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An improved coverslip method for investigating epipelic diatoms

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The traditional coverslip method for harvesting motile diatoms was improved and standardized by determining optimum times for diatom harvesting; by using opaque Petri dish bases as experimental chambers; and by preparing coverslips by burning. Three experiments were conducted to evaluate the harvesting efficiency of the coverslip method. Experiment 1 was performed to reveal the spatial distribution of epipelic diatoms on the coverslips after exposure periods ranging from 2 to 36 h. The transect count results were supplemented using an Inverse Distance Weighted algorithm and showed that after 8 h exposure the epipelic diatoms began to accumulate at the coverslip margin. Therefore, 8 h was identified as the optimum time for the coverslip method to capture epipelic diatoms efficiently for the samples. However, the optimum time could vary between sites because of differences in diatom composition, light intensity and other environmental conditions. Experiment 2 revealed that the numbers of epipelic diatoms harvested from opaque chambers were 15.6% higher than those harvested from transparent chambers, indicating that opaque sides and base reduce multi-directional diatom migration. Experiment 3 indicated that the burning method was more efficient than traditional method (directly counting fresh diatoms) because it makes identification easier and yielded higher densities of diatoms. All experiments demonstrated that the coverslip method harvested more motile diatoms, made identification easier than traditional method and enabled permanent slides to be created. In addition, the method makes it possible to estimate *in situ* epipelic cell densities, if the sediment area is known.

Key words: coverslip method, epipelic diatoms, migration

Introduction

Surfaces in shallow freshwater environments are usually colonized by micro-organisms, particularly benthic algae, creating a biofilm that can extend over rocks, sand and submerged plants as well as over soft sediments. One biofilm component, the motile diatom community, was named the epipelon by Round (1956), who defined it as 'consisting of free-living algae on submerged sediments'. Epipelic diatoms are motile, biraphid taxa that secrete exopolymeric substances (EPS) during locomotion (Edgar & Pickett-Heaps, 1984). EPS is an important as a carbon source for other microbes (e.g. Underwood *et al*., 1995). In freshwaters, epipelic diatoms often reach high biomass and productivity, especially in oligo-mesotrophic lakes (Gruendling, 1971; Cyr, 1998) and can contribute substantially to whole-lake primary production (e.g. Wetzel, 1964; Liboriussen & Jeppesen, 2003; Poulíčková *et al*., 2008; Whalen *et al*., 2008).

However, the epipelon is a relatively poorly investigated community probably because of sampling difficulties (Round, 1990; Poulíčková *et al*., 2008). Poulíčková *et al*. (2008) summarized materials for collecting epipelic diatoms, including the use of glass, microspheres and lens tissues. Sampling has traditionally entailed the use of the lens tissue method. Eaton and Moss (1966) recommended this method specifically to investigate the motile diatoms in epipelic communities. The method uses double layers of lens tissues to trap migrating epipelic diatoms. Following removal and placing in 40% glycerol (in Lugol's iodine solution) the diatoms within the tissues are identified and counted in the light microscope (Eaton & Moss, 1966). Non-motile diatoms, other algae and detritus are excluded; it has been widely used and further developed to harvest motile epipelic diatoms from a variety of aquatic environments (e.g. Paterson *et al*., 1998; Thornton *et al*., 2002; Ribeiro *et al*., 2003). The method was further modified to include the use of an *in situ* coverslip. The coverslip method was first introduced by Round (1953) and widely used to trap epipelic diatoms in lakes and streams (Eaton & Moss, 1966; Mann, 1984*a*, *b*; Cox, 1985, 1988). Coverslips are laid directly on the wet sediment (Round, 1953; Eaton & Moss, 1966) and removed and mounted

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directly on slides (Round, 1953), or in drops of 40% glycerol on slides (Eaton & Moss, 1966). As Eaton and Moss (1966) pointed out, one difficulty of the coverslip method is contamination from sediment, making counting difficult and inaccurate. Mann (1984*b*) modified the coverslip method by putting a layer of lens tissue between the sediment samples and coverslips to collect diatoms for life history studies, rather than ecological investigations. The coverslips are mounted with a drop of filtered water onto slides and sealed with petroleum jelly (Mann, 1984*b*) making a clean preparation of living diatoms for observation in the light microscope. To obtain permanent preparations of motile diatoms from different substrata, gentle treatment with hydrogen peroxide has been tried for diatoms on coverslips (Cox, 1990). For studies of the frustules of diatoms attached to coverslips, coverslip burning in a muffle furnace at approximate 600°C was also attempted (Mann, 1984*a*).

Compared with the lens tissue method, the coverslip method is less used, as diatoms on the coverslips tend to migrate to the edge after several hours exposure to light, resulting in an uneven diatom distribution (Eaton & Moss, 1966). However, quantitative aspects of coverslip distributions involving experimental exposure time, were not investigated, especially regarding the rates at which epipelic diatoms migrate. In our study, a 36-h experiment (experiment 1) was performed to determine the optimum time (under standard conditions) to harvest epipelic diatoms on coverslips. If the time taken before motile diatoms begin to move significantly to coverslip edges can be defined, then harvesting motile diatoms using the coverslip method could be quantitative.

The details of experimental chambers used in previous studies were not described but we assume that transparent experiment chambers, such as glass or plastic Petri dishes, were used. However, transparent dishes could enlarge diatom migration towards the chamber sides and thus decrease the number of diatoms harvested from the upper surface of sediment samples. We evaluated this potential effect (experiment 2).

A further difficulty of the traditional coverslip method concerns identification precision of fresh samples based on ×400 magnification observation of cell shape and chloroplast configuration compare to ×1000 identified finer features. Although the burning coverslip method has been attempted by Mann (1984*a*), no quantitative comparison between direct counting of fresh material and after burning was made. This is carried out here in experiment 3.

Materials and methods

Research site, field method and materials

Fresh littoral surface sediment for the experiments was obtained from a Scottish upland loch, the Round Loch of Glenhead. The loch is small (12.5 ha), relatively shallow (mean water depth 4.28 m). The water is acidic (pH ca. 5.0) and brown (DOC ca. 5.1 mg l^{-1}) and has been described elsewhere (e.g. Flower *et al.*, 1987; Yang *et al.*, 2009). The benthic diatom communities are well developed and acidophilous diatom taxa such as *Frustulia rhomboides* var. *saxonica* (Rabenhorst) de Toni are common (Jones & Flower, 1986). Undisturbed sub-aquatic surface sediment samples (to about 1 cm depth) were collected using a shallow-water surface-sediment sampler at approximately 1 m water depth (Yang & Flower, 2009). The sampled sediment was placed in a cold box and transported back to the laboratory within 24 h. Although the samples were collected from a relative homogeneous mud-surface in the loch, it is almost unavoidable that some samples from the natural environment occasionally contained sand, small stones or plant detritus. To ensure good homogenization of the samples small stones, macrophyte roots and leaves and any other visible organic and inorganic detritus were removed (Poulíčková *et al.*, 2008). The sediments were then mixed thoroughly and allowed to settle overnight in an air-conditioned room (air temperature 12°C). To reduce the influence of water content, we removed surplus water by pipette. The subsequent three experiments were carried out at around 12°C.

A minimum of 300 diatom cells were counted for each sample using × 1000 magnification under oil immersion. Identifications were made to the highest possible taxonomic level using standard diatom floras (e.g. Krammer & Lange-Bertalot, 1997).

Experimental methods

Experiment 1. Diatom migration over 36-h light exposure. This experiment used a period of 36 h to determine how exposure time affects the accumulation of motile diatom cells onto coverslips and the time taken for the motile diatoms to concentrate significantly at the coverslip edges. The carefully mixed fresh sediment was poured into a large white opaque plastic tray (33 × 51 cm) and flattened using a *spatula* to make the sediment an even thickness. Squares of lens tissue were firmly placed on the sediment, avoiding air pockets and creases. Thirty-six 19-mm diameter coverslips were applied carefully over the lens tissue avoiding trapped air pockets. Two coverslips were collected each 2 h, and the experiment lasted for 36 h, from 8 am on the 21 October to 8 pm on the 22 October 2006. To reduce the influence of light intensity, the experiment was conducted in an enclosure under constant light conditions (1.5 μ mol m^{-2} s^{-1}) supplied by a daylight bulb fixed 1.5 m above the tray. After the coverslips were taken off the lens tissues, they were air-dried, burnt and mounted onto slides (see experiment 3).

To assess the development of diatom distribution patterns on the coverslips during the 36-h period all

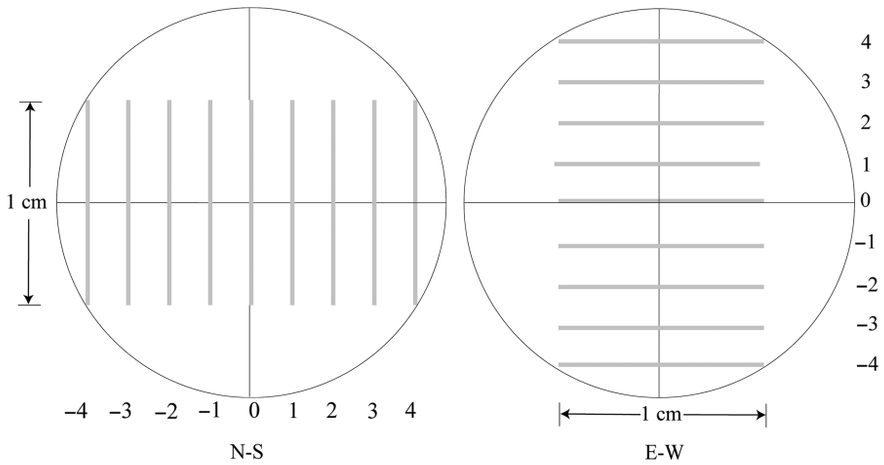

Fig. 1. Diatom counting methodology used for each coverslip in the 36-h motile diatom harvesting experiment (experiment 1). Each coverslip was divided into nine 1-cm long 0.184-mm wide traverses along the diameter in both North–South (N–S) and East–West (E–W) directions. The traverses were numbered from West (–4) to East (4) and from South (–4) to North (4) and 0 referred to the middle of the coverslip. Diatoms were counted along the traverses in the N–S and E–W directions at × 1000 magnification under oil immersion. (For more details, see Experiment 1).

diatoms on each coverslip should ideally be counted. However, such counting is time-consuming and can be impractical, especially for dense diatom samples. Instead, the coverslips were divided into nine 10-mm long and 0.184-mm wide (i.e. the width of the field of view at ×1000 magnification) traverses parallel to the diameter in both North–South (N–S) and East–West (E–W) directions (Fig. 1). The positions and lengths of the traverses were accurately determined using a pair of vernier calipers. The traverses were numbered from West (–4) to East (4) and from South (–4) to North (4) and 0 referred to the middle of the coverslip. Diatoms were counted along the traverses in N–S and E–W directions. In order to reduce the influence of differences in diatom cell density (cells cm^{-2}) between N–S and E–W directions and between different exposure times, percentages of diatoms in each traverse compared to all nine traverses in N–S or E–W direction were calculated. To predict the percentages of uncounted diatoms on the entire coverslip, the Inverse Distance Weighted (IDW) method was used (Johnston *et al*., 2003). The IDW uses the measured values surrounding the prediction site and weights the points closer to the prediction site greater than those farther away.

The diatom percentage at location S_o is predicted using the following formula (Johnston *et al*., 2003):

$$\hat{z}(s_0)\sum_{i=1}^{N}\lambda_i Z(s_i) \quad (1)$$

where N is the number of measured sample locations surrounding the prediction location S_o; λ_i is the weight assigned to each measured location; $Z(s_i)$ is the measured diatom percentage at the location s_i.

The weights λ_i are determined by the following formulas (Johnston *et al*., 2003):

$$\lambda_i = d_{i0}^{-p} / \sum_{i=1}^{N} d_{i0}^{-p} \quad (2)$$

$$\sum_{i=1}^{N}\lambda_i = 1 \quad (3)$$

where d_{i0} is the distance between the prediction location s_0 and each of the measured locations s_i. p is a power factor. The weight decreases exponentially with increase in distance.

The IDW interpolation was performed using ArcGIS 9.0 (Johnston *et al*., 2003) and the interpolated surfaces are plotted in Fig. 2.

Experiment 2. Comparison of harvesting efficiencies between opaque and transparent experimental chambers. This experiment used petri dishes as experimental chambers and tested the effect of using transparent versus opaque dishes on diatom migration efficiency. One set of Petri dishes was normal (transparent) (Fig. 3*a*). The other set of dishes was made opaque by applying black paint to the sides and base (to a height of 1 cm) (Fig. 3*b*). Sediment was poured into the transparent and opaque Petri dishes after mixing well. The volumes and depths of sediments in the transparent and opaque petri dishes were carefully adjusted to make them as similar as possible. Lens tissue squares (3 × 10 cm) were placed firmly on the upper sediment surface, avoiding air pockets and creases. Three 19 mm diameter coverslips were then placed on the lens tissue. Based on the results of experiment 1, 8 h was selected as the optimum exposure time. The experiment was conducted on a sunny day between 8 am and 4 pm after which time the coverslips were removed, air-dried, burnt and mounted onto glass slides.

Experiment 3. Comparison in identification precision between direct counting and burning methods. This experiment was conducted to assess if the coverslip burning method is more efficient than the traditional direct counting method. Opaque petri dishes were used as experimental chambers as above. After 8 h exposure on

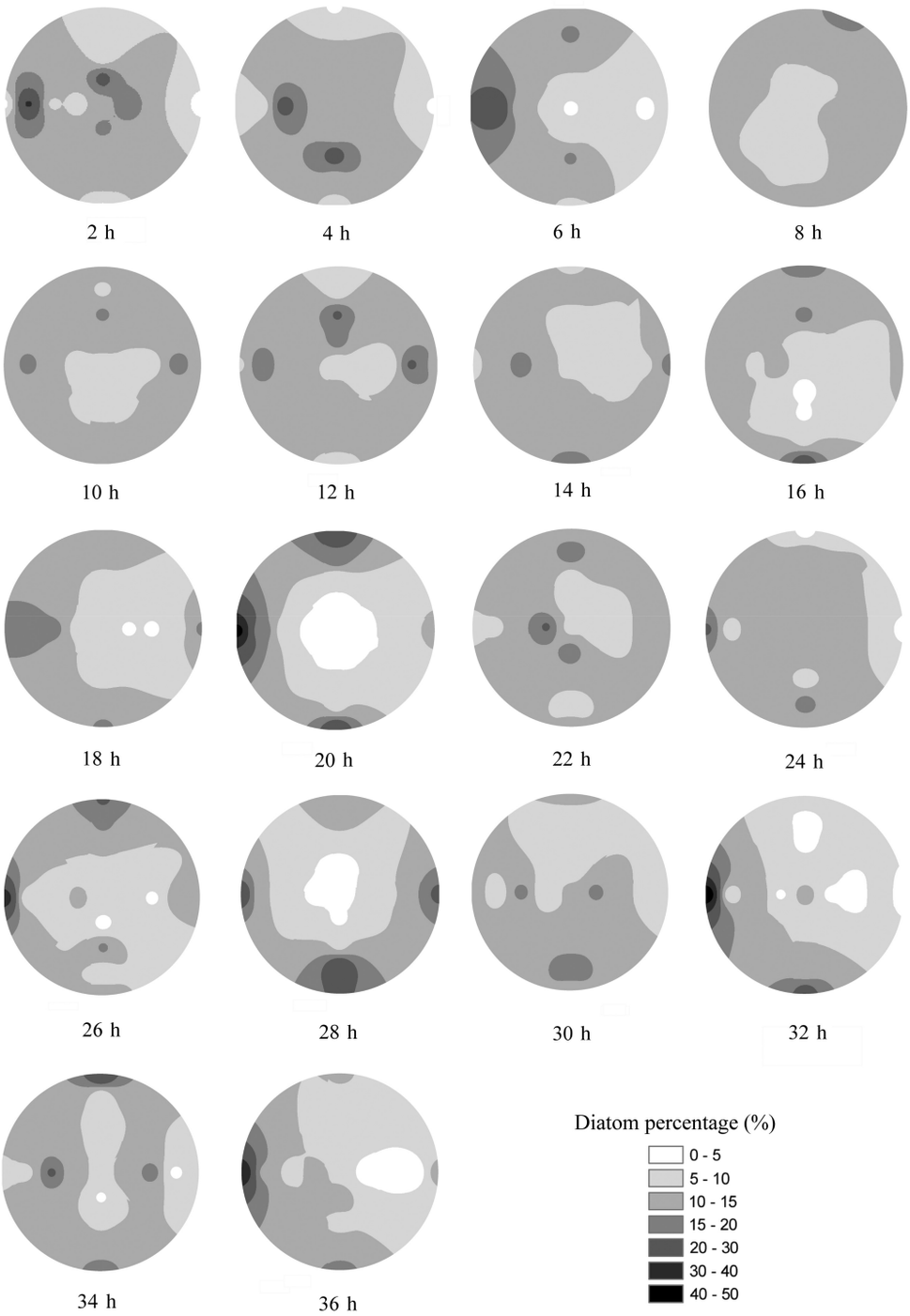

Fig. 2. Spatial distribution of motile diatom percentage on coverslips under different exposure times. This experiment was carried out over 36 h and coverslips were removed and counted at 2-hour intervals. The Inverse distance weighted (IDW) method was used to interpolate the diatom percentage in the non-enumerated coverslip areas. The greyscale represents the percentage of diatoms in each tranverse to all nine traverses along the coverslip diatmeter. (For more details, see Fig. 1 and Experiment 1).

a sunny day from 8 am to 4 pm, the coverslips were removed, air-dried overnight and burnt for a few seconds in a Bunsen burner flame to remove the organic matter from the diatoms. The coverslips were mounted onto slides using Naphrax® mounting medium and the diatoms were counted. In a parallel exercise diatoms were counted using the 'traditional method' (direct counting fresh living diatoms mounted in drops of glycerol (Eaton & Moss, 1966), following 8-h exposure in the same experimental chambers.

Statistical methods

To estimate the similarity of different experimental treatments in experiments 2 and 3, cell densities were

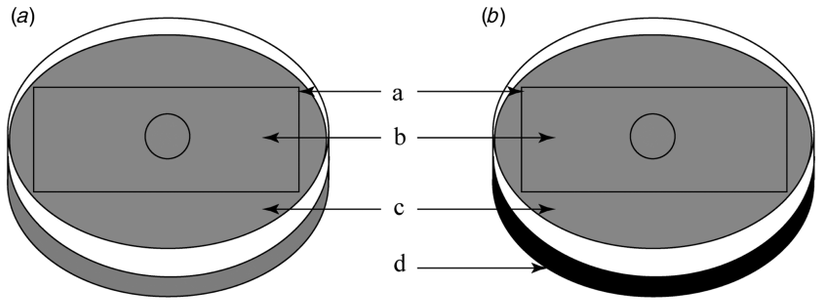

Fig. 3. Transparent (normal) and opaque (painted black in the sides and bases) plastic petri dishes as experimental chambers containing fresh surface sediment surface sediment samples. Key: A: Transparent petri dish; B: Opaque Petri dish; a: a layer of lens tissue (3 cm × 10 cm) placed directly on the sediment surface; b: three 19-mm diameter coverslips placed on the lens tissue; c: surface sediment (grey colour); d: black painted bases and sides (up to height of 1 cm to the lower end of the Petri dish). Clear lids were applied to both dishes. (For more details, see Experiment 2).

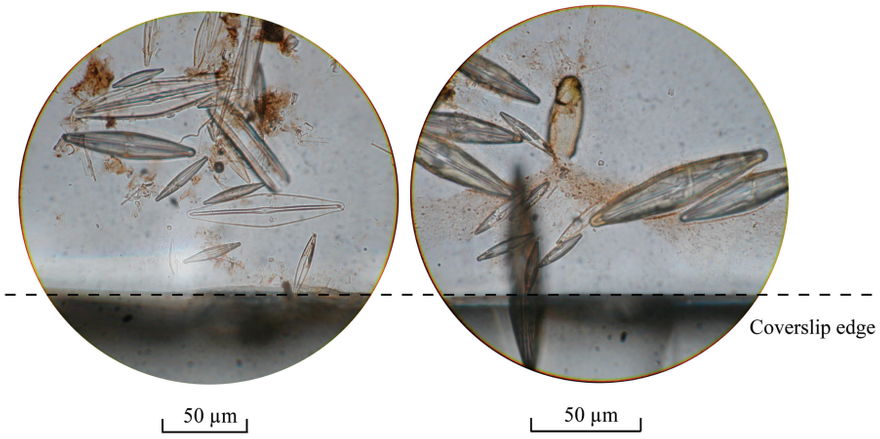

Fig. 4. Light microscope photography to show motile epipelic diatoms accumulated at the coverslip margin after 8 h (left) and 10 h (right) accumulation. Note *Frustulia rhomboides* var. *saxonica* leaving the coverslip (right).

compared and diatom composition was also compared using Bray–Curtis (BC) similarity indices. Diatom cell densities were calculated by dividing the counted diatom cells by the area of the field of view. The BC similarity indices based on percentage abundance of diatoms were calculated using the following formula (Bray & Curtis, 1957):

$$\mathrm{BC} = 1 - \frac{\sum_{i=1}^{n} |X_{ij} - X_{ik}|}{\sum_{i=1}^{n} (X_{ij} + X_{ik})} \qquad (4)$$

where X_{ij} is the abundance of the ith species in the jth sample, X_{ik} is the abundance of the ith species in the kth sample and n is the number of species. The index ranges from 0 to 1, where 0 indicates that the two samples have no taxa in common, whereas 1 indicates that both samples share the same taxa in the same rank order of abundance.

Results and discussion

Optimum time to harvest motile diatoms

The distribution of diatoms on the coverslips at each time step during the 36-h experiment is expressed as percentage of motile diatoms in each traverse to all nine traverses along the coverslip diameter interpolated (created new data points within the range of a discrete set of known data points) using the IDW algorithm in ArcGIS in Fig. 2. The light colour corresponds to low diatom percentages, while the dark colour corresponds to high percentages. The spatial distribution of diatoms is shown to be uneven after 2–6 h exposure. Higher percentages occurred around the central areas of the coverslip at 2 and 4 h. After 6 h, the diatoms began to move to the west edge and 2 h later a very uneven distribution developed with higher percentages at the coverslip edges and lower percentages in the central areas, so that a 'hole' (lighter colour) was formed at the coverslip centre. These results indicated that the motile diatoms migrated to the margins after an exposure of 8 h (Fig. 2). This uneven distribution persisted from 10–20 h, but diatoms increased again around the central area after 22 h. This typically uneven distribution pattern almost disappeared over the subsequent exposure time, although a 'hole' appeared at 28 h and diatom percentages increased along some sides of the coverslips at 24, 26, 32, 36 h.

The light microscope photograph (Fig. 4) clearly shows that some diatoms had migrated to the

coverslip margin, and one diatom (*F. rhomboides* var. *saxonica*) had left the coverslip edge after 10 h. Diatom cell densities on the coverslip changed from 920 to 2978 cells cm^{-2} (average of 1717) over the 36-h exposure period, with higher values from 14–22 h (Fig. 5).

Eaton and Moss (1966) pointed out that motile diatoms can migrate to the edge of coverslips after several hours and causing an uneven distribution. However, they did not indicate the time taken for the process to occur, probably because of the difficulty in assessing the spatial distribution of motile diatoms on a coverslip. In this experiment we estimated this process and used IDW to model the changing patterns of diatom accumulation on coverslips. IDW is simple, fast and easily programmed and has been successfully used in spatial interpolation in different situations (e.g. Xu *et al*., 2001; Johnston *et al*., 2003). The present work is the first attempt to interpolate diatom patterns on a coverslip using IDW.

The result showed that there was a significantly uneven distribution with more diatoms at the edges and fewer diatoms in the centre after 8 hours accumulation (Fig. 2). Diatom movement to the coverslip edge made diatom counting difficult and inaccurate and the escape of motile diatoms from the coverslip reduced diatom cell density. Consequently, the experiment suggested that 8 h is the optimum time for the coverslip method to harvest motile diatoms efficiently in a given period of time for these samples. In addition, using a combination of normal illumination and autofluorescence, viewed under an epifluorescent microscope, it is possible to detect live cells and this could be used to check the veracity of the method and also act as a control. The optimum time for samples from other sites may vary according to the composition of the diatom assemblage, light intensity and other environmental conditions. Poulíčková *et al.* (2008) have suggested that exposure time experiments should be conducted for each specific sample location. For example, research on epipelic diatoms from fishponds in Central and Northern Moravia, Czech Republic, showed that 24 h was necessary to trap some larger diatoms, such as *Pinnularia* and *Amphora* (Lysáková *et al*., 2007). In our samples, the epipelic diatoms were *Navicula*, *Frustulia*, *Pinnularia* and *Brachysira*. All taxa, irrespective of size, migrated up to the coverslip within 8 h.

The reason for diatoms migrating to the coverslip edges is unclear. One possibility is that a CO_2 concentration gradient develops between the middle and the edges of the coverslip. Light and CO_2 are two indispensable elements for diatom photosynthesis (e.g. Kirk, 1994). Light was equally available across the transparent coverslip, but that would not be the case for CO_2. CO_2 concentration at the centre of a coverslip is likely to be reduced creating a CO_2 concentration gradient between the middle and edges of the coverslip. We hypothesize this is the cause of the diatom migration to coverslip edges.

In the experiment, the non-homogeneity persisted from 8 to almost 20 h. After 20 h, the typically uneven distribution disappeared (Fig. 2). The reason for this is unknown but it could be related to migration from the sediment stimulated by the light and CO_2 gradients.

To avoid the influence of daylight variation, constant light ($1.5\,\mu mol\,m^{-2}\,s^{-1}$) supplied by a daylight bulb was used in experiment 1. However, there was still a significant increase in diatom cell densities between 14 and 22 h (Fig. 5). Round and Eaton (1966) also found some increase in epipelic diatom numbers in Abbots Pond even after 72 h in the constant light condition. Although this could indicate that diatoms are under the control of a 'biological clock mechanism' independent of light (Round & Eaton, 1966), more studies are needed to investigate diatom migration. In the present study, we adopted 8 h for routine sampling as the balance between the time needed for migration

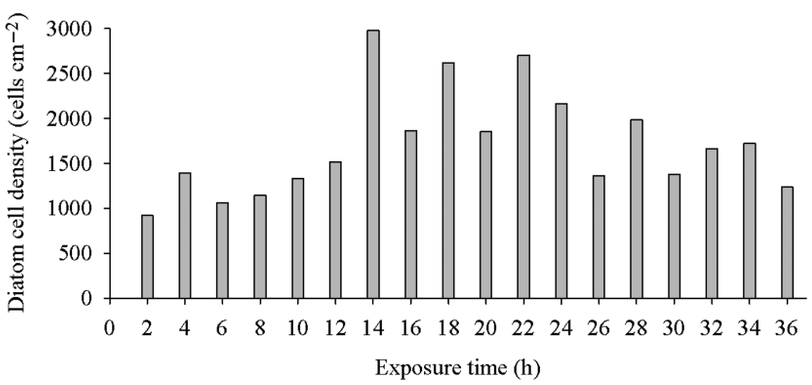

Fig. 5. Variation of diatom cell densities (cells cm^{-2}) over 36 h constant light exposure.

on the one hand and the loss of cells from the coverslip margins on the other.

Harvesting efficiency of opaque experimental chambers

Diatom cell densities on coverslips in the opaque (painted) Petri dishes were 27.2%, 10.0% and 9.5% higher than those in the transparent dishes (Table 1). This indicates that the opaque Petri dishes had on average a 15.6% higher harvest efficiency compared to the transparent dishes.

The same diatoms were found to be most abundant in both the opaque and transparent Petri dishes: *Navicula leptostriata* Jørgensen, *Navicula madumensis* Jørgensen/*Navicula hoefleri* Cholnoky and *F. rhomboides* var. *saxonica* in samples 1 and 2, and *N. leptostriata* and *F. rhomboides* var. *saxonica* in sample 3. The BC similarity indices based on diatom species percentage abundances between samples from obscured and transparent Petri dishes were high, especially for sample 2 (BC = 0.92).

When transparent dishes are used as experimental chambers light can penetrate not only the upper surface, but also through the sides. Because of positive phototaxis (e.g. Eaton & Moss, 1966), some diatoms, therefore, have the potential to migrate sideways as well as upwards resulting in lower harvest efficiency at the upper surface. Despite no significant difference in species composition between opaque and transparent petri dishes, the opaque sides and base of treated Petri dishes appear to reduce multi-directional diatom migrations and thus more motile epipelic diatoms migrate upwards. The opaque dishes used in experiment 2 therefore indicate that harvest efficiency is improved by restricting illuminated surfaces.

Identification accuracy of coverslip burning method

Diatom cell densities counted on coverslips prepared using the burning method were 5.8%, 6.0% and 8.8% higher than counts obtained using the traditional method (Table 2). However, diatoms counted using both methods had the same dominant species: *N. leptostriata*, and *F. rhomboides* var. *saxonica* in all three samples. The BC similarity indexes between diatoms from the two methods were very high, 0.99, 0.96 and 0.95 in samples 1, 2 and 3, respectively.

After removal of the coverslip from the lens tissue, coverslips can be directly placed on glass slides (Round, 1953), mounted in drops of 40% glycerol (Eaton & Moss, 1966) or petroleum jelly (Mann, 1984*b*) and examined at ×400 magnification (Table 3). The fresh diatoms can then be directly identified based on the shape of the cell

Table 1. Comparison of main epipelic diatom percentages (%), diatom cell density (cell cm^{-2}) and the Bray–Curtis similarity indexes of diatoms harvested in triplicate using opaque (O) (painted black in sides and bases) and transparent (T) Petri dishes after 8 h accumulation under natural light condition.

Samples	Methods	*Navicula leptostriata* (%)	*Navicula madumensis/ hoefleri* (%)	*Frustulia rhomboides* var. *saxonica* (%)	*Pinnularia* spp. (%)	*Brachysira* spp. (%)	Cell density (cells cm^{-2})	Bray–Curtis similarity indices
1	O	58.9	6.2	24.8	0.9	0.9	3690	
	T	59.4	20.8	7.9	0.0	0.0	2889	0.77
2	O	72.7	5.5	13.6	0.8	0.0	3146	
	T	74.0	3.0	19.0	2.0	1.0	2860	0.92
3	O	56.7	1.9	38.5	1.0	0.0	2975	
	T	67.4	1.1	31.6	0.0	0.0	2717	0.89

Table 2. Comparison of main epipelic diatom percentages (%), diatom cell density (cell cm^{-2}) and the Bray–Curtis similarity indexes of diatoms harvested from coverslip exposed in opaque petri dishes for 8 h under natural light condition. Coverslip diatom cell densities were counted using a burning method (B) and traditional method (T) (directly counting fresh diatoms).

Samples	Methods	*Navicula leptostriata* (%)	*Navicula madumensis/ hoefleri* (%)	*Frustulia rhomboides* var. *saxonica* (%)	*Cymbella* spp. (%)	*Brachysira* spp. (%)	Cell density (cells cm^{-2})	Bray–Curtis similarity indices
1	B	93.7	0.9	5.4	0.0	0.0	25 286	
	T	94.0	0.0	5.5	0.5	0.0	23 913	0.99
2	B	90.3	0.0	8.7	0.5	0.5	22 311	
	T	94.6	0.0	5.4	0.0	0.0	21 053	0.96
3	B	91.2	1.6	4.0	0.0	1.6	3576	
	T	96.5	0.0	3.5	0.0	0.0	3289	0.95

Table 3. Comparison of different coverslip methods according to this study and published methods.

Methods	Experimental chambers	The contact of coverslip and sediment	Treatment of coverslip	Mounting method	Samples preservation time
Round (1953)	Normal Petri dishes	Direct	Fresh sample	Directly on slides	About 15 min
Eaton and Moss (1966)	Normal Petri dishes	Direct	Fresh sample	Mounted in drops of 40% glycerol on slides	Several h
Mann (1984*a*)	Normal Petri dishes	Direct	Burn	Mounted to slides with Naphrax® medium	Permanent
Mann (1984*b*)	Normal Petri dishes	A layer of lens tissue	Fresh sample	Sealed onto the slide with petroleum jelly	6–12 h
Cox (1990)	Normal Petri dishes		Gentle treatment with H_2O_2	Mounted to slides with Naphrax® medium	Permanent
This study	Painted Petri dishes	A layer of lens tissue	Burn[a]	Mounted to slides with Naphrax® medium	Permanent

[a]Ignition of exposed coverslip in a Bunsen burner flamer.

and the configuration of the chloroplast (Cox, 1996). However, these preparations are not permanent. It is also difficult to identify some diatoms accurately to a high taxonomic level because of masking by organic matter and the lower magnification. In an attempt to resolve this problem, Cox (1990) gently treated the living diatoms using hydrogen peroxide and Mann (1984*a*) burned the coverslip to destroy the organic matter to make permanent slides (Table 3). Following Mann's method, we ignited the organic matter using a flame and mounted the coverslip in Naphrax® enabling us to identify species at ×1000 magnification. A quantitative comparison of counts between the two methods (Table 2) showed that the burning method gave an increase in abundance of almost 7%. A further advantage is that the slides are permanent (Table 3).

The method could readily allow *in situ* epipelic cell densities to be estimated, if known areas of sediment are sampled, settled over a standard area and sampled from a standard sub-area. Overall, the coverslip method harvested more motile diatoms, made identification and counting easier, and allowed permanent slides to be created.

Conclusions

After accumulation for 8 h, diatoms significantly migrated towards the edges of coverslips, possibly due to a CO_2 concentration gradient developing between the coverslip middle and edges. For the samples, the optimum harvesting time is 8 h.

In addition, 8 h is the maximum recommended exposure for obtaining an even distribution of diatoms in the samples of this Scottish upland loch. Opaque Petri dishes suppressed diatom migration to the side and therefore, increased diatom cell density to the upper surface of the sediment samples. When the organic matter of diatoms and other organisms on the coverslip were burnt, diatom identification was easier than identification from fresh samples. After burning, the coverslips were mounted onto slides using Naphrax® enabling the slides to be made permanent. The modified Petri dishes and burning coverslip method improved the harvest efficiency of the coverslip method. Allowing 8 h for the diatoms to migrate from the sample to the coverslip was found to be the optimum time for our samples. However, this may not be the case for samples from other sites. We recommend that migration experiments, similar to the one described here, should be carried out to assess the optimum exposure time for other samples on a site-specific basis.

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