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. 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. It is based on the protocol described in the revised TDI Manual (Kelly *et al*., 2001), which is itself based on Battarbee (1986) and is compatible with the draft European standard EN 14407.

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.

- **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. For the DARES project, counts should be transferred, as soon as possible, to an Excel spreadsheet by the person responsible for performing the count.

- **Facilities for verifying the identity of difficult specimens**. This can take several forms with high quality photomicrographs or captured video images as the preferred option. 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 (such as an England Finder) that enables the absolute position of the specimen to be recorded may be useful.

4.3 Preliminary steps

4.3.1 Taxonomic criteria

The minimum acceptable level of taxonomy is the checklist of taxa provided in the Appendix. This is the level of taxonomy that is covered by DARES Quality Assurance protocols. Taxa that are found in DARES samples but which are not included in this checklist should also be identified, if possible. If identification is not possible then, for the purposes of DARES analyses, they will be treated as genus-level identifications.

4.3.2 Sample size

At least 300 valves of non-planktonic taxa should be counted. If one taxon represents more than one third of the total number of valves, then the sample size should be increased until at least 200 valves of other non-planktonic taxa have been counted. A stratified counting procedure (see 4.4) may be appropriate where one taxon is overwhelmingly dominant. Any such steps must be recorded.

Planktonic taxa can be abundant downstream of impoundments and in some lowland rivers. For the purposes of this study, planktonic taxa are: *Acanthoceras, Asterionella, Aulacosira, Chaetoceras, Cyclostephanos, Cyclotella, Fragilaria crotonensis, Skeletonema, Stephanodiscus, Thalassiosira* and *Urosolenia*. Some other taxa can be facultatively planktonic (e.g. *Nitzschia acicularis)* and these should be identified to species even when you suspect that they are inwashed plankton in the sample under investigation. 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.

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.

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

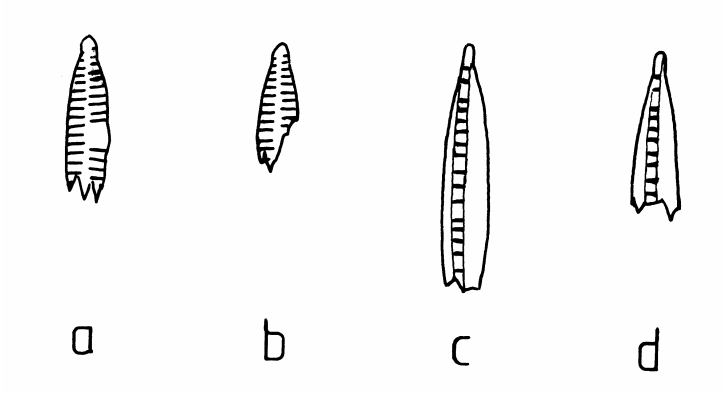

Fig. 4.1. Two examples of how to treat broken valves during counts: *Fragilaria vaucheriae* (a and b) and *Nitzschia dissipata* (c and d): "a" shows a broken valve of *F. vaucheriae* with one pole and a central area, which should be included in a count; whilst "b" shows a broken valve with one pole but 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.

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 individuals that cannot be assigned with confidence to one of the taxa listed in the appendix.** 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" (i.e. the density of some diatom taxa is greater at the edge of the strew than in the centre).

   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 in the appendix represents the minimum level of taxonomy that is required. Additional taxonomic information should also be recorded where possible as this may prove to 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 one taxon comprises more than a third of all recorded valves, 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 benthic taxa except this taxon until the revised target is reached. For DARES, this revised target is 200 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.

Appendix: Provisional taxon list for DARES project

This list is derived from the taxon list for the first phase of the diatom CD-ROM project and should contain all genera found in UK freshwaters, along with all taxa found frequently in rivers. Taxonomic conventions for the CD-ROM have not been finalised, but will follow Round *et al.* (1990) and subsequent generic changes, for the most part (not all of which are included in this list).

Genus	species
CENTRIC DIATOMS	
Actinocyclus	
Aulacoseira	
Cyclostephanos	
Cyclotella	
Cyclotella	*meneghiniana*
Ellerbeckia	
Melosira	
Melosira	*varians*
Skeletonema	
Stephanodiscus	
Thalassiosira	
Urosolenia	
Acanthoceras	
ARAPHID DIATOMS	
Asterionella	
Centronella	
Ctenophora	
Ctenophora	*pulchella*
Diatoma	
Diatoma	*ehrenbergii*
Diatoma	*hyemale / hyemalis*
Diatoma	*mesodon*
Diatoma	*moniliforme / moniliformis*
Diatoma	*tenue / tenuis*
Diatoma	*vulgare / vulgaris*
Distrionella	
Distrionella	*Asterionelloides*
Fragilaria	
Fragilaria	*bidens*
Fragilaria	*capucina (vars capucina & rumpens)*
Fragilaria	*capucina var. gracilis*

Fragilaria *capucina var. radians*
Fragilaria *capucina var. perminuta*
Fragilaria *capucina KLB Taf. 112 fig. 11*
Fragilaria *capucina* var *mesolepta*
Fragilaria c*rotonensis*
Fragilaria o*ldenburgiana*
Fragilaria v*aucheriae*
Fragilaria *vaucheriae var. capitellata*
Fragilariforma
Fragilariforma b*icapitata*
Fragilariforma c*onstricta*
Fragilariforma *virescens*
Hannaea
Hannaea *arcus*
Martyana
Martyana *martyii*
Meridion
Meridion *anceps*
Meridion *circulare*
Meridion *circulare var. constrictum*
Oxyneis
Oxyneis *binalis*
Pseudostaurosira
Pseudostaurosira *brevistriata*
Staurosira
Staurosira *construens*
Staurosira *elliptica*
Staurosirella
Staurosirella *lapponica*
Staurosirella *leptostauron*
Staurosirella *pinnata*
Synedra
Synedra *acus*
Synedra *capitata*
Synedra *parasitica*
Synedra *parasitica* var. *subconstricta*
Synedra *rumpens*
Synedra *tenera*
Synedra *ulna*
Tabellaria
Tabellaria *fenestrata*
Tabellaria *flocculosa*
Tabellaria *quadriseptata*
Tabellaria *ventricosa*
Tabularia
Tabularia *fasiculata*
Tetracyclus
MONORAPHID DIATOMS
Achnanthes
Achnanthes c*onspicua*
Achnanthes g*rana*
Achnanthes l*utheri*

Achnanthes	o*blongella*
Achnanthes	r*upestris*
Achnanthidium	
Achnanthidium	*biasolettiana*
Achnanthidium	*biasolettiana var. subatomus*
Achnanthidium	*microcephalum*
Achnanthidium	*minutissimum*
Planothidium	
Planothidium	*delicatulum*
Planothidium	*frequentissimum*
Planothidium	*lanceolatum*
Planothidium	*rostratum*
Planothidium	*dubium*
Karayevia	
Karayevia	*clevei*
Karayevia	*laterostrata*
Kolbesia	
Kolbesia	*ploenensis*
Lemnicola	
Lemnicola	*hungarica*
Psammothidium	
Psammothidium	*helveticum*
Psammothidium	*lauenburgianum*
Psammothidium	*levanderi*
Psammothidium	*subatomoides*
Eucocconeis	
Eucocconeis	*flexella*
Cocconeis	
Cocconeis	*pediculus*
Cocconeis	*placentula*
Cocconeis	*placentula var. euglypta*
Cocconeis	*placentula var. klinoraphis*
Cocconeis	*placentula var. lineata*
Cocconeis	*placentula var. pseudolineata*
Rossithidium	
BIRAPHID DIATOMS	
Ampipleura	
Amphipleura	*Pellucida*
Amphora	
Amphora	*inariensis*
Amphora	*libyca*
Amphora	*normanii*
Amphora	*ovalis*
Amphora	*pediculus*
Amphora	*veneta*
Aneumastus	
Aneumastus	*tusculus*
Aneumastus	*pseudotusculus*
Anomoeoneis	
Anomoeoneis	*sphaerophora*
Bacillaria	
Bacillaria	*paradoxa*

Brachysira	
Brachysira	*serians*
Brachysira	*vitrea*
Caloneis	
Caloneis	*bacillum*
Caloneis	*amphisbaena*
Caloneis	*salina*
Caloneis	*silicula*
Campylodiscus	
Campylodiscus	*clypeus*
Cavinula	
Cavinula	*cocconeiformis*
Cavinula	*variostriata*
Craticula	
Craticula	*accomoda*
Craticula	*cuspidata*
Craticula	*ambigua*
Craticula	*halophila*
Cymatopleura	
Cymatopleura	*elliptica*
Cymatopleura	*solea*
Cymbella	
Cymbella	*affinis*
Cymbella	*cesatii*
Cymbella	*cuspidata*
Cymbella	*delicatula*
Cymbella	*helvetica*
Cymbella	*microcephala*
Cymbella	*naviculiformis*
Cymbella	*cistula*
Cymbella	*ehrenbergii*
Cymbella	*lanceolata*
Cymbellonitzschia	
Cymbellonitzschia	
Denticula	
Denticula	*tenuis*
Diadesmis	
Diadesmis	*confervacea*
Diadesmis	*contenta*
Diatomella	
Diatomella	*balfouriana*
Didymosphenia	
Didymosphenia	*geminata*
Diploneis	
Diploneis	*elliptica*
Diploneis	*oblongella*
Encyonema	
Encyonema	*caespitosum*
Encyonema	*gracile*
Encyonema	*minutum*
Encyonema	*prostratum*
Encyonema	*reichardtii*

Encyonema	*silesiacum*
Epithemia	
Epithemia	*adnata*
Epithemia	*sorex*
Epithemia	*turgida*
Eunotia	
Eunotia	*bilunaris*
Eunotia	*curvata*
Eunotia	*exigua*
Eunotia	*formica*
Eunotia	*incisa*
Eunotia	*minor*
Eunotia	*pectinalis*
Eunotia	*subarcuatoides*
Fallacia	
Fallacia	*pygmaea*
Fallacia	*subhamulata*
Frustulia	
Frustulia	*rhomboides*
Frustulia	*vulgaris*
Gomphocymbella	
Gomphocymbella	*ancylii*
Gomphonema	
Gomphonema	*acuminatum*
Gomphonema	*angustatum*
Gomphonema	*angustum*
Gomphonema	*augur*
Gomphonema	*bohemicum*
Gomphonema	*clavatum*
Gomphonema	*clevei*
Gomphonema	*gracile*
Gomphonema	*grovei* v. *lingulatum*
Gomphonema	*minutum*
Gomphonema	*olivaceoides*
Gomphonema	*olivaceum*
Gomphonema	*parvulum*
Gomphonema	*parvulum var. exilissimum*
Gomphonema	*pseudoaugur*
Gomphonema	*pumilum*
Gomphonema	*tergestinum*
Gomphonema	*truncatum*
Gomphonema	*truncatum var. capitatum*
Gyrosigma	
Gyrosigma	*acuminatum*
Gyrosigma	*attentuatum*
Hantzschia	
Hantzschia	*amphioxys*
Luticola	
Luticola	*goeppertiana*
Luticola	*mutica*
Luticola	*ventricosa*
Mastogloia	

Mastogloia	*elliptica*
Mastogloia	*smithii*
Navicula	
Navicula	*angusta*
Navicula	*bryophila*
Navicula	*capitata*
Navicula	*capitatoradiata*
Navicula	*cari*
Navicula	*cincta*
Navicula	*claytonii*
Navicula	*cryptocephala*
Navicula	*cryptotenella*
Navicula	*decussis*
Navicula	*digitoradiata*
Navicula	*exilis*
Navicula	*gregaria*
Navicula	*lanceolata*
Navicula	*menisculus*
Navicula	*minima*
Navicula	*oblonga*
Navicula	*protracta*
Navicula	*pseudogregaria*
Navicula	*radiosa*
Navicula	*reichardtiana*
Navicula	*reinhardtii*
Navicula	*rhynchocephala*
Navicula	*schönfeldii*
Navicula	*slesvicensis*
Navicula	*subrhynchocephala*
Navicula	*tenelloides*
Navicula	*tripunctata*
Navicula	*trivialis*
Navicula	*veneta*
Navicula	*viridula*
Navicula	*atomus*
Navicula	*ignota*
Navicula	*lacunolaciniata*
Navicula	*minuscula*
Navicula	*minusculoides*
Navicula	*molestiformis*
Navicula	*saprophila*
Navicula	*subminuscula*
Navicula	*submolesta*
Navicula	*submuralis*
Navicula	*subrotundata*
Navicula	*Utermöhlii*
Neidium	
Neidium	*affine*
Neidium	*productum*
Neidium	*dubium*
Nitzschia	
Nitzschia	*acicularis*

Nitzschia	*amphibia*
Nitzschia	*angustata*
Nitzschia	*capitellata*
Nitzschia	*clausii*
Nitzschia	*constricta*
Nitzschia	*dissipata*
Nitzschia	*epithemoides*
Nitzschia	*fonticola*
Nitzschia	*frustulum*
Nitzschia	*gracilis*
Nitzschia	*inconspicua*
Nitzschia	*linearis*
Nitzschia	*microcephala*
Nitzschia	*palea*
Nitzschia	*paleacea*
Nitzschia	*perminuta*
Nitzschia	*pusilla*
Nitzschia	*recta*
Nitzschia	*sigma*
Nitzschia	*sigmoidea*
Nitzschia	*sinuata*
Nitzschia	*sociabilis*
Nitzschia	*subacicularis*
Nitzschia	*vermicularis*
Peronia	
Peronia	*fibula*
Pinnularia	
Pinnularia	*abaujensis* (= *gibba*)
Pinnularia	*appendiculata*
Pinnularia	*borealis*
Pinnularia	r*upestris*
Pinnularia	*subcapitata*
Pinnularia	v*iridis*
Placoneis	
Placoneis	*clementis*
Placoneis	*elginensis*
Reimeria	
Reimeria	*sinuata*
Reimeria	*uniseriata*
Rhoicosphenia	
Rhoicosphenia	*abbreviata*
Rhopalodia	
Rhopalodia	*gibba*
Sellaphora	
Sellaphora	*bacillum*
Sellaphora	*pupula*
Sellaphora	*seminulum*
Semiorbis	
Semiorbis	*hemicyclus*
Simonsenia	
Simonsenia	*delognei*
Stauroneis	

Stauroneis	*anceps*
Stauroneis	*gracile*
Stauroneis	*kriegeri*
Stauroneis	*phoenicenteron*
Stauroneis	*smithii*
Stenopterobia	
Stenopterobia	*curvula*?
Surirella	
Surirella	*angusta*
Surirella	*brebissonii*
Surirella	*crumena*
Surirella	*islandica / roba*
Surirella	*linearis*
Surirella	*minuta*
Surirella	*ovalis*
Tryblionella	
Tryblionella	*acuminata*
Tryblionella	*acuta*
Tryblionella	apiculata
Tryblionella	*debilis*
Tryblionella	*hungarica*