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## Sampling littoral diatoms in lakes for ecological status assessments: a literature review

Science Report SC030103/SR1

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**Authors:**

Lydia King<sup>12</sup>, Gina Clarke<sup>3</sup>, Helen Bennion<sup>3</sup>, Martyn Kelly<sup>2</sup>, Marian Yallop<sup>4</sup>

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**Research Contractors:**

<sup>1</sup> Lancaster Environment Centre, Lancaster University, Lancaster LA1 4YQ

<sup>2</sup> Bowburn Consultancy, 11 Montaigne Drive, Bowburn, Durham DH6 5QB, UK.

<sup>3</sup> ECRC-ENSIS, Department of Geography, University College London, 26 Bedford Way, London WC1H 0AP.

<sup>4</sup> Department of Biological Sciences, University of Bristol, Woodland Road, Bristol BS8 1UG.

**Project Manager:**

B. J. Jamieson, Ecosystems Science, Wallingford

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# Summary

The Water Framework Directive has created a statutory obligation for EU Member States to monitor phytobenthos in lakes. Although there has been a considerable amount of work using phytobenthos to monitor river water quality in Europe, there is much less on the use of phytobenthos in lakes. This review summarises the existing literature and provides a theoretical basis plus practical recommendations for sampling littoral phytobenthos from UK standing waters.

# Contents

<b>Summary</b>	<b>4</b>
<b>Contents</b>	<b>5</b>
<b>1 Introduction</b>	<b>6</b>
<b>2 Sampling issues</b>	<b>9</b>
2.1 Spatial variability	9
2.2 The effect of light on community composition	10
2.3 Successional patterns of phytobenthos	12
2.4 Seasonality	20
2.5 Substratum specificity	22
<b>3 Recommendations</b>	<b>24</b>
<b>References</b>	<b>25</b>

## Figures

Figure 1.	Schematic representation of stages in response of photosynthetic community to increase in nutrient loading.	7
Figures 2 – 7	Scanning electron micrograph showing colonisation of an artificial substratum submerged in Wastewater, English Lake District, after various time scales...	13-15
Figure 2	...after one week	13
Figure 3.	...after two weeks	13
Figure 4.	...after three weeks	14
Figure 5.	...after four weeks	14
Figure 6.	...after five weeks	15
Figure 7.	...after six weeks	15
Figure 8.	Ecological strategies and associated adaptive traits described in text.	17
Figure 9.	Diagram showing possible pathways of development of communities dominated by species showing the ecological strategies R, S, C and D.	17

## Tables

Table 1.	Average similarity for epilithic algal communities from studies of spatial and temporal variability	10
Table 2.	Number of algae-smothered cobbles to be included in DARES/DALES samples.	19

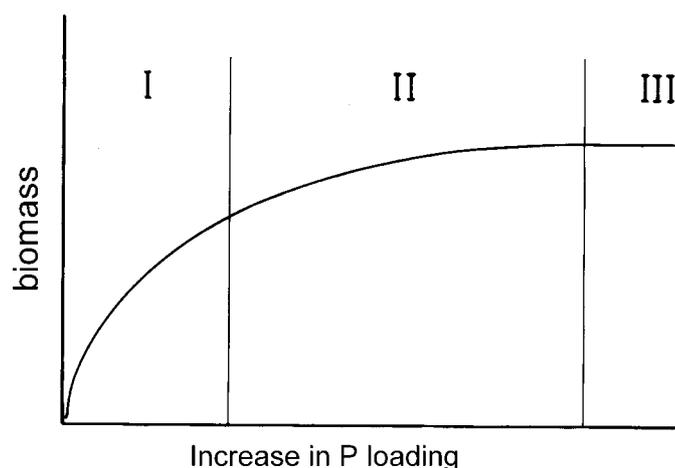
# 1 Introduction

The Water Framework Directive created a statutory obligation for EU Member States to monitor phytobenthos in lakes, as one part of ecological status assessments. Although a considerable amount of work has been done using phytobenthos to monitor river water quality in Europe (Whitton and Rott, 1996; Prygiel *et al.* 1999), there has been considerably less on the use of phytobenthos in lakes. Most of the work in rivers has focussed on diatoms, which are often the most diverse division of photoautotrophs present in freshwaters. This work resulted in the production of a number of diatom indices for use in water monitoring programmes, (see Prygiel *et al.* 1999) based on a common sampling method (Kelly *et al.* 1998). These, in turn, led to the development of standard methods for the collection and analysis of material throughout Europe (CEN, 2003, 2004).

Research on diatoms in lakes tended to focus on the dynamics of contemporary plankton (e.g. Reynolds, 1984) and on palaeoecological studies. The latter, with its focus on environmental reconstruction, provide strong theoretical bases for the use of diatom assemblages to reflect pressures such as pH (Battarbee *et al.* 1999) and nutrients (Bennion *et al.* 1996) and for establishing 'reference conditions' (Bennion *et al.* 2004). However, the focus of these studies was on the assemblage in profundal sediments, which is assumed to contain diatoms representative of all habitats within the lake and provides an integrated flora, both in time and space (DeNicola, 1986; Cameron, 1995), whereas monitoring contemporary phytobenthos will require additional sampling of live assemblages from the littoral zone.

The principles that underlie sampling diatoms and phytobenthos for rivers are broadly applicable to sampling diatoms from littoral zones in lakes. The purpose of this report is to review the literature, drawing from both lentic and lotic sources, to provide justification for the DALES (diatom assessment of lake and loch ecological status, UK; EA project number SC030103) sampling protocol. A key assumption underlying DALES is that the diatom species composition from a single substratum, obtained from analyses of cleaned valves, is a valid proxy of the signal for the entire phytobenthos assemblage present in the littoral zone of a lake. This assumption in DALES is being tested elsewhere. Studies of lake phytobenthos have addressed a wide range of theoretical and applied issues and a variety of methods were employed. Many of the conclusions from these studies are relevant to sampling for routine monitoring purposes. While the focus of this review is on diatoms, the reality in the lake littoral zones is that the diatoms live in close proximity, and compete for resources, with many other photoautotrophs and it is necessary to include references to studies that embrace these. Similarly, while our focus is on the species composition of the littoral diatom assemblage, many of the factors that affect this diatom assemblage also influence the biomass of algae present.

The relationship between maximum possible biomass of algae and P loading is summarised in Fig. 1 (based on a model that describes the behaviour of phytoplankton in lakes; Sas, 1989). This conceptual model recognises thresholds above which the system is saturated with nutrients and further changes will not occur. From the point of view of this study, the key threshold is between zones I and II in Fig. 1. This shows the point beyond which no further changes in species composition take place and at which there is only a potential increase in the biomass of nutrient-tolerant taxa. Methods based on the analysis of cleaned diatom samples are effective only as far as this threshold. Methods for assessing the biomass are not considered here, largely because several studies have shown that the actual biomass of phytobenthos does not correlate well with nutrient loading. This is due to the importance of factors such as grazing and, at waterbodies with higher trophic status, competition with phytoplankton (Cattaneo, 1987; Lalonde and Downing, 1991). The curve therefore represents potential, rather than actual, biomass. Bearing in mind the normative definitions given in the WFD, we suggest that the boundaries between high and good ecological status, and between good and moderate ecological status will lie within zone I in Fig. 1, but that other boundaries may be positioned within zones II or III.



**Figure 1. Schematic representation of stages in response of photosynthetic community to increase in nutrient loading. (modified from Sas, 1989).** The response to P loading depends upon the zone in which the initial P concentration is found: I, Increase in biomass and compositional change in response to increased P loading; II, Increase in biomass but no compositional change; III, No biomass response to increase in P loading

This review concentrates on the literature related to the sampling of epilithic and epiphytic diatoms in lakes. The epipellic (mud-associated) and epipsammic (sand-associated) diatoms are not considered in any detail here because these are often thought to be less representative of the

environmental conditions prevailing within the lake. For example, in highly eutrophic (nutrient rich) systems the muds and silts are often highly nutrient enriched and the diatoms may respond to the sedimentary chemistry rather than to the chemical conditions of the overlaying waters. This may limit their value as indicators of ecological status in lakes (Hansson, 1992; Scheffer, 1998; Siver, 1999). Furthermore, the epipelagic diatom community in the littoral zone of most lowland lakes is relatively homogeneous, often being dominated by small *Fragilaria (sensulato)* taxa. These taxa take advantage of the favourable light conditions in the shallow water of littoral zones, but they are poor indicators of water quality having a broad tolerance to nutrient concentrations (e.g. Bennion *et al.* 2001). Their low sensitivity suggests that they are unlikely to be suitable for the classification tool development.

# 2 Sampling issues

## 2.1 Spatial variability

Few quantitative data evaluate the spatial variability of phytobenthos assemblages within lakes. Some studies focussed on the effect of depth on the distribution of benthic algal communities (Kingston *et al.* 1983; Stevenson *et al.* 1991) while others concentrated on macroscopic differences along the shoreline caused by differences in substratum (Kann, 1958, 1959, 1982; Maltais and Vincent, 1997). Changes in microscopic community composition along the shore of a lake have been investigated in the littoral zone of Windermere, where differences in community composition were found to result mostly from changes in the substratum available for colonisation (Godward, 1937). Hofmann (1994) studied the diatom communities from nine different locations around Chiemsee in Germany, concentrating on epilithic and epiphytic samples and related the differences found to variations in nutrient concentrations.

Both the abundance and species composition of phytobenthic diatoms may vary around the littoral area of a lake owing to differences in water quality, local variation of runoff from the shore and differences in substratum and levels of disturbance. To circumvent this, diatom samples are almost always a composite obtained by pooling material from a number of stones (typically a minimum of three stones), usually with a minimum water depth requirement (typically about 40-50 cm). Jones and Flower (1986) noted that spatial variability in epilithic sample assemblages was greater than any effect of temporal variability at the Round Loch of Glenhead (see also Jørgensen, 1948).

In a study of epilithon from lakes in North West England, King *et al.* (2000, see also King 2000) devised a sampling strategy to control for variation in physical variables that may influence the epilithic algal community composition, to examine the spatial and temporal variation in algal assemblages. Sampling locations were open to full sun for most of the day and, at each of these, stones were removed from the same sampling depth throughout the study. Spatial variability was studied at two lakes, Wastwater (oligotrophic) and Bassenthwaite (mesotrophic) and the similarity between species composition was high within samples from each lake (Table 1).

It is possible that some of the spatial variability in phytobenthos within the lake littoral zones was missed by the adherence to a rigorous sampling protocol (very similar to that advocated by the DALES project). However, these data do demonstrate that epilithic samples collected from a standard habitat are highly reproducible. While a similar approach of combined samples has also been used by other workers (e.g. Cattaneo, 1990), others have treated every stone as a single sample (e.g. Maltais and Vincent, 1997). However, such an approach is very time consuming and, consequently, composite samples are commonly used in periphyton research (Mueller, 1994; Vymazal and

Richardson, 1995), because periphyton development is often patchy. As a result of the likely small-scale spatial heterogeneity we advocate sampling and combining the epilithon from different stones at the sampling location. Combining the epilithon from a number of stones results in samples that are both representative of the habitat and cost-effective.

Different forms of brushing or scraping have been used to remove algae from hard substrata (Aloi, 1990). Often only qualitative samples were taken (Godward, 1937; Hofmann, 1994), since for quantitative sampling the stone surface has to be more or less flat. Quantitative data are easier to obtain from artificial substrata of known area; however, the developing communities often differ from the natural ones, with the rarer taxa, in particular, missing (King, 2000). Cattaneo and Amireault (1992) and Kelly *et al.* (1998) discuss the use of artificial substrata in greater detail.

**Table 1. Average similarity for epilithic algal communities from studies of spatial and temporal variability (modified from: King *et al.* 2002a, b) assessed using the Similarity Index of Hoagland *et al.* (1982) with values that ranged from 0 (= no similarity) to 1 (= high similarity).**

Waterbody	Spatial (12 locations per lake)	Temporal (24 month)
Wastwater (oligotrophic)	0.85 spring 0.89 autumn	0.87
Coniston Water (mesotrophic)		0.74
Bassenthwaite Lake (mesotrophic)	0.95 autumn	
Malham Tarn (meso-eutrophic)		0.61
Esthwaite Water (eutrophic)		0.61

## 2.2 The effect of light on community composition

Benthic diatoms need light to photosynthesise, and variability in their growth patterns can be determined by light fluctuations and availability. As most benthic diatoms are sessile, their ability to move to areas of greater light intensity and duration is limited. Areas with dense macrophyte growth or where the water column is highly turbid are likely to have different diatom assemblages than clear water zones because of the differing light regimes (Brown 1976; Nygard 1994; Tesolin and Tell, 1996). Little is known of the light requirements of most benthic diatoms species, as most ecological studies

were restricted to the phytoplankton (Hill, 1996). A number of studies have been carried out to investigate the effects of varying light regimes on community composition of benthic algae but these proved difficult to interpret because of the potential indirect effects of competition for space and temperature in the growing algal matrix (Stevenson *et al.* 1996).

In general, higher irradiance levels support higher proportions of filamentous members of the *Zygnematales* (e.g. *Spirogyra*, *Mougeotia*), whereas lower light conditions result in a greater absolute and relative abundance of diatoms and desmids (Bothwell *et al.* 1994). Diatom species may benefit from shading from the filamentous algae, possibly by reduction of the damaging effects of UV radiation (Bothwell *et al.* 1994) or photoinhibition. Also, large colonies of filamentous green algae can affect the underlying benthic community by reducing the transfer of nutrients to and from the sediments (Turner *et al.* 1995). In addition, high concentrations of dissolved organic carbon (DOC), can act like a light filter and may influence the growth of benthic algae communities.

Stevenson *et al.* (1985) studied the distribution of attached diatoms along a depth gradient in 20 soft water lakes in New Hampshire and found that shifts in species composition could be attributed to the loss of light with depth. They found that the diatom *Pinnularia* had its highest densities at some of the lowest light levels, and suggested that these organisms might be heterotrophic. A prostrate diatom species, *Planothidium rostratum*, can survive for long periods (> 30 days) in a heterotrophic state (Tuchman *et al.* 1994). Diatom species that are facultatively heterotrophic (utilising various sources of DOC), may have a selective advantage under conditions of low irradiance caused by overlying cells in a benthic mat or by bankside vegetation or turbid water (Hill, 1996). Such an ability has been demonstrated in two common benthic diatom genera, *Navicula* and *Nitzschia* (Admiraal and Peletier 1979).

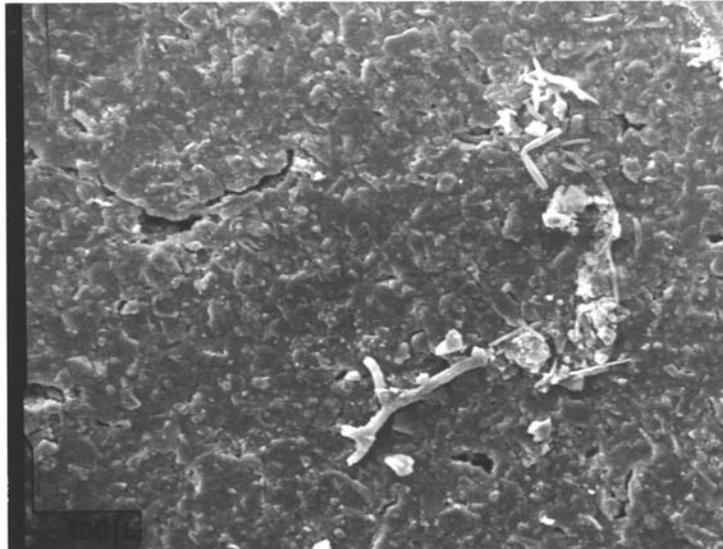
Reliable biomass estimates are extremely difficult to obtain from phyto-benthic communities. Over short time scales there may be a clear relationship between irradiance and photosynthesis, but over the long-term factors other than light may act as constraints that limit biomass accrual. Accrual of biomass is the integration of both gains and losses. Losses may arise through a number of factors such as grazing, physical disturbance and pathogens (Hill *et al.* 1992). Hill *et al.* (1995) reported that maximum photosynthesis rates were much greater for benthic algae in streams in unshaded habitats compared to those in shaded habitats. However, heavy grazing pressures occurred in the unshaded habitats and so differences in photosynthetic rates were not realised in measurements of algal standing crop. Furthermore, the responses of benthic algae to light may be modified by changes in temperature. The frequent correlation between light and temperature means that partitioning the effects of these two variables is problematic. A direct relationship was found between light intensity and biomass in epilithic assemblages at four acidic lakes in northern Michigan (Pillsbury and Lowe 1999). However, other lake studies in which the relationship between algal biomass and depth were investigated, indicate a lack of exponential decline. Stevenson and Stoermer (1981) observed that epipellic diatoms were scarce

at shallow depths (< 9 m), increased in abundance at depths between 9 and 15 m and declined again at depths in excess of 23 m. Wave action may be an important limitation on biomass accrual in shallow depths (Evans and Stockner, 1972). High irradiance at lake margins may also result in photoinhibition and thereby reduce algal biomass.

## 2.3 Successional patterns of phytobenthos

The composition of a diatom assemblage on a substratum changes over time and this, too, can be a source of error when making comparisons or ecological status assessments. Although it is often difficult to determine the age of diatom communities, different successional stages can be distinguished. Colonisation of newly submerged substratum by periphyton begins with cells passively transported by the water current attaching and multiplying in cracks on rocks and at edges and in depressions, where they are sheltered (Hamilton and Duthie, 1984; King, 2000). Hoagland *et al.* (1982) studied the three-dimensional structure of periphyton communities colonising glass slides and plexiglass tabs in small shallow eutrophic reservoirs and could describe different stages of succession. The first week involved 'conditioning' of the substrate by colonisation by bacteria and an associated organic coating. Following this, opportunistic diatoms arrived. Hoagland *et al.* (1982) observed *Gomphonema parvulum* during this period, but other researchers have reported genera such as *Staurosira*, *Pseudostaurosira* and *Staurosirella* in early biofilms, along with *Achnantheidium* spp with short stalks. This pioneer phase is followed by the establishment of long-stalked diatoms (e.g. *G. olivaceum*), large diatom rosettes (e.g. *Fragilaria vaucheria*) and filamentous green algae such (e.g. *Stigeoclonium* spp.) Some of these organisms can themselves serve as substrata for smaller species, and motile species can colonise and move around this community (Acs *et al.* 2000). Finally, chain-forming species may attach themselves to this outer layer. This series of events is referred to as microsuccession and is comparable to the process of succession in higher plant communities and marine macroalgal communities.

The timescale of the succession may depend upon the trophic state of the water body. Colonisation and development of periphyton in the littoral zone of Wastwater (oligotrophic) was much slower than at the eutrophic Esthwaite Water. At Wastwater from week two through to week four the community stayed in the second stage described above, with small adnate or short stalked diatoms gradually filling up all available space (Figs. 2-5). *G. parvulum* reaches high abundances. This seems to be a ubiquitous diatom that colonises largely independently of nutrient status, but that is rather susceptible to competition for space and/or grazing later on. As long as sufficient resources are available, smaller forms with higher growth rates have a competitive advantage. However, when space becomes less readily available, competition for resources such as light and nutrients increases close to the surface and long, upright taxa (taxa with long stalks and filamentous forms) become more common. In weeks five and six, when long-stalked diatoms appear, the microsuccession enters the third stage (Figs. 6-7).



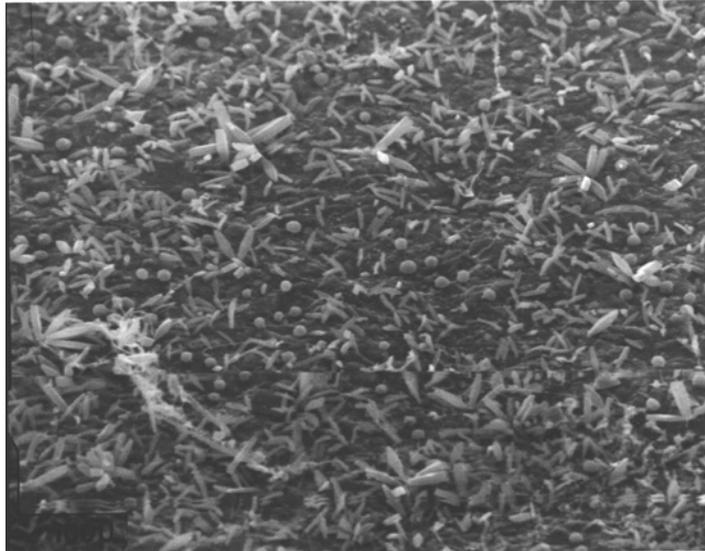
King, 2000

**Figure 2.** Scanning electron micrograph showing colonisation of an artificial substratum submerged in Wastwater, English Lake District, after one week. Scale bar: 100  $\mu\text{m}$ . Very few algae are present.



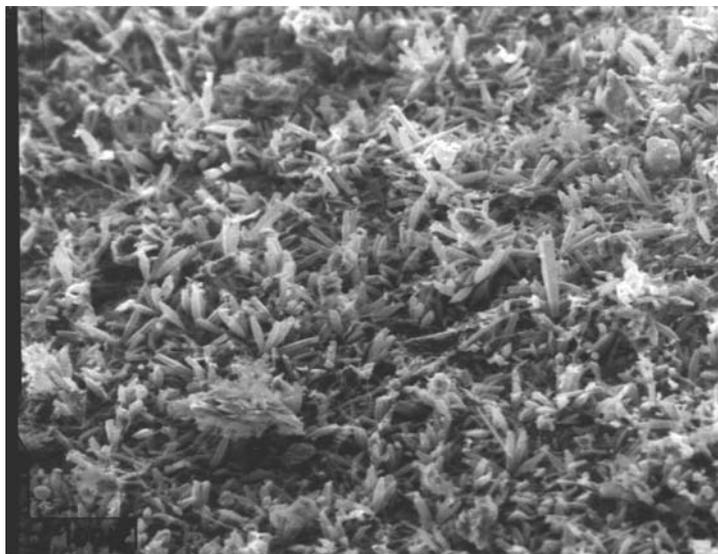
King, 2000

**Figure 3.** Scanning electron micrograph showing colonisation of an artificial substratum submerged in Wastwater, English Lake District, after two weeks. Scale bar: 100  $\mu\text{m}$ . A thin layer of algae, dominated by *Achnanthydium*, *Fragilaria* and *Gomphonema*, is present.



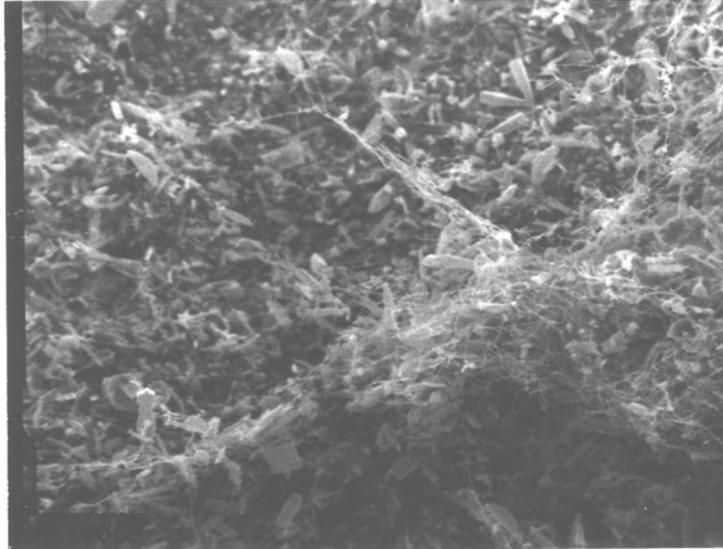
King, 2000

**Figure 4.** Scanning electron micrograph showing colonisation of an artificial substratum submerged in Wastwater, English Lake District, after three weeks. Scale bar: 100  $\mu\text{m}$ . A thin layer of algae, dominated by *Achnanthidium*, *Fragilaria* and *Gomphonema*, is present on about a quarter of the available surface. *Tabellaria flocculosa* chains form on top.



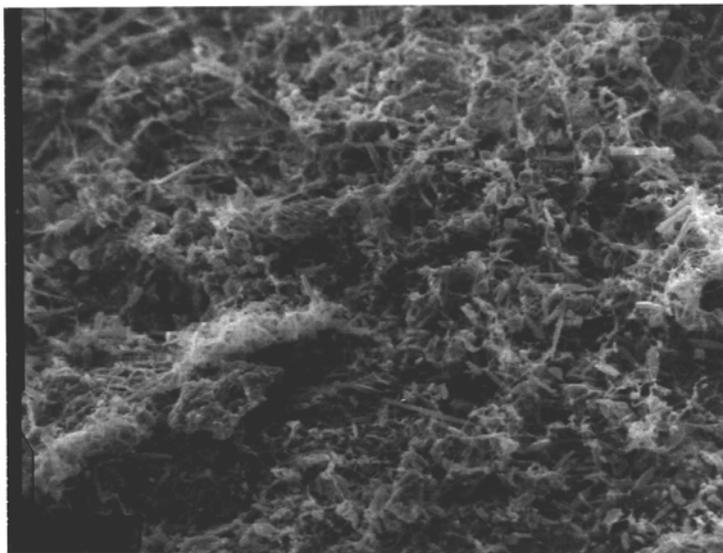
King, 2000

**Figure 5.** Scanning electron micrograph showing colonisation of an artificial substratum submerged in Wastwater, English Lake District, after four weeks. Scale bar: 100  $\mu\text{m}$ . The entire surface is now covered and short-stalked *G. parvulum* cells are often found in groups. Some rosette-forming *Fragilarias* are also found.



King, 2000

**Figure 6.** Scanning electron micrograph showing colonisation of an artificial substratum submerged in Wastwater, English Lake District, after five weeks. Scale bar: 100  $\mu\text{m}$ . Longer-stalked forms of *Gomphonema* (*G. acuminatum*, *G. gracilis*) and *Encyonema silesiacum* are now growing above the dense bottom layer into the water column. Unfortunately, because of the preparation method, most seem to be lying on top of the bottom layer.



King, 2000

**Figure 7.** Scanning electron micrograph showing colonisation of an artificial substratum submerged in Wastwater, English Lake District, after six weeks. Scale bar: 100  $\mu\text{m}$ . The biofilm is now very dense and contains even more large, long-stalked forms.

The stage of dominance of filamentous green algae might not be reached at oligotrophic waterbodies within six weeks, although in eutrophic waterbodies, such as Esthwaite Water, the successional process happened more quickly. In this lake, the microsuccession was largely completed after two weeks, with filamentous green algae starting to build an overstorey above the diatom layer. Species with high growth rates have a competitive advantage under the higher nutrient conditions experienced at Esthwaite Water, but many algae are lost here by week four through grazing. This disturbance brought the community back to the earlier stage of succession with only one layer of periphyton. Conditions suitable for a higher periphyton carrying capacity are found at Esthwaite Water compared to Wastwater, but the available biomass soon supports a large number of grazers that reduce the algal cover to a thin layer of tightly attached forms.

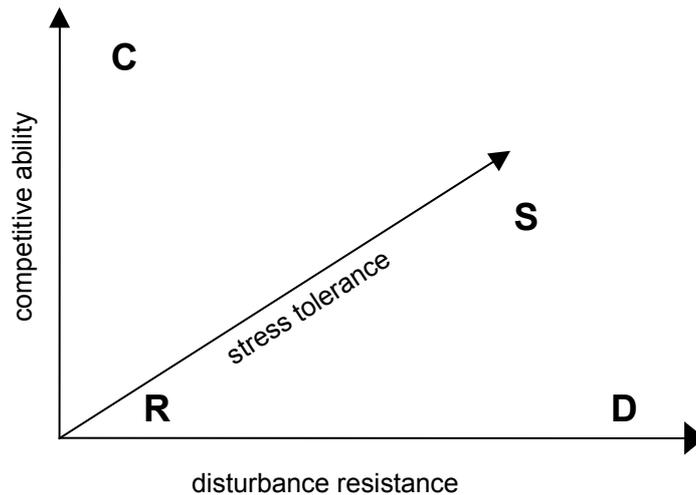
Experimental evidence from both lotic and lentic ecosystems shows that in some circumstances a reduction in biomass is not seen in the presence of herbivores. Reasons implicated for a lack of decline may be that

- (1) That the density of grazers and their rate of consumption is less than the rate of increase in algal biomass (Steinman et al. 1987)
- (2) The mouth parts of the grazer may not be compatible with the dominant algal morphologies (Karouna and Fuller, 1992)
- (3) Resources limit the algal biomass.

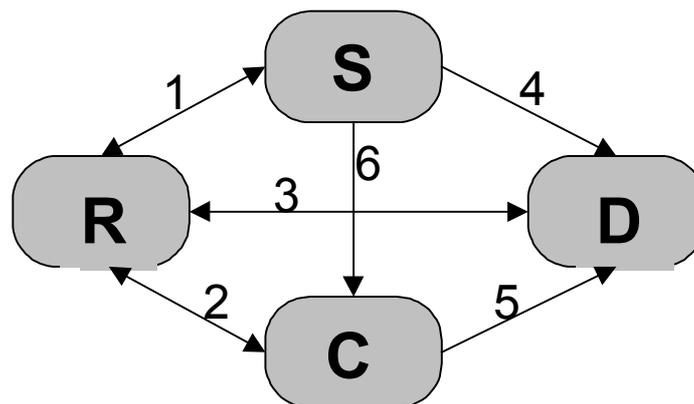
Less commonly, when grazer density is low, grazing may promote an increase in algal biomass (Swamikannu and Hoagland, 1989).

### **2.3.1 Resource competition and species co-existence**

McCormick (1996) applied the idea of Grime (1979) about competition within higher plant communities (the species with the better ability to sequester resources effectively will have a competitive advantage), to epilithic algal communities as their structure shows many similarities to those of higher plants. McCormick (1996) placed four types of algae into a three dimensional space determined by disturbance resistance, competitive ability and stress tolerance (Fig 8). In Fig. 8 C-species are those adapted to maximise resource capture and growth rate, S-species are stress tolerant (e.g. adapted to utilise resources to maintain positive growth rates under conditions of low resource availability), D-species are resistant to disturbance through adaptations that prevent removal by scouring and herbivory and R-species are adapted to colonise disturbed sites where resource supply is high and density-dependent interactions are low.



**Figure 8. Ecological strategies and associated adaptive traits described in text.** C, competitors adapted to maximise resource capture and growth rate; S, stress-tolerant species, for example, those adapted to utilise resources efficiently to maintain positive growth rates under conditions of low resource availability; D, disturbance-resistant species possessing morphological adaptations that prevent removal by scouring or herbivory; R, ruderal species adapted for colonising disturbed sites where resource supply rates are high and density-independent interactions are weak (modified from McCormick, 1996).



**Figure 9. Diagram showing possible pathways of development of communities dominated by species that show the ecological strategies R, S, C and D.** Arrows indicate pathways that join the various community types. See text for more details (After King, 2000).

Any epilithic assemblage could be placed in this three dimensional space according to the contributions of algal species that belong to these different groups. In theory, community development can follow different pathways, as shown in Fig. 9. On a newly submerged surface a community with many R-species colonises if resources are ample. Such a community can be characterised by high numbers of small, green, spherical cells and flagellates, thin blue-green filamentous forms, *Achnantheidium minutissimum* and *G. parvulum*. A transition from a community dominated by R-species to one that is characterised by S-species (Path 1) is expected under oligotrophic conditions. Species found in such conditions include *Brachysira* spp. or *Frustulia rhomboides* var. *saxonica/crassinerva*.

Under eutrophic conditions, competition for resources quickly becomes a problem and the development proceeds from a community dominated by R-species to one with a high abundance of C-species (Path 2). This is usually the case when biomass is high and filamentous forms are abundant.

A transition from R- to D-species (Path 3) is theoretically possible, if grazers were already present during the colonisation phase. However, usually the colonisation by grazers is slower than the colonisation by phyto-benthos. More realistic is that a colonisation by algae, predominantly S- or C-species depending on nutrient status, is then followed by the invasion of grazing organisms that shape the community to be dominated finally by D-species (those most resistant to disturbance: Paths 4 or 5). Increasing eutrophication may lead from a community dominated by S-species to a community that comprises mainly C-species, a transition accompanied by increasing mat thickness (Path 6).

Processes such as wave action or senescence and sloughing, may remove a large amount of the biofilm, and development starts again with a community dominated by R-species. However, these categories (R, S, D and C) are not mutually exclusive and some species may have multiple strategies for survival and could potentially be found in each of the communities.

### **2.3.2 Implications for monitoring phyto-benthos**

The model described has two implications for practical monitoring in which spatial and temporal comparisons of diatom assemblages are made. First, if phyto-benthos assemblages are undergoing cycles of changes, as described above, the assemblage contained in a sample depends, to some extent, on the point of the cycle at which it was collected. As a result, there will be natural variation in the proportions of R- C- S- and D- species present. Consequently, there is no single 'reference state' for littoral phyto-benthos in waterbodies, but rather a cluster of possible outcomes positioned within the space defined by the axes on Fig. 8. These outcomes depend upon a number of factors, including the time since the previous disturbance, the time of year and availability of resources. The scale of this natural variability varies between waterbodies and possibly between sampling locations within a waterbody (depending upon the amount of exposure, for example).

This in turn will determine the statistical properties of a sample and therefore, the confidence with which a deviation from Good Ecological Status can be detected.

The second implication is that disturbance may vary within a lake's littoral zone or reach of a river depending upon properties such as the size of the substratum. Smaller cobbles might be expected to be rolled by the current more frequently than larger boulders, leading to floras dominated by R- or D-species, while the latter are able to develop floras dominated by C-species such as filamentous green algae or, if resources are scarce, S-species. The result is an inherent patchiness, often visible to the naked eye, in lake littoral and stream phyto-benthos assemblages that, if not appreciated in sampling protocols, may lead to samples being collected that represent only part of the diversity present at a sampling location. We argue that, if this patchiness is to be reflected in phyto-benthos samples, substrata with and without filamentous algae should be selected in approximate proportion to their abundance at the sampling location. For DARES and DALES, a simple procedure is recommended in which the cobbles with and without filamentous algae cover are sampled in proportion to their representation at the location. The percent cover of filamentous algae at the sampling location in question is assessed to the nearest five per cent and the number of cobbles with filamentous algae cover to include in the sample is determined according to Table 2.

**Table 2. Number of algae-smothered cobbles to be included in DARES/DALES samples.**

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

## 2.4 Seasonality

Diatom productivity and assemblage composition vary throughout the year. Spring diatom blooms are commonly seen in the plankton of freshwater systems, often followed by a less intense late autumn bloom (Werner, 1977; Crumpton and Wetzel 1982; Hinder *et al.* 1999; Simona *et al.* 1999; Rautio *et al.* 2000;), but seasonality of the attached community has not been studied so intensively that such general patterns can be identified. Nonetheless, the pattern of diatom maxima in spring and autumn and greater abundance of green and blue-green algae during the summer months has also been found in periphyton communities (Castenholz, 1960; Godward, 1937). Of the diatom genera, *Fragilaria*, *Diatoma* and *Tabellaria* have been shown to follow seasonal patterns. Long, thin forms of *Fragilaria* are especially common in spring and autumn, *Diatoma* shows maxima in spring and *Tabellaria* is common in early autumn (Hofmann, 1994). A re-occurring *Diatoma* bloom in spring is also typical of Malham Tarn (King, 2000). However, temporal variability was lower in oligotrophic lakes such as Wastwater (Table 1).

If, as indicated above, phytobenthos, like phytoplankton does experience seasonal succession and is exposed to changing chemical and physical conditions throughout the year, several samples collected over different seasons may be necessary to characterise lake phytobenthos (U.S. Environmental Protection Agency 2003). Alternatively, it is important that comparisons between samples collected from different lakes take the time of sample collection into account.

A number of studies demonstrated marked seasonal changes in the biomass of epipellic diatoms. Round (1960) observed three peaks of growth of epipellic diatoms in Blelham Tarn and Lake Windermere, with generally low production occurring in the late summer, early autumn and winter. He also noted marked seasonal variation over three years in the epipellic samples from two small ponds in Birmingham (Round, 1972). He concluded that the majority of English lakes show distinct seasonal changes in their benthic epipellic diatom communities, characterised by low winter growth, high spring growth, variable (sometimes negligible) summer growth and moderate autumn growth. Cox (1984) also found that seasonal patterns played a role in diatom production of the epipellic samples in Plußsee, Northern Germany, mainly related to the seasonal shading of the substrata by the deciduous catchment vegetation during leaf growth and drop causing light limitation.

While there are clearly seasonal patterns in phytobenthic diatom productivity, Jones and Flower (1986) found no evidence of seasonal succession in species composition in either the epipsammon or epilithon samples from the oligotrophic Round Loch of Glenhead. This is in contrast to the clear seasonal species succession observed in the phytoplankton (Reynolds 1984) and the epipellic (Stockner and Armstrong 1971) of more productive lakes. They attributed this to the fact that the waterbody has relatively stable physico-chemical conditions. Similarly, a clear seasonal periodicity could not be seen in any of the species found by Nygaard (1994) in his study of diatom assemblages growing on the benthic plant *Nitella flexilis* in Grange Langsø,

Denmark. Furthermore, Kahlert *et al.* (2002) observed temporal variation in the biomass of epilithic diatoms in Lake Erken, Sweden, but the changes in species composition appeared to be related less to time of year and more strongly to differences in nutrient status and wind action.

Seasonal effects may be emphasised, however, when comparing lakes from different geographical locations and of different types. For example, with the changes in season, issues of shading by phytoplankton, macrophytes and emergent vegetation become important, particularly in lowland waterbodies with greater within lake and catchment vegetation. Shading may directly reduce the diatom cell density when leaves appear and when leaf drop occurs, causing blanketing of the substrata and light limitation (Cox 1984). This is unlikely to be a factor that affects many oligotrophic upland waterbodies, but conversely ultraviolet intensity may influence diatom assemblages in these waterbodies (see section 2.1). The seasonal effect on diatom species composition as opposed to biomass is poorly understood and requires further study.

Diatoms are an important component of the diet of a range of grazers (Round, 1981), especially chironomid larvae (Botts, 1993) and thus grazers can alter the floristic assemblage of the diatom community (Allan, 1995). The effect of grazers on the benthic diatom community is hard to enumerate and few studies have attempted to examine this in standing waters. Nevertheless, grazing pressure is likely to vary over the course of the year and may therefore seasonally affect the diatom assemblage.

Finally, it is useful to consider this section on seasonality alongside the previous section on succession. While there are some clear patterns of seasonal change, it is not always clear how many of these are the result of natural selection favouring taxa with particular physiological traits to thrive at specific times of the year and how much is simply a greater probability of finding certain organisms at certain points in the resource-disturbance space at certain times of year? While some taxa have clear preferences for low temperature (e.g. *Meridion circulare*, Cox, 1993) and are consequently more common in spring, it is also likely that the more turbulent environment of late-winter and spring may lead to a greater probability of finding R-species at this time, while a grazer-tolerant species such as *Cocconeis placentula*, is more likely to be abundant in the summer and early autumn.

## 2.5 Substratum specificity

The physical and chemical properties of the substratum influence the composition of attached communities. The boundary layer that surrounds the substratum and the microtopography are important features of the substratum during initial colonisation, when microcrevices offer refugia for the settlement of cells with a reduced risk of dislodgement (Dudley and Dantonio, 1991). The stability of the substratum is another important feature that influences the colonisers. The relationship between macrophytes or soft sediment and the attached community has received much attention and led to often contradictory conclusions (Cattaneo *et al.* 1979; Wetzel, 1983), but has recently been reported to be rather mechanistic than mutualistic (B. Moss, personal communication). Stones are normally regarded as inert substrata and epilithic communities depend on nutrients either from the overlying water column or from recycling processes within the mat (Burkholder, 1996). Many investigations used artificial substrata, often for purely logistic reasons, although the first of Aloi's (1990) recommendations is: "sampling periphyton on naturally occurring substrata rather than artificial substrata when measuring biomass, primary productivity or species composition".

A study of benthic diatoms in the littoral zone of 34 ultra-oligotrophic lakes in Arctic Canada was conducted to establish the impact of habitat type on species assemblage, independent of water chemistry (Michelutti *et al.* 2003). It was shown that several taxa exhibited strong habitat preferences to sediment, moss (epiphyton) or rock (epilithon), and these authors also found significant differences in diatom composition among the three habitats. However, many of the species shown to be habitat specific in this study were found to inhabit different habitats in other Canadian arctic studies. For example, *Cyclotella pseudostelligera* was identified as moss-specific by Michelutti *et al.* (2003) and as rock-specific by Lim *et al.* (2001), which highlight the complexities of comparing habitat specificity across studies and suggests that perhaps the taxa do not exhibit such strong substratum specificity as originally thought.

However, the arctic environment represents a limnological extreme in terms of lake characteristics and diatom ecology, with complex ice/-climate interactions that limit habitat availability, and extremes of light intensity also exist within such systems. Such limitations are unlikely to be as pronounced in UK systems. A number of studies indicated that epiphytic communities vary depending on their host macrophyte (e.g. Gough and Gough, 1981). Blindow (1987) studied the influence of the host plant on both epiphyton density and composition. She found that taxa such as *Navicula cryptocephala*, *Anomoeoneis exilis* and *Cymbella microcephala* were evenly distributed across the samples. Some taxa occurred in patches without any preference for a particular substratum, while a third group was more abundant on particular plant types. She also found that some species could migrate from a 'preferred habitat' to a 'less preferred habitat'. For instance, the density of *Cocconeis placentula* was significantly higher on *Nitellopsis obtusa* growing close to *Potamogeton pectinatus* than on *Nitellopsis* growing alone or

associated with other characeans. She therefore concluded that submerged macrophytes were not a neutral substratum for epiphyton.

Eminson and Moss (1988) also indicated that high host specificity existed with four different types of macrophyte in an oligotrophic lake system. However, this host specificity seems to be less pronounced in more eutrophic systems, possibly because of the reduced concentration of nutrient excretion by plants in productive lakes in comparison to the overlying water (Wetzel 1969). The degree of shading of the phytobenthic community by the host macrophyte may depend on the morphology of the latter, and therefore different plant species may have different shading effects.

In contrast, Cattaneo and Kalff (1978) found similar epiphytic density, and importantly composition, on natural and artificial plants and concluded that macrophytes are neutral substrata for the epiphyton. There appears to be confusion in the literature, therefore, on the degree to which substratum influences diatom composition and diatom indices. Hofmann (1994) suggested that any differences in species composition among samples from different substrates (stones and plants) did not lead to different indications of trophic status. However, more recently, Poulíčková *et al.* (2004), studying seven perialpine lakes in Austria, found that indices calculated from samples from different substrata indicated significantly different trophic status. They concluded that epiphyton was the most useful indicator, while Danilov and Ekelund (2000), working in central Sweden, found that epiphyton was unsuitable for assessing eutrophication in lakes. These latter two studies were each based on a small number of lakes and only serve to underline the confusion in the literature about the extent to which substratum affects the composition of assemblages and trophic indices.

### 3 Recommendations

Importantly, this literature review indicates that the magnitude of spatial and temporal variation of the epilithon within a given waterbody during the sampling period is small, relative to the variation among waterbodies, especially for waterbodies with low nutrient concentrations. If the sampling location is chosen carefully, within-waterbody variability in diatom composition should be less than between-waterbody variability. Locations likely to be affected by point-source contaminants or stream in-flow factors should be avoided. Any sampling programme should ensure that samples are collected from the same location within a waterbody on each visit, to reduce spatial variability.

On the basis of the literature review, it is recommended that epilithon samples be sampled in preference to epiphyton samples in waterbodies where stones and rocks represent the dominant habitat for benthic diatoms. This is likely to be the case in the relatively nutrient-poor, upland waters of the UK. This sampling strategy avoids the problem of host specificity or substratum variability. Nevertheless in the soft, muddy, more productive waterbodies of lowland UK, where epilithic habitats maybe lacking or limited and do not represent a significant component of the lake phytobenthos, an epiphytic sample should be collected. The sample should be taken from the emergent macrophytes and ideally should be standardised to a single species, such as *Phragmites australis*. When sampling emergent macrophytes, we recommend that five healthy established stems from different individuals of the same species are selected, and that dead material and new shoots are avoided. Sampling in this way reduces the effect of colonisation stage on the community composition. The epipelon should be avoided, as this may more closely reflect sediment properties than the water column physico-chemistry.

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