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Validation of diatoms as proxies for phytobenthos when assessing ecological status in lakes

Science Report SC030103/SR2

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Authors:
Lydia King[1], Martyn Kelly[2]

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Research Contractor:
[1] Lancaster Environment Centre, Lancaster University, Lancaster LA1 4YQ
[2] Bowburn Consultancy, 11 Monteigne Drive, Bowburn, Durham DH6 5QB,

Project Manager:
Jane Jamieson, Ecology and Soil, Science Group, Wallingford

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Executive Summary

Research to develop tools to assess the ecological status of phytobenthos, as required in Annex V of the Water Framework Directive, is focussing on diatoms. Diatoms are often the most abundant and diverse group of algae within the phytobenthos and have been widely used for other monitoring purposes. However, few data at present justify the use of diatoms as proxies for the phytobenthos. This report re-examines a dataset compiled largely from littoral samples from standing waters in the Lake District and compares transfer functions for total phosphorus, dissolved inorganic carbon, conductivity and calcium concentration generated from diatoms and non-diatoms separately and together. The results show that transfer functions generated from diatoms alone are as powerful as transfer functions generated from diatoms and non-diatoms combined, while transfer functions generated from non-diatoms alone are less effective. These results provide support for the use of diatoms as proxies for phytobenthos when ecological status is being assessed.

Contents

1. Introduction

Annex V of the Water Framework Directive (WFD; European Union, 2000) provides definitions of ecological quality in rivers and lakes based on four biological quality elements:

- phytoplankton;
- macrophytes and phytobenthos;
- benthic invertebrate fauna;
- fish fauna.

Assessment methods based on both macrophytes and algae have been developed in recent years in Europe, stimulated by the increased interest in aquatic eutrophication and leading to the development of European standards EN 14184 (CEN, 2003), EN 13946 (CEN, 2003) and EN 14407 (CEN, 2004). Methods to assess the phytobenthos have tended to focus on diatoms, which often form a large part of the algal diversity at sites and have the added advantage of being relatively easy to analyse in the laboratory. While the problems of diatom identification should not be underestimated, the presence of a rigid, non-biodegradable cell wall (or 'frustule') is a distinct advantage over the soft-bodied organisms that prevail in other groups. A method based on diatoms alone is, therefore, particularly attractive as it may provide an estimate of ecological status at a significantly lower cost per sample than one based on all the taxa present.

This focus on diatoms was not a problem when the main purpose of monitoring was to evaluate the intensity of particular pressures, such as eutrophication. However, the WFD has shifted the focus by requiring the assessment of biota present at a site in relation to that expected in the absence of pressures. While it is possible to do this using a single group within the biological element, such a step would be based on an initial assumption that any such variation within this group mirrored changes within the element as a whole. As there are no good published data on which to support such an assumption, this is one issue that has had to be addressed within the Diatom Assessment of Lake and Loch Ecological Status (DALES).

This report analyses data collected and analysed by King *et al*. (2000) to see whether diatoms are an effective proxy for the entire epilithic algal community or whether data for non-diatom species might provide additional information and should therefore be included in biological water quality assessments, despite the additional costs. This data set consists of the relative abundance of diatom species and genera of other algal groups from 51 samples at 17 sites in the English Lake District, together with measurements of a number of environmental variables. The performance of transfer functions for total phosphorus (TP), conductivity, calcium ion concentration and dissolved inorganic carbon (DIC), established under four different scenarios, are compared. The scenarios considered are:

- diatoms identified to species and other algae to genus (this is the approach adopted in King *et al.,* 2000);
- diatoms identified to species and other algae identified to the lowest level possible (some taxa identified to species; filamentous algae placed in width categories);
- diatoms only (identified to species);
- other algae only (identified to the lowest level possible).

2. Methods

2.1 Study sites and sampling dates

For the study, 17 sites on 16 lakes, mostly in the English Lake District, were selected and the epilithon and physicochemical parameters investigated three times between June 1997 and September 1998. A map showing the location of the sampling sites is given in *Figure 1* and information about the lakes is summarised in *Table 1*. Two sites on Windermere were chosen – one each in the North and South Basins.

2.2 Physicochemical measurements

Temperature, conductivity and dissolved oxygen concentration were measured at the same time and place as the epilithon samples were collected, at 40–70 cm depth (depending on water level and wave action). Temperature and conductivity were measured at the sampling sites using a combined temperature–conductivity metre (HACH Model 44600). Dissolved oxygen measurements were made with a YSI 57 combined oxygen–temperature meter and probe.

To determine pH and DIC, three 120 ml glass bottles were filled completely with water for transport in a cool box back to the laboratory, where DIC and pH were determined immediately. DIC was determined by Gran titration, following the procedure described in Mackereth *et al*. (1978), and using a Radiometer PHM82 standard pH meter. The initial pH value was recorded as the pH of the lake water.

In addition, two 250 ml acid-washed polyethylene bottles were filled with water at the sampling site, transported back to the laboratory in a cool-box and processed the same day. One of these was frozen, unfiltered, to determine TP, while the other was frozen after filtration through a Whatman GF/C glass-fibre filter. The filter was used to determine chlorophyll *a* (Chl *a*) in the water column. All spectrophotometric measurements were made with a Perkin Elmer UV/VIS Spectrometer Lambda 14 instrument.

For TP analysis, the unfiltered, frozen water was left to defrost in a fridge at 4 ºC. After an acid/persulfate digestion in the autoclave (Menzel and Corwin, 1965), the free orthophosphate was converted into a complex with molybdenum, which was then detected spectrophotometrically at 880 nm (Murphy and Riley, 1962).

Magnesium and calcium content were determined by measuring an appropriate dilution of the filtered water samples on a Perkin Elmer 280 Atomic Absorption Spectrophotometer. Magnesium was measured using an oxidising flame at a wavelength of 284.2 nm in an acetylene–air mixture. For calcium, 422.0 nm and a reducing flame were used.

Sodium and potassium contents were measured using filtered water and flame photometry (Corning Flame Photometer 410).

Table 1: Sampling sites and dates and trophic status of the lakes ranked according to ascending mean TP value.

Lake	Abbreviation	Grid reference	Sampling dates			Trophy
			1997	1997	1998	
Buttermere	Bu	NY 15423 19161	30.06.	20.09.	16.09.	Oligo-
Wastwater	Wa	NY 05076 14973	24.06.	11.09.	17.09.	Oligo-
Ennerdale Water	En	NY 14977 11232	24.06.	20.09.	16.09.	Oligo-
Coniston Water	Co	SD 95228 30971	24.06.	11.09.	17.09.	Oligo-Meso-
Derwent Water	De	NY 22727 26460	30.06.	20.09.	16.09.	Oligo-Meso-
Crummock Water	Cr	NY 18305 16276	30.06.	20.09.	16.09.	Oligo-Meso-
Brotherswater	Br	NY 13003 40215	25.06.	18.09.	15.09.	Meso-
Bassenthwaite Lake	Ba	NY 27349 22196	30.06.	09.10.	17.09.	Meso-
Winderemere South	Ws	SD 87002 37979	24.06.	09.10.	15.09.	Meso-
Elterwater	El	NY 03944 33769	26.06.	18.09.	15.09.	Meso-
Ullswater	Ul	NY 18849 38736	25.06.	18.09.	15.09.	Meso-
Rydal Water	Ry	NY 06343 35616	26.06.	18.09.	15.09.	Meso-
Loweswater	Lo	NY 21216 12682	30.06.	20.09.	16.09.	Meso-
Windermere North	Wn	SD 96301 38929	26.06.	11.09.	15.09.	Meso-
Grasmere	Gr	NY 06081 34273	26.06.	18.09.	15.09.	Meso-
Blelham Tarn	Bl	NY 00628 36803	26.06.	11.09.	17.09.	Meso-
Esthwaite Water	Es	SD 96475 36341	24.06.	11.09.	17.09.	Eu-

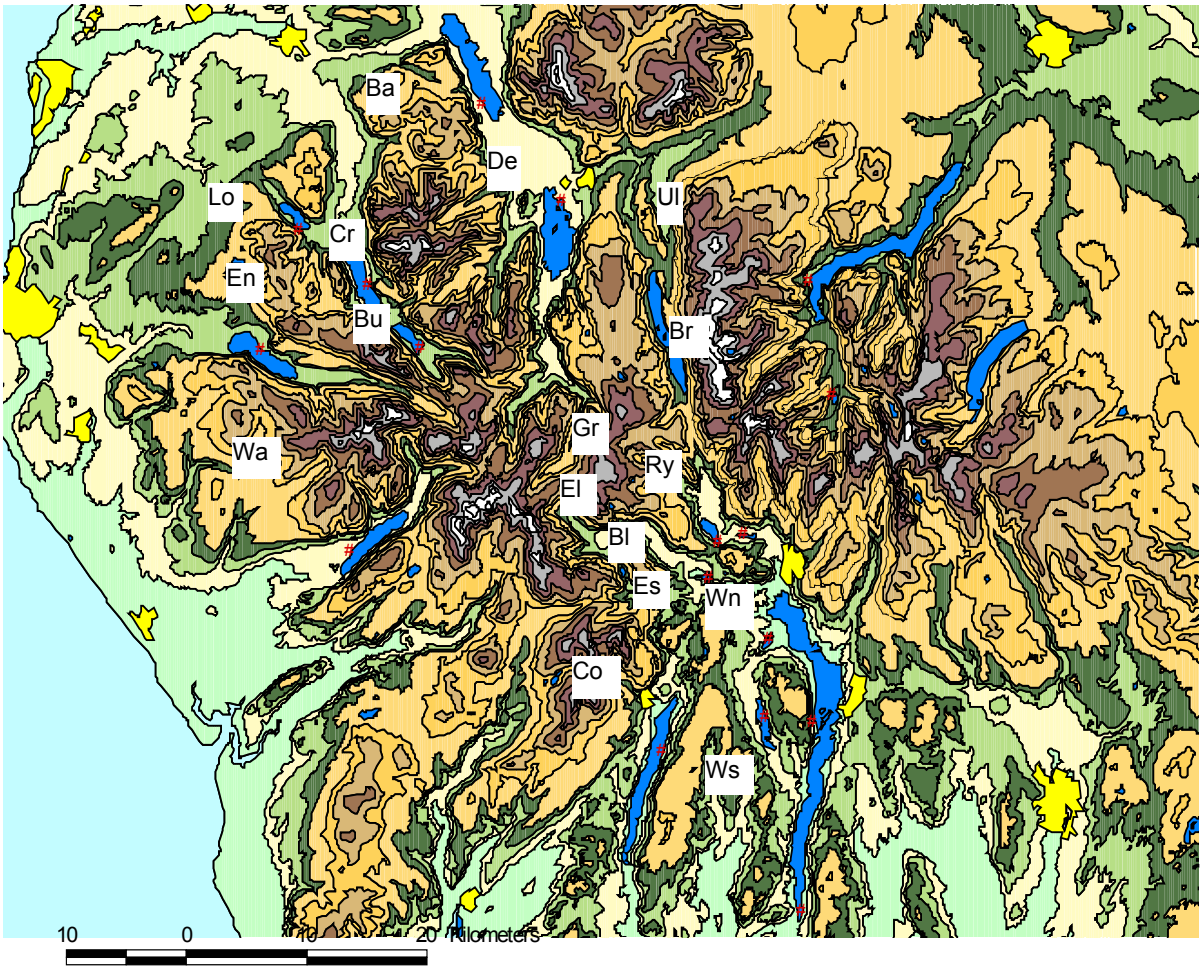

Figure 1: Map of the lakes included in this study and situation of the sampling sites: Bu, Buttermere; Wa, Wastwater; En, Ennerdale; Co, Coniston Water; De, Derwent Water; Cr, Crummock Water; Br, Brotherswater; Ba, Bassenthwaite Lake; Ws, Windermere South; El, Elterwater; Ul, Ullswater; Ry, Rydal Water; Lo, Loweswater; Wn, Windermere North; Gr, Grasmere; Bl, Blelham Tarn; Es, Esthwaite Water.

2.3 Epilithon sampling and preparation

2.3.1 Sampling procedure

From the same site of the lake at a water depth of 40–70 cm, depending on the water level, 10 stones were chosen at random. A syringe–toothbrush sampler (modified from Aloi, 1990; see *Figure 2*) was used to brush the enclosed epilithon from a defined area of the top of the stones by turning the plunger eight times. The algae were washed into a beaker using a wash bottle that contained Milli-RO water and the volume adjusted to 300 ml. After thoroughly mixing, the suspension was divided into three aliquots in 100 ml sampling jars – one for Chl *a* analysis, one for algal enumeration and one to prepare permanent slides for diatom identification (*Figure 3*).

An attempt was made to assess the performance of the syringe–toothbrush sampler by repeating the sampling procedure and investigation of scraped area under an incident light microscope. However, because natural stones are not completely flat, it was not possible to obtain a 100% seal between the stone and syringe and so more algae leaked in after the plunger had been removed for the first time. The performance also depended on the roughness of the stones and on how strongly the biofilm was attached. In general, the periphyton was harder to remove under increased grazing pressure. Where cell numbers are given, they should been taken as estimates only. Cattaneo and Roberge (1991) tested a similar brush sampler and found a consistent overestimation of Chl *a* of lake epilithon removed by brushing compared to the amount found from direct extractions by immersing the substrate in ethanol.

Figure 2: Syringe–toothbrush sampler.

2.3.2 Chlorophyll *a* analysis

The algal suspension was transported back to the laboratory in a cool box, where it was filtered onto a Whatman GF/C filter. This was then placed in a Sterilin tube and frozen at –20 ºC. Before the analysis was carried out, the samples were left to defrost overnight in a fridge at 4 ºC. Then 10 ml industrial methylated spirits (IMS, 96% ethanol and 4% methanol) were added to each tube. After a further 24 hours in the fridge, a dilution of the extract was measured using a fluorometer (10-AU Fluorometer Turner Designs, Sunnyvale, California). Filters to determine Chl *a* in the water were handled in the same way.

The formula used to calculate Chl *a* concentration of the epilithon was:

$$Y = \frac{a \bullet b}{c} \bullet 10$$

Y Chl *a* per unit area ($mg \cdot m^{-2}$)

a Chl *a* concentration of the extract ($\mu g \cdot ml^{-1}$)

b amount of IMS used for extraction (ml)

c area of stones from which algae were removed (cm^2)

To determine the Chl *a* concentration of the phytoplankton, the formula was adapted as follows:

$$Y = \frac{a \bullet b}{c}$$

Y Chl *a* per unit volume ($\mu g \cdot l^{-1}$)

a Chl *a* concentration of the extract ($\mu g \cdot ml^{-1}$)

b amount of IMS used for extraction (ml)

c volume of water filtered (l)

2.3.3 Algae counts

The aliquot for counting all algae was preserved in the field using Lugol's solution. Samples were stored cool and dark until enumeration, normally within three months of sampling. The sample was mixed thoroughly by shaking and a sub-sample settled overnight in an Utermöhl settling chamber. The following day, two transects were counted at a magnification of 400x using an inverted transmitted light microscope (Zeiss Axiovert 35; Lund *et al.*, 1982). Most forms were identified to genus level using mainly Streble and Krauter (1988), Prescott (1978), Canter-Lund and Lund (1995) or Bourrelly (1985). Counting units were cell numbers wherever possible: however, blue–green filamentous forms were enumerated by length using eye-piece graticule units and colonial forms were

counted as colonies. All cells that were whole, healthy and identifiable were enumerated in this way.

Cell numbers per unit area were calculated following this formula:

$$Y = \frac{y \bullet a \bullet b \bullet x}{c \bullet v}$$

Y cells per unit area (cm^2)

y cells counted

x volume of sample settled (ml)

v volume sample was adjusted to (ml)

a area of stones from which algae were removed (cm^2)

b area of settling chamber (cm^2)

c area of settling chamber counted (cm^2)

2.3.4 Diatom counts

The sample aliquots used to identify diatoms were preserved in the field with Lugol's solution, stored cool and dark until required. Permanent slides were normally prepared, following Battarbee (1986), within a month. The volume of the sample was reduced to about half after settling using a vacuum pump. Exactly half of the remaining sample was put into a high 250 ml glass beaker and about 25 ml of H_2O_2 added. After heating for two hours, the samples were left to cool, before washing them at least three times. The final volume was adjusted to, in most cases, 50 ml and 0.5 ml of the diatom suspension was used for slide preparation. Naphrax (refractive index 1.69) was used as the mounting medium.

At least 500 frustules were counted and identified following the *Süßwasserflora von Mitteleuropa* (Krammer and Lange-Bertalot, 1986, 1988, 1991a, 1991b) and Lange-Bertalot (1994). Frustules were only counted when more than half was conserved and so a clear identification was possible. Where cells (e.g., if in girdle view) could not be identified to species level, they were still included at genus level. Unidentifiable specimens and girdle bands were recorded, but were not included in counts. The results of these counts were transformed into percentage abundances and incorporated into the algae counts.

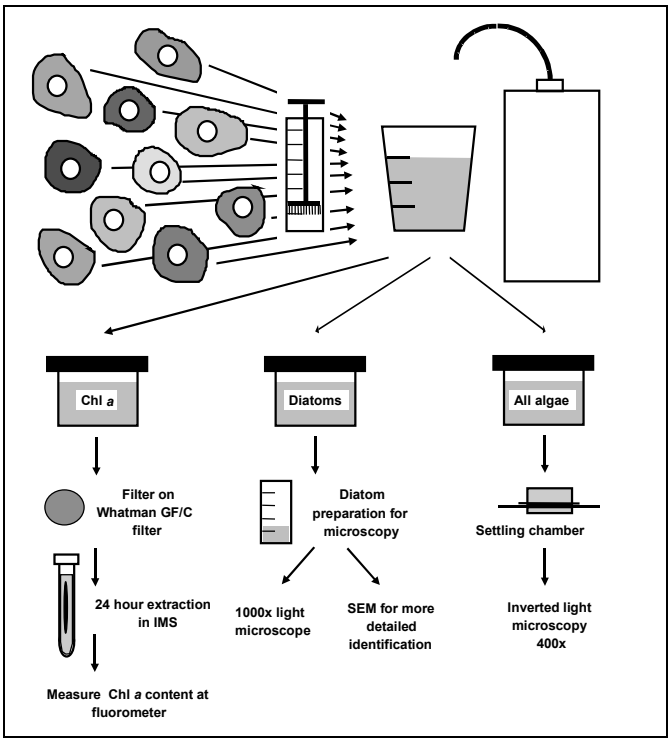

Figure 3: Diagram summarising sampling procedure.

2.4 Data analysis

The relationship between species composition and environmental factors was explored using the data collected during the three surveys. Before calculating optima by weighted-averaging using the computer program C2, a number of screening methods were used. Only species with more than three occurrences were included, the relative abundances of species were used and not transformed. Of the 17 environmental variables measured (*Table 2*), all but pH, temperature and dissolved oxygen concentration were ln(x+1) transformed because of their highly skewed distribution.

2.4.1 Unimodal species – environment response models

In the original analysis (King *et al.*, 2000), although highly correlated, Ca, DIC and conductivity, each considered separately, explained a high percentage of variance and had high eigenvalue ratios. Therefore transfer functions for all three parameters were developed. The only other environmental variable that showed a similar high correlation was TP, which allowed a transfer function for inferring TP from epilithic algal communities to be established as well. Models with and without tolerance downweighting were tested. Tolerance downweighting takes into consideration that species with a smaller variance (equivalent to tolerance) around their ecological optimum occupy a smaller ecological niche and are therefore theoretically better indicators. However, rare species are then often assigned a very small tolerance and their importance is overestimated (Köster *et al*, 2004).

Table 2: Number, mean, standard deviation, minimum and maximum of all environmental variables.

No	Environmental variable	Units	Mean	Standard deviation	Min	Max
1	Dissolved oxygen concentration	mg/l	10.3	1.1	7.2	15.0
2	Temperature	ºC	13.9	1.6	11.2	18.1
3	pH		6.85	0.65	5.90	8.80
4	Conductivity	µS/cm	66	23	38	124
5	Dissolved inorganic carbon	mg/l	2.7	1.7	0.5	8.6
6	Total phosphorus	µg P/l	14.6	9.9	0.8	49.2
7	Soluble reactive phosphorus	µg P/l	11	8	*	57
8	Total oxidised nitrogen	µg N/l	237	122	46	498
9	Ammonium	µg N/l	20	27	*	139
10	Silicate	µg Si/l	344	252	32	1139
11	Chlorophyll *a* epilithon	mg/m^2	10.1	9.9	1.3	55.6
12	Chlorophyll *a* water	µg/l	8.1	10.2	0.6	47.2
13	Colour	Pt. mg/l	18	11	4	59
14	Sodium	(mg/l)	4.3	1.1	2.7	7.4
15	Potassium	(mg/l)	0.4	0.2	0.2	1.0
16	Magnesium	(mg/l)	1.0	0.7	0.5	4.4
17	Calcium	(mg/l)	5.6	2.7	2.2	13.0

*Below detection limit.

Four different scenarios were investigated:

- **Scenario 1:** diatoms identified to species and other algae to genus (this is the approach adopted in King *et al.,* 2000);

- **Scenario 2:** diatoms identified to species and other algae identified to the lowest level possible (some taxa identified to species, filamentous algae placed in width categories);

- **Scenario 3:** diatoms only (identified to species);

- **Scenario 4:** other algae only (identified to the lowest level possible).

3. Results and discussion

Results are summarised in *Table 3*. These show clearly for all variables and for all models tested that transfer functions established using the relative abundances of non-diatom algae alone, at the taxonomic level considered here, perform less well than models that include diatoms. However, the differences in the performance of the three models that include diatoms are only slight. For weighted averaging without tolerance downweighting, there is a trend of increased model performance from diatoms only to diatoms and other algae at genus level to diatoms and other algae at higher resolution. This might be expected as the number of categories included in the analysis increases from 103 for diatoms only to 138 and 158 when considering diatoms and other algae together. However, looking at the results of weighted averaging with tolerance downweighting, this trend disappears (*Figure 4*). For DIC the model performance is worse when all algae are included than when diatoms are considered alone. The opposite is true for TP, and the differences in model performances for calcium and conductivity are very small.

Obviously, the comparison would be fairer if all taxa could have been identified to the same level. However, several problems prevent other algae being identified to species level at the moment:

1. The cleaning of material and preparation of permanent slides make the analysis of diatoms straightforward. Samples can easily be preserved, exchanged and re-analysed (e.g. for solving taxonomic problems later on).

2. While numerous publications are available for the identification of benthic diatoms, the taxonomic literature for the other algal groups is not so readily available.

3. Besides these more logistical problems, many filamentous forms can only be identified to species level if the reproductive structures are examined. However, these are rarely available in natural samples and therefore identification requires culture of these species. This is laborious and impossible if samples were preserved in the field or if only small amounts of the algae in question are available.

4. Databases with detailed description and digital photographs of diatoms are becoming established worldwide (e.g., European Diatom Database Initiative, National Diatom Database Canada). This development is at a less advanced stage for other benthic algae. Detailed taxonomic studies of British benthic algae other than diatoms and the establishment of such a database would be necessary before these species could be included in routine analysis of a high number of samples. In a comparative study in Germany, analysis of a diatom sample to species level required between two and four hours effort, whereas the study of other benthic algae at the same level of detail required, on average, about 18 hours of effort (Schaumburg et al.,2004).

Table 3: Root mean square error (RMSE) and r^2 between measured and inferred values of environmental parameters of interference models established under four different scenarios: Scenario 1, diatoms at species level and other algae at genus level; Scenario 2, diatoms at species level and other algae at genus level divided into size classes; Scenario 3, diatoms only; Scenario 4, other algae only.

Total Phosphorus		**Scenario 1**		**Scenario 2**		**Scenario 3**		**Scenario 4**	
		***n* = 138**		***n* = 158**		***n* = 103**		***n* = 55**	
	code	**RMSE**	**r^2**	**RMSE**	**r^2**	**RMSE**	**r^2**	**RMSE**	**r^2**
1	WA_Inv	0.4336	0.60	0.4096	0.64	0.4362	0.59	0.5034	0.46
2	WA_Cla	0.5619	0.60	0.5123	0.64	0.5675	0.59	0.7463	0.46
3	WATOL_Inv	0.4175	**0.63**	0.3852	**0.68**	0.4142	**0.63**	0.5262	**0.40**
4	WATOL_Cla	0.5281	**0.63**	0.4669	**0.68**	0.5215	**0.63**	0.8275	**0.40**
DIC		**Scenario 1**		**Scenario 2**		**Scenario 3**		**Scenario 4**	
		***n* = 138**		***n* = 158**		***n* = 103**		***n* = 55**	
	code	**RMSE**	**r^2**	**RMSE**	**r^2**	**RMSE**	**r^2**	**RMSE**	**r^2**
1	WA_Inv	0.2428	0.70	0.2343	0.72	0.2528	0.68	0.3099	0.51
2	WA_Cla	0.2899	0.70	0.2757	0.72	0.3074	0.68	0.4324	0.51
3	WATOL_Inv	0.2322	**0.73**	0.2481	**0.69**	0.2326	**0.73**	0.3296	**0.45**
4	WATOL_Cla	0.2724	**0.73**	0.2990	**0.69**	0.2730	**0.73**	0.4914	**0.45**
Conductivity		**Scenario 1**		**Scenario 2**		**Scenario 3**		**Scenario 4**	
		***n* = 138**		***n* = 158**		***n* = 103**		***n* = 55**	
	code	**RMSE**	**r^2**	**RMSE**	**r^2**	**RMSE**	**r^2**	**RMSE**	**r^2**
1	WA_Inv	0.1875	0.64	0.1747	0.69	0.1959	0.61	0.2204	0.50
2	WA_Cla	0.2350	0.64	0.2108	0.69	0.2518	0.61	0.3112	0.50
3	WATOL_Inv	0.1851	**0.65**	0.1815	**0.66**	0.1840	**0.65**	0.2399	**0.41**
4	WATOL_Cla	0.2299	**0.65**	0.2231	**0.66**	0.2278	**0.65**	0.3753	**0.41**
Calcium		**Scenario 1**		**Scenario 2**		**Scenario 3**		**Scenario 4**	
		***n* = 138**		***n* = 158**		***n* = 103**		***n* = 55**	
	code	**RMSE**	**r^2**	**RMSE**	**r^2**	**RMSE**	**r^2**	**RMSE**	**r^2**
1	WA_Inv	0.2077	0.72	0.2083	0.71	0.2252	0.67	0.2646	0.54
2	WA_Cla	0.2455	0.72	0.2465	0.71	0.2761	0.67	0.3608	0.54
3	WATOL_Inv	0.2042	**0.72**	0.2184	**0.69**	0.2120	**0.70**	0.2907	**0.44**
4	WATOL_Cla	0.2398	**0.72**	0.2639	**0.69**	0.2528	**0.70**	0.4372	**0.44**

Although this study does demonstrate that diatoms can act as proxies for the entire epilithic phytobenthos, the range of lakes included here is limited and it is possible that algae other than diatoms might become more important in lakes with higher nutrient levels. The lakes included here were all circumneutral in character, and it would also be interesting to repeat the study over a wider pH gradient. Nonetheless, results from this study complement those of Kelly (2005) and other studies within Diatom Assessment of River and Ecological Status (DARES) to provide some justification for the use of diatoms as proxies for phytobenthos.

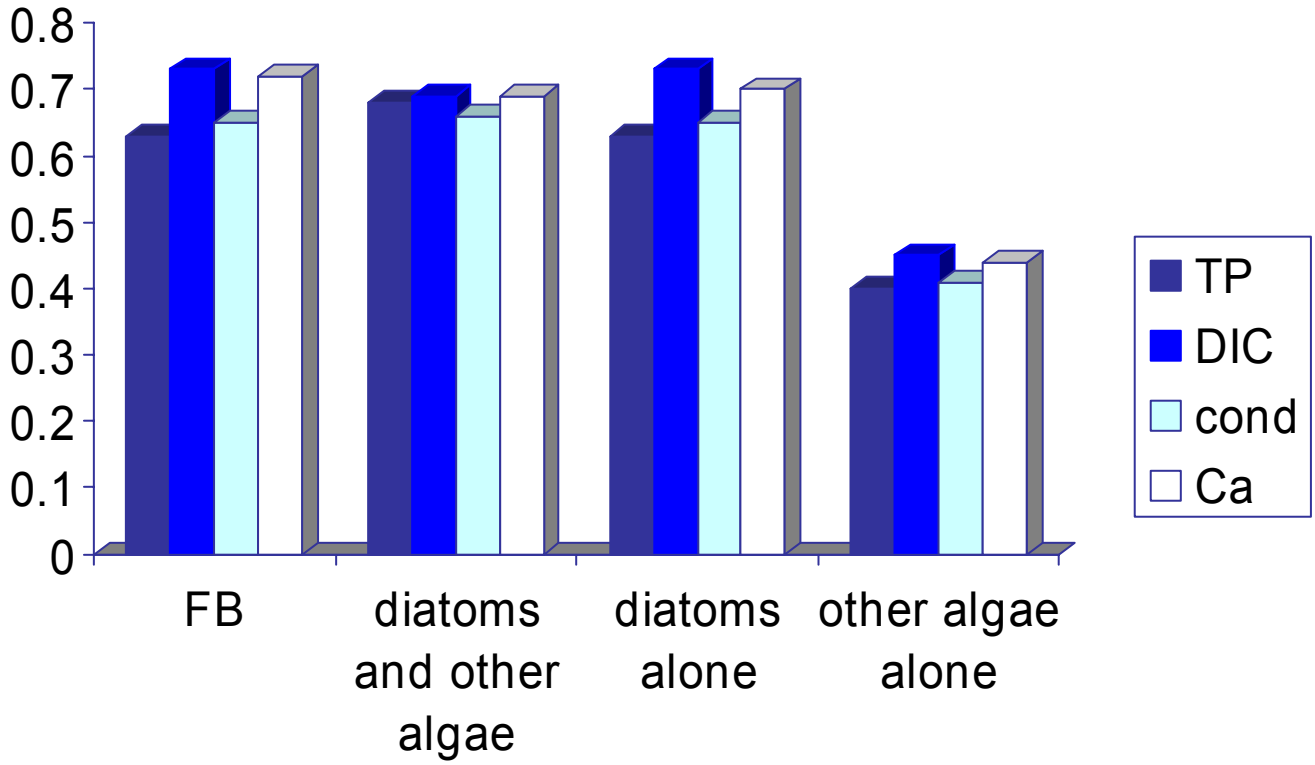

Figure 4: Correlation between measured values of total phosphorus (TP), dissolved inorganic carbon (DIC), conductivity (cond.) and calcium ion concentration (Ca) between values estimated by weighted averaging with tolerance downweighting and measured values under four different scenarios (see text for details).

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