Biological techniques of still water quality assessment

Workshop Briefing Pack

10th December 1997

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BIOLOGICAL TECHNIQUES OF STILL WATER QUALITY ASSESSMENT

PROJECT OUTLINE AND AIMS

The aim of EA R&D Project A(05) 94 is to develop still water biological quality assessment method(s) which can be used as the basis for a biological classification system for GQA and other reporting purposes.

The project has three phases:

Phase 1 (1995-1996) Scoping study

A Phase I scoping study, which was completed in 1996, and aimed to:

(i) consider broad approaches to biological assessment of still waters for use in General Quality Assessment and the establishment of Water Quality Objectives,

(ii) evaluate the existing range of biological assessment methods used for monitoring still waters,

(iii) recommend a still water monitoring method, or methods, for further evaluation and testing in Phase 2 and 3.

Phase 2 (1997-1998) Project development and testing (I)

Phase 2, which is currently underway, involves:

(i) trialling multimetric assessment methods in two still waterbody types (canals and ponds) using macroinvertebrate and aquatic plant assemblages for quality assessment,

(ii) investigating the potential of (i) other biotic assemblages (fish and diatoms) for use in multimetric testing (ii) other uses of multimetrics, such as use for Biodiversity Action Plan monitoring,

(iii) further evaluation of methods which can be used for diagnosing environmental degradation.

Phase 3 (1998-1999) Project development and testing (II)

Phase 3, which starts in 1988, will include further development and testing of the multimetric method. Details have yet to be finalised.

PROJECT PHASE 1: DEVELOPMENT OF A STILL WATER MONITORING METHOD

General approaches to monitoring: a rationale

Evaluation of EA requirements for a GQA monitoring method shows a need for biological methods which can provide two different types of output. The main requirement is for a *general ecosystem quality* assessment method which will evaluate the *overall* condition of the waterbody. However, there is also a need for methods with a *diagnostic potential* to enable the causes of ecosystem degradation to be determined.

The biotic characteristics required to achieve these two end-points are very different. The ultimate aim of any *general ecosystem assessment* method is to encapsulate and summarise the overall quality of the ecosystem. Methods which are likely to be effective in doing this will almost invariably need to be broadly based in terms of the taxa and attributes that are measured. Suitable taxa for monitoring are likely to be major assemblages which span a number of trophic levels, occupy a variety of waterbody habitats and include taxa which are long-lived, so that they can provide a temporally and spatially integrated measure of the current ecosystem state.

In contrast, diagnostic methods must single out causes rather than integrate them and are, typically, reductionist rather than broadly applicable. Ideal techniques are therefore more likely to be based on a limited range of indicator species or taxa, or on individual attributes which show a strong and discriminatory relationship with a particular stress. Because of the wide range of potential impacts and the required specificity of indicators, it follows that no one diagnosis indicator or method is likely to be applicable in all situations. Thus, in any water quality assessment programme, there will be a need for an array of complementary indicators that can be flexibly tailored to help diagnose the source(s) of degradation. These may be biologically based but other complementary approaches (desk studies of historical data, hydrological investigations or chemical monitoring) are likely to be equally relevant.

Combining assessment methods: a protocol

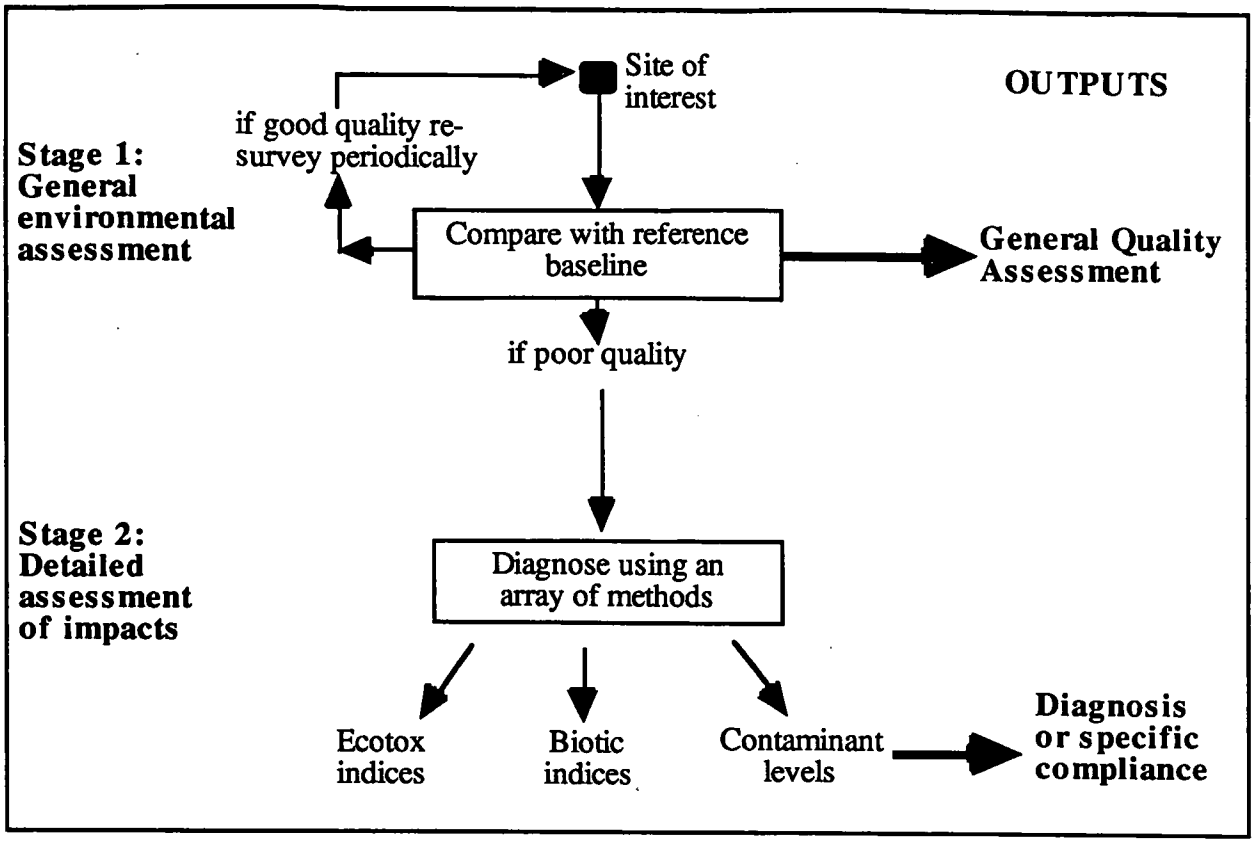
Trying to combine general ecosystem assessment and diagnosis into a single method is likely to compromise the effectiveness of both. So, where both general ecosystem assessment and diagnosis is required at a site, a rational approach is to consider these different processes as part of a two-stage protocol:

Stage 1 *General ecosystem assessment*, which evaluates the net effect of all forms of degradation,

Stage 2 *Diagnosis*, a more detailed follow-up investigation, used where damage is evident, and employing one or more of an array of appropriate techniques.

Separating out the different functions of biological techniques does not preclude re-analysis of data to fulfil more than one role however. Thus, there is potential for data already collected for general ecosystem assessment, to be analysed separately, providing additional and independent indices relevant to specific impacts (eutrophication, biocides, acidification, habitat damage etc.). This double use of data has the potential to provide a cost-effective method of monitoring and assessing water quality.

Figure 1 Biological assessment techniques: a framework



Theoretical framework for a GQA monitoring method

The essential requirement for the development of a GQA method is, as discussed above, that it should represent and summarise the overall existing biological quality and integrity of a water body. Biological integrity is a wide-ranging concept, so more accurate measures of integrity are likely to be derived where a number of biodiversity elements (e.g. genes, species) and attributes (e.g. taxa richness, trophic structure) are used for assessment.

To assess *all* aspects of biological integrity is not an economically viable option. A narrower range of (i) groups (taxa) or (ii) attributes (species-richness, rarity etc.) needs to be selected which, however, *still represents* the overall integrity of the system.

Narrowing down the range of taxa to use in assessments

Overall, it is clear that, given the range of stressors with the potential to affect waterbodies, monitoring of a wide range of taxa will normally be required to detect these impacts. It is therefore possible that even a major assemblage (macroinvertebrates, macrophytes etc.) will not have the capacity to adequately represent all ecosystem stresses.

Choosing the assemblages most suitable for water quality monitoring involves evaluation of many variables; scientific, practical and economic. Matrix analysis provides a means of rationalising and quantifying such variables. This approach was taken, in the scoping study, to determine the relative merits of each assemblage type and to identify the best combination of assemblages to use for evaluating ecosystem integrity (see later sections).

Cutting down the attributes

The most important characteristic of useful attributes (richness, rarity etc.) is that they are direct correlates of ecosystem degradation and can, therefore, be used to clearly discriminate between sites of differing water quality. The list of attributes which could potentially be used is extensive. Refining the choice of 'best attributes' is made easier by information from a wide range of empirical studies world-wide, which suggests that many attributes (e.g. taxa richness, percentage of exotics, proportion of functional feeding groups, ratio of predators/herbivores and rarity etc.) show consistent relationships with degradation gradients.

Empirical studies can, however, only provide us with a set of likely candidates - a rough indication of the range of attributes which should be investigated further. For practical monitoring of any waterbody type or any assemblage, knowledge of the attributes which will prove most useful in tracking degradation, can only be derived from field data gathered from the region of interest.

Thus, although choice of 'best taxa' may be rationalised from a knowledge of assemblage characteristics, determining best ways to measure those taxa will ultimately rely on the collection and analysis of field data. In principle, the attributes initially investigated in field trials should be as extensive as possible, spanning a wide range of community features and interactions (e.g. species/family richness, wet weight, disease, proportion of sensitive taxa).

Currently, most European monitoring methods use a relatively restricted set of biotic measures for water quality assessment - typically, diversity, relative abundance or taxa richness. There is, however, the potential to use a much wider range of attributes. Thus, even from simple taxa lists it would be possible to derive measures such as proportion of functional feeding groups, ratio of predators/herbivores, rarity etc. Such measures, if proven relevant in analysis, have the potential to considerably broaden assessments of ecosystem integrity with little extra resource requirement.

Establishing a baseline reference condition

A central question in the development of any general biological assessment method is: 'how is good biological water quality to be defined?' What bench marks or information do we use for comparison, and what are acceptable and unacceptable deviations from those bench marks?

It is now widely accepted by ecologists, and by an increasing number of regulatory authorities (e.g. the EU and US EPA), that physical, chemical and biological conditions in all waterbodies should, where possible *'...resemble those of similar waterbodies with insignificant anthropogenic disturbance'* (CEU 1994).

Although this is a simple concept, in practice there are at least five different ways in which reference condition can be defined (see Annexe 2):

(1) Comparisons with the best available present-day reference sites,
(2) Reconstruction of waterbody histories using paleolimnological techniques,
(3) Modelling approaches (including hindcasting),
(4) Historical data,
(5) Professional consensus.

For biological data in general, the most viable choice for that baseline is comparison with 'least impacted' present-day sites.

Classification

Still waters vary naturally in their physical, chemical and biological characteristics. Classification of baseline reference sites, and subsequent comparison of impaired sites within the framework of a classification is therefore an essential part of general ecosystem assessment. Their use both minimises the confounding effects of natural variation and allows degradation gradients to be identified more easily.

Putting a method together: the US concept of multimetrics

Desk studies of methods used for water quality assessment show that the concept of *multimetric assessment of ecological integrity*, now used routinely in the United States has many similarities with the theoretical framework for an EA monitoring method outlined above.

Using this system, ecosystem integrity is assessed on the basis of multiple attributes ('metrics') which are known to be related to ecosystem degradation. Each attribute (i.e. factors such as taxa richness, percentage functional feeding groups, health etc.) is scored separately according to the extent to which it deviates from an undisturbed baseline condition. Metrics are then divided into simple 'rating' (e.g. 1-5) categories and summed to give a single index.

The principle benefit of this system is that it enables a wide range of ecosystem measures to be combined in a single index. This considerably increases the potential for biological assessments to represent the integrity of the community as a whole. In addition, multimetric indices are very flexible in that new metrics can be added at any stage without undermining the entire concept.

The main shortcoming of the multimetric approach as practised in the US, is that the classification groups used for any water body type are arrived at subjectively (although within the context of natural regions).

The more advanced multivariate statistical techniques now routinely used in Britain (e.g. the RIVPACS methodology) have not yet been applied in the United States. Uniting the two approaches has the potential to give the best of both worlds.

Summary of the approach

In summary the recommended approach involves adoption of a multimetric method of water quality monitoring based on assessment of biotic integrity and using a range of assemblage attributes. This approach involves four main steps:

1. Comparing selected biotic assemblages with least impacted present-day reference assemblages predicted using multivariate techniques.
2. Assessing the extent to which biotic assemblages deviate from the reference state using a variety of metrics (e.g. taxon richness, percentage sensitive groups, functional feeding groups).

   Together these metrics aim to summarise the integrity of the freshwater system.
3. Normalising metric data against the baseline and dividing it into simple scoring categories (1 = very poor to 5 = good).
4. Combining individual metric values to give a site integrity score. This score provides the basis for water quality assessment.

Details of the steps involved in method development and testing are described in Annexe 1.

Advantages of the scheme

The multimetric assessment approach proposed should fulfil most major EA operational and policy requirements for a biological assessment method for use in still waters. In particular:

1. The scheme is flexible, it can be applied across any region or area and adapted for use on any still waterbody type.
2. The wide range of parameters used to assess water quality can be summed, without loss of information, to give a single score which can form the basis for GQA assessment and the establishment of Water Quality Objectives.
3. The method is founded on principles of biodiversity and sustainability, so the scheme addresses the EA's pollution monitoring responsibilities, its general duty to have regard to the conservation of aquatic flora and fauna and its shared responsibility for Biodiversity Action Plans.
4. In terms of legislative requirements, the methodology can be applied to fulfil the biotic components of the draft Ecological Quality of Water Directive, including the requirement for comparisons with minimally impacted baseline conditions.

The objective of the method proposed, is to assesses the *overall* condition of freshwater ecosystems. The system does not, in itself, aim to provide a diagnosis of the cause of degradation. Indeed it is considered inappropriate for a general quality assessment method to be biased towards evaluation of a single or small number of pollutant impacts. However, there is considerable potential for data which is collected using this scheme to be re-interpreted to diagnose the causes of degradation. This may be achieved both by inspection of individual metrics which make up the total integrity score, or by reanalyses to give pollution indices, such as trophic scores or acidification indices.

Re-use of data in this way, to provide information which will fulfil multiple end points, has the potential to make the scheme highly cost-effective. In addition, the method can be built up incrementally, minimising risk and initial costs in method development.

More detail: Choosing which taxa to uses in multimetric assessments

Identifying the assemblages most suitable for water quality monitoring involves evaluation of many, sometimes conflicting, variables. Matrix analysis was used to facilitate objective comparisons of the major taxonomic assemblages (phytoplankton, periphyton, marginal macrophytes, submerged aquatic macrophytes, microinvertebrates, macroinvertebrates, fish, amphibians, birds and mammals). Separate matrices were completed for each of the main waterbody types (lakes, ponds, temporary ponds, ditches, canals, brackish waters).

Within the matrix analysis biotic assemblages were assessed in three general areas (Annexe 4):

1. The ecological relevance of the group: including (i) the extent to which the group is representative of overall biodiversity; and (ii) how well each assemblage is likely to respond to, and integrate, the wide range of anthropogenic stresses which may affect waterbody integrity.

2. The practical suitability of the group: including questions relating to 'catchability' i.e. the abundance of individuals in waterbody types, and consideration of whether taxa are *naturally* found in all physico-chemical variants of each waterbody type.

3. The cost of collecting and analysing data for each assemblage: including (i) the cost of equipment and consumables (ii) the time required to undertake field surveys, laboratory work and data analysis, and (iii) the time required to train staff to become proficient in the use of methods.

Evaluation of ecological relevance and practical suitability was undertaken using a simple ranking system on a five-point scale (e.g. 0 = very poor to 4= very good). Costs were estimated and entered in monetary terms, and were therefore assessed independently.

Matrix results

The results of matrix analysis indicate that there are several candidate assemblages which are likely to be valuable in water quality and integrity assessment (Table 1). However, no one assemblage is able to fully represent all aspects of biotic integrity and to integrate the effects of all possible stresses. In general, the reliability and validity of assessments would therefore be enhanced by use of two biological assemblages. For lakes, which are both large waterbodies and virtually prohibitively difficult to restore once degraded, monitoring on the basis of at least two biotic assemblages is recommended.

In general, the best combination of two taxonomic groups in most waters is likely to be: (i) a faunal assemblage - preferably invertebrates, but possibly fish in permanent waters and (ii) a floral assemblage - either aquatic macrophytes or diatoms. Together these groups span a complimentary range of trophic levels, habitats and pollutant sensitivities and can effectively represent the integrity of the ecosystem. The assemblages specifically recommended as a basis for monitoring in each waterbody type are:

Lakes	Macroinvertebrates	+	Aquatic macrophytes (Diatoms + Fish)[1]
Ponds	Macroinvertebrates	+	Aquatic macrophytes (or Diatoms)
Canals	Macroinvertebrates	+	(Diatoms or Fish)
Ditches	Macroinvertebrates	+	Aquatic macrophytes (or Diatoms)
Temporary waters	(Macroinvertebrates, Microinvertebrates, Macrophytes, Diatoms)		
Brackish waters	(Macroinvertebrates, Microinvertebrates, Macrophytes, Diatoms)		

In practice, of these assemblages, only macroinvertebrate communities could be considered to be an 'ideal' assessment group. Macrophytes are considered to be sub-optimal because their use

[1] Assemblages in parenthesis are those for which methodological viability had not been fully established.

is limited by poor temporal characteristics and the paucity of species found in naturally shallow, turbid and shaded waterbodies.

Periphyton (particularly diatoms) and fish are both promising assemblages for assessing biotic integrity, but both require further investigation to ensure their practical viability.

Brackish waters and temporary waters are inherently species-poor habitats. This combined with the paucity of information regarding their communities and impact sensitivity, makes it difficult to predict which (or how many) assemblages will have sufficient resolution to enable waterbody degradation to be adequately assessed.

It is clear that the groups recommended above vary considerably in their potential for immediate development and testing. Thus macroinvertebrate assemblages could be rapidly applied as a basis for pond or ditch assessment. In contrast, a diatom-based assessment would require a more prolonged set-up period during which the potential of the group is evaluated.

Table 1 Summary of taxonomic groups with the highest matrix scores based on ecological viability and practical relevance

	Lakes	Canals	Ditches	Ponds	Temporary ponds	Brackish waters
Phytoplankton		-	-	-	-	-
Periphyton	[*]	[*]	*	[*]	*	*
Aquatic macrophytes	*	[*]	[*]	[*]	-	-
Emergent macrophytes,	-	-	-	-	*	-
Microinvertebrates	[*]	[*]	*	[*]	*	[*]
Macroinvertebrates	*	*	*	*	*	*
Fish	*	*	[*]	-	-	-
Amphibians	-	-	-	-	-	-
Birds	-	-	-	-	-	-
Mammals	-	-	-	-	-	-

* = within the top 25% of the range of matrix scores

[*] = borderline i.e. within top 30% of the range of matrix scores

PHASE 2: METHOD DEVELOPMENT AND TESTING

Based on Phase 1 scoping recommendation a multi-track approach was adopted to further methodological development of the multimetric method. Phase 2 tracks included:

Track 1. Multimetric testing and development:

Testing and development of a multimetric method based on macroinvertebrate assemblages of two still water body types (ponds, canals) using a regional data set.

Track 2. Investigate the viability of other assemblages:

(i) investigation of the potential of diatom communities for application as a floral assemblage to assess the quality of lakes, ponds, canals, ditches.

(ii) use desk study information to investigate the potential for fish metrics to be developed for use in lakes and canals.

Track 3. Investigate diagnosis methods

Evaluation of the most promising methods for diagnosing environmental degradation identified by general quality assessment.

The following sections of the briefing pack focus on Track 1 development of the multi-metric method for canals and ponds.

Testing the multimetric method on canal and pond data-sets

SURVEY REGIONS

Ponds

The regional survey area used in the study comprised a broad transect (approximately 300 x 110 km) extending from Kent to mid-Wales. This area represents approximately 20% of the land area of England and Wales, is geologically varied and includes considerable topographic range (20 m - 500 m asl). Land areas in the transect area are impacted by a range of the main degradation factors affecting still waters, including eutrophication, acidification, biocide pollution, hydrological stress and urbanisation effects.

Canals

The choice of the canal survey region was constrained by their existing distribution. In order to gain a good geological and land-use spread for the canal survey, the survey region was larger than for ponds and covered an area of about 250 x 200 km, extending from Surrey in the south to the Cheshire Plain.

The survey included: the main Midland canals (e.g. Grand Union, Oxford Canal), which are impacted by a range of agricultural, urban and industrial impacts, together with a number of operational rural systems (e.g. Kennet and Avon) with moderate boat traffic and good water quality (e.g. Llangollen Branch of the Shropshire Union) which are considered of high nature conservation interest.

Baseline reference sites

Ponds

The Phase I Scoping Study recommended that characterisation of reference conditions for ponds should use minimally impacted present day reference sites.

Pond survey data utilised ponds from Pond Action's existing National Pond Survey (NPS) data base. The NPS data set is based upon minimally impaired present day reference sites located in areas of semi-natural land use.

Canals

For canals (which are man-made freshwater systems with a specific societal purpose), a combination of minimally impacted sites and professional consensus techniques were deemed more appropriate.

We proposed that the 'unimpaired' canal reference sites should be based on the concept of 'appropriate waterbody conditions' and that ideally this should be defined as canal sites which have: (i) good chemical water quality e.g. GQA Chemical Class A or B (ii) a semi-natural bank structure and (iii) 'moderate' boat traffic. This was modified after consultation with the EA Project Board and British Waterways to include a number of sites with little or no boat traffic and a number of replicate sites which sampled contrasting bank type.

Summary of data sets used

Canals

The canal data set included macroinvertebrate and physico-chemical data collected from 70 canal sites in the survey area. This included 12 replicate samples taken from sites of contrasting bank type (reinforced and semi-natural). In total 30 canal samples were used to form the 'minimally impaired' baseline classification. Fifty-two variably impacted and degraded sites were used for metric development. Additional use was made of EA water chemistry data, together with British Waterways information on sediment quality, water flow, dredging records and boat-movements.

Ponds

Pond survey data used c.100 ponds from Pond Action's existing National Pond Survey (NPS) and ROPA data bases. This included:

- Species level macroinvertebrate and macrophyte data, plus physico-chemical data.
- Approximately equal number of undisturbed and variably impacted sites.

Compatible field data were also collected from an additional 20-30 ponds as part of the project. These ponds were strategically located to ensure representative coverage of all geologies and ITE land classes within the survey region.

New field data were collected for a selection of smaller lakes included in Lake Classification R&D project.

BIOTIC METHODOLOGY: MACROINVERTEBRATES

The invertebrate survey methods used for the study were based on standard 3 minute hand-net sampling methods. Samples were undertaken in the summer season (June and July). The main features were:

Ponds and small lakes

- 3 minute habitat *type* related sampling (not habitat *area* related). 180 seconds divided equally amongst the main mesohabitats present,
- exhaustive live laboratory sorting,
- identification and enumeration of specimens at levels shown below.

Canals

Canals are steep sided and relatively deep waterbodies, so the area-related hand-net sampling methodologies appropriate for rivers (e.g. typical RIVPACS sampling) cannot be directly applied to canals. In particular: (i) in canals most species are concentrated in a very thin band at the edge so that an area-based sampling method would considerably under-sample invertebrate diversity, (ii) hand-net methods are difficult to apply to the deepest open water areas of canals.

Discussion with EA biologists suggested that the methodology currently used to take canal samples was modified from the 3-minute hand-net river techniques. However, no formal adaptation of the river methodology has been made. Similarly, although IFE had a project to survey canal invertebrate communities, in April 1997 when sampling for the current project began, they had not yet developed a specific sampling methodology.

In the absence of existing methods we developed a standard canal survey technique based on a hybrid between the '3 minute hand-net sample' currently used for sampling shallow rivers, and the '1 minute hand-net sample + dredge hauls' method which IFE recommend for sampling deep rivers. The method comprised:

1. A two minute semi-continuous hand-net sampling of the canal margin, shallows and any emergent or aquatic plant habitats present.
2. Four net or dredge hauls of deeper bottom sediments, elutriated on site to wash out the bulk of muds and fine sands.
3. A brief (1 minute) additional search.

For both practical and information purposes, samples from the margins and deep bottom samples were retained and sorted separately.

Table 2 Macroinvertebrate taxa included in canal and pond surveys.

Taxa	*ID level*	*Taxa*	*ID level*
Tricladida	Species	Hemiptera	Species
Gastropoda	Species	Coleoptera	Species
Bivalvia	Species	Plecoptera	Species
Crustacea (Malacostraca)	Species	Lepidoptera	Species
Hirudinea	Species	Trichoptera	Species
Ephemeroptera	Species	Oligochaeta	Class[1]
Odonata	Species	Diptera	Family[1]
Megaloptera (inc. spongeflies)	Species		

[1]Groups retained for identification to lowest practical level if necessary at later stage

BIOTIC METHODOLOGY: MACROPHYTES

Aquatic and marginal macrophytes were surveyed at all ponds, but were not specifically included in the canal assessment, where diatoms are the preferred plant assemblage. The principle survey method employed was walking or wading along the waterbody shoreline. Deeper water areas were sampled either by grapnel thrown from shallow water or from a boat. A standard wetland plant species list was used to aid searching in the field and provide the basis for species richness estimates. Vegetation abundance was recorded as percentage cover for each species.

BIOTIC METHODOLOGY AND SURVEY: DIATOMS

Diatom methodology development

The Phase I Scoping Study identified diatoms as a promising assemblage for water quality assessment. However, sampling method development was required before their suitability could be properly evaluated.

To progress the use of diatoms as a water quality assessment method in still waters, an EA workshop was held at UCL in April 1997. The proposed methodology for ponds, and a preliminary method for canal surveys, which were outputs of the workshop, are summarised in Annex 7.

Diatom survey data trial

The diatom survey methodology was trialled in 92 survey ponds for which macrophyte, macroinvertebrate and physiochemical data was already held. When analysed, the diatom data set has the potential to be used to (i) create diatom-based multimetrics, and (ii) look at the merits of macrophytes and diatoms as alternative plant assemblages for water quality monitoring.

BIOTIC METHODOLOGY AND SURVEY: FISH

In the Scoping Study fish were identified as a promising biotic assemblage for inclusion in multimetric assessments of lakes and canals. The report noted, however, that although fish assessment methods are well developed, fisheries monitoring programmes have been largely undertaken for fish stock assessments rather than as a method for evaluating water quality. The Scoping Study therefore recommended that the potential for development of fish metrics using existing fisheries data should be further evaluated.

Discussion with regional fisheries staff, as part of the Phase 2 project indicate that the EA currently undertakes a range of fisheries surveys in still waters: approximately 100 lakes per year are surveyed, and routine fish surveys are undertaken for canals in Severn Trent and Anglian regions. The parameters measured (and collection methods) vary between regions but include: species richness, species composition, biomass, disease, age and growth rates.

In theory these data should allow potential metrics to be developed based on factors such as species richness, relative abundance, proportion of tolerant/intolerant species, growth rates and health. However, the major ecological disadvantage of fish as a quality indicator is that their populations are often strongly influenced by stocking and fisheries management. In practice, this makes it difficult to identify natural community baselines with which other sites can be compared. A proposed compromise would be to use a mix of indicator species (e.g. salmonid spp.) metrics where relevant, and non-species based metrics such as biomass, growth rates and parasite loads.

PHYSICO-CHEMICAL PARAMETERS

Selection of physical and chemical variables to be measured

Physical and chemical data from the canals and ponds were collected for the project in order to:

1. Form the basis of biotic assemblage predictions developed with reference to minimally impaired baseline sites (cf. RIVPACS).
2. Assist in the derivation of viable metrics based on physico-chemical impairment.

Where possible, physico-chemical variables were chosen so as to maximise similarity with RIVPACS and the EA Lakes project.

A summary of the variables measured is given in Annex 6.

Multimetric biological assessment using an Index of Biotic Integrity

The main analytical stages in the development of a predictive multimetric Index of Biotic Integrity (IBI) for standing waters are outlined below. The example is developed with reference to canal macroinvertebrate assemblages, but all the main points also apply to pond aquatic macrophyte and macroinvertebrate assemblages.

Development of the method is best seen in two main stages:

(i) development of fauna/flora prediction techniques

(ii) development of the Index of Biotic Integrity.

(i) Development of methods to predict the minimally impaired baseline assemblage

The baseline assemblage prediction method that was used was essentially the same as that used for RIVPACS, with only minor methodological differences.

Classification of the unimpaired baseline sites on the basis of their macroinvertebrate assemblages using TWINSPAN

Classification of the unimpaired baseline sites, on the basis of their macroinvertebrate assemblages, was undertaken using TWINSPAN.

Canal sites for the 'minimally impaired' baseline classification were primarily in terms of water quality (e.g. BOD, suspended solids, nutrients, heavy metals). We also included some sites with moderate boat traffic and vertical banks. The decision as to what the term 'degradation' means in canals (should heavy boat traffic be seen as degradation for example), is ultimately a professional consensus decision, which needs to be taken by the EA in consultation with BW and others. Minimally impaired canal sites were drawn from the following canals: Oxford, Kennet & Avon, Ashby, Grantham, Brecon, Shropshire, Grand Union and Taunton.

The canal classification mainly used species-level macroinvertebrate data (with abundance categories), with some taxa at higher levels (i.e. Oligochaeta, Chironomidae, other Diptera), (see Table 2). The classification was based on a total of 179 taxa, with TWINSPAN run in its abundance categories mode. Annexe 8 shows the canal TWINSPAN endgroups.

Prediction of TWINSPAN endgroup membership using MDA (Multiple Discriminant Analysis)

MDA (Multiple Discriminant Analysis) was used to identify environmental variables that could predict the TWINSPAN endgroup (or groups) a given site should fall into. Most sites have one TWINSPAN group they are most likely to fall into, followed by several others for which they have a lower probability of membership.

MDA works by deriving a series of discriminant functions to predict the TWINSPAN group to which a site belongs. Real environmental data are then substituted into the discriminant functions to predict the TWINSPAN group membership of individual sites. Preliminary assessments of the success of MDA was made by 'backpredicting' the TWINSPAN endgroup of the sites used to derive the original TWINSPAN classification, and comparing the prediction with the original TWINSPAN classification. In the present study, around 90% agreement was obtained for the canal sites between TWINSPAN and the MDA prediction. For ponds the agreement was a minimum of 70% - 80%. Similar levels of agreement were seen at the equivalent stages in the development of RIVPACS (see Annexes 9a, 9b, 9c). As the same sites are used for the prediction and the original classification a relatively high level of success is expected. In subsequent stages of the project (e.g. Phase 3) a second, and more robust, test of

the MDA functions will be required using a new set of test sites which have not been previously classified.

Prediction of fauna

Knowing which TWINSPAN endgroup(s) a site is predicted to belong to, and knowing the typical species composition of each endgroup (in terms of the proportion of sites in which individual species occur in that group), the fauna of the site can be predicted.

For each species *i*, the expected probability p_i of occurrence at a new site is estimated by:

$$p_i = \Sigma\, G_j S_{ij}$$

where G_j is the probability of the new site belonging to a particular TWINSPAN endgroup, and S_i is the proportion of reference sites in group *j* with species i[1].

Annexe 10 shows an example of a predicted and observed species list for an Oxford Canal site.

(ii) Development of a trial Index of Biotic Integrity (IBI)

Identification and testing of potential metrics

Identification of trial metrics

Trial metrics were identified from the literature and from general ecological principles. Any metric which has been shown to have a measurable relationship with environmental degradation can potentially be included in an IBI, provided that a baseline value can be calculated.

Metrics have initially been confined to those which could be predicted from the species lists generated by the TWINSPAN/MDA prediction of fauna. Three main classes of potential metric were tested: species richness metrics, intolerant species metrics and trophic structure metrics.

Annexes 11-13 lists viable metrics for canal invertebrates, pond aquatic macrophytes and pond invertebrates respectively. Each of the metrics listed were significantly correlated with environmental degradation (e.g. heavy metal pollution, BOD, nutrient concentrations, aquatic vegetation loss, boat traffic, bank structure) at $p<0.001$ (Spearman rank correlation). Approximately three times this number of metrics were explored but either had less significant relationships, or did not significantly correlate with degradation factors. Note that invertebrate metrics are at a variety of taxonomic levels.

In ponds, identification of viable metrics to use in a biotic integrity assessment is relatively straightforward based on correlations with catchment degradation and pollutants. Choice of the most appropriate *canal* metrics is, however, complicated by the potential to include or exclude anthropogenic impacts such as bank structure. For example if the main aim of canal assessments is to look at water quality, then metrics based on EPT taxa would be most effective (see Annexe 11). If boat traffic and hard bank structure are seen as environmental degradation, then parameters based on taxon richness or bug and beetle species richness would be included in the IBI.

Potential metrics excluded

Note that ratios (e.g. ratio of crustacea to beetles) were excluded from the analysis to avoid use of metrics with quantities which vary together. It is better to consider the two quantities separately (Jim Karr pers. comm.). Diversity indices (e.g. Shannon-Weiner index) were also excluded as they are now widely accepted to have little biological basis.

[1] Clarke, R.T., Furse, M.T., Wright, J.F and Moss, D. (1996). Derivation of a biological quality index for river sites: comparison of the observed with the expected fauna. *Journal of Applied Statistics*, 23, 311-332.

Further rationalisation of metrics

Further rationalisation to choose the most effective of the viable metrics to use in an IBI needs to be undertaken by balancing a number of concepts. It is important to choose metrics which respond to a very wide range of degradation gradients and it is also valuable to include metrics which have some diagnostic potential. Using metrics which reinforce each other gives confidence that the degradation assessment is correct but equally it is important to avoid too much redundancy, so that a degradation signal indicated by only one metric is not lost in the final IBI calculation.

Calculation of the IBI

Using the predicted species list, derived from the TWINSPAN/MDA, the predicted metric values (e.g. species richness, %EPT, damselfly richness) are calculated for the sites of interest.

The predicted values are compared with the observed values of the metrics for the site. Sites which are minimally impaired will show no significant deviations from the baseline values. Metrics are transformed to a 1 to 5 scale to enable them to be compared.

Biological techniques of Still Water assessment: Phase 3 Options

Possible future directions

Further development of the multimetric method could be taken in one or more of five major areas. The main options are:

- Undertake further development and testing of data sets within the existing survey area
  This could include:
  (i) validating the existing canal and pond classifications using species-level data.
  (ii) trialling the multi-metric method using additional canal and pond data, potentially using data collected by EA biologists.
  (iii) developing a 'front-end' for the program to enable site quality predictions to be made by staff at regional level.
- Extend the survey *area* for ponds and canals to cover all of England and Wales.
- Extend the survey *season* for ponds and canals.
- Extend the *assemblage types* used for existing waterbody types, particularly trialling of diatoms in canals.
- Develop the method for *other* still waterbody types (lakes, ditches, temporary brackish waters).

Further discussion of each of these options is given below.

Option 1 Undertake further development and testing of data sets within the existing survey area

There are a number of possible directions here:

(i) Validate the existing canal and pond classifications using species-level data from additional sites in the regional survey area.

(ii) Trial the multi-metric method using additional canal and pond data.
It would be particularly useful to have the method trialled by EA biologists, in order to (a) get feed-back on the field methodology and (b) provide data which could be used, in conjunction with EA water chemistry, to investigate the validity of the results. This would require:

- collection of hand-net (+dredge) invertebrate samples identified to family or species level.
- recording of a limited number of environmental variables (bank type, secchi depth etc.).

(iii) developing a 'front-end' for the program to enable site quality predictions to be made by staff at regional level.

By the end of the Phase 2 project, it will be possible to make preliminary biotic assessments of canal and pond quality in the relevant survey regions. By adding a front-end to the model it would be possible for regional staff to try the methods for themselves.

Option 2 Extend the survey *area* for ponds and canals

The existing data sets for canals and ponds are based on a limited survey area covering approximately 20% of the land area of England and Wales. Extension of the survey area has clear advantages for making the method most widely applicable for EA monitoring. For ponds this would be a relatively cost effective option since, by 1998, Pond Action will hold macroinvertebrate and plant data from ca. 300 baseline and impacted ponds across England and Wales.

Option 3 Extend the survey *season* for ponds and canals

The current canal and pond data sets were gathered in a single season (spring and summer respectively). It would be difficult to justify use of the survey methodology far outside these index periods; further samples taken in other seasons would, therefore, help to broaden the temporal viability of the method.

Option 4 Extend the assemblage types used for existing waterbody types

Multi-metric assessment for canals is currently based only on macroinvertebrate data. It would be useful to have plant-based biotic information to widen the scope of integrity assessment. Diatoms are likely to be the most appropriate plant assemblage to use, and since a diatom sampling methodology has recently been developed, it would be relatively simple to gather a data set from the spring 1997 canal survey sites. The main drawback to diatom method development is likely to be the cost of sample analysis, which could be considerable.

Option 5 Develop the method for other still waterbody types

The legislative requirements proposed in the current draft of the Water Framework Directive includes a requirement for 'representative' monitoring of still waters. This suggests a need for biotic assessment methods which can be used to monitor a wide range of waterbody types. Having initiated survey methods for ponds and canals, there is an argument for extending the methods to the other major still waterbody types including lakes, temporary ponds, ditches and brackish waters.

Lakes

Lakes are a strong candidate for biotic method development, partly because they have a high biodiversity value, and partly because, once impacted, lakes are prohibitively difficult to restore. There is therefore a considerable imperative for lake bio-monitoring to ensure their protection. Lake quality assessment is currently being addressed through the EA Lake Classification project. Biotic methods would provide a useful compliment to this essentially chemical approach to lake monitoring.

The main obstacle to developing a biotic assessment method for lakes is that standard sampling methodologies are poorly defined for most recommended survey taxa (invertebrates, fish, diatoms, macrophytes). For example: are littoral macroinvertebrate samples appropriate or should sub-littoral and benthic samples also be included? How should aquatic plants be sampled? The choice of methodology, and in particular the necessity for boat use, have considerable resource implication for the cost of the final monitoring method.

Possible options include (i) preliminary field investigation of sampling techniques and sample variability (ii) guestimation of appropriate techniques and sub-sample collection to investigate variability within the data-set, or (iii) guestimation of appropriate techniques (e.g. for littoral

invertebrate samples), and later development and addition of other metrics to fill gaps (e.g. for benthic invertebrates).

Other waterbody types

In contrast to lakes, development of multi-metric assessment methods for ditches, temporary ponds and brackish waters would be relatively straightforward using modifications of existing river and pond sampling methods.

ANNEXES

Annexe 1 Definitions of still waterbody types included in the assessment

Lakes	A body of water greater than 2 ha in area (Johnes *et al.* 1994). Includes reservoirs, gravel pits, meres and broads
Permanent and semi permanent ponds	Waterbodies between 1 m^2 and 2 ha in area which usually retains water throughout the year (Collinson *et al.* 1995). Includes both man-made and natural waterbodies.
Temporary waters	Waterbodies with a predictable dry phase, usually in the order of 3-8 months (Ward 1992).
Brackish waters	Pools and lagoons containing between 500 and 30,000 mgl^{-1} sodium chloride (Allaby 1985).
Canals	Artificial channels originally constructed for navigation purposes.
Ditches	Man-made drainage channels. Includes drains and rhines.

Annexe 2 Comparison of techniques for characterising reference conditions

	Present-day reference sites	Paleolimnology	Modelling	Historical data	Professional consensus
Strengths	• Applicable to all types of still water body. • All physical, chemical and biological characteristics can be measured.	• Provides historical time series data for diatom assemblages, chrysophytes, and, to a lesser extent, some crustaceans and some insects. • Water quality can be inferred from assemblage data.	• Can be used when no paleo-limnological or historical data are obtainable. • Works well for water quality.	• Gives actual historical information on status. • Inexpensive to obtain.	• Can be used when no data are obtainable. • Relatively inexpensive. • Can be better applied to biological assemblages than models. • Common sense and experience can be incorporated.
Weaknesses	• Even best sites subject to human impacts. • Inclusion of degraded sites can lower standard of reference sites.	• Restricted to sites with good sediment record • Preservation of fish, invertebrates, macrophytes, and non-diatom algae is poor. • Pre-settlement status might be unrealistic and unobtainable in a present-day context.	• Community and ecosystem models not useful. • Extrapolation beyond known data and relationships is risky. • Can be expensive. • Not testable	• Unlikely to be many sites with good data. • Data not usually collected for status monitoring so likely to be inappropriate. • Human impacts present in historical times were sometimes severe. • Difficulties in determining when the 'natural' state occurred.	• Qualitative descriptions of "ideal" communities. • Might be unrealistic and unobtainable. • Experts might have strong bias.

Annexe 3. Stages in the development of a multimetric assessment method for still waters

Choice of sites and survey techniques for the creation of a minimally impacted baseline dataset

For any waterbody type (pond, canal etc.), a minimally impacted baseline data set needs to be created for assemblage groups to be used in the assessment. The preferred method, is to use minimally impacted present-day reference sites. The major concern in selection of these reference sites is to ensure that they are as unaffected as possible by major anthropogenic influences, and not moderately disturbed, producing mediocre expectations.

Selection of reference sites, on whatever basis, needs to consider the principle natural chemical, physical and biotic parameters likely to be acting upon each waterbody type (e.g. longitude and latitude, geology, watershed characteristics, depth, shade). The number of regional reference sites chosen should be a function of regional variability and the desired level of detectable change. In practice, the ideal also needs to be balanced against budget realities.

Methods used to collect and analyse the reference data, will inevitably form the basis of subsequent methodologies (as was the case with RIVPACS, for example). Poor choices at this stage will, therefore, be perpetuated in all future surveys.

Collection of data and classification of unimpaired reference sites

Selection of reference sites is followed by:

- Collection of appropriate biological data from these sites, together with sufficient physical and chemical information to characterise them.
- Classification of biological communities based on this data to minimise natural variation and give better within-class impairment resolution.
- Analysis to identify the natural environmental parameters which characterise (i.e. can be used to predict) each community type.

Survey data for a range of variably impaired sites

Surveys of *impaired* sites (good to very poor) are also essential in order to determine degradation gradients for metric discrimination. This survey may be undertaken consecutively with or following collection of baseline data set. There may also be potential for using existing data from 'impacted' sites, where they exist, providing data is fully compatible in terms of survey methodology and quality.

Identification and development of viable metrics

To determine the discriminatory power of metrics within a waterbody class potential metrics are chosen for assessment. The list of potential metrics should initially be extensive, and include parameters relating to a wide range of community interactions and health (e.g. species/family richness, proportion of functional feeding groups, wet weight, proportion of sensitive taxa etc.).

These variables are tested against the range of best quality and impaired data to identify parameters which show a significant relationship with damage. Clearly, metrics which show a strong monotonic gradient to degradation are likely to be the most effective in accurately expressing degradation through the range of impact intensities. Metrics are rejected if they:

- show high variability in response to *natural* environmental stress,
- they are cost prohibitive,

- have superior measures.

All successful metrics are normalised against the baseline sites and divided to give simple scoring categories (i.e. 1= good, 2=fair, 3=poor, 4=very poor). The process of normalisation provides a mean of combining scores across metrics despite their initially dissimilar values. The division of sites on what is, in reality, a quality continuum, can be undertaken in a number of ways (simple division of the frequency distribution of data into percentiles; proportion of maximum levels etc.).

6.2.5 Combining metrics

Use of the metric data in practice involves combining the normalised metric results to give a single score which represents the overall integrity of the system. This score can be derived from the metrics of a single assemblage, or from the combined results of a number of taxonomic groups. Individual metrics may be weighted if appropriate.

Since metrics are not combined until the final analysis, new metrics or new assemblages can be developed independently, over different timescales, and added into the system as they become available. This gives a very flexible methodology which can be improved and refined without undermining the rationale for the method as a whole.

Testing

A trial phase, during which metrics are tested against new sites, is required to validate and refine the methodology. If there is evidence of poor performance this is most likely to indicate that the initial data set was not adequate to reflect natural variability and will suggest a need to collect further data.

Further use of data to provide additional information on the causes of degradation

The approach described above does not aim to determine the specific causes of degradation, although clearly the assessment will suggest factors which may be important. Investigating the cause(s) of degradation is, conceptually, a separate stage, which is likely to require application of a wide array of methods to disentangle the complexities of causation.

It is clear, however, that the data already gathered for multimetric analysis may have additional potential in providing clues to the causes of impairment. Thus, component parameters can be examined for their individual effects on the aggregated values providing further insight into the factors responsible for degradation. In addition, there is considerable potential for correlation of individual metrics with specific pollutants or other data from impaired sites (collected either during biological surveys, or from other EA sampling programmes). The results of such analysis (e.g. development of trophic ranking scores etc.) may offer a considerable diagnostic capability.

Annexe 4 Criteria used to evaluate suitability of plant and animal assemblages for GQA monitoring

Ecological relevance

Species-richness in the waterbody type

Range of trophic levels at which the group occurs

Range of waterbody habitats that the group occupies

Extent to which the group reflects aquatic/wetland (as opposed to terrestrial) influences

General interest in, and concern about, the group (ecological, conservation, public)

The ability of the group to integrate the environmental quality spatially

The ability of the group to integrate the environmental quality temporally

The responsiveness of the group to anthropogenic impacts including:

- Nutrient enrichment
- Acidification/pH changes
- Deoxygenation
- Biocides and other micro-organics
- Metals
- Turbidity
- Water level changes
- Physical habitat damage
- Biological impacts e.g. nuisance spp.

Practical suitability

How well is the taxonomy of the group known?

Does the group occur throughout the range of water chemistry regimes naturally present in the waterbody type?

Does the group occur throughout the range of physical variants naturally present in the waterbody type?

The typical abundance of individuals

The extent to which the group shows:

- temporal persistence in the waterbody
- intra-season stability in community types
- intra-habitat homogeneity within the waterbody

Costs

Cost of equipment items and consumables

Time required for staff training

Time required to undertake field surveys, laboratory work and data inputting

Annexe 5.1 Potential invertebrate metrics

- Number of taxa, families, species
- Ephemeroptera, Plecoptera, Trichoptera (EPT) taxa: number, proportion
- Ephemeroptera, Trichoptera, Odonata (ETO) taxa: number and proportion
- Crustacea and Mollusca (as gastropods and bivalves) taxa: number and proportion, including intolerant taxa
- Chironomidae and Oligochaeta: abundance and proportion
- % non-insects
- % individuals of numerically dominant taxa
- Relative abundance of individual species
- Types of functional-feeding groups (shredders, scrapers, suspension feeders, collector-filterers, predators, omnivores and scavengers): percentage and ratios
- Ratio and number of trophic specialists/generalists
- Mean number of individuals per taxon
- Presence of intolerant species
- Endemic/exotic species: number and proportion
- BMWP score
- Rare Species Score; Species Rarity Index
- Conservation value, based on rarity and richness attributes

Annexe 5.2 Potential plant metrics

Species richness	based on number of: • submerged species • floating species (rooted) • floating species (free-floating) • all aquatic species • ratios between these groups.
Vegetation cover	based on percentage cover of plant groups
Species rarity	Species Rarity Score and index for: • submerged species • floating species • marginal species
Conservation value	a categorisation based on a combination of rarity and richness
Endemic/exotic species	based on the number, percentage and cover of exotic species
Key species	the occurrence, relative abundance and dominance of key species and families: i.e. charophytes, *Sphagnum*, *Callitriche*, *Lemna* etc.

Annexe 6 Physico-chemical data gathered from water bodies

Ponds

Lithology
Water depth
Drawdown
Catchment size
Pond area
Shade
Fish
Mesohabitats
Water quality
Sediment depth and type
Permanence
Water source and inflows
Margin complexity
Age
Grazing and trampling
Vegetation cover
Surrounding land use
Adjacent wetlands

Canals

Location
Flow (BW data)
Base
Depth
Shade
Bank type
Boat movements
Management (BW data)
Water quality (EA & PA data)
Altitude
Turbidity
Sediment
Width
Vegetation cover
Bank angle
Mesohabitats
Surrounding land use
Sediment quality (BW data)

Annexe 7 Diatom Field Sampling Protocols

The following diatom sampling protocols were developed during an EA workshop held at UCL in April 1997 and pond field sampling day in Oxford in August 1997.

1. Pond protocol

(i) Aim

To collect representative diatom samples from all appropriate pond microhabitats (see Table A7) For each microhabitat approximately 10 sub-samples should be taken from around the pond. These are combined together in a single microhabitat sample. Ponds typically have between 3 and 8 microhabitats.

(ii) Timing

Sampling should be undertaken in summer or autumn (up to November). Spring is not ideal because of the presence of atypical species which alter abundance ratios for diatom assemblages.

(iii) Field methods

If possible, habitats should be chosen from sunlit places which will have more species and the greatest abundance of diatoms. Filamentous algae should be avoided, or if necessary, more Lugol's solution added to preserve the sample if filamentous algae is abundant. (Refer to Table A7 overleaf for detailed methodologies for each microhabitat).

Once samples have been collected and preserved from each microhabitat they should be placed in a large labelled plastic bag. All samples should be kept dark and stored in a cold room.

2. Canal protocol

(i) Aim

Canals are a relatively uniform habitat type in comparison with other waterbody types with regular occurrence of locks and mooring places. Hard surfaces are always available as diatom sampling substrate. Diatom sampling in canals can therefore use samples collected from a single habitat substrate / hard bank surfaces.

(iii) Field methods

Approximately 10 sub-samples should be collected from along a 5m length of stone canal bank and combined together into a single microhabitat sample. Wooden structures (e.g. lock gates) should be avoided because they have anomalous communities with high saprobic tolerance.

3. Equipment list

(i) General

Lugol's solution
plastic zip-close bags of different sizes
small plastic tray
toothbrush
cool box
wash bottle

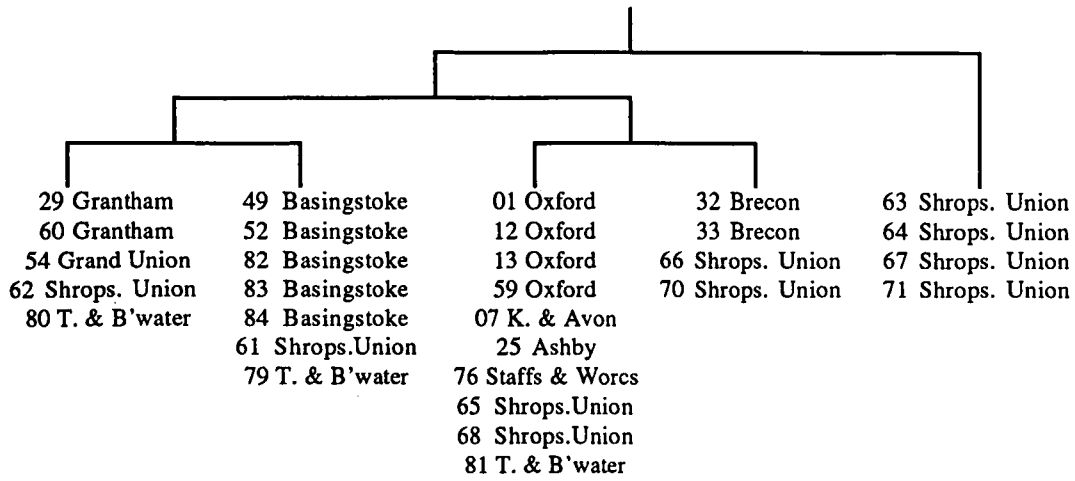
(ii) Epipelon

perspex tube
pert dishes with lids
lens tissue (Whatman 105)
pasteur disposable pipettes (150mm)
30 ml and 60 ml sterilin tubes

Table A7 Microhabitat field sampling methodology for diatoms

Microhabitat	Diatom sampling methodology
Epiphyton	Diatoms should be present on permanently submerged stems and leaves of tall emergents. Older plants (including brown or decaying plants) should be sampled as they are more likely to have well developed communities. New growth should be avoided. **Submerged macrophytes** Take submerged portions of plants a few centimetres in length from various parts of the pond (approximately 10 samples). Place in a labelled zip-end plastic bag. Add a pipette full of Lugol's solution, seal bag and mix solution. **Floating plants** Floating plants, including *Lemna*, can be sampled for diatoms. Sample and preserve as above. **Tall emergents** Diatoms can be collected by brushing the plant surface with a toothbrush and collecting the material in a small plastic tray. The material should look slightly brown if diatoms are present. The sample should be transferred to a sterilin tube and 4 drops of Lugol's added.
Roots	Roots can be sampled for diatoms as long as they are growing within the water column (e.g. willow roots) and are not from the sediment. Sample and preserve as above.
Fallen leaves	Approximately 10 submerged leaves (not fresh) should be placed in a labelled zip-end plastic bag and preserved as above.
Epilithon	Rock surfaces can be sampled for diatoms by brushing with a toothbrush and preserving with a few drops of Lugol's solution. Particular attention should be paid to cracks where diatoms may be abundant (the collection of mineral matter should be avoided).
Episammon	Sand or gravel can be sampled for diatoms by placing in a sterilin tube and adding half a pipette full of Lugol's solution.
Epipelon	Mud from beneath the drawdown zone can be sampled for diatoms. Place thumb over the top of a 1.5m length perspex tube. Put tube into water at sediment surface. Gently ease pressure of thumb and draw tube across the sediment surface. Mud is drawn up into the sediment tube. Collect 10 sediment samples from different areas of the pond and add to a 60 ml sterilin tube. Do not add Lugol's solution. Leave to settle for 15 minutes and then pour away water. Make sure there is enough sediment to half fill a 30 ml sterilin tube. Keep samples dark and cool and return them to the laboratory for processing in the evening using the method described in Eaton & Moss (1966) as follows. (i) shake sediment tube and pour out into a petri dish. Leave for a couple of hours for the sediment to settle (ii) remove excess water using a pipette or vacuum pump. Place a square of lens tissue (double layer) over sediment, add petri lid and label sample. (iii) leave on windowsill overnight for diatoms to move up into the lens tissue. At 8.00 to 9.00 am remove lens tissue (plus diatoms) and place in a labelled sterilin tube. (iii) add Lugol's solution, put on lid and shake a little to distribute the solution. Reference: J.W. Eaton and B. Moss (1966) The estimation of numbers and pigment content in epipelic algal population. *Lim. & ocean.* vol. 11 no. 4 pp584-595.

Annexe 8 TWINSPAN dendrogram for the canal baseline sites



ANNEXE 9 MDA (Multiple Discriminant Analysis) prediction of canal TWINSPAN endgroups from environmental variables alone

Overall, 90% of canal sites are placed into the correct TWINSPAN endgroup by MDA prediction using environmental data alone. The variables used to predict canal site TWINSPAN group membership are shown in the table below.

A9a Prediction of canal endgroups

	Predicted classification					
Observed classification	1	2	3	4	5	
Group						% Correct
1	4	1				80
2		7				100
3		1	8		1	80
4				4		100
5					4	100
Total						**90**

Variables used to predict canal site TWINSPAN groups

Map data
1. Northing
2. Altitude (m)

Field data
3. Secchi depth (cm)
4. Total vegetation cover in the sampling area (%)
5. % earth bank in the sampling area
6. Angle of bank at edge (degrees)
7. No. of submerged plant species
8. Average sediment depth (measured at three points from bank: 1m, 2m, 3m)

Discriminant functions for the MDA predictions

Variable	Root 1	Root 2	Root 3	Root 4
NORTHING	-.000047	.00018	-.000031	-.000005
ALTITUDE	-.003453	-.01253	-.004729	-.013566
SECCHI	.010194	-.00188	.006140	-.000990
%VEG_SA	.013502	.01287	.004771	-.001619
%EARTH	-.032540	.03424	.016686	-.021883
B_ANGLE	-.008347	.05072	-.012700	-.027326
SED_TOT	1.801573	-.36165	.031201	4.760631
SUB_SPP	.499133	.10185	-.311104	-.307707
Constant	1.413269	-8.57181	.651336	3.517700

A9b Prediction of pond aquatic plant endgroups

	Predicted classification					
Observed classification	1	2	3	4	5	
Group						% Correct
1	4					100
2		13	1			93
3			10			100
4				6	1	86
5		1	1		10	83
Total						**91**

Variables used to predict pond aquatic plant TWINSPAN groups

Map data
1. Easting
2. Northing

Field data
3. Pond base: clay (%)
4. Pond base: gravel (%)
5. Water source: groundwater (%)
6. Water source: runoff (%)
7. Shade (% of pond area)
8. Drawdown (% area of water remaining)
9. Permanence (1-4 scale)
10. Wood and scrub in 5-25m zone around pond (%)
11. Wetland in 5-25m zone around pond (%)
12. Wood and scrub in 25-100m zone around pond (%)
13. Wetland in 25-100m zone around pond (%)
14. Deciduous woodland in 0-100 zone around pond (%)
15. Heathland in 0-100 zone around pond (%)
16. Connections to other waterbodies (0-5 scale)
17. Age (rank)
18. Grazing (% pond margin)
19. Inflow (presence/absence)
20. Turbidity (1-4 scale)
21. Average silt depth (cm)
22. Conductivity (uS cm^{-1})

Discriminant functions for the MDA predictions

Variable	Root 1	Root 2	Root 3	Root 4
EASTING	-.00555	.00649	-.004626	.00536
NORTHING	-.00423	-.00209	-.002828	-.00115
BASEC	.02070	.00894	.005110	.00386
BASEG	.03977	.00377	-.012649	.00827
WS_G	-.01825	.00394	.019527	-.00949
WS_R	-.00232	.00824	-.014062	-.01896
SH_PA	.00554	-.01619	.007492	-.00312
DRAW%	-.02401	-.01456	-.038247	-.00362
PERM	.25230	.25312	-.560116	-.13431
WS_25M	.01099	.02217	-.011020	-.01255
WET_25M	.11238	.00025	.086499	-.00559
WS_100	.00982	-.01930	.028074	-.02048
WET_100	-.13784	.05098	-.106922	.01264
DECID	-.05661	.00698	-.026583	.06018
HEATH	-.01353	-.03449	-.000979	.00750
CONNECT	-1.37746	-.10321	-.659982	-.70054
R_AGE	-.38217	.18850	.179184	1.43338
%GRAZED	.02384	-.00402	-.010892	-.01392
INFLOW	.62324	-.19990	-.935688	.39861
TURBID	.76249	.16480	-.442887	.65592
AV_SILT	.02306	-.01326	.013070	-.01177
AV_COND	.00060	-.00057	.001095	.00033
Constant	1.68249	-2.33308	7.799126	-5.26732

A9c Prediction of pond invertebrate endgroups

	Predicted classification						
Observed classification	1	2	3	4	5	6	
Group							% Correct
1	6						100
2		8					100
3	1	2	14	1	1		74
4				18		1	95
5			1	2	8		73
6				2		5	71
Total							**84**

Variables used to predict pond invertebrate TWINSPAN groups

Map data
1. Easting
2. Northing

Field Data
3. Pond base: clay (%)
4. Pond base: gravel (%)
5. Water source: groundwater (%)
6. Water source: runoff (%)
7. Pond margin complexity index (1-10 scale)
8. Shade (% of pond area)
9. Shade (% of pond margin)
10. Permanence (1-4 scale)
11. Wood and scrub 0-5 m zone around pond
12. Catchment woodland and scrub (%)
13. Deciduous woodland in 0-100 m zone around pond (%)
14. Heathland in 0-100 m zone around pond (%)
15. Unimproved grassland in 0-100 m zone around pond (%)
16. Grazing (% pond margin)
17. Grazing intensity (1-5 scale)
18. Trampling intensity (1-5 scale)
19. Inflow volume (l s^{-1})
20. Average depth (cm)

Discriminant functions for the MDA predictions

Variable	Root 1	Root 2	Root 3	Root 4	Root 5
EASTING	.00221	.00910	-.007231	.004858	-.004144
NORTHING	-.00130	.00086	-.000714	-.002923	-.003063
BASEC	-.00022	-.00629	-.003467	.010523	-.009149
BASEG	.00010	-.01450	.008460	.010506	-.007007
WS_G	-.01002	.00590	-.014683	-.031951	.012045
WS_R	-.00621	.00673	-.009061	-.029892	.000658
PMC	.11721	-.24250	.160815	.177810	-.398124
SH_PA	.00419	.03859	-.010149	.003135	.017207
SH_PM	.00515	-.00937	-.020056	-.018505	-.025229
PERM	-.56866	.08749	-.129863	-.178814	.220145
M_WS	-.01187	-.01701	.038783	-.029300	-.005862
CMT_WS	.02801	.01954	-.011647	.006530	.015316
DECID	-.01241	-.02574	.022234	.002857	-.032987
HEATH	.01831	.03986	.028930	.002371	-.017935
UNIMP	.02327	-.01387	.009889	-.008255	-.016878
%GRAZED	-.00432	.01043	-.000765	-.009360	.022268
GRAZE_1	-.00905	.00582	.007411	-.007529	.013716
TRAMP_1	.12732	-.21571	-.102323	.311027	.062679
IN_VOL	.69037	.35990	-.692570	-.269949	.194884
AV_DEPTH	.01364	.00610	.005859	-.008853	.009505
Constant	-2.17073	-4.46216	2.874076	1.825772	3.199368

Annexe 10 Example comparison of predicted and observed macroinvertebrate fauna at Wolvercote, Oxford Canal

Predicted species	Probability	Observed
Anisus vortex	1.00	✔
Asellus aquaticus	1.00	✔
Bithynia tentaculata	1.00	✔
Crangonyx pseudogracilis	1.00	✔
Ischnura elegans	1.00	✔
Gyraulus albus	0.90	✔
Limnephilus lunatus	0.90	✔
Sphaerium corneum	0.90	✔
Anacaena limbata	0.85	
Hippeutis complanatus	0.85	✔
Lymnaea stagnalis	0.83	
Planorbis carinatus	0.83	
Athripsodes aterrimus	0.80	✔
Lymnaea peregra	0.78	✔
Sialis lutaria	0.76	✔
Laccophilus hyalinus	0.73	✔
Noterus clavicornis	0.71	
Sphaerium lacustre	0.68	
Coenagrion puella	0.66	
Gerris lacustris	0.66	✔
Triaenodes bicolor	0.66	
Erpobdella octoculata	0.61	
Hyphydrus ovatus	0.61	
Caenis horaria	0.61	
Haliplus fluviatilis	0.59	✔
Helobdella stagnalis	0.59	
Sigara dorsalis	0.59	✔
Bithynia leachi	0.56	✔
Cloeon dipterum	0.56	
Enallagma cyathigerum	0.54	
Haliplus lineolatus	0.51	
Nepa cinerea	0.51	
Sigara falleni	0.51	✔
Valvata piscinalis	0.51	✔
Physa acuta	0.51	
TOTAL	**25.32**	**19**

Annexe 11 Examples of canal metrics which have significant relationships with environmental degradation

Variable	Factors correlated
SNAIL SP%	Salts
CRUST SP%	-ve vegetation cover; boats, -ve Secchi
MAY SP%	-ve WQ
BUG SP%	Hard edge and vegetation abundance
BEETLE SP%	Bank type, angle and water depth.
CADDIS SP%	All -ve: salts, heavy metals, sediment quality
SPP_RICH	Banks, vegetation, boats, Secchi depth
FAM_RICH	Banks, vegetation, Secchi depth
SNAIL_SP RICH	Banks and vegetation
Lymnaeidae_ri	Bank vegetation, depth
Planorbidae_ri	Vegetation, boats, Secchi
CRUST_SP RICH	(-ve): antimony and organic matter.
DAMS_SP RICH	Vegetation cover, ss, secchi.
Coenagrionidae_ri	Vegetation, Secchi, Boats
DRAG_SP RICH	Vegetation
MAY_SP RICH	Boats, Secchi (r= 0.48 +ve), WQ (-ve)
Baetidae_ri	Mostly vegetation; strong -ve with boats, Secchi
MAY_FAM RICH	Boats and Secchi
BUG_SP RICH	Similar to beetles.
Pred. bugs_ri	Banks, vegetation, Secchi
BUG_FAM RICH	Banks, vegetation
BEETLE_SP RICH	Bank, vegetation cover (SA and 50m), Secchi.
Haliplidae_ri	Vegetation and banks
Hydrophilidae_ri	Banks, vegetation, depth.
Small Dytiscids	Banks and vegetation
BEETLE_FAM RICH	Banks, vegetation
CADDIS_SP RICH	Bank, salts, sed. qual. (not heavy metals)
Leptoceridae_ri	Banks, vegetation, Secchi (0.5), salts
Limnephilidae_ri	Banks, marginal vegetation, depth, salt, sed_qual.
CADDIS_FAM RICH	Salts (partly with bank)
Lepidoptera	Mostly vegetation and boats
EPT SP	Bank, boats, salt, metals (i.e. caddis + mayfly)
EPT SP%	All -ve: salts, heavy metals
EPT_FAM	WQ, salts, ammonia
DETRITIVORES_FAM	Banks, Secchi depth, metals, PAH, WQ
PREDATORS_FAM	Banks, vegetation (weaker with WQ and metals)
ASPT	(-ve) Heavy metals, salts, WQ.
BMWP	Sus. solids, heavy metals, WQ (all -ve)
TAXA	Arsenic, selenium, SS.

Annexe 12 Pond plant metrics which have significant relationships with environmental degradation

Variable	Factors correlated
AQUATIC PLANT SPECIES RICHNESS	Overall pollution index (-ve), agricultural drainage (-ve), agricultural surrounds (-ve), semi-natural catchment and surrounds.
AQUATIC PLANT SRI	Overall pollution index (-ve), semi-natural surrounds and catchment, surrounding wetlands, intensive landuse and agriculture in surrounds and catchment (-ve) suspended solids (-ve).
AQUATIC PLANT SRS	Overall pollution index (-ve), agricultural drainage (-ve), agricultural surrounds (-ve), semi-natural catchment and surrounds.
TROPHIC RANKING SCORE (TRS)	Heathland surrounds (-ve), intensive catchment.
FLOATING-LEAVED SPECIES RICHNESS	Ducks
FLOATING-LEAVED SRS AND SRI	Ducks
SUBMERGED PLANT SPECIES RICHNESS	Intensive surrounds and catchment, particulalry agriculture (-ve), overall pollution index, agricultural runoff, agricultural surrounds and catchment (-ve), semi-natural surrounds and catchment.
SUBMERGED PLANT SRS	Intensive surrounds and catchment, particulalry agriculture (-ve), overall pollution index, agricultural runoff, agricultural surrounds and catchment (-ve), semi-natural surrounds and catchment.
SUBMERGED PLANT SRI	Overall pollution index (-ve), intensive agricultural surrounds and catchment (-ve), waterbodies and wetlands in the surrounds, semi-natural land use and catchment.

Annexe 13 Examples of pond invertebrate metrics which have significant relationships with environmental degradation

Variable	Factors correlated
SPP_RICH	Unimproved catchment and surrounds, overall pollution, wetlands (5-25m), ducks (-ve), turbidity (-ve), ammonia (-ve)
FAM_RICH	Unimproved catchment and surrounds, wetlands in the area, ducks (-ve), pH
SNAIL_SP RICH	Alkalinity, pH.
SNAIL_FAM RICH	pH
DRAG_SP RICH	Intensive agriculture (-ve), runoff from intensive land (-ve), heathland catchment
DRAG_FAM RICH	Runoff from intensive land (-ve), heathland catchment,
MAY_SP RICH	Wetland surrounds
BUG_SP RICH	Semi-natural catchment and surrounds
BUG_FAM RICH	Semi-natural catchment and surrounds
BEETLE_SP RICH	Ducks (-ve)
BEETLE_FAM RICH	Fish (-ve)
EPT_SP	Semi-natural surrounds and catchment, other wetlands nearby, Overall pollution index, intensive catchment, surrounds, and polluted runoff (-ve), intensive agricultural surrounds (-ve)
EPT_FAM	Semi-natural surrounds and catchment, other wetlands nearby, intensive catchment, surrounds, and runoff (-ve), intensive agricultural surrounds (-ve)
ETO_SP	Semi-natural surrounds and catchment, other wetlands nearby, Overall pollution index, intensive catchment, surrounds, and polluted runoff (-ve), intensive agricultural surrounds (-ve), Mg, K
ETO_FAM	Semi-natural surrounds and catchment, other wetlands nearby, Overall pollution index, intensive catchment, surrounds, and polluted runoff (-ve), intensive agricultural surrounds (-ve), Mg, Total P
DETRITIVORE_ Hemiptera	Semi-natural land-use and catchment, fish(-ve)
SPECIES RARITY SCORE	Unimproved catchment, wetlands in the surrounds, semi-natural surrounds and catchment, overall pollution index, intensive land use (5-25m)(-ve), turbidity (-ve), Ducks (-ve).
SPECIES RARITY INDEX	Intensive catchment and surrounds, particularly urban (-ve), semi-natural catchment and surrounds, overall pollution index, Alkalinity (-ve), pH (-ve).