2. SAMPLE COLLECTION

2.1 Introduction

This document describes the procedures that must be followed when collecting diatom samples for use in the DARES project. It is very similar to that described in the *TDI Manual* and is compliant with EN 13946:2003 (*Water quality – Guidance Standard for the routine sampling and pre-treatment of benthic diatom samples from rivers*). There are, however, a few differences in detail between the *TDI manual* and this DARES method and it is essential that samplers read this document thoroughly before collecting any samples for the DARES project.

The main difference lies in the overall sampling strategy: the *TDI Manual* was written with the needs of the Urban Wastewater Treatment Directive in mind. As this required assessments of eutrophication to be made relative to conditions upstream of discharges, a strong emphasis was placed on consistency within a study, but with sufficient flexibility to allow the method to be adapted to all situations likely to be encountered. The Water Framework Directive (WFD), however, requires methods that give comparable results throughout the country and, indeed, elsewhere in Europe. For this reason, the methods described in this protocol may seem more prescriptive than those described in the *TDI Manual.*

A further difference is that there is no option here for the use of introduced substrates. Assessment of 'good ecological status' requires assessment of the biota that occurs naturally at a site and the flora that develops on an introduced substrate, whilst reflecting prevailing environmental conditions, is clearly not natural.

2.2 Health and Safety considerations

Before using this protocol it is essential that fieldworkers are familiar with the risks and hazards associated with sampling from flowing waters. For Environment Agency staff, the Health and Safety guidance is given in the document *Generic Risk Assessment for Fieldwork* (currently in draft form) and the work must be undertaken in accordance with the specified safety controls.

2.3 Principle of method

Benthic diatom samples are collected from submerged surfaces in rivers in order to produce representative samples of the taxa present at the site. The preferred substrate is cobbles, but if these or other hard surfaces are not characteristic of the river type, then macrophytes are sampled instead.

2.4 Overview of sampling strategy

Diatom communities in rivers are neither simple, nor homogeneous. 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. In addition, phytoplankton cells such as *Cyclotella meneghiniana* might become trapped within the mucus, along with benthic taxa that have been dislodged from sites further upstream

In general, communities in the upper reaches of rivers, where current velocity is high, tend to consist of diatoms closely adpressed to rocks (e.g. *Cocconeis placentula*) or with short, flexible stalks (e.g. *Achnanthidium minutissimum*). 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 in determining the benthic diatoms found at a site; 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.

Three 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 are found within the main flow of the river in reaches that are not heavily shaded. In the absence of cobbles and small boulders, emergent macrophytes should be sampled instead and, if these too are not available, then submerged macrophytes should be sampled. If none of these are available, then no sample should be collected but a field record form must still be completed, and the reason for the lack of a sample should be noted.

   There are a few situations where there are no natural hard surfaces suitable for sampling, but there are permanent man-made structures such as weirs and bridge supports. These can be sampled, using a modified protocol (2.10) and special apparatus (2.7, Fig. 2.2).

2. Substrates should be submerged for at least four weeks prior to sampling. All depths that can be easily sampled wearing thigh 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. In deeper rivers, it may be necessary to collect samples from the edges.

3. Notes on the prevailing conditions must also be made to aid subsequent interpretation of the data (a field record form is provided on the DARES website.

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 if cobbles and small boulders are scarce. The main requirement for a sampling site is that it is typical of the reach being assessed, with riffles recommended in waters with classic riffle-run-pool sequences. However, "runs" and "glides" are also suitable if these have suitable substrates.

For the 2004 sampling program, diatom samples should be collected from the same locations as GQA macro-invertebrate samples, unless macro-invertebrate sites are deemed unsuitable (given the rationale above). In such cases, diatom samples should be collected form a nearby more suitable site, where available, and a note giving the reasons should be made on the sample record form.

2.5 Choice of substrate

The WFD requires comparison of the biota found at a site with 'type-specific reference conditions'. In order to collect diatom samples that are suitable for these assessments, identical substrates must be sampled at all sites within a river 'type'. Unfortunately, the final typology for river diatoms in the UK will not be available until the end of the DARES project and some assumptions, based on the DARES team's own experiences of UK rivers have had to be made at the outset.

Cobbles are the recommended substrate, as these balance stability (allowing diatom communities to develop) with manoeuvrability. These should be available at most river types. Large 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 most of hard surfaces are smothered with growths of *Cladophora* or other filamentous algae. Under such circumstances, the sampling protocol needs to be modified in order to ensure that samples are representative of the site, (see below). In the absence of cobbles or other inorganic surfaces, then samples should be collected from macrophytes. The films found on submerged portions of the stems of emergent macrophytes such as *Sparganium* and *Phragmites* are preferred (so long as they are not contaminated by bottom sediments) but in the absence of such surfaces, then submerged macrophytes such as *Ranunculus* should be used. The flow chart (Fig. 2.1) is designed to guide users to the most appropriate means of sampling

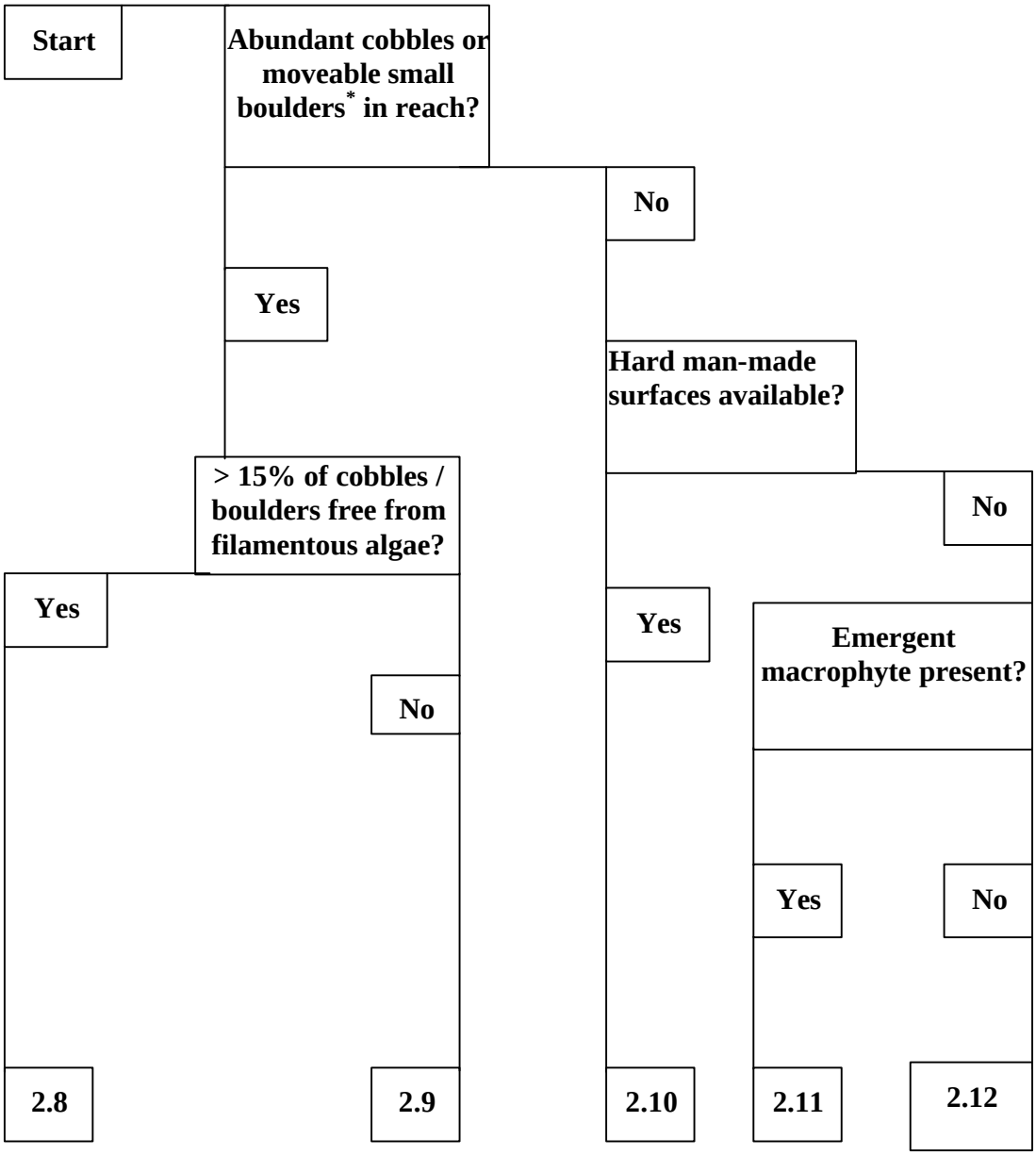

Fig. 2.1. Flow chart for selecting appropriate sampling strategies for collecting benthic diatoms for environmental-monitoring studies. * Under some circumstances (e.g. chalk streams), large pebbles may be substituted for cobbles. Increase the number of pebbles sampled accordingly.

under various circumstances and tries to balance the competing interests of standardisation and typicality of the substrate. Any deviations from these methods must be fully documented in order to inform subsequent data interpretation. The field record form also includes boxes for a rough indication of the period of time since the last spate.

2.6 Timing of surveys

Samples should be collected during the Spring and Autumn GQA surveys and the date noted on both the field record form and the sample bottle. Spring samples should not be collected before mid-April.

2.7 Apparatus

- Thigh waders (and associated water safety equipment).
- 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 (if necessary). 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. Modified Lugol's Iodine is recommended. If samples are to be processed immediately on return to the laboratory, the preservative should be omitted; however, as many 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 processing. The preservative should be added immediately on return to the laboratory, following appropriate Health and Safety guidelines. Lugol's Iodine can be prepared by dissolving 2 g potassium iodide and 1 g 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

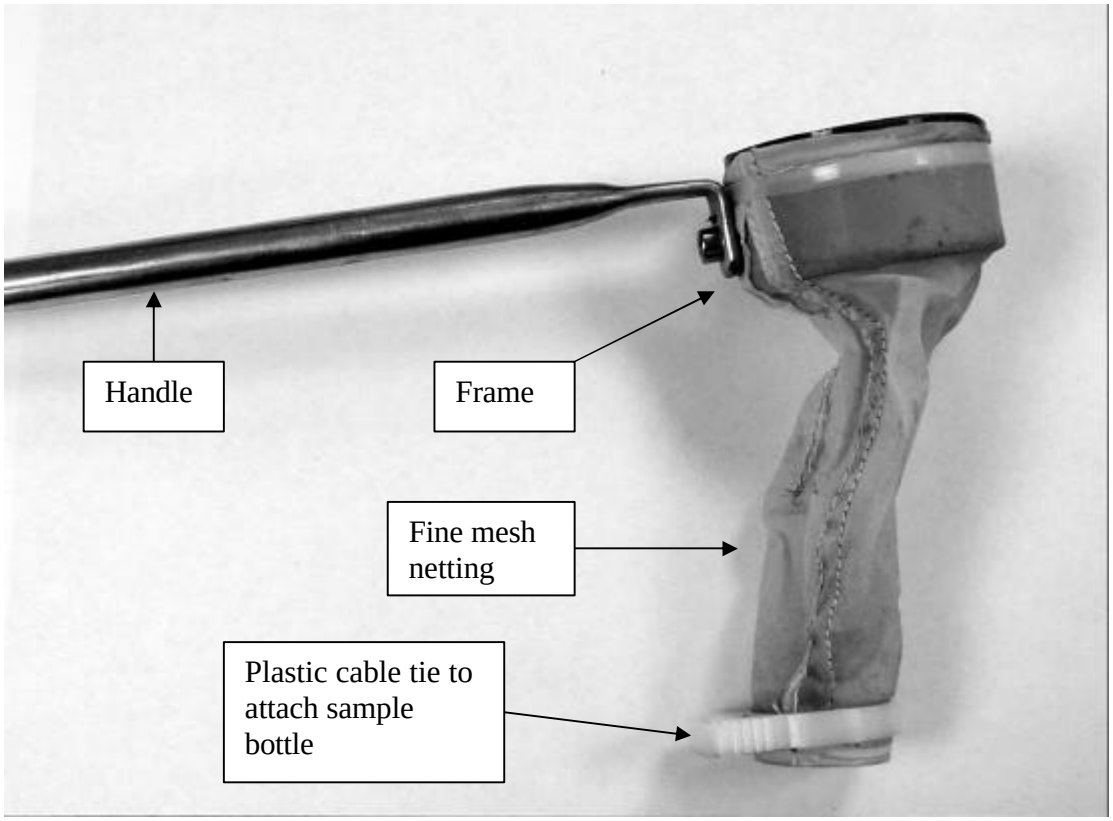

Fig. 2.2. Photograph of sampler for vertical hard surfaces.

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.

2.8 Method for sampling cobbles and boulders (without significant filamentous green alga cover)

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 2.4 above),

2. Any loosely attached surface contamination on the biofilm should be removed by gentle agitation in the stream water (the biofilm itself will not be dislodged by this process). This surface contamination might include small particles of organic matter or sediment. 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.

4. 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.

5. 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, sampler's initials and the name of any preservative that has been added to the sample.

6. 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.

7. 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.

8. Batches of samples for the 2004 sampling season should be sent by courier to the School of Biological Sciences (attn: Dr M. Yallop), University of Bristol, Woodland Road, Bristol BS8 1UG.

2.9 Modified method for sites where filamentous green algae are abundant

1. If filamentous green algae such as *Cladophora* are abundant at a sampling site, then the sampling protocol needs to be modified in order to reflect this.

2. Assess the percent cover of filamentous algae at the reach in question to the nearest five per cent. Use the result of this assessment to determine the number of cobbles with filamentous algae cover to include in the sample according to Table 2.1.

3. Suitable cobbles or small boulders should be removed from the water, following the same criteria as outlined in 2.6. After a brief wash of the cobble (see 2 above), the upper surface of the cobble (including the filamentous algae) should be brushed with a toothbrush to remove both the diatoms from the cobble surface and those attached to the algae. Any filaments not dislodged in the cleaning process can be removed and placed in the sample pot. Note this sampling strategy on the sample record form.

   Note: this procedure is different to the procedure for sampling rocks smothered with filamentous algae described in the TDI Manual.

4. See 2.8 points 5 to 8 for instructions on labelling, preservation, transport and storage of samples.

Table 2.1 Number of algae-smothered cobbles to be included in DARES samples

Percent cover of filamentous green algae	Number of cobbles
< 15%	0
≥ 15 < 29	1
≥ 30 < 44	2
≥ 45 < 59	3
≥ 60 < 75	4
≥ 75	5

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

1. The apparatus (see 2.7; Fig. 2.2) 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 2.8). 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 2.8 points 5 to 8 for instructions on labelling, preservation, transport and storage of samples.

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

2.11 Sampling emergent macrophytes

1. In general, samples should be collected from an emergent macrophyte species such as *Sparganium erectum, Glyceria maxima or Phragmites australis,* only if there are portions that remain permanently submerged but which are not contaminated by the bottom sediments. Details of the species of macrophytes used should be included with field sampling notes.

2. Care must be taken to avoid touching the piece of stem to be sampled for diatoms as the diatom film is often very fragile and easily lost. At least five stems should be cut below water level (ideally from different individuals of the same species) 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 upright. Diatoms should be brushed from the stem into the tray using a toothbrush, as described above.

3. See section 2.8 points 5 to 8 for guidance on labelling, transfer to the laboratory and storage.

2.12 Sampling submerged macrophytes

1. Samples should be collected as far as possible from a single macrophyte species or genus that is sufficiently abundant at the reach to facilitate sampling of the same species in the future. Genera such as *Myriophyllum*, *Ranunculus*, *Elodea*, *Ceratophyllum* and *Potamogeton* are all suitable

2. Replicate samples from five different plants of the same species should be taken. Samples of plants growing in the main flow of the river (avoiding edges and side-arms) should be placed into a plastic bag along with about 50 ml of stream water. 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. The plants should be shaken vigorously in the plastic bag in order to dislodge attached diatoms. The result should be a brown suspension that can then be poured into a bottle.

4. See section 2.6 points 5 to 8 for guidance on labelling, transfer to the laboratory and storage.