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Dinophyta Characterise Nitrogen Scarcity More Strongly than Cyanobacteria in Moderately Deep Lakes

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Abstract. A survey of the summer phytoplankton communities of thirty-six moderately-deep north temperate lowland lakes showed that the proportions of Dinophyta and non-heterocyst-bearing cyanobacteria taxa, measured as biovolume, were inversely related to the total nitrogen:total phosphorus (TN:TP) ratio and that these groups were predominant in lakes where available nitrogen fell to undetectable concentrations. The proportion of heterocyst-bearing cyanobacteria was positively correlated to the TN:TP ratio and nitrate. Dinophyta and/or non-heterocystous cyanobacteria were prevalent in lakes with the highest epilimnion nutrient concentrations, whilst heterocystous cyanobacteria predominated in lakes with moderate nutrient concentrations. It is argued that the ability of Dinophyta to migrate vertically and to supplement their nutrient requirements though heterotrophy may enable them to be at least as successful as cyanobacteria in high nutrient lakes and in overcoming nitrogen-scarcity. Our findings provide evidence that Dinophyta can be used as indicators of water quality.

Key words: Lakes, Shropshire and Cheshire Meres, phytoplankton, seasonality, nutrients, vertical movement.

INTRODUCTION

Recent studies into the frequency of occurrence of nitrogen and phosphorous scarcity in lakes suggest that nitrogen limitation might be as common as phosphorus limitation (Elser 2007, Lewis and Wurtsbaugh 2008). Eutrophication is often attributed to increased phosphorus loading, leading to a reduction in the ratio of nitrogen:phosphorus (N:P), further promoting nitrogen (N) limitation, a decrease in phytoplankton diversity, and a phytoplankton assemblage predominantly of cyanobacteria (Tilman *et al.* 1982, Harper 1992, Levine and Schindler 1999, Dokulil and Treubner 2000). Redfield (1958) noted that algae required N:P in a ratio around 7:1 (by weight). At ratios lower than this, algae that are capable of fixing their own nitrogen, (many cyanobacteria and others having cyanobacterial symbionts) are advantaged. Large populations of cyanobacteria are sometimes a nuisance and can result in toxic blooms that pose a health risk to recreational users and animals drinking the water (Howard *et al*. 1996).

The proposed links among eutrophication, low N:P ratios and cyanobacterial dominance were emphasised by Schindler (1974) who showed that addition of phosphorus (P) to one lobe of a lake, separated by a nylon curtain from the rest of the lake, resulted in increased

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chlorophyll concentrations and a phytoplankton population dominated by N-fixing cyanobacteria. Whilst many field and laboratory studies have shown cyanobacterial domination to be greater in waters with low N:P ratios, there is growing evidence that such dominance is more highly correlated with other indicators of trophic status (Prepas and Trimbee 1988, Reynolds and Petersen 2000). A survey of 99 lakes (Downing *et al.* 2001) showed cyanobacterial population to be correlated with total phosphorus (TP), total nitrogen (TN) and phytoplankton production, rather than to ratios of N:P. Indeed, the proportion of nuisance cyanobacteria, in conjunction with total chlorophyll, has been proposed as a means of monitoring nutrient enrichment in lakes under the Water Framework Directive (WFD UKTAG 2008).

The lack of a clear relationship between cyanobacteria and measures of N-limitation in some studies may be a product of the diverse requirements of this group. Blomquist *et al.* (1994) argue that it is more appropriate to analyse the links among cyanobacteria, trophic status and low N:P ratio by considering N-fixing and non N-fixing species separately. Non N-fixing cyanobacteria will be favoured by the presence of ammonium, while low concentrations of nitrogen in general will promote those cyanobacteria capable of fixing N (Blomquist *et al*. 1994). The ability of some cyanobacteria, such as the Nostocales, to fix nitrogen can be recognised by their ability to produce heterocysts, (recently called heterocytes, which is etymologically correct, but the original term remains most widely used and understood). N-fixing capabilities are not confined to these taxa however, and some species within the genera of *Oscillatoria*, *Lyngbya* and *Phormidium*, all of which are non-heterocystous cyanobacteria, are also capable of N-fixation (Paerl 1988), though generally under microaerophilic conditions that are more associated with sediments and metalimnia than surface waters. In open waters, the frequency of heterocysts is generally taken to be a reasonable guide to the potentiality for nitrogen-fixation. Investigations into how to recognise which nutrient limits phytoplankton biomass are becoming more important now that there is increasing evidence that phosphorus limitation may not be so universal as once thought and that nitrogen limitation or co-limitation by both N and P may be at least as common (Maberly *et al*. 2002, James *et al.* 2003, Elser *et al*. 2007, Lewis and Wurtsbaugh 2008).

Recognising N-limitation from in-lake nutrient concentrations is challenging, though Hameed *et al*. (1999) showed that interpretation of in-lake concentrations were just as reliable as the results of a series of physiological tests of nutrient limitation. The “Redfield ratio” (Redfield 1958) suggests that N deficiency is likely to occur at N:P ratios < 7:1 (by weight). Absolute concentrations are also important, as if the concentrations of both bio-available nutrients are high, the ratio becomes irrelevant (Reynolds 1999) and ratios are incalculable if concentrations are below laboratory detection levels. Furthermore, the Redfield ratio reflects the average nutrient requirement of algae while different species may have different nutritional requirements (Rhee 1978, Klausmeier *et al.* 2004). In addition nutrient limitation is likely to be stoichiometric (Kilham 1990, Kim *et al.* 2007), and while it has been questioned if two or more nutrients can be simultaneously limiting to the same species (Droop 1973) it is certain that different species of algal will be limited by different nutrients, such as N, P, silica or carbon, at the same time. It is insufficient to rely on annual mean nutrient concentrations to determine limitation, but sustained periods in which the bioavailable form of a nutrient is undetectable indicates which nutrient is *potentially* limiting (Maberly *et al*. 2004), though phytoplankton may be able to take-up nutrients at concentrations far lower than laboratory detection limits and be able to use luxury uptake to overcome periods when one or more nutrient may be limiting. This raises the issue of whether algal groups can be used to indicate nutrient limitation, and whether cyanobacteria are effective indicators of nitrogen scarcity, despite strong conventional beliefs in their efficacy.

Cyanobacteria are associated with moderate but not the highest nutrient concentrations, where green algae, particularly Chlorococcales predominate, at least in shallow unstratified lakes (Jensen *et al*. 1994). The position in deeper lakes is less clear, but the predominance of cyanobacteria in moderately deep, nutrient-rich lakes seems to depend on their ability either to fix N or to migrate between deeper, more nutrient-rich waters and the illuminated surface waters using regulation of their content of gas vesicles (Reynolds and Walsby 1975, Ganf and Oliver 1982). Such migrations are possible also for flagellate algae. A clear indicator of nitrogen scarcity would be useful for monitoring purposes owing to the high cost of direct investigations and the number of lakes now requiring monitoring under the European Water Framework Directive. In this study we investigated if other algae capable of vertical migration, such as the Dinophyta, are as effective as heterocystous cyanobacteria, at indicating nitrogen scarcity in moderately deep lakes. We used extensive correlation among lakes in the UK that are generally severely eutrophicated and lakes in Poland where agriculture has been less intensive to investigate this hypothesis.

MATERIALS AND METHODS

Study sites

Thirty-six moderately deep ($Z_{max} > 3$ m) lowland freshwater lakes were studied. Sixteen were among the North-West Midlands meres area of England, nine in the Lublin Uplands of Eastern Poland and eleven in the Mazurian Lake District of North-East Poland (Table 1). Most of the lakes are glacial in origin. The UK Meres are a cluster of over sixty lakes situated in the hummocky topography of fluvio-glacial sands, gravels and moraine which overlay Triassic sandstone (Reynolds 1979). They are among the most important natural freshwater habitats in Britain for both ecological and scientific reasons (Luther and Rzoska 1971, Twigger and Haslam 1991). Hanmer Mere, Cole Mere, Crose Mere and Rostherne Mere are Sites of Special Scientific Interest (SSSI), and the latter is also a Ramsar site and a National Nature Reserve (NNR). The Meres have few surface drainage channels, and lie at the approximate height of the water table. As a result the meres generally have long residence times (mean of 1.3 years^{-1}, n = 18) and react slowly to changes in precipitation and drainage. The lakes lie in a region of mild, sub-oceanic conditions, and the local land use is dominated by dairy farming and arable agriculture. Agricultural intensification and land drainage has led to soil erosion and the leaching of nutrients to the meres, causing eutrophication (Reynolds and Sinker 1976, Reynolds 1979, Moss *et al*. 1994, Moss *et al*. 2004).

The lakes sampled in the Łęczyńsko-Włodawskie region lie to the north and east of Lublin. Drift sediments are fluvio-glacial and lacustrine in origin (Balaga 1982) and land use is a mosaic of deciduous and pine forests, arable land, pasture, and numerous villages and hamlets. The wetland area has been reduced as a result of land drainage, and agriculture, which is less intensive than in the United Kingdom. This area of Eastern Poland is landlocked and dominated by a continental climate with cold, dry winters, and warm summers when the majority of the precipitation falls.

The Mazurian Lakeland is more undulating than that of the Łęczyńsko-Włodawskie region, and drift deposits also derive from fluvio-glacial activity (Bajkiewicz-Grabowska 1983). Approximately 10% of the area is made up of lakes (Mikulski 1966) and is dominated by two large lake complexes centred around Jezioro (‘lake’) Dargin and Jezioro Śniardwy. Natural and man-made navigable sailing routes interconnect many of the lakes, and they receive both groundwater and surface inflows (Bajkiewicz-Grabowska 1983). Municipal sewage is discharged into some of the larger lakes, while the intensification of agriculture is having a marked impact on the water quality of many of the sites (Zdanowski *et al*. 1984). The Mazurian Lakeland has a more maritime climate than that of the Lublin region but again more than half of the precipitation falls in the summer (Bajkiewicz-Grabowska 1983).

Field sampling

The north-west midland meres were visited regularly seven times, throughout the growing season (May until October 2000) and integrated water samples were taken from the top one metre at the centre of each lake, with the exception of Bomere where samples were taken from a jetty. The Polish lakes were visited once, between July and August 2001, and three replicate water samples were taken from wading depth from the shore, or from a jetty where possible, using a 2 m long extended sampler.

Laboratory analysis

One litre samples were filtered through Whatman GF/C glass-fibre filters on the day of collection and the filter frozen for chlorophyll analyses. Chlorophyll-*a* concentration was determined by placing the filters in 90% acetone for extraction overnight at 4°C, and the abstracted chlorophyll-*a* measured at 665 nm on a Jenway spectrophotometer (Model 6405). Filtered water samples were analysed on the day of sampling for soluble reactive phosphorus (SRP), nitrate (NO_3-N) and ammonium (NH_4-N) concentrations using standard methods outlined in Mackereth *et al*. (1978). In Poland, nitrate-nitrogen and ammonium were analysed using colorimetric CHEMets kits within 10 hours of sample collection (CHEMetrics from GALGO(UK) Ltd). SRP was measurable to 5 μg l^{-1}, and nitrate and ammonium to a minimum detection limit of 10 μg l^{-1} N^{-1} for both the laboratory and kit analyses. At high ammonia concentrations (> 50 μg l^{-1} NH_4-N) the resolution of the test kits was reduced to 50 μg l^{-1} as the kits colour comparison, provided for these higher ammonia concentrations, were only available at these lower resolutions. The accuracy of the CHEMets kits in comparison with laboratory analyses were tested on natural water samples before the Polish fieldwork. Unfiltered water samples were preserved with 0.147 mM (final concentration) mercuric chloride (Klingaman and Nelson 1976, German Chemists Association 1981), and refrigerated in the dark for the analysis of total nitrogen (TN) and total phosphorus (TP) concentrations, following microwave digestion (Johnes and Heathwaite 1992). Point measurements of pH, alkalinity and conductivity were also taken on the day of sampling though, due to equipment constraints, pH values of the Polish water samples were not measured.

Sites with mean TN:TP ratios lower than 7 or, where available nitrogen (nitrate and ammonium) was undetectable on at least one sampling occasion, during the growth-season, were considered potentially N-limited. Those samples in which mean TN:TP ratios were more than 15 or where SRP concentrations were below detection limits on at least one sampling occasion were considered potentially P-limited (Table 1).

Phytoplankton

A 10 ml subsample of unfiltered water was preserved with 5 drops of Lugol’s iodine solution. Phytoplankton cells were settled in sedimentation chambers before being identified (species shown in Table 4) and counted using an inverted microscope at × 400 magnification. A minimum of 400 organisms was counted per sample and the length, width and diameter of randomly selected cells of each species were measured in order to calculate biovolumes. Biovolumes were calculated using standard formulae for the volumes of known geometric shapes (Jones 1979). The biovolumes of those species occurring rarely in the samples were obtained from the literature (Prescott 1962). Cyanobacteria were divided into those that have heterocysts and potentially fix nitrogen in the open waters, and those without heterocysts that are unlikely to do so.

Table 1. Summary of morphological, geographical and mean growth season nutrient and physico-chemical variables of the thirty-six study lakes. Growth-season physico-chemical and nutrient variables for the UK are a mean from 7 sampling occasions, and for Poland are a mean from three samples taken on one sampling occasion. Last column – available N (nitrate and ammonia) and P (SRP) below detectable concentrations (BDC) on at least one sampling occasion. ? – available N and P detectable on all sampling occasions and therefore unknown if either fell below detectable concentrations during the growth season, n/a = no data.

Lake name	Z max	Z mean	Area	Latitude	Longitude	Altitude	TN:TP	TP	TN	SRP	NO_3-N	NH_4-N	pH	Alkalinity	N or P
	(m)	(m)	(ha)	N		(m)	(weight)	($\mu g\ l^{-1}$)	($mg\ l^{-1}$)	($\mu g\ l^{-1}$)	($\mu g\ l^{-1}$)	($\mu g\ l^{-1}$)		(mequiv l^{-1})	BDC
Alkmund Park Pool	5.1	2.0	4.4	52° 44′ 5″	2° 53′ 9″ W	70	6	390	2.3	15	260	77	7.9	6.97	N
Betton Pool	10.9	3.6	6.4	52° 40′ 0″	2° 46′ 8″ W	70	17	97	1.5	33	60	66	7.5	2.46	N
Blake Mere	14.1[a]	3.9[a]	8.4	52° 54′ 1″	2° 52′ 1″ W	91	22	62	1.4	4	20	27	8.0	5.96	N+P
Bomere	15.2	5.1	10	52° 40′ 2″	2° 45′ 7″ W	55	23	49	1.0	7	20	24	7.2	3.99	N
Budworth Mere	5.9[a]	2.2[a]	39	53° 17′ 3″	2° 31′ 0″ W	16	6	361	1.7	278	370	91	8.2	9.25	?
Cole Mere	11.5	3.3	28	52° 53′ 7″	2° 50′ 7″ W	91	6	262	1.4	152	30	37	8.6	6.50	N
Crose Mere	9.3	4.8	15	52° 52′ 3″	2° 51′ 0″ W	87	29	55	1.3	19	180	129	8.3	6.17	N+P
Ellesmere	18.8	8.0	46	52° 54′ 7″	2° 53′ 2″ W	91	1	894	1.2	761	20	32	8.4	6.04	N
Hanmer Mere	6.5[a]	2.1[a]	18	52° 56′ 8″	2° 48′ 9″ W	76	1	1590	1.3	1240	20	36	8.3	6.10	N
Marbury Big Mere	8.0	3.0	11	53° 0′ 3″	2° 39′ 2″ W	76	13	251	2.6	105	360	176	8.7	8.37	N+P
Newton Mere	16.4[a]	4.1[a]	8.3	52° 54′ 2″	2° 51′ 4″ W	90	7	196	1.3	97	50	6	8.1	3.03	N
Pick Mere	9.2[a]	3.2[a]	18	53° 17′ 5″	2° 28′ 5″ W	16	12	96	1.0	63	150	46	8.3	9.88	?
Redes Mere	5.2[a]	1.5[a]	17	53° 14′ 5″	2° 13′ 6″ W	80	27	146	3.0	70	1140	65	8.2	6.24	?
White Mere	14.5	4.4	26	52° 53′ 6″	2° 52′ 2″ W	91	2	694	1.4	587	20	30	8.7	5.26	N
Mere Mere	8.1	2.8	16	53° 19′ 8″	2° 24′ 1″ W	50	25	87	1.8	9	270	106	7.5	1.73	N
Rostherne Mere	27.5	13.6	49	53° 21′ 3″	2° 26′ 8″ W	31	12	127	1.5	65	470	96	8.6	2.17	?
Piaseczno	38.8[b]	12.6[b]	85	51°23′ 1″	23° 01′ 8″ E	170	39	14	0.5	35	030	50	n/a	0.32	?
Rogóźno	25.4[b]	7.4[b]	57	51° 22′ 7″	22° 58′ 4″ E	168	40	22	0.9	36	70	50	n/a	1.82	?
Zagłębocze Włodawskie	25[b]	7.3[b]	59	51° 25′ 9″	23° 01′ 3″ E	167	63	17	0.9	35	30	50	n/a	1.52	?
Białe Włodawskie	33.6[b]	14.1[b]	110	51° 29′ 9″	23° 32′ 0″ E	159	24	31	0.7	40	< 10	< 10	n/a	1.87	N
Czarne Włodawskie	11.4[b]	3[b]	24	51° 29′ 9″	23° 30′ 9″ E	160	15	102	1.4	43	< 10	< 10	n/a	2.17	N
Bialskie Sosnowickie	18.2[b]	4[b]	32	51° 32′ 3″	23° 01′ 1″ E	160	36	30	1.1	62	< 10	42	n/a	2.23	N
Czarne Sosnowickie	15.6[b]	5.1[b]	39	51° 31′ 1″	23° 01′ 8″ E	161	38	42	1.6	54	< 10	100	n/a	3.17	?
Maśluchowskie	9.4[b]	4.6[b]	27	51° 28′ 1″	22° 56′ 8″ E	160	31	43	1.4	19	< 10	800	n/a	0.70	?
Krasne	33[b]	10.8[b]	n/a	51° 25′ 7″	22° 57′ 7″ E	164	29	45	1.3	18	< 10	467	n/a	1.60	?
"Gąsior"[c]	n/a	n/a	n/a	53° 43′ 7″	21° 33′ 0″ E	n/a	24	28	0.6	26	< 10	100	n/a	0.08	?
Majcz Wielki	16.4[b]	6[b]	160	53° 46′ 9″	21° 27′ 4″ E	125	31	14	0.4	2	< 10	100	n/a	2.45	?
Gołdopiwo	26.9[b]	11.8[b]	860	54° 07′ 1″	21° 56′ 9″ E	117	25	33	0.8	1	< 10	100	n/a	2.88	N
Stręgiel	12.5[b]	5.6[b]	400	54° 11′ 8″	21° 50′ 4″ E	116	17	92	1.5	2	< 10	5	n/a	3.32	?
Mamry (Północne)	43.8[b]	11.9[b]	2,500	54°10′ 9″	21° 41′ 8″ E	116	32	22	0.7	1	< 10	67	n/a	2.68	?
Rydzówka	16.7[b]	6.2[b]	490	54° 14′ 1″	21° 34′ 9″ E	83	19	47	0.9	6	< 10	33	n/a	3.02	?
Silec	14[b]	5.9[b]	120	54° 10′ 7″	21° 32′ 0″ E	98	8	184	1.5	38	< 10	25	n/a	2.60	?
Śniardwy	23.84[b]	5.8[b]	11,000	53° 44′ 5″	21° 45′ 0″ E	116	14	76	1.0	5	< 10	< 10	n/a	2.88	N
Białoławki	36.1[b]	9.8[b]	210	53° 44′ 1″	21° 50′ 0″ E	116	36	24	0.9	3	< 10	100	n/a	2.75	?
Orzysz	36[b]	7[b]	1,100	53° 49′ 3″	22° 01′ 7″ E	120	40	21	0.8	3	< 10	100	n/a	3.27	?
Inulec	10.1[b]	4.6[b]	180	53° 48′ 3″	21° 28′ 7″ E	122	28	35	1.0	9	< 10	100	n/a	3.33	?

[a] Lake depths calculated from measurements made from between 4 and 7 transects across each lake.

[b] Depths taken from Jańczak *et al.* (1999).

[c] A small lake 500m south of Gąsior.

Statistical analysis

Ordinations were carried out using CANOCO for Windows version 4 (ter Braak and Šmilauer 1998). Linear ordination techniques were chosen after examination of the gradient lengths of the species response curves showed species responses to environmental gradients to be linear. The gradient lengths were viewed after running detrended correspondence analysis (DCA) and selecting detrending-by-segments. Jongman *et al*. (1995) note that, where gradient lengths are less than 2 standard deviation units, the response curves will generally be monotonic, and therefore linear ordination techniques should be used. Where gradient lengths are greater than 4 standard deviation units the data tend towards unimodal (Jongman *et al*. 1995). We decided to employ the linear techniques of principal components analysis (PCA) and redundancy analysis (RDA) since the gradient lengths of the species data were found to be lower than 3 standard deviation units.

All phytoplankton data were expressed as percentages of total biovolume, at both the level of division and species. This was used in preference to absolute biovolumes to ensure that statistical relationships between phytoplankton and nutrients focussed on phytoplankton composition rather than total biomass. All ordinations were 'species-centred' so that phytoplankton with the greatest variance and, therefore normally those with greatest abundance, dominated the ordinations (Jongman *et al.* 1995). Environmental variables were standardised 'to zero mean and unit variance' (Jongman *et al.* 1995). For ordinations, sites were plotted according to the mean phytoplankton biomass calculated from the seven samples taken during the growth season and from the three samples taken on the one sampling occasion from the Polish sites, and expressed as a percentage of the total growth season biovolume.

Phytoplankton percentage data were arcsin transformed and the environmental variables were transformed using a natural log where analysis showed the data to be skewed. This and other statistical tests, including correlation coefficients, means, standard errors and comparisons of means using Kruskall-Wallis test and ANOVA, were carried out in SPSS version 15 (IBM, 2006).

RESULTS

Phytoplankton and productivity

Mean growth season phosphorus concentrations varied greatly among sites, from less than $14\ \mu g\ l^{-1}$ total P (TP) to nearly $1{,}600\ \mu g\ l^{-1}$, while total N (TN) varied from just over $1\ mg\ l^{-1}$ to over $3\ mg\ l^{-1}$ (Table 1). Twelve of the lakes can be conventionally considered to be hyper-eutrophic ($P > 100\ \mu g\ l^{-1}$), seventeen eutrophic ($25 < P < 100\ \mu g\ l^{-1}$) and seven mesotrophic ($8 < P < 25\ \mu g\ l^{-1}$) from their TP concentrations (Table 1) (Vollenweider and Kerekes 1981). The larger, deeper, less nutrient-rich lakes tended to be the Polish sites, while the smaller lakes with higher total phosphorus concentrations occurred in the UK (Table 1). Cyanobacteria, Dinophyta and Chlorophyta made up the bulk of the phytoplankton biovolume and the greatest proportions (medians 24%, 13% and 15% respectively) in the thirty-six lakes studied (Table 2).

The proportion of Dinophyta biovolume was also significantly positively correlated with TP ($p < 0.01$) and TN ($p < 0.1$) concentrations (Table 3). Non-heterocystous cyanobacteria were positively correlated to TP ($p < 0.05$), SRP ($p < 0.1$) and nitrate concentrations ($p < 0.05$) while heterocystous cyanobacteria were negatively correlated with TP (both $p < 0.05$) and nitrate ($p < 0.01$) concentrations (Table 3). There was no significant correlation ($p < 0.05$) between cyanobacteria, either in total, or split into heterocystous and non-heterocystous forms, and ammonia (Table 3). Other than the proportion of Chlorophyta, diatoms and Cryptophyta, which were inversely correlated with total or available N and P (all $p < 0.05$ and higher), no other phytoplankton group was significantly ($p < 0.05$) correlated with growth-season nutrient concentrations (Table 3).

Flagellated algae such as *Ceratium hirundinella* (O.F. Müller) Bergh. (Dinophyta), *Cryptomonas* spp. (Cryptophya) and *Mallomonas* spp. (Chrysophyta), were associated with higher than average (centre of ordination) concentrations of TN, TP, SRP and nitrate-N (Fig. 1).

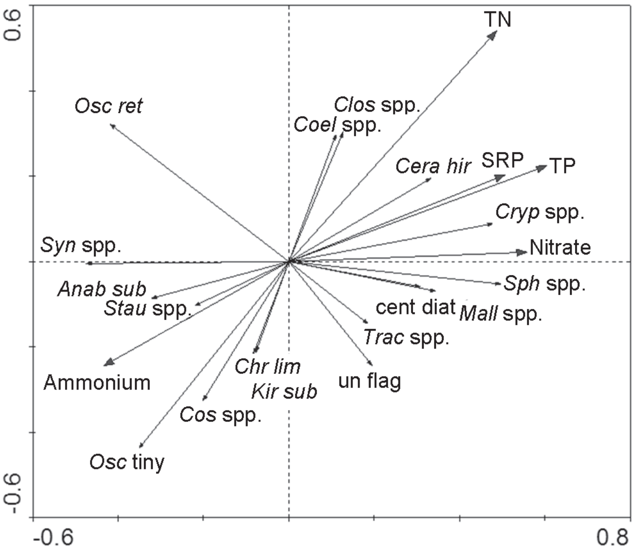

Fig. 1. Redundancy Analysis (RDA) of the mean proportion of phytoplankton species composition expressed as proportion of biovolume, $\lambda 1 = 0.094$, $\lambda 2 = 0.051$. Key to species in Table 4, λ – eigenvalue.

Table 2. The mean growth season proportions of phytoplankton in each Division and chlorophyll-*a* concentrations in the thirty-six study lakes. Data is expressed as absolute (abs) total biovolume (× 10^{-4} μm^{-3} ml^{-1}) and percentage (%) of total phytoplankton biovolume.

	Cryptophyta		Chlorophyta		Total Cyanobacteria		Heterocystous Cyanobacteria		Diatoms		Euglenophyta		Chrysophyta		Dinophyta		Chlorophyll-*a*
	abs	%	abs	%	abs	%	abs	%	abs	%	abs	%	abs	%	abs	%	(μg l^{-1})
Alkmund Park Pool	140	5	485	18	1,009	37	4	40	145	5	24	1	30	1	896	33	72
Betton Pool	36	6	449	74	12	2	1	0	15	2	0	0	6	1	86	14	27
Blake Mere	30	3	620	65	233	24	16	37	68	7	0	0	2	0	5	0	38
Bomere	34	3	160	16	654	64	1	7	9	1	0	0	32	3	135	13	11
Budworth Mere	95	7	110	8	163	13	13	21	684	53	72	6	0	0	172	13	22
Cole Mere	87	2	189	5	504	13	1	5	862	22	0	0	50	1	2,155	56	43
Crose Mere	82	2	233	7	2,528	71	0	0	47	1	0	0	7	0	686	19	14
Ellesmere	30	1	167	5	1,850	60	5	92	64	2	0	0	29	1	926	30	33
Hanmer Mere	37	1	328	9	1,771	51	9	159	104	3	10	0	192	6	1,011	29	49
Marbury Big Mere	75	2	579	13	2,753	61	10	275	183	4	10	0	21	0	869	19	66
Newton Mere	44	2	99	4	1,187	43	4	47	1,300	47	15	1	7	0	94	3	31
Pick Mere	45	2	134	6	1,635	68	8	131	44	2	21	1	5	0	503	21	13
Redes Mere	76	10	116	16	34	5	0	0	176	24	26	4	62	9	233	32	20
White Mere	68	3	121	5	73	3	2	1	68	3	9	0	78	3	2,225	84	41
Mere Mere	43	1	111	3	63	2	2	1	54	2	6	0	38	1	2,900	90	65
Rostherne Mere	23	4	138	22	48	8	8	4	144	23	0	0	17	3	261	41	25
Piaseczno	15	14	72	60	31	24	15	5	2	1	0	0	0	0	0	0	17
Rogóźno	16	5	27	8	6	2	1	0	18	5	13	3	43	12	252	67	1
Zagłębocze Włodawskie	16	9	88	44	11	5	3	0	64	33	13	7	5	3	0	0	0
Białe Włodawskie	5	4	531	62	30	2	2	1	173	31	1	0	3	1	8	0	22
Czarne Włodawskie	8	1	197	19	221	21	18	40	136	13	21	3	4	1	467	43	0
Bialskie Sosnowickie	6	0	292	12	1,363	74	73	995	124	7	10	0	45	3	69	3	2
Czarne Sosnowickie	26	1	552	7	5,127	88	87	4,460	120	3	20	0	0	0	136	2	0
Maśluchowskie	1	0	6,449	83	2,058	12	6	123	243	5	8	0	0	0	0	0	0
Krasne	6	0	99	5	1,227	75	70	859	158	9	10	1	0	0	194	10	15
"Gąsior"[a]	4	1	129	23	406	61	36	146	38	5	42	9	11	2	0	0	6
Majcz Wielki	5	2	221	37	82	12	12	10	139	36	0	0	4	2	23	11	2
Gołdopiwo	12	0	490	15	1,191	39	30	357	301	11	0	0	2	0	892	35	20
Stręgiel	24	1	253	7	1,694	51	49	830	1,336	37	164	4	3	0	18	0	61
Mamry (Północne)	13	4	68	19	46	12	12	5	230	62	0	0	10	3	0	0	9
Rydzówka	17	1	407	23	106	7	6	6	191	10	16	1	0	0	978	59	24
Silec	4	0	186	4	791	14	14	111	148	3	2	0	0	0	4,120	79	123
Śniardwy	3	0	342	18	170	7	1	2	2,231	75	1	0	0	0	0	0	29
Białoławki	8	2	73	21	211	42	35	74	63	17	0	0	41	7	62	11	15
Orzysz	34	2	88	5	1,252	83	81	1,014	148	9	0	0	0	0	0	0	18
Inulec	10	1	194	18	648	59	51	330	158	14	56	7	0	0	16	1	26
Median	24	2	187	15	455	24	9	39	141	8	9	0	6	1	154	13	21
Lower quartile (Q1)	8	1	111	6	71	7	2	3	63	3	0	0	0	0	14	0	11
Upper quartile (Q3)	43	4	358	22	1,280	60	21	150	185	23	17	1	31	3	874	33	34

[a] See Table 1.

Table 3. Significant correlation coefficients among the proportions of phytoplankton, calculated as percentage of total phytoplankton biovolume and depth (Z), altitude, TN:TP, total N (TN), total P (TP), soluble reactive phosphorus (SRP), nitrate (NO_3-N), ammonia (NH_4-N) alkalinity and chlorophyll-*a* concentrations. Only environmental variables and phytoplankton groups which were found to be statistically significantly correlated are shown (n = 36).

	Cryptophyta	Chlorophyta	Total Cyanobacteria	Heterocystous Cyanobacteria	Non-heterocystous Cyanobacteria	Diatoms	Euglenophyta	Chrysophyta	Dinophyta
Z max					** −0.44		** −0.43		
Z mean					** −0.47		** −0.38		
Altitude					** −0.4		* −0.33		
TN:TP		* 0.35		* 0.3	** −0.39				** −0.4
TP		** −0.40		* −0.32	** 0.39				** 0.47
TN		* −0.28							** 0.38
SRP					** 0.38	* −0.31			* 0.29
Nitrate	** −0.41			*** 0.48	* −0.33				
Ammonium						** −0.42			
pH					** 0.47				*** −0.66
Alkalinity		** −0.4					* −0.31		* 0.32
Chlorophyll-*a*								* −0.27	* 0.27

* $p < 0.5$, ** $p < 0.01$, *** $p < 0.001$.

Table 4. Phytoplankton species and their codes as shown in Figs 1, 2 and 3.

Species name	Code	Division
Amphora spp.	*Amp* spp.	Diatom
Anabaena subcylindrica	*Anab sub*	Cyanophyta
Aphanizomenon flos-aquae	*Aph flaq*	Cyanophyta
Asterionella formosa	*Ast form*	Diatom
Ceratium hirundinella	*Cera hir*	Dinophyta
Chroococcus limneticus	*Chr lim*	Cyanophyta
Closterium spp.	*Clos* spp.	Chlorophyta
Coelastrum spp.	*Coel* spp.	Chlorophyta
Cosmarium spp.	*Cos* spp.	Chlorophyta
Cryptomonas spp.	*Cryp* spp.	Cryptophyta
Cymbella spp.	*Cym* spp.	Diatom
Fragilaria crotonensis	*Frag* cro.	Diatom
Fragilaria spp.	*Frag* spp.	Diatom
Kirchneriella subsolitaria	*Kir sub*	Chlorophyta
Mallomonas spp.	*Mall* spp.	Chrysophyta
Oscillatoria retzii	*Osc ret*	Cyanophyta
Oscillatoria tiny	*Osc* tiny	Cyanophyta
Oscillatoria spp.	*Osc* spp.	Cyanophyta
Pediastrum boryanum	*Ped bory*	Chlorophyta
Phormidium spp.	*Phor* spp.	Cyanophyta
Sphaerocystis spp.	*Sph* spp.	Chlorophyta
Staurastrum spp.	*Stau* spp.	Chlorophyta
Synura spp.	*Syn* spp.	Chrysophyta
Tabellaria spp.	*Tab* spp.	Diatom
Trachelomonas spp.	*Trac* spp.	Euglenophyta
Unidentified centric diatom	cent diat	Diatom
Unknown flagellate	un flag	?
cyanobacteria, heterocystous	Cyan-het	Cyanophyta
cyanobacteria, non-heterocystous	Cyan-non	Cyanophyta

PCA separated the lakes into those dominated (1) by Dinophyta, (2) by heterocystous cyanobacteria and (3) a group where Chlorophyta, diatoms and Cryptophyta were co-dominant (Figs 2 and 3). The proportion of Dinophyta was correlated with axis 1 (λ1 = 0.377), which explains 38% of the species data, while heterocystous cyanobacteria were weekly correlated to axis 1 and 2. The north-west midland meres tended to be more highly associated with either heterocystous cyanobacteria or Dinophyta, while there was no clear trend within the Polish sites as a whole (data not shown).

Phytoplankton and nitrogen scarcity

The proportion of Dinophyta and non-heterocystous cyanobactera were inversely correlated to the TN:TP ratio and the proportion of heterocystous cyanobacteria was positively correlated to the TN:TP ratio and nitrate concentrations (all $p < 0.05$, Table 3). The proportion of Dinophyta and non-heterocystous cyanobacteria was positively correlated to lake nutrient status; Dinophyta to both total nitrogen and total phosphorus and non-heterocystous cyanobacteria to total phosphorus (all $p < 0.05$, Table 3). The predominance of Dinophyta was negatively correlated to pH ($p < 0.001$) and heterocystous cyanobacteria was positively correlated to pH ($p < 0.01$) (Table 3) though pH data only exists for the UK sites and there were no significant relationships between the proportions of these phytoplankton divisions and alkalinity which included data from Poland and the UK. The majority of the sites associated with high proportions of heterocystous cyanobacteria such as *Anabaena subcylindrica* Borge and *Aphanizomenon flos-aquae*, and Chlorophyta, had TN:TP ratios of > 15. Indeed, the proportions of heterocystous cyanobacteria and Chlorophyta were significantly lower in sites with a mean TN:TP ratio of < 7 than in sites where mean TN:TP ratio was > 15 (both $p < 0.01$). Lakes which had a TN:TP ratio of < 7 were dominated by non-heterocystous cyanobacteria, such as *Phormidium* spp. and *Oscillatoria* spp., Dinophyta such as *Ceratium hirundinella*, and diatoms (Fig. 2a, b).

Dinophyta and non-heterocystous cyanobacteria were most predominant in lakes where available nitrogen became undetectable in the water column (Fig. 3a), though when the proportions of these algae in such lakes were compared with those in lakes where nitrate and ammonium and SRP, or neither nutrient, had dropped below detectable limits, no statistically significant differences ($p < 0.05$) were found. Only diatoms and Euglenophyta accounted for a significantly greater proportion of the biomass in lakes where neither nutrient became undetectable when compared with lakes where both dropped below detectable concentrations (both $p < 0.05$). In lakes where nitrate and ammonium fell below detectable concentrations concurrently *Ceratium hirundinella*, araphid diatoms such as *Tabellaria* spp., *Asterionella formosa* and centric diatoms, were predominant (Fig. 3b). Lakes where both available N and P, or where neither nutrient dropped below detectable concentrations, were not clearly associated with a characteristic phytoplankton flora (Fig. 3b).

DISCUSSION

Phytoplankton and productivity

The phytoplankton of the sites consisted predominately of Chlorophyta, Dinophyta and cyanobacteria, both in terms of relative proportions and absolute biovolumes (Table 2). The thirty-six lakes can be grouped

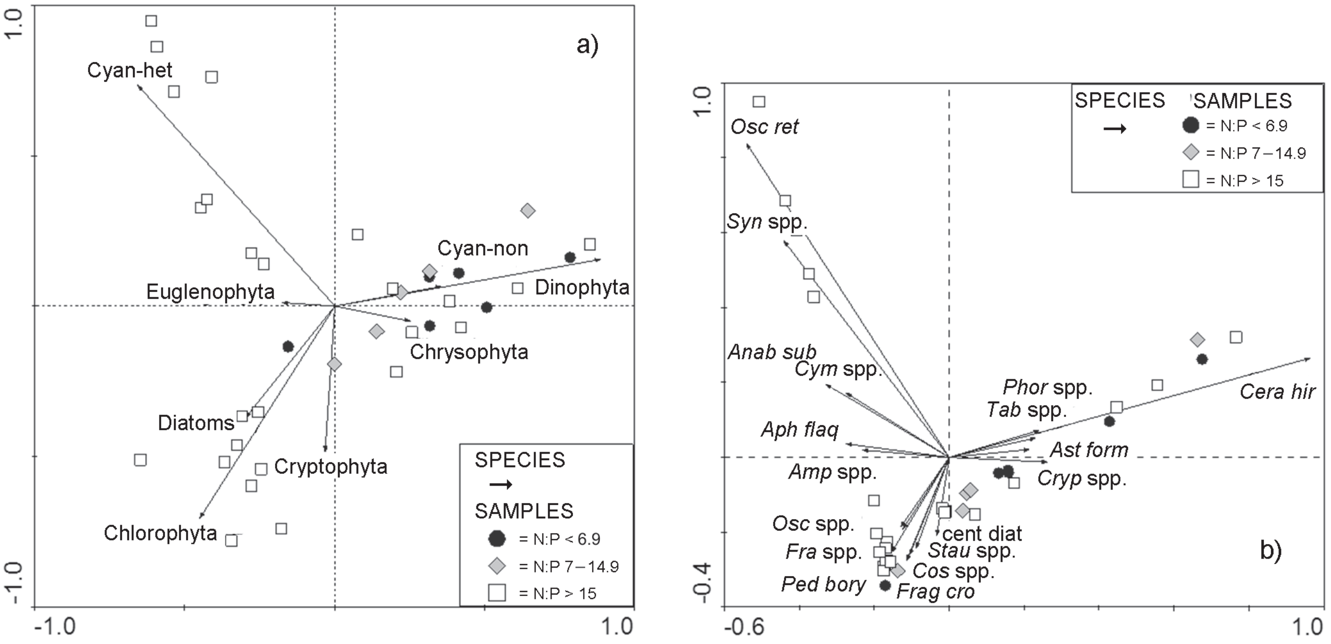

Fig. 2. PCA of lakes ordinated in relation to mean growth season composition of phytoplankton, expressed as proportion of biovolume. **a** – phytoplankton divisions, λ1 = 0.377, λ2 = 0.237; **b** – phytoplankton species, λ1 = 0.418, λ2 = 0.198, key to species shown in Table 4. Nutrient ratios are TN:TP ratios and symbols are shaded to show mean TN:TP ratios at each site.

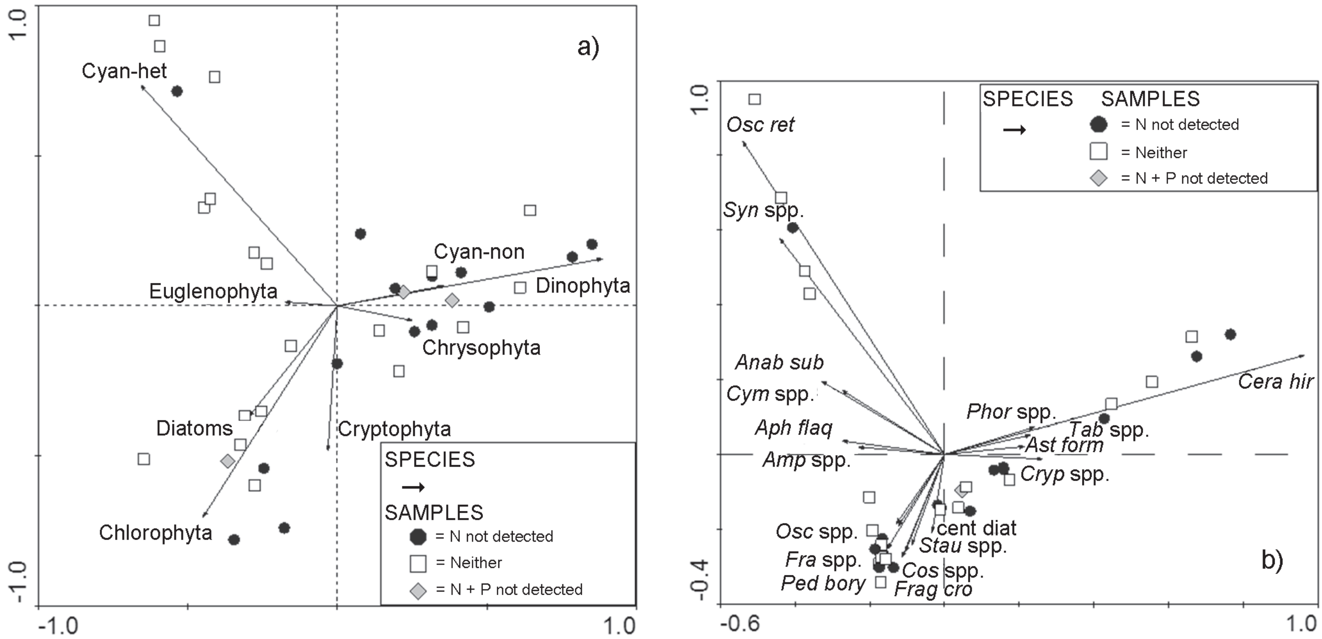

Fig. 3. PCA of lakes ordinated in relation to mean growth season composition of phytoplankton, expressed as proportion of biovolume. **a** – phytoplankton divisions; **b** – phytoplankton species, eigenvalues are the same as in Fig. 2. Symbols shaded to show N:P ratio or to denote where available N or P fell below laboratory detection limits in the growth seasons samples collected (key to symbols in Table 4).

into those whose phytoplankton communities were dominated by Dinophyta, those by heterocystous cyanobacteria and those where other divisions predominated (Fig. 2a).

Many studies have related cyanobacteria predominance in lakes to high nutrient concentrations (Trimbee and Prepas 1987, Dokulil and Treubner 2000, Reynolds and Petersen 2000, Downing *et al*. 2001, for example). In this study however, Dinophyta and non-heterocystous cyanobacteria tended to dominate in the lakes with the highest concentrations of P and N (Table 3; Fig. 1) suggesting that these have low competitive ability for N and P and predominate where there are higher concentrations of bioavailable nutrients. Both the proportion of Dinophyta and non-heterocystous cyanobacteria were correlated to the TN:TP ratio which can be indicative of *potential* N-limitation, and the proportion of non-heterocystous cyanobacteria were also negatively correlated to nitrate concentrations (Table 3), again suggesting predominance where N is potentially limiting.

At the species level, *Ceratium hirundinella* was the most abundant species at the highest nutrient concentrations while *Oscillatoria retzii* and a form of *Oscillatoria* with filament width of < 2 µm were abundant in sites with lower nutrient concentrations (Fig. 1b). This suggests that *C. hirundinella* is adapted to nutrient scarcity in lakes with high trophic status.

Unicellular Dinophyta move faster than many other freshwater algae (Taylor 1987), *Ceratium* can migrate at speeds up to $0.97\ \mathrm{mhr}^{-1}$ (Whittington *et al.* 2000). Dinophyta are able to migrate to the water surface prior to dawn (Lieberman *et al.* 1994) and move between areas of different nutrient concentration both vertically and horizontally, which may give this division a competitive advantage over other phytoplankton (Lieberman *et al*. 1994), although the mobility of Dinophyta varies depending on the genus (Blasco 1978). The vertical motility of *Ceratium* is not hindered by thermal stratification of the water column (Frempong 2006), which was common during the sampling period in many of the UK study sites (Fisher, unpublished) and is known to be a feature of the Polish lakes in the Mazurian Lakeland district (Zdanowski 1984).

The contrasting association between heterocystous and non-heterocystous cyanobacteria and nutrient concentrations (Table 3; Figs 2 and 3) agrees with the approach of Blomquist *et al*. (1994) who argue that heterocystous and non-heterocystous cyanobacteria should be treated separately. The lack of significant correlations between ammonia and any of the phytoplankton divisions suggests that the high mean growth-season concentrations of ammonia (> 50 µg l^{-1} NH_4-N) in some of the lakes (Table 1) was exceeding demand. Could the correlation between the heterocystous cyanobacteria and nitrate concentrations be a result of these algae fixing and therefore providing N? Horne (1971) showed that N-fixation contributed approximately 40% of the nitrogen input to an eutrophic lake, and the presence of available nitrogen, especially ammonium, has been shown to inhibit N-fixation and the development of heterocysts (Fogg 1971). It is likely that the cause and effect relationship between in-lake nitrogen concentration and N-fixing cyanobacteria may be too complex to be represented by correlations between cyanobacteria biomass and N concentrations in lake water samples, partly as a result of the time-lag between available nutrient concentration and resultant changes to the phytoplankton composition.

Phytoplankton and nitrogen scarcity

Low TN:TP ratios, the occurrence of both nitrate and ammonia falling below test detection limits and the results of nutrient bioassay studies (Carvalho and Moss 1995, James *et al*. 2003, Fisher *et al*. 2009) suggest that nitrogen was potentially limiting in more of these lakes than phosphorus. It is generally assumed that cyanobacteria will be more predominant in lakes which are N-limited (Schindler 1977, Smith 1983, Levine and Schindler 1999) and reduction of N-loading has been shown to lead to an increase in N-fixing activity (Schindler *et al*. 2008, Vrede *et al*. 2009) in certain lake types. Some researchers argue that N-fixing cyanobacteria only increase in response to relative N deficiency in certain lakes where the physical and chemical environment is suitable (Conley *et al*. 2009, Paerl 2009) and that their N-fixing may not be sufficient to maintain phytoplankton standing stocks (Scott and McCathy 2010). In contrast, in the UK and Polish lakes, the proportion of heterocystous cyanobacteria was positively correlated to nitrate concentrations and they predominated in lakes which did not have low N:P ratios and where available nitrogen was detectable on all sampling occasions (Figs 2a, 3a; Table 3). The lakes which were most likely to be N-limited (mean growth season N:P ratios of lower than 7 and where available nitrogen was below detectable limits) were associated with either non-heterocystous cyanobacteria or Dinophyta populations (Figs 2a, 3a). The presence of non-heterocystous cyanobacteria in lakes which appear to be potentially N-limited does not necessary mean that fixation was not taking place by the genera *Oscillatoria, Lyngbya* and *Phormidium* that are sometimes capable of N-fixation (Paerl 1988), but it is unlikely given that phytoplankton samples were taken from oxygenated upper water layers. There is evidence that cyanobacteria may not gain their competitive dominance in N-limited lakes through N-fixation alone, but through their highly competitive ability to sequester ammonium. Ferber *et al*. (2004), for example, found that a mixed cyanobacteria population, including *Aphanizomenon flos-aquae, Planktothrix agaradhii* and *Microcystis* spp., obtained most of their N via uptake of ammonia rather than through N-fixing in a N-limited pond in Vermont, USA.

The ability of N-fixing cyanobacteria to predominate may be prevented by light limitation and light has been shown to be a potential limiting factor in Rostherne Mere, one of the lakes included in this study (Reynolds 1978, Reynolds and Bellinger 1992, Barker 2006). Pinto and Litchman (2010) have shown that N-fixing activity is not related to N:P ratios where light is limiting. The occurrence of undetectable concentrations of available nutrients, despite high total nutrient concentrations in some of the lakes, however, indicates that biological uptake of nutrients was high and not limited by light in these sites. In the other sample lakes, the persistence of available concentrations of nutrients may be a result of light limitation. Indeed, light limitation may be a feature of these eutrophic moderately deep lakes, either through self-shading as a result of the high phytoplankton biomass or due to mixing of the water column to depths below the euphotic zone. The vertical migration ability of Dinophyta and bloom forming cyanobacteria, which predominate, enables them to overcome light limitation under thermally stable condition. Eleven of the UK meres were found to be thermally stratified during the growth-season (Fisher, unpublished data).

Levine and Schindler (1999) found that Dinophyta were particularly successful in lakes with low N:P ratios. In this study Dinophyta reached their greatest proportion in lakes where available N alone became exhausted (Fig. 3a, b) and the proportion of Dinophyta was inversely correlated to TN:TP ratios (Table 3). Some field experiments show that Dinophyta have a high requirement for available nitrogen (Sakka *et al*. 1999, Chapman and Pfiester 1995). Despite this, Reynolds (1973a, b) found that *Ceratium hirundinella* dominated, and the growth of cyanobacteria was restricted, when nitrate became undetectable in the eplimnion during a study of Crose Mere, and Lieberman *et al*. (1994) found that blooms of the dinoflagellate *Gymnodinium* spp. occurred during periods of low N and high P.

The dinoflagellate species present in these lakes may have been able to overcome N-limitation. Heaney and Eppley (1981) found that increased N-depletion led to the downward migration of Dinophyta over a vertical distance of 2 metres, and the avoidance of high irradiance at the surface, during laboratory experiments, which would enable them to exploit the nutrient-rich hypolimnion. Their rapid migration to water layers of higher nutrient concentrations and their ability to maintain a steeper nutrient concentration gradient round their cells, through their mobility and action of the transverse flagellum (Pollingher 1988), may also enable them to out-compete other species when nitrogen is low. The vertical migration of Dinophyta and cyanobacteria has been shown to occur at all depths in the water column in Rostherne Mere and Mere Mere (The Mere) (Barker 2006). During periods of N-limitation, some Dinophyta may be able to supplement their nutrient requirements through phagocytosis (Sanders 1991, Hansen and Calado 1999, Stoecker 1999). It is unknown whether the particular individuals of *Ceratium hirundinella*, predominant in these lakes, were capable of mixotrophy, but it has previously been reported for freshwater Dinophyta, including *Ceratium hirundinella* (Dodge and Crawford 1970, Sanders 1991). Several dinophyte genera are also known to contain N-fixing symbionts, although this has yet been reported for marine rather than freshwater species (Gordon *et al*. 1994) though Wehr (2003) reports the *Ceratium* may also injest cyanobacteria which may thus provide an addition nitrogen source.

If heterotrophy is important for Dinophyta to overcome N-limitation, it might also be expected to allow Dinophyta to avoid P-limitation. Like cyanobacteria, Dinophyta are known to have a capacity for luxury uptake and storage of phosphorus for use during periods when this nutrient is low (Serruya and Berman 1975, Healey 1982). Dinophyta were not predominant in lakes with high TN:TP ratios, or where both available P and N became undetectable however (Figs 2a, b; 3a, b). While Dinophyta are known to migrate down in response to P scarcity (Taylor 1987) Dinophyta tend to have lower affinities and lower uptake rates for P than other phytoplankton groups, and therefore do not have competitive advantage when this nutrient is limiting (Berman and Dubinsky 1985). The ability of Dinophyta to overcome low epilimnion P concentrations by migration to the bottom lake sediment can also be limited by low oxygen concentrations in the hypolimnion (James *et al*. 1992). Therefore while Dinophyta can migrate to overcome P scarcity, P sequestrations may be hindered by bottom water anoxia, which is known to occur in most of the UK meres sampled and in moderately deep lakes in the Mazurian Lakeland (Zdanowski *et al*. 1984, James *et al*. 2003).

Using Cyanobacteria for monitoring nutrient concentrations in lakes

This study has contributed evidence that cyanobacteria are not reliable indicators of low TN:TP ratios or of nitrogen scarcity in moderately deep, lowland, eutrophic lakes. Nor was the proportion of cyanobacteria clearly related to trophic status. In fact, the proportion of heterocystous cyanobacteria was inversely correlated to TP concentrations (Table 3), while only the proportion of non-heterocystous cyanobacteria were significantly correlated to TP. This has particular significance in the context of the European Water Framework Directive, where it has been suggested that the proportion of nuisance cyanobacteria, in conjunction with chlorophyll concentrations, can be used as a measure of nutrient enrichment (Solheim *et al*. 2008, WFD UKTAG 2008). The nuisance cyanobacteria include the heterocystous *Anabaena* and *Aphanizonemon*, which, in this study, were inversely associated with nutrient concentrations, including TP (Fig. 1b; Table 3). It may be the lack of relationship between cyanobacteria and trophic state in this study is a result of these lakes comprising predominantly eutrophic to hyper-eutrophic sites, rather than spanning the whole trophic spectrum, though such high nutrient concentrations are a common occurrence across much of lowland Europe.

While the lakes sampled in this study were predominately eutrophic or hypereutrophic, the concentration of available nutrients in the surface layers became undetectable in some of the lakes, bringing about potential nutrient limitation. Given that these lakes are moderately deep, the vertical separation of light and nutrients enabled Dinophyta to predominate, and their occurrence appeared to be more highly related to trophic status and potential N scarcity than the proportion of heterocystous cyanobacteria. It is concluded that motility and nutritional flexibility of the Dinophyta may enable them to become at least as successful as heterocystous cyanobacteria in overcoming nitrogen scarcity in such moderately deep lakes.

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