletons (Schindler, 1988). Crayfish also suffer increased parasitism and egg mortality, and reduced recruitment in response to acidification (Mierle *et al.,* 1986).

Elsewhere, littoral invertebrate assemblages were shown to be different in an experiment where control lakes were compared to artificially acidified lakes (Stephenson, 1994). Calcium-poor and acidic lakes contained fewer oligochaetes, mayflies, and *Cryptochironomus* and *Stictochironomus* (chironomids), and more Odonata, Trichoptera, *Crangonyx* (Amphipoda), and *Chironomus*, *Conchapelopia*, *Microtendipes* and *Procladius* (chironomids). Equally, the liming of acidified lakes resulted in an increased pH and a shift in the community to one with increased numbers of Chironomidae, Ephemeroptera (including *Caenis* spp.), Odonata, Trichoptera and Sphaeriidae (Bradt, 1996). Palaeolimnological evidence from Scottish lochs indicated that taxa other than chironomids, namely Ephemeroptera and Trichoptera, respond to acidification (Brodin & Gransberg, 1993).

Littoral fauna

The taxa found in lake littoral zones that are sensitive to acidification overlap with the sensitive taxa found in running waters (see Davy-Bowker *et al.,* 2003 for running water taxa). In 37 lakes, the existing littoral sampling method collected 64% of the taxa that are used in theAWICS index and the entire range of AWICS scores was encountered (Table 2.2). These data suggest that the existing EA/SEPA method does have the potential to provide data that are sensitive to changes in acidification.

Table 2.2 The number of AWICS scoring taxa collected in 2003 by the Environment Agency using the existing littoral sampling methodology at 37 standing waters

AWICS score	Number of riverine scoring taxa nationally	Lake littoral invertebrate scoring taxa	% of scoring taxa found in lake survey
1	4	4	100
2	3	2	67
3	3	1	33
4	7	5	71
6	34	21	62

As data on the presence or absence of macrophytes at the sample locations were not provided it was impossible to determine whether the key functional groups associated with shifts from macrophyte to phytoplankton dominance were present.

Profundal fauna

Profundal communities have been shown to respond to acidification and it is possible to trace acidification events using chironomid sub-fossils (Brodin, 1993. Classification of lakes based on chironomid communities includes chironomid species of naturally acidic lakes (Lindegaard, 1995). Profundal invertebrates may be slower to exhibit recovery from acidification after liming than littoral species, due to the accumulation of aluminium hydroxides in the profundal (Lindegaard, 1995). As chironomid species are routinely collected in grab samples, the existing EA/SEPA method should be suitable for detecting changes in acidification status.

Measurement scale

Sampling methods may provide qualitative, semi-quantitative or quantitative data, all of which are suitable for tool development. In relation to benthic invertebrate sampling, qualitative data consist of records of the presence or absence of species at a site; semi-quantitative data consist of species (relative) abundance per unit sampling effort; quantitative data are measures of abundance per unit area (see Jongman *et al.,* 1987 for further explanation of scales).

All three data types are suitable for use in multivariate analysis. Multimetric analysis is usually a form of least squares regression and theoretically requires data measured on a continuous scale (quantitative data). Nevertheless it is also routinely applied to data recorded on integer scales and has been successfully applied to data based on ordinal scales (see, for example, Clarke *et al*., 2002). Therefore, the two main approaches to data analysis can be applied to two of the three data forms. Alternative regression techniques could be applied potentially to qualitative data to develop predictive models, eg logistic regression.

The sensitivity of a sample to changing conditions increases with the degree of quantification. However, quantitative sampling methods take a considerable amount of time to process. To avoid excessive processing times, where quantitative methods are applied, usually only small areas are sampled. In turn, however this may compromise statistical power where densities of animals are extremely low, as in the case of oligotrophic systems. Where sites are severely impacted, qualitative methods may be insensitive at the start of recovery, as change is first detected by changes in the abundance of tolerant species.

The existing littoral method is semi-quantitative and represents a good compromise between collecting enough animals across a range of site types and providing a degree of quantification. However, it was felt that the method may be insensitive in reservoirs suffering from severe eutrophication.

The profundal method was quantitative because a defined surface area of sediment was sampled but, as mentioned earlier, so few animals are collected at some oligotrophic sites that statistical power may be compromised.

2.4 Revising current methods

An extensive review of existing methods was not required. However, both littoral and profundal sampling techniques have been reviewed recently, (Jackson 1997, Murphy *et al* 2002).

2.4.1 Littoral method

As the existing littoral method could not be carried out at all standing waters in the UK, the method has been divided into two, one for sampling hard substrate and the other for sampling vegetated areas. Both methods should be used at all sites if possible. In addition, both new methods are thought to be sufficiently similar to the existing method to allow comparison with existing data. They also have the same attributes of the old method – of being cost effective and requiring no new investment in equipment and little in training. They are semi-quantitative, producing data suitable for tool development, the same sensitive groups are collected and, in addition, key indicator groups are now collected from submerged macrophytes.

To reduce noise, clear guidance is given directing field workers to choose sites which should be, as far as possible, similar in character and homogeneous. For the hard substrate method, the ideal sample station should have a substrate composed predominantly of gravel, pebble or cobble. Larger and smaller substrate particles and macrophytes may also be present at the station. However, the ideal station for using this method is one without submerged or emergent vegetation, mud or sand and, if at all possible, such stations should be chosen. The minimum requirement is that sufficient suitable substrate exists to carry out a three-minute kick sample. That area can be split into patches. At some stations, a fine layer of sand or silt may cover the gravel, pebble and/or cobble layer. This is acceptable, provided the shape of the coarser cobble, gravel or pebble layer is discernible.

For the vegetated areas, the sample station should be dominated by submerged macrophytes with minimal areas of exposed substrate. The station should be relatively homogeneous in character. In the absence of submerged macrophytes, emergent or floating macrophytes may serve as substitutes, but this should be noted. The minimum requirement is that there are enough macrophytes present to take the sweep sample.

The implications of this proposed change in sampling are that while field workers are still able to sample habitats present at a site in proportion to their presence, the amount of noise generated by this activity should be significantly reduced since sites should now be more homogeneous. There is one exception to the freedom to sample habitats in proportion to their occurrence, however, and that is that the substrate under plants should not be sampled. This element was added to reduce sorting time. Emphasis is also placed on spending time in the field cleaning samples in the net.

To reduce noise further and still allow for the collection of as many taxa as possible, the kick/sweep part of the sample and the search should be stored separately. This allows data from the kick/sweep sample to be used in tool development on their own and in combination with the search-derived data.

It is anticipated that the hard substrate method will be most applicable in wave-swept lakes in Scotland and the Lake District. The vegetated area method should be most applicable in shallow lowland lakes dominated by submerged macrophytes. In the future, it should be possible to use the single more suitable method at a given water body. However, the need for both methods is likely to remain in order that all water bodies of interest can be monitored, unless the agencies consider methods which do not use pond nets.

The use of formaldehyde for sample preservation is still the preferred option, and where samples must be preserved without the use of formaldehyde, they must be identified as soon after collection as possible to limit the influence of sample deterioration.

After the initial evaluation of the revised method, other possible methods may need to be considered. For example, as noted above, the existing sampling method is limited to wader depth for safety reasons, restricting the sampling zone firmly in the wave-washed zone, where the fauna is likely to be specialist and relatively depauperate compared to the deeper littoral (Lindegaard, 1992). There is also the belief that the deeper littoral is more homogeneous than the wave-washed zone (Brierley pers. comm.), which, if true, would suggest that the littoral zone should be sampled at greater depth.

And indeed there is some published evidence to support these assertions. The range of substrates is likely to be more limited in the deeper littoral, as exposure exerts less control on substrate composition. For example, samples of aquatic macrophytes and sediment in Loch Lomond taken with an Eckman grab have revealed a relationship between exposure and the amount of fine substrate to a depth of around 3 m (Freissinet *et al.,* 2002). Loch Lomond's littoral zone is not extensive, being limited by water clarity. However, in lakes where the littoral zone is deeper, sediment may be less prone to influence from exposure. The influence of trees and other objects are known to influence the littoral community in near shore areas by affecting adult behaviour (Harrison & Hildrew, 1998a). How far this influence extends into the littoral zone is not known but work on an off-shore littoral reef in Loch Lomond suggested that early Trichoptera instars could travel large distances (up to 100 m) from the only available oviposition sites (Weerekoon, 1956).

2.4.2 Profundal method

As with the littoral method, the suggested changes to the existing EA/SEPA method are minor. We suggest that a suitable winch be employed on deeper lakes to prevent injury to field workers. Normal safety procedures must be put in place when operating from boats and EA/SEPA guidelines should be followed in this regard.

The number of replicates has not been changed, so as to provide sufficient samples from oligotrophic waters for data analysis. However, this number of samples will produce excess material in eutrophic water bodies and sub-sampling may be necessary. A sub-sampling

procedure should be applied by taking the first 50 animals in proportion to their occurrence and then removing additional taxa. Once the data from this study have been processed, the number of samples taken can, and should, be revised.

Because benthic invertebrates are patchily distributed, replicates are unlikely to contain the same fauna. The study will enable the number of samples required to be representative of the fauna to be determined. As the samples are collected from one site, the number of samples required will be determined by the heterogeneity of the habitat and the patchiness of the fauna. To increase sample area, and hence representation, it may be possible to pool samples and sort the pooled sample rather than all replicates individually. This should not happen until after the initial study. Therefore, it is important that replicates are kept separately to calculate the required number of replicates. In the long term, this will allow us to reduce the amount of work involved in collecting samples.

The method has been amended to suggest 10 replicates be taken per basin**,** at multi-basin sites, in order to allow information on variability at sampling locations to be compared across water bodies. Sampling at the deepest point in a lake is not necessary. Anywhere within the profundal zone is acceptable but, preferably, it should be in a flat area and steeply sloping sides should be avoided.

As with littoral sampling, formaldehyde remains the best option for sample preservation.

The existing EA/SEPA method does not mention seasonal sampling; we recommend that samples are collected before and after stratification (early summer or spring and late summer) as hypolimnetic oxygen concentrations are likely to influence the benthic fauna.

3 Lake sampling strategy

3.1 Introduction

In order to comply with the goals of the WFD, any sampling methodology used for lake littoral and profundal invertebrate communities must provide data that can be used to construct a sufficiently robust classification of a given water body to allow the assessment of its ecological status (as defined in Annex V of the WFD). The reliability of data must, therefore, be evaluated relative to all sources of variation which are likely to arise. Variation among samples can be attributable to a) inherent between- and within-site differences that may change with season, and b) differences in operator-sampling and subsequent laboratory or field effort. Statistical confidence is affected by sample size (including sub-sampling procedures), within-site replication, habitat sampled, taxonomic resolution and statistical treatment of data.

These realities present particular challenges with respect to the monitoring of spatially and seasonally variable invertebrate communities. The purpose, and goals, of monitoring of surface water, as required under Article 8 of the WFD, are outlined in Annex V of the WFD and elaborated by ECOSTAT (2003). The information obtained from monitoring needs to be sensitive to defined pressures in order to assess impact.

This chapter reviews sampling strategies for littoral and profundal invertebrates in lakes, with particular emphasis on both the existing protocols proposed by the Environment Agency and WFD compliance.

3.1.1 Littoral invertebrates

Littoral invertebrate communities pose a significant challenge to monitoring under the WFD because:

1) with few exceptions (eg acidity index (SWEPA, 2000)), the relationship between pressures and state is poorly understood (Effect-Load-Sensitivity (ELS) models as discussed by Håkanson, 2001)

2) littoral invertebrates have high temporal and spatial patchiness, and community structure within and between lakes may vary with region (Johnson, 1998, 2000) or lake type (Tolonen *et al*., 2001; White and Irvine, 2003);

3) there can be significant variation of macroinvertebrate communities in different years (Hämäläinen *et al*., 2003);

4) statistical techniques can have a low predictive ability and pose a high risk of misclassification (Håkanson, 1999).

Furthermore, ELS is expected to vary between lake types, supporting the need for an Ecological Quality Ratio (EQR), as required by the WFD, which is lake type-specific. The lack of understanding of the fundamental relationship in the littoral zones of lakes is striking, and it has been argued that the inherent heterogeneity apparent in the littoral zones of lakes prevents meaningful use of invertebrate communities for ecological assessment (Rasmussen, 1988; Johnes *et al*., 1996; Harrison & Hildrew, 1998b; Koskenniemi, 2000). Nevertheless, White & Irvine (2003) sampled 15 meso-habitats while Johnson & Sandin (2001) identified a total of 13 meso-habitats, yetboth of these teams considered that littoral macroinvertebrates are relevant for lake monitoring. The lack of a CEN standard method

might be a consequence of this uncertainty over the development of robust methods for sampling littoral invertebrates in lakes. Evaluation of compliance of the proposed EA/SEPA method with published CEN standards is not possible at the present time.

Littoral macroinvertebrates are used in routine monitoring of many Scandinavian, acidified lakes (Fjellheim & Raddum, 1990; Wiederholm and Johnson, 1997). In addition, the response of littoral communities to other pressures has been employed in monitoring, but is generally site-specific (Milbrink, 1978; Reynoldson *et al*., 1997), and the use of littoral invertebrates in widespread regional monitoring is limited. The Danish faunal index categorises invertebrates based on their tolerance to eutrophication and organic pollution (SEPA, 2000), but is predominantly used in the assessment of rivers. In Sweden, a RIVPACStype species-environment model for macroinvertebrates is under development (Johnson & Sandin, 2001;); however, misclassifications of individual sites can be high. In the US, a multimetric approach to bioassessment is used by more than 90% of state agencies (Barbour & Yoder, 2000), and commonly used biometric models are described by Karr *et al*. (1986), MacCormick *et al*. (2000), Barbour *et al*. (2000) and Paulson *et al*. (1998). However, such biometric models generally have not been used for the assessment of water quality, ecological quality or conservation value of lakes (Resh & Jackson 1993; Maitland, 1997), although biometric criteria are included in technical guidance (USEPA, 1998). Resh *et al*. (2000) reported that multimetric assessment can produce a low probability of correctly assessing impairment of a site, while metrics based on taxa richness were less prone to error than more complicated metrics that incorporated measures of abundance. Resh *et al*. (2000) suggested that multimetric assessment would benefit from the incorporation of multivariate approaches.

Recent work in Ecoregion 17 (White, 2000; Neale, submitted) explored the use of littoral invertebrates for the classification of lakes. White used both multivariate and multimetric models, identified potentially useful indicator taxa, and assessed seasonal and habitat variability among lakes. Neale was able to produce a discriminant function model to predict the probability of biological group membership based on altitude, surface area, pH and conductivity. Both demonstrated that species-level identification was important for discriminating lake status. Further work in Ecoregion 17 (Little, in prep) demonstrated that meaningful biological reference communities can be discriminated using a relatively restricted typology based primarily on three classes of alkalinity and two of depth, with lake area possibly of additional importance. Development of suitable methods to meet the needs of the WFD for the assessment of littoral macroinvertebrates in lakes should consider both multimetric and multivariate approaches and investigate further the potential application of artificial intelligence techniques (Walley & Fontama, 2000).

To develop a well-focused and cost-effective sampling regime that fulfils the needs of monitoring as outlined in Annex V of the WFD (see Irvine, 2004 for discussion), it is worth considering the purpose of surveillance, operational and investigative monitoring: is a comprehensive method that records all taxa necessary, or is it sufficient to monitor taxa that are sensitive to defined pressures? The later approach suggests that sampling a limited number of well-defined meso-habitats may best meet the objectives of monitoring. In addition, the complex habitat structure and generally low predictive power of the relationship between environmental pressure and impact may make the use of a narrow range of taxa necessary. Such an approach could be cost-effective if the chosen taxa are sufficiently sensitive to change. Examples of candidate taxa are corixids (Jansson 1987), chydorids (de Eyto & Irvine 2002; de Eyto *et al*., 2003), and mixed groupings of littoral macroinverebrates (Brodersen *et al*., 1998; White, 2000; White *et al*., submitted). While pre-selection of these groups is outside the scope of the current project, it would be ill-advised to discount them from the thinking in the development of robust sampling strategies for lakes.

3.1.2 Profundal invertebrates

Profundal indicator taxa have been used extensively in the assessment of the trophic status of lakes. Some of the earliest attempts to classify lakes according to productivity using biological indicators were based on bottom fauna (eg Thienemann, 1909, 1915, 1931; Naumann, 1931; Saether, 1979), and such approaches have been used as the basis for assessment of eutrophication and recovery of lakes (Lang, 1990; Lang & Redmond 1992, 1996). Profundal chironomids are the basis of the Benthic Quality Index (BQI), an index developed to assess lake trophic state in arctic regions (Wiederholm, 1980). More recently, profundal communities have been identified as useful in the assessment of Irish lakes (Irvine *et al*., 2001), and the use of chironomid pupal exuviae for the classification of lakes has been promoted by Ruse (2002).

BEAST is a multivariate statistical approach that was developed to compare lake benthic communities in impacted and unimpacted sites in the Laurentian Great lakes (Reynoldson, 1994; Reynoldson *et al*., 2000), but has also been tested in rivers (Reynoldson *et al*., 1997). BEAST compares a site against only those reference sites which belong to the same groupas the test site, whereas RIVPACS compares the test site with all reference sites, applying a probability weighting (Reynoldson *et al*., 1997). Further discussion of the use of biological parameters in relation to reference state is found in Bailey *et al*. (2003).

3.2 Comment on proposed sampling strategy

3.2.1 Uncertainty analysis and sampling frequency

The EA/SEPA proposal raised some fundamental questions relating to sampling and monitoring strategies. These issues were related directly to the ability to provide a robust methodology and assess uncertainty, discussed extensively in ECOSTAT (2003). However, in order to 'quantify uncertainty', additional field tests must be conducted within the EA/SEPA sampling programme. This will require increased sampling effort at a limited number of sites, as has been discussed with EA/SEPA and recommended in the sampling protocol for acid lakes (see Annex II).

Without sufficient understanding of the influence of sample and site variation it will not be possible to undertake statistically robust monitoring. Variation affects both required sample frequency (see Annex V, 1.3.4 of WFD) and intensity. Evidence from Clarke *et al*. (2002), Halse *et al*. (2002) and Neale (submitted), indicate that samples collected from two seasons produce a significantly more robust model than those from a single season, but that the addition of a third season provides relatively little increased reliability of information. In addition, Halse *et al*. (2002) found that two replicates from different lake sectors accounted for 75% of estimated total species richness, while a change of more than 20% in community composition could be detected by ordination.By comparison, 10 samples were required to detect the same degree of change using species richness. However, the UK acid waters monitoring network found it necessary to increase the number of littoral invertebrate samples from three to five to detect system change (Monteith & Evans, 2000).

Previously, the RIVCOMS group devised a methodology for determining inter-operator error for river invertebrate samples (Furse *et al*., 1995). The method required one person to take at least two replicate samples which can then be compared with one or more samples taken by another operative. This test is included in the recommended protocol as described in Annex II. As sampling procedures for the lake littoral zone are at an early stage of development, the sampling strategy will require further testing to ensure it is cost effective. The EA/SEPA programme can, and should, provide an important contribution to this process.

3.2.2 Sampling location

The proposed EA/SEPA method did not provide a clear description of how to choose a site and how to spread sampling effort. This has been improved by describing two well-defined sampling areas, namely a hard substrate area and a macrophyte dominated area. These should be sampled in preference to all other shore types. Any inconsistency between lakes in the habitats sampled is likely to obscure, rather than enhance, the value of community information. In practice it is impossible to sample representative habitat space in lakes within the constraints of any reasonably envisaged routine sampling. It is preferable to confine samples to well-defined habitats. Routine sampling from identified habitat types is likely to be of greater value for lake classification than that from varied and different habitats. Samples taken from a limited number of meso-habitats have been found to be representative of the lake as a whole (White, 2002; White & Irvine, 2003), thus removing the need to include a large range of meso-habitats in any sampling protocol. This conclusion, however, does merit further testing across a range of lake types and pressures. Nevertheless, the extent of visually obvious meso-habitats should be recorded within all sectors of lake samples, irrespective of whether samples are collected from those sites. Change in meso-habitat distribution over time can be a powerful, and simply estimated, metric of changes in environmental pressures (including hydromorphological).

In view of the above, we suggest that that the search element of sampling, in addition to the kick sample, be maintained at this stage. The investigator should now spend half their time sampling in macrophytes and the other half in an unvegetated area. It may transpire that information from either of these two mesohabitats may be sufficient to provide data for model development, but this remains to be tested. Data collected from rocky/gravely areas are likely to be less variable than from macrophyte-covered areas and less time-consuming to process.

3.2.3 Sampling duration

The time taken to collect a sample has varied markedly among workers, and ranged from around 10 seconds to several minutes. As the number of species collected increases towards an asymptote with increased sample time, this may have implications for the determination of reference conditions and classification (Halse *et al.,* 2002; Neale, submitted). Questions remain regarding how sampling effort affects measures of taxa richness across productivity gradients. Whilst large sampling times (ca. 3 minutes) may be necessary for oligotrophic waters, such times may increase markedly the time required to sort samples collected from eutrophic waters. In eutrophic situations it may be necessary to sub-sample or collect the sample over a shorter sampling period. Tests of sampling intensity should be incorporated into the early phase of sampling. Such a test could be confined to a restricted number of lake types (eg two) across a range of impacts (low, moderate, high). Tests should be conducted in July-August when likelihood of detection of any effect is at a maximum. Suggested sampling times are 10, 20, 40, 80 and 160 seconds.

3.2.4 Identification of type-specific EQRs

Type-specific EQR is of fundamental importance for sampling strategy. Knowledge of the underlying mechanisms that relate biological elements to specific pressures is more advantageous than a purely empirical approach, as it facilitates a targeted programme of measures related to the causative pressure. Reference state of the invertebrate community varies across lake types and within habitat types. Lowland European lakes are unlikely to be in reference state. The identification of reference communities/species is a major challenge that requires further research. It is, therefore, especially important that the lakes sampled represent the full range of lake types and pressures. The draft list of lakes to be sampled is unlikely to allow sufficient discrimination of response to pressures. Selection of lakes should be undertaken using a ‘bottom-up’ approach that takes into account the draft typology and

specific pressures, and includes the need to replicate across both these axes. The recently developed GIS classification of UK lakes will assist site selection greatly.

3.3 Recommendations

To develop an operationally effective sampling regime it is recommended that:

- the list of lakes to be sampled should be revised to increase the range of lakes within types that lie across pressure gradients;
- development of methodology should be – as far as possible and without compromising validity of sampling – compatible with existing data;
- Initially, all lakes should be sampled twice (spring/early summer and late summer/autumn) without replication, but from two meso-habitats within a site (ie unvegetated or vegetated) that are collected and processed separately. The method has been revised to include this concept by dividing the kick sample between these two meso-habitats (1.5 minutes each). The extent of visually obvious meso-habitats should be recorded within all sectors of lake samples on each visit, irrespective of whether samples are collected from those sites. The search element of the proposed EA/SEPA methodology should be included pending further evaluation.
- A test of replication should be undertaken,using acid lakes as outlined in Annex II.
- Optimal sampling time requires testing. It is recommended that tests are conducted within two lake types, across a range of impacts (ie low, moderate, high), at one time the year (July-August), and for sampling periods of 10, 20, 40, 80 and 160 seconds;
- The identification of reference communities/species is a major challenge that requires further research. The pilot programme should estimate both environmental pressures and the risk of failure to meet environmental objectives for each sub-set of working lake types.

4 Essential environmental parameters

4.1 Task

To determine and specify the essential environmental parameters needed for the development of lake littoral and profundal benthic macroinvertebrate prediction, classification and diagnostic tools, in consultation with experts and ecologists undertaking the sampling work. Also to review other pertinent datasets.

4.2 Introduction

This chapter is divided into four sections, the first of which discusses the hydro-morphological and physico-chemical parameters that are required by the WFD for assessing ecological status of a water body. Section two details the environmental parameters required for site-specific prediction of the expected macroinvertebrate fauna.Section three describes a range of environmental parameters that may prove useful in the development of diagnostic tools. The concluding section constitutes an overview of the previous three sections, to identify the degree of overlap and allow comparison with the parameters recommended for collection by the Environment Agency during their current sampling operations. The chapter concludes with a summary list of the environmental parameters recommended for collection during the forthcoming sampling programme.

4.3 WFD requirements

Whilst Annex V of the WFD prioritises biological communities for assessing the ecological status of surface waters, an assessment of the hydro-morphological and physico-chemical characteristics of a water body is required also in order to determine its ecological status. The characteristics required for the assessment of lakes are specified in section 1.1.2 of Annex V of the Directive, and fall into four broad categories; hydrological regime, morphological conditions, general water chemistry and specific pollutants.

It would be an obvious benefit if the parameters that are specified in the WFD – and therefore that must be collected for each lake – could be used in the development and operation of predictive and diagnostic tools. These parameters are detailed below and their potential use in biological assessment tools is considered.

Hydrological regime

The WFD states (Annex V, Section 1.3.4) that the hydrological regime of lakes should be monitored on a monthly basis. The definition of ‘high’ status for the hydrological regime is that “the quantity and dynamics of flow, level, residence time, and the resultant connection to groundwaters, reflect totally or nearly totally undisturbed conditions”. As with many of the requirements of the Directive, there is no specific guidance on how to assess the hydrological regime of a lake, but, irrespective of how the hydrological regime is assessed, any information it provides will be of little value in a predictive model.

This is because the hydrological regime of a water body may be altered by humans, and it is an important requirement of predictive models that predictor variables are not affected by anthropogenic activities (Clarke *et al*., 2003). Nevertheless, in the development of a predictive model, the hydrological regime of a lake should be used as an important

parameter in identifying potential reference sites. Additionally, water level fluctuations in Scottish lochs have been associated with major changes in floral and faunal communities (Smith *et al*., 1987), and such information about the hydrological regime of a lake could be useful in the development of diagnostic tools. As previously stated, the WFD requires the collection of monthly information on the hydrological regime of lakes, and this frequency of sampling could provide high quality data detailing the range and frequency of water level fluctuations.

Morphological conditions

The Directive also states (Annex V, Section 1.3.4) that morphological conditions are to be assessed at least every six years. The definition of 'high' status for morphological conditions is that "lake depth variation, quantity and structure of the substrate, and both the structure and condition of the lake shore zone correspond to totally or nearly totally to undisturbed conditions". This is a category of parameters with similar properties to the hydrological parameters – and again there is no specific guidance on how to assess the morphological conditions of a lake. The use of morphological parameters as predictor variables in predictive models is undesirable as the morphology of lakes could be significantly altered by human activity. However, information on lake morphology could be used in identifying potential reference sites and in developing diagnostic tools. As the minimum frequency for assessment of morphological conditions is six years, temporal incongruence may be an issue if this minimum frequency is applied.

Water Quality

The general water quality parameters required by the WFD are specified in more detail than hydro-morphological parameters, the Directive stateing that information on nutrient concentrations, salinity, pH, oxygen balance, acid neutralising capacity, transparency and temperature should be collected initially on a minimum three-monthly basis. Here 'high' status is defined as "not showing signs of anthropogenic disturbance and remain[ing] within the range normally associated with undisturbed conditions". Again, the use of such parameters as predictor variables in predictive models is undesirable, but they could be used in identifying potential reference sites and in developing diagnostic tools. Similarly, the concentrations of 'priority substances' and 'other substances' can provide information for the selection of reference sites and the development of diagnostic tools, but will be of little use in a predictive model.

In summary, information on the hydro-morphological and physico-chemical characteristics discussed above should be available for each lake, as they are specific requirements of the WFD. Moreover, this information should be utilised, though its use may be restricted to the identification or confirmation of potential reference sites and the development of diagnostic tools.

4.4 Classification parameters

An important requirement of this work is the identification of the environmental parameters needed for the prediction of site-specific reference conditions. This approach was pioneered by John Wright and colleagues (Wright, 2000) and led to the development of the RIVPACS system for lotic waters in the UK. The system works by offering site-specific predictions of the macroinvertebrate fauna expected in the absence of significant environmental stress, based on a limited number of environmental variables. An important consideration is that it is undesirable to include predictor variables that have been, or could be, altered by environmental stress (Clarke *et al*., 2003).

For example, measures of water chemistry, which are often correlated with macroinvertebrate communities, show much variation in response to pollution and other environmental stress. Despite this, measures of water chemistry, along with other hydro-morphological parameters that are subject to anthropogenic impacts, have been used extensively in the development and operation of RIVPACS-type predictive models, (Biggs *et al*., 2000; Clarke *et al*., 2003; Johnson & Sandin, 2001; Johnson & Wiederholm, 1989; Neale, submitted). Comparison of the predicator variables used in such predictive models show a core suite of common variables (Table 4.1). Among these are variables associated with site location (latitude, longitude, altitude) and the surface area of the water body. Given the importance of these parameters in existing predictive models, their collection for sample sites in this study is highly recommended. It is advantageous that these are map-based variables derived from desk study, rather than requiring field sampling programmes.

Table 4.1 Comparison of the environmental variables used in a range of predictive models[1]

Parameter	Pond Action (Biggs *et al*., 2000)	SWEPAC Johnson & Sandin (2001)	RIVPACS (Clarke *et al*., 2003)	Neale (Submitted)
Latitude	Y	Y	Y	N
Longitude	Y	Y	Y	N
Altitude	Y	Y	Y	Y
Surface Area	Y	N	Y	Y
pH	Y	N	N	Y
Alkalinity	N	N	Y	N
Conductivity	N	N	N	Y
Substrate	Y	Y	Y	N
Water Colour	N	Y	N	N
Chloride	N	Y	N	N
Vegetation	Y	Y	N	N
Nitrogen	N	Y	N	N
Inflow	Y	N	N	N

[1] Note: Some of the RIVPACS variables are omitted as they are specific to running water

Problems associated with using water chemistry parameters as predictive variables could be overcome by including information on the geology of the catchments. For example, Gibson *et al*. (1995) reported a close relationship between underlying solid geology and water chemistry of lakes in Northern Ireland. In contrast, evidence from the Republic of Ireland suggests a poor relationship between catchment geology and measures of water chemistry (Irvine, pers. comm..). It is proposed that detailed information on the geology of lake catchments should be collected, to enable an assessment of whether such information could provide a suitable surrogate for background (unimpacted) water chemistry. A possible approach is to determine the percentage of calcareous and siliceous base geology within the lake catchments from GIS. Additionally, the percentage cover of peat may prove to be a useful predictor variable, as it is often associated with lakes of naturally low pH.

The importance of water chemistry in the structuring of aquatic macroinvertebrate communities is well documented (Biggs *et al*., 2000; Clarke *et al*., 2003; Johnson & Sandin, 2001; Johnson & Wiederholm, 1989; Neale, submitted). If measures of geology are not sufficiently powerful to act as surrogates for background water chemistry, the inclusion of direct measures of water chemistry as predictor variables may be required. The inclusion of any water chemistry variables will require careful consideration, to reduce potential confusion between cause and effect.

The substrate characteristics of the sample site consistently feature in the predictive models compared above. Under undisturbed conditions the particle size of sediments at any given site is a function of the wave exposure (Barton & Carter, 1982). However, the substrate

characteristic at a site can be altered by anthropogenic activity and, therefore, a measure of exposure at the sample site may prove more appropriate. The aspect and fetch of a sample site may prove useful as a coarse measure of exposure as prevailing winds in the UK are relatively uniform. The effect of substrate may be reduced if macroinvertebrate sampling is restricted to gravel/wave washed shores. Together with exposure, the slope of the shoreline should be estimated. The current Environment Agency method of measuring the distance from waters edge to 75 cm depth should provide an indication of the nature of the littoral zone.

If the requirement to use predictor variables that are unaltered by environmental stress is strictly maintained, the following predictor parameters remain:

- longitude
- latitude
- altitude
- surface area (may encounter problems with reservoirs)
- % calcareous geology in catchment
- % siliceous geology in catchment
- % organic drift geology in catchment
- aspect (and fetch) of study site
- slope of shoreline

The advantage of this list of predictor variables is that they are relatively timeant and many of them can be derived from maps or GIS.

As recognised by Clarke *et al*. (2003), predictive models based on predictor variables restricted to characteristics that are not likely to be altered by environmental stress may not provide the best possible statistical performance. There is no obvious solution to this problem – either models are developed with potentially low statistical power, or else predictor variables that may be altered by anthropogenic impact are included.

4.5 Parameters for diagnostic tools

This section will focus on the environmental parameters required to develop diagnostic tools for acidification and eutrophication – pressures identified as most important to the Environmental Agency. Nevertheless, the potential impacts of other environmental pressures should not be ignored.

The environmental parameters required by the WFD to enable the determination of ecological status (Section 1) can also be used in the development of diagnostic tools. Among the water chemistry parameters specified in the Directive, information is required on nutrient concentrations and pH. Provided that naturally low pH lakes are taken into account, pH can be correlated with measures of macroinvertebrate communities to investigate the impact of acidification on these communities. A similar exercise can be conducted with nutrient concentrations, typically phosphorus and nitrogen (Carvalho *et al*., 2002). Biggs *et al.* (2000) and White *et al*. (submitted) have also reported associations between measures of the littoral macroinvertebrate community and phosphorus and nitrogen concentrations, and their use in diagnostic tools should be investigated further.

The Directive also requires information on the hydrological regime of lakes, which could again be used in diagnostic tools. Smith *et al*. (1987) recorded major changes in the floral and faunal communities of Scottish lochs in response to water level fluctuations. Hence, the macroinvertebrate community could provide a useful diagnostic tool, particularly for lakes used as reservoirs.

White *et al*. (submitted) reported significant correlations between macroinvertebrate community measures and CORINE land use categories within the catchment. They recognised that these correlations reflect both natural catchment geology and anthropogenic impact. Therefore, information on the land use in each lake catchment could prove useful in diagnostic tool development. This parameter may be derived from GIS and could be expressed as the proportion of different land uses or as human population density.

The development of successful diagnostic tools is dependent on identifying macroinvertebrate community measures that show strong relationships with environmental degradation, with little variation or 'noise'. It is likely that some of these environmental parameters will be correlated with each other (for example, land use and phosphorus) and, as a result, some could be disregarded in the diagnostic tools.

4.6 Overview

Table 4.2 compares the environmental parameters that are required by the WFD with those recommended for collection in the proposed Environment Agency sampling protocols. As anticipated, there is much overlap between the different requirements and it is beneficial if data collected for one purpose can be used for others. However, the methods used to collect information regarding these environmental parameters must be suitable for the primary intended use of the data.

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Table 4.2 Comparison of the environmental parameters required for different aspects of the project

Parameter	WFD	Predictive tools	Diagnostic tools	Proposed EA
Hydrological regime	Y	N	Y	Y
Morphological conditions	Y	N	Y	Y
Water Chemistry	Y	N	Y	Y
Map location	N	Y	N	Y
Surface area	N	Y	N	N
Geology	N	Y	N	Y
Aspect	N	Y	N	Y
Shoreline slope	N	Y	N	Y
Land use	N	N	Y	Y

Where resources allow, it would be prudent to collect as many environmental variables as is possible at study sites. The list of environmental variables will be interrogated using iterative statistical analyses to identify the most important variables for the construction of predictive and diagnostic tools. Unimportant parameters will be excluded at a later stage of development. The following parameters are recommended for collection at each study site:

General

- longitude
- latitude
- altitude
- surface area
- % calcareous geology in catchment
- % siliceous geology in catchment
- % organic drift geology in catchment
- catchment land use
- population densities
- CORINE Land Use categories

- water level fluctuation
- lake morphology
- depth – mean and maximum

Sample site characteristics

- aspect of study site (for littoral sampling)
- getch (for littoral sampling)
- slope of shoreline (for littoral sampling)
- depth of sample site (for profundal sampling)
- stratification regime (for profundal sampling)
- oxygen regime (for profundal sampling)
- substrate composition
- shoreline modification score (RHS)

Water chemistry

- phosphorus (total P, soluble P)
- nitrogen (total N)
- chlorophyll concentration
- water colour
- pH
- salinity
- oxygen concentration
- alkalinity
- conductivity
- transparency – Secchi depth
- temperature
- priority substances – concentrations

Another parameter that may be useful in explaining some of the inter-annual variation in macroinvertebrate communities is the North Atlantic Oscillation (NAO). Hamalainen *et al*. (2003) and Bradley & Ormerod (2001) suggested that macroinvertebrate communities may be linked with large scale climatic variation; incorporation of the NAO may allow us to differentiate natural inter-annual variation from anthropogenic impact.

5 Appropriate taxonomic resolution

5.1 Task

The specific objective of this chapter is to determine the most appropriate level of taxonomic resolution required to enable the identification of a variety of possible pressures – including acidification and eutrophication – on macroinvertebrates collected from lakes in the UK. Also to evaluate costs and benefits of identifying lake macroinvertebrate samples to family and mixed taxon by literature search and consultation with experts, and to identify the risks of identifying lake macroinvertebrates only to family level. Finally, if a programme of testing is needed, to incorporate this into the proposed sampling strategy and take account of budget limitations.

5.2 Introduction

Different levels of taxonomic resolution of benthic macroinvertebrates are used to assess the biological condition of watercourses, ie species, genus or family. Reynoldson & Wright (2000) concluded that decisions on taxonomic resolution are largely dependent on the objectives of the study, but that identification to species/genus level is not always necessary. The choice of taxonomic level of identification has important consequences for future uses of the data. In relation to the taxonomic resolution of lake macroinvertebrates the key issue of this study is how to maximise the information derived for the minimum effort/cost. Recommendations need to be based on the data required for robust model development, which take costs into account. For water quality managers responsible for implementing the WFD in lakes, this is an important issue to resolve.

The key questions are:

- What taxonomic resolution is best for distinguishing between reference sites (in both littoral and profundal habitats) to produce a site classification and for predicting the expected fauna (based on site classification)?

- Does species/genus identification add significantly more information than family or higher level identification?

- What taxonomic resolution is most appropriate for detecting deviation from reference conditions, ie for detecting the effects of environmental pressures (acidification and eutrophication) and their severity? Is detection easier with species/genus-level identification than identification to family or higher level?

- Are the extra resources and costs to identify macroinvertebrates to species/genus level worthwhile?

5.3 Best taxonomic resolution for classifying lake sites and predicting the expected fauna

In the scientific literature, which is largely biased towards lotic rather than lentic environments, there is contrasting evidence regarding the level of taxonomic resolution required for biological assessment (Bailey *et al*., 2001). Furse *et al*. (1984), while developing RIVPACS, concluded that the use of species data produced a more reliable categorisation of sites and more accurate prediction of fauna (see also Hawkins *et al*., 2000). However, other authors have claimed that identification to higher taxonomic levels provides as much

information as species level identification, but at a much reduced cost. The issue of taxonomic resolution is complicated by natural variation in macroinvertebrate communities across different biotic regions, and lake and habitat types (eg littoral and profundal habitats), as well as by differences in the treatment of rare taxa, the use of mixed taxonomic levels in species-level datasets, and by the process of sub-sampling. The effects of these latter three procedures have been reviewed recently (Neale, submitted); the conclusions are summarised below.

5.3.1 Removal of rare species

Rare species are often removed from datasets before analysis, for largely technical considerations related to a perceived need to eliminate large sparse datasets with numerous zero values and to achieve approximate multivariate normality. Cao *et al*. (2001) consider these justifications to be weak given recent advances in computing technology. The removal of rare species can influence the results of multi-variate analysis in a similar way to taxonomic resolution. This is because many rare taxa are from families represented by more abundant taxa. Therefore, the removal of rare species results in the analysis resembling a family-level analysis (Lenat & Resh, 2001). In other words, the removal of rare taxa can lead to species- and family-level datasets that are essentially the same, producing similar model performances. A further example of this phenomenon was provided by Johnson & Sandin (2001) who developed SWEPAC models for assessing lake littoral assemblages, calibrated using two levels of taxonomic resolution, where 'species-level' resolution consisted of 85 mixed taxa and 'family-level' resolution consisted of 85 families plus other levels of resolution. The species-level data set comprised taxa present in >3% of the total number of sites, whereas the family-level models included all families with 1 observation.

Mixed taxonomic levels

The inclusion of many non-species identifications in 'species-level' datasets – a consequence of difficulty in the identification of groups such as Chironomidae, Oligochaeta and Sphaeriidae (characteristic macroinvertebrate groups of the profundal benthos) – can produce similar results to those derived from genus/family-level datasets (see Hewlett, 2000; Johnson & Sandin, 2001).

5.3.2 Sub-sampling

The practice of sub-sampling, where only a fraction of a sample is sorted, is often carried out in an effort to reduce the time spent sorting samples. Lenat & Resh (2001) argue that this has the same impact as removing rare species, as rare species are more likely to be missed in sorting (see Hewlett, 2000).

Northern Ireland case study

Neale (submitted) investigated the impact of taxonomic resolution on the classification of sample sites based on littoral macroinvertebrate fauna, using a RIVPACS-style model developed to classify and assess the ecological status of lakes in Northern Ireland. The results were compared using four different levels of taxonomic resolution, namely: species, species with rare taxa removed (defined as occurring at two sites or less), genus and family. Lakes were selected on the basis of their chemistry and immediate land use, to ensure minimal impacted conditions. Verification that these results reflected reference conditions rather than the effects of impacts was demonstrated by the study lakes being grouped together, firstly by altitude, and then by surface area, using both divisive and agglomerative classification techniques and by ordination. Further supporting evidence was provided by the fact that a discriminant function model, based only on surface area and altitude, was nearly

as successful as a model with water chemistry variables in predicting the reference sites (Neale, submitted).

Table 5.1 Effect of taxonomic treatment on the number of taxa, classification strength and predictive success of a discriminant function model (cross-validation test)

Taxonomic treatment	Number of taxa	Classification strength	Predictive success
Species	154	0.641	82.6%
Species (rare taxa removed)	92	0.632	69.6%
Genus	108	0.567	78.3%
Family	62	0.535	73.9%

Source: Neale (submitted)

The results indicated:

- number of taxa decreased with lowering of taxonomic resolution;
- different taxonomic treatments produced largely similar site classifications to those produced by the species-level dataset, although the strength of each classification decreased with decreasing taxonomic resolution;
- predictive success of the discriminant function models decreased with the level of taxonomic resolution;
- species-level littoral macroinvertebrate data produced the most effective predictive model with which to distinguish between reference sites.

The main conclusion from this case study was that, although site classifications based on littoral macroinvertebrate fauna were similar at different taxonomic levels, species-level data were the most effective at distinguishing between reference sites (Neale, submitted).

In general, the diversity of the profundal benthos of lakes is relatively limited compared to that found in the littoral zone, where the more complex and diverse habitat conditions result in a greater variety of macroinvertebrates. Environmental conditions tend to be more uniform and predictable in profundal sediments. The profundal fauna is usually restricted to chironomids, oligochaetes and sphaeriid mussels. Therefore, a high degree of taxonomic resolution (ie species- or genus-level) will be necessary to separate reference sites. Nevertheless, recent work in Ireland suggested that profundal macroinvertebrates could be used for lake monitoring without detailed taxonomic resolution because of the limited range of taxa recorded in profundal areas (Irvine, pers. comm.). The CIT profundal index developed for the Irish EPA, works by counting and identifying the oligochaete family, Tubificidae, plus four distinctive genera: *Peloscolex, Aulodrilus* (both tubificids), *Procladius* and *Chironomus* (both chironomids). The CIT index can be summarised as follows:

CIT = total tubificids (excluding *Aulodrilus* & *Peloscolex*) + *Procladius* + 0.1 *Chironomus*

As it is quantitative, the method requires a sufficient number of replicates to be accurate. Nevertheless, the total density of tubificid worms from 5 or 6 replicate profundal samples may be sufficient to characterise a lake (Irvine, pers. comm.).

5.4 Taxonomic resolution required to detect the nature and severity of environmental stress

While RIVPACS-type models can predict the macroinvertebrate fauna to be expected in the absence of environmental stress, they cannot predict faunal response to an environmental stressor. To produce a model that can diagnose the nature and severity of an environmental stress requires data from observed communities at impacted sites to be linked with specific types of stressors (de Pauw, 2000).

Predictions of 'expected' fauna at test sites can be made at different taxonomic levels and the deviation of 'observed fauna' from the target 'expected' fauna forms the basis of a biological index, such as the Ecological Quality Index (EQI). Here, the question is whether data resolved to family level are as informative as species-level data. The literature suggests that species-level data are more reliable and effective in detecting environmental stresses (eg Hawkins *et al*., 2000). Lenat and Resh (2001) commented that whilst family-level data may detect grossly polluted sites, smaller, more, subtle changes in water quality may remain undetected. Ferraro & Cole (1991) and Bailey *et al*. (2001) stressed that the variation in ecological characteristics and their responses to stress among species within families make it important to monitor individual species.

Where there is ecological diversity within any particular taxonomic level, reduced resolution will produce a less robust classification and risk errors in the identification of an environmental stressor (Hawkins and Norris, 2000). However, a compromise may be necessary between the increased amount of information provided by species-level identification and the resources required to obtain it (Bowman and Bailey, 1997). This may be the case in widespread operational quality assessment schemes; the Environment Agency conducts routine assessments of river water quality based on family-level data (Hemsley-Flint, 2000).

An alternative to the above RIVPACS-style approach to the biological classification of water quality is the use of artificial intelligence (AI). While RIVPACS is based on a single reference state, the AI approach takes a more holistic view of the biology of impacted and non-impacted waters. Walley and Fomtama (2000) claimed that AI techniques could define a biological model spanning all river qualities and could provide a more reliable classification of river quality than EQIs.

The AI approach works on the premise that no single measure of water quality is more important than any other (in contrast to the use of EQIs in the RIVPACS approach) and incorporates characteristics of the data that are normally ignored or poorly represented. Specifically, these include the inherent uncertainty in its meaning, the evidence offered by absent taxa, and the fact that different abundance categories of a taxon may indicate different water qualities (Walley and Fontama, 2000). The utilisation of data on frequency distribution might help describe the non-linear relationships that exist between taxa and river water quality, and to define the probabilities of the macroinvertebrate communities typical of each river quality class or grade (Walley & Fontama 2000). Species-level identification may provide a more discriminating frequency distribution than family-level identification, although the scale used to score abundance will also have an impact. Using AI techniques based on pattern recognition and plausible reasoning, Staffordshire University, in conjunction with the Environment Agency, have developed a computer-based system that can diagnose and predict river health using macroinvertebrate and environmental data (Walley *et al*, 2002). No equivalent system has yet been developed for lakes.

5.4.1 Eutrophication/acidification pressures

Carvalho *et al*. (2002) reviewed the use of benthic macroinvertebrates to indicate eutrophication pressures. Historically, approaches to establishing relationships between benthic macroinvertebrates and nutrient concentrations have concentrated on the profundal fauna of deep stratifying lakes (>3 m depth). As a consequence of the relatively uniform and predictable environmental conditions in these deeper waters, the profundal benthos tends to be limited to Chironmidae, Oligochaeta and Sphaeriidae. Chironomids have great potential for the definition of reference conditions, due to the preservation of their head capsules in lake sediments (Carvahlo *et al*., 2002).

Chironomid larvae have been used as trophic state indicators as:

- they have a high species richness compared to other benthic macroinvertebrate groups;
- they occur over the whole spectrum of nutrient conditions;
- as individual species have highly specific environmental tolerances, species composition changes in tandem with changing lake trophic status (Rosenberg 1992).

To distinguish the relatively subtle differences between lakes using the composition and abundance of chironomids and oligochaetes requires a high degree of taxonomic resolution (compared to that required for river surveys). Family-level identifications are not sufficient for this task. Sampling requires the use of a boat, in addition to which, the process of sorting samples and identifying chironomid (and oligochaete) samples to species level can be very time-consuming (Moss *et al*., 1996).

In contrast, to profundal habitats, littoral habitats are more diverse and are influenced by a range of abiotic and biotic factors making it difficult to link benthic macroinvertebrate communities to eutrophication and acidification pressures. However, the chironomid pupal exuviae method (CHIRON) developed by the Environment Agency (Ruse, 2002), correlated the distribution and abundance of chironomid pupal exuviae with chemical and physical factors – albeit based on study set of only thirty lakes in England and Wales. This method produces a sample that integrates the quality of a whole lake, using both profundal and littoral taxa. It is recommended that fourmonthly samples are collected between April – October o provide sufficient coverage of species emergence (Ruse, 2002).

The CHIRON method relies on the collection of chironomid pupal exuviae, which accumulate on leeward shores after pupae emerge from the surface of lake. The theory is that pupal exuviae represent a passively accumulated sample (integrating species emergence variation in time and space) of the species present in the lake, which in turn reflect the conditions in the water column and sediments. Alkalinity and lake volume were found to be the best predictors of chironomid assemblages in lakes, although this may reflect different pressures rather than actual reference state (Irvine, pers. comm.). Ruse (2002) found that 97% of the 30 lakes studied were correctly classified using a subset of indicator taxa comprising 15 profundal species (classification derived from total of 208 littoral and profundal taxa), while 73% were correctly classified using only 7 genera. A high degree of taxonomic expertise is required to analyse exuviae to species level; less expertise is required to analyse these to genus level, although this results in a reduction in discriminatory power. Family-level identifications are likely to be of little discriminatory value.

***Advantages[3] of the CHIRON method*:**

- integrates over space & time
- can use sediment records to define reference conditions
- chironomid species present in all lakes
- could be used to assess profundal macroinvertebrates (supplement littoral macroinvertebrate schemes)

***Disadvantages of the CHIRON method*:**

- nutrient response difficult to interpret
- requires specialist training in chironomid taxonomy

5.5 Costs *versus* benefits of identifying macroinvertebrates to species/genus-level

The cost associated with using macroinvertebrates to assess and water quality depends on both the cost of field sampling and sample processing – sorting, identifying and enumerating. The time and cost involved in sample processing will depend on the breadth of taxonomic groups examined plus the level of taxonomic resolution. The table below summarises the estimated time CEH consider to be realistic to process and identify macroinvertebrate samples from the littoral and profundal zones of lakes. The table indicates that increased taxonomic resolution results in increased time required to identify samples, but that the time to sort samples remains the same. The level of expertise required to identify macroinvertebrate taxa to species level will also increase and will therefore result in additional costs (staff with the skills to identify macroinvertebrate taxa to species level generally costing more than staff whose expertise is limited to family/mixed).

Table 5.2 Estimated time to process and identify lake macroinvertebrate samples

		Sort (hrs)	Expert ID (hrs)	Total (hrs)
Littoral	Family	5	2	7
	Mixed	5	10	15
	Species	5	18	23
Profundal	Family	5[1]	2	7
	Mixed	5[1]	5	10
	Species	5[1]	13	18

[1]sorting times for profundal samples may be reduced if a sieving machine is used

5.6 Summary: advantages of different levels of taxonomic resolution

Advantages of identification to species/genus:

- significantly better discriminatory power and for the detection of sites suffering from a range of environmental stresses – especially in areas where families are represented

[3] Note. These advantages and disadvantages are not exclusive to this method

by many species differing in their ecological requirements and tolerances (eg Chironomidae in profundal benthos[4]

- closely related species respond differently to environmental conditions and are preferable as a biomonitoring tool – may detect small differences between sites or between sampling dates and may indicate type of environmental pressure;
- increased confidence in biomonitoring results used to guide management decisions;
- greater flexibility to predict target macroinvertebrate communities/assemblages, either at species level or at a higher taxonomic level (as shown by RIVPACS);
- more valuable historical data for other scientific studies, such as the conservation evaluation of sites.

Advantages of identification to family:

- requires less taxonomic expertise;
- will result in significant savings, in terms of time and resources, in any extensive biomonitoring programme – though may also result in less ecological information;
- may not be significantly different from species/genus-level in providing ecological information and in detecting sites suffering from a range of environmental stresses – especially in taxa with few species per family (eg Ephemeridae or Perlodidae in littoral benthos);[5]
- sufficient if goal is to indicate relatively large between site differences – for example, to separate reference sites from impaired sites or separate into broad water quality categories;
- already routinely used for river water quality assessments (including by the Environment Agency);
- close correlation between family (BMWP family) and species richness. Family level data can be used to focus species surveys on areas which are species rich .

5.7 Conclusions

- Reference macroinvertebrate communities in lakes need to be linked to specific, well defined, habitat types, ie the benthos of littoral stony/pebbly wave-washed shores should be separated from the benthos of vegetated/silty littoral habitats;
- It may be possible to adopt different strategies (in terms of taxonomic resolution) for littoral and profundal zones;
- In the profundal zone, because of limited diversity of benthic macroinvertebrate groups, identification should be to a high taxonomic resolution (species/genus-level) in order to differentiate between reference sites;
- In the littoral zone, because of much greater diversity of benthic macroinvertebrate groups, it may be possible to adopt lower taxonomic resolution (family-level) to differentiate between sites. This is akin to the strategy already adopted for the routine water quality assessments of UK rivers (Hemsley-Flint, 2000). However, any

[5] Hawkins & Norris (2000) argue that the adequacy of family level models is inversely related to the amount of adaptive radiation occurring within the families

recommendation to use lower taxonomic resolution would need the support of robust analysis.

- As a precautionary principle the species-level is advocated for tool development, with the recommendation that lower resolution be considered in the future to reduce sorting time.

Abbreviations

AI	artificial intelligence
AWICS	Acid Water Indicator Community
BEAST	Benthic Assessment of Sediments
BMWP	Biological Monitoring Working Party
BQI	Benthic Quality Index
CEH	Centre for Ecology and Hydrology
CEN	European Committee for Standardisation
CHIRON	Chironomid Pupal Exuviae method
CORINE	Cordination Of Information on the Environment
EA	Environment Agency
ELS	Effect-Load-Sensitivity
EPA	Environmental Protection Agency
EQI	Ecological Quality Impact
EQR	Ecological Quality Ratio
GIS	Geogpraphical Information Systems
IMS	Industrial Methylated Spirits
NAO	North Atlantic Oscillation
NERC	Natural Environment Research Council
RIVPACS	River Invertebrate Prediction and Classification System
SEPA	Scottish Environmental Protection Agency
SWEPAC	Swedish Prediction and Classification System
WFD	Water Framework Directive

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Annex 1: proposed sampling methods

WATER FRAMEWORK DIRECTIVE STILL WATER SAMPLING PROTOCOL 2004

THE COLLECTION OF MACROINVERTEBRATES FROM A) VEGETATED AND B) STONY LITTORAL AREAS

Introduction

The methods described in this document are a modification of the standard RIVPACS method for rivers. Many of the principles and procedures are the same but some have been altered significantly.

This document is a revision (by J. Murray-Bligh, R. Guthrie and M. O'Hare, 5th July 2003 and subsequently M. O'Hare, K. Irvine, M. Neale and I. Gunn, 5th April 2004) of that produced by Peter Hale in April 2002 and presented to the UK Technical Advisory Group's Lakes Task Team for the Water Framework Directive on 15 May 2002 as paper TAG/LTT 29. The methodology itself is a modification of sampling procedures implemented in the British Isles for the collection of river macroinvertebrates described in BT001 (Murray-Bligh, 1999a) and ISO 7828 (ISO, 1985).

Aim

To sample macroinvertebrates from vegetated and stony littoral areas of still waters in a way that will provide reproducible samples suitable for developing classification and prediction tools.

Health and Safety Statement

The main health and safety risks associated with collecting macroinvertebrate samples from the littoral areas of still waters are given in the Generic Risk Assessment for Fieldwork and supplemented by the Generic Risk Assessment for Water Framework Directive Sampling of Still Waters. Sampling must be undertaken in accordance with the specified safety controls.

Where circumstances dictate, line managers should ensure a Supplementary Risk Assessment is undertaken to record additional hazards and controls. This Supplementary Risk Assessment can then be used to develop additional working instructions for each sampling location.

All staff should be issued with the appropriate Personal Protective Equipment, as specified in the Generic Risk Assessments, as well as any additional items determined by their line manager.

Scope

The procedures describe methods for collecting aquatic macroinvertebrates from A) vegetated and B) stony littoral areas of still water bodies specifically for water quality and ecological assessment purposes. The requirement for this sampling is inherent in the Water Framework Directive 2000/60/EC (Official Journal of the European Union, 2000).

The methods are based on the collection of a single sample from A) a station located in a vegetated area of shore and B) a station located in a stony area of shore. Samples are subdivided into a search and a sweep element.

The methods are considered suitable for collecting 'semi-quantitative' aquatic macroinvertebrate samples from lakes and other bodies of standing water. Samples are taken using a standard mesh size pond net (FBA pattern). Semi-quantitative data are produced through the standardisation of the sampling time period.

The methods are suitable for use to wading depth. Samples must be collected by wading from the shore and not by boat.

The methods are not suitable for generating quantitative samples. Further, they are unsuitable for collecting profundal macroinvertebrates from lakes and other bodies of standing water for which other sampling methodologies are more appropriate.

Equipment

See BT001 (Murray-Bligh, 1999a), except that sampling equipment is the pond net only. A compass or GPS will be required to determine the aspect of the shore. A measuring tape will be needed to determine the slope of the shore.

Dry suits or chest waders are recommended. Chest waders must not be used on boats. Standard life jackets (150 N) may be used with chest waders.

Procedure

Site Selection: General

The sample station is a section of shoreline from which a sample will be taken. As a rule of thumb, a minimum of 15m of shoreline is required to carry out the sampling effectively. The station is bordered by the shore and an imaginary line parallel to it that extends to the maximum wadeable depth of approximately 75cm.

The same safety and etiquette considerations should be given to locating and gaining access to lake sites as river sites. "Quaking bog" and soft, sinking littoral sediments are more likely to be encountered when searching for access to appropriate lake sampling stations. As with rivers, alternative sampling locations should be considered when conditions are deemed to be unsafe. Sampling should not be attempted if do so would compromise safety.

Examine the sampling station closely to ensure there are no hidden hazards. Use a wading pole to ensure there are no exceptionally soft patches of sediment; these are more likely to be encountered in lakes than rivers. If necessary consider an alternative sampling station.

Ensure from both maps and an inspection of the site, that there are no localised lotic influences at the station. If necessary, find a more representative sampling station remote from localised influences of streams, flushes and other inputs.

The following locations should be avoided:

1. Areas with artificial substrate. Where the water body itself is artificial this may be impossible and samples should be taken from areas as similar as possible to naturally vegetated or stony areas (according to the method used).

2. Areas near main inflows and smaller influent streams. Remember that river flows may hug the shoreline of a lake for some distance before mixing with the main body of water.

3. Areas near outflows; sites here may be riverine.

4. Areas dominated by (close to) fish farms, sewage effluents and farms (this would be equivalent to sampling in rivers within effluent plumes). The station should be located where the effects of such features are integrated with the main body of water.

5. Harbours, dams, and jetties.

6. Areas poached by cattle.

7. Very exposed locations.

Locations on promontories *are* acceptable.

You should not extend the area of the sample station in order to increase the range of habitats sampled and so the number of taxa caught. This is consistent with RIVPACS sampling in rivers, but differs from PSYM for canals.

Site Selection: A) Vegetated Littoral Areas

The sampling method uses a sweep sampling technique for three minutes augmented by a one minute search. The ideal sample station is dominated by submerged macrophytes with minimal areas of exposed substrate. The station should be relatively homogeneous in character. In the absence of submerged macrophytes, emergent or floating macrophytes may serve as substitutes, but should be noted. The minimum requirement is that there are enough macrophytes present to take the sweep sample.

Site Selection: B) Stony Littoral Areas

The sampling method uses an active kick-sampling technique for three minutes augmented by a one minute search. The ideal sample station will have a substrate composed predominantly of gravel, pebble or cobble. Larger and smaller substrate particles and macrophytes may also be present at the station. However, the ideal station for using this method is one without submerged or emergent vegetation, mud or sand and, if at all possible, such stations should be chosen. The minimum requirement is that sufficient suitable substrate exists to carry out the three minute kick sample. That area can be split into patches. At some stations a fine layer of sand or silt may cover the gravel, pebble and / or cobble layer. This is acceptable provided the shape of the coarser cobble, gravel, pebble layer is discernible.

In addition to the locations to avoid noted above, the following areas should also be avoided:
1. Areas without movable (kickable) substrate between boulders.
2. Areas where the presence of boulders would compromise safety.

Biological Sampling Protocol: A) Vegetated Littoral Areas

The sample consists of material collected at the site by actively sampling for three minutes augmented by a one minute search. The approach is similar but not the same as that prescribed for rivers (BT001, Murray-Bligh, 1999a), ponds and canals (Williams *et al*, 1998) or small lakes (Biggs *et al*, 2000). Macrophytes within the area are sampled with effort in proportion to their cover but the bed substrate is avoided. In addition, animals captured during the sweep are stored separately from those captured during the search.

Before collecting a sample, examine the pond net closely to ensure it is free from holes or tears and any organisms from the previous sample. If necessary, replace the net.

The Search

The search is for individual specimens of species that are unlikely to be captured in the main sample. In contrast, for the main sample, the sampler concentrates on sampling particular habitats and collects whatever animals are there. The principles are the same as those described for rivers in BT001 (Murray-Bligh, 1999a) except the bed substrate is avoided.

The search is divided into two parts:

1. a search for surface-dwellers before collecting the main sample; and

2. a search for animals attached to objects after collecting the main sample.

The proportions of the one-minute allocated to each of these parts will depend upon the nature of the site, notably the presence submerged and emergent macrophytes that may harbour attached animals.

Thoroughly examine the water surface within the sampling area for the presence of surface dwelling invertebrates such as pond skaters, whirligig beetles and water measurers. These should be captured by scooping with the net before entering the water. Any disturbance will alert these animals to your presence; their evasive action may make them difficult or impossible to locate later during sampling. Store and mark this sample separately labelling with the words ‘vegetation search’. The station is then actively sampled by sweeping the net for three minutes.

Vegetation ineffectively sampled during the three-minute sweep is subsequently searched. This involves manually searching submerged macrophytes and removing strongly attached specimens that were unlikely to be collected during the sweep procedure. These animals are added to the sample marked ‘vegetation search’.

The Three-minute Sweep

If more than one species of macrophyte is present at the sample station, divide your time proportionately between them. The sample should be preserved separately from the search parts of the sample. Label the container ‘sweep’.

To optimise the area of lake that is sampled, a zigzag sampling pattern is recommended, moving from shallow to deep to shallow and so forth. This will minimise any effects of water level fluctuation on the littoral benthic fauna. It is important to cover as much of the lakeshore as possible in the sampling time.

The sampling method involves dislodging macroinvertebrates from submerged vegetation and sweeping the net through the resulting area of disturbance. It should be recognised that this method is aimed at sampling the macroinvertebrates inhabiting the submerged macrophytes and not the macrophytes themselves. Therefore, the collection of macrophytes

within the sweep sample is actively discouraged. Similarly, disturbing and sampling the substrate beneath the vegetation with the net should be avoided. To reduce the amount of vegetation collected in a sample, it is acceptable to rinse the macroinvertebrates from macrophytes caught in the net, ensuring that the mouth of the net is above the water level to ensure that no animals may escape. Washed vegetation may be discarded. The rinsing procedure does not form part of the three-minute sweep time and the stopwatch should be paused during this process. As a result, the total time required to take a sample from a vegetated area may be as much as 30 minutes.

Maintain a current through the net whenever it is in the water by constantly moving it through the water. Whenever you stop moving the net, lift its opening out of the water. Take the mouth of the net out of the water when you are not moving it to prevent animals swimming out. Take the net completely out of the water when moving from one part of the sampling station to another. “Wash-back” from the net is a particular problem in standing waters; inserting a cobble in the net prior to sampling will help prevent this by effectively holding the net in place. Empty the net frequently to prevent sample material from blocking the mesh. If the net becomes too full or blocks the material should be emptied into the individual pre-labelled sample bucket or similar container which is used to transport the sample back to the laboratory.

The material retained in the pond net should be washed vigorously in the waterbody in order to produce a discrete “pellet” in the bottom of the net; this will enable efficient removal of the sample. Wash the collected material in the net into a labelled transport container. Examine the net for any attached invertebrates. If invertebrates are attached, these should be washed-off or manually removed and added to the material in the labelled transport container. Thoroughly wash any residual material from the net and ensure it is as clean as possible before leaving the site.

Do not collect samples during floods, i.e. when the water rises above the normal high water level. Give the invertebrates time to redistribute after a flood event. Ten days is sufficient.

Do not sample exposed sites on very windy days because the invertebrates will take refuge deep in the substrate (if it is stony) or elsewhere on the lake and be less likely to be captured. This is equivalent to sampling rivers in spates, which is avoided for the same reasons.

Biological Sampling Protocol: B) Stony Littoral Areas

The sample consists of material collected at the site by actively sampling for three minutes augmented by a one-minute search. The approach is similar but not the same as that prescribed for rivers (BT001, Murray-Bligh, 1999a), ponds and canals (Williams *et al*, 1998) or small lakes (Biggs *et al*, 2000). Stony substrates within the area are sampled with effort in proportion to their cover but macrophytes and fine sediment are avoided. In addition, animals captured during the three-minutes of kick sampling are stored separately from those captured during the search.

Before collecting a sample, examine the pond net closely to ensure it is free from holes or tears and any organisms from the previous sample. If necessary, replace the net.

The Search

The search is for individual specimens of species that are unlikely to be captured in the main sample. In contrast, for the main sample, the sampler concentrates on sampling particular habitats and collects whatever animals are there. The principles are the same as those described for rivers in BT001 (Murray-Bligh, 1999a) except macrophytes and fine sediment are avoided.

The search is divided into two parts:

1. a search for surface-dwellers before collecting the main sample; and

2. a search for animals attached to objects after collecting the main sample.

The proportions of the one-minute allocated to each of these parts will depend upon the nature of the site, notably the presence of substrates such as stones and submerged logs that may harbour attached animals.

Thoroughly examine the margins and pools within the sampling area for the presence of surface dwelling macroinvertebrates such as pond skaters, whirligig beetles and water measurers. These should be captured by scooping with the net before entering the water. Any disturbance will alert these animals to your presence; their evasive action may make them difficult or impossible to locate later during sampling. Store and mark this sample separately labelling with the words ‘substrate search’. The station is then actively kick sampled for three minutes.

Habitats ineffectively sampled during the three-minute sweep are subsequently searched. This involves manually washing or picking off specimens from, for example, boulders and logs. These animals are added to the sample marked ‘substrate search’. Specimens that adhere tightly to stones (e.g. freshwater limpets and leeches) can be removed manually and placed in a watertight specimen tube for identification in the laboratory. If used, the specimen tube should be included with the rest of ‘substrate search’ element of the sample.

The Three-Minute Kick

Allocate the three minutes of active sampling in proportion to the extent of the individual microhabitats present within sample station; avoid macrophytes and areas of fine sediment. The sample should be preserved separately from the search part of the sample. Label the container ‘kick’

To optimise the area of lake that is sampled, a zigzag sampling pattern is recommended, moving from shallow to deep to shallow and so forth. This will minimise any effects of water level fluctuation on the littoral benthic fauna. It is important to cover as much of the lakeshore as possible in the sampling time.

The pond net should be held firmly on the bed immediately behind your feet. Rigorously disturb the substrate with the toe and heel of your boot to dislodge the benthic fauna and pass the net through the area of disturbance. Where possible, seek to sample within the substrate matrix and not just at the surface. Catch the invertebrates that you have disturbed by passing the net through the plume of material disturbed by kicking. Allow most of the stones and sand to drop out of suspension but be quick enough not to let the animals do the same. Time spent puddling should not be included in the three minutes of sampling, as puddling constitutes sample processing rather than collection.

Maintain a current through the net whenever it is in the water by constantly moving it through the water. Whenever you stop moving the net, lift its opening out of the water. Take the mouth of the net out of the water when you are not moving it to prevent animals swimming out. Take the net completely out of the water when moving from one part of the sampling station to another. “Wash-back” from the net is a particular problem in standing waters; inserting a cobble in the net prior to sampling will help prevent this by effectively holding the net in place. Empty the net frequently to prevent sample material from blocking the mesh. If the net becomes too full or blocks the material should be emptied into the individual pre-labelled sample bucket or similar container which is used to transport the sample back to the laboratory.

The material retained in the pond net should be washed vigorously in the waterbody in order to produce a discrete “pellet” in the bottom of the net; this will enable efficient removal of the sample. Wash the collected material in the net into a labelled transport container. Examine the net for any attached invertebrates. If invertebrates are attached, these should be washed-off or manually removed and added to the material in the labelled transport container. Thoroughly wash any residual material from the net and ensure it is as clean as possible before leaving the site.

The sample can be examined in the field by placing the sample into a white photographic tray.

Do not collect samples during floods, i.e. when the water rises above the normal high water level. Give the invertebrates time to redistribute after a flood event. Ten days is sufficient.

Do not sample exposed sites on very windy days because the invertebrates will take refuge deep in the substrate (if it is stony) or elsewhere on the lake and be less likely to be captured. This is equivalent to sampling rivers in spates, which is avoided for the same reasons.

Sampling Seasons

Sampling should be undertaken over three seasons until sufficient information is available for this to be re-evaluated. The seasons are ‘spring’ (March, April and May), ‘summer’ (June, July and August) and ‘autumn’ (September, October and November).

Sample Labelling and Documentation

Follow BT001 (Murray-Bligh, 1999a) but, replace ‘site’ with ‘station’ and store and process the search and sweep or kick elements of samples separately. Label the search element of samples ‘vegetation search’ or ‘substrate search’ according to the method used. Label the net sweep element of the sample ‘sweep’ and the kick part of the sample ‘kick’ according to the method used.

Fixing / Preserving Samples

In line with the COSHH Risk Control Hierarchy, ‘best practice’ is to fix and / or preserve samples on return to the laboratory. Where this is not practicable, a Safe System of Work (SSoW) should be developed to cover adding chemicals on site and the transport of stock solutions and fixed and / or preserved samples in vehicles. As a result of local differences in the types and concentrations of chemicals and the types of sample pots used, a range of SSoWs will need to be developed at a local level.

Sample Analysis

Follow BT001 (Murray-Bligh, 1999a).

Quality Assurance

Follow BT001 (Murray-Bligh, 1999a) and BT003 (Murray-Bligh, 1999b).

Samples collected using the procedures described above should be included in AQC and audit schemes for standard river macroinvertebrate samples. However, laboratories unfamiliar with analysing lake samples should consider additional checking of analyses for a period until analysts are confident they can recognise all the new taxa that they encounter. If still water samples are sent to CEH for audit, it is important to indicate that the samples should subsequently be retained by CEH.

References – Method 1

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WFD Lake Sampling Field Data

Waterbody Identification Number[1]: Waterbody Name:
Date:

Vegetated Littoral Macroinvertebrate Sample

Site Name:		Site NGR:			
Time sample taken:		Sampler:			
Substrate Composition (%)[2]					
Silt/Clay (<0.06mm)		**Gravel/Pebble (2 – 64mm)**			
Sand (0.06 – 2mm)		**Cobbles/Boulders (>64mm)**			
Mesohabitat Composition (%)[3]					
Amphibious plants		Submerged fine-leaved macrophytes			
Emergent macrophytes		Filamentous algae			
Floating-leaved macrophytes (rooted)		Phytobenthos			
Submerged broad-leaved macrophytes		Leaf litter			
Distance from waterline to 75cm depth (m) ➡		**1.**	**2.**	**3.**	
Trashline to waterline vertical distance (m) ➡		**1.**	**2.**	**3.**	
Site aspect (º) ➡		**1.**	**2.**	**3.**	
Shading of site (%)[4]		**Site accessible to livestock (y/n)**			

Macrophyte species sampled (%)[5]		
1.	**2.**	**3.**
4.	**5.**	**6.**
7.	**8.**	**9.**
10.	**11.**	**12.**

Stony Littoral Macroinvertebrate Sample

Site Name:		Site NGR:		
Time sample taken:		Sampler:		
Substrate Composition (%)				
Silt/Clay (<0.06mm)		**Gravel/Pebble (2 – 64mm)**		
Sand (0.06 – 2mm)		**Cobbles/Boulders (>64mm)**		
Mesohabitat Composition (%)				
Amphibious plants		Submerged fine-leaved macrophytes		
Emergent macrophytes		Filamentous algae		
Floating-leaved macrophytes (rooted)		Phytobenthos		
Submerged broad-leaved macrophytes		Leaf litter		
Distance from waterline to 75cm depth (m) ➡		**1.**	**2.**	**3.**
Trashline to waterline vertical distance (m) ➡		**1.**	**2.**	**3.**
Site aspect (º) ➡		**1.**	**2.**	**3.**
Shading of site (%)		**Site accessible to livestock (y / n)**		

Phytobenthos Sample

Site Name:		Sample point NGR:	
Time sample taken:		Sampler:	
Substratum Sampled. If emergent macrophyte, name species.			
Cobbles / Boulders (✓):	**Emergent macrophyte:**		
Substrate Composition (%)			
Silt/Clay (<0.06mm)		**Gravel/Pebble (2 – 64mm)**	
Sand (0.06 – 2mm)		**Cobbles/Boulders (>64mm)**	
Trashline to waterline vertical distance (m) ➡		**1.**	
Water clarity (%)[6] ➡	Clear:	Cloudy:	Turbid:
If change in water level during previous month, estimate vertical distance (m):			
Other Photosynthetic Organisms (% and dominant taxa)			
Filamentous algae			
Other algae			
Bryophytes			
Higher plants			

WFD Lake Sampling Field Data

Waterbody Identification Number: Waterbody Name:
Date:

Additional Comments[7]

Field Data Completion Notes

Record the percentage areal coverage for each type. The total should equal 100%.

Record the percentage areal coverage for each type. The total does not need to equal 100%.

Unshaded = None, Light = <25%, Moderate = 25 - 50%, Heavy = >50%

Record the percentage areal cover for each species sampled. The total does not need to equal 100%.

Procedure for the collection of aquatic benthic macroinvertebrates from fine sediments of standing waters for ecological quality assessment

Draft prepared by Robin Guthrie
Revised M O'Hare

1. Health and safety

No attempt is made in this procedure to detail health and safety requirements. Specifications will vary between organisations and operatives must ensure that they are following the procedures specified by their organisation.

2. Scope

This procedure describes a method for the collection of aquatic benthic invertebrates from areas of fine sediment of lakes. The method described is particularly suitable for sampling the profundal zones of lakes.

The specification given is intentionally very restricted in scope in order to provide a repeatable and consistent methodology on which to base resource estimates and to ensure inter-comparability between surveys.

The methodology described is intended to provide a practical sampling methodology which will provide estimates of the densities of the commoner taxa of the profundal macroinvertebrate fauna of most types of lakes with a reasonable degree of precision, but without unduly excessive resource implications.

3. Principle

The methodology is derived from procedures described for sampling river invertebrates in deep rivers. (HMSO 1983.)

Benthic macroinvertebrates are collected from fine sediments by means of a miniature Van Veen Grab or Ekman type grab which is lowered from a boat on a rope or cable.

A fixed number of replicate sampling units per sample is specified. The number specified is ten. This is considered to be a number of replicates which will enable the capture of the most common elements of the fauna at a sampling location and enable estimations of abundance with reasonable precision.

4. Personnel

Typically a minimum of two operators will be required. These will require to be trained in boat handling according to the requirements of their organisation.

5. Equipment

The following is a list of basic sampling equipment, but must be supplemented by other appropriate equipment, especially health and safety equipment as laid down in organisations individual procedures. A power winch is especially useful and although its use is not mandatory when sampling deeper areas of water it should be seriously considered and the health and safety implications of not using one fully understood, i.e. back strain.

Boat
Miniature Van Veen Grab or Ekman type grab.
Rope or cable marked at 1 metre intervals.
Means for accurately determining position on lake e.g. GPS
Depth gauge e.g. echo sounder.
Buckets with lids or plastic bags large enough to fit over the jaws of the grab.
Sample analysis tray.
Waterproof labels.
Waterproof marker pen.
Notebook and pencil.

6. Location of sampling sites

When taking profundal samples, sites should be located on a flat plain of the profundal zone. The aim is to avoid sloping areas where the grabs will not function well. When lake bathymetry is not available locals' knowledge may help or alternatively one can aim for the deepest part of the lake (again if this is known) as it is likely to be on the profundal plain. Where lakes have more than one distinct basin, consideration should be given to sampling each basin separately, i.e. 10 replicates per basin.

Areas close to the littoral zone should also be avoided as a transitional fauna may be encountered.

7. Sampling procedure

The grab should be deployed from a stationary boat to ensure that the grab hits the bottom perpendicularly.

When sampling in shallower waters it may be possible to anchor the boat to ensure that the boat remains on station. In deeper water the boat will have to be kept on station using the engines or oars, as applicable. Checks should be made using the available positioning system and depth gauge to ensure that the boat remains as close on station as possible.

Ten replicate grabs are taken at each sampling site.

Van Veen Grab

The grab is cocked by drawing apart the arms of the grab and hooking the jaws open with the latch located on the bottom of one of the arms which hooks on to the other arm.

As soon as the hook is in place tension must be maintained on the lowering rope, otherwise the jaws will close prematurely.

The grab is lowered slowly to the bottom and when tension on the rope is released the jaws close. The sample is taken as the grab is raised. Particular care must be taken during the final stages of descent to ensure that the grab descends slowly. Too rapid a descent can

lead to a pressure wave below the descending grab which can cause the displacement of fine sediments.

The grab is raised to the surface and checked to ensure that the contents are intact. The contents are put into an appropriate bag or bucket. Alternatively the sample may be emptied into a sample analysis tray of appropriate size, inspected, and subsequently transferred to bag or bucket. This latter option reduces the risk of sample spillage.

Ekman Grab

The grab is cocked by drawing back the jaws and fixing them to the release mechanism.

The grab is lowered slowly to the bottom and the messenger weight is released to trigger the jaws. Particular care must be taken during the final stages of descent to ensure that the grab descends slowly. Too rapid a descent can lead to a pressure wave below the descending grab which can cause the displacement of fine sediments.

The grab is raised to the surface and checked to ensure that the contents are intact. The contents are put into an appropriate bag or bucket. Alternatively the sample may be emptied into a sample analysis tray of appropriate size, inspected, and subsequently transferred to bag or bucket. This latter option reduces the risk of sample spillage.

8. Sample handling

A waterproof label giving the following information is placed inside the sample bag or bucket:-

Name of waterbody
Date
Sampling site location
Replicate number
Sampler
Depth sample taken from
Any relevant observations on appearance/smell of sediment
Any other comments

A label duplicating this information should be attached to the outside of the bag/bucket. The field notebook should also be written up with the same information.

Samples should be returned to the laboratory for fixing with formaldehyde solution. The aim should be to achieve a 4%-6% concentration of formaldehyde in the sample. Operators must follow their organisation's health and safety requirements for handling formaldehyde. If molluscs are present in the sample they should be removed, when possible, before fixing in formaldehyde, as formaldehyde is acidic and can damage shells. They can then be stored separately in 70% IMS. If gastropods are present they are best killed by first immersing in deoxygenated water which allows the soft tissues to remain relaxed. Boiled water or carbonated water (commercial mineral water) are both deoxygenated. A jar or similar which can be filled to the brim with deoxygenated water is needed for this procedure. The procedure can take up to 12 hours.

9. Sample processing

Samples should be left in the formaldehyde fixative solution for at least twelve hours to allow adequate fixation. This will help minimise damage to specimens when they are elutriated.

After fixation samples are emptied into a 250 µm sieve and elutriated with a cold water spray, under fume extraction, to remove fine particles and all traces of fixative.

Individual replicates are processed separately.

Each replicate is placed with water in a white tray for picking out of specimens.

A maximum of three hours is usually adequate to elutriate and then pick out all the individuals in each replicate. The number of invertebrates in each replicate will typically vary between 0 for some replicates from oligotrophic lochs to 200 in eutrophic lochs. Higher numbers may be encountered, particularly in impacted conditions and it may be necessary to sub-sample.

To sub-sample remove the first 50 animals in proportion to their occurrence and then remove any additional taxa from the sample.

Specimens removed from the samples can be stored in preservative consisting of 70% industrial methylated spirits, 10% glycerol and 20 % water prior to identification.

10. Identification of specimens

Detailed procedures for the preparation of specimens for identification can be found in the various keys and identification references listed below.

Specimens should be identified to the lowest possible taxonomic level to which they can readily be ascribed.

Voucher specimens should be retained for each taxon.

11. Resource implications

Approximate times to take, process and identify samples are given below.

Sampling

- to take one set of ten replicates from a lake requires 2 operators for approximately 3 hours each. i.e. 6 hours per set of replicates.

Sample processing

- to elutriate and pick over each replicate takes approximately 3 hours

Identification of specimens

- the time taken to identify specimens from each replicate is extremely variable and is dependant on the composition of the fauna and experience of the operator. It is estimated that a set of ten replicates should take a maximum of 40 hours to identify.

Reporting etc.

- to compile data and report 2 hours

Total for one set of 10 replicates from one sample site - approximately 78 hours.

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Annex II: sampling programme to assess uncertainty in lake littoral sampling methodology

Objective

Quantify uncertainty for reporting to meet the needs of the Water Framework Directive

Focus

Acid Sensitive Lakes

Aim

To determine variation:

1. among operators
2. Within station
3. Among stations within a lake
4. Across two levels of pressure, high (3 lakes) and low (3 lakes)
5. Among replicate lakes

Sample Design

Number of lakes to be sampled in total: 6

Sampling method is 'Hard Substrate Method'

- The sampling should be carried out in a single season, either in spring or autumn.
- Within each lake there is a random (albeit constrained by logistics and access) selection of 3 'hard substrate method' stations, e.g. stations dominated by pebbly, gravel, cobble substrate
- Each station is sampled using the 'hard substrate' method
- Within each station two samples are collected by each operator A and B. (This means that with each lake six samples are collected by each A and B)
- Total samples per lake 3 stations x 4 samples = 12

	Calculations	**Totals**
Total samples for high pressure lakes	12 samples per lake x 3 lakes	36
Total samples for low pressure lakes	12 samples per lake x 3 lakes	36
Overall Total		72

Important

- It does not matter if the same or different teams are used across the six lakes
- An additional benefit can be had if the three lakes within low pressure are paired, in terms of e.g. size and altitude

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Environment Agency
Rio House
Waterside Drive, Aztec West
Almondsbury, Bristol BS32 4UD
Tel: 0870 8506506
Email: enquiries@environment-agency.gov.uk
www.environment-agency.gov.uk