FDT Protocol

EPILITHIC DIATOMS

(Standing and running waters)

Aim

To monitor changes in the species composition of epilithic diatom communities in standing or running waters.

Rationale

Diatoms are a diverse group of unicellular siliceous algae and are well-established as sensitive biological indicators of surface water quality due to the narrow tolerance of individual species to environmental parameters such as pH and nutrient availability (Round 1993). Diatom epilithon grow attached to submerged stones within the photic zones of lakes and streams and are easy to sample in most freshwaters in a replicable manner. The sampling procedure adopted by ECN is simple and follows that used for the United Kingdom Acid Waters Monitoring Network (Patrick *et al.* 1995).

For the purposes of ECN, epilithic diatom samples will be sent to the Environmental Change Research Centre (ECRC) laboratories (see Appendix I, page 85), where they will be prepared by hydrogen peroxide digestion and mounted on microscope slides (see Battarbee 1986). The slides will be archived in anticipation of the availability of funding for analysis at some time in the future. Analysis will involve examination by x 1000 microscopy, with 500 diatom valves from each sample being identified to species level. Data will be summarised for each sample as a list of species present and their percentage abundance.

Method

Equipment

A toothbrush, small plastic funnel, sample tray, 30 ml screw-top Sterilin® polystyrene vials, acidified or non-acidified Lugol's iodine, distilled or filtered water in a wash bottle, penknife.

Location

- **Standing waters**
  Three spatially discrete littoral locations, which are not unduly influenced by inflow streams or localised catchment disturbance, heavy shade etc, are selected around the shore. The locations are recorded on a sketch map to assist in future re-location and grid references of each sampling location are recorded, usually to a resolution of 30–40 m. Sampling is carried out preferably three times each year, in March–April, June–July and September–October. Once a sampling schedule has been determined, sampling dates should be adhered to as closely as possible in future years. If only annual sampling is possible, this should be carried out during September.

- **Running waters**
  A 50 m length of stream is selected to coincide with part of that used for ECN macrophyte sampling. Three sampling locations, which are not unduly influenced by inflow streams, localised catchment disturbance, heavy shade, etc, are selected along this length – the first near the upstream end (Location 1), the second at its centre (Location 2), and the third near its downstream end (Location 3). Grid references of each sampling location are recorded to a resolution of 10 m (or better if possible). Sampling is carried out preferably three times each year, in March–April, June–July and September–October. Once a sampling schedule has been determined, sampling dates should be adhered to as closely as possible in future years. If only annual sampling is possible, this should be carried out during September.

Sampling

- **Standing waters**
  At each location five permanently submerged cobble-sized stones, ideally from a depth >30 cm, are selected and epilithic diatoms removed and preserved. For this purpose a cobble is defined as a large stone which can be held in one hand; if this size of stone is unavailable, smaller or larger stones may be substituted and a record made so that a similar size is used on each subsequent sampling occasion. Stones covered in bryophytes or macro-algae, (eg *Cladophora)*, should be avoided. Epilithic diatoms, usually discernible as a brownish slimy cover on the upper surface of submerged stones, are removed from the stones into a tray by vigorous brushing with a clean toothbrush. Alternatively, the blade of a penknife may be used to scrape the stones. The stones should be rinsed 2–3 times with a few drops of distilled or filtered water and re-brushed. The bulk sample for each sampling location (ie from the 5 stones) is homogenised in the tray, (eg by stirring with the toothbrush), and a subsample is then decanted to fill a plastic vial and preserved with 2–3 drops of Lugol's iodine (see Appendix II, page 85). The remaining solution may be discarded. Each tube is labelled as described below.

  The toothbrush should be thoroughly cleaned between sampling different sites.

- **Running waters**
  At each of the three sampling locations five cobble-sized stones are selected and removed from the water, preferably from stretches of at least moderate flow and at a depth below that of minimum flow. For this purpose a cobble is defined as a large stone which can be held in one hand; if this size of stone is unavailable, smaller or larger stones may be substituted and a record made so that a similar size is used on each subsequent sampling occasion. Stones covered in bryophytes or macro-algae, (eg *Cladophora)*, should be avoided. Epilithic diatoms, usually discernible as a brownish slimy cover on the upper surface of submerged stones, are removed from the stones into a tray by vigorous brushing with a clean toothbrush. Alternatively, the blade of a penknife may be used to scrape the stones. The stones should be rinsed 2–3 times with a few drops of distilled or filtered water and re-brushed. The bulk sample for each sampling location (ie from the 5 stones) is homogenised in the tray, (eg by stirring with the toothbrush), and a subsample is then decanted to fill a plastic vial and preserved with 2–3 drops of Lugol's iodine (see Appendix II, page 85). The remaining solution may be discarded. Each tube is labelled as described below.

  The toothbrush should be thoroughly cleaned between sampling different sites.

Unavailability of epilithic habitats

At sites where a suitable habitat for epilithon is unavailable, such as lowland nutrient-rich lakes with thick reed fringes or streams with silty or clay/mud beds, an epiphytic diatom sample is substituted as follows.

At each of the three sampling sites, remove three small pieces of permanently submerged macrophyte material using a penknife, place in a plastic vial with a little distilled or filtered water, and preserve with several drops of Lugol's iodine. The stems of reeds and rushes and the undersides of lilies and broadleafed species provide particularly favourable habitats for epiphytic diatoms but they should be checked to ensure that they have the brownish, slimy cover indicative of diatom communities before they are selected. The macrophyte species should be sufficiently abundant at the sample location to facilitate sampling of the same species in the future, and a brief description of the selected macrophyte should be added to the vial label.

Labelling

Each tube is identified uniquely by:

- the ECN Measurement Code (FDT)
- the ECN Site ID Code (eg Lochnagar L09)
- the internal location code* (eg S01 – see below)
- the collection date (eg 10-Mar-95)

where 'X' is either S = Stone substrate (epilithic) or P = Plant substrate (epiphytic) and 'nn' is a numeric code: 01, 02 03, etc. For running waters, 01 is used for upstream location, 02 for centre location and 03 for downstream location of the ECN stream section. For standing water sites, codes are assigned as seems most appropriate.

Where the sample is epiphytic, information about the selected macrophyte should be included.

Samples should be stored in the dark in a cool environment and, if necessary, sent as soon as possible to a ECRC (see Appendix 1, page 85) adequately sealed, protected with padding and accompanied by any appropriate field notes. Grid references of each sampling location should also accompany the first batch of samples.

* The internal location code identifies the particular sampling point within the ECN Site, and takes the form: Xnn

Author

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References

Battarbee, R.W. 1986. Diatom analysis. In: *Handbook of Holocene palaeoecology and palaeohydrology,* edited by B.E.Berglund, 527–570. Chichester: Wiley.

Patrick, S.T., Waters, D., Juggins, S. & Jenkins, A. eds. 1995. *The United Kingdom Acid Waters Monitoring Network. Site descriptions and methodology report.* London: ENSIS Ltd.

Round, F.E. 1993. *A review and methods for the use of epilithic diatoms for detecting and monitoring changes in river water quality.* London: HMSO.

Wetzel, R.G. & Likens, G.E. 1991. *Limnological analyses.* New York: Springer-Verlag.

Appendix I

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Appendix II

Lugol's iodine is prepared as follows (Wetzel & Likens 1991).

Acidified

Dissolve 20 g potassium iodide and 10 g iodine crystals (caution: toxic) in 200 ml distilled water containing 20 ml concentrated glacial acetic acid.

Non-acidified

Dissolve 20 g potassium iodide and 10 g iodine crystals in 200 ml distilled water.

To preserve samples with Lugol's iodine add 0.3 ml of the solution to 100 ml of sample and store in the dark. For long-term storage add 0.7 ml of the solution per 100 ml of sample and buffered formaldehyde to a minimum of 2.5% final concentration after
1 hour.

Specification of results and recording conventions

The measurement variables listed below are those required for each FDT sampling location at an ECN Site. Sites submitting data to the ECNCCU should refer to the accompanying Data Transfer documentation for the specification of ECN dataset formats, available on the restricted access Site Managers' extranet. Contact ecnccu@ceh.ac.uk if you need access to this documentation.

The first 4 key parameters uniquely identify a sample or recording occasion in space and time, and must be included within all datasets:

• Site Identification Code (e.g. R10)	Unique code for each ECN Site
• Core Measurement Code (e.g. FWC)	Unique code for each ECN 'core measurement'
• Location Code (e.g. 01)	Each ECN Site allocates its own code to replicate sampling locations for each core measurement (e.g. FWC 01, FWC 02 for different surface water collection points)
• Sampling Date (/time)	Date on which sample was collected or data recorded. This will include a time element where sampling is more frequent than daily

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Core measurement: Epilithic diatoms (FDT protocol)

Samples should be taken at a recommended frequency of 3 times a year for both rivers and lakes.

At the present time, diatom samples are archived at the ECRC, University College London, for future analysis, and no data specifications apply. ECN acquires information on sites, locations and sampling dates for diatom sampling directly from the ECRC. Please refer to the FDT protocol (page 81) for information on labelling of samples.