

Diatom fossils in mires: a protocol for extraction, preparation and analysis in palaeoenvironmental studies

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SUMMARY

When fossil diatoms are preserved in mires, they can be excellent indicators of ecological conditions and very useful in palaeoenvironmental reconstruction, both alone and in combination with other siliceous fossils. Processing techniques for diatom frustules have varied and been modified according to the sediment from which the diatoms are being extracted. They differ from those used for palynological studies, which destroy the siliceous component. A procedure that was developed for diatom studies in the French Massif Central is presented, variations used by different workers are described, and some applications in palaeoenvironmental reconstruction and contemporary peatland science are reviewed.

KEY WORDS: fen, frustules, methods, palaeoenvironment, palaeohydrology, peatland.

1. INTRODUCTION

Diatoms (Bacillariophyceae) are colonial, unicellular, yellow-green to golden-brown microalgae whose cell walls—known as frustules or valves—come in a variety of shapes and sizes with an even wider variety of ornamentation, enabling them to be identified to species level. The frustules are composed of amorphous silica (SiO₂) which, if conditions are right, can be preserved in the sediments. Diatom frustules are abundant in minerotrophic mires but scarce (except in the surface sediments) in ombrotrophic *Sphagnum* mires with a pH lower than 5. At le Dignonnière, Cubizolle (unpublished data) found that they disappear due to silica dissolution at pH 4.

Diatoms have a variety of life forms which were classified by Denys (1991/2) as follows: euplanktonic (living in open waters), tychoplanktonic (present in the plankton but derived from other habitats), epontic (attached to a substratum) and benthic (also attached to a substratum, but less strongly). Changes in the representation of these different categories are used to reconstruct environmental change. In the case of peatlands, the changes that are most often examined are hydrological changes in fens. Such palaeohydrological information is also relevant to archaeological studies of land use within mire basins (Cubizolle *et al.* 2004, Cubizolle *et al.* 2011) where human activity can be detected (Cubizolle *et al.* 2005). A wider review of research questions that have been addressed using diatoms in peatlands is presented in Section 9.

In palaeoenvironmental reconstruction, diatom

analysis is usually carried out in conjunction with analyses of other microfossils, usually pollen. However, because diatoms are often destroyed by pollen extraction techniques (which are generally unsuitable for diatom studies), different extraction techniques are needed. Other siliceous fossils (phytoliths, sponge spicules and Chrysophyte cysts and scales) which are found on diatom slides can provide additional information about the site.

Detailed accounts of diatom extraction methods are seldom given in research articles. Here, common extraction techniques are reviewed (e.g. Battarbee 1986) and the protocol developed by our group for studying diatoms as indicators of palaeoecological conditions in mires (fens) located in the eastern Massif Central (France) (Cubizolle *et al.* 2003, Cubizolle *et al.* 2005, Cubizolle *et al.* 2011) is described. This is based on both literature and personal experience. The different variations of the extraction technique used by other diatomists are also described.

2. RETRIEVAL OF PEAT CORES

In conjunction with coring, which is described elsewhere in this volume (De Vleeschouwer *et al.* 2010), samples of present-day diatoms are collected and the physical and chemical conditions in which they are found are recorded. This information is used as a basis for ecological interpretation of the fossil diatom communities found within the peat. Smol (2008) provides several chapters on coring and dating sediments, reading the diatom records

found in the sediments, and calibrating indicators of environmental variables using surface-sediment training sets; but this treatment focuses on lake sediments rather than peat.

3. SAMPLE PREPARATION

One important factor that should be taken into consideration before any sample is processed is the cleanliness of glassware, especially if samples from several different sites are to be processed in the same laboratory. Glassware and spatulas should be scrubbed vigorously and then rinsed several times in distilled or demineralised water. After cleaning it is important to invert the glassware for storage to prevent contamination. Before processing, it is also necessary to decide whether or not diatom concentrations in the sediment are to be calculated. If they are, the wet volume or dry weight of each sub-sample must be ascertained, and the final volume of liquid in which the diatoms are suspended must be known.

Depending on the site, it may be necessary to remove up to three types of components from the sub-samples, namely: soluble salts, organic matter and minerogenic matter (see Table 1).

There are several variations of this method, particularly when it is necessary to remove organic matter, which may give better results in some cases. Julius & Theriot (2010) used concentrated nitric acid (HNO_3) with potassium dichromate (KCr_2O_7) as a catalyst, but the dissociation of chromium makes this technique hazardous. Scherer (1988) used a combination of HNO_3 and hydrogen peroxide (H_2O_2). Battarbee (1986) heated the sample in a beaker with 30 % hydrogen peroxide (H_2O_2), having first removed any coarse organic matter by sieving through a 0.5 mm screen; but the sieving process can lead to contamination if the sieves are not cleaned properly. In Van der Werff's (1955) method, which is used by many Dutch diatomists (Denys & van Straaten 1992, Van de Vijver 1996, Van de Vijver 1997, Denys 2006), the sample is heated to 80 °C with 37 % H_2O_2 for about one hour, the reaction is completed by adding a saturated solution of potassium permanganate (KMnO_4) until it starts to precipitate, then the precipitate is removed by adding small amounts of hydrochloric acid (HCl) in dilute H_2O_2 .

Another technique for removing minerogenic matter is flotation in heavy liquids, but this process requires a lot of time with repeated rinsing and the samples usually have to be processed with H_2O_2 beforehand (Battarbee *et al.* 2001).

4. SLIDE PREPARATION

Before mounting the diatom sample, it is advisable to treat it in an ultrasonic bath (Battarbee *et al.* 2001). This helps to break up chain diatoms and separate single frustules, and often gives a better distribution for observation using a light microscope (fewer valves on top of one another). However, prolonged sonication can lead to extensive breakage of the frustules, which makes them more difficult to identify. Therefore, it is advisable to make a preliminary slide to verify the condition of the frustules before using this technique. If required, the sample is placed in the ultrasonic bath for a maximum time of ten seconds.

If the diatom concentration in the sediment is to be calculated, the diatoms are suspended in a known volume of water. Then, 0.2 ml of the suspension is pipetted onto a clean coverslip and spread out evenly, the coverslip is covered or placed in a closed cupboard (to avoid airborne contamination), and the water is allowed to evaporate at room temperature. For this, Battarbee & Kneen (1982) developed a technique using microspheres while others have used evaporation trays (Battarbee 1973) and other techniques (Bodén 1991, Scherer 1994). When the coverslip is dry, it is mounted in a resin with refractive index (RI) 1.7, such as Naphrax, Hyrax, Zrac or Clearax. A drop of the resin is placed on a glass slide and heated on a hotplate to drive off the resin solvent, then the coverslip is placed upside-down on the resin. The slide is then removed from the heat and the coverslip is pushed gently to remove air bubbles. After cooling, push the coverslip with a fingernail to make sure that it does not move; if it does, reheat the slide. It is advisable to prepare a single slide first, and use it to check that the concentration of diatoms is not so high that many of them overlap such that identification is difficult or impossible. If this is the case, the sample dilution must be increased. This verification may also indicate that further cleaning of the sample is necessary, particularly when clay particles are present, as these can mask diatom features that are important for identification.

5. LIGHT MICROSCOPE OBSERVATIONS

Diatoms are most readily observed using a microscope with differential interference contrast. Instruments which have been used successfully by the authors are the Nikon Eclipse 80i with D.S. camera and NIS-Elements 3D software (Nikon, version 3.03) which enables the user to control both

Table 1. Diatom preparation for peatland samples, modified by the authors from Battarbee's (1986) method for removing soluble salts, organic matter, and minerogenic matter.

A: Soluble salts

1. Using a pre-cleaned spatula, which must be cleaned again after handling each sample, weigh out a peat sample of suitable size (or take a known volume if doing a quantitative study). Place the sample in a pre-cleaned beaker.
2. If the presence of carbonate, metal salts or oxides, and especially iron are suspected, slowly add 10 % HCl. It is important to add the reagent slowly because any effervescence that follows can cause the loss of material over the side of the beaker; this is a good general rule for all of the chemical treatments. Heat the sample on a hotplate at very low temperature (maximum 25 °C) until effervescence stops, usually in about 15 minutes. Be careful not to let the sample dry out completely.
3. After heating, either centrifugation or settling can be used to concentrate the remaining material. Centrifugation (at 1,200 r.p.m. for three minutes) may break some of the more delicate diatoms, particularly in subsurface material. Allowing the diatoms to settle in the beaker (usually overnight) will help eliminate this problem. Although settling increases the total processing time, it shortens the amount of active preparation time required per sample. It is important to cover the beakers during settling in order to prevent contamination of the samples.
4. Decant the liquid, preferably into a hooded sink, taking care not to re-suspend the settled diatoms.
5. Rinse the residue one or more times with distilled or demineralised water before continuing.

B: Organic matter

1. Add a small amount of distilled or demineralised water to the sample, then slowly add 30 % hydrogen peroxide (H₂O₂). Again, take care that the effervescence does not overflow the rim of the beaker. If this is a risk, add a little more distilled or demineralised water. Heat again at low temperature (25 °C) until very little liquid is left. As the amount of liquid decreases, watch the beaker and make sure that it does not bounce off the hotplate.
2. Some organic material may prove very difficult to break down using this method. Larger pieces can be picked out using clean forceps. For small particles, the sample can be reprocessed using a combination of sulphuric and nitric acids (H₂SO₄ and HNO₃).
3. After digestion of the organic matter, rinse the sample repeatedly (three times or more) with distilled or demineralised water until all traces of the chemical reagents have been removed.

C: Minerogenic matter

1. Particles larger than 62 µm (1/16 mm)—such as grains of sand—fall at 20 cm min⁻¹. Suspend the sample in distilled or demineralised water, stand for the time needed for settling, then pour off the liquid containing the diatoms. This process should be performed several times. Then proceed to removal of mineral particles that are smaller than 62 µm (Step 2).
2. Add a few drop of ammonium hydroxide (NH₄OH) or a very dilute solution of fabric softener (laundry product). The amount required depends on the volume of solution in the beaker. This helps to prevent clumping of the diatom valves.
3. Diatoms are usually larger than 5 µm in length or 4 µm (1/256 mm) in diameter. Suspend the sample in distilled or demineralised water, cover the beaker and allow to stand for the time necessary for particles larger than 4 µm to settle (4 µm particles settle at a rate of 10 cm in 3 hours 12 minutes). Then decant off the supernatant from the diatoms. Again, repeat this process several times. It is important to check the decanted liquid (using a microscope) to make sure that diatoms are not being removed.

the microscope and the camera, and facilitates imaging and data management; and the Zeiss Axioskop 2 Plus with Canon camera and software. To identify the very small species that are commonly found in peat, examination at high ($\times 100$) magnification is required. In some cases, it may be necessary to resort to scanning electron microscopy (Figure 1).

6. TAXONOMIC IDENTIFICATION

The use of local diatom floras is highly recommended, and these will obviously vary from region to region across the world. However, there are several important general references including Hustedt (1927–1966, 1930), Patrick & Reimer (1966, 1975), Germain (1981), Krammer & Lange-Bertalot (1986, 1988, 1991a, 1991b, 2000). Other useful sources include Barber & Haworth (1994), Hartley *et al.* (1996) and Coste (1999). There are also several relevant periodical series, for example those published by A.R.G. Ganter Verlag K.G. including *Iconographia Diatomologica*, *Diatom Monographs* and *Diatoms of Europe*; along with *Bibliotheca Diatomologica* published by J. Cramer.

7. ANALYSIS AND STATISTICS

Because diatom valves can be broken or corroded before sampling or during cleaning, a decision on how to count them must be made. If more than half a valve is found, it can be counted as one valve; similarly, central fragments of centric or pennate diatoms can be counted as one valve. If a central fragment is counted as one valve, then the ends of pennate diatoms should not be counted. For long thin diatoms like *Ulnaria* species, it is often better to count ends and then divide the total number by two. Ends (instead of centres) can also be counted for pinnate diatoms (Batterbee 1986, Straub 1990, Kuehlthau-Serieyssol 1993, Batterbee *et al.* 2001).

Batterbee *et al.* (2001) found that “there are marked differences in the percentage counts between 10 and 200 a count, while there is little change between 400 and 500. A count between 300 and 600 may therefore be recommended”. Fabri & Leclercq (1984) state that 300–1000 frustules are counted, whereas Scherer (1988) counted a minimum of 300 valves, Van Dam (1996) 400, and Kingston (1982) 500–600. In fact, the number of frustules that it is necessary to count varies with the species diversity of the sample; the more taxa present, the more frustules must be counted. The

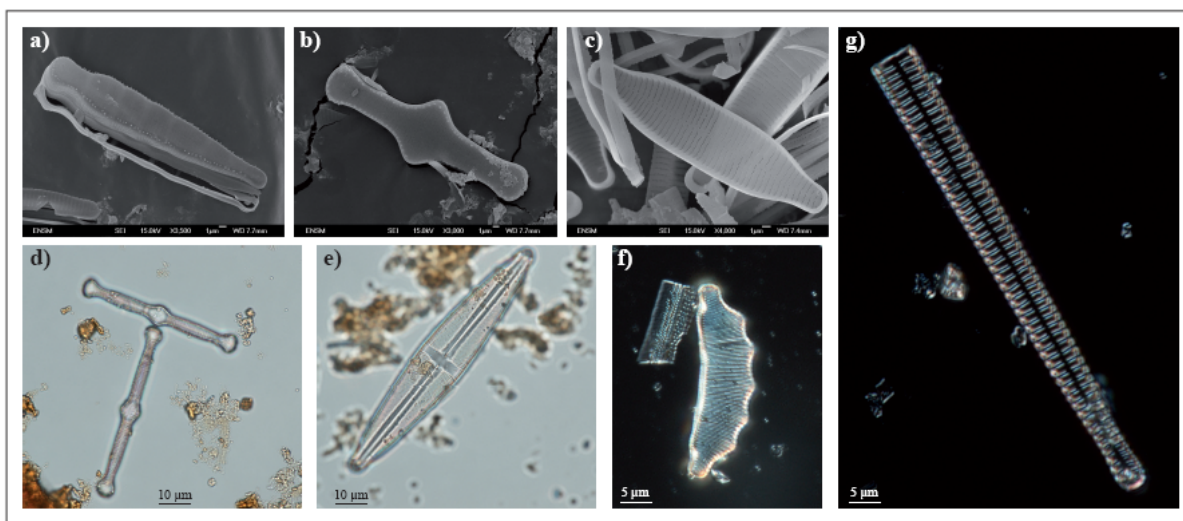


Figure 1. Photomicrographs of some diatom taxa (Sandra Chatelard, UMR 5600 CNRS - ISTHME, St-Etienne): a) *Meridion circulare* var. *constrictum* (Ralfs) Van Heurck (SEM $\times 3,500$); b) *Tabellaria flocculosa* (Roth) Kützing (SEM $\times 3,000$); c) *Fragilaria virescens* Ralfs (SEM $\times 4,000$); d) *Tabellaria* cf. *fenestrata* (Lyngbye) Kützing (optical microscope $\times 1,000$); e) *Stauroneis anceps* Ehrenberg (optical microscope $\times 1,000$); f) *Eunotia* cf. *hexaglyphis* (optical microscope with differential interference contrast $\times 1,000$); g) *Ulnaria ulna* (Nitzsch) Compère (optical microscope with differential interference contrast $\times 1,000$).

number of valves that should be counted in an individual sample can be determined by plotting the number of different species recorded against the number of valves counted (cumulative richness curve). Sufficient valves have been counted when the curve reaches a plateau. For the samples used to construct Figure 2, the plateau was reached when 350 valves had been counted.

Once the relative percentage of each taxon within each of the samples is known, a whole series of different statistical techniques can be employed to analyse the data. Birks (2010) gives an extensive discussion about the use of these techniques. One approach involves carrying out canonical correspondence analysis (CCA) on the dataset, in order to determine which environmental variables are most closely correlated with the observed differences in species composition. Hierarchical cluster analysis, k-means partitioning and two-way indicator species analysis group samples with similar diatom composition, but do not take account of any available geographical, environmental or

stratigraphical information. Ordination analyses such as principal components analysis (PCA), correspondence analysis (CA), detrended correspondence analysis (DCA) and principal coordinates analysis (PCoA) are often used to produce two-dimensional plots in which samples that are closer together are more similar in composition than samples that are farther apart. Thus, the ordination programs explore how abiotic environmental variables influence the biotic composition and then the ordination diagrams are interpreted using whatever environmental information is available. According to ter Braak (1995), there are several advantages to these methods. First, the species composition of the different samples is easy to determine; secondly, the relationships of individual species to environmental conditions are often unpredictable whereas the general patterns of several species are more useful for detecting species-environmental relationships; and thirdly, “the ordination approach is less elaborate and gives a global picture”.

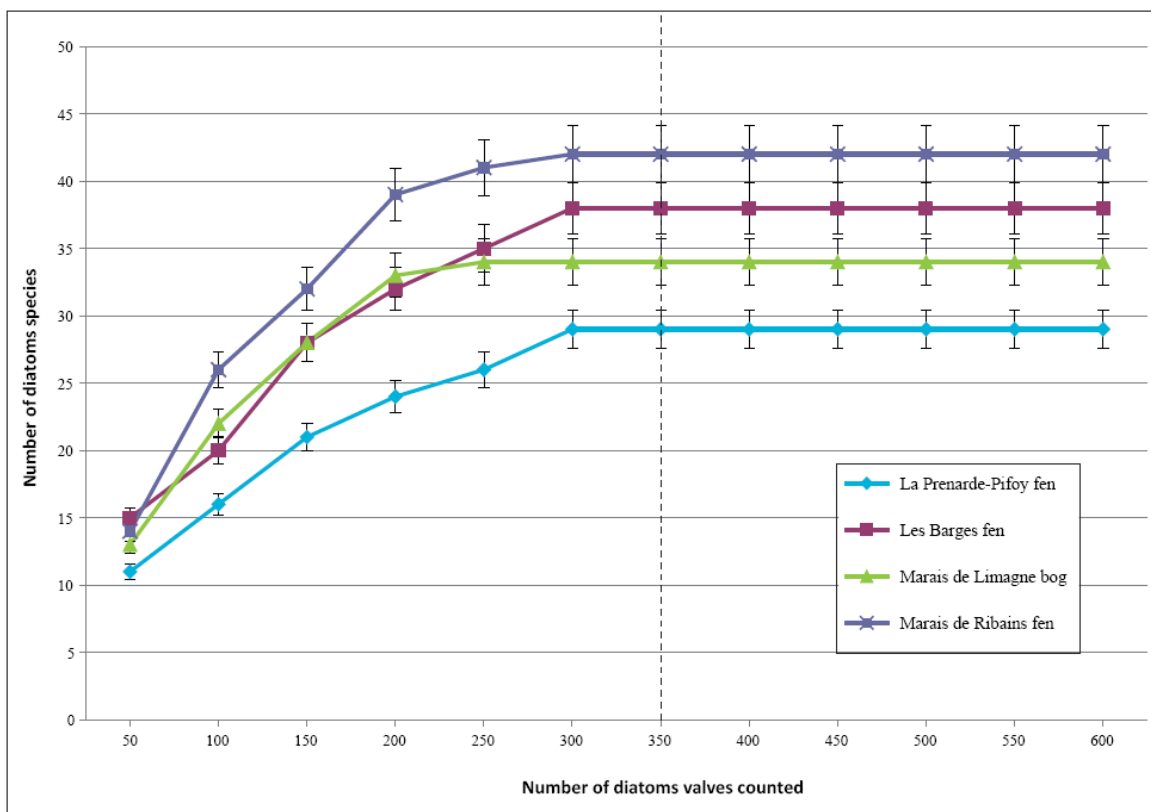


Figure 2. Relationships between the numbers of valves counted and diatom species recorded (cumulative richness curves). The samples represented were collected from four mires located in the eastern part of the French Massif Central, namely: La Prenarde Fen (les Monts du Forez), altitude 1125m; Les Barges Fen (Les Bois Noirs), altitude 710m; Marais de Limagne Bog, altitude 1090m; and Marais de Ribains Fen, altitude 1100m. The dotted line indicates the minimum number valves that it is necessary to count.

8. THE USE OF DIATOM DATA IN RECONSTRUCTION OF PAST HYDROLOGICAL CONDITIONS

Knowledge of the modern distribution of diatoms within a region enables better interpretation of fossil communities and a better understanding of palaeoecological conditions. Modern statistical methods (Bruno & Lowe 1980, Earle & Duthie 1986, ter Braak 1986) allow the establishment of quantitative relationships between diatom assemblages and a range of physico-chemical environmental variables. Some examples of physico-chemical influences that have been associated with changes in diatom assemblages are the type of habitat and water depth (Wolin & Duthie 1999), water depth and land use changes (Cubizolle *et al.* 2005), salinity changes (Fritz *et al.* 1999), pH (Birks *et al.* 1990, Battarbee *et al.* 1999), nutrient enrichment (nitrogen oxides, phosphates, silica) and trophic changes (Hall & Smol 1999). Vos & de Wolf (1993) present a method for reconstructing sedimentary environments based on diatoms in coastal wetlands.

An understanding of the ecology of the different species present can be used in combination with statistical analyses to develop an understanding of changes that are occurring or have occurred in the past. For example, changes in the relative abundance of planktonic, periphytic and/or aerophytic species can be used to indicate changes in water depth (e.g. an increase in planktonic species indicates increased water depth).

Diatom valves may be scarce or totally lacking in the sediment because they have been corroded or transformed by dissolution either in the water column or after deposition in the sediment (Calvert 1974, Barron 1987, Flower 1993). Especially in such cases, it is advisable to combine diatom data with information about other proxies such as pollen, phytoliths, sponge spicules and other macrofossils that can help build a reliable representation of past conditions.

9. SOME PEATLAND STUDIES

Florin (1970) studied diatoms from an ice-block depression known as Kirchner Marsh in south-eastern Minnesota and found four stages in the late-glacial development of the basin. Diatom Zone 1 had mainly dry-habitat forms (*Hantzschia amphioxys*, *Pinnularia borealis* and *Melosira roeseana*), representing the pre-limnic stages. These diatoms lived in mosses growing on soil and

wood in a spruce forest that had developed on aeolian silt. The silt was originally deposited on dead-ice, and when the ice melted, the sedimentary layer was deposited and formed the basal deposits of the marsh. The absence of aquatic diatoms confirmed the dry condition, and the fact that many of the large diatom frustules were still articulated indicated very gentle conditions or very short transport. Zone 2 contained a greater variety of species belonging to different ecological groups suggesting an increase in wetness, and the appearance of acidophilous taxa—in particular eight species of *Eunotia*—indicated the development of marsh-like conditions. At this time, the area was probably a mixture of hummocks and pools, and the dead-ice block was beginning to melt. Littoral diatoms, many of which live in alkaline waters, appeared in Zone 3, reflecting the onset of lacustrine conditions when the entire basin above the remaining ice may have been gradually filling with water. The full development of the lake was marked by Zone 4, with more than 50 % of the flora composed of pelagic diatoms (*Cyclotella caspia*, *Cyclotella comta*, *Cyclotella kützingiana* var. *schumannii*, *Stephanodiscus astreae* var. *minuta*).

In another study, Kingston (1982) examined surface samples from peatlands in northern Minnesota. Three diatom assemblages were identified. The first of these had many co-dominant species from *Eunotia*, *Navicula*, *Cymbella*, *Frustulia*, *Pinnularia* and *Surirella*, and was found in hollows on rich and poor fens. The second assemblage came from wet hollows on poor fens and bogs and contained common taxa, mainly *Eunotia*, *Navicula* and *Pinnularia*. The third was dominated almost exclusively by *Eunotia exigua* associated with *Sphagnum*, and came from hummocks in bogs, poor fens and rich fens. The fens had more species than hummocks or bog hollows, and the diatoms corresponded significantly with position in the hummock-hollow microtopography and the trophic status of the groundwater. Diversity and richness were lower on hummocks than in hollows and also decreased with the decline in minerotrophy from rich fen to bog conditions.

The 3000-year diatom succession of a 7.7 m core from the Second Marsh on the north shore of Lake Ontario described by Earle & Duthie (1986) indicated a history of recurrent extreme fluctuations in the abundance of several periphytic species. Three major successional events were observed. The first was an increase in epiphytic species ca. 2800 years BP, when a sand bar may have reduced wave action and current velocity within the marsh, allowing emergent vegetation to colonise the site. A

second event marked by a large increase in epiphytic species was associated with the lowering of lake levels that occurred in central North America ca. 1700 years BP. The third event occurred within the following 100 years, when diatom assemblages dominated by *Staurosira construens* and *Staurosira pinnata* were replaced by communities in which *Achnanthes minutissimum* and *Achnanthes deflexum* were dominant. This change is attributed to the formation of a sand barrier due to increased erosion resulting from deforestation. Recurring phases were observed between these three major successional events. One was characterised by low diatom abundance but a large number of species that prefer fast-flowing water conditions, and another by extremely high abundance of diatoms mostly belonging to species that are adapted to still-water conditions. The most recent assemblage included diatoms with a preference for sewage contamination and marked the recent development of pollution from a water treatment plant.

In describing diatom assemblages at the Okefenokee swamp/marsh, Scherer (1988) obtained similar results to Kingston (1982), but developed the interpretation by conducting down-core analysis. Five major diatom assemblages were found, and their distributions were related to patterns of streamflow and water input from the uplands. Assemblage 1 developed in the most stagnant and isolated locations and was dominated by *Eunotia exigua*. In Assemblage 2, *Frustulia rhomboides* var. *saxonica* (also known as *Frustulia saxonica*) was dominant and *Eunotia exigua* was common. This assemblage had higher diatom diversity than Assemblage 1 and was associated with the water lily *Nymphaea odorata*. It was interpreted as occurring in a less restrictive environment with slightly higher inflow. Assemblage 3 was strongly dominated by *Asterionella ralfsii*, and denoted a very specific environment of open, still pools or ponds with very little aquatic macro-vegetation and slow peat accumulation. Assemblage 4, dominated largely by *Aulacoseira nygaardii*, was found in regions of upland inflow; its distribution followed the streamflow patterns and appeared to be associated with higher nutrient concentrations and more silica input. The last assemblage (Assemblage 5) was dominated by *Eunotia carolina* and included a series of other taxa, namely: *Fragilaria javanica*, *Eunotia pectinalis* var. *rostrata*, *Neidium iridis* var. *ampliatum*, *Pinnularia pogoi* and *Anomoeoneis paludigena*. This assemblage had the highest diversity and occurred in locations with the highest rates of streamflow, especially along major drainage channels. However, direct comparison of surface

assemblages was complicated by dissolution which led to an increase in representation of the more robust forms. Even though the subsurface assemblages were not complete, the distributions of the three species *Eunotia exigua*, *Eunotia carolina* and *Aulacoseira nygaardii* could be used to interpret the changes within the cores.

The relationship between epiphytic diatoms and bryophytes was explored by Bertrand *et al.* (2004), who found that certain diatom taxa were specific to certain bryophytes but the relative abundance of the diatoms was very small (0.7–3.3 %). Four groups of bryophytes were identified, and their characteristic diatom taxa were given as follows:

- 1) *Sphagnum* communities without *Sphagnum rubellum* and *Sphagnum denticulatum*, characterised by the diatom species *Kobayasiella subtilissima*, *Eunotia glacialis*, *Chamaepinnularia mediocris* and *Eunotia steineckii*;
- 2) the moss *Warnstorfia exanulata*, with characteristic diatoms *Frustulia saxonica*, *Brachysira brebissonii*, *Eunotia hexaglyphis* and *Fragilaria constricta*;
- 3) another moss *Fontinalis squamosa* characterised by *Fragilaria capucina* var. *septentrionalis*; and
- 4) the two mosses *Sphagnum rubellum* and *Rhynchosstegium riparoides* with characteristic diatoms *Fragilaria capucina* and *Diatoma mesodon*.

This study also revealed an association of certain diatom species with three water level classes, namely: 'immersed' at depths of -10–0 cm below the water level; 'emergent' at 0–50 cm above the water level; and 'extremely emergent' at 90 cm above the water level. Interestingly, the 'extremely emergent' group had the greatest number of characteristic species (the eight species *Fragilaria virescens*, *Eunotia intermedia*, *Fragilaria capucina*, *Diatoma mesodon*, *Cocconeis placentula*, *Eunotia minor*, *Meridion circulare* and *Hantzschia amphioxys*), the 'immersed' group had six characteristic species (*Eunotia incise*, *Tabellaria flocculosa*, *Fragilaria constricta*, *Frustulia saxonica*, *Cymbella gracilis* and *Fragilaria capucina* var. *septentrionalis*), and the 'emergent' group had only two (*Eunotia paludosa* var. *trinacria* and *Pinnularia rupestris*). The authors felt that this result might be an artefact of the way they counted the diatoms but also speculated that, because these species came from many different environments, the diatoms might have been blown in by the wind. This type of information could be useful in interpreting down-core changes in water level and peatland dynamics.

Diatoms have also been used for monitoring in the context of conservation and restoration of

marshlands, particularly in Belgium and The Netherlands. Recent acidification of Dutch moorlands has been associated with declining abundances of *Eunotia bilunaris*, *Frustulia rhomboides* var. *saxonica* and *Navicula subtilissima* and an increase of *Eunotia exigua* (Van Dam & Kooyman-Van Blokland 1978, Van Dam *et al.* 1981, Van Dam 1987). More recently, Denys & van Straaten (1992) used diatom data to assess the present condition of 47 lentic heathland water bodies, mostly moorland pools, in order to determine their management needs. They concluded that management should conserve the remaining refugia and prevent eutrophication.

The Virennies fenland study (Cubizolle *et al.* 2005) illustrated how extensive human habitation has changed the environment in the upper catchment of the River Loire, in the Livradois Mountains (France). Peat accumulation began during the second half of the Subboreal (3294 ± 50 BP), when only diatom fragments were found within the sediments. It was not until the Second Iron Age (between 2214 ± 40 BP and 1336 ± 45 BP), when a cooling trend forced humans to increase their use of the area and this in turn provoked changes in the environment, that diatoms started to be preserved in the fen. *Abies*, *Quercus* and *Fagus* forests regressed and *Betula* and *Alnus* increased, indicating more humid ground conditions. Deforestation increased runoff, possibly carrying more nutrients into the fenland. Intermediate water depths were registered by the diatoms, and high percentages of oligotrophic (poor in nutrients) and oligosaprobic (highly oxygenated water with little organic material) species were present during this period. During the High Middle Ages (1336 ± 45 BP), the peatland expanded and started accumulating on a rock bench. This was shown to be associated with an irrigation ditch and an increase in cereal pollen. The diatom assemblages indicated higher water levels with oligotrophic and oligosaprobic conditions. Moist subaerial diatoms also increased and it is most likely that these were eroded from the fen deposits on the slope. Around 564 ± 40 BP (Lower Middle Age), cultivation declined and forest cover expanded, and an increase in erosion was suggested by the presence of sand. The diatoms indicated a maximum water depth as well as a maximum in oligotrophic and oligosaprobic species, along with an increase in meso-eutrophic (indicative of higher nutrient levels) species. This was followed by a cyclic pattern of increasing and decreasing cultural indicators (cereal, ruderal and weed pollen), showing a high level of land use until the 20th century. This intensive use of the land as pasture and for grazing caused a change in water conditions, which was again registered by

the diatoms. Dystrophic species (that live in water with high humic matter) and polysaprobic species (that live in oxygen-poor water with high biological oxygen demand) increased and water levels decreased. These changes could be explained by one or several factors, such as increased drainage of the fen, changes in the amount of precipitation, increased numbers of animals living next to the fen, and/or increased water retention within the catchment. At the end of the 20th century, water levels decreased with the introduction of *Picea* forests and the related decline in agricultural land use along with abandonment of the irrigation system, causing an increase in subaerial diatoms indicating dryer conditions and lower water levels. This continued until recently, when a new farmer took up residence in the village, introduced cows and re-opened the irrigation ditches. Again, the diatoms registered these changes with an increase in aquatic, eutrophic and α -meso-polysaprobic species, indicating higher water levels and more nutrient-rich water with lower oxygen content.

10. CONCLUSIONS

Diatoms are useful palaeo-bioindicators because they are sensitive to variations in a number of physical, chemical and biological attributes of the environment including pH, salinity, water chemistry (nitrogen and phosphorous compounds), oxygen saturation, trophic status, saprobity and moisture conditions; and change in any of these factors causes the diatom community to change (Stoermer & Smol 2010). Thus, they can provide information about a whole variety of different changes that might occur within the environment. In most cases, they are aboriginal and respond more quickly than other bio-indicators to changes in hydrology, and have the potential to provide evidence for low-amplitude physical and chemical changes that are often not reflected by local vegetation changes.

11. ACKNOWLEDGEMENTS

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