The Trophic Diatom Index: A User's Manual. Revised Edition

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ENVIRONMENT AGENCY

The Trophic Diatom Index: A User's Manual. Revised edition

R&D Technical Report E2/TR2

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Statement of Use
This report guides users in appropriate methods of collection, preparation, identification and, enumeration of benthic diatoms from running waters and of calculation and interpretation of the trophic diatom index.

Keywords
Algae (Diatoms), Streams (In Natural Channels), Water Quality (Natural Waters), Monitoring, Eutrophication, Trophic Diatom Index.

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FOREWORD

Initial stages of development of the Trophic Diatom Index were funded by the National Rivers Authority (NRA). Following the formation of the Environment Agency on 1 April 1996, the project was absorbed into the Agency's R&D programme.

The first version of the TDI introduced benthic diatom analyses to many biologists within the Agency without first-hand experience of diatoms. There was a steep learning curve for all concerned, adapting the principles outlined in the manual to a wide range of circumstances. For the most part, ideas contained in the first edition of the TDI manual were robust and, indeed, many of the principles of sampling, preparation and enumeration have been included into CEN Guidance Standards that are currently under development. However, after five years, it was time to take a new look at the manual and to incorporate as much new experience as possible.

One major change of this updated manual is that the number of authors has increased from one to nine, indicating the much wider pool of experience on which to draw, compared with the situation when the first edition of the manual was published. This bodes well for the future, not just for monitoring eutrophication, but also looking towards the monitoring requirements of the Water Framework Directive.

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EXECUTIVE SUMMARY

The Trophic Diatom Index (TDI) is a new index developed in response to the monitoring needs of the Urban Wastewater Treatment Directive (UWWTD).

This document guides users of the TDI in appropriate methods of collection, preparation, identification and enumeration of benthic diatom samples and calculation and interpretation of the index. It is based on an earlier manual (R&D Technical Report E2) but updated to include more recent experiences and incorporating elements of Guidance Standards under development by CEN.

The TDI is recommended for widespread use by the Environment Agency and other regulatory bodies to assess the trophic status of rivers and streams.

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1. INTRODUCTION

1.1 Purpose and scope

This manual sets out the procedures for collecting, examining, analysing and interpreting benthic diatom samples for use in the assessment of trophic conditions in streams and rivers with respect to the Urban Wastewater Treatment Directive (UWWTD; European Community, 1991). All staff undertaking such work must follow these procedures. This includes staff within the Environment Agency and contractors working for it.

Methods described in this manual may also be appropriate for other monitoring purposes. It is recommended that these methods are used whenever benthic diatom samples are collected in order to ensure consistency within and between laboratories, and comparability of survey results. Good management practices must be followed at all stages to ensure that the results meet this criterion. If procedures in this manual are not followed, comparisons of data may be misleading and could lead to erroneous conclusions.

Before embarking on any practical work, all readers of the manual must familiarise themselves with the procedures laid down in Appendix C: Risk assessments for Activities associated the Trophic Diatom Index.

1.2 Performance characteristics

Biota sampled

Benthic diatom communities

Habitats sampled

Streams and rivers

Basis of operation

Collection of benthic diatom films from natural or artificial substrates within a 10-metre study reach. Preparation of permanent slides from this material. Identification and estimate of relative proportion of each taxon present on slide. Calculation of index of eutrophication and associated measures of reliability for each sample.

Form of data

Lists of relative proportions of taxa present plus index showing degree of eutrophication ("Trophic Diatom Index", TDI) and reliability of this measure for each sample. TDI indicates floristic changes in response to increased nutrient concentrations. In addition, permanent slides are lodged with national herbaria.

Limitations of method

Availability of suitable substrates **or** positions to leave artificial substrates.
Water depth (low light may limit diatom growth in deep water).
River flow (high flows may limit access to sites and scour away diatom films).

Seasonal factors can override water quality in determining community composition. These factors vary in intensity between sites and are described in more detail in chapter 6.

Performance of the TDI is reduced when other pollutants are present and when inorganic nutrient levels are sufficiently high that phosphorus is no longer the limiting nutrient. Other environmental stresses (e.g. periodic inundations with brackish water) have not been tested, but are also likely to influence results.

Efficiency of method

Sampling natural substrates is quick and easy and can be combined with visits to sites for other purposes (i.e. macrophyte survey, invertebrate kick samples etc.). Sampling artificial substrates requires a preliminary visit in addition to the above, to place the substrates. Data interpretation should be based on a series of samples from the same sites.

Logistics of method

Sampling by one operator (two recommended at main river sites, for safety reasons). Length of time at site: approximately 10 minutes. Preparation of permanent slides: allow 3-4 hours for a batch of 6-8 samples. Microscopic analysis: 30 mins to 1 h per slide depending upon complexity of material and operator skill. As a rule of thumb, allow 2-3 h per sample for preparation and counting. 1 in 10 samples (1 in 5 for newcomers to technique) must be submitted for analytical quality control/audit.

1.3 Background

The trophic diatom index (TDI) was developed as part of a National Rivers Authority Research Fellowship at the University of Durham between 1992 and 1995 (Kelly & Whitton, 1995). Part of the objective of this Fellowship was to review existing methods of using plants to monitor rivers in light of the existing and anticipated needs of the NRA (Whitton & Kelly, 1995). Early on in this work, several NRA staff mentioned concerns about increasing levels of eutrophication in rivers (mainly from diffuse sources) coupled with an inability to monitor eutrophication using existing invertebrate-based tools. These concerns were given a sharper focus as the implications of the UWWTD became clear.

In particular, Article 5 of the Directive calls for more stringent treatment (usually interpreted as phosphorus stripping) of wastewaters that discharge into "sensitive areas". These are defined as freshwater bodies "which are found to be eutrophic or which in the near future may become eutrophic if protective action is not taken" (Annex II, UWWTD, European Community, 1991). Sewage discharges into such areas of greater than 10,000 population equivalent require nutrient stripping "unless it can be demonstrated that removal [of phosphorus] will have no effect on the level of eutrophication." A workshop, organised in Durham as part of the Fellowship, highlighted the need for new monitoring tools to identify sites that were vulnerable to

eutrophication and to monitor the effectiveness of nutrient stripping, where this was installed (Kelly and Whitton, 1995a).

Diatoms were one group identified during the Fellowship as having particular potential for monitoring eutrophication in running waters. The development of a Trophic Diatom Index (TDI) started with an evaluation of a number of existing techniques, including a zoning system (Round, 1993), along with general pollution indices developed in France (Indice de polluosensibilité, IPS; Indice Diatomique Generique, GDI, Coste *et al.*, 1991; Rumeau and Coste, 1988) and two earlier TDIs developed in Germany (Schiefele and Kohmann, 1993).

An important point that emerged was that there was a high correlation between the IPS, based on identification to species and the GDI which required identification only to genus (Coste *et al*, 1991; Kelly *et al.*, 1995). Examination of the relationship between IPS and GDI suggested that part of the scatter was caused by occasional differential responses of a species (e.g. *Amphora pediculus*) from other members of a genus. By expanding a generic index to include these species, it was hoped to improve the sensitivity of the index further. A further observation was that neither of the two German TDIs were particularly effective in the UK and, for this reason, a new TDI was developed specifically for UK conditions (Kelly & Whitton, 1995a). The first version of the TDI included 86 taxa (genera plus indicator species) and produced numerical values from 1 (indicating low nutrient concentrations) to 5 (indicating high nutrient concentrations; Kelly and Whitton, 1995b).

The relative effectiveness of generic-based indices was significant, because monitoring of river eutrophication was likely to be performed largely by biologists already employed in the water industry, few of whom were trained in diatom taxonomy. The Biological Monitoring Working Party (BMWP) score, used routinely in the UK for assessment of organic pollution using macroinvertebrates (Chesters, 1980; Armitage *et al.*, 1983), was used as a benchmark for development of a practical index that balanced scientific integrity with ease of use (Kelly *et al.*, 1996).

No attempt was made at this stage to differentiate between the response of P and N. As trophic variables are often highly correlated it was considered to be better to model a broad response to "nutrients" or "eutrophication" using a single variable (e.g. molybdate-reactive P ≡ filtrable reactive phosphorus (FRP) ≅ "orthophosphate") as a proxy. The underlying assumption was that P, rather than N, was the limiting nutrient in most rivers. The validity of this assumption is discussed in Chapter 6.

A particularly important feature of the first version was a facility to separate nutrient rich waters from those that are organically-polluted. Organic pollution is frequently associated with high nutrient concentrations, but under such conditions, nutrients were not necessarily the primary factor influencing community composition (Table 1.1; Kelly *et al.*, 1996). For this reason, the sum of taxa tolerant to organic pollution (e.g. *Nitzschia palea, Gomphonema parvulum*) was used alongside the TDI value to indicate the reliability of the TDI as a measure of eutrophication. This feature has proved to be very useful, but is not without problems. Firstly, the term "percent pollution tolerant valves" (%PTV) focussed attention on organic pollution whereas experience from the subsequent five years has indicated that a range of other water quality issues can lead to elevated %PTV values upstream as well as downstream of STWs and, consequently, interfere with interpretation of the TDI. Secondly, the term "%PTV" leads to

erroneous use of the measure as an index of organic pollution, rather than strictly as a measure of the reliability of the TDI.

The first version of the TDI was then subjected to an evaluation exercise by the Environment Agency, as a result of which a number of changes were made to the original TDI. The most notable of these was the change in the scale of the index so that it now extends from 0 (indicating very low nutrient concentrations) to 100 (indicating very high nutrient concentrations). This change was made to aid interpretation by biologists familiar with other biological indices in use in the UK. In addition, taxa such as *Cyclotella* and *Stephanodiscus* which were principally planktonic were removed from the calculation (see Chapter 4 for explanation).

A final development, recommended by the Regional Biologists of the Environment Agency, was to adopt a common scale for both diatom and macrophyte indices of water quality used in UWWTD assessments. This involved inverting the scale of the TDI so that low scores corresponded to high nutrient concentrations and high scores to low nutrient concentrations. In order to avoid further confusion, this "new" index was referred to as the "Diatom Quality Index" (DQI). Where diatoms were used in conjunction with the macrophyte Mean Trophic Rank (Holmes *et al.*, 1999) the DQI was recommended for use, with the TDI being retained for situations where comparison with other measures of eutrophication were necessary.

Table 1.1. Effects of organic and nutrient pollution on plants and the ecophysiological adaptations required for tolerance (from Kelly *et al.*, 1996).

Chemical effect of pollution	Ecological response of tolerant plant
elevated P concentration	competitive advantage at high P concentrations and/or low N:P ratio
Elevated ammonium concentration	tolerant to high ammonium concentration
reduced oxygen concentration	capable of respiration at low oxygen concentrations
elevated suspended solids	shade tolerant or motile
abundant organic matter	capable of heterotrophic growth

Routine use of diatoms for water quality monitoring in the UK at this point was further complicated by the lack of clear guides to taxonomy and identification in English. As a result, the first version of the TDI manual also included keys to the genera along with illustrations and descriptions of the key species.

This was the situation when the first edition of the TDI was published in 1996. Subsequently, the TDI has been used by the Environment Agency throughout England and Wales, and by the Scottish Environment Protection Agency in Scotland and diatom evidence was included in submissions for the second tranche of UWWTD "sensitive area" designations in 1997 (Harding & Kelly, 1999) and for the third tranche in 2001. In addition, there have been developments elsewhere in Europe, with new trophic indices being developed in Germany (Coring *et al.*, 1999) and Austria (Rott *et al.*, 1999) along with a combined pollution/eutrophication index developed in Italy (Dell'Uomo, 1999). Furthermore, there has been collaboration at a European level to harmonise

methods, with the first stage being harmonisation of sampling techniques (Kelly *et al.*, 1998). The output from these discussions and workshops is a draft European standard (BS EN 13946) for sampling, with further standards covering other aspects of methodology available soon.

1.4 Changes since the first edition of this manual

The combination of the extensive experience gained in using diatoms in the UK along with the additional European perspectives, provided the impetus to revise and update the TDI manual. Changes, compared to the first edition, include:

Sample collection (Chapter 2): modifications to bring chapter in line with draft European Standard.

Preparation of permanent slides (Chapter 3): inclusion of method for using hot hydrogen peroxide, alongside the sulphuric-oxalic-potassium permanganate method recommended in the first edition.

Identification of common diatoms (Chapter 4 in the first edition) was included to enable Agency biologists to use the TDI without recourse to standard floras. The chapter has been revised and extensively tested before publication in 1999 as a Field Studies Council "AIDGAP" key (Kelly, 2000). As this key is now available separately, Chapter 4 is not included in this revised version.

Enumeration (Chapter 5 in 1st edition, Chapter 4 in revised edition): modified to include guidelines for handling circumstances where diversity is very low. New procedures include a stratified counting protocol. More detailed guidelines on sample size are also given.

Calculation of the TDI (Chapter 6 in 1st edition, Chapter 5 in revised version): includes TDI values for a few taxa (e.g. *Achnanthes oblongella, A. conspicua*) whose previous inclusion in generic categories of the index could obscure potentially interesting patterns. Also, the %PTV measure has been replaced by a broader index of reliability based on the percent of valves that belong to motile taxa.

Interpretation of the TDI (Chapter 7 in 1st edition, Chapter 6 in revised version): extended and rewritten in light of experience gained over the past five years.

Quality assurance (Chapter 8 in 1st edition, Chapter 7 in revised version): a new method for auditing permanent slides has replaced the method in the 1st edition, which proved to be difficult to use in practice.

Health and safety (now collected together in Appendix C) More details of risk associated with procedures outlined in this manual have been included in this edition, along with examples of "best practice" for managing these risks.

None of these changes affect the integrity of data collected following procedures outlined in the first version of the manual although, in a few instances, samples may need to be reanalysed in order to use the modifications to the index proposed here. The revised TDI can be used alongside chemical and macrophyte data to prepare robust cases for the designation of sensitive areas by providing information about:

- the state of the river upstream of the sensitive area (with respect to inorganic nutrients and organic pollution);
- whether or not there is a floristic change downstream of a qualifying discharge;
- whether or not this change can be attributed to nutrients alone or to other components of the discharge; and,
- whether or not nutrient removal is likely to lead to a floristic change.

2. SAMPLE COLLECTION

2.1 Overview of sampling strategy

Diatom communities in rivers are neither simple, nor homogenous. Although some workers have attempted to define discrete "epilithon", "epiphyton", "episammon" and "epipelon" communities, in practice these tend to represent extremes of continua. Thus an epilithic community might contain filaments of green algae such as *Oedogonium* or *Cladophora* that bear epiphytic *Rhoicosphenia abbreviata*. The epilithic diatoms themselves produce mucus that traps sediment - permitting epipelic taxa such as *Navicula gregaria* to invade - as well as phytoplankton cells such as *Cyclotella meneghiniana*.

In general, communities in the upper reaches of rivers, where current velocity is high, tend to consist of diatoms closely adpressed to rocks. Further downstream, upright and stalked taxa become more common along with motile taxa. However, within a reach, community composition can vary depending upon current velocity and boulder size (related to susceptibility to movement during storms). Other factors such as heavy shade may also be important. Similar processes govern community composition on other substrates. There is a widespread assumption that water quality overrides physical factors; however, physical variability between sampling sites should be avoided wherever possible by adhering strictly to these guidelines, and by a consistent approach to sampling at all sites.

Four principles provide a framework:

1. Samples should be collected, **as far as possible,** from cobbles and small boulders that are free from filamentous algae and found within the main flow of the river in reaches that are not heavily shaded.

2. Artificial and natural substrates must be submerged for at least four weeks prior to sampling. All depths that can be easily sampled wearing waders are suitable, so long as they remain in the euphotic zone. Within these limits, the precise depth is unimportant so long as the sampler is sure that the surfaces have not been exposed to air.

3. Workers must collect samples from similar substrates upstream and downstream of discharges in order to minimise the effect that substrate has on community structure. Ideally, a single type of substratum should be used at all sites included in a survey.

4. Notes on the prevailing conditions must also be made to aid subsequent interpretation of the data (a model field record form is given in Appendix B).

A segment of river that has suitable substrates for sampling should be selected and defined in relation to permanent physical features so that it can be revisited in the future. As a general rule, it should be at least 10 m long, but longer lengths may be appropriate, depending upon the nature of the river. "Riffles" are recommended as these tend to have a good variety of natural hard surfaces, but shallow "runs" and "glides" are also suitable. In deeper rivers, or where no hard substrates are available, consideration should be given to using artificial substrates within the euphotic zone.

2.2 Choice of substrate

Diatoms can be found growing on most submerged surfaces; however, the composition of the community varies according to the nature of the substrate. Ideally, a single type of substrate should be used at all sites included in a survey. If this is not possible, then the use of artificial substrates is recommended (see below).

Cobbles are the recommended substrate, as these balance stability (allowing diatom communities to develop) with manoeuvrability. Pebbles and small boulders can also be used if cobbles are not available. If these are not available, then vertical faces of man-made structures such as quays and bridge supports should be sampled (so long as these are not made from wood). Other man-made hard surfaces, such as bricks can also be sampled, if these have been submerged for at least four weeks prior to sampling.

In many lowland reaches, the majority of hard surfaces are smothered with growths of *Cladophora* or other filamentous algae. Under these circumstances, those few cobbles and boulders that lack *Cladophora* are clearly not "representative" and a modified sampling protocol should be adopted under such circumstances (see below).

Further options include the use of introduced ("artificial") substrates and sampling from macrophytes. The disadvantage of introduced substrates is that two visits to each site are required in order to deploy and collect the substrates, and there are often losses due to interference by river users. However, they do enable a consistent sampling policy to be adopted over wide areas where suitable natural substrates are either lacking altogether or not available at all sites.

Whilst macrophytes can be sampled (see 2.8 and 2.9), they are not recommended unless it is not possible to either sample hard surfaces or deploy artificial substrates.

The flow chart (Fig. 2.1) is designed to guide users to the most appropriate means of sampling under various circumstances and tries to balance the competing interests of standardisation and typicality of the substrate. The method chosen should be applied to all sites included in a particular survey. Although the chart should cover most situations, there may be circumstances where *ad hoc* approaches not described in this chapter need to be adopted. Such methods must be fully documented and should also be supported by preliminary experiments to provide a firm basis for subsequent data interpretation.

2.3 Timing of surveys

It is difficult to give precise guidelines on the time of sampling that apply to all circumstances; however, the following principles should be followed:

1. Samples collected for specific comparisons (i.e. upstream and downstream of major discharges) should be collected on the same day.

2. Repeat sampling should take place at approximately the same time each year. Some discretion is required here: major spates scour away much growth and "reset" communities to pioneer stages, largely regardless of the time of year. For this reason, time of sampling may differ by several weeks between years, depending on

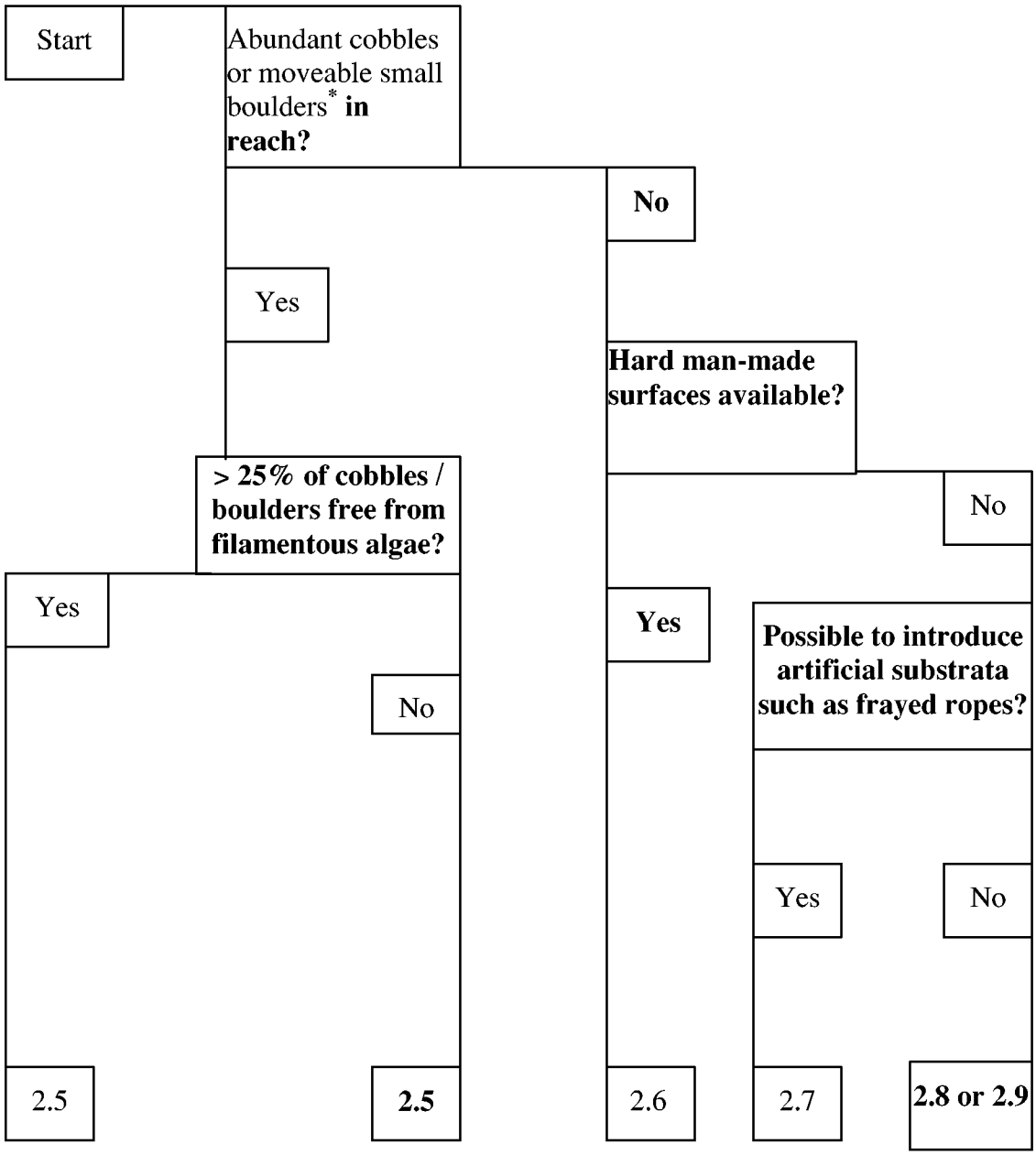

Fig. 2.1. Flow chart for selecting appropriate sampling strategies for collecting benthic diatoms for pollution-monitoring studies. * Under some circumstances (e.g. chalk streams), large pebbles may be substituted for cobbles. Increase the number of pebbles sampled accordingly.

3. the intensity of late winter or spring storms. However, as a general rule, repeat sampling should take place in the same season each year and not within four weeks of a storm or within two weeks of other periods of high flow.

4. The ability to detect a change in TDI between two sites is dependent upon the size of the dataset (see Chapter 6) and the collection of two or three samples per year from a site should be considered in order to increase the sensitivity.

5. The first survey at a particular site should be treated as exploratory and strategies may need to be modified in future surveys in order to maximise sensitivity. See Chapter 6 for more details.

6. Although diatom samples can be collected at any of time of the year, sampling in winter is not recommended, as cell growth rates are lower. Because of this, the diatom communities will have less opportunity to reflect prevailing environmental conditions.

2.4 Apparatus

- Thigh waders (and associated water safety equipment – see BT001 *Procedures for Collecting and Analysing Macroinvertebrate Samples* for more details).

- Toothbrush (this must be cleaned in stream water before and after each sample is collected to minimise cross-sample contamination)

- Plastic tray (large enough to contain a small boulder and at least 2 cm in depth)

- Wide-mouthed plastic sample bottles with watertight lids (approximately 100 ml or greater)

- Waterproof marker pen (or other means of labelling samples).

- Special equipment for scraping hard surfaces. A small funnel of net (8 cm width at mouth; 100 µm mesh size) attached to a metal frame, with a bottle to collect diatom material attached to the bottom using a Jubilee clip. This allows the bottle to be removed in order to decant the material or to be replaced with another sample bottle (Fig. 2.2). The net is attached to a wooden or metal handle approximately 45 cm in length.

- Preservative (The type of preservative is less critical than for many groups of algae where it is important to preserve delicate structures such as flagella). Formalin, alcohol (industrial methylated spirits, IMS) or Lugol's Iodine are all suitable, although formalin is not recommended for health and safety reasons. **Read Health and Safety Appendix before using any of these preservatives.** If samples are to be prepared immediately on return to the laboratory, the preservative should be omitted; however, as most samples are likely to be collected from lowland rivers with high bacterial loads, some form of preservative is recommended whenever samples are to be stored before preparation. Note that Lugol's Iodine can cause distortion of delicate frustules such as those of *Nitzschia acicularis*.

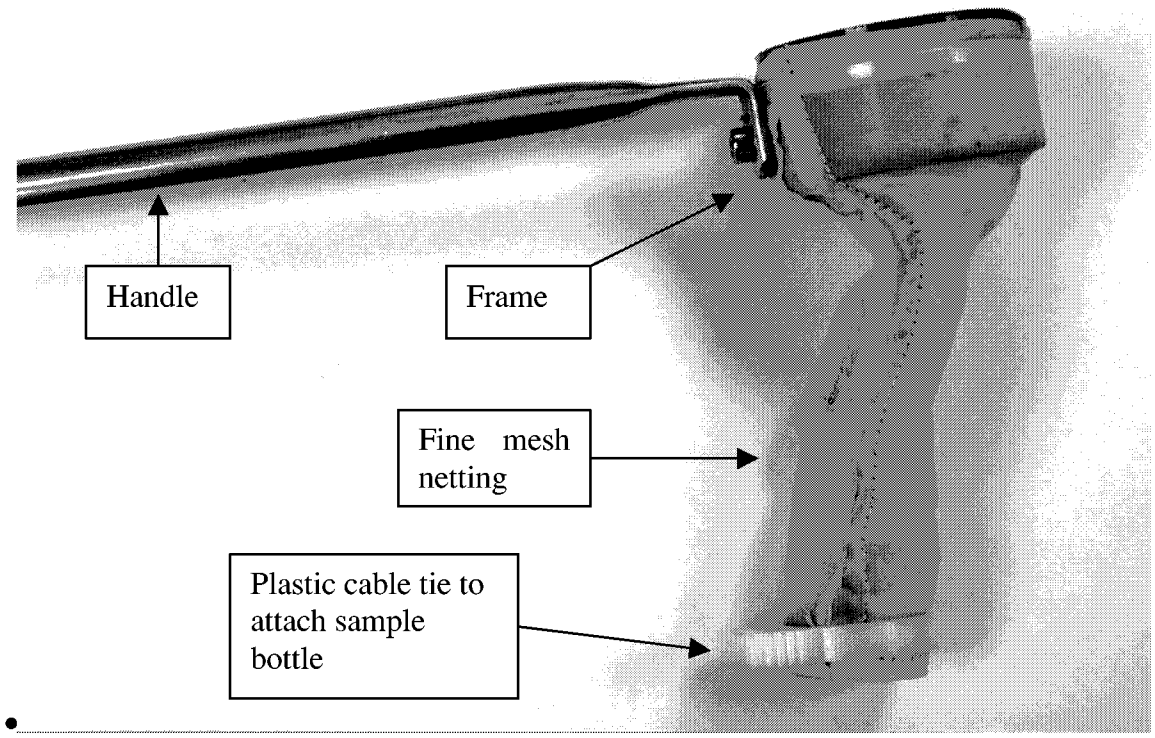

Fig. 2.2. Photograph of sampler for vertical hard surfaces.

To prepare **buffered formalin (40% formaldehyde),** add 200 g of the organic base examine (hexamethylene-tetramine) to each litre of undiluted formaldehyde. The buffer is necessary to prevent dissolution of the silica frustules

Lugol's Iodine can be prepared by dissolving 2g potassium iodide and 1g iodine crystals in 300 ml water. The resulting liquid should be straw-coloured. Some other recipes also include acetic acid or glutaraldehyde to prevent the loss of flagella. These reagents are not necessary when the solution is only to be used for diatoms. Note that iodine sublimates from most types of container, so should not be used for long-term storage. Samples should be stored in air-tight containers in a cool, dark place (such as a refrigerator) until they can be cleaned.

2.5 Method for sampling cobbles and boulders

1. At least five cobbles (> 64, ≤ 256 mm) or small boulders (> 256 mm) should be collected without bias to one side of the river or the other from areas which fulfil the microhabitat conditions described above and which have an obvious diatom film (detected by either its brown colour or slimy texture). Where suitable substrates are very abundant, random or stratified sampling strategies may be appropriate within the defined reach. Stones should be selected, as far as possible, from unshaded areas within the main flow and free from obvious filamentous algae or siltation (see above).

2. Any loosely attached surface contamination (e.g. organic debris) should be removed by washing the stone briefly in the stream water. The stones should be placed in a tray, along with approximately 50 ml of river water.

3. Wash a stiff toothbrush in clean river water and rub it on waders or a similar surface in order to remove any diatom contamination from previous samples. Brush the upper surface of the stone vigorously to remove the diatom film, rinsing the toothbrush periodically in the water in order to transfer the diatoms. If there are filamentous algae or silt deposits on the stone, try to remove diatoms from those parts of the stone which are free of such contaminants.

   Sites should be sampled in an upstream- downstream sequence. This means that the small number of diatoms transferred between sites on toothbrushes will be those that might be washed down the river naturally.

4. If > 75% of stones are smothered with filamentous algae, these should be sampled in preference to stones lacking such growths. Remove as many of the filaments as possible before brushing or scraping. See 3 above. Note this sampling strategy on the sample record form.

5. Replace the stone in the stream, and repeat the process for the other replicate stones. Transfer the water (which should now be brown and turbid due to the presence of diatoms) from the tray into the sample bottle.

6. All sample containers must be labelled, and labels should be applied before the container gets wet. Lids should not be labelled, as they can become separated from the rest of the container when the sample is being prepared in the laboratory. Labels must include watercourse name, site name, date of sampling and sampler's initials.

7. Transfer the sample to the laboratory in a cool, dark place. If samples are brought to the laboratory within 24 h and these precautions are followed, it is not necessary to add preservative in the field.

8. If samples are to be stored, add sufficient preservative (for Lugol's Iodine) either at this stage or immediately on return to the laboratory. The sample label must include details of any preservatives that have been used.

   Appropriate quantities of preservative:
   IMS: approximately 10% (v/v) in sample (i.e 10 ml per 100 m);
   Lugol's iodine: approximately 10 ml l^{-1} (about 6-8 drops with a Pasteur pipette);
   Formalin: approximately 2-4% (v/v) in sample (i.e 2-4 ml per 100 ml).

9. If samples are to be stored for some time, the suspension can be concentrated by allowing it to settle overnight, decanting the supernatant and transferring the sediment to a smaller (e.g. 60 - 100 ml) bottle

2.6 Method for sampling vertical man-made structures *in situ*

1. The apparatus (see 2.4) should be scraped along the surface to be sampled at a depth of about 30 cm (to allow for fluctuating water levels and wave action). The diatom film, dislodged as a result of this scraping, falls into the net and bottle.

2. This scraping should take place at five different places (to simulate the five replicates in 2.5). The total area covered must be at least 10 cm^2. If the diatom film is sparse, then this area should be increased.

3. See 2.5 points 6 to 9 for instructions on labelling, preservation, transport and storage of samples.

4. The net and collecting bottle (if used) must be well rinsed between samples.

2.7 Method for installing and using frayed polypropylene rope as an artificial substrate

Precise details of the deployment of rope substrates cannot be given. This is an area where the ingenuity of the sampler needs to be exploited to the full. The following points represent principles that must be followed on all occasions. These are followed by some examples of "best practice" developed within the Agency over the past five years and from the literature (Cattaneo & Amireault, 1992; Kelly *et al.*, 1998).

1. Artificial substrates must be left in the river for a minimum of four weeks. Longer periods of exposure may be appropriate under some circumstances (i.e. very oligotrophic conditions, low temperatures, heavy shade).

2. At least five replicate substrates should be sampled. However, extra, replicate substrata should be deployed, to allow for potential losses due to spates or vandalism. Care should be taken with the design and deployment of artificial substrates to ensure that they do not interfere with the activities of legitimate river users and to minimise risks of vandalism.

3. Where artificial substrates are to be used for comparative studies in the same watercourse (e.g. upstream and downstream of a sewage outfall), it is important that all are exposed to identical conditions. Both the length of exposure and the start date must be the same (to allow for the impact of spates upon the developing diatom community).

4. At the end of the exposure period, the final 5 cm of the rope should be removed with a pair of scissors and placed in a sample container.

5. See section 2.5 points 6 to 9 for guidance on labelling, preservatives, transport and storage.

6. There are two options for treatment prior to analysis. If samples are prepared using the hot hydrogen peroxide method, then entire rope samples should be placed directly in flasks and hydrogen peroxide added directly. Alternatively, the ropes should be either shaken vigorously in either stream water or distilled water to dislodge attached diatoms or they should be scraped using a toothbrush. The rope

should then be removed and the suspension decanted into a sample bottle. This procedure can take place either in the laboratory or the field.

Best practice for the deployment of ropes

The aim of rope substrates is to simulate fine-leaved aquatic macrophytes such as *Ranunculus* or *Potamogeton*. The length of rope required will depend upon water depth (i.e. sufficient to allow sufficient rope to be sampled (see 2.7 point 4) to be suspended just under the water surface Rope with a diameter of about 7 mm is recommended. Polypropylene rope, available from DIY stores, has been used extensively, but other types of rope may also be suitable. No tests have been performed on the relative performance of different types of rope.

Attachment: different Regions have experimented with the use of bricks (to anchor one end of the rope) and stakes to attach the rope to the river bed. Scan the river bed first to ensure that there are no buried electric cables. Further options include attaching ropes to bridge supports or to buoys (in the case of navigable rivers). The ropes should be placed far enough away from the bank that they do not become entangled with bankside vegetation, and are not heavily shaded. A nail, pushed through the rope about 30 cm from the end, has also been used as a means of keeping the end of the rope submerged; however, this practice may not be suitable in all rivers, particularly if there is a risk of injury to the public. The function of the nail is to keep the rope floating just under the surface; the weight should not be sufficient to bring the rope into contact with the river bed.

Minimising losses: where possible, inform river users of the use of the ropes. In one Region, bailiffs were consulted for advice on the best places for deployment and also asked to "keep an eye" on the ropes during the exercise. Local angling clubs were informed of the Agency's activities, with emphasis given to the long-term goal of restoring fisheries in the catchment. A little extra effort to place the ropes out of sight of footpaths and away from places with easy access to the river is well worthwhile.

2.8 Sampling emergent macrophytes

In general, samples should be collected from emergent macrophytes such as *Sparganium* only if there are portions that remain permanently submerged but which are not contaminated by the bottom sediments.

1. As far as possible, comparative studies in rivers should be based on samples collected from the same macrophyte species (or group of closely-related species). .

2. Details of the species of macrophytes used should be included with field sampling notes.

3. At least five stems should be cut at water level and a plastic sampling bottle or glass jar put upside down on the underwater stem. The stem should be cut below the mouth of the bottle, then the bottle plus stem turned upside down and closed. Diatoms should be removed from the stem in the laboratory by stirring, scraping or gentle brushing.

   If the diatom film is not delicate, it may be possible to cut 5-6 stems and transfer

these directly to a sampling container without the need to invert bottles over the stem.

4. See section 2.5 points 6-9 for guidance on labelling, transfer to the laboratory and storage.

2.9 Sampling submerged macrophytes

1. As far as possible, comparative studies in rivers should be based on samples collected as far as possible from a single macrophyte species (or group of closely-related species) that is sufficiently abundant at the reach to facilitate sampling of the same species in the future. Genera such as *Myriophyllum*, *Ranunculus* and *Potamogeton* are all suitable.

2. Five replicates should be sampled and placed into plastic bags for transfer to the laboratory. Each replicate should consist of a single stem plus associated branches of the plant from the lowest healthy leaves to the tip. Submerged leafless stems should not be included. Diatom epiphytes should be present as a brown floc or film associated with the macrophytes.

3. In the laboratory, the plants should be stirred or shaken vigorously in some distilled water in a large beaker to dislodge attached diatoms. The macrophytes should then be removed from the beaker and the diatoms allowed to settle, before the supernatant is poured off.

4. Alternatively, some lengths should be cut at random from submerged plants using a knife or scissors and the sections placed into a sampling bottle. These can be fractionated further in the laboratory if required and the macrophyte sections plus attached diatoms placed directly in a flask for cleaning (6.4.2).

5. See section 2.5 points 6-9 for guidance on labelling, transfer to the laboratory and storage.

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3. PREPARATION OF DIATOMS FOR MICROSCOPY

3.1 Introduction

This chapter describes how to prepare permanent slides from benthic diatom samples collected from rivers. For ease of identification, all internal contents of the frustule (chloroplast, cytoplasm etc.) need to be removed, along with extraneous organic materials that were included in the sample. The "cleaned" valves (digestion usually separates the two valves of the frustule) are then mounted in a special mountant with a high refractive index in order to make it easier to see surface ornamentation such as striae.

Permanent slides should be stored in herbaria for future reference. They can also be easily posted from one laboratory to another so that identifications of "difficult" taxa can be checked and so that slides can be subjected to audits.

There is not a "right" way to clean diatom frustules. All diatomists have their own particular method. All agree in the use of one or more oxidizing agents, which are, by their very nature, highly reactive and/or explosive. **For this reason those preparing diatoms for microscopy must be fully conversant with the appropriate health and safety guidelines before they start (see Appendix C) and Good Laboratory Practice must be followed at all times.** Methods are not inherently dangerous if proper precautions are followed; however, many Agency biologists will have had little experience of using strong mineral acids and oxidising agents. Appropriate safety training must take this into account. Two methods are described here; however, other methods based on strong oxidising agents are also suitable. References for the two methods included are Hendey (1974) and Battarbee (1986), whilst Barber and Howarth (1981), Round (1993) and the forthcoming CEN standard (draft BS EN 13946) provide alternative approaches.

Note that samples rich in organic matter in addition to diatoms need stronger oxidation than samples from relatively "clean" sites and the optimum ratio of sample to oxidants may need to be tested first. Solid carbonates can cause problems and may influence the type of treatment and the sequence of oxidants used (for example, in carbonate-rich waters the carbonates have to be removed by HCl before using strong sulphuric acid in order to prevent formation of gypsum). Treatment with dilute HCl will also be necessary for samples that are rich in iron.

Note that it is extremely important to avoid contaminating samples with diatoms from elsewhere. For this reason, glassware must be scrupulously clean and if glass rods are used for stirring samples, these must not be used in more than one sample, in order to prevent diatoms being transferred from one tube to another. Workers must follow a strict protocol to ensure that no cross-contamination of samples occurs.

3.2 Cold acid (permanganate) method

This method is suitable for all types of sample described in Chapter 2, with the exception of circumstances where entire rope or macrophytes samples need to be digested.

3.2.1 Reagents and apparatus required

Figures in square brackets give some indication of the quantities of reagents required for a "typical" sample. All of these reagents are stable under normal conditions and if you are planning to look at lots of diatom samples in the next few months then it is worth preparing large batches of the reagents.

The limiting step in terms of efficient use of time is centrifugation. A batch size that is equal to or divisible by the number of buckets available on the centrifuge is recommended. Use of centrifuge tubes as digestion vessels is recommended.

Analytical grade reagents are not necessary for this procedure.

- Dilute (e.g. 0.1 M) hydrochloric acid (another mineral acid will do if HCl is not available) [about 5 ml per sample];

- Concentrated sulphuric acid [5 ml per sample];

- A saturated solution of potassium permanganate [a few drops, approximately 1 - 2 ml per sample];

- Saturated oxalic acid (dissolve approx. 10 g oxalic acid in approx. 100 ml distilled water over gentle heat whilst stirring. Allow to cool. Crystals of oxalic acid should precipitate out. If not, add a further 10 g (approximately) of oxalic acid and reheat. [10 ml per sample]

- Distilled water. Ultra-high purity water can also be used, but is not necessary.

- Centrifuge and appropriate tubes (these must be large enough to contain all the reagents listed above, plus space for dilution with distilled water. 30 - 50 ml capacity is recommended. Check, if using plastic tubes, that they are resistant to the reagents involved before starting).

  A further word of advice: if you do use a centrifuge, don't leave it in the fume cupboard when you are not preparing diatoms and periodically strip it down and clean and grease all moving parts. Exposure to corrosive vapours can shorten its life significantly.

- A means of pipetting 5 - 10 ml volumes of acids (an automatic pipette is suitable, if it is periodically stripped and cleaned in order to prevent corrosion).

- Glass stirring rods (one per sample : you can also use disposable Pasteur pipettes)

- Disposable Pasteur pipettes.

- Labelled sample vials with secure lids. A capacity of 10-20 ml is recommended. The label must be waterproof and any writing on the label must use a waterproof pen.

- Basic laboratory safety equipment (fume cupboard, gloves, lab. coats etc.)

3.2.2 Preliminary sample preparation

1. Scan material and remove large pieces of silt and sand. If necessary, pass sample through a 1 mm sieve to remove coarse particles. Homogenise the sample by shaking and transfer 5 – 10 ml of the suspension into a centrifuge tube. Always keep some of the sample in reserve, in case of disaster.

2. If calcareous material is present, add a few drops of dilute HCl and shake gently. The material should effervesce as the carbonates are reduced to CO_2. Continue adding dilute HCl, and agitate the tube gently until effervescence stops.

   This stage can be omitted if you are sure that the sample does not come from a site with any calcareous rock in the catchment.

3. Add distilled water to 1 cm below the rim of the centrifuge tube and centrifuge to remove the acid.

   The purpose of centrifugation is to separate solid and liquid phases. The speed and time are not critical. A speed of 3000 – 5000 rpm for five minutes has been used successfully in past but the efficiency depends upon the gravitational force exerted (which is a function of the rotor properties as well as the speed) and users must satisfy themselves that these conditions are suitable for their own equipment before proceeding.

3.2.3 Sample oxidation

1 Pour off all of the supernatant and treat the waste liquid appropriately.

2 Add 5 ml concentrated sulphuric acid and agitate gently.

3 Add a few drops of a saturated solution of potassium permanganate and agitate gently. After this step, the suspension should have a purple colour. If this is not the case, add a few more drops.

4 Add 10 ml saturated oxalic acid slowly to the sample by trickling it down the wall of the centrifuge tube. There will be much fizzing and effervescence, which is why this must be done slowly. The end product should be a clear solution with a bleached precipitate at the bottom of the tube. If this is not the case, and organic matter has not been fully oxidised, add more oxalic acid until the colour change is complete.

   Do not add more than 5 ml additional oxalic acid after the first 10 ml. If there is still unoxidised organic matter, you should centrifuge the sample, then repeat steps 1 to 5. Alternatively, the procedure should be repeated with a smaller quantity of material.

5 Add distilled water to 1 cm below the rim of the centrifuge, mix the sample and centrifuge as before. Decant the supernatant and treat the waste liquid appropriately. Repeat this centrifugation stage twice more. After the supernatant has been decanted for the third time, test the pH of the remaining suspension with indicator paper. If it is still strongly acid, then the centrifugation step should be

repeated. (Note that ultrapure water typically has a pH of about 5-6, so it is not possible to obtain a completely neutral solution)

6 When the acid has been removed, transfer the cleaned material to a small glass vial, using a clean Pasteur pipette. Add a small amount of distilled water to wash material from the wall of the tube. This sample must be labelled with waterbody name, reach name and sample date as a minimum and should be stored prior to slide preparation. A preservative (a few drops of formaldehyde or alcohol) should be added to prevent microbial growth.

7 See 3.5 for details of slide preparation.

3.3 Hot peroxide method

This method is suitable for all types of sample described in Chapter 2, including circumstances where entire rope or macrophytes samples need to be digested.

3.3.1 Reagents and apparatus required

Figures in square brackets give some indication of the quantities required for a "typical" sample. All of these reagents are stable under normal conditions and if you are planning to look at lots of diatom samples in the next few months then it is worth preparing large batches of the reagents.

The limiting step in terms of efficient use of time is the number of digestion vessels that can fit on the hotplate.

Analytical grade reagents are not necessary for this procedure.

- 30% (100 volume) hydrogen peroxide (H_2O_2) solution [20 ml per sample; more if digesting rope or macrophytes samples – see note after 3.3.2 point 1]
- Dilute (e.g. 1 M) hydrochloric acid (HCl) [about 5 ml per sample]
- Distilled water. Ultra-high purity water can also be used, but is not necessary.
- Basic laboratory safety equipment (fume cupboard, gloves, lab. coats etc.)
- Beakers (or conical flasks; one per sample; 100 ml capacity recommended)
- Hot plate, with a thermostat. The hot plate must be capable of heating to 90 (± 5) º

  (Note that it is also possible to perform the digestions using a water bath in place of a hot plate, and boiling or centrifuge tube replacing the beakers)
- Means of measuring 20 ml volumes of oxidising agents (e.g. small measuring cylinder)
- Clean Pasteur pipettes
- Glass stirring rods (one per sample: you can also use disposable Pasteur pipettes)

- Centrifuge plus appropriate tubes (these need to be large enough to contain all the reagents listed above, plus space for dilution with distilled water. 30 - 50 ml capacity is recommended. Check, if using plastic tubes, that they are resistant to the reagents involved before starting).

- Sample vials for long-term storage.

3.3.2 Method

Note that one advantage of this method is that rope and macrophyte samples can be placed directly into beakers for digestion, removing the need for pre-treatment described in section 2.7 and 2.8. If this approach is adopted, then coarse material should be removed after 30 minutes oxidation.

1 Homogenize the sample by shaking, then transfer 5 - 10 ml of the suspension to a beaker. Add approximately 20 ml of hydrogen peroxide and heat on a hotplate set at 90 (± 5) ºC until all the organic material has been oxidized (typically 1-3 hours). Care is needed while pouring cold concentrated hydrogen peroxide onto organic-rich material and aquatic plants, and also during the heating process.

   Note: If rope or macrophytes samples are placed directly into beakers for digestion, then the volume of oxidising agent may need to be increased to about 50 ml per sample.

   The oxidation process involves a reaction between hydrogen peroxide and carbon atoms bound into organic molecules to produce water plus carbon dioxide. The reaction is complete when the evolution of bubbles of carbon dioxide ceases. If there is still organic matter present, then more hydrogen peroxide should be added.

2 Remove the beaker from the heat and allow to cool in the fume cupboard. Run a few drops of hydrochloric acid down the side of the beaker to remove any remaining hydrogen peroxide and any carbonates (This stage can be omitted if the sample comes from a region where carbonates are unlikely to be present in the water). Wash down sides of beaker with distilled water.

3 Transfer contents of the beaker to a centrifuge tube, top up with distilled water to 1 cm below the rim of the centrifuge tube and centrifuge.

   The purpose of centrifugation is to separate solid and liquid phases. The speed and time are not critical. A speed of 3000 – 5000 rpm for 3 - 5 minutes has been used successfully in past but the efficiency depends upon the gravitational forces exerted (which is function of the rotor properties as well as the speed) and users must satisfy themselves that these conditions are suitable for their own equipment before proceeding.

4 Decant the supernatant, treating the waste liquid appropriately. If the reaction is complete, then the hydrogen peroxide should all be oxidised, so supernatant can be washed down the sink with plenty of water Resuspend pellet with distilled water to 1 cm below the rim of the centrifuge tube and repeat centrifugation stage twice more.

5 When all traces of hydrogen peroxide and acid have been removed, transfer the cleaned material to a small glass vial, using a clean Pasteur pipette. Add a small amount of distilled water to wash material from the wall of the tube. This sample must be labelled with waterbody name, reach name and sample date as a minimum and should be stored prior to slide preparation. A preservative (a few drops of formaldehyde, alcohol or hydrogen peroxide) should be added to prevent microbial growth.

3.4 Preparation of permanent slides

3.4.1 Apparatus and reagents

Brand new glassware (slides, coverslips, Pasteur pipettes) must be used to minimise risks of contamination.

- **Naphrax**. This is a diatom mountant, supplied in toluene. It can only be bought from:
  Northern Biological Supplies,
  3 Betts Avenue,
  Martlesham Heath,
  Ipswich IP5 7RH.
  (tel: 01473 623995; fax: 01473 612148)

  At the time of writing, it costs £5.00 (+ VAT and P&P) for 15 ml.

- **Microscope slides**. Note that most microscope slides are washed in detergent prior to sale. Slides for the preparation of permanent diatom mounts should not be washed. **Either** purchase orders should specify unwashed slides, **or** slides should be washed in ethanol prior to use to remove any residues of detergent.

- **Coverslips**. Shape and size are not critical, but for long term storage it is necessary for the mountant to cover the entire coverslip. This is easiest to achieve using small (e.g. 19 mm or less), round coverslips but square coverslips can also be used so long as this criterion is fulfilled. The thinnest practical coverslips (thickness 0) are recommended as these minimise the distance between objective and diatom, but thickness 1 and 1½ can also be used.

- **Disposable Pasteur pipettes**

- **Ethanol**

- **Forceps** (spade ended are best for holding coverslips)

- **A source of heat,** such as a Bunsen burner, spirit lamp or hotplate. Different stages of the process require different intensities of heat, so more than one heat source is recommended.

3.4.2 Method

As for other stages in the preparation of diatom slides, there is no "right" way to prepare permanent slides, so long as the finished slides fulfil certain criteria. There is a definite "knack" to preparing slides, especially in judging the density of suspensions from which the slides are prepared. This comes with experience, and beginners may have to experiment to develop their technique.

The principle is that a drop of the cleaned suspension, prepared according to section 3.2 or 3.3 is evaporated gently onto a coverslip, which is then mounted onto a slide. This slide will then be used for the analysis of the diatom assemblage and calculation of the TDI and, for this reason, it is important that the final suspension is of a low enough density to permit easy identification and counting, and that factors which contribute to non-random distributions (such as edge effects) are minimised.

1. Shake a vial of material, cleaned according to section 3.2 or 3.3, and hold it up to the light. Fine particles should be just visible in the suspension. If the suspension appears milky or turbid, distilled water should be added to reduce their concentration. If no particles can be seen, then allow the suspension to settle and then carefully decant off some of the excess liquid. Note that ethanol can be used to dilute the suspension (but not more than 5-10% v/v final concentration); this reduces the density of the solution and allows the drop to spread out more on the coverslip.

2. Use a Pasteur pipette to remove some of the shaken suspension from the vial and put a drop onto a clean cover slip (Note: if you "huff" on the coverslip first, the drop will spread out more.). Room temperature (or room temperature supplemented by gentle warming from a desk lamp's bulb) is recommended. Evaporation should take place in a warm, dust-free environment and takes upwards of one hour (depending upon ambient conditions). Once all liquid has been evaporated, the result should be a thin, whitish-grey film over about half of the cover slip.

   Common reasons for uneven strews of diatoms following evaporation include too rapid evaporation (caused by high temperatures) or because there is still some residual acid left in the suspension. Some clumping of diatoms is acceptable, so long as all valves can be identified reliably, and density criteria are not exceeded.

3. The density of valves should now be checked by placing the coverslip face down on a slide and examining this under medium power objective (i.e. 400 ×). Assuming a maximum final density of about 15 valves per field of view at 1000 × magnification, this dry mount should have a maximum of roughly 30 valves per field of view. If the density is too high, repeat step 2 but with a more dilute suspension.

   Note that very gritty samples may necessitate dilute suspensions to ensure that the valves are not obscured by mineral particles.

4. Once a satisfactory density of valves has been achieved, put a small drop of naphrax onto a warm slide (e.g. using a warm hotplate) and place the coverslip face down on top of it. Heat the slide until the naphrax spreads and just begins to bubble. Apply gentle pressure (e.g. with the end of a pair of blunt forceps) to remove air bubbles. Repeat this 3 - 4 times then allow to cool. Ensure that the mountant has been properly cured by checking to see if the coverslip moves when pushed with a

fingertip. If it cannot be moved, then check the valve density again under a microscope.

5. When a satisfactory mount has been prepared, it must be labelled with the site location and date of collection and any information (i.e. site codes) to permit cross-reference with computer records.

3.5 Archiving diatom slides and samples

Diatom slides provide a permanent historical record of conditions at a site and should be stored in order to ensure that they can be accessed for future analyses. It is recommended that at least two slides are prepared from each sample. One of these should be lodged in the appropriate national herbarium, for future reference. These are:

England and Wales: The Natural History Museum, Cromwell Road, London SW7 5BD.

Scotland: The Royal Botanic Gardens, Edinburgh EH3 5LR

Northern Ireland: Botanic Gardens, Ulster Museum, Belfast BT9 5AB.

Samples to be lodged with herbaria should be labelled with the following information (use a fine-tipped pen), to make the task of curation easier:

Name of collector (if several are involved, one name plus "*et al.*" is appropriate) "Environment Agency" is adequate.
Date of collection
Locality (place name, not code)
Substratum from which sample obtained(i.e. rock, plant, sediment, artificial substrate.)
Date the mount was made
Name of mountant (Nphx for naphrax, the preferred mountant)

Storage of suspensions permits additional slides to be prepared in the future, and for further analyses (e.g. by scanning electron microscope) to be performed. For long-term storage, diatom material should be allowed to settle, the supernatant decanted and the material resuspended in methanol or ethanol. Suspensions should be stored in airtight glass vials.

4. ENUMERATION OF DIATOM SAMPLES

4.1 Introduction and scope

This Chapter describes a protocol for the identification and enumeration of relative proportions of diatom taxa on prepared slides with the intention of calculating the TDI. A high power light microscope is used to identify benthic diatoms, cleaned of cell contents and mounted in a medium with a high refractive index, until an appropriate sample size has been obtained.

The technique is, in principle, very straightforward. The analyst puts a slide on the microscope stage, focuses, finds the edge of the patch of diatoms and then slowly moves the stage horizontally underneath the objective, recording every valve seen. If the far side of the patch of diatoms is reached before the required sum is obtained, the analyst moves down (or up) the slide and starts again. The results for each taxon are then totalled and expressed as a percentage of the total number of valves counted.

In practice there are, of course, many subtleties of which a beginner needs to take account. Some of these are discussed below.

4.2 Apparatus

- **Compound light microscope**, equipped with a mechanical stage and 1000× oil-immersion lens. Use of a phase contrast or differential interference (Nomarski) condenser is recommended. The microscope must incorporate facilities for measurements (e.g. an eyepiece graticule) with a resolution of at least 1 µm. Apparatus for photomicroscopy or video capture are useful for taking pictures of difficult specimens.

- **Floras, identification guides and iconographs** appropriate to the habitats under consideration. The Field Studies Council's AIDGAP key (Kelly, 2000) was written specifically for users of the TDI. Other useful identification guides are listed in Table 4.1

- **Immersion oil** and dispenser

- **Lens tissue**

- **Facilities for recording data as it is collected.** This can be a *pro forma* count sheet with a list of taxon names and space beside each on which the counts can be made or a laboratory notebook organised in such a way that taxon identities and numbers can be clearly recorded, or a computer program with facilities for direct entry of data.

- **Facilities for verifying the identity of difficult specimens.** This can take several forms: high quality photomicrographs or captured video images may suffice. However, it is also useful to be able to relocate actual specimens if a second opinion is not available immediately. If taxonomic assistance is available "in house", noting co-ordinates on the microscope's Vernier scale may be sufficient. If another microscope is likely to be used, then a facility to record the absolute position of the

specimen may be necessary. One such device is an England Finder which ensures that the same point on a slide can be located on a different microscope, which means that a slide could be posted to another diatomist (who also needs to have an England Finder) for a second opinion. England Finders are available from:

Graticules Limited
Soverign Way,
Tonbridge,
Kent
(Tel: 01732 359061)

4.3 Preliminary steps

4.3.1 Taxonomic criteria

The minimum acceptable level of taxonomy is the checklist of TDI taxa provided in Chapter 5. This is the level of taxonomy that is covered by Quality Assurance protocols outlined in Chapter 7. Identification to species is not necessary for calculation of the TDI, but may be appropriate for some other purposes. It may also aid subsequent data interpretation.

4.3.2 Sample size

The sample size quoted in the first edition of the TDI manual was 200 valves. This guideline has been changed and the recommendation now is that the sample size should be 300 valves of which at least 200 must contribute information on the trophic status of a reach. Taxa that would not contribute to the target of 200 include phytoplankton (as in the first edition) but also some other taxa with broad ecological tolerances (such as *Cocconeis placentula* and *Navicula lanceolata*) that can dominate the flora under some conditions. This strategy is necessary because such taxa can obscure patterns of less-common taxa that may convey useful environmental information. When these taxa are overwhelmingly dominant, then either the total should be increased, or a stratified counting procedure should be adopted (see 4.4). Any such steps must be noted and explained in reports. Chapters 5 and 6 explain how such counts should be treated and interpreted.

Planktonic taxa are a particular problem downstream of impoundments and in some lowland rivers. For the purposes of this study, planktonic taxa are: *Acanthoceras, Asterionella, Aulacosira, Chaetoceras, Cyclostephanos, Cyclotella, Skeletonema, Stephanodiscus, Thalassiosira* and *Urosolenia*. Under some conditions, *Fragilaria crotonensis,* some *Tabellaria* spp. and *Nitzschia acicularis* can also fall into this category. In practice, only *Cyclostephanos, Cyclotella* and *Stephanodiscus* are likely to be of quantitative importance in rivers, although other taxa can be abundant immediately downstream of lakes and impoundments at certain times of the year.

4.3.3 Preparation of microscope

The eyepiece graticule, or other measuring equipment, must be calibrated against a stage micrometer prior to the analysis. The results of this calibration must be displayed in a position where users of the microscope can easily consult them.

Table 4.1 Keys and identification guides suitable for use with the TDI. Many older keys are also useful; this table lists those that are currently in print.

Author(s)	Comments
Hartley *et al.* (1996)	Drawings of most species of diatom recorded from the British Isles. No keys but a very useful compendium of illustrations.
Cox (1996)	Keys are designed for use with live, rather than cleaned material. However, it is still a useful adjunct to publications adopting a more traditional approach.
Kelly (2000)	Includes all TDI taxa and subjected to Field Studies Council's stringent tests prior to publication.
Krammer and Lange-Bertalot (1986-1991)	Standard European flora in four volumes. Written in German but well-illustrated. Many nomenclatural differences to the TDI.
Prygiel and Coste (2000)	Well illustrated key to most common taxa, designed for use with French Indice Biologique Diatomées. Includes a CD-ROM with an electronic version of the key.
Hustedt (1930)	An old key with line illustrations of most of the common taxa. Available in facsimile edition from Koeltz Scientific Books, PO Box 1360, D-61453, Königstein, Germany.

The second eyepiece may be equipped with a second graticule to aid enumeration. This can take several forms: including a square grid, H-shape, Whipple field etc. The important point is that this is linked to a "house rule" that ensures that no diatom is counted more than once. Two options for enumeration are:

1. (Recommended for routine use) A slow vertical or horizontal traverse is performed, with each diatom identified and added to the total as it passes one of the lines on the eyepiece graticule; or,

2. All diatoms visible in a field of view (or within the grid of a graticule) are identified and counted before **either** moving along a horizontal or vertical traverse to the next field **or** selecting a new field of view at random.

In both cases, the procedure is repeated until the total has been reached.

A further "house rule" is needed to cover situations where a diatom is only partially inside a defined counting area. Such a rule might include taxa that are only partially visible at the upper but not the lower margin (in the case of horizontal traverses) or the left but not the right margin (in the case of vertical traverses). The precise form of this rule is less important than consistency in its use when analysing samples.

The field of view visible at each magnification must be calibrated against the Vernier scale on the microscope's mechanical stage. Whether a horizontal or vertical traverse is used, it is important that each subsequent traverse does not overlap with the previous one. The distance that the stage is moved on each occasion must also account for any diatoms only partially visible in a field of view. If sample analysis is unlikely to be completed in a single session, then it is useful to record the position of each traverse using the Vernier scale. This ensures that subsequent traverses do not overlap with those already completed. (Additional precautions are required if more than one microscope is likely to be used for analysis, as positions on Vernier scales may differ between microscopes.)

4.3.4 Treatment of broken and other unidentifiable diatoms

Include a valve in a count only if both the central area and one pole are present (see Fig. 4.1). Otherwise omit. For *Asterionella, Nitzschia, Diatoma* and other taxa with no clear central area, count the poles and divide by two. This avoids the possibility of double counting.

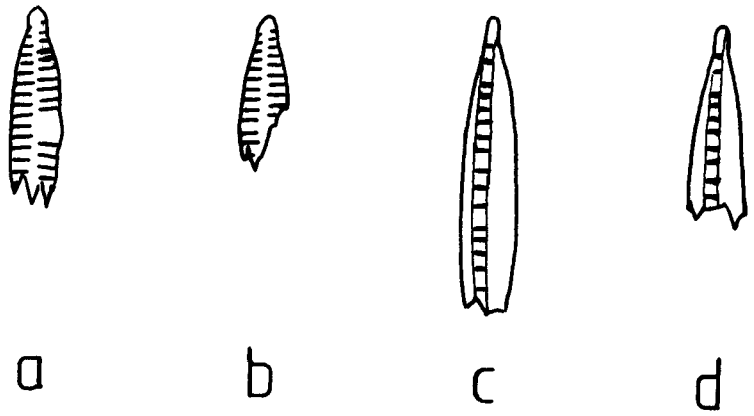


Fig. 4.1. Two examples of how to treat broken valves in TDI analyses: *Fragilaria vaucheriae* (a and b) and *Nitzschia dissipata* (c and d): "a" shows a broken valve of *F. vaucheriae* with one pole and a central area, which should be included in a count; whilst "b" shows a broken valve with one pole but no central area. This should not be included. *N. dissipata* does not have an obvious central area. Comparison with intact specimens will indicate that the specimen in diagram "c" should be included, whilst that in "d" should be excluded.

Because physical damage during sampling and preparation is likely to be negligible, the presence of many small fragments of diatoms may indicate that dead diatoms are being washed in from upstream sites so a note to this effect should be made on the count sheet/lab notebook.

A diatom may not be identifiable for a number of reasons. If overlying material obscures many valves, new slides should be prepared using more dilute suspensions.

Some taxa are recognisable from girdle views, either because the girdle view is particularly characteristic (e.g. *Rhoicosphenia abbreviata*) or because the girdle view can be assigned with confidence to a particular taxon by "matching" it with corresponding valve views of taxa found in the sample. However, this is not always possible and, if in doubt, the analyst should record the girdle views at the lowest level to which they can be assigned with confidence (e.g. "unidentified *Gomphonema* sp.", "unidentified pennate girdle view").

This convention should also be applied to any other individuals found on the slide but not identifiable by the analyst. A large number of such individuals may indicate a problem with either slide preparation or the identification skills of the analyst. **Not more than five per cent of the total count should be composed of unidentifiable individuals.** If this limit is exceeded, then the analyst should seek help to remedy the problem (e.g. by asking a more experienced analyst to examine the problem valves).

4.4 Analytical procedure

1. Make a preliminary check of the microscope and, if necessary, clean the eyepiece lenses using a piece of lens tissue.

2. Place a slide on the stage and copy relevant information from the slide label to the record sheet or computer program. The minimum information is sample number, river name, site name, sample date. Other essential data are the date of the analysis and analyst's name.

3. Select an appropriate starting position on the slide. The edge of the dried sample suspension is recommended if horizontal or vertical traverses are used, but if this approach is adopted, ensure that there are no significant "edge effects".

   An alternative to the use of traverses is to use random fields. If this approach is adopted, then random fields should be located using the Vernier scales on the microscope in conjunction with either tables of random numbers or random number functions within computer programs or electronic calculators.

4. Using a 100× objective, identify all valves present in the first field of view. Use the fine focus mechanism to differentiate between a single valve and an intact frustule. Record an intact frustule as two units.

   An intact frustule will have two distinct planes of focus when the striae, raphe and other structures will be clearly visible. Careful use of the fine focus mechanism should enable these to be differentiated. An intact frustule also often has different optical properties to a single valve.

The list of taxa used in the TDI represents the minimum level of taxonomy that is required. Additional taxonomic information should also be recorded where possible as it may be useful in the future.

Beginners and less-experienced analysts may find it useful to scan the slide carefully and make a list of all taxa encountered before they start the analysis. They should take particular care to finding both raphe and non-raphe valves of *Achnanthes* and *Cocconeis* species, and their relatives.

Occasional filaments should be recorded as the corresponding number of valves. If large numbers of valves are found in filaments, a new preparation, using a more aggressive mix of oxidising agents, should be considered.

5. If a diatom unit cannot be identified for any reason, follow conventions outlined in 6.4. Photographs, "captured" digital images or detailed drawings should be made. Notes should also be taken of shape and dimensions of the diatom unit, striae density and arrangement (at centre and poles), shape and size of central area, number and position of punctae and arrangement of raphe endings. The position of the specimen on the microscope slide should also be recorded, using the microscope's Vernier scale or an alternative means.

6. Once all taxa within the first field of view have been recorded, the count should continue until the preliminary target of 300 valves has been reached. At this point, the analyst must decide whether this target is sufficient, or if a larger count is required. If the sample is overwhelmingly dominated by a cosmopolitan taxon, then a stratified procedure should be adopted. Once the initial target has been reached, the proportion of the dominant taxon is noted and the analyst continues counting, including all taxa except this taxon until the revised target is reached. For the TDI, this revised target is 300 valves, excluding the dominant taxon from the sum. The numbers of the dominant taxon are then scaled up, in proportion to its representation after the first target had been achieved.

7. For some purposes, it is useful to continue to scan the slide after the required number of diatom units has been counted, and any taxa encountered that were not included in the count should be identified and recorded as "present". A further scan using a medium power magnification (e.g. 400 ×) may also be appropriate in order to note any larger taxa (e.g. *Gyrosigma, Didymosphenia*) which can escape detection at higher magnifications.

8. At the end of the analysis, the slide should be removed from the mechanical stage and excess immersion oil wiped from the objective and slide.

5. CALCULATING THE TROPHIC DIATOM INDEX

5.1 Introduction

This chapter describes how the TDI is calculated from data analysed as described in the preceding chapters. Full details of how the original version of the TDI was derived are given elsewhere (Kelly and Whitton, 1995b; Kelly, 1998). Since then there have been a number of nomenclatural changes which affect freshwater diatoms (summarised in Whitton *et al.*, 1998). Experience gained over the past five years has also indicated that some taxa, previously lumped into broad generic categories, are sensitive indicators under certain environmental conditions. These two factors have resulted in a number of new categories in the TDI.

The TDI is based on the weighted average equation of Zelinka and Marvan (1961):

$$index = \frac{\sum_{j=1}^{n} a_j s_j v_j}{\sum_{j=1}^{n} a_j v_j}$$

where a_j = abundance or proportion of valves of species j in sample, s_j = pollution sensitivity (1 - 5) of species j and v_j = indicator value (1-3). Values of sensitivity (s) are as follows:

1 = favoured by very low nutrient concentrations
2 = favoured by low nutrient concentrations
3 = favoured by intermediate concentrations of nutrients
4 = favoured by high concentrations of nutrients
5 = favoured by very high concentrations of nutrients

In addition, a few taxa have TDI sensitivity values of zero. These include a few taxa that are relatively rare in freshwaters and whose ecological preferences are not well defined, along with planktonic taxa, which are routinely excluded from calculations. Chapter 6 describes situations when further taxa might be excluded under certain circumstances in order to improve the sensitivity of the TDI.

Calculating this equation gives the "weighted mean sensitivity" ("WMS") of the taxa present in the sample. This varies from 1 (for sites with very low nutrient concentrations) to 5 (for sites with very high nutrient concentrations). TDI is the WMS expressed on a scale from 0 to 100. It is calculated as follows:

$$TDI = (WMS \times 25) - 25$$

The WMS can be calculated using either the number of valves of each taxon recorded, or the percent that each taxon contributes to the total. The former is quicker, but the latter allows differences between sites to be more readily compared. It is recommended that raw numbers are entered directly into databases or spreadsheets used for calculations, in order to avoid calculation errors.

5.2 Nutrient sensitivities and indicator values

The list of sensitivities and indicator values for each taxon included in the TDI is given in Table 5.1, based on Kelly and Whitton (1995a). Changes from the previous version of the TDI are printed in bold. These include incorporation of recent nomenclatural changes in order to harmonise the TDI with the latest taxonomic checklist (Whitton *et al.*, 1998) and splitting off some species from broad generic categories in order to increase sensitivity under certain circumstances. A pragmatic approach has been adopted, in order to balance the sensitivity of the index with ease of use. The genus *Rossithidium*, for example, was split from *Achnanthes* (Round & Bukhtiyarova, 1996), but contains no representatives that are common in rivers so, rather than add to the taxonomic burden, this genus has been retained within *Achnanthes* (other). Similarly, not all of the new genera that have been split from *Navicula* in recent years (e.g. *Hippodonta, Geissleria, Mayamaea, Eolimna, Fistulifera*) are distinguished in the TDI and users should continue to use either *Navicula* (other) or *Navicula* (small) for these, as appropriate.

Table 5.1. Nutrient sensitivities (s) and indicator values (v) for taxa included in the TDI. No entry in comment column indicates no change since previous version of index.

Taxon	s	v	Comment
Achanthoceras	0	0	Moved from *Attheya* (**plankton**)
Achnanthes clevei			**See under *Karayevia***
Achnanthes conspicua	**5**	**2**	**New taxon**
Achnanthes lanceolata type			**Moved to *Planothidium***
Achnanthes lauenburgiana			See under *Psammothidium lauenburgianum*
Achnanthes oblongella	**1**	**2**	**New taxon**
Achnanthes ploenensis			See *Kolbesia*
Achnanthes subatomoides			See under *Psammothidium subatomoides*
Achnanthidium	2	2	**Previously *Achnanthes minutissima*-type.** Includes *A. minutissimum, A. microcephalum, A. biasolettiana*
Achnanthes (other)	3	1	Excluding taxa now moved to *Achnanthidium, Planothidium, Psammothidium, Karayevia, Kolbesia* but including *Rossithidium*
Amphipleura	1	3	**Motile**
Amphora pediculus	5	2	
Amphora (other)	5	1	
Anomoeoneis	4	1	**Motile**
Asterionella	0	0	**Plankton**
Attheya			see *Achanthoceras*

Taxon	s	v	Comment
Aulacosira	**0**	**0**	**Plankton**
Bacillaria paxillifer	**4**	**1**	**New taxon Motile**
Brachysira	1	3	**Motile**
Caloneis	3	1	**Motile**
Ceratoneis arcus			see *Hannaea*
Chaetoceras	0	0	**plankton**
Cocconeis pediculus	4	2	
Cocconeis placentula	3	2	
Cocconeis (other)	2	2	
Craticula	**5**	**1**	**New taxon. Motile.** Split from *Navicula* (other)
Ctenophora pulchella	2	1	Formerly *Synedra pulchella*
Cyclostephanos	0	0	**Plankton**
Cyclotella	0	0	**Plankton**
Cymatopleura	4	1	
Cymbella affinis	1	3	
Cymbella delicatula	1	3	
Cymbella microcephala	1	2	
Cymbella minuta / silesiaca			Transferred to *Encyonema*
Cymbella sinuata			See *Reimeria sinuata*
Cymbella (large forms)	4	2	Nominally > 70 µm. Includes
C. caespitosa, C. lanceolata			
Cymbella (other)	2	1	Includes *Encyonema* spp.
Denticula tenuis	2	2	Other *Denticula* spp are halophilous.
Diadesmis	**5**	**1**	**New taxon.** Split from *Navicula* (small). Same s and v values, but *Diadesmis* is tolerant to desiccation.
Diatoma mesodon	**2**	**2**	**New taxon**
Diatoma moniliforme	**3**	**1**	**New taxon**
Diatoma tenue	2	2	
Diatoma vulgare	5	3	
Diatoma (other)	2	1	
Didymosphenia geminata	2	3	
Diploneis	1	1	

Taxon	s	v	Comment
Ellerbeckia arenaria	4	2	
Encyonema minutum / silesiacum	3	2	moved from *Cymbella*
Encyonema (other)			see *Cymbella* (other)
Epithemia	1	2	
Eucocconeis	3	1	
Eunotia	1	3	
Fragilaria brevistriata			see *Pseudostaurosira*
Fragilaria capucina	2	2	
Fragilaria crotonensis	**0**	**0**	**New taxon. Plankton**
Fragilaria pinnata			see *Staurosirella*
Fragilaria vaucheriae	3	2	
Fragilaria (other)	2	1	*Staurosira* and *Fragilariforma* now placed in separate categories.
Fragilariforma	**2**	**1**	**New taxon.** Formerly included in *Fragilaria* (other)
Frustulia	1	2	**Motile**
Gomphocymbella	2	1	
Gomphoneis			see *Gomphonema* -other
Gomphonema angustatum	1	2	
Gomphonema minutum	**4**	**2**	**New taxon**
Gomphonema olivaceoides	2	3	
Gomphonema olivaceum	**5**	**2**	
Gomphomema parvulum	5	3	
Gomphonema (other)	3	1	
Gyrosigma	5	2	**Motile**
Hannaea arcus	1	3	
Hantzschia	5	1	**Motile**
Karayevia	**4**	**2**	**New taxon.** Split from *Achnanthes* (other)
Kolbesia	**4**	**2**	**New taxon.** Split from *Achnanthes* (other)
Luticola	**5**	**2**	**New taxon.** Genus split from *Navicula* (other) **Motile**
Martyana	5	2	formerly freshwater species of *Opephora*.

Taxon	s	v	Comment
Melosira varians	4	2	
Meridion circulare	2	3	
Navicula capitoradiata	**3**	**2**	**New taxon. Motile**
Navicula cryptotenella-type	5	2	**Motile.** Includes *N. menisculus, N. reichardtiana*
Navicula gregaria	5	1	**Motile**
Navicula lanceolata	5	2	**Motile**
Navicula tripunctata	4	2	**Motile**
Navicula (other)	4	1	excluding small species (see below). Including related genera such as *Cavinula* etc. **Motile**
Navicula (small species)	5	1	nominally < 12 µm. Including small species of *Sellaphora*, *Diadesmis* and related genera.
Neidium	2	3	
Nitzschia acicularis	4	1	**Motile**
Nitzschia amphibia	5	3	**Motile**
Nitzschia dissipata	5	2	**Motile**
Nitzschia fonticola	**3**	**2**	**New taxon. Motile**
Nitzschia inconspicua	**5**	**1**	**New taxon. Motile**
Nitzschia palea	**5**	**1**	**New taxon. Motile**
Nitzschia pusilla	4	2	**Motile**
Nitzschia section *Sigmoideae*	4	2	generally large (> 100 µm) forms. **Motile**
Nitzschia (other)	4	1	includes *Psammodictyon*. **Motile**
Opephora			see *Martyana*
Peronia fibula	1	3	
Pinnularia	1	3	**Motile**
Planothidium	**5**	**2**	**New taxon.** Formerly *Achnanthes lanceolata*-type, but now including *P. delicatulum* (formerly *A. delicatula*)
Pseudostaurosira brevistriata	2	2	
Psammothidium (other)	**3**	**1**	**New taxon.** Split from *Achnanthes* (other)
Psammothidium lauenburgianum	**4**	**2**	**New taxon.** Split from *Achnanthes* (other)
Psammothidium subatomoides	**2**	**2**	**New taxon.** Split from *Achnanthes* (other)

Taxon	s	v	Comment
Psammodictyon			see *Nitzschia* (other)
Reimeria sinuata	4	3	
Rhizosolenia			see *Urosolenia*
Rhoicosphenia abbreviata	5	1	
Rhopalodia	1	1	
Rossithidium			See *Achnanthes* (other)
Sellaphora			Included with small *Navicula*.
Semiorbis	1	3	
Skeletonema	0	0	**Plankton**
Stauroneis	5	2	**Motile**
Staurosira	**4**	**1**	**New taxon.** Previously included with *Fragilaria* (other)
Staurosirella	4	1	
Stenopterobia	1	1	**Motile**
Stephanodiscus	0	0	**plankton**
Surirella islandica	**1**	**2**	**New taxon. Motile**
Surirella (other)	3	1	**Motile**
Synedra pulchella			see *Ctenophora pulchella*
Synedra ulna	3	1	
Synedra (other)	4	1	
Tabellaria	2	3	
Tabularia	5	2	
Tetracyclus	1	1	
Thalassiosira	0	0	**plankton**
Tryblionella	**4**	**1**	Formerly included within *Nitzschia* (other). **Motile**
Urosolenia	0	0	**Plankton**

Table 5.2. Summary of changes to the updated version of the TDI.

Taxon	Change
Achnanthes (Achnanthidium) lanceolata-type	Moved to *Planothidium*.
Achnanthes (Achnanthidium) minutissima-type	Renamed *Achnanthidium*.
Achnanthes conspicua	New taxon added
Achnanthes oblongella	New taxon added
Fragilaria crotonensis	New taxon added
Psammothidium (other)	Genus split from *Achnanthes* (other). This broad category has the same values as *Achnanthes* (other).
Psammothidium lauenburgianum	New taxon added
Psammothidium subatomoides	New taxon added
Kolbesia	Genus split from *Achnanthes* (other). Only one common species (K. *ploenensis*)
Karayevia	Genus split from *Achnanthes*. Only one common species (*K. clevei*)
Bacillaria paxillifer	New taxon added
Craticula	Genus split from *Navicula* (other)
Diadesmis	Genus split from *Navicula* (other)
Diatoma mesodon	New taxon added
Diatoma moniliforme	New taxon added
Gomphonema minutum	New taxon added
Fragilariforma	Genus split from *Fragilaria* (other)
Luticola	Genus split from *Navicula* (other)
Navicula capitoradiata	New taxon added
Nitzschia fonticola	New taxon added
Nitzschia palea	New taxon added
Nitzschia inconspicua	New taxon added
Fragilariforma	Genus split from *Fragilaria* (other)
Staurosira	Genus split from *Fragilaria* (other)
Surirella islandica	New taxon added

5.3 Calculation of the TDI and DQI

1. Calculate, for each taxon, (abundance (a) × v) and (a × s × v)
2. Add together all values for av to give Σav
3. Repeat for asv to give Σasv
4. Calculate Σasv ÷ Σav to give WMS.
5. Calculate TDI as (WMS × 25) - 25
6. Add up the number of valves belonging to motile taxa and calculate this as a percentage of the total.

Calculation of the index can be easily automated via a spreadsheet or database package.

For some reporting purposes, results should be presented as the "Diatom Quality Index" (DQI), which is 100 - TDI.

See Chapter 6 for details of how to interpret these results.

5.4 Worked example

A practical example (River Browney, upstream of Browney Sewage Treatment Works) is presented below.

Table 5.3. Data used in calculation of TDI and DQI for site on River Browney on 5 July 1994.

Taxon	count (a)	s	v	asv	av	comment
Achnanthidium minutissimum	8	2	2	32	16	
Amphora pediculus	23	5	2	230	46	
Cocconeis pediculus	38	4	2	304	76	
Cocconeis placentula	54	3	2	324	108	
Ctenophora pulchella	1	2	1	2	1	
Cyclotella meneghiniana	34	0	0	0	0	**plankton**
Diatoma sp.	4	3	1	12	4	
Diatoma vulgare	3	5	3	45	9	
Encyonema silesiacum	1	3	2	6	3	
Gomphonema olivaceum	1	5	2	10	5	

Taxon	count (a)	s	v	asv	av	comment
Gomphonema parvulum	4	5	3	60	12	
Navicula cryptotenella	2	5	2	20	4	
Navicula gregaria	5	5	1	25	5	**Motile**
Navicula lanceolata	11	5	2	110	22	**Motile**
Navicula (small)	2	5	1	10	2	) **Motile**
Navicula tripunctata	1	4	2	8	2	**Motile**
Nitzschia amphibia	1	5	3	15	3	**Motile**
Nitzschia dissipata	7	5	2	70	14	**Motile**
Nitzschia (other)	10	5	1	50	10	**Motile**
Planothidium lanceolatum	4	5	2	40	8	
Reimeria sinuata	7	4	3	84	21	
Rhoicosphenia abbreviata	15	5	1	75	15	
Stauroneis sp.	1	5	2	10	2	**Motile**
Grand total	**237**					
Total less plantonic taxa	**203**			**1542**	**388**	
Total motile valves	**38**					

WMS = Σasv / Σav = 1528 / 388 = 3.97

TDI = (WMS × 25) - 25

= (3.97 × 25) - 25

= 99.2 -25

= 74.2 ≈ 74

DQI = 100 - TDI

= 100 - 74

= 26

%motile taxa = (38 / 203) × 100 = 18.7

Interpretation

The data indicates a fairly eutrophic site (TDI = 74). See Chapter 6 for more details on interpretation.

6. INTERPRETING THE RESULTS

6.1 Introduction

In this chapter a brief overview is given of how diatoms - through the TDI - can be used to help make decisions for the management of nutrients in rivers. Our understanding of eutrophication in running waters, as opposed to lakes, is relatively crude and we have little experience of using phosphorus stripping on large sewage works in this country. Whilst a great deal of spatial monitoring has taken place since the first edition of this manual was published in 1996, there has been little opportunity for rigorous analysis of situations before and after the installation of nutrient removal. This chapter, whilst benefiting from the experience of the past five years, should therefore be regarded as providing guidance rather than authoritative advice.

The aim of biological monitoring is to aid decision-making. Typically, data are used to infer aspects of river quality that cannot be obtained by direct physical and chemical measurements. Thus, when monitoring eutrophication, biological data are not just concerned with the amount (concentration or load) of nutrients available, but also with how the impact of those nutrients are manifested in the biota. We assume a positive relationship between the index and nutrient concentrations but also understand that part of the variation in the TDI is due to other factors (see 6.2). These factors must be taken into account when interpreting the data.

This chapter is based on the assumption that the TDI is being used to provide evidence to support a designation of a "eutrophic sensitive area" under the Urban Wastewater Treatment Directive (European Community, 1991), following guidelines issued by DETR and the Welsh Office (DETR / Welsh Office, 2000). This means that the emphasis of the chapter is on assessing large STWs (>10,000 population equivalent) discharging predominately to lowland rivers. The assumption underlying this chapter is that temporal trends in eutrophication can be inferred from predominately spatial data. (i.e. changes in eutrophication over time are measured by longitudinal differences in TDI) This assumption is valid so long as the limitations of the TDI (described in this chapter) are recognised However, issues such as the scale of within-site variation, and the influence of non-nutrient factors including seasonality and substrate also need to be considered. This interpretation, in turn, requires an understanding of basic river and algal ecology.

Further details of survey design and data interpretation are given in Wyatt *et al.* (1998), which is based around software packages available within the Environment Agency and SEPA.

Section 6.7 considers other situations where the TDI may be useful. In addition, some of the broader issues covered in the chapter may also be relevant when using other indices such as the MTR.

6.2 Influence of non-nutrient factors

6.2.1 General comments

Each organism has a unique "niche" controlled by various factors. Some factors are related directly to water quality (e.g. nutrients, ammonia) whilst others (e.g. current speed, grazing) are not. Similarly, it is also important to distinguish between factors that are related directly to the variable of interest and those caused by other variables (which might be auto-correlated with that variable). Some examples of variables that might influence the diatom assemblage and, as a result, the TDI, are listed in Table 6.1. Some of these will vary at a site on time scales ranging from days to months.

6.2.2 Assessing the reliability of the TDI

The first edition of the manual included the TDI along with a second value, Percent Pollution Tolerant Valves (%PTV). The original rationale was that %PTV helped to identify sites where the primary factor controlling community composition was organic pollution. The taxa which contributed to the %PTV were *Gomphonema parvulum*, *Navicula gregaria*, *N. lanceolata*, small *Navicula* species and *Nitzschia* (other). This proved to be useful up to a point; however, it soon became clear that the terminology was flawed, and that %PTV was being used by some as an index of organic pollution, rather than as an indication of the reliability of the TDI as an estimate of eutrophication. This was compounded by the fact that some of the taxa included in the %PTV sum were also abundant at sites where organic pollution was not a serious problem. Such instances included samples where *Navicula lanceolata* was abundant (see 6.5.1 below) and some upland sites where *Gomphonema parvulum* thrived. In addition, *Nitzschia* (other) was a very broad "dumping ground", largely due to the difficulties associated with identifying *Nitzschia* spp. This category included taxa such as *N. palea* that favoured organic pollution along with taxa such as *N. fonticola* which preferred cleaner conditions. Despite these limitations, the principle of using a second characteristic of the diatom assemblage to assess the confidence with which the TDI can be interpreted seemed fundamentally sound. However, this update of the TDI uses a slightly different criterion – the percentage of valves belonging to motile taxa (%motile) – and the rationale for using this criterion is explained below.

There is some evidence that the growth forms of diatoms can provide insights into factors influencing community composition. Sediments, for example, are typically dominated by motile diatoms which are able to avoid burial by fresh depositions (Round *et al.*, 1990). The close proximity of oxidising and reducing conditions within sediments along with often relatively high concentrations of organic matter favours the evolution of traits such as tolerance to toxins and the capacity for facultative heterotrophy within sediment-dwelling taxa. By contrast, headwater streams are typically dominated by growth forms adapted to withstand or recover rapidly from disturbances (Molloy, 1992). Within a reach there will also be temporal and spatial heterogeneity in relative abundance of growth forms, related to local hydrological conditions (Marker, 1976). Local spatial heterogeneity should not be a significant issue for users of the TDI, if the guidelines in chapter 2 (sample collection) are followed. However, if there are differences in average hydrological conditions between two reaches, then a shift in dominant growth forms might be encountered even if water

Table 6.1. Some non-nutrient factors which can influence the composition of the diatom assemblage.

Factor	Response	Examples of taxa favoured by these conditions
1. nature of substrate	• hard surfaces favoured by attached forms	*Gomphonema*
	• silty surfaces favour motile forms	*Navicula, Nitzschia*
2. presence of filamentous algae or other macrophytes	• certain taxa are characteristically epiphytic	*Rhoicosphenia*
3. current speed	• sluggish currents favour loosely-attached filaments	*Melosira*
	• fast currents favour closely-adpressed forms	*Cocconeis*
4. grazing pressure	• some species are more resistant to grazing than others	*Cocconeis placentula*
5. organic pollution	• some species are capable of heterotrophic growth	*Nitzschia palea*
6. heavy metals	• some species have morphological aberrations in the presence of heavy metals	*Fragilaria capucina*
	• some species can develop tolerance	*Achnanthidium minutissimum*
7. eutrophication	• some taxa have a competitive advantage at low nutrient concentrations	many *Eunotia* spp.
	• others grow well at higher concentrations	*Diatoma vulgare*
8. salinity	• some taxa are characteristic of brackish conditions	*Pleurosigma, Melosira nummuloides*
9. pH	• some taxa favoured by low pH	*Eunotia exigua*
10. temperature	• some taxa have a competitive advantage at low temperatures	*Navicula lanceolata, N. gregaria*

Hints for data interpretation 1: use of % motile valves

- ✓ This criterion should be used as a guide to factors controlling the composition of the diatom assemblage and not as an absolute property of the assemblage. Use the criterion as a guide to inform interpretation and comment on those taxa which are responsible for the differences
- ✓ Use a difference of 20% as a threshold when considering whether the change in % motile valves between two sites is significant. If the difference is <20%, assume that a comparison between two TDI values is valid (bearing in mind issues considered elsewhere in this chapter)
- ✓ % motile taxa usually increases downstream of a sewage discharge.
- ✓ Exclude planktonic taxa from the sum before calculating % motile taxa, but do not exclude any benthic taxa, even if using a modified TDI (see 6.2.3)

quality remained unchanged. As it is not possible, at this stage, to eliminate fully the effect of such a change on the TDI, it is important that users are aware of this possibility when interpreting results.

It is recommended that % motile valves is used to make broad comparisons between samples which may highlight non-nutrient factors influencing the flora, rather than as an absolute measure of a community characteristic.

6.2.3 Modification of TDI calculation

Where one or two taxa dominate a sample for reasons other than water quality, there is a good case for excluding them from the TDI calculation (but bear in mind provisos given in 4.3.2) This practice was recommended in the first edition of this manual for samples with a large proportion of phytoplankton. In this revised version, the same principle is extended to include "weedy" species such as *Cocconeis placentula* and *Navicula lanceolata*.

The justification for this comes from theoretical studies on species diversity, which suggest that unpredictable, unstable and severe environments will be dominated by one or a few very common species and a smaller number of less abundant species (May, 1975; Sanders, 1968). By contrast, greater diversity may be expected in situations where one or a few environmental resources control the relative abundance of species (Magurran, 1988; May, 1975). A diatom assemblage developing under low-flow conditions in summer with intense grazing is a plausible scenario (though not as yet tested) for the development of dominance by *Cocconeis placentula*. Similarly, an assemblage from early spring dominated by *Navicula lanceolata* may reflect this taxon's ability to thrive at relatively low temperatures. Interpretation of the TDI under circumstances such as these should proceed with caution as the primary assumption that nutrients are the main variable influencing community structure has been violated. There are two possibilities: either adjust the sample date or modify the TDI calculation. The former is preferable, but is not always possible. It is considered in more detail in 6.5.1. The latter option is considered here.

As a rule of thumb, any situation where extended counting protocols are invoked (see 4.4 point 6) is a candidate for modification of the TDI. The assumption underlying the modification is that sub-dominant and other taxa present in such a sample are more likely to give an indication of the trophic status than the dominant organism. The TDI is therefore recalculated with the dominant taxon removed. This strategy is worth following so long as the following conditions are met:

- The count size must be increased (or a stratified counting strategy used – see section 4.4 point 6) so that the modified TDI calculations are based on a statistically valid sample size;

- TDI results must be presented with and without modification and the methods section of any report must state clearly what has been done;

- Any report should also include an ecological justification for the modification, written in terms that non-biologists can understand;

- It is also useful to present a scatter chart showing the relationship between the original and modified TDI.

However, there are also circumstances where diversity is low, but the trophic status indicated by the diatom assemblage is clear. Assemblages with low diversity but dominated by *Eunotia* or *Achnanthidium* typically represent relatively low nutrient concentrations (within the context of UWWTD-related monitoring) whilst those dominated by small *Naviculas* and relatives represent typically nutrient rich conditions. Under such circumstances, a stratified count and modified TDI calculation may not be necessary. If in doubt, seek expert advice.

Hints for data interpretation 2: influence of non-nutrient factors

✓ When examining changes between sites, consider not just the change in the TDI, but also changes in the dominant growth forms at the two sites.

✓ Always compare "like" with "like": valid spatial comparisons can only be made on samples from similar substrates collected at the same time of year.

✓ Be aware of any known sources of other pollutants at sites included in the survey.

✓ Ensure that sample record forms include estimates of the abundance of substrate types and filamentous algae.

✓ Consider modifying the TDI calculation in order to increase sensitivity, particularly when the TDI shows little longitudinal variation despite the presence of known point sources of nutrients.

6.3 Threshold of sensitivity

Those attempting to control nutrients in rivers can learn from the experience of nutrient control in lakes. As a result of observations on a large number of lakes where efforts to reduce nutrient loading had been made, four distinct zones were recognized within which the response to reductions in P loading could be evaluated (Sas 1989). The

principles of this descriptive model are also applicable to rivers (Fig. 6.1) insofar as thresholds are recognized above which the system is saturated with nutrients and further ecological changes will not occur (Kelly, 1998). The effort required to produce an observable change will be in direct proportion to the concentration of phosphorus before nutrient reduction starts. An obvious conclusion from Fig. 6.1 is that a community-based index of eutrophication will have an upper limit of responsiveness to P (the zone I – zone II boundary on Fig. 6.1), although it is possible that the apparent relationship with P will continue above this threshold because of cross-correlations between P and other limiting factors.

The nutrient concentrations corresponding to this threshold has not been established yet in a manner that is widely applicable. However, preliminary data suggest that it may be as low as 0.3 mg l^{-1} "available" P (i.e. as "orthophosphate" or "soluble reactive P"). It is important, however, that the threshold is considered on a case-by-case basis. Two useful indicators that the threshold might be exceeded are:

- Concentrations of other nutrients, in particular N. This can be evaluated by examining the N:P ratio (based on "available" forms). Although N:P ratios are relatively crude, values > 15 can generally be regarded as indicating P-limited conditions, whilst values < 10 typically indicate N-limited conditions. An example from a lowland river is given in Fig. 6.2. The lowest N:P typically occurs in the summer, when algal growth is most prolific. More importantly, Fig. 6.2 gives an indication, for this particular river system, of target "available" P concentrations (assuming N remains unchanged) if a floristic change is to be expected. If diatom assemblages are to change in response to P reduction, then post-stripping concentrations should be low enough to ensure that N:P > 15 for most of the year (including the summer).

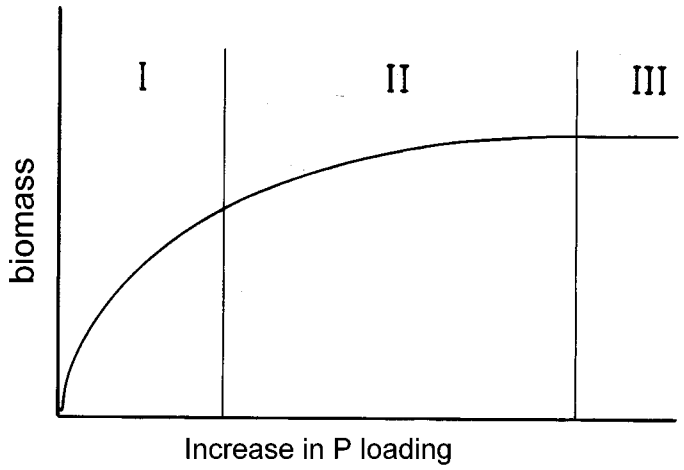


Fig. 6.1. Schematic representation of stages in response of photosysnthetic community to increase in nutrient loading. Modified from Sas (1989). The response to P loading depends upon the zone in which the initial P concentration is found: I, Increase in biomass and floristic change in response to increased P loading; II, Increase in biomass but no floristic change; III, No biomass response to increase in P loading

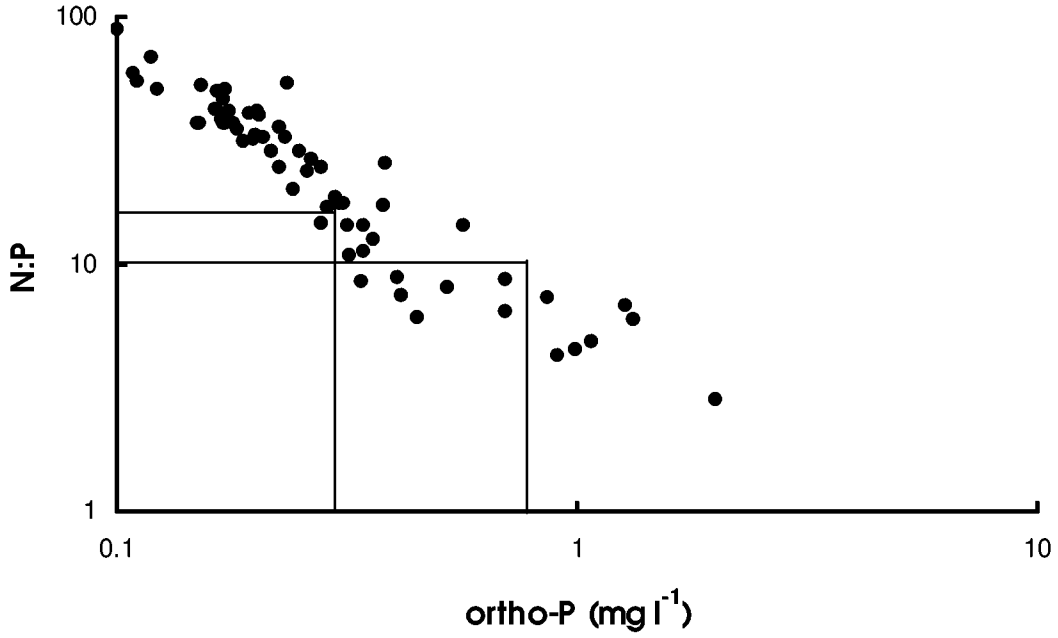


Fig. 6.2. Relationship between ortho-P concentration and N:P (as TON and ortho-P respectively) in the River Stour above and below Ashford STW, Kent. Vertical lines indicate P concentrations representing N:P ratios of 10 and 15. See text for more details.

- Factors, other than inorganic nutrients, that might influence competitive interactions between species. The first edition of this manual stressed the importance of distinguishing the direct response of the flora to nutrients from responses to factors that were correlated with nutrients. As the main source of P in lowland rivers is from sewage discharges, it is particularly important to recognise the influence of other components of sewage effluents on benthic diatom assemblages (Table 1.1; Kelly *et al.*, 1996). In broad terms, the TDI is more likely to be sensitive to change

- following nutrient removal in rivers where the levels of other physical and chemical stresses are minimal. This corresponds to General Quality Assessment biological and chemical classes A and B.

6.4 Variation in space

Changes in the longitudinal distribution of diatom taxa along stream gradients occur even in the absence of human impacts (Allen, 1995; Molloy, 1992). It is important, when using indices such as the TDI that such changes are not confused with changes due to human impacts. Most comparisons involving the TDI take place over a relatively short spatial scale (i.e. comparisons above and below a STW might use two sites < 1 km apart) in which case it should be possible to assume that changes due to non-human factors are negligible. However, this assumption must be evaluated separately for each comparison. Factors such as a major change in physical attributes (slope, current speed, temperature) between sites might invalidate such an assumption.

Hints for data interpretation 3: threshold of sensitivity

- ✓ Analyse results from TDI surveys alongside chemical data from the same sites. Do not just consider annual means, but concentrate on data from the main growing season.
- ✓ Consider N:P ratios as well as P concentrations in isolation. Use N:P > 15 as an approximate indication of a P-limited, rather than an N-limited system.
- ✓ Consider output from modelling programs such as AARDVARK to estimate the likelihood of post-stripping conditions fulfilling the criteria when a change in TDI should be expected.
- ✓ Bear in mind the influence of non-nutrient factors on diatom assemblages downstream of sewage discharges. Use information from other sources (chemical analyses, invertebrate surveys) and assume that other stress factors will be significant unless the water course falls into GQA biological and chemical classes A or B.

The issue of spatial variation is illustrated in Fig. 6.4. A strict interpretation of the UWWTD would involve a decision based on samples from sites B and C only, as these would indicate the effect of the discharge on the receiving water. However, there are three practical problems with a sampling scheme based on sites B and C only:

- If site B has nutrient concentrations that are high enough to place it in zone I or II of Fig. 6.1 then nutrient control at the STW is unlikely to have a noticeable impact on the ecology of the river at site C. If nutrient concentrations here are high enough to place site B in zone III of Fig. 6.1, then the biomass may change at site C, but the TDI will not detect this.
- Sites immediately upstream of STWs (site B in Fig. 6.4) are typically downstream of the urban area served by the STW and are often influenced by urban runoff, storm sewers and trade effluents in the town.
- Sites immediately downstream of STWs (site C in Fig. 6.4) often have high levels of other constituents of the sewage effluent (see Table 6.2) that will exert their own influence on the benthic diatom flora.

The recommendation for monitoring programs is to include an additional site upstream of the urban area (site A in Fig. 6.4) in order to indicate the contribution of the town itself to water quality at the upstream site, plus an additional site further downstream from site C (site D in Fig. 6.4). Site D should be replaced in the "recovery zone" and it should be located on the basis of local knowledge. Such a design may not always be practicable (e.g. in highly urbanised areas) and may be further complicated by confluences and other factors which affect water quality. It may not even be necessary at all sites: in some cases sites immediately upstream and downstream of STWs (sites "B" and "C") show little influence of urban runoff or serious organic pollution, perhaps reflecting efficient water collection and/or treatment and/or good dilution ratios. However, this situation must not be assumed.

A "look-up" table (Table 6.3) is provided to help with data analysis. Adjacent sites are entered into the appropriate boxes on the table and joined by arrows. A vertical movement then indicates a change in the TDI, whilst a horizontal movement indicates changes due to other factors. Table 6.4 shows an example of the look-up table as applied to the situation described in Fig. 6.3. TDI values that fall in the left-hand column should be considered as "reliable" estimates of the level of eutrophication at a particular site, although such conclusions need corroboration from replicate surveys (see section 6.5).

Look-up charts have been proved to be useful in many situations and Harding and Kelly (1999) contain examples of "look-up" charts that have been used to prepare UWWTD designations. However, they should be used as tools for exploring spatial patterns in the data rather than as standalone "evidence" of the effect of nutrients on a river.

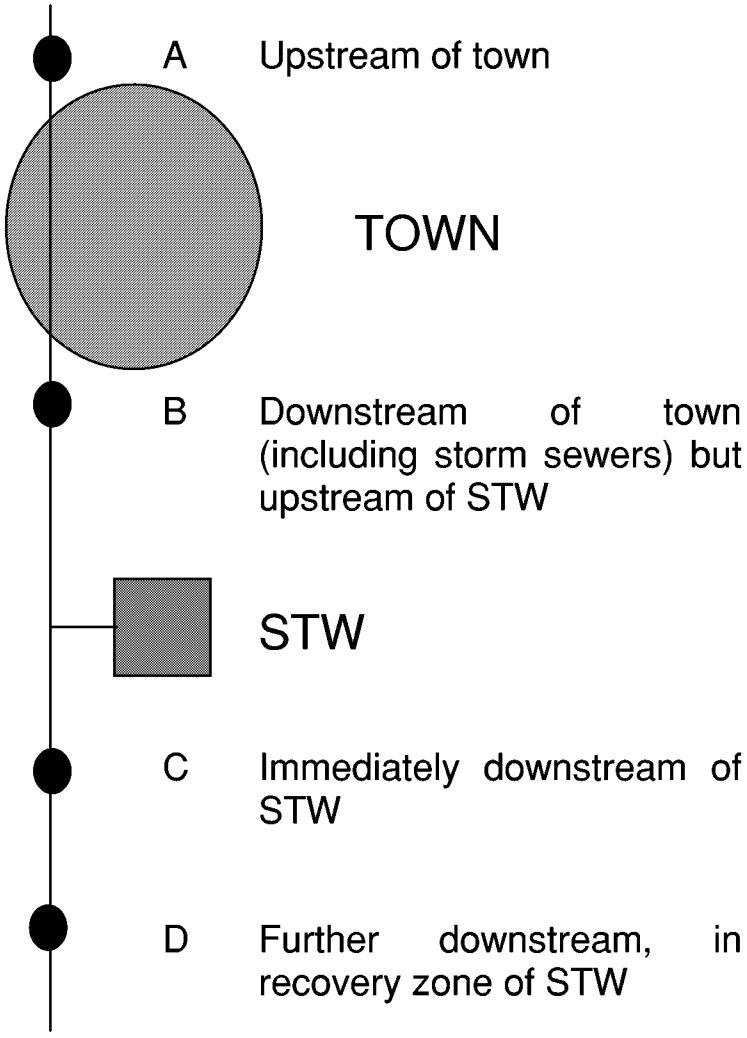


Fig. 6.3. A catchment-based approach to detecting major sources of nutrients. A - D: sampling stations. STW, sewage treatment works. See text and Kelly (1998) for more details.

Hints for data interpretation 4: spatial variation in TDI

- ✓ Prior to a comparison, test the assumption that there is no reason to expect the flora at the sites involved to be different, if there were no human impacts on the river.
- ✓ Variation in space needs to be considered in light of issues raised in 6.3 concerning the limits of sensitivity of the TDI.
- ✓ Consider the possible impact of urban runoff, storm sewers and trade effluents on sites upstream of STWs when designing a survey
- ✓ Be aware that the flora at sites immediately downstream of STWs may be impacted by factors other than nutrients. Place additional sampling sites in the "recovery zone" if necessary.
- ✓ Sample collection and storage is relatively cheap, so it is better to collect too many samples from a river, rather than too few
- ✓ Treat the first survey on a stretch of river as provisional and be prepared to change locations of sampling sites in light of experiences.
- ✓ Consider the use of "look-up" charts to aid data interpretation.

6.5 Variation in time

6.5.1 Timing of surveys

Although samples can be collected from any season (although winter should be avoided: see Chapter 2), the ideal time for a survey will depend upon local circumstances. The main ecological factor influencing sampling is the seasonal dominance of "weedy" taxa which tend to obscure real patterns of variation within the reach. Two taxa – *Cocconeis placentula* and *Navicula lanceolata* - cause particular problems under certain circumstances, detailed below. Other taxa – *Melosira varians, Navicula gregaria, Nitzschia* (other) – can cause similar problems. Unfortunately, in other situations they contribute valuable information to the TDI, so these taxa cannot be excluded from the TDI altogether. Two options for handling such circumstances are either to plan the survey to minimise the problem or to manipulate the TDI calculation subsequently (see section 4.4 point 6 and section 6.2.2). The former option is preferable but requires prior knowledge of the system under consideration.

Cocconeis placentula has a broad ecological range and is found in most running waters except those with low nutrients or acidic conditions. It is tolerant of moderate organic pollution and also extends into brackish waters. It is abundant on rocks, but is also found on other surfaces such as filamentous algae and macrophytes. There is also evidence (Jacoby, 1987; Rosemund *et al.*, 1993; Biggs & Lowe, 1994) that this species thrives under heavy grazing, which may explain why it is dominant in some types of UK river during the late summer (Fig. 6.4a). Under favourable conditions, valves of *C. placentula* can constitute >80% of the total. For these rivers, samples from spring may be more sensitive to nutrients than samples from summer or autumn.

Navicula lanceolata also has a broad ecological range, but differs from *C. placentula* in being motile and having a stronger tendency towards organically enriched conditions. However, it also appears to be able to grow at low temperatures and is often the dominant organism, across a wide range of water qualities, in late winter and early spring (Fig. 6.4b). For these rivers, samples from early to mid summer may be more sensitive to nutrients.

In general, all circumstances where diversity is low should be treated with caution, and extra help sought when interpreting data. The reason for low diversity may not be seasonality, but this should be borne in mind.

The size of the dataset is an important factor influencing the confidence with which diatom-based data can underpin decision-making. If possible, more than one sample per year may be collected from each site. Ideally, these replicate samples should be collected during different seasons; however, the issues discussed above may make this difficult. If very little time elapses between collection of samples from a site, then the samples do not represent "independent" observations in statistical terms and are "pseudoreplicates" rather than true replicates (see Hairston, 1989). For this reason, it is recommended that at least one month should separate replicate samples collected from the same site during the same calendar year.

For a statistical test to be valid, all replicates must be truly independent. This can never be the case in the types of pollution studies under consideration here, as the upstream and downstream sites will always form two distinct "blocks" of observations. This is overcome by the use of a paired sample test. A further assumption is that all the samples within each block are independent of one another. There are sufficient "catastrophic" events for this assumption to be valid between years (i.e. surfaces are scoured back to colonisation stages after each winter) and probably between seasons as well. The shorter the gap between samples, the greater the probability that the composition of the first sample influences that of the second sample, so violating the assumptions underpinning the statistical analysis

Hints for data interpretation 5: timing of surveys

✓ See comments on timing of surveys in 2.3.

✗ Do not base decisions on data from a single survey.

✓ .Treat the first survey on a new stream / reach as exploratory and be prepared to adjust the sampling program in subsequent years in order to maximise sensitivity.

✓ Minimise the influence of seasonality by always sampling at approximately the same time of year.

✓ If more than one sample is collected during a calendar year, try to collect these from different seasons and ensure that at least one month elapses between sample dates.

Table 6.3. A "look-up" chart for interpreting results of the Trophic Diatom Index. Enter values for sites above and below discharge into appropriate boxes and join adjacent sites by arrows. A vertical movement on the chart indicates a change due to nutrients; horizontal movement indicates change due to other factors.

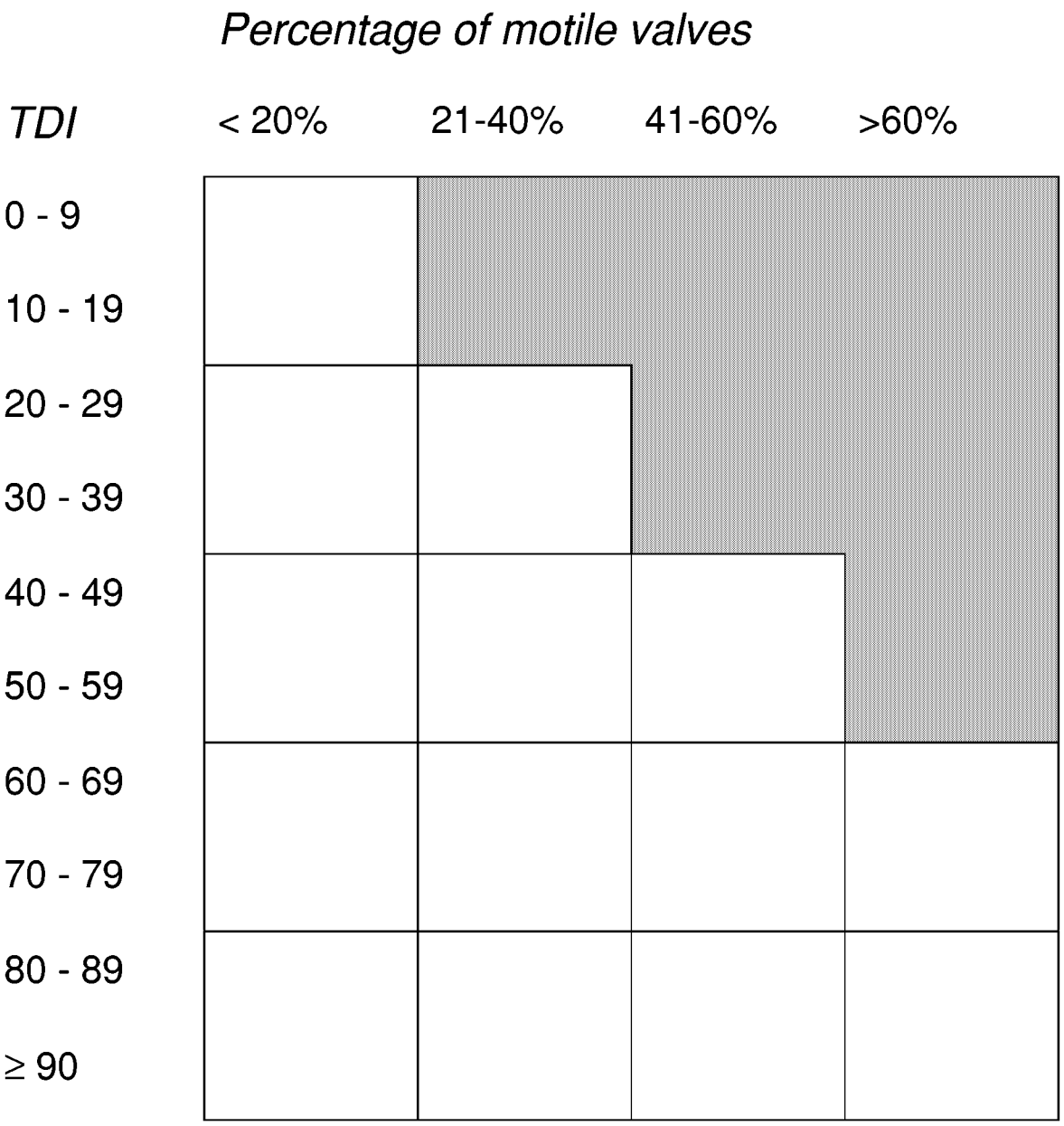
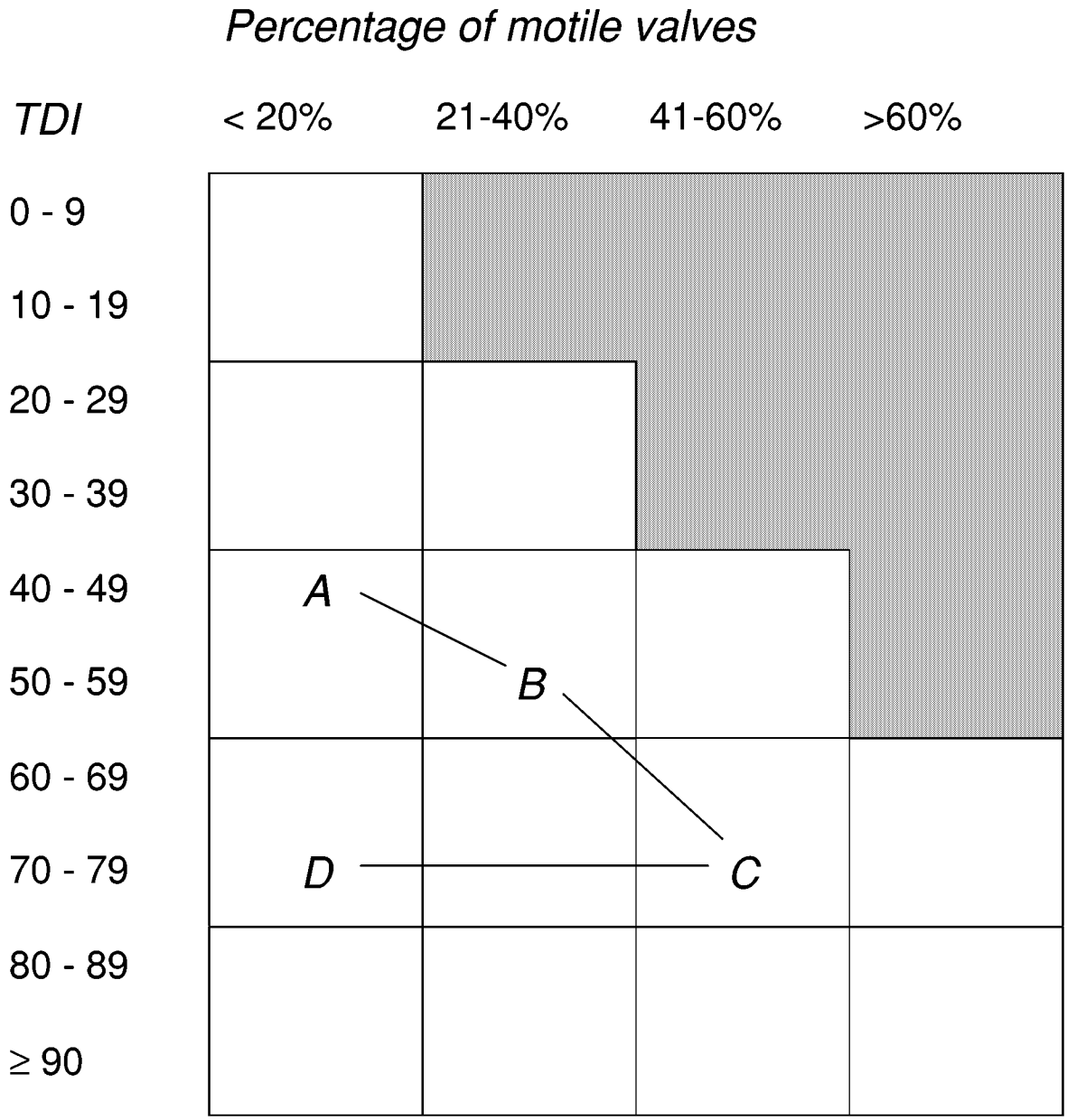
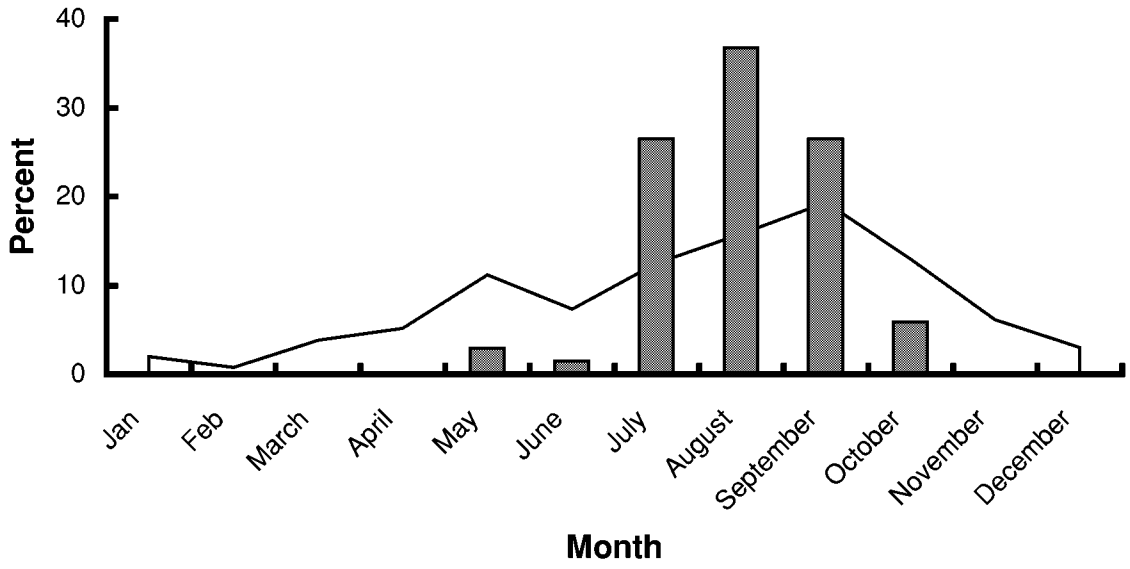


Table 6.4. Example of "look-up" chart as applied to the situation described in Table 6.3. See text for further details.



a) Cocconeis placentula ≥ 50%



b) Navicula lanceolata ≥ 25%

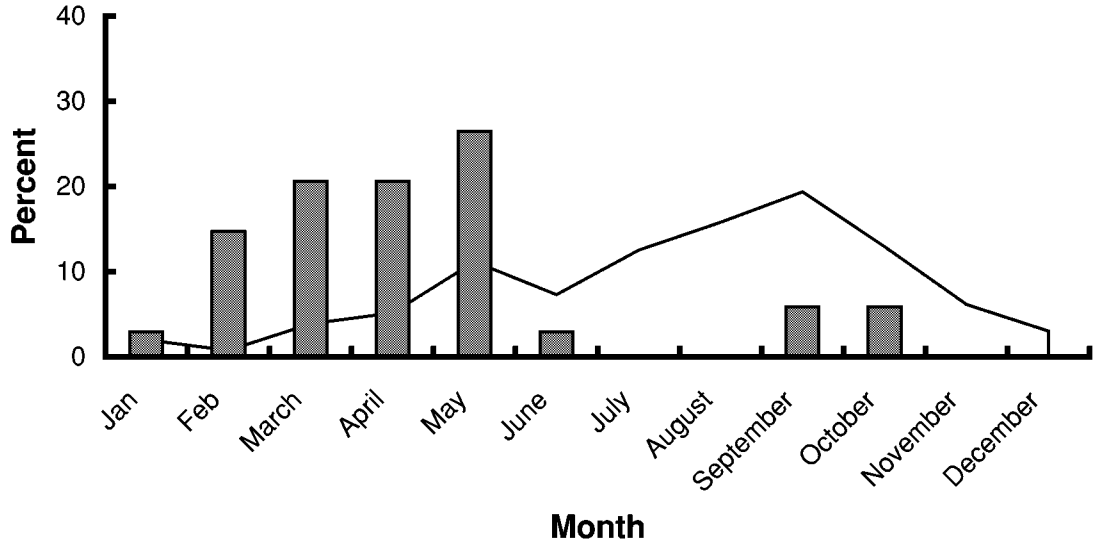


Fig. 6.4 Seasonal occurrence of high proportions of *Cocconeis placentula* and *Navicula lanceolata* in benthic diatom samples from the UK. Shaded area indicates the seasonal occurrence of all samples in the dataset (1861 samples) and represents relative sampling effort. Solid bars indicate percentage of occurrences exceeding threshold values (set at roughly the upper 5 percentile). In both cases, there is a statistically significant relationship between sampling effort and the distribution pattern of the diatom in question.

6.5.2 Handling between-survey variation

Between surveys variation is apparent even if guidelines in sections 2.3 and 6.4 are followed closely. This reflects the complexity of river and stream systems, and the influence of non-nutrient factors. Although most spates, treated individually, have relatively little influence on benthic diatom ecology (Stevenson, 1990) or the value of diatom indices (Kelly *et al.*, 1995), these observations must be placed into a broader hydrological, chemical and ecological context. Thus, high flows and low temperatures in late spring one year might favour *Navicula lanceolata* to dominate in April or May, whilst warmer, drier conditions the following year might lead to a different dominant taxon on the same date. High flows will also influence nutrient concentrations and the development of macroalgal, macrophyte and invertebrate assemblages, all of which may in turn interact with the diatom assemblage.

From the point of view of a monitoring biologist providing information to underpin decisions on water management, the problem lies in separating this "noise" driven by non-nutrient factors from genuine spatial or temporal changes in nutrient concentrations.

Interpretation of diatom-based data for UWWTD designations should always be based on more than one set of samples (sections 2.3, 6.5.1). Additional samples will improve the precision of any estimates; however, the required level of precision will vary from case to case and will depend upon:

1. the difference in the TDI before and after nutrient removal (or above and below the discharge);

2. the reliability of the TDI as an indicator of eutrophication (see Fig. 6.6); and,

3. the variance of the data.

Point 1 presents a problem. The magnitude of the TDI "before" can be estimated relatively easily at sampling sites both upstream and downstream of the STW. The magnitude "after" at a site upstream of STW may be assumed to be the same as that at the site "before" the change, but the magnitude "after" downstream of the discharge is harder to predict and, at this stage, it is not possible to model an "expected" diatom assemblage for a given nutrient concentration. Point 2 is discussed in more detail in section 6.2.2. The number of samples required is linked to points 1 and 3. A consistently large difference in TDI between two sites is more likely (assuming that other conditions are fulfilled) to generate a statistically valid difference based on a small number of samples than if the relative difference in TDI was small.

This is related to the concept of statistical power. The null hypothesis implicit in studies of the impact of point sources on rivers is that there is no change between upstream and downstream sampling stations. However, if no statistically significant difference was found between the upstream ("control") and downstream ("treatment") sites, then this may either mean that there is genuinely no difference between the two sites, or it may mean that the sample size was too small, or that the methods were too coarse for the difference to be detected (Peterman & M'Gonigle, 1992). Conventional statistics test only the first of these possibilities (i.e. that there is genuinely no difference between the two sites) and a separate technique – statistical power analysis – is

necessary to ensure that the latter two options are not responsible for the observed results. This manual does not include a detailed description of how to perform the necessary calculations, but provides users with a broad overview of the potential of power analysis. They should then be in a position to seek specialist help to perform the necessary analyses.

The most useful role of power analysis for UWWTD-related monitoring is to estimate the number of samples required to make a reliable statistical inference. This can take several forms:

1) Estimating the required number of samples in advance of collecting any data;

2) Testing the validity of a statistical analysis based on existing data; and,

3) Using existing data to estimate how many additional samples are required

In practice, the first option is difficult at present because this requires knowledge of the scale of within-site variation over time. This characteristic is itself highly variable, with some sites having a very predictable flora whilst others have a range of possible assemblages, depending upon prevailing conditions. The recommendation at this stage is that the number of samples required is predicted on the basis of existing data.

The recommendation to collect all samples for a particular survey on the same day (section 2.3) means that repeated sampling will result in a series of paired TDI values (i.e. upstream and downstream in survey 1, upstream and downstream in survey 2, and so on) for which the most valid statistical test is a "paired sample" t-test, which is widely available in spreadsheets and statistical packages such as Minitab. This will test the null hypothesis that the mean difference between upstream-downstream pairs of samples is zero. If the null hypothesis is retained, then this t-test should be followed by a power analysis. Whilst the null hypothesis is usually accepted or rejected on a criterion of 95% confidence, a statistical power of 80% is usually regarded as acceptable.

By providing the program with the number of pairs of samples, the standard deviation of the mean difference between pairs and the size of difference that you want to detect, it is possible to calculate the statistical power of the analysis.

Alternatively, by providing the program with the standard deviation of the mean difference between pairs, the size of difference that you want to detect and the acceptable power (i.e. 80%), it is possible to estimate how many additional pairs of samples are required. The question of what scale of difference in the TDI is acceptable is considered in example 2 below.

Example 1

The first Qualifying Discharge (QD) on the River Wear enters between diatom sampling points at Bradley (28.5 km from source) and u/s Gaunless (43.5 km from source). Five TDI values were available from which to evaluate changes in trophic status. These were as follows:

Date	Jun-93	Jul-94	Jun-95	Sep-95	Jul-96
Bradley	36	48	49	66	48
U/s Gaunless	54	60	60	67	76
Difference	14	12	11	1	28

A paired sample t-test was performed, with the null hypothesis that there was no significant difference between the two sites. The resulting t value was 5.153, which was highly significant ($p < 0.01$). The TDI does, in this example, support the case for designation (which was eventually made in 1998). (Note that there are valid grounds for eliminating the September 1995 samples, as they were taken during a long period of low flow and were not typical; however, it has been included for the sake of this exercise.).

Example 2

A large non-qualifying discharge enters the River Wear above the first QD, and diatom samples were collected from above and below this works in order to examine its contribution to eutrophication upstream of the sensitive area. Four TDI values were common to both sites:

Date	Jun-93	Jul-94	Jun-95	Jul-96
Wolsingham	36	34	38	49
D/s Bradley	36	48	48	48
Difference	0	14	10	-1

Once again, a paired-sample t-test was performed with the same null hypothesis as above. However, on this occasion, there was no significant difference between the two sites ($t = 2.960$, $P > 0.05$). However, this immediately raises the question of whether or not the sample size was adequate to detect a difference, if one was there, and this is where statistical power analysis can play a role.

The standard deviation of the difference between the two sites was 7.32. Under these circumstances, and using a one-sided t-test (because it is very unlikely, on theoretical grounds, that the TDI will fall downstream of a discharge), a sample size of four has a 21% chance of detecting a 10% change in TDI (i.e. 4 units), a 35% chance of detecting a 15% change (6 units) and a 51% chance of detecting a 20% change (8 units). The effect of increasing sample size is illustrated in Fig. 6.5. A statistical power of 80% is usually considered to be acceptable (Fairweather, 1991; Peterman & M'Gonigle, 1992).

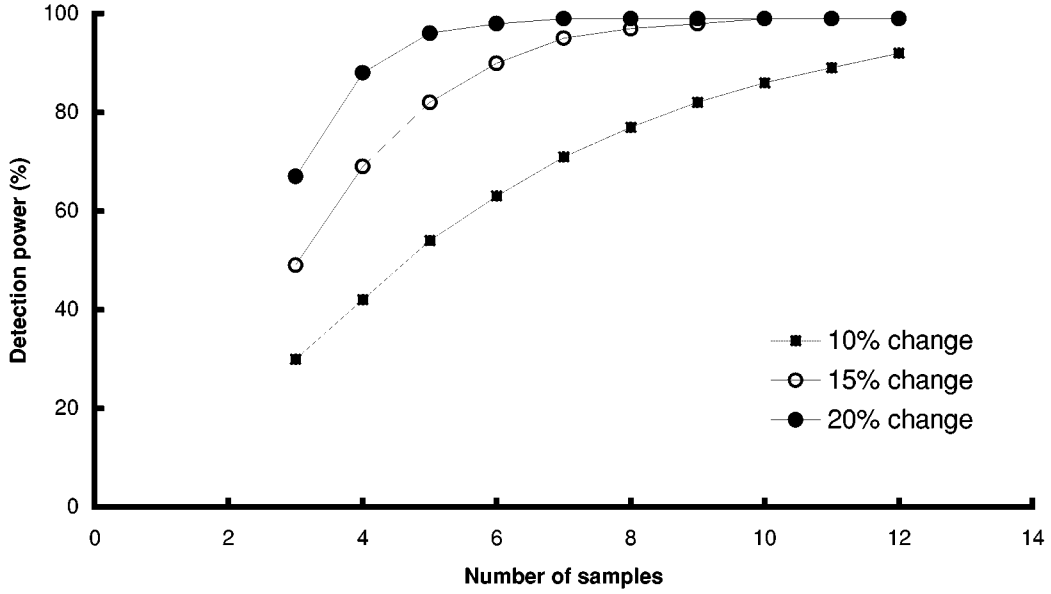


Fig. 6.5. Statistical power analysis as applied to the difference in TDI values between Wolsingham and Bradley, River Wear, Co. Durham.

This, in turn, raises the question of what constitutes a significant change in the TDI. This will vary from case to case, and it will also depend upon the level of the TDI upstream. For this reason, the scale of difference that should be considered to be significant is better quoted as a percentage rather than as an absolute number. The standard approach to interpretation of MTR results suggests a downstream change of 15% (Holmes *et al.*, 1999) and this is probably a reasonable criterion for most TDI surveys although in some circumstances (such as example 2), a difference of 20% might be more realistic (Fig. 6.5). However, any "rule of thumb" needs to be tested for each individual case and supported by appropriate power analyses.

6.5.3. Deciding whether to continue monitoring at a site

- If the upstream-downstream difference is not significant and the statistical power is >80%, then there is no benefit for monitoring to continue, if the UWWTD is the sole motivation. If designation goes ahead despite the diatom evidence (e.g. on the basis of MTR or chemical evidence), then continued diatom monitoring might be of interest to see if any changes occur once nutrient removal is installed.

- If the upstream-downstream difference is not significant and the statistical power is <80%, then continued diatom monitoring is recommended, until sufficient samples are available to make a robust analysis.

- If the upstream-downstream difference is significant, then continued monitoring should continue in order to strengthen the baseline dataset against which changes post-stripping can be measured (see also section 6.6)

Fig. 6.6 summarises the steps involved in interpreting TDI results for the purposes of UWWTD designations.

Methods for detecting changes once stripping has been installed are beyond the scope of this manual.

6.6 Use of other techniques in UWWTD evaluations.

Several multivariate statistical techniques – such as Two-way Indicator Species Analysis (TWINSPAN) and Canonical Correspondence Analysis (CCA) - may play valuable roles alongside the TDI in demonstrating temporal as well as spatial changes. The longitudinal distribution of end-groups resulting from a TWINSPAN classification of samples collected from the River Wear candidate Sensitive Area between 1993 and 1997 is given in Fig. 6.7. End-group A was found at sites all along the river and closer analysis revealed this to be primarily associated with samples collected in September 1995 following a prolonged period of unusually low flow. The other end groups are divided into those found primarily upstream of 40 km and those found primarily downstream of 50 km (the 10 km in the middle have a mixture of end-groups). Interestingly, the sensitive area designation for the River Wear starts close to km 40. Nutrient stripping has not yet been installed on the River Wear but we can predict, on the basis of these data, that the range of end-groups E, F, G and H should extend further downstream in the years following.

In this particular case, TWINSPAN is used alongside the TDI to highlight spatial patterns and, in time, to demonstrate temporal change as the effects of nutrient removal are manifest. A good examples of how ordination techniques can be used in a similar manner can be found in Lancaster *et al.* (1996).

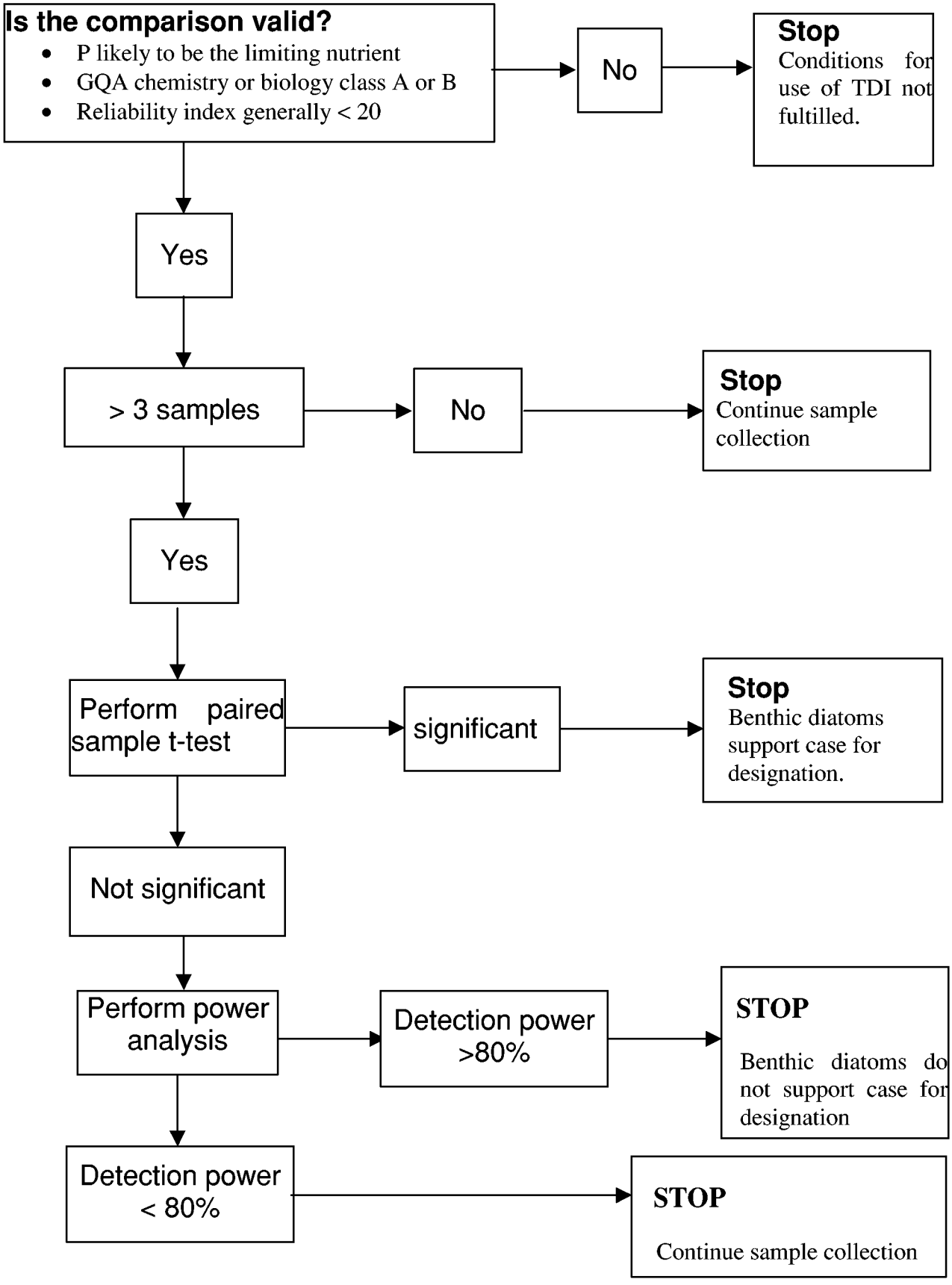
6.7 Use of the TDI for other purposes

In addition to UWWTD designations, the TDI has also been used to investigate eutrophication and general water quality problems in a wide variety of situations. The criteria discussed above apply in general to these situations: the main difference is that the focus of analyses is not a large qualifying discharge. In the case of small rivers, the TDI can be used to examine the influence of STWs smaller than those covered by the UWWTD, as well as the influence of fish farms and other sources of nutrients.

Diffuse nutrient pollution, from agricultural runoff is a major problem in some areas. The TDI can be used to study the impact of diffuse pollution; however, the manifestation of this problem necessitates a more liberal sampling policy. When water quality in the upper Hampshire Avon was examined, samples were collected from above and below numerous small village STWs and fish farms in order to eliminate each of these individually as causes of step changes in the TDI. Under such circumstances, the TDI can provide evidence to demonstrate the impact of diffuse pollution, but this should complement other sources of data (e.g. direct chemical analyses).

In addition to studies on nutrients, benthic diatoms have also been used to investigate a range of other water quality problems. Examples of recent uses in the UK include:

Fig. 6.6 Decision tree for interpreting TDI values above and below major STWs.



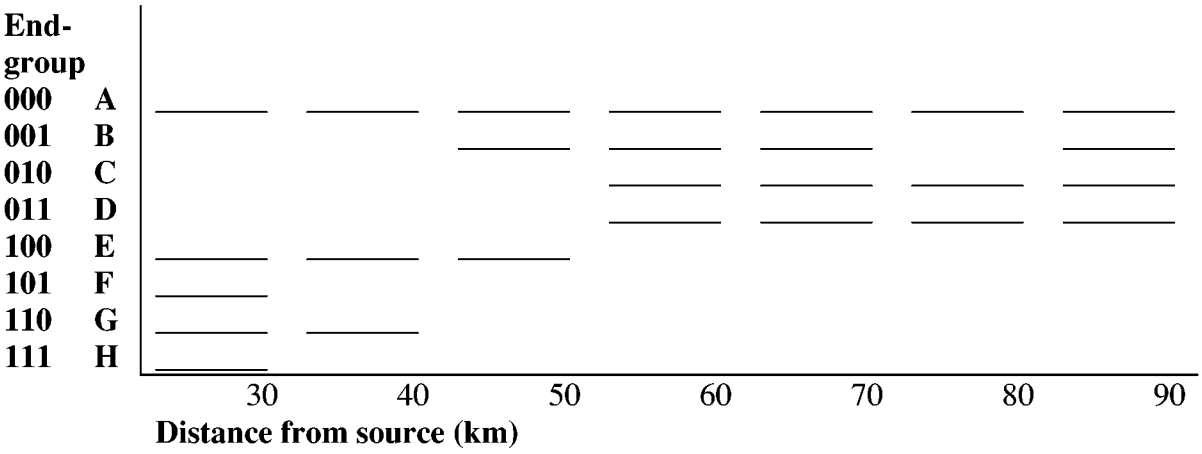


Fig. 6.7. Longitudinal distribution of TWINSPAN end-groups in the River Wear, based on sampling by Northumbria Area between 1993 and 1997. The river is divided into 10 km lengths, and the horizontal lines indicate the occurrence of a TWINSPAN end-group within that length. The sensitive area designation starts at approx. km 40 and there are 9 Qualifying Discharges between here and the tidal limit.

- Investigations of the impact of mining and quarrying, including the provision of baselines against which future changes due to remediation work may be measured.

- Investigation of the impact of other types of land use, including forestry and military training.

For situations such as these, all species (not just TDI taxa) in a sample should be identified. This will provide greater flexibility in data analysis. A further potential use that is being explored is to use existing and new data to track the impact of changing flow regimes (complementing the LIFE index; Extence *et al.*, 1999). Finally, the Framework Directive on Water Resources (WFD, 2000/60/EC) requires good ecological status to be defined in terms of the status of a number of separate components of the biota, one of which is the phytobenthos, for which the diatoms are an obvious candidate. Development of appropriate methods applicable across Europe is hoped to start soon.

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7. QUALITY ASSURANCE

7.1 Introduction

The TDI should only be used within the context of a quality assurance (QA) system so that managers and users of the data can be assured of its integrity. A robust analytical quality control (AQC) procedure is central to this but, it must be emphasised, this is only a guarantee that the quality assurance procedure as a whole is working.

Definitions of relevant terminology – audit, analytical quality control and quality assurance – are given in the glossary.

The QA system includes:

- specifications for training
- standard operating procedures, including specifications for apparatus
- systems of internal and external audit

Standard operating procedures are dealt with elsewhere in this manual (chapters 2-4): in this chapter other elements of the quality system will be explained.

This is one area where the protocols outlined in the previous version of the manual have been improved and updated. In particular, the audit protocol has been replaced by a new one, based on the Bray-Curtis similarity measure. No Europe-wide harmonisation of methods has yet been attempted, so these protocols may have to change again as this process continues within CEN.

7.2 Training requirements

During the original evaluation of the TDI, "open learning" materials were developed to enable Agency staff to learn enough about diatoms to use the TDI. These proved highly successful and have subsequently been used by many Agency and SEPA staff. They are recommended as a cost-effective means for non-specialists to develop a basic awareness of aspects of diatom taxonomy. Individuals can learn at their own pace and "on the job", and the prepared slides supplied with them can act as a "reference collection" for future use. However, some form of additional help is also recommended, particularly for some of the more "difficult" groups. As users have already gained some basic awareness through the open learning materials, this can take the form of a highly focussed one or two day tutorial to an individual or small group.

A suggested training protocol for complete beginners is as follows:

1. Gain familiarity with genera and common species of benthic diatoms using the Open Learning course (which takes approximately 30 hours to complete, although some of the time is spent collecting and analysing samples relevant to the individual's work).

2. Attend one day on-site training towards end of Open Learning course to iron-out any particular problems and queries.

3. Start routine use of TDI, submitting 1 in 5 slides for external quality audit until a satisfactory standard is reached (section 7.3.3) (dropping to 1 in 10 once a satisfactory standard is reached).

Periodic "ring tests" are organised by Bowburn Consultancy and are recommended as "refresher" exercises for staff who use the TDI only sporadically. These tests are also useful "pre-qualification" exercises for staff with some experience of diatom taxonomy or for potential contractors.

Periodic "ring tests" are organised by Bowburn Consultancy and are recommended as "refresher" exercises for staff who use the TDI only sporadically. These tests are also useful "pre-qualification" exercises for staff with some experience of diatom taxonomy or for potential contractors. All staff who are not able to analyse at least 30 audited samples per year should perform this test of competence.

Note that the emphasis here is on "training" rather than "qualifications". The ultimate demonstration of "quality" is a satisfactory success rate in the external quality audit. More importantly, the value of the training falls unless the skill is exercised on a regular basis.

The Open Learning materials and slides for the tests of competence are available from:

Bowburn Consultancy,
11 Monteigne Drive,
Bowburn,
Durham DH6 5QB.

tel: 0191 377 2077

e-mail: Bowburn_Consultancy@compuserve.com

7.3 Quality control and audit procedures

7.3.1 Introduction

The frequency of diatom analyses performed in most Agency laboratories is too small to justify formal "quality control" measures as used for benthic invertebrate samples and the boundaries between internal quality control and external audit are, consequently, blurred. However, because diatom samples are routinely made into permanent mounts, replicate analyses, either on site or elsewhere, are relatively straightforward and these are recommended as the basis of quality control for the TDI.

The method described here is appropriate for independent evaluation of the quality of the analysis of a diatom slide by a third party. It has evolved gradually over the past five years and further explanation can be found in Kelly (1999) and Kelly (2001). It consists of a preliminary analysis of the quality of the slide preparation, followed by a duplicate count, which is then compared with the original count by various statistics. These provide an objective comparison between the two samples and are supplemented by notes, made by the auditor, to help the analyst address any problems encountered with the slide.

Quality management is the responsibility of a designated Quality Manager, who may be the relevant Team Leader. This person does not have to be trained in diatom analyses but does need an awareness of general QA and AQC issues.

7.3.2 Ensuring the quality of permanent slides

The facility to produce permanent slides in TDI analyses is an important benefit. These slides, if correctly prepared and stored, represent an archive of conditions that can be consulted or even re-analysed in the future. For this reason, it is important that the slides themselves are subjected to quality assurance procedures. At least 10% of slides must be subjected to this audit. Two semi-quantitative scales are used for this, to evaluate the quality of the preparation itself and the density of valves. These scales are as follows:

Quality of preparations

1 (v. poor)	Organic matter not completely removed from valves **and/or** mountant not properly cured **and/or** many air bubbles in mountant **and/or** mountant not spreading right to edge of coverslip **and/or** distribution of valves on coverslip clumped **and/or** large quantities of foreign matter that cause problems in identification or enumeration of the specimens.
3 (acceptable)	Mountant is properly cured and spread to edges of coverslip; air bubbles absent; distribution of valves shows some variability (including edge effects) but any clumping is not so serious that it causes problems when counting or identifying diatoms.
5 (v. good)	Even distribution of valves with minimal edge effects and no valves obscured by detritus or other foreign matter.

At present the definition of intermediate classes is subjective, and scores of 3 or more are considered acceptable for routine purposes. Some of the criteria (such as the presence of large quantities of foreign matter) may be beyond the control of the analyst, and are not necessarily grounds for rejecting a slide. The auditor must make a note of the reasons for rejection.

Density of valves

1	Too sparse. Less than one valve per field of view. Counting is time consuming and/or it is not possible to count 300 valves.
2	Sparse. 1 – 5 valves per field of view. Presents no problems for counting.
3	Good. 5 - 15 valves per field of view
4	Dense. 16 - 25 valves per field of view. Some difficulties in enumeration and identification due to overlapping valves etc.
5	Very dense > 25 valves per field of view.

The density of valves should be assessed by scanning the slide using a high power objective. The appropriate score should be based on a count of valves visible in at least five separate fields of view. When making judgements, auditors must place themselves in the position of the primary analyst, and avoid any areas of the slide that are unusually dense.

Classes 2 - 4 are acceptable for routine purposes, with 3 preferred. Class 1 may indicate a problem with the sample beyond the control of the analyst and is not necessarily grounds for rejection, whilst slides in Class 5 should be rejected as counts based on very dense slides may be prone to human errors.

7.3.3 Ensuring the quality of analyses

The audit is based on a comparison between the original analysis (termed the "primary count") and a replicate analysis by an independent auditor (termed the "audit count"). Results of the two analyses are compared using the Bray-Curtis similarity measure. The equation for this is:

$$D_{1,2} = \Sigma\, q_i$$

where $D_{1,2}$ is the similarity between samples 1 and 2 and q_i is the smaller of the two relative abundances of species *i*. A comparison between two identical samples will result in a Bray-Curtis value of 100%, whereas two totally different samples will result in a value of 0%. Samples that share several common taxa in roughly the same proportions will have high scores and can be treated as replicates. The question of what constitutes a "replicate" will vary depending upon the system being examined, but a Bray-Curtis score of > 60% is often quoted (Engelberg, 1987; Spellerberg, 1991) and evidence suggests that this also applies to benthic diatoms (Kelly, 2001). However, Bray-Curtis similarity values also depend on the diversity of the sample, with low diversity (i.e. where one species is strongly dominant) giving either very low or very high similarity values (depending upon whether the dominant species was identified correctly) whilst replicate analyses of diverse samples rarely achieve very high similarity values.

In addition to the Bray-Curtis similarity value, the following parameters should be calculated:

- Hill (1973)'s N_2 measure of diversity should be calculated. This is the reciprocal of Simpson (1949)'s index and measures the extent to which a sample is dominated by a single taxon. The calculation is:

  $$N_2 = 1 / \Sigma\, P_i^2$$

  where P_i is the relative abundance of the *i*th species (Hill, 1973). A species diversity measure is preferred to a measure of species richness as the latter would be skewed by the large number of rare taxa that are frequently encountered when analysing diatom samples.

- The difference in TDI between primary and audit counts (ΔTDI).

- The numbers of "gains" and "losses". A "gain" is defined as a species recorded in the audit count that was not recorded in the primary count, whilst a "loss" is a taxon recorded in the primary count, but not in the audit count. Only taxa that comprise more than 2% of either the primary or audit count are included in the number of gains or losses.

Procedure

1. One in ten of all samples per analyst (one in five during the early stages), selected at random, are submitted for audit. These ten samples represent a "batch" that must be formally linked in some means (by a numbering system, for example) to the audit sample.

2. A replicate count ("audit count") is made under identical conditions to the primary count. If a stratified count procedure was adopted for the primary count, then this must also be used for the audit count.

3. Results from the primary and audit counts are entered into a spreadsheet and used to calculate the following statistics: Bray-Curtis similarity; Hill's N_2 diversity, "gains"; "losses"; difference in TDI (ΔTDI). If the TDI calculation was modified by removing one or more dominant taxa, then the statistics should be calculated both with and without this modification.

4. A sample is considered to have "acceptable" quality if the Bray-Curtis similarity > 60% and "gains" and "losses" are each $\leq$2. If such conditions are fulfilled then ΔTDI is usually $\leq$ 5. If Hill's N_2 diversity is <3, then the quality is acceptable if Bray-Curtis similarity is >70%. These criteria should also apply to modified TDI calculations, if appropriate.

5. Audit results of failed batches that are reanalysed should not be used when detecting lab quality. Reanalysed samples must be subject to audit in normal way.

6. A summary of the audit results must be returned to the primary analyst, along with comments from the auditor on possible reasons for any deviations from acceptable quality. A copy of the audit results should also be sent to the line manager.

7. All samples belonging to the batch from which this sample was drawn are considered to be verified.

8. A sample that is deemed not to have acceptable quality is returned to the original laboratory for re-checking. Taxonomic queries may be handled simply by a qualitative check of the slide although a partial or complete re-count may be necessary under some circumstances.

9. All samples in a batch from which a sample with unacceptable quality was drawn must be considered to be suspect until the causes of rejection have been addressed. This may involve qualitative or quantitative re-examination of some or all samples and submission of at least one extra sample (along with resubmission of the failed sample) for audit.

It is recommended that analysts "new" to diatom analysis submit one in five of all samples for audit. Once a satisfactory success rate has been achieved (no more than 1 failure in 20 samples is the figure usually quoted in audit studies), then the proportion of

all samples submitted can drop to one sample in ten. It has been suggested (Cheeseman and Wilson, 1978) that **it is better to obtain 10 - 20% fewer results of a known accuracy rather than more results of unknown accuracy**.

As the audit exercise forms the latter part of the "learning curve" for biologists involved with the TDI it is essential that possible reasons for a samples failure are passed back to the analyst. The audit procedure must be seen as part of an overall QA program, and as the culmination of a training exercise, rather than as a *post hoc* "check" on performance. The experience from the evaluation exercise was that running the audit program in this way engendered a spirit of friendly co-operation which made it easy for problems to be accepted and ironed-out. It is the experience from invertebrate audit procedures that audit failures decrease as more confidence and experience is gained.

7.3.4 Ensuring the quality of computer records

At least ten percent of all computer-based records must be selected at random and compared to the original count sheet. Any discrepancies should be noted.

7.4 Use of contractors

QA procedures must be modified for any diatom analyses performed by contractors rather than Agency staff.

Selection of contractors: As diatom identification skills are less common than skills in identifying other taxonomic groups (e.g. invertebrates and macrophytes) a pre-selection stage is recommended before tender documents are issued. Potential contractors must be able to demonstrate that the analysts concerned have experience of working with river diatom assemblages. This information can be obtained from other Regions or Areas that have let diatom analysis contracts, or by asking potential contractors to perform a pre-qualification exercise (7.2).

Management of contract: The contract should be organised in such a way that the contractor can supply slides and results in regular batches (i.e. 20 slides per batch) so that audits can detect and correct problems as they arise, rather than at the end of the project. Audits for diatom analyses undertaken by contractors should be managed via a separate contract between the Agency and auditor and not by a sub-contract from the contractor. This avoids conflicts of interest that may arise if audits reveal poor quality. Copies of audit results sheets should be sent to the Agency project manager and a copy forwarded to the contractor. Descriptions of what is considered to be an acceptable standard of work must be included in tender specifications.

LIST OF ABBREVIATIONS AND ACRONYMS

ΔTDI	Difference in TDI (parameter used in AQC protocol)
AIDGAP	Aids to Identification in Difficult Groups of Animals and Plants
AQC	Analytical Quality Control
BMWP	Biological Monitoring Working Party
CCA	Canonical Correspondence Analysis
CEN	Comité Européen de Normalisation (European Committee for Standardization)
DETR	Department of Environment, Transport and the Regions (now Department of the Environment, Food and Rural Affairs)
DQI	Diatom Quality Index
FRP	Filtrable Reactive Phosphorus
GDI	Indice Diatomique Generique
IMS	Industrial methylated spirits
IPS	Indice de polluosensibilité
LIFE	Lotic Invertebrates for Flow Evaluation
MTR	Mean Trophic Rank (macrophytes-based index of water quality)
NRA	National Rivers Authority
%motile	Percent of valves belonging to motile taxa
%PTV	Percent Pollution Tolerant Valves
QA	Quality Assurance
QD	Qualifying Discharge
s	Sensitivity of taxon to nutrients (variable in weighted average equation)
SEPA	Scottish Environment Protection Agency
STW	Sewage Treatment Works
TDI	Trophic Diatom Index
TWINSPAN	Two-way Indicator Species Analysis
UWWTD	Urban Wastewater Treatment Directive
v	Indicator value (variable in weighted average equation)
WFD	Water Framework Directive (Framework Directive on Water Resources)
WMS	Weighted Mean Sensitivity

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9. GLOSSARY OF TERMS

Definitions are provided for words in bold type.

Analytical Quality Control (AQC)	Procedures to control errors in laboratory analyses within specified limits.
Audit	An independent measurement of the quality of the laboratory analysis of samples or the quality of the **AQC** inspection.
Benthos (n), benthic (adj)	Those organisms which live on the bottom of a lake, river or other water body. Cf **plankton**
BMWP score	Biological Monitoring Working Party score, a biotic index of organic pollution based on the presence or absence of benthic invertebrate taxa in a sample.
Boulder	Stones with a diameter > 256 mm
Centric diatom	A diatom showing radial ("pill box" or "petri-dish") symmetry. In the latest classification, represented by the Class Coscinodiscophyceae.
Cobble	Stones with a diameter > 64, ≤ 256 mm
Diatom Quality Index (DQI)	A biotic index of environmental quality calculated as 100 – **TDI**.
Epilithon	The community living on the surface of a rock. Literally "on rock"
Epipelon	The community living on soft surfaces (mud and silt).
Ephiphyton	The community living on (but not usually fed by) aquatic plants. Literally "on plant".
Episammon	The community living attached to sand grains. Literally "on sand".
Eutrophic	An ecosystem containing high concentrations of those inorganic nutrients which usually limit plant growth (e.g. nitrogen or phosphorus). Cf. **oligotrophic**.
Eutrophication	The biological effects of an increase in concentration of plant nutrients – usually nitrogen and phosphorus, but sometimes others such as silicon, potassium, calcium, iron or manganese – on aquatic ecosystems (Harper, 1992)
Formaldehyde	Popular name for methanal, HCHO. A gas that is highly soluble in water and widely used as a preservative. It is

	highly toxic.
Formalin	A 40% solution of formaldehyde.
Frustule	The collective term for the silicious components of a diatom cell. Includes **valves** and **girdle bands**. A complete **frustule** is composed of two **valves** plus a number of **girdle bands**.
Girdle band	Silicious bands wrapped around the outside of a **frustule**. The number depends upon the species and can vary from 2 to 50 or more. They enclose and protect the cell whilst permitting an increase in volume during the cell cycle.
Girdle view	The side view of a diatom **frustule**, in which the **mantle** and **girdle bands** may be visible, but not the **valve** face.
Glide	A term describing a river reach where water moves effortlessly in a "smooth" fashion (Raven *et al.*, 1998).
Heterotrophic	Applied to organisms which have a mode of metabolism that requires a supply of organic material from the environment.. Some diatoms are facultative heterotrophs with respect to carbon and/or nitrogen.
Macrophyte	Any aquatic plant recognisable with the naked eye. Includes a few larger algae (e.g. *Cladophora, Lemanea*) plus bryophytes and vascular plants.
Mantle	Side part of the **valve**
Mean Trophic Rank (MTR)	A biotic index based on the relative abundance of **macrophyte** taxa in a survey length (typically 100 m).
Oligotrophic	An aquatic ecosystem containing very low concentrations of inorganic nutrients (cf. **eutrophic**)
Pebble	Stones with a diameter > 16, ≤ 64 mm
Pennate diatom	A diatom showing longitudinal ("date box") symmetry.
Percent Pollution Tolerant Valves (%PTV)	A measure of reliability of the **Trophic Diatom Index** used in the first version, but not in this update.
Phytoplankton	Those photosynthetic taxa found primarily in the **plankton**.
Plankton (n), planktonic (adj)	Those organisms which live suspended in the water column. Cf **benthos**
Quality Assurance	Procedures to quantify and control or reduce errors.
Quality Control	See **Analytical Quality Control**.

Raphe	Longitudinal slit or furrow found in many **pennate diatoms**, through which mucus, for support or motility, is extruded. Divided into two parts, arranged end-to-end with a gap at the centre.
Rapid	An area of broken standing waves, forming distinctive whitewater conditions, normally over cobble or boulder substrate. Associated with steep gradient rivers and streams (Raven *et al.*, 1998).
Riffle	Shallow, fast-flowing water with a distinctly disturbed surface, forming upstream-facing unbroken standing waves, usually over gravel substrate (Raven *et al.*, 1998).
Run	Generally fast moving water with a rippled surface. Often associated with a rapid or riffle just upstream, or where the channel narrows and therefore speeds up the flow (Raven *et al.*, 1998).
Sensitive area (eutrophic)	A waterbody which is "found to be eutrophic or which in the near future may become eutrophic if protective action is not taken" (European Community, 1991)
Trophic Diatom Index (TDI)	A biotic index of **eutrophication** based on the percentages of benthic diatom taxa in a sample.
Valve	A structural component of the diatom **frustule**. Two **valves** fit together (like the two halves of a date box or petri dish) to form (along with associated **girdle bands**) a **frustule**.

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APPENDIX A CHANGES TO THIS EDITION OF TDI MANUAL

Change	Rationale
Chapter 1	
Purpose and scope added	Following practice of other Agency manuals.
Performance characteristics modified	Mainly minor changes in light of experience.
Background revised and updated	Original version details development of TDI until 1996. New version includes developments subsequent to this date.
Main changes to this version described	For information.
Chapter 2	
Three principles increased to four	Update in line with CEN standard (the new principle was implied but not stated in the previous edition)
"suffix of confidence" dropped	Not necessary. The %PTV provides a measure of the reliability of the TDI as a measure of trophic status
Flow chart for selecting sampling strategy updated	To incorporate other changes (see below)
Preferred artificial substrata changed from tiles to ropes	Based on experience within the Agency over the past five years.
Guidelines on sampling vertical man-made structures included (2.6)	*Ad hoc* methods adopted in some regions need rationalisation. The method used here is that used in Thames Region.
Guidelines on use of emergent (2.8) and submerged (2.9) macrophytes included	Based on request from Anglian Region. Adopted methods are taken from CEN Standard.
More detailed guidelines on time of sampling	Based on experience within the Agency. Not included in the CEN standard, as best time for sampling varies between different eco-regions.
General tightening of guidelines to bring TDI manual into line with CEN standard	These all involve changes in wording from 1st edition in order to provide a clearer explanation of what is required.

Chapter 3

Inclusion of hot peroxide method as alternative to cold acid method	Widely used alternative to method in 1st edition. Included in CEN standard.
Several minor changes in wording	Reflecting experience over the past 5 years, and the CEN standard.
Added new section on archiving samples	Mainly based on material in 1st edition but emphasising the importance of diatom slides as a long-term record of conditions.

Chapter 4

Was Chapter 5, but is now Chapter 4.	Chapter 4 from the first edition (Identification) is now available as a separate publication (published by Field Studies Council)
Comments on suitable floras for calculation of the TDI now included here	To provide users with information on appropriate floras.
Inclusion of health and safety note	Modified from a protocol used at Blandford Forum
Recommendations on count size changed	Experience since first edition
Stratified counting procedure included for use under certain conditions	To overcome problems of overwhelming dominance of some taxa.
Various minor changes	To bring manual in line with recommendations in draft European standard.

Chapter 5

Reference to Diatom Quality Index deleted from chapter title	Advice of Project Board. DQI to be recommended only for reporting for UWWTD purposes. Information on calculation of DQI is included in 5.3.

Chapter 6

Rewritten from scratch	Many new insights gained through widespread use of the TDI.

Chapter 7

Training requirements rewritten and expanded, to cover situations when external contractors are involved.	A greater reliance on external contractors than envisaged when the manual was first written.

Audit procedure extended to cover quality of slide preparation	To ensure that the slide archive is of high quality and to minimise enumeration problems caused by poor quality slides.
New audit procedure for comparison of slides	Previous system proved to be unworkable in practice. This new system has been tested extensively.
Audit procedure for checking data added	As used in other procedural manuals
QA issues when using contractors added	More widespread use of contractors than envisaged when 1st edition written

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APPENDIX B: FIELD RECORD FORM

River: _____ Site: _____ Date: _____
NGR: _____ Sample collected by: _____

Physical records (measure as for RIVPACS)

Width _____ **Depth:** _____

Substrate (record estimated percentage)

bedrock ☐ boulders/cobbles ☐ pebbles/gravel ☐
sand ☐ silt/clay ☐ peat ☐

Estimate percentage of boulders and cobbles covered by:

Cladophora and other filamentous algae: ☐ other macrophytes ☐

Shading (record estimated percentage)

Left bank None ☐ Broken ☐ Dense ☐
Right bank None ☐ Broken ☐ Dense ☐

Habitat Pool ☐ Run ☐ Riffle ☐ Slack ☐

Water clarity Clear ☐ Cloudy ☐ Turbid ☐

Bed stability Firm ☐ Stable ☐ Unstable ☐ Soft ☐

Time since last spate

< 3 days ☐ 3 - 7 days ☐ 7 - 14 days ☐ > 14 days ☐
not known ☐

Photograph Facing upstream _____ Facing downstream ___

Use the reverse of this sheet for sketch map and other comments

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APPENDIX C. RISK ASSESSMENTS FOR ACTIVITIES ASSOCIATED WITH THE TROPHIC DIATOM INDEX

This section of the manual includes a series of Task Risk Assessments (and associated COSHH Risk Assessments) covering all aspects of the procedures outlined in chapters 2-4. These have been prepared in line with the method described in the Environment Agency's Health and Safety Risk Management Manual, which provides a straightforward way of assessing and recording a wide range of risks using the five main steps as recommended by the Health and Safety Executive:

- Identify hazards;
- Identify all those who may be affected by the hazards; estimate the level of risk, taking into account the adequacy and reliability of existing or planned risk control measures;
- Decide if new risk control measures are needed and investigate what new measures can be introduced, having regard for good practice;
- Prioritise the measures to be introduced and plan their implementation.

The Task Risk Assessments included in this manual have been developed as *best practice* and are necessarily generic in nature. It is the responsibility of managers to produce Task Risk Assessments that are relevant to local circumstances and to implement the appropriate arrangements for safe working in their work locations.

Task Risk Assessments are sub-divided into a number of columns:

Column	**Comment**
1. Item	
2. Description of task elements.	Common to all laboratories performing diatom analyses.
3. Identification of hazard, harmful effect.	Common to all laboratories performing diatom analyses.
4. Identification of person(s) affected	Common to all laboratories performing diatom analyses.
5. Initial risk assessment	Common to all laboratories performing diatom analyses.
6. Suggested risk control measures	Others may also apply.
7. Level of risk	This is based on a preliminary evaluation, bearing in mind responses in columns 3-6.
8. Risk control measures adequate?	If the risk assessment in column 7 is greater than

Column	Comment
	that in column 5 then the response must be"no".
9. Options for improved risk control	If the response in column 8 is "no", then remedial action should be described here.
10. Priority of action(s) required	Prioritise actions based on evaluation of all responses in column 9.
11. Action plan reference number	Refers to relevant Region / Area documentation

The following Task Risk Assessments are included:

1. Collection of diatom samples from freshwaters (including deployment of polypropylene rope for diatom colonisation).
2. Addition of preservative to diatom sample.
3. Digestion of diatom samples using sulphuric and oxalic acids and potassium permanganate.
4. Digestion of diatom samples using hot hydrogen peroxide.
5. Preparation of permanent microscope slides.
6. Identification and enumeration of diatoms from permanent microscope slides.

Task Risk Assessments are followed by example COSHH Assessment Forms for specific activities:

1. Addition of iodine to diatom samples and subsequent laboratory procedures involving diatom samples preserved in iodine solutions.
2. Laboratory and field procedures involving use of 4% formaldehyde solution (diluted from 40% stock solution).
3. Decanting stock solution of methylated spirit (industrial).
4. Decanting 37% hydrochloric acid from stock to glass dropping bottle and subsequent addition to samples during chemical digestion process.
5. Preparation of saturated oxalic acid. Decanting saturated oxalic acid into glass dropping bottle and subsequent use in chemical digestion of diatom samples.
6. Addition of potassium permanganate crystals to beaker containing rope + sulphuric acid and chemical digestion of samples.
7. Decanting sulphuric acid into glass dropping bottle and subsequent use in chemical digestion of diatom samples.
8. Use of hydrogen peroxide in chemical digestion of diatom samples.

C1. Fieldwork

PART 1. WORK ACTIVITY/TASK IDENTIFICATION SHEET	**1.1.1 REF DI 1.1 to 1.3**
1. REGION/AREA	Insert name of Region and Area
2. TEAMS/JOB TITLES	Biology/Team Leader Biology/ Biologists/Student Biologists/ Temporary Biologists
3. TASK SUMMARY	Collection of diatom samples from freshwaters (including deployment of polypropylene rope for diatom colonisation).
4. TASK COMMENCES	Loading vehicle.
5. TASK FINISHES	Unloading vehicle.
6. TOOLS & EQUIPMENT	Polypropylene rope (artificial colonisation samples only), scissors. White tray and toothbrush (samples from natural substrates only). Mobile telephone. Life jacket, throwing line, hand wipes, first aid kit. Waterproof clothing, thigh/chest waders, heavy duty rubber gloves. Anti-bacterial hand-wipes. Warrant card + Leptospirosis card.
7. LOCATIONS	Streams, rivers and ditches.

8. ASSESSORS	Insert name of Team Leader Biology
9. DATE	Insert date
10. REVIEW PERIOD	12 months
11. AUTHORISATION	CHECKED BY SIGNED DATE …../…./…

PART 2. RISK ASSESSMENT SHEET

1. NO.	2. DESCRIPTION OF TASK ELEMENTS	3. IDENTIFICATION OF HAZARD, HARMFUL EFFECTS	4. IDENTIFICATION OF PERSONS AFFECTED	5. INITIAL RISK LEVEL H/M/L
1	Loading/unloading sampling/safety equipment from vehicle	Road traffic hazards. Lifting injuries.	Biology Team	M(3)
2	Driving to/from sampling sites	Road traffic accidents/ breakdown.	Biology Team	H(6)
3	Walking from vehicle to sampling site	Road traffic hazards. Attacks/trampling by livestock. Hostile landowners/pets. Fractures etc from tripping/falling (including crossing fences, gates). Hypothermia/frost bite. Sunburn/skin cancer/over-heating/heat exhaustion.	Biology Team	M(3)
4	Accessing sampling site	Steep banks, deep and fast - flowing water. Fractures/drowning from tripping/falling/slipping.	Biology Team	M(4)
5	Deploying/collecting polypropylene rope/natural substrates (collecting only) in/from watercourse	Cuts from scissors. Tripping/falling on uneven river bed. Cuts from glass etc on river bed. Submersion. Poisoning/infection through contact with contaminated/ polluted water. Weil's Disease.	Biology Team	M(3)
6	Scrubbing of polypropylene rope/natural substrates to remove diatoms	Cuts from sharp edges of natural substrates (stones etc) Poisoning/infection through contact with contaminated/ polluted water. Weil's Disease.	Biology Team	M(3)

1. NO	6. SUGGESTED RISK CONTROL MEASURES	7. LEVEL OF RISK H/M/L	8. RISK CONTROL MEASURES ADEQUATE YES/NO	9. OPTIONS FOR IMPROVED RISK CONTROL	10. PRIORITY OF ACTIONS REQUIRED H/M/L	11. ACTION PLAN REF. NO.
1	High visibility jackets. Training in manual handling.					
2	Regularly serviced/maintained vehicles. Authorised drivers only (annual licence checks). Field-worker monitoring procedure.					
3	High visibility jackets. Hostile situations training. Dog scarer. Risk Reminders For Fieldwork. Appropriate clothing (inc. waterproofs). Waders with high-grip soles. Double-manning. Field-worker monitoring procedure.					
4	Water Safety training course. Risk Reminders For Fieldwork. Double-manning. Life-jacket. Waders with high-grip soles. Field-worker monitoring procedure.					
5	Water Safety training course. Risk Reminders For Fieldwork. Waders with high-grip soles. Life-jackets. Heavy duty rubber gloves. Immunisation against tetanus/hepatitus A & B. Leptospirosis card/letter to GP. Double-manning. Anti-bacterial hand-wipes. Field-worker monitoring procedure.					
6	Heavy duty rubber gloves. Immunisation against tetanus/hepatitus A. Leptospirosis card/letter to GP. Anti-bacterial hand-wipes.					

C2. Addition of preservative to diatom sample

PART 1. WORK ACTIVITY/TASK IDENTIFICATION SHEET	REF DI 1.4
REGION/AREA	Insert as appropriate
1. TEAMS/JOB TITLES	Biology/Team Leader Biology/Biologists/Student Biologists/Temporary Biologists
2. TASK SUMMARY	Addition of preservative to diatom sample.
3. TASK COMMENCES	Opening sample container.
4. TASK FINISHES	Sealing sample container following addition of preservative.
5. TOOLS & EQUIPMENT	Sample container + sample. Preservative Pipette.
6. LOCATIONS	Entire task should be undertaken in a fume cupboard unless formaldehyde is used. As formaldehyde is heavier than air a local exhaust ventilation system (which draws air away at the bottom) is required.

7. ASSESSORS	Insert name of Team Leader Biology
8. DATE	Insert date
9. REVIEW PERIOD	12 months
10. AUTHORISATION	CHECKED BY SIGNED DATE …../…./…

PART 2. RISK ASSESSMENT SHEET

1. NO.	2. DESCRIPTION OF TASK ELEMENTS	3. IDENTIFICATION OF HAZARD, HARMFUL EFFECTS	4. IDENTIFICATION OF PERSONS AFFECTED	5. INITIAL RISK LEVEL H/M/L
1	Opening sample container. Addition of iodine solution to sample container. Sealing of sample container.	Splashing/spillage of preservative – refer to COSHH assessment.	Biology Team	L(2)

1. No	6. SUGGESTED RISK CONTROL MEASURES	7. LEVEL OF RISK H/M/L	8. RISK CONTROL MEASURES ADEQUATE YES/NO	9. OPTIONS FOR IMPROVED RISK CONTROL	10. PRIORITY OF ACTIONS REQUIRED H/M/L	11. ACTION PLAN REF. NO.
1	Restricted access to working area. Laboratory CoP. Laboratory induction procedure. Activity undertaken by authorised personnel only. Chemical inventory/storage arrangements. Chemical spillage procedures. Fume cupboard or local exhaust ventilation facilities COSHH Risk Assessment for preservative Heavy duty rubber/latex gloves, safety spectacles, laboratory coat. Eye wash facilities/safety shower.					

C3 Digestion of diatom samples using sulphuric and oxalic acids and potassium permanganate

PART 1. WORK ACTIVITY/TASK IDENTIFICATION SHEET	REF DI 2.0
1. REGION/AREA	Insert as appropriate
2. TEAMS/JOB TITLES	Biology/Team Leader Biology/Biologists/Student Biologists/Temporary Biologists
3. TASK SUMMARY	Chemical digestion of diatom samples in preparation for mounting on microscope slides. Process involves addition of chemical oxidising agent and acid to samples.
4. TASK COMMENCES	Removal of sample from preservative.
5. TASK FINISHES	Final re-suspension of pellet following centrifugation (prior to preparation of microscope slide).
6. TOOLS & EQUIPMENT	Sample – diatoms (+ polypropylene rope) in iodine solution. Centrifuge tube or 100 ml glass beaker. Concentrated Sulphuric acid Oxalic acid crystals. Potassium permanganate crystals. Hotplate Distilled water. Hydrochloric acid (specific gravity 1.18) Glass dropping bottles. Centrifuge
7. LOCATIONS	Entire task should be undertaken in fume cupboard, within defined laboratory area.

8. ASSESSORS	Insert name of Team Leader Biology
9. DATE	Insert date
10. REVIEW PERIOD	12 months
11. AUTHORISATION	CHECKED BY SIGNED DATE …../…./…

PART 2. RISK ASSESSMENT SHEET

1. NO.	2. DESCRIPTION OF TASK ELEMENTS	3. IDENTIFICATION OF HAZARD, HARMFUL EFFECTS	4. IDENTIFICATION OF PERSONS AFFECTED	5. INITIAL RISK LEVEL H/M/L
1	Dissolve oxalic acid crystals in distilled water, whilst stirring and heating.	Contact with / swallowing oxalic acid crystals and splashing / spillage of solution containing oxalic acid – refer to COSHH assessment.	Biology Team	H(6)
2	Decanting oxalic acid, sulphuric acid and hydrochloric acid into glass dropping bottles.	Splashing/spillage of oxalic acid, sulphuric acid and hydrochloric acid solutions – refer to COSHH assessments.	Biology Team	H(6)
3	Scan sample and remove large pieces of silt and sand. Sieve if necessary.	Splashing/spillage of solution containing iodine – refer to COSHH assessment.	Biology Team	L(2)
4	Add hydrochloric acid if any calcareous material present.	Splashing/spillage of hydrochloric acid and solution containing iodine + hydrochloric acid – refer to COSHH assessments.	Biology Team	H(6)
5	Pour off supernatant and decant sample into glass beaker.	Splashing/spillage of solution containing iodine + hydrochloric acid - refer to COSHH assessments	Biology Team	H(6)
6	Addition of sulphuric acid to glass beaker/centrifuge tube containing sample.	Splashing/spillage of sulphuric acid – refer to COSHH assessment.	Biology Team	M(4)
7	Addition of potassium permanganate crystals to beaker containing sample + sulphuric acid/ hydrochloric acid.	Contact with / swallowing potassium permanganate crystals , inhalation of vapours and splashing / spillage of solution containing – refer to COSHH assessment.	Biology Team	H(6)
8	Addition of oxalic acid to beaker containing sample, sulphuric acid and potassium permanganate.	Splashing/spillage of oxalic acid/beaker containing mixture of chemicals – refer to COSHH assessment.	Biology Team	M(4)
9	Transfer of contents of beaker to centrifuge tube and subsequent centrifugation.	Splashing/spillage chemical mixture within beaker – refer to COSHH assessments. Physical injury from incorrect operation of centrifuge. Electric shock.	Biology Team	M(4)

1. NO.	6. SUGGESTED RISK CONTROL MEASURES	7. LEVEL OF RISK H/M/L	8. RISK CONTROL MEASURES ADEQUATE YES/NO	9. OPTIONS FOR IMPROVED RISK CONTROL	10. PRIORITY OF ACTIONS REQUIRED H/M/L	11. ACTION PLAN REF. NO.
1	Restricted access to working area. Laboratory CoP. Laboratory induction procedure. Activity undertaken by authorised personnel only. Chemical inventory/storage arrangements. Chemical spillage procedures. Hot plate operating instructions. Electrical testing. Fume cupboard. Equipment maintenance record. Oxalic acid COSHH Risk Assessment. Latex gloves, heat resistant gloves, safety spectacles, laboratory coat. Eye wash facilities/safety shower.					
2	See control measures given in 1 above, + hydrochloric acid, sulphuric acid and oxalic acid COSHH Risk Assessments.					
3	See control measures given in 1 above, + COSHH Risk Assessment for appropriate preservative..					
4	See control measures given in 1, 2 and 3 above + Hydrochloric acid COSHH Risk Assessment.					
5	See control measures given in 1, 2 and 3 above.					
6	See control measures given in 1, 2 and 3 above.					
7	See control measures given in 1, 2, 3 and 7 above + Potassium permanganate COSHH Risk Assessment.					
8	See control measures given in 1, 2, 3 and 7 above.					
9	See control measures given in 1, 2, 3 and 7 above + centrifuge operating instructions.					

C4. Digestion of diatom samples using hot hydrogen peroxide

PART 1. WORK ACTIVITY/TASK IDENTIFICATION SHEET	REF DI 4.0
1. REGION/AREA	Insert as appropriate
2. TEAMS/JOB TITLES	Biology/Team Leader Biology/Biologists/Student Biologists/Temporary Biologists
3. TASK SUMMARY	Chemical digestion of diatom algae samples in preparation for mounting on microscope slides. Process involves addition of chemical oxidising agent and acid to samples.
4. TASK COMMENCES	Removal of sample from preservative.
5. TASK FINISHES	Final re-suspension of pellet following centrifugation (prior to preparation of microscope slide).
6. TOOLS & EQUIPMENT	Sample – diatoms (+ polypropylene rope) with preservative. Centrifuge tube or 100 ml glass beaker. Hydrogen peroxide Hotplate Distilled water. Hydrochloric acid (specific gravity 1.18) Glass dropping bottles. Centrifuge
7. LOCATIONS	Entire task should be undertaken within defined laboratory area.

8. ASSESSORS	Insert as appropriate
9. DATE	Insert date
10. REVIEW PERIOD	12 months
11. AUTHORISATION	CHECKED BY SIGNED DATE …../…./…

PART 2. RISK ASSESSMENT SHEET

1. NO.	2. DESCRIPTION OF TASK ELEMENTS	3. IDENTIFICATION OF HAZARD, HARMFUL EFFECTS	4. IDENTIFICATION OF PERSONS AFFECTED	5. INITIAL RISK LEVEL H/M/L
1	Addition of up to 5 ml dilute hydrochloric acid (1M) to sample	Causes burns – see COSHH risk assessment	Biology Team	L
2	Addition of 20-50 ml 30% (v/v) hydrogen peroxide to sample	Causes burns – see COSHH risk assessment	Biology Team	L
3	Possible breakage of glass whilst handling	Cuts	Biology Team	M
4	Accidental spillage of digestion chemicals	Risk of fume inhalation and burns	Biology Team	H
5	Hot surface of hot plate	Burns from touching hot surfaces. Danger from spillage of hydrogen peroxide onto hot plate	Biology Team	H
6	Leaving hot plate unattended	Burns to unaware personnel	Biology Team	L
7	Use of centrifuge	Mechanical injury	Biology Team	L
8	Transfer of sample to centrifuge and of supernatant to waste	Burns	Biology Team	M
9	Disposal of unwanted supernatant	Problems with fumes. Risk of noxious fumes and burns.	Biology Team	L

1. NO.	6. EXISTING/PLANNED RISK CONTROL MEASURES	7. LEVEL OF RISK H/M/L	8. RISK CONTROL MEASURES ADEQUATE YES/NO	9. OPTIONS FOR IMPROVED RISK CONTROL	10. PRIORITY OF ACTIONS REQUIRED H/M/L	11. ACTION PLAN REF. NO.
1	Restricted access to working area. Laboratory CoP. Laboratory induction procedure. Activity undertaken by authorised personnel only. Chemical inventory/storage arrangements. Chemical spillage procedures. Hot plate operating instructions. Electrical testing. Fume cupboard. Equipment maintenance record. Latex gloves, heat resistant gloves, safety spectacles, laboratory coat. Eye wash facilities/safety shower. Carry out preparation in active fume cupboard. COSHH risk assessments for hydrochloric acid and hydrogen peroxide					
2	See control measures given in 1 above plus: Ensure bench area is clear					
3	See control measures given in 1 above plus: Have ready supply of water swab and dilute any spillage.					

1. NO.	6. EXISTING/PLANNED RISK CONTROL MEASURES	7. LEVEL OF RISK H/M/L	8. RISK CONTROL MEASURES ADEQUATE YES/NO	9. OPTIONS FOR IMPROVED RISK CONTROL	10. PRIORITY OF ACTIONS REQUIRED H/M/L	11. ACTION PLAN REF. NO.
4	See control measures given in 1 above plus: Clear bench area around and work away from hot plate. Place and retrieve sample glassware on hot plate when hot plate is cool.					
5	See control measures given in 1 above plus: Place warning notice by hot plate and inform others of hazard					
6	See control measures given in 1 above plus: Only use centrifuge if trained by experienced staff Do not attempt to open the lid prior to the machine stopping					
7	See control measures given in 1 above.					
8	See control measures given in 1 above plus: Wash away supernatant with water and run tap for a few minutes afterwards in fume cupboard sink.					

C5. Preparation of permanent microscope slides

PART 1. WORK ACTIVITY/TASK IDENTIFICATION SHEET	REF DI 3.0
1. REGION/AREA	Insert as appropriate
2. TEAMS/JOB TITLES	Biology/Team Leader Biology/Biologists/Student Biologists/Temporary Biologists
3. TASK SUMMARY	Preparation of microscope slides from chemically-digested diatom samples.
4. TASK COMMENCES	Removing vial containing chemically-digested sample from storage area.
5. TASK FINISHES	Labelling of prepared microscope slides.
6. TOOLS & EQUIPMENT	Vial containing chemically-digested sample. Microscope slides and coverslips. Hotplate Distilled water. Pasteur pipette. NBS NAPHRAX High Resolution Diatom Mountant (dissolved in toluene). Compound microscope.
7. LOCATIONS	Entire task should be undertaken within defined laboratory area. Steps involving Naphrax should take place in fume cupboard due to the presence of toluene..

8. ASSESSORS	Insert name of Team Leader Biology
9. DATE	Insert date
10. REVIEW PERIOD	12 months
11. AUTHORISATION	CHECKED BY SIGNED DATE …../…./…

PART 2. RISK ASSESSMENT SHEET

1. NO.	2. DESCRIPTION OF TASK ELEMENTS	3. IDENTIFICATION OF HAZARD, HARMFUL EFFECTS	4. IDENTIFICATION OF PERSONS AFFECTED	5. INITIAL RISK LEVEL H/M/L
1	Shaking of vial and removal of 2-3 drops to a cover slip.	Splashing/spillage of any preservatives added to the sample vial – refer to COSHH assessment.	Biology Team	M(3)
2	Heating of coverslip over hotplate.	Contact of skin/eyes with hydrogen peroxide solution – refer to COSHH assessment. Electric shock/scalding from hotplate. Cuts from breaking cover slip.	Biology Team	L(3)
3	Addition of Naphrax to microscope slide and application of coverslip to slide.	Splashing/spillage of Naphrax solution – refer to toluene COSHH assessment. Cuts from breaking microscope slide and/or coverslip.	Biology Team	L(2)
4	Examination of microscope slide under compound microscope.	Electric shock. Cuts from breaking microscope slide and/or coverslip.	Biology Team	L(3)
5	Label microscope slide and store until Naphrax has set.	Cuts from breaking microscope slide and/or coverslip.	Biology Team	L(2)

1. NO.	6. SUGGESTED RISK CONTROL MEASURES	7. LEVEL OF RISK H/M/L	8. RISK CONTROL MEASURES ADEQUATE YES/NO	9. OPTIONS FOR IMPROVED RISK CONTROL	10. PRIORITY OF ACTIONS REQUIRED H/M/L	11. ACTION PLAN REF. NO.
1	Restricted access to working area. Laboratory CoP. Laboratory induction procedure. Activity undertaken by authorised personnel only. Chemical spillage procedures. Fume cupboard. Equipment maintenance record. Hydrogen peroxide – COSHH Risk Assessment. Eye wash facilities/safety shower. Latex gloves, safety spectacles, laboratory coat.					
2	As 1 above, + Electrical safety testing. Hot plate operating instructions.					
3	As 1 above, + Naphrax (toluene) COSHH Risk Assessment.					
4	Restricted access to working area. Laboratory CoP. Laboratory induction procedure. Activity undertaken by authorised personnel only. Equipment maintenance record. Electrical testing.					
5	Restricted access to working area. Laboratory CoP. Laboratory induction procedure. Activity undertaken by authorised personnel only.					

C6. Identification and enumeration of diatoms from permanent microscope slides.

PART 1. WORK ACTIVITY/TASK IDENTIFICATION SHEET	REF DI 4.0
1. REGION/AREA	Insert as appropriate
2. TEAMS/JOB TITLES	Biology/Team Leader Biology/Biologists/Student Biologists/Temporary Biologists
3. TASK SUMMARY	Identification and enumeration of diatoms from previously-prepared microscope slides.
4. TASK COMMENCES	Removal of microscope slide from storage container.
5. TASK FINISHES	Return of slide to storage container following analysis.
6. TOOLS & EQUIPMENT	Microscope slides and coverslips. Microscope immersion oil. Count sheet + pen/pencil. Compound microscope.
7. LOCATIONS	Entire task should be undertaken within defined laboratory area.

8. ASSESSORS	Insert as appropriate
9. DATE	Insert date
10. REVIEW PERIOD	12 months
11. AUTHORISATION	CHECKED BY SIGNED DATE …../…./…

PART 2. RISK ASSESSMENT SHEET

1. NO.	2. DESCRIPTION OF TASK ELEMENTS	3. IDENTIFICATION OF HAZARD, HARMFUL EFFECTS	4. IDENTIFICATION OF PERSONS AFFECTED	5. INITIAL RISK LEVEL H/M/L
1	Removal/return of microscope slide from/to storage container.	Cuts from breaking microscope slide and/or coverslip.	Biology Team	L(1)
2	Addition/removal of microscope immersion oil.	Cuts from breaking microscope slide and/or coverslip. Splashing/spillage of microscope immersion oil – refer to COSHH Risk Assessment.	Biology Team	L(2)
3	Identification/enumeration of diatoms using compound microscope.	Electric shock. Eye strain. Back strain.	Biology Team	M(3)

1. NO.	6. EXISTING/PLANNED RISK CONTROL MEASURES	7. LEVEL OF RISK H/M/L	8. RISK CONTROL MEASURES ADEQUATE YES/NO	9. OPTIONS FOR IMPROVED RISK CONTROL	10. PRIORITY OF ACTIONS REQUIRED H/M/L	11. ACTION PLAN REF. NO.
1	Restricted access to working area. Laboratory CoP. Laboratory induction procedure. Activity undertaken by authorised personnel only.					
2	See control measures in 1 above, + Chemical inventory/storage arrangements. Chemical spillage procedures. Microscope immersion oil – COSHH Risk Assessment. Latex gloves, safety spectacles, laboratory coat.					
3	See control measures in 1 above, + Equipment maintenance record. Electrical testing.					

C7. Example COSHH assessment forms

Note that step 3 makes reference to the following table, relating information on quantity and dustiness/volatility of the identified health hazard group (see page 3 COSHH Risk Assessment Procedure "Applying the five step process") to find the level of control required.

LIKELIHOOD OF EXPOSURE	HEALTH HAZARD A	B	C	D	E
SMALL QUANTITY & LOW DUSTINESS/VOLATILITY	1	1	1	2	4
SMALL QUANTITY & MED DUSTINESS/VOLATILITY	1	1	2	3	4
SMALL QUANTITY & HIGH DUSTINESS/VOLATILITY	1	1	2	3	4
LARGE QUANTITY & LOW DUSTINESS/VOLATILITY	1	1	2	3	4
LARGE QUANTITY & MED DUSTINESS/VOLATILITY	1	2	3	4	4
LARGE QUANTITY & HIGH DUSTINESS/VOLATILITY	2	2	3	4	4

SUBSTANCES HAZARDOUS TO HEALTH RISK ASSESSMENT

REGION		**ASSESSED BY**	
AREA		**DATE**	
TEAMS	BIOLOGY	**REVIEW PERIOD**	12 months

STEP ONE

TASK DESCRIPTION AND SUBSTANCES USED (OR GENERATED)	Addition of iodine solution (1% w/v in potassium iodide) to diatom samples and subsequent laboratory procedures involving diatom samples preserved in iodine solution.
DESCRIBE WHY IT IS NOT REASONABLY PRACTICABLE TO ELIMINATE THE SUBSTANCE	Necessary to preserve samples between time of collection and analysis – dictated by seasonality of sampling.
DESCRIBE WHY IT IS NOT REASONABLY PRACTICABLE TO USE A SAFER SUBSTITUTE	None identified.
LIST OF ASSOCIATED ACTIVITIES AND POSSIBLE CAUSES OF EXPOSURE	Spillage/spillage clean-up. Breakage/leakage of stock solutions.
DESCRIBE ANY HAZARDOUS PROPERTIES OTHER THAN THOSE CAUSING RISKS TO HEALTH AND CONFIRM THAT CONTROLS ARE SPECIFIED IN THE TASK RISK ASSESSMENT	None identified.
DESCRIBE ANY HAZARDOUS PROPERTIES WHICH COULD CAUSE HARM TO THE ENVIRONMENT	None identified.

STEP TWO

IDENTIFY THE GROUPS OF EMPLOYEES UNDERTAKING THE TASK	Biology Team. Temporary staff/students have less experience of working with chemicals.
IDENTIFY OTHER PERSONS WHO MAY BE INVOLVED OR AFFECTED BY THE ASSOCIATED ACTIVITIES OR POSSIBLE CAUSES OF EXPOSURE	Others in lab. (e.g. building contractors, cleaners, visitors).

STEP THREE

IDENTIFY THE TYPE OF RISK TO HEALTH THAT THE SUBSTANCE PRESENTS AND TICK THE APPROPRIATE GROUP, PLUS THE S GROUP IF CONTACT WITH SKIN AND EYES IS HARMFUL

A	B	C	D	E	S
		✔			✔

DECIDE IF THE AMOUNT OF THE SUBSTANCE PER OCCURRENCE OF THE ACTIVITY CAN BE DESCRIBED AS:
SMALL (grams or millilitres)
OR, LARGE (kilograms or litres)
AND TICK THE APPROPRIATE BOX

SMALL	LARGE
	✔

IDENTIFY HOW DUSTY OR VOLATILE THE SUBSTANCE IS, AND TICK THE APPROPRIATE BOX

	DUSTINESS OF SOLID	OR	VOLATILITY OF LIQUID
LOW			✔
MEDIUM			
HIGH			

REFER TO THE TABLE ON P. 94 TO FIND THE LEVEL OF CONTROL YOU NEED

TICK THE APPROPRIATE BOX TO IDENTIFY THE CONTROL LEVEL NEEDED

1	2	3	4
	✔		

IF YOU ALSO TICKED BOX S IN THE HEALTH HAZARD GROUPS YOU WILL NEED TO CONSIDER	SAFE USE OF PPE EYE PROTECTION SKIN PROTECTION

STEP FOUR

RISK CONTROL LEVEL	RISK CONTROL APPROACH
1	**GENERAL VENTILATION** Natural or forced ventilation within the range of 5 and 15 air changes per hour to dilute the contaminant to a safe level. Working procedures defined and training and information provided to employees. Restricted access to working area.
2	**ENGINEERING CONTROL** Local exhaust ventilation applied at source to capture contaminants.
3	**CONTAINMENT** Closed systems possibly under negative pressure, with safe discharge arrangements for extracted air. Typically requiring permit to work maintenance procedures.
4	**SPECIAL CONTROLS** Controls designed by experts in occupational hygiene to provide site specific solutions for cases where an extreme risk to health exists.
S	**PROTECTION OF SKIN & EYES** Special attention to control measures protecting the individual and ensuring the effective use of items of personal protective equipment.

GIVE DETAILS OF THE CURRENT OR PROPOSED METHODS OF CONTROL	Restricted access to working area. Laboratory CoP. Laboratory induction procedure. Activity undertaken by authorised personnel only. Chemical inventory/storage arrangements. Chemical spillage procedures. Fume cupboard. Eye wash facilities/safety shower. PPE – heavy duty rubber/latex gloves, laboratory coat, safety spectacles.

DOES THE CURRENT METHOD OF CONTROL COMPLY WITH THE APPROACH IDENTIFIED IN THE RISK CONTROL LEVELS, ABOVE ?	**YES**	✔	**NO**	

GIVE DETAILS OF THE IMPROVED METHODS OF CONTROL THAT NEED TO BE IMPLEMENTED	

STEP FIVE

IMPLEMENTATION OF FURTHER ACTIONS		
ACTION	**BY WHOM**	**TARGET DATE**

SUBSTANCES HAZARDOUS TO HEALTH RISK ASSESSMENT

REGION		**ASSESSED BY**	
AREA		**DATE**	
TEAMS	BIOLOGY	**REVIEW PERIOD**	12 months

STEP ONE

TASK DESCRIPTION AND SUBSTANCES USED (OR GENERATED)	Laboratory and field procedures involving use of 4% formaldehyde solution (diluted from 40% stock solution).
DESCRIBE WHY IT IS NOT REASONABLY PRACTICABLE TO ELIMINATE THE SUBSTANCE	Need to fix/preserve samples between time of collection and analysis – seasonality of workload dictates that samples cannot be analysed immediately following collection.
DESCRIBE WHY IT IS NOT REASONABLY PRACTICABLE TO USE A SAFER SUBSTITUTE	Only effective substance for both fixing **and** preserving samples – experience shows that using alcohol preserves samples but does not fix them adequately for subsequent identification.
LIST OF ASSOCIATED ACTIVITIES AND POSSIBLE CAUSES OF EXPOSURE	Addition to and rinsing from biological samples. Road transport of samples containing. Handling/analysis of samples containing (including by contractors). Spillage/spillage clean-up. Breakage/leakage of stock solutions.
DESCRIBE ANY HAZARDOUS PROPERTIES OTHER THAN THOSE CAUSING RISKS TO HEALTH AND CONFIRM THAT CONTROLS ARE SPECIFIED IN THE TASK RISK ASSESSMENT	Combustible and incompatible with oxidising chemicals and acids – storage arrangements specified in task risk assessment.
DESCRIBE ANY HAZARDOUS PROPERTIES WHICH COULD CAUSE HARM TO THE ENVIRONMENT	Contamination of waste water and atmosphere.

STEP TWO

IDENTIFY THE GROUPS OF EMPLOYEES UNDERTAKING THE TASK	Biology Team. Temporary staff/students have less experience of working with chemicals.
IDENTIFY OTHER PERSONS WHO MAY BE INVOLVED OR AFFECTED BY THE ASSOCIATED ACTIVITIES OR POSSIBLE CAUSES OF EXPOSURE	Others in lab (e.g. building contractors, cleaners, visitors). Emergency Services and other EA staff in rest of building (in event of a "major" spill).

STEP THREE

IDENTIFY THE TYPE OF RISK TO HEALTH THAT THE SUBSTANCE PRESENTS AND TICK THE APPROPRIATE GROUP, PLUS THE S GROUP IF CONTACT WITH SKIN AND EYES IS HARMFUL

A	B	C	D	E	S
		✔			✔

DECIDE IF THE AMOUNT OF THE SUBSTANCE PER OCCURRENCE OF THE ACTIVITY CAN BE DESCRIBED AS:
SMALL (grams or millilitres)
OR, LARGE (kilograms or litres)
AND TICK THE APPROPRIATE BOX

SMALL	LARGE
	✔

IDENTIFY HOW DUSTY OR VOLATILE THE SUBSTANCE IS, AND TICK THE APPROPRIATE BOX

DUSTINESS OF SOLID		VOLATILITY OF LIQUID
	LOW	
	MEDIUM	✔
	HIGH	

REFER TO THE TABLE ON P. 94 TO FIND THE LEVEL OF CONTROL YOU NEED

TICK THE APPROPRIATE BOX TO IDENTIFY THE CONTROL LEVEL NEEDED

1	2	3	4
		✔	

IF YOU ALSO TICKED BOX S IN THE HEALTH HAZARD GROUPS YOU WILL NEED TO CONSIDER	SAFE USE OF PPE EYE PROTECTION SKIN PROTECTION

STEP FOUR

RISK CONTROL LEVEL	RISK CONTROL APPROACH
1	**GENERAL VENTILATION** Natural or forced ventilation within the range of 5 and 15 air changes per hour to dilute the contaminant to a safe level. Working procedures defined and training and information provided to employees. Restricted access to working area.
2	**ENGINEERING CONTROL** Local exhaust ventilation applied at source to capture contaminants.
3	**CONTAINMENT** Closed systems possibly under negative pressure, with safe discharge arrangements for extracted air. Typically requiring permit to work maintenance procedures.
4	**SPECIAL CONTROLS** Controls designed by experts in occupational hygiene to provide site specific solutions for cases where an extreme risk to health exists.
S	**PROTECTION OF SKIN & EYES** Special attention to control measures protecting the individual and ensuring the effective use of items of personal protective equipment.

GIVE DETAILS OF THE CURRENT OR PROPOSED METHODS OF CONTROL	Restricted access to working area. Laboratory Code of Practice. Laboratory induction procedure. Activity undertaken by authorised personnel only. Chemical inventory/storage arrangements. Chemical spillage procedures. LEV facility – annual testing/certification. Equipment maintenance record. Personal exposure monitoring. PPE – heavy duty rubber gloves, safety spectacles, laboratory coat. Information requested on safety precautions in contractor's laboratories. Field applications undertaken in "open air", wearing heavy duty rubber gloves and safety spectacles.

DOES THE CURRENT METHOD OF CONTROL COMPLY WITH THE APPROACH IDENTIFIED IN THE RISK CONTROL LEVELS, ABOVE ?	**YES**	✔	**NO**	

GIVE DETAILS OF THE IMPROVED METHODS OF CONTROL THAT NEED TO BE IMPLEMENTED	

STEP FIVE

IMPLEMENTATION OF FURTHER ACTIONS		
ACTION	**BY WHOM**	**TARGET DATE**

SUBSTANCES HAZARDOUS TO HEALTH RISK ASSESSMENT

REGION		**ASSESSED BY**	
AREA		**DATE**	
TEAMS	BIOLOGY	**REVIEW PERIOD**	12 months

STEP ONE

TASK DESCRIPTION AND SUBSTANCES USED (OR GENERATED)	Decanting stock solution of methylated spirit (industrial).
DESCRIBE WHY IT IS NOT REASONABLY PRACTICABLE TO ELIMINATE THE SUBSTANCE	Need to preserve biological specimens for reference, quality control etc without deterioration.
DESCRIBE WHY IT IS NOT REASONABLY PRACTICABLE TO USE A SAFER SUBSTITUTE	No lower risk alternative identified. IMS is a lower risk substitute for formaldehyde which could be used for the same purpose.
LIST OF ASSOCIATED ACTIVITIES AND POSSIBLE CAUSES OF EXPOSURE	Spillage/spillage clean-up. Breakage/leakage of stock solutions.
DESCRIBE ANY HAZARDOUS PROPERTIES OTHER THAN THOSE CAUSING RISKS TO HEALTH AND CONFIRM THAT CONTROLS ARE SPECIFIED IN THE TASK RISK ASSESSMENT	Highly flammable. Vapour/air mixture explosive. Incompatible with various chemicals – storage arrangements specified in task risk assessment.
DESCRIBE ANY HAZARDOUS PROPERTIES WHICH COULD CAUSE HARM TO THE ENVIRONMENT	None identified.

STEP TWO

IDENTIFY THE GROUPS OF EMPLOYEES UNDERTAKING THE TASK	Biology Team. Temporary staff/students have less experience of working with chemicals.
IDENTIFY OTHER PERSONS WHO MAY BE INVOLVED OR AFFECTED BY THE ASSOCIATED ACTIVITIES OR POSSIBLE CAUSES OF EXPOSURE	Others in lab (e.g. building contractors, cleaners, visitors). Emergency Services and other EA staff in rest of building (in event of "major" spill or fire).

STEP THREE

IDENTIFY THE TYPE OF RISK TO HEALTH THAT THE SUBSTANCE PRESENTS AND TICK THE APPROPRIATE GROUP, PLUS THE S GROUP IF CONTACT WITH SKIN AND EYES IS HARMFUL	**A** ☐ **B** ✔ **C** ☐ **D** ☐ **E** ☐ **S** ✔
DECIDE IF THE AMOUNT OF THE SUBSTANCE PER OCCURRENCE OF THE ACTIVITY CAN BE DESCRIBED AS: SMALL (grams or millilitres) OR, LARGE (kilograms or litres) AND TICK THE APPROPRIATE BOX	**SMALL** ☐ **LARGE** ✔

IDENTIFY HOW DUSTY OR VOLATILE THE SUBSTANCE IS, AND TICK THE APPROPRIATE BOX	DUSTINESS OF SOLID		VOLATILITY OF LIQUID
	☐	**LOW**	☐
	☐	**MEDIUM**	☐
	☐	**HIGH**	✔

REFER TO THE TABLE ON P. 94 TO FIND THE LEVEL OF CONTROL YOU NEED

TICK THE APPROPRIATE BOX TO IDENTIFY THE CONTROL LEVEL NEEDED	**1** ☐	**2** ✔	**3** ☐	**4** ☐

IF YOU ALSO TICKED BOX **S** IN THE HEALTH HAZARD GROUPS YOU WILL NEED TO CONSIDER	SAFE USE OF PPE EYE PROTECTION SKIN PROTECTION

STEP FOUR

RISK CONTROL LEVEL	RISK CONTROL APPROACH
1	**GENERAL VENTILATION** Natural or forced ventilation within the range of 5 and 15 air changes per hour to dilute the contaminant to a safe level. Working procedures defined and training and information provided to employees. Restricted access to working area.
2	**ENGINEERING CONTROL** Local exhaust ventilation applied at source to capture contaminants.
3	**CONTAINMENT** Closed systems possibly under negative pressure, with safe discharge arrangements for extracted air. Typically requiring permit to work maintenance procedures.
4	**SPECIAL CONTROLS** Controls designed by experts in occupational hygiene to provide site specific solutions for cases where an extreme risk to health exists.
S	**PROTECTION OF SKIN & EYES** Special attention to control measures protecting the individual and ensuring the effective use of items of personal protective equipment.

GIVE DETAILS OF THE CURRENT OR PROPOSED METHODS OF CONTROL	Restricted access to working area. Laboratory Code of Practice. Laboratory induction procedure. Activity undertaken by authorised personnel only. Chemical inventory/storage arrangements. Chemical spillage procedures. Fume cupboard. Equipment maintenance record. PPE – heavy duty rubber gloves, safety spectacles, laboratory coat.

DOES THE CURRENT METHOD OF CONTROL COMPLY WITH THE APPROACH IDENTIFIED IN THE RISK CONTROL LEVELS, ABOVE ?	**YES**	✔	**NO**	

GIVE DETAILS OF THE IMPROVED METHODS OF CONTROL THAT NEED TO BE IMPLEMENTED	

STEP FIVE

IMPLEMENTATION OF FURTHER ACTIONS		
ACTION	**BY WHOM**	**TARGET DATE**

SUBSTANCES HAZARDOUS TO HEALTH RISK ASSESSMENT

REGION		**ASSESSED BY**	
AREA		**DATE**	
TEAMS	BIOLOGY	**REVIEW PERIOD**	12 months

STEP ONE

TASK DESCRIPTION AND SUBSTANCES USED (OR GENERATED)	Decanting 37% hydrochloric acid from stock to glass dropping bottle and subsequent addition to samples during chemical digestion process.
DESCRIBE WHY IT IS NOT REASONABLY PRACTICABLE TO ELIMINATE THE SUBSTANCE	Chemical digestion is an essential pre-cursor to microscopic examination of diatom samples.
DESCRIBE WHY IT IS NOT REASONABLY PRACTICABLE TO USE A SAFER SUBSTITUTE	Chemical digestion process requires use of acid and strong oxidising agents. Chemicals used are the only ones which are suitable for polypropylene rope samples.
LIST OF ASSOCIATED ACTIVITIES AND POSSIBLE CAUSES OF EXPOSURE	Spillage/spillage clean-up. Breakage/leakage of stock solution.
DESCRIBE ANY HAZARDOUS PROPERTIES OTHER THAN THOSE CAUSING RISKS TO HEALTH AND CONFIRM THAT CONTROLS ARE SPECIFIED IN THE TASK RISK ASSESSMENT	Storage (away from heat, flame and direct sunlight and in cool, dry and well-ventilated area) specified in task risk assessment.
DESCRIBE ANY HAZARDOUS PROPERTIES WHICH COULD CAUSE HARM TO THE ENVIRONMENT	None identified.

STEP TWO

IDENTIFY THE GROUPS OF EMPLOYEES UNDERTAKING THE TASK	Biology Team. Temporary staff/students have less experience of working with chemicals.
IDENTIFY OTHER PERSONS WHO MAY BE INVOLVED OR AFFECTED BY THE ASSOCIATED ACTIVITIES OR POSSIBLE CAUSES OF EXPOSURE	Others in lab. (e.g. building contractors, cleaners, visitors).

STEP THREE

IDENTIFY THE TYPE OF RISK TO HEALTH THAT THE SUBSTANCE PRESENTS AND TICK THE APPROPRIATE GROUP, PLUS THE S GROUP IF CONTACT WITH SKIN AND EYES IS HARMFUL

A	B	C	D	E	S
		✔			✔

DECIDE IF THE AMOUNT OF THE SUBSTANCE PER OCCURRENCE OF THE ACTIVITY CAN BE DESCRIBED AS:
SMALL (grams or millilitres)
OR, LARGE (kilograms or litres)
AND TICK THE APPROPRIATE BOX

SMALL	LARGE
✔	

IDENTIFY HOW DUSTY OR VOLATILE THE SUBSTANCE IS, AND TICK THE APPROPRIATE BOX

DUSTINESS OF SOLID		VOLATILITY OF LIQUID
	LOW	
	MEDIUM	✔
	HIGH	

REFER TO THE TABLE ON P. 94 TO FIND THE LEVEL OF CONTROL YOU NEED

TICK THE APPROPRIATE BOX TO IDENTIFY THE CONTROL LEVEL NEEDED

1	2	3	4
	✔		

IF YOU ALSO TICKED BOX S IN THE HEALTH HAZARD GROUPS YOU WILL NEED TO CONSIDER	SAFE USE OF PPE EYE PROTECTION SKIN PROTECTION

STEP FOUR

RISK CONTROL LEVEL	RISK CONTROL APPROACH
1	**GENERAL VENTILATION** Natural or forced ventilation within the range of 5 and 15 air changes per hour to dilute the contaminant to a safe level. Working procedures defined and training and information provided to employees. Restricted access to working area.
2	**ENGINEERING CONTROL** Local exhaust ventilation applied at source to capture contaminants.
3	**CONTAINMENT** Closed systems possibly under negative pressure, with safe discharge arrangements for extracted air. Typically requiring permit to work maintenance procedures.
4	**SPECIAL CONTROLS** Controls designed by experts in occupational hygiene to provide site specific solutions for cases where an extreme risk to health exists.
S	**PROTECTION OF SKIN & EYES** Special attention to control measures protecting the individual and ensuring the effective use of items of personal protective equipment.

GIVE DETAILS OF THE CURRENT OR PROPOSED METHODS OF CONTROL	Restricted access to working area. Laboratory CoP. Laboratory induction procedure. Activity undertaken by authorised personnel only. Chemical inventory/storage arrangements. Chemical spillage procedures. Fume cupboard. Eye wash facilities/safety shower. PPE – heavy duty rubber/latex gloves, safety spectacles, laboratory coat. Use of glass dropping bottles reduces risk and volume of any spillage during chemical digestion process.

DOES THE CURRENT METHOD OF CONTROL COMPLY WITH THE APPROACH IDENTIFIED IN THE RISK CONTROL LEVELS, ABOVE ?	**YES**	✔	**NO**	

GIVE DETAILS OF THE IMPROVED METHODS OF CONTROL THAT NEED TO BE IMPLEMENTED	

STEP FIVE

IMPLEMENTATION OF FURTHER ACTIONS

ACTION	BY WHOM	TARGET DATE

SUBSTANCES HAZARDOUS TO HEALTH RISK ASSESSMENT

REGION		**ASSESSED BY**	
AREA		**DATE**	
TEAMS	BIOLOGY	**REVIEW PERIOD**	12 months

STEP ONE

TASK DESCRIPTION	Preparation of saturated oxalic acid. Decanting saturated oxalic acid into glass dropping bottle and subsequent use in chemical digestion of diatom samples.
SUBSTANCE IDENTIFICATION	Oxalic acid dihydrate.
DESCRIBE ANY HAZARDOUS PROPERTIES OF THE SUBSTANCES CAUSING RISKS TO HEALTH	Contact with skin and eyes causes severe irritation, and possible eye burns. Causes gastrointestinal tract burns. Causes severe pain, nausea, vomiting, diarrhoea and shock. May cause haemorrhaging of digestive tract. Overexposure can cause hypocalcemia and kidney injury. May cause severe irritation of respiratory tract with pain, burns and inflammation.
DESCRIBE ANY HAZARDOUS PROPERTIES OTHER THAN THOSE CAUSING RISKS TO HEALTH AND CONFIRM THAT CONTROLS ARE SPECIFIED IN THE TASK RISK ASSESSMENT (fire, explosion, environmental etc.)	Incompatible with combustible materials and certain other chemicals, including oxidising agents - storage conditions specified in task risk assessment.
DESCRIBE ANY HAZARDOUS PROPERTIES WHICH COULD CAUSE HARM TO THE ENVIRONMENT	Contamination of waste water.
DESCRIBE WHY IT IS NOT REASONABLY PRACTICABLE TO ELIMINATE THE SUBSTANCE	Chemical digestion is an essential pre-cursor to microscopic examination of diatom samples.
DESCRIBE WHY IT IS NOT REASONABLY PRACTICABLE TO USE A SAFER SUBSTITUTE	Chemical digestion with oxalic acid and sulphuric acid has been identified as the safest option and replaces the previous use of hydrogen peroxide.
LIST OF ASSOCIATED ACTIVITIES AND POSSIBLE CAUSES OF EXPOSURE	Spillage/spillage clean-up. Breakage/leakage of stock solution.

STEP TWO

IDENTIFY THE GROUPS OF EMPLOYEES UNDERTAKING THE TASK	Biology Team. Temporary staff/students have less experience of working with chemicals - only staff deemed competent will be permitted to undertake tasks using this substance.
IDENTIFY OTHER PERSONS WHO MAY BE INVOLVED OR AFFECTED BY THE ASSOCIATED ACTIVITIES OR POSSIBLE CAUSES OF EXPOSURE	Others in lab (e.g. building contrators, cleaners, visitors) - access to lab restricted to authorised personnel only.

STEP THREE

IDENTIFY THE TYPE OF RISK TO HEALTH THAT THE SUBSTANCE PRESENTS AND TICK THE APPROPRIATE GROUP, PLUS THE S GROUP IF CONTACT WITH SKIN AND EYES IS HARMFUL

A ☐ **B** ☑ **C** ☐ **D** ☐ **E** ☐ **S** ☑

DECIDE IF THE AMOUNT OF THE SUBSTANCE PER OCCURRENCE OF THE ACTIVITY CAN BE DESCRIBED AS:
SMALL (grams or millilitres)
OR, LARGE (kilograms or litres)
AND TICK THE APPROPRIATE BOX

SMALL ☑ **LARGE** ☐

IDENTIFY HOW DUSTY OR VOLATILE THE SUBSTANCE IS, AND TICK THE APPROPRIATE BOX

DUSTINESS OF SOLID		VOLATILITY OF LIQUID
☐	**LOW**	☐
☑	**MEDIUM**	☐
☐	**HIGH**	☐

REFER TO THE TABLE ON P. 94 TO FIND THE LEVEL OF CONTROL YOU NEED

TICK THE APPROPRIATE BOX TO IDENTIFY THE CONTROL LEVEL NEEDED

1 ☑ **2** ☐ **3** ☐ **4** ☐

IF YOU ALSO TICKED BOX S IN THE HEALTH HAZARD GROUPS YOU WILL NEED TO CONSIDER	SAFE USE OF PPE EYE PROTECTION SKIN PROTECTION

STEP FOUR

TICK THE APPROPRIATE BOX TO IDENTIFY THE CONTROL LEVEL NEEDED	1	✔	2		3		4		S	✔

GIVE DETAILS OF ENGINEERING CONTROLS REQUIRED	Operation should be undertaken within fume cupboard.
GIVE DETAILS OF PPE REQUIRED (If you also ticked box s in the health hazard groups you will need to consider this)	Chemical resistant gloves, safety spectacles & laboratory coat.
GIVE DETAILS OF ANY RPE REQUIRED	Only required for spillage clean-up: facemask + appropriate filter.
GIVE DETAILS OF ANY OTHER METHODS OF CONTROL REQUIRED (Procedures, etc)	Restricted access to working area. Laboratory CoP. Laboratory induction procedure. Activity undertaken by authorised personnel only. Chemical inventory/storage arrangements. Chemical spillage procedures. Eye wash facilities/safety shower. Use of glass dropping bottle reduces risk of spillage during chemical digestion process.

IS EXPOSURE MONITORING REQUIRED	**YES**		**NO**	✔
IS HEALTH SURVEILLANCE REQUIRED	**YES**		**NO**	✔

EMERGENCY RESPONSE? STORAGE?	Spillage procedures are outlined in the laboratory's "Chemical Spillage Procedures". Correct storage arrangements are identified in the laboratory's "Chemical Inventory & Storage Arrangements".

STEP FIVE

IMPLEMENTATION OF FURTHER ACTIONS		
ACTION	**BY WHOM**	**TARGET DATE**
No further actions required - all identified risks are covered by existing procedures etc.		

SUBSTANCES HAZARDOUS TO HEALTH RISK ASSESSMENT

REGION		**ASSESSED BY**	
AREA		**DATE**	
TEAMS	BIOLOGY	**REVIEW PERIOD**	12 months

STEP ONE

TASK DESCRIPTION	Addition of potassium permanganate crystals to beaker containing rope + sulphuric acid and chemical digestion of samples.
SUBSTANCE IDENTIFICATION	Permanganic acid, potassium salt (100%).
DESCRIBE ANY HAZARDOUS PROPERTIES OF THE SUBSTANCES CAUSING RISKS TO HEALTH	Contact with skin and eyes causes severe irritation and possible burns. May causes gastrointestinal tract irritation and kidney damage. Inhalation causes respiratory tract irritation. Chronic exposure may cause defatting and dermatitus.
DESCRIBE ANY HAZARDOUS PROPERTIES OTHER THAN THOSE CAUSING RISKS TO HEALTH AND CONFIRM THAT CONTROLS ARE SPECIFIED IN THE TASK RISK ASSESSMENT (fire, explosion, environmental etc.)	Incompatible with combustible materials and certain other chemicals, including oxidising agents, formaldehyde and concentrated hydrochloric acid - storage conditions specified in task risk assessment.
DESCRIBE ANY HAZARDOUS PROPERTIES WHICH COULD CAUSE HARM TO THE ENVIRONMENT	Contamination of waste water.
DESCRIBE WHY IT IS NOT REASONABLY PRACTICABLE TO ELIMINATE THE SUBSTANCE	Chemical digestion is an essential pre-cursor to microscopic examination of diatom samples.
DESCRIBE WHY IT IS NOT REASONABLY PRACTICABLE TO USE A SAFER SUBSTITUTE	Chemical digestion with oxalic acid, sulphuric acid and potassium permanganate has been identified as the safest option and replaces the previous use of hydrogen peroxide.
LIST OF ASSOCIATED ACTIVITIES AND POSSIBLE CAUSES OF EXPOSURE	Spillage/spillage clean-up. Breakage/leakage of stock solution.

STEP TWO / THREE

IDENTIFY THE GROUPS OF EMPLOYEES UNDERTAKING THE TASK & ESPECIALLY VULNERABLE GROUPS/INDIVIDUALS	Biology Team. Temporary staff/students have less experience of working with chemicals - only staff deemed competent will be permitted to undertake tasks using this substance.
IDENTIFY OTHER PERSONS WHO MAY BE INVOLVED OR AFFECTED BY THE ASSOCIATED ACTIVITIES OR POSSIBLE CAUSES OF EXPOSURE	Others in lab (e.g. building contrators, cleaners, visitors) - access to lab restricted to authorised personnel only.

IDENTIFY THE TYPE OF RISK TO HEALTH THAT THE SUBSTANCE PRESENTS AND TICK THE APPROPRIATE GROUP, PLUS THE S GROUP IF CONTACT WITH SKIN AND EYES IS HARMFUL

A	B	C	D	E	S
	✔				✔

DECIDE IF THE AMOUNT OF THE SUBSTANCE PER OCCURRENCE OF THE ACTIVITY CAN BE DESCRIBED AS:
SMALL (grams or millilitres)
OR, LARGE (kilograms or litres)
AND TICK THE APPROPRIATE BOX

SMALL	LARGE
✔	

IDENTIFY HOW DUSTY OR VOLATILE THE SUBSTANCE IS, AND TICK THE APPROPRIATE BOX

DUSTINESS OF SOLID		OR		VOLATILITY OF LIQUID
	LOW		LOW	
✔	MEDIUM		MEDIUM	
	HIGH		HIGH	

STEP FOUR

TICK THE APPROPRIATE BOX TO IDENTIFY THE CONTROL LEVEL NEEDED	1	✔	2		3		4		S	✔

GIVE DETAILS OF ENGINEERING CONTROLS REQUIRED	Operation should be undertaken within fume cupboard.
GIVE DETAILS OF PPE REQUIRED (If you also ticked box s in the health hazard groups you will need to consider this)	Chemical resistant gloves, safety spectacles & laboratory coat.
GIVE DETAILS OF ANY RPE REQUIRED	Only required for spillage clean-up: facemask + appropriate filter.
GIVE DETAILS OF ANY OTHER METHODS OF CONTROL REQUIRED (Procedures, etc)	Restricted access to working area. Laboratory CoP. Laboratory induction procedure. Activity undertaken by authorised personnel only. Chemical inventory/storage arrangements. Chemical spillage procedures. Eye wash facilities/safety shower. Use of glass dropping bottle reduces risk of spillage during chemical digestion process.

IS EXPOSURE MONITORING REQUIRED	**YES**		**NO**	✔
IS HEALTH SURVEILLANCE REQUIRED	**YES**		**NO**	✔

EMERGENCY RESPONSE?	Spillage procedures are outlined in the laboratory's "Chemical Spillage Procedures".
STORAGE?	Correct storage arrangements are identified in the laboratory's "Chemical Inventory & Storage Arrangements".

STEP FIVE

IMPLEMENTATION OF FURTHER ACTIONS		
ACTION	**BY WHOM**	**TARGET DATE**
No further actions required - all identified risks are covered by existing procedures etc.		

SUBSTANCES HAZARDOUS TO HEALTH RISK ASSESSMENT

REGION	
AREA	
TEAMS	BIOLOGY

ASSESSED BY	
DATE	
REVIEW PERIOD	12 months

STEP ONE

TASK DESCRIPTION	Decanting sulphuric acid into glass dropping bottle and subsequent use in chemical digestion of diatom samples.
SUBSTANCE IDENTIFICATION	Sulphuric acid (95-98.0%)
DESCRIBE ANY HAZARDOUS PROPERTIES OF THE SUBSTANCES CAUSING RISKS TO HEALTH	Contact with skin and eyes causes burns, possible irreversible eye damage and tissue necrosis (continued contact). Causes gastrointestinal tract burns. Possible severe and permanent damage. Causes irritation and haemorrhaging of digestive tract. Can be fatal. Chronic exposure may cause kidney and lung damage, foetal effects and cancer.
DESCRIBE ANY HAZARDOUS PROPERTIES OTHER THAN THOSE CAUSING RISKS TO HEALTH AND CONFIRM THAT CONTROLS ARE SPECIFIED IN THE TASK RISK ASSESSMENT (fire, explosion, environmental etc.)	Contact with combustible materials may cause fire. Contact with water can cause violent liberation of heat and splattering of the material. Containers may explode when heated or if contaminated with water. Contact with metals may evolve flammable hydrogen gas. Storage conditions are specified in task risk assessment.
DESCRIBE ANY HAZARDOUS PROPERTIES WHICH COULD CAUSE HARM TO THE ENVIRONMENT	Runoff from fire control or dilution water may cause pollution.
DESCRIBE WHY IT IS NOT REASONABLY PRACTICABLE TO ELIMINATE THE SUBSTANCE	Chemical digestion is an essential pre-cursor to microscopic examination of diatom samples.
DESCRIBE WHY IT IS NOT REASONABLY PRACTICABLE TO USE A SAFER SUBSTITUTE	Chemical digestion with oxalic acid and sulphuric acid has been identified as the safest option and replaces the previous use of hydrogen peroxide.
LIST OF ASSOCIATED ACTIVITIES AND POSSIBLE CAUSES OF EXPOSURE	Spillage/spillage clean-up. Breakage/leakage of stock solution.

STEP TWO/THREE

IDENTIFY THE GROUPS OF EMPLOYEES UNDERTAKING THE TASK & ESPECIALLY VULNERABLE GROUPS/INDIVIDUALS	Biology Team. Temporary staff/students have less experience of working with chemicals - only staff deemed competent will be permitted to undertake tasks using this substance.
IDENTIFY OTHER PERSONS WHO MAY BE INVOLVED OR AFFECTED BY THE ASSOCIATED ACTIVITIES OR POSSIBLE CAUSES OF EXPOSURE	Others in lab (e.g. building contractors, cleaners, visitors) - access to lab restricted to authorised personnel only.

IDENTIFY THE TYPE OF RISK TO HEALTH THAT THE SUBSTANCE PRESENTS AND TICK THE APPROPRIATE GROUP, PLUS THE S GROUP IF CONTACT WITH SKIN AND EYES IS HARMFUL

A ☐ **B** ☐ **C** ☑ **D** ☐ **E** ☐ **S** ☑

DECIDE IF THE AMOUNT OF THE SUBSTANCE PER OCCURRENCE OF THE ACTIVITY CAN BE DESCRIBED AS: SMALL (grams or millilitres) OR, LARGE (kilograms or litres) AND TICK THE APPROPRIATE BOX

SMALL ☑ **LARGE** ☐

DUSTINESS OF SOLID OR VOLATILITY OF LIQUID

REFER TO THE TABLE ON P. 94 TO FIND THE LEVEL OF CONTROL YOU NEED

IDENTIFY HOW DUSTY OR VOLATILE THE SUBSTANCE IS, AND TICK THE APPROPRIATE BOX

☐	**LOW**	☑
☐	**MEDIUM**	☐
☐	**HIGH**	☐

STEP FOUR

TICK THE APPROPRIATE BOX TO IDENTIFY THE CONTROL LEVEL NEEDED	1	✔	2		3		4		S	✔

GIVE DETAILS OF ENGINEERING CONTROLS REQUIRED	Operation should be undertaken within fume cupboard.
GIVE DETAILS OF PPE REQUIRED (If you also ticked box s in the health hazard groups you will need to consider this)	Chemical resistant gloves, safety spectacles & laboratory coat.
GIVE DETAILS OF ANY RPE REQUIRED	Only required for spillage clean-up: facemask + appropriate filter.
GIVE DETAILS OF ANY OTHER METHODS OF CONTROL REQUIRED (Procedures, etc)	Restricted access to working area. Laboratory CoP. Laboratory induction procedure. Activity undertaken by authorised personnel only. Chemical inventory/storage arrangements. Chemical spillage procedures. Eye wash facilities/safety shower. Use of glass dropping bottle reduces risk of spillage during chemical digestion process.
IS EXPOSURE MONITORING REQUIRED	**YES** ☐ **NO** ✔
IS HEALTH SURVEILLANCE REQUIRED	**YES** ☐ **NO** ✔
EMERGENCY RESPONSE? STORAGE?	Spillage procedures are outlined in the laboratory's "Chemical Spillage Procedures". Correct storage arrangements are identified in the laboratory's "Chemical Inventory & Storage Arrangements".

STEP FIVE

IMPLEMENTATION OF FURTHER ACTIONS		
ACTION	**BY WHOM**	**TARGET DATE**
No further actions required - all identified risks are covered by existing procedures etc.		

SUBSTANCES HAZARDOUS TO HEALTH RISK ASSESSMENT

REGION		**ASSESSED BY**	
AREA		**DATE**	
TEAMS	BIOLOGY	**REVIEW PERIOD**	12 months

STEP ONE

TASK DESCRIPTION	Use of hydrogen peroxide in chemical digestion of diatom samples.
SUBSTANCE IDENTIFICATION	Hydrogen peroxide, 30% v/v.
DESCRIBE ANY HAZARDOUS PROPERTIES OF THE SUBSTANCES CAUSING RISKS TO HEALTH	
DESCRIBE ANY HAZARDOUS PROPERTIES OTHER THAN THOSE CAUSING RISKS TO HEALTH AND CONFIRM THAT CONTROLS ARE SPECIFIED IN THE TASK RISK ASSESSMENT (fire, explosion, environmental etc.)	
DESCRIBE ANY HAZARDOUS PROPERTIES WHICH COULD CAUSE HARM TO THE ENVIRONMENT	
DESCRIBE WHY IT IS NOT REASONABLY PRACTICABLE TO ELIMINATE THE SUBSTANCE	Chemical digestion is an essential pre-cursor to microscopic examination of diatom samples.
DESCRIBE WHY IT IS NOT REASONABLY PRACTICABLE TO USE A SAFER SUBSTITUTE	
LIST OF ASSOCIATED ACTIVITIES AND POSSIBLE CAUSES OF EXPOSURE	

STEP TWO

IDENTIFY THE GROUPS OF EMPLOYEES UNDERTAKING THE TASK	Biology Team. Temporary staff/students have less experience of working with chemicals - only staff deemed competent will be permitted to undertake tasks using this substance.
IDENTIFY OTHER PERSONS WHO MAY BE INVOLVED OR AFFECTED BY THE ASSOCIATED ACTIVITIES OR POSSIBLE CAUSES OF EXPOSURE	Others in lab (e.g. building contrators, cleaners, visitors) - access to lab restricted to authorised personnel only.

STEP THREE

IDENTIFY THE TYPE OF RISK TO HEALTH THAT THE SUBSTANCE PRESENTS AND TICK THE APPROPRIATE GROUP, PLUS THE S GROUP IF CONTACT WITH SKIN AND EYES IS HARMFUL

A	B	C	D	E	S
					✔

DECIDE IF THE AMOUNT OF THE SUBSTANCE PER OCCURRENCE OF THE ACTIVITY CAN BE DESCRIBED AS:
SMALL (grams or millilitres)
OR, LARGE (kilograms or litres)
AND TICK THE APPROPRIATE BOX

SMALL	LARGE
✔	

IDENTIFY HOW DUSTY OR VOLATILE THE SUBSTANCE IS, AND TICK THE APPROPRIATE BOX

	DUSTINESS OF SOLID	OR	VOLATILITY OF LIQUID
LOW			✔
MEDIUM			
HIGH			

REFER TO THE TABLE ON P. 94 TO FIND THE LEVEL OF CONTROL YOU NEED

TICK THE APPROPRIATE BOX TO IDENTIFY THE CONTROL LEVEL NEEDED

1	2	3	4
✔			

IF YOU ALSO TICKED BOX S IN THE HEALTH HAZARD GROUPS YOU WILL NEED TO CONSIDER	SAFE USE OF PPE EYE PROTECTION SKIN PROTECTION

STEP FOUR

TICK THE APPROPRIATE BOX TO IDENTIFY THE CONTROL LEVEL NEEDED	1		2		3		4		S	

GIVE DETAILS OF ENGINEERING CONTROLS REQUIRED	Operation should be undertaken within fume cupboard.
GIVE DETAILS OF PPE REQUIRED (If you also ticked box s in the health hazard groups you will need to consider this)	Chemical resistant gloves, safety spectacles & laboratory coat.
GIVE DETAILS OF ANY RPE REQUIRED	Only required for spillage clean-up: facemask + appropriate filter.
GIVE DETAILS OF ANY OTHER METHODS OF CONTROL REQUIRED (Procedures, etc)	Restricted access to working area. Laboratory CoP. Laboratory induction procedure. Activity undertaken by authorised personnel only. Chemical inventory/storage arrangements. Chemical spillage procedures. Eye wash facilities/safety shower. Use of glass dropping bottle reduces risk of spillage during chemical digestion process.

IS EXPOSURE MONITORING REQUIRED	**YES**		**NO**	✔
IS HEALTH SURVEILLANCE REQUIRED	**YES**		**NO**	✔

EMERGENCY RESPONSE? STORAGE?	Spillage procedures are outlined in the laboratory's "Chemical Spillage Procedures". Correct storage arrangements are identified in the laboratory's "Chemical Inventory & Storage Arrangements".

STEP FIVE

IMPLEMENTATION OF FURTHER ACTIONS		
ACTION	**BY WHOM**	**TARGET DATE**