Arch. Hydrobiol. 137 3 363–384 Stuttgart, September 1996

Annual profiles of photosynthetic lipophilic pigments in four freshwater lakes in relation to phytoplankton counts as well as to nutrient data

Peter Woitke, Toralf Schiwietz, Katrin Teubner and **Johannes-Günter Kohl**[1]

Humboldt-University of Berlin

With 6 figures and 7 tables in the text

Abstract: Phytoplankton structure and dynamics of four eutrophic lakes of Berlin were studied in 1992. The biomass change of relevant classes – Cyanophyceae, Bacillariophyceae, Cryptophyceae, and Chlorophyceae – is well represented by HPLC-analysis of photosynthetic lipophilic pigments simultaneously carried-out. Nevertheless, an estimation of algal class biovolumes or phytoplankton composition, on the basis of chromatographically recorded pigment data is restricted to a semi-quantitative consideration. Different pigment amounts in individual species of the same algal class or varying marker pigment/chlorophyll-a ratios are discussed as a reason for the misleading pigment-based quantification of phytoplankton dynamics. However, the analysis of the obtained data points to the possibility of using HPLC-aided pigment determinations in connection with microscopic cell counting for a detection of variable physiological states under field conditions; provided that mass developments of algae are dominated by a single species as common in eutrophic lakes. For time periods with dominating *Planktothrix agardhii* or *Microcystis* spec. a positive correlation was found between their content of the cyanophyte specific pigment echinenone and increasing nitrogen availability. In contrast, no relations could be detected between the changing fucoxanthin content of *Aulacoseira* spec. and any of the measured environmental parameters (nutrient concentrations or light climate).

Introduction

Algae and photosynthetic procaryotes such as blue-green algae (cyanobacteria) contain, in contrast to higher plants, some specific lipophilic pigments

[1] **Authors' address:** Institute of Biology/Ecology, Humboldt-University of Berlin, Luisenstraße 53, D-10117 Berlin, Germany.

0003-9136/96/0137-0363 $ 5.50

(GOODWIN 1988). Apart from several chlorophylls, a diverse spectrum of oxygen containing carotenoids (xanthophylls) is well known. Some of these are specific for individual algal classes. Therefore, these substances are used as chemotaxonomic markers (PALERMO et al. 1991, FAWLEY 1991, HALLEGRAEFF et al. 1991), and their chemical stability allows pigment determinations even in sediment cores, which can contribute to the study of lake history (LEAVITT et al. 1989, HURLEY & ARMSTRONG 1990). At present, the determination of photosynthetic lipophilic pigments is considered a helpful tool for the study of phytoplankton populations in aquatic ecosystems.

A promising approach, based on individual determinations of selected marker xanthophylls, is the estimation of phytoplankton composition. GIESKES & KRAAY (1983) concluded a dominance of Cryptophyceae in the central North Sea from chromatographically recorded alloxanthin concentrations, which had not been observed by microscopic investigations. Furthermore, they found a good correlation between measured fucoxanthin concentrations and diatom counts implying a rare abundance of other fucoxanthin containing taxa. EVERITT et al. (1990) used fitted pigment ratios for calculating the contribution of different algal classes to total chlorophyll-a in the western equatorial Pacific. The above mentioned investigations can also be carried out in freshwater systems as demonstrated by WILHELM et al. (1991). These authors compared their method of estimating phytoplankton assemblage, based on pigment determinations, with the classical Utermöhl cell counting technique and found very similar results. This approach, to determine the composition of phytoplankton communities, presumes relatively stable pigment patterns of the species independent of their physiological status.

Nevertheless, a variety of studies carried out mainly with unialgal cultures, show a change in pigmentation between different species within the same algal class or under varying light regime and nutrient supply (cf. KOHL & NICKLISCH 1988, for review). SENGE & SENGER (1990 a), for example, investigated the adaptation of green algae to high and low light intensities. Their results show different chlorophyll and carotenoid concentrations and different ratios of lutein or violaxanthin to chlorophyll-a between the studied taxa as well as under irradiance changes. Changes in pigment content or ratios caused by varying light regimes can occur in other algal classes too, as demonstrated for Raphidophyceae (KOHATA & WATANABE 1988), for several Cyanophyceae (MILLIE et al. 1990, RÜCKER et al. 1995), and for Bacillariophyceae (GILSTAD et al. 1993).

Nutrient availability is considered to be another factor influencing the amount of chlorophylls and carotenoids in algal cells (LAWS & BANNISTER 1980). WILHELM & MANNS (1991) monitored pigment/chlorophyll-a ratios during growth and stationary phase of four phytoplankton species, and discussed variations in these ratios with nutrient limitations. RÜCKER et al. (1995)

showed adaptations of the photosynthetic pigment apparatus of three species of planktic Cyanophyceae by investigating cultures grown under N- and P-limitation, both under limiting and saturating light supply. Therefore, varying pigment patterns may be used to indicate different physiological states within plankton communities and for detecting light and/or nutrient limitation under field conditions (RÜCKER & KOHL 1994).

Four eutrophic shallow lakes were studied intensively in 1992 comparing their ecological status. Investigations carried out during the closed seasonal cycle included the characterization of phytoplankton dynamics, the determination of several hydrophysical and hydrochemical data, and the chromatographic determination of lipophilic algal pigments. The data collected offer the possibility to characterize the phytoplankton dynamics based on pigment determinations, as well as to investigate, if and how selected physical and chemical parameters can influence algal pigment patterns measured in these lakes.

Materials and methods

Four lakes near Berlin were under investigation within this project – Lake Müggelsee, Lake Langer See, Lake Seddinsee and Lake Flakensee. All lakes are polymictic eutrophic lakes and part of the river/lake system of the Spree River and the Dahme River. Several hydrologic and morphometric parameters of these lakes are listed in Table 1.

Sampling was carried out twice a month from March to December 1992. Samples were taken with a Friedinger sampler from at least 3 sampling stations per lake and from every 2 m depth intervall. To get a representative of the mostly well-mixed lake water, these samples were mixed together (about 100 l), making the number of depth samples proportional to lake volume of the respective strata. Several subsamples, out of this mixed sample, were prepared for phytoplankton counting, hydrochemical analysis, and determination of photosynthetic lipophilic pigments. Vertical light attenuation (PAR) was measured using a photometer with two spherical quantum sensors placed in a distance of 0.5 m from each other (LiCor Li 193SA).

Table 1. Hydrologic and morphometric parameters of lakes under investigation.

Parameter	Lake Müggelsee	Lake Flakensee	Lake Langer See	Lake Seddinsee
area [km^2]	7.18	0.76	1.94	2.25
maximal depth [m]	7.8	9.8	6.5	7.0
mean depth [m]	4.9	4.2	3.5	3.2
volume [km^3]	0.037	0.003	0.007	0.007
mean residence time [d]	68.3	17	3.77	13.5

Table 2. Hydrochemical parameters and methods of determination.

Parameter	Sample		Methods
phosphate	filtered	–	ion-chromatography
nitrate	filtered	–	ion-chromatography
nitrite	filtered	–	ion-chromatography
ammonia	filtered	–	flow injection analysis
silicate	filtered	–	flow injection analysis
DIN	–	dissolved inorganic nitrogen	sum of nitrate, nitrite and ammonia
TN	unfiltered	total nitrogen	peroxydisulfate-digestion, flow injection analysis as nitrate
TP	unfiltered	total phosphorous	peroxydisulfate-digestion, flow injection analysis as phosphate

Hydrochemical analysis

Samples for hydrochemical analysis were divided into two subsamples. One part was immediately filtered (Sartorius cellulose acetate filters, 0.45 µm, with glass microfibre prefilter, Whatman) for the following measurements of dissolved nutrients. The other part was stored unfiltered at –20 °C for determination of total nitrogen and total phosphorus. The hydrochemical data as well as the procedures used for their determination are listed in Table 2.

Phytoplankton counting

Appropriate subsamples were fixed with Lugols solution and phytoplankton was counted in sedimentation chambers using an inverted microscope (SEDIVAL Hydrobiology, Carl Zeiss Jena). The different taxa were identified and their biovolumes were calculated as described by UTERMÖHL (1958). The biovolumes of filamentous bluegreen algae and *Aulacoseira* spec., fitted best to cylindric bodies, were determined from length sum and width of filaments. Chroococcales were carefully determined from countings of single cells or from the estimation of the packed volume of cells within dense colonies, regarding *Microcystis wesenbergii* and *Microcystis viridis* colonies as planes of one to two cell layers and *Microcystis aeruginosa* as cell aggregations arranged in globes and globe-like sub-colonies. Both procedures resulted in comparable estimates. The cells of unicellular algae and oligocellular coenobia were counted and the biovolume of these cells was determined from size parameters important for calculating the volume of bodies fitted best to the special cell shape. This was done very carefully, e. g. Cryptomonades were fitted to flattened instead of regular rotation ellipsoids. If species or a species group, indistinguishable in the inverted microscope without preparation (unicellular or filamentous centric diatoms), exhibited a heterogeneous size distribution, then single size clusters were counted and treated in the biovolume calculation separately.

Determination of pigments

Usually 250 ml of the mixed lake water sample were filtered onto GF/C filters (Whatman). The filters were freeze-dried and stored at −20 °C for analysis. For pigment extraction, dried filters were ground in a mortar under dim light with 2 ml of cold acetone (90 %), and then sonicated in 10 ml plastic centrifuge tubes for 30 s (50 W, 5 s intervals, ice-bath). After centrifugation (15 min, 20,000 rpm), the particulate residue was re-extracted twice with 1 ml acetone (90 %). The collected extracts were mixed with IPR-solution (10 : 1, IPR-solution: 15 g/l tetrabutylammoniumacetate in 77 g/l ammoniumacetate solution) and 300 µl were filtered through 0.2 µm-syringe-filters into low volume inserts of Waters' autosampler tubes. Usually, 100 µl of this solution were injected for HPLC-analysis. The system used for all separations consisted of a Waters 600E ternary gradient module with a low pressure mixing chamber, a Waters 717 autosampler and a Waters 991 diodenarraydetector. All separations were controlled by Waters Powerline software. Chromatography was carried out with a gradient system following MANTOURA & LLEWELLYN (1983). A Waters Novapak C 18 steel column (3.9 × 300 mm) was used, protected with an appropriate pre-column (Waters Guard-Pak system). The flow rate was set to 1.0 ml/min. A linear gradient from 100 % eluent A (methanol/water/IPR-solution = 80 : 10 : 10, v/v/v) to 100 % eluent B (methanol/acetone = 60 : 40, v/v) within 40 min was used, followed by a 15 min isocratic hold of 100 % eluent B. Peak areas were monitored at 440 nm, and UV/Vis-spectra were recorded online in the range from 350 nm to 600 nm for pigment identification purposes.

Results and discussion

Pigment chromatography

The chromatograms displayed in Fig. 1 demonstrate the separation of lipophilic algal pigments and represent the pigment pattern in July, with variable amounts of these substances, due to different phytoplankton communities in the investigated four lakes. The identified pigments and the used calibration factors, determined with pigment standards or calculated from literature (GOODWIN 1976), are listed in Table 3. Unfortunately, the separation procedure was not able to resolve the xanthophyll pairs lutein/zeaxanthin and myxoxanthophyll/diadinoxanthin, respectively. This problem was resolved at a later date by using a modified gradient and a non-endcapped C18-RP-column (WOITKE et al. 1994).

Phytoplankton dynamics and marker pigment content

Chlorophyll-a, the ubiquitous photosynthetic pigment in all algae, is widely used in limnology as a rough equivalent of phytoplankton (TOLSTOY 1979). Chromatographically recorded chlorophyll-a concentrations are shown in Fig. 2 together with microscopically determined phytoplankton biovolumes. In

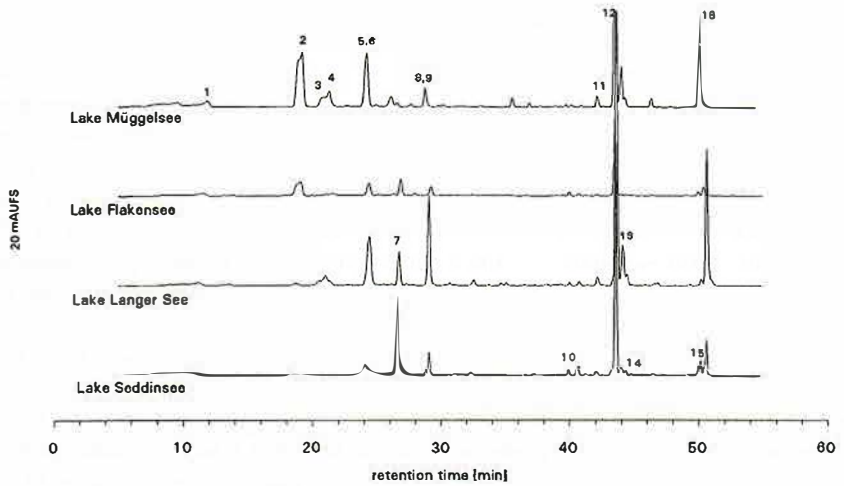

Fig. 1. Chromatograms of pigment extracts at the beginning of July, 1992; peak identification as in Table 3.

Table 3. Chromatographic and spectral properties of algal pigments separated by HPLC.

No.	Ret.-time [min]	Pigment	Abbr.	Spectral data* Maxima [nm]			Published data Maxima [nm]			Factor** [ng/(AU·min)]
1	10.6	Chlorophylls-c	Chl-c	444.9			444			(2280)
2	19.1	Fucoxanthin	Fuc	447.5			446			7331
3	21.1	Neoxanthin	Neo	412.4	437.1	465.7	410	436	464	4879
4	21.7	Violaxanthin	Vio	416.3	441.0	469.6	416	440	470	4411
5	24.4	Myxoxanthophyll	Myx	452.2	474.8	507.3	448	473	504	15327
6	24.4	Diadinoxanthin	Did	424.0	447.5	476.1	424	446	476	4077
7	26.8	Alloxanthin	Allo		452.7	481.3	430	452	481	(4500)
8	29.1	Lutein	Lut	422.0	446.2	473.5	424	446	474	4552
9	29.2	Zeaxanthin	Zea		451.4	477.4	424	450	478	5159
10	39.9	Chlorophyll-b	Chl-b	459.2			464			2284
11	42.1	Chlorophyll-a allometer	Chl-a allo	431.9			428			(21684)
12	43.5	Chlorophyll-a	Chl-a	430.6			430			21684
13	44.0	Echinenone	Ech	461.8			464			5435
14	44.5	Chlorophyll-a′	Chl-a′	431.9			430			(21684)
15	50.1	α-Carotene	a-Car	426.7	447.5	474.8	423	444	473	(4500)
16	50.6	β-Carotene	b-Car		454.0	477.4	422	448	472	4170

* On-line recorded.

** For pigment quantification, determined from calibration curves (values in brackets are approximates based on standardized pigments with comparable spectra).

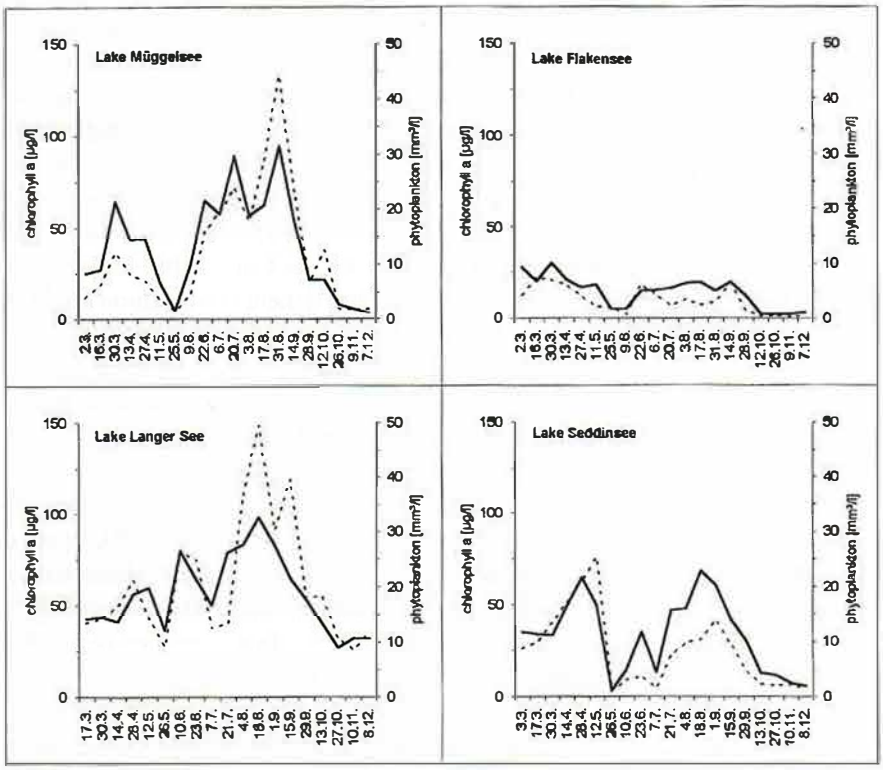

Fig. 2. Concentration profiles of chromatographically recorded chlorophyll-a (——) and microscopically determined phytoplankton biovolumes (- - - -) in the investigated lakes in 1992.

Lake Müggelsee, Lake Langer See, and Lake Seddinsee mean values of 40 µg/l, 56 µg/l, and 34 µg/l chlorophyll-a and summer maxima of 94 µg/l, 98 µg/l, and 69 µg/l were recorded, respectively. Clear-water periods at the end of May with very low chlorophyll-a contents of the lake water below 5 µg/l were determined for Lake Müggelsee and Lake Seddinsee, but not for Lake Langer See. However, Lake Flakensee exhibits a very low yearly average of only 14 µg/l chlorophyll-a with the spring maximum of not more than 30 µg/l. The very similar annual profiles of chlorophyll-a and phytoplankton biovolume in all investigated lakes are reflected by a significant correlation between both parameters (see Table 4). This indicates a low variation of the chlorophyll-a content of the algae compared to their biovolume fluctuations. Therefore, chlorophyll-a can be used as a rough biovolume equivalent. However, there are some deviations between both parameters which will be discussed later. Also, the specific relations between both parameters cannot be directly applied to other lakes with a different dominance structure of the phytoplankton.

The comparison of the algal class specific pigments and the composition of the phytoplankton with respect to the different algal classes reveals more specific insights into phytoplankton dynamics. The phytoplankton counting data revealed that only Bacillariophyceae and Cyanophyceae developed higher biovolumes, but Cryptophyceae and Chlorophyceae were, mostly, much less frequent during the whole sampling period. Only in Lake Flakensee these groups were relatively more abundant because of a lower level of diatoms and bluegreens abundance. A contribution of Dinophyceae or Chrysophyceae common in other lakes to overall biovolume as well as to pigment composition could be neglected because the amount of counted dinophyceaen or chrysophyceaen species did not exceed 5 % of the whole phytoplankton biovolume for any sampling date. This agrees well with measured pigment data, because peridinin, a xanthophyll specific for Dinophyceae, could not be found in the chromatograms at any date.

Similar results regarding the phytoplankton composition for Lake Müggelsee have already been found for a longer period by TÄUSCHER (1980), KOHL et al. (1985, 1991) and NIXDORF & HOEG (1993). But, some characteristic changes have been recorded within the dominant species (KOHL et al. 1985). Whereas Lake Müggelsee showed the typical *Aphanizomenon flos-aquae/Microcystis* spec. dominance after the clear-water period during summer in 1992, Lake Langer See was characterized by the long lasting dominance of *Planktothrix agardhii,* accompanied by *Limnothrix redekei* in spring. This so-called "*Oscillatoria*"-regime was typical for Lake Müggelsee until 1978 and episodically again in 1988 and 1989.

In order to get an indicatory value for the different algal groups present in the phytoplankton, the application of chlorophyll-a as a rough biomass equivalent should be supplemented by the use of the different pigments, specifically related to different algal classes. Due to their chromatographic behaviour the following pigments were chosen: echinenone (Cyanophyceae), alloxanthin (Cryptophyceae), fucoxanthin (Bacillariophyceae) and chlorophyll-b (Chlorophyceae). Fucoxanthin and alloxanthin were also used by GIESKES & KRAAY (1983) and by WILHELM et al. (1991) as marker pigments for diatoms and cryptophytes, respectively. GIESKES et al. (1988) monitored phytoplankton composition in the marine environment based on fucoxanthin (diatoms) or chlorophyll-b (green algae). Nevertheless, the functional integration of these pigments is different, for example, fucoxanthin and chlorophyll-b are constituents of the antennae pigment protein-complexes and echinenone and alloxanthin are membran-integrated xanthophylls (WILHELM 1990). Despite their different integration, the adaptation of the cell content of echinenone and fucoxanthin, the marker pigments of the most important algal classes, is regulated in constant proportion to chlorophyll-a, whereas the chlorophyll-b/chlorophyll-a ratio is highly variable (JEFFREY & VESK 1977, VESK & JEFFREY

1977, RÜCKER et al. 1995, GROTJOHANN et al. 1992, SENGE & SENGER 1990a, b).

The microscopically determined biovolume values of the most important algal classes are given in Fig. 3 together with the annual concentration profiles of their chosen marker pigments. The rough comparison of the annual change of both parameters reveals good correspondence only for the blue-greens and the diatoms. This is confirmed by the results of a linear regression between both parameters for each algal class and the pooled data of all lakes (Table 4). The data reveal very close correlations between echinenone and cyanophycean biovolume, as well as fucoxanthin and bacillariophyceaen biovolume. Although the correlation coefficients are also significant for alloxanthin and cryptophycean biovolume and chlorophyll-b and chlorophycean biovolume, respectively, the relatively high variances have to be taken into account (Table 4), and the reasons for that have to be clearified before the specific pigments can be used as a semiquantitative parameter for the biovolume of these algal groups. The high variances can be ascribed to different pigment content and different physiological adaptation states of the various algal species present at different sampling dates and localities. Furthermore, the counting data of subdominant algal species may be doubtful, too. For example, the overall biovolume sum of green algae can be estimated only with low confidence because this algal class is present in low abundances and consists of as much as 40 different species.

The high concentrations of the xanthophyll echinenone (Fig. 3 A) in all investigated lakes except Lake Flakensee from May till September is related to the development of only a few cyanophycean species. This enables its comparison with species dominance. The relatively high content of echinenone in Lake Müggelsee in May is related to a short development of *Planktothrix agardhii,* whereas the previous higher development of *Limnothrix redekei* is only expressed in the biovolume curve. At the end of July the development of

Table 4. Linear regression analysis for calculating algal class biovolumes as a function of marker pigment concentration (regression forced through the origin: biovolume = pigment concentration * factor, d.f. = 78; tabulated correlation coefficient: $r^2 = 0.0836$, $P<0.01$).

Algal class [mm^3/l]		Marker pigment concentration [µg/l]		Slope [$mm^3/\mu g$]	r^2	Relative standard deviation of slope [%]
Phytoplankton	=	chlorophyll-a	*	0.330	0.7494	4.19
Cyanophyceae	=	echinenone	*	9.778	0.7451	5.33
Bacillariophyceae	=	fucoxanthin	*	0.712	0.8201	3.90
Cryptophyceae	=	alloxanthin	*	0.411	0.1717	8.41
Chlorophyceae	=	chlorophyll-b	*	0.249	0.2054	13.63

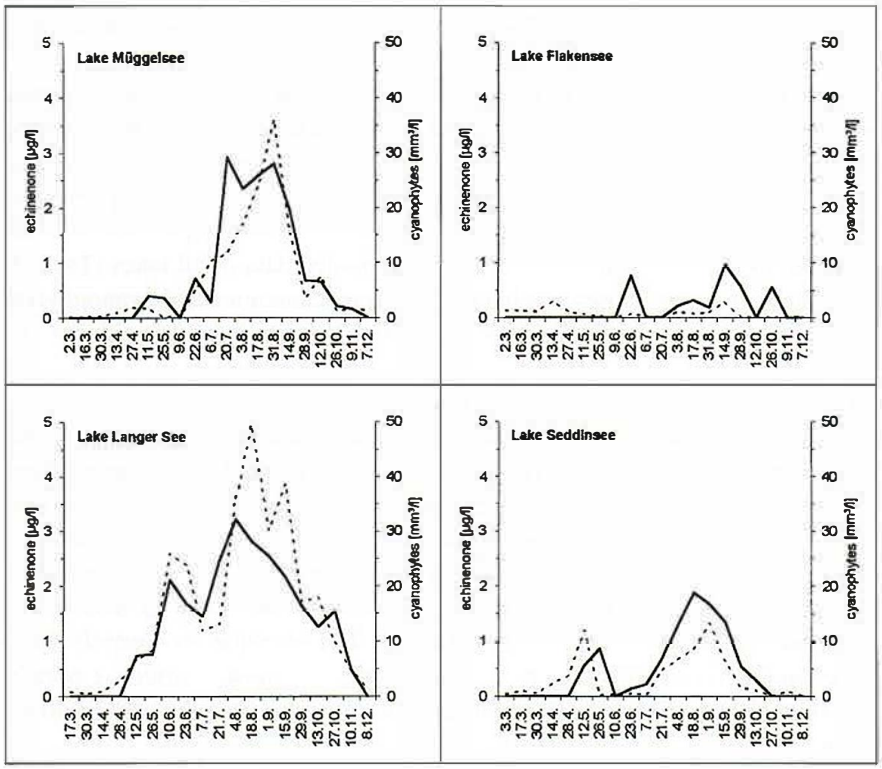

Fig. 3 A.

Aphanizomenon flos-aquae is related to high echinenone content relative to the biovolume followed by the dominance of *Microcystis* spec., which is equally expressed in biovolume and echinenone content. These data suggest that different species dominance may be related to different echinenone content per biovolume. However, the deviations in echinenone and biovolume curves in Lake Langer See are not related to species succession because *Planktothrix agardhii* dominates the blue-green algae from the end of May by more than 95 %. The deviations afterwards may be related to physiological adaptations or to analytical and counting errors.

The highest diatom biovolume, and therefore the highest concentrations of their specific pigment fucoxanthin, were found in all investigated lakes in spring and autumn. Only in Lake Flakensee relatively constant diatom biovolumes were counted over the whole sampling period (Fig. 3 B). Lake Müggelsee shows an additional diatom peak in summer. The deviations between biovolume and fucoxanthin curves cannot be easily related to changes in species composition, because a diverse spectrum of species are present at most sam-

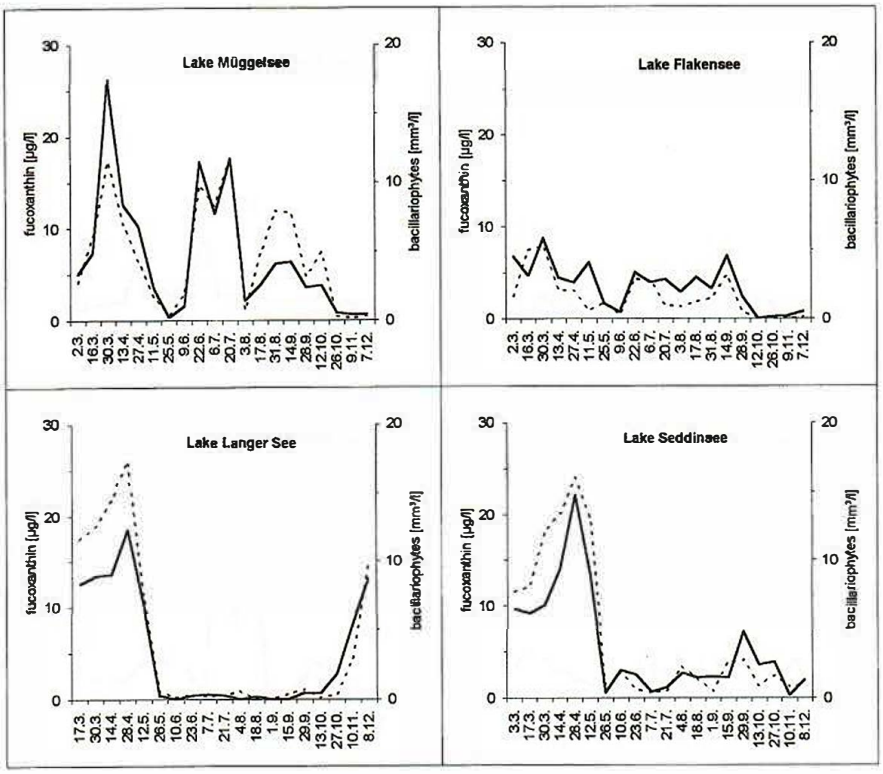

Fig. 3 B.

pling dates. Nevertheless, the lower fucoxanthin content in spring is related to the dominance of solitary centric diatoms in all lakes, whereas the summer peak of Lake Müggelsee consists of more than 90 % of *Melosira* and *Aulacoseira* species.

Cryptophytes were present during the whole sampling period (Fig. 3 C), which could also be concluded from the determined concentrations of the xanthophyll alloxanthin, with a distinct summer maximum in Lake Seddinsee (after the clear-water period). The maxima are sometimes well represented in pigment content, but they are mostly underrepresented in the alloxanthin content in spring. Relations to different species composition are not visible.

Fig. 3 D displays the biovolume data of the Chlorophyceae together with the measured chlorophyll-b concentrations in the lakes under investigation. These profiles show differences in all lakes in spring with very high chlorophyll-b contents and remarkable differences in chlorophyll-b and biovolume peaks in summer. The exceptional occurrence of higher abundances of green algae in Lake Müggelsee, immediately after the clear-water period, occurs

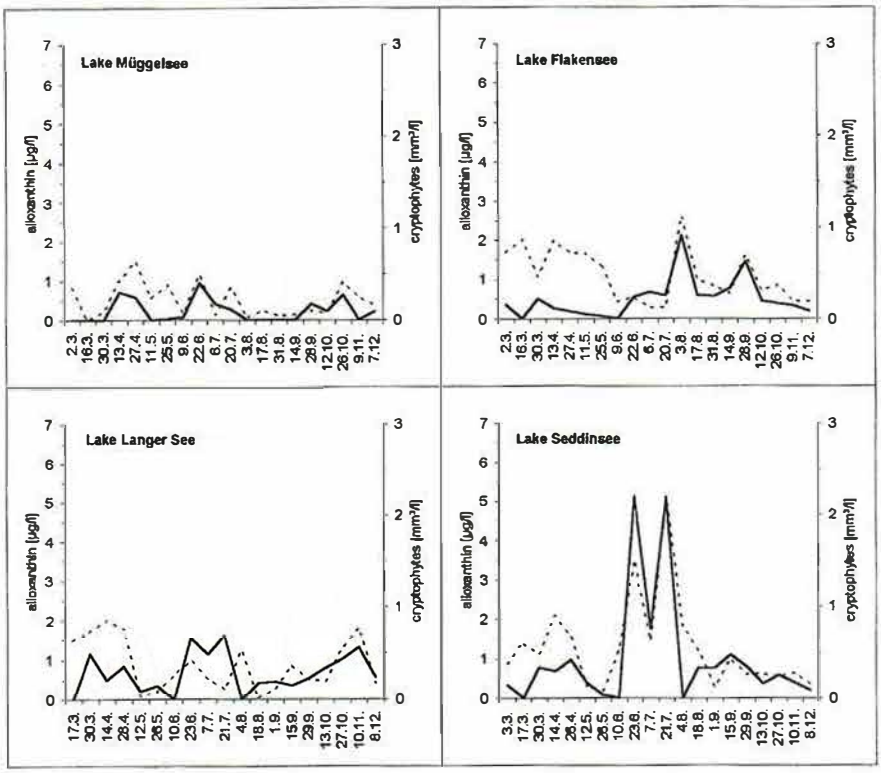

Fig. 3 C.

regularly (KOHL et al. 1991) and is well expressed also in the chlorophyll-b peak. In Lake Flakensee and Lake Seddinsee comparable chlorophyceaen maxima were found, which are not equally represented by the measured chlorophyll-b concentrations.

Marker pigment based analysis of phytoplankton composition

The highly significant correlation between overall biovolume of the most represented algal classes and their specific marker pigments points to an application of correlation analysis for simulating phytoplankton dynamics on the basis of marker pigment concentrations.

Fig. 4 displays microscopically counted phytoplankton data (Fig. 4 A) and calculated algal class biovolumes (Fig. 4 B) using the equations of Table 4 for Lake Müggelsee as an example. A second approach is based on a multiple regression analysis of the chlorophyll-a concentration in dependence of the selected marker pigments (GIESKES et al. 1988). Table 5 summarizes the results of this regression for the used pigments echinenone, fucoxanthin, alloxanthin

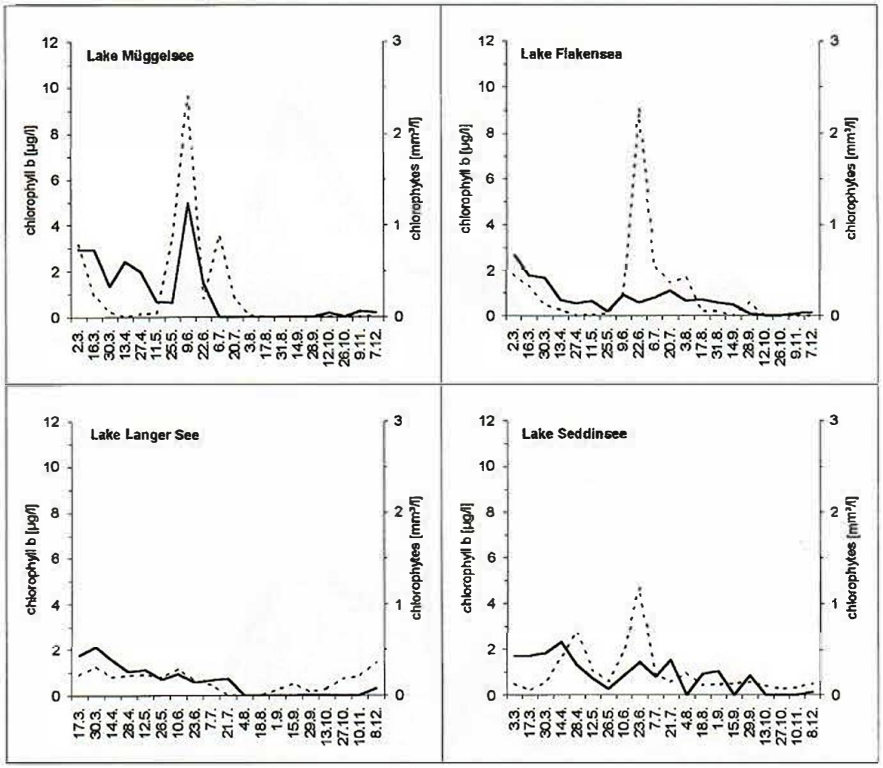

Fig. 3 D.

Fig. 3. Concentration profiles of chromatographically recorded marker pigments (——) and microscopically determined phytoplankton biovolumes of relevant algal classes (- - - -) in the investigated lakes in 1992. **A** – echinenone (Cyanophyceae); **B** – fucoxanthin (Bacillariophyceae); **C** – alloxanthin (Cryptophyceae); **D** – chlorophyll-b (Chlorophyceae).

and chlorophyll-b. In Fig. 5 the predicted chlorophyll-a concentrations are plotted against the chromatographically determined ones. As can be concluded from the high correlation coefficient of the multiple regression function (Table 5) as well as from the similarity between calculated and measured chlorophyll-a values (Fig. 5) the content of this common pigment can be described by the contribution of the different algal classes represented by the specific marker pigments of Cyanophyceae (echinenone), Cryptophyceae (alloxanthin), Bacillariophyceae (fucoxanthin) and Chlorophyceae (chlorophyll-b). The determination is good for the dominant groups (Cyanophyceae and Bacillariophyceae) but lower in the case of Cryptophyceae and Chlorophyceae, as the relative standard deviations of the individual regression coefficients suggest (Table 5). The contribution of other algal classes are insignificant, because the

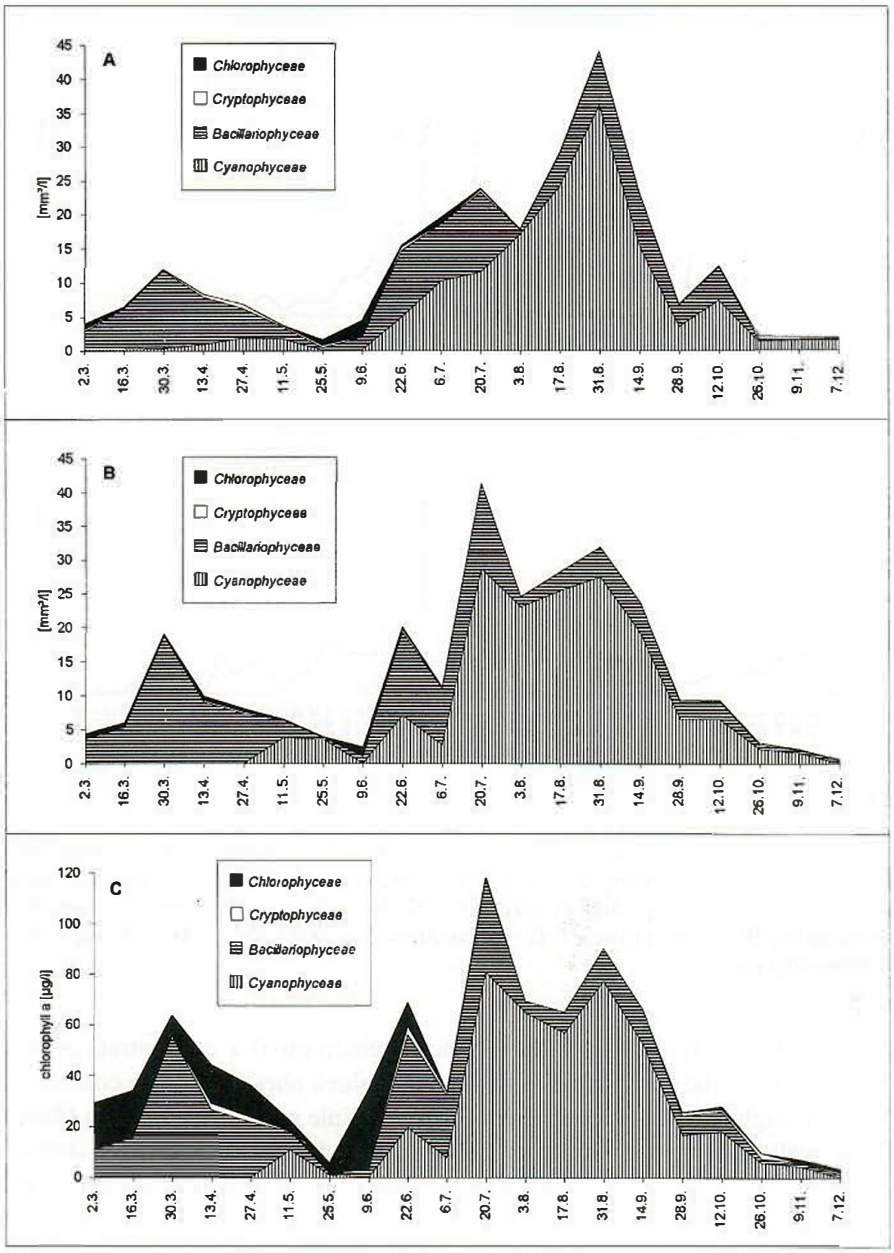

Fig. 4. Comparison between microscopically determined (**A**) and marker pigment based calculated phytoplankton succession (**B** and **C**) in Lake Müggelsee in 1992. **B** – estimation of algal class biovolumes, according to a simple linear regression (see Table 4); **C** – contribution of algal classes to total chlorophyll-a, according to a multiple regression (see Table 5).

Table 5. Multiple linear regression analysis of chlorophyll-a in dependence of selected marker pigment concentrations (regression forced through the origin: Chl-a = b_1 * Ech + b_2 * Fuc + b_3 * Allo + b_4 * Chl-b, r^2 = 0.9233, d. f. = 75; tabulated correlation coefficient: r^2 = 0.0871, P<0.01).

Marker pigment		Coefficient	Significance	Relative standard deviation of coefficient	Partial correlation coefficient (r_i^2)
echinenone	(Ech)	b_1 = 27.51	P<0.01	2.82 %	0.6972
fucoxanthin	(Fuc)	b_2 = 2.11	P<0.01	6.50 %	0.4775
alloxanthin	(Allo)	b_3 = 3.42	P<0.01	25.37 %	0.2520
chlorophyll-b	(Chl-b)	b_4 = 6.27	P<0.01	14.33 %	0.3025

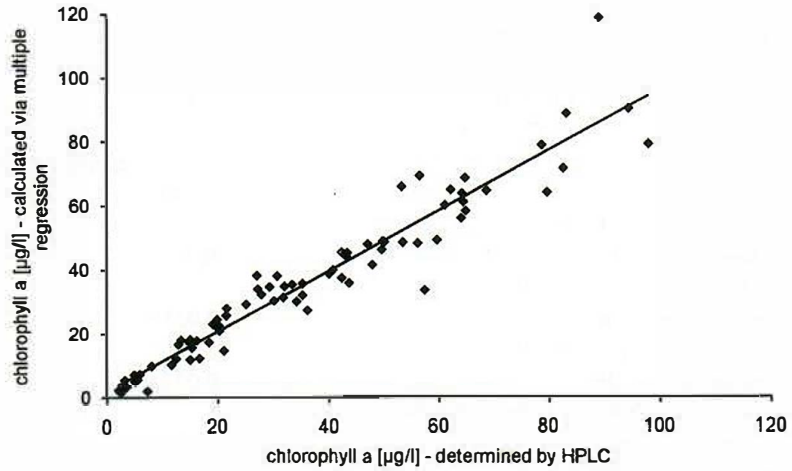

Fig. 5. Plot of calculated chlorophyll-a concentrations (see Table 5) for all data of 1992 against chromatographically determined concentrations of chlorophyll-a (regression function: y = 0.941 * x + 1.81, r^2 = 0.9239, d.f. = 78; tabulated correlation coefficient: r^2 = 0.0836, P<0.01).

regression function calculates the chlorophyll-a concentration with low residuals (see Fig. 5). For the individual algal classes, estimates of the marker pigment/chlorophyll-a ratios are a further result of this multiple regression (the reciprocal values of the coefficients in Table 5), but these values are means for all lakes and the whole sampling period and include, therefore, all species of an algal class present in these lakes. A comparison of these ratios with those determined with algal cultures is shown in Table 6 for Bacillariophyceae and for Cyanophyceae. The ratios determined via multiple regression analysis of lake samples fit well to the range of these parameters for the individual cultures (Table 6), demonstrating the applicability of the approach for describing pigment relations. Following this approach, it should be possible to determine

Table 6. Comparison of marker pigment/chlorophyll-a ratios [μg/μg] of several species of the Bacillariophyceae and the Cyanophyceae.

Bacillariophyceae	fucoxanthin/chlorophyll-a
Cyclotella meneghiniana (WILHELM et al. 1991)	0.627
Phaeodactylum tricornutum (BERKALOFF et al. 1990)	0.479
Skeletonema costatum (GILSTAD et al. 1993)	0.529
Synedra acus (NICKLISCH & WOITKE, unpubl. results)	0.349
Stephanodiscus hantzschii (NICKLISCH & WOITKE, unpubl. results)	0.414
multiple regression of pigment fingerprints (GIESKES et al. 1988)	0.581
mean value of all lakes in 1992 (as a result of multiple regression, see Table 5)	0.475
Cyanophyceae	echinenone/chlorophyll-a
Microcystis aeruginosa (STRANSKY & HAGER 1970)	0.170
Anabaena variabilis (STRANSKY & HAGER 1970)	0.347
Synechococcus elongatus (STRANSKY & HAGER 1970)	0.0215
Planktothrix agardhii (NICKLISCH & WOITKE, unpubl. results)	0.0274
Limnothrix redekii (NICKLISCH & WOITKE, unpubl. results)	0.0037
Microcystis aeruginosa (strain HUB 5-3)	0.0523
Microcystis aeruginosa (strain HUB 524) (NICKLISCH & WOITKE, unpubl. results)	0.0480
mean value of all lakes in 1992 (as a result of multiple regression, see Table 5)	0.0363

the percentage contribution of an algal class to total chlorophyll-a (GIESKES et al. 1988, WILHELM et al. 1991). For Lake Müggelsee, the results are displayed in Fig. 4 C for comparison with the microscopically counted phytoplankton composition (Fig. 4 A), and along with the results of the linear regression for simulating phytoplankton dynamics (Fig. 4 B). Both simulation approaches show a certain similarity to the directly determined phytoplankton dynamics. Regarding the most prominent algal classes, the same deviations in both simulation approaches are visible. They are obviously related to different pigment contents of the species dominant at the given sampling date.

A variety of discrepancies is evident on several sampling dates regarding the most prominent algal classes. For example, a contribution of Cyanophyceae to total chlorophyll-a started only in May, as estimated on the basis of the marker pigment echinenone. Whereas, the counting technique yielded cyanophyceaen species already at the beginning of the sampling period. This may be caused by the presence of *Limnothrix redekii* within the Cyanophyceae until May and their outstanding low echinenone/chlorophyll-a ratio (see Table 6). The same differences in echinenone content may indicate the different reflection of the biovolumes in the pigment content during the succession from *Aphanizomenon flos-aquae* to *Microcystis* spec. during summer. Furthermore, the Bacillariophyceae achieved a higher percentage of phytoplankton, when microscopically determined in comparison to the pigment-based calculated values during the sampling period. The opposite case can be observed for the Chlorophyceae for most of the sampling dates, as found by WILHELM et al. (1991), too. Taking into account that the multiple regression calculates the contribution of an algal class to total chlorophyll-a, these findings indicate a higher chlorophyll-a content (per biovolume) of green algae in comparison to diatoms. This corresponds well with results regarding the chlorophyll-a content of dominating algal classes found by NIXDORF & HOEG (1993) for Lake Müggelsee from 1979 to 1990 and is known from the literature (TOLSTOY 1979).

Effects of the physiological status

Any approach to substitute phytoplankton counts by marker pigment methods requires relatively constant marker pigment/chlorophyll-a or marker pigment/biovolume ratios for the considered algal class independent of the species composition or their physiological state. Despite a variety of problems regarding the microscopic observations (counting errors, fixation problems etc.), as discussed in detail by GIESKES & KRAAY (1983) or by WILHELM et al. (1991), the following reasons may be responsible for the unsatisfactory coincidence between pigment-based calculated and microscopically counted phytoplankton composition.

First, the species composition within a considered algal class is different between the investigated lakes or is changing during the sampling period. The cyanophytes in Lake Langer See, for example, were dominated by *Planktothrix agardhii* from June to November. For this time period, a mean ($\pm$ 95 % c.l.) echinenone content in this algal class of $0.094 \pm 0.023\ \mu g/mm^3$ ($n = 11$) was calculated. In all other lakes the cyanophyceaen population consisted of some other species (i.e. *Microcystis* spec., *Aphanizomenon* spec., *Limnothrix redekii*) with different echinenone/chlorophyll-a ratios (Table 6) or different echinenone contents. The highest echinenone content ($0.44\ \mu g/mm^3$) was de-

termined in Lake Müggelsee at the end of May. At that time, the cyanophyceaen population was dominated by *Aphanizomenon* species. In autumn the *Aphanizomenon* species were completely displaced by *Microcystis* species decreasing the cyanophyceaen echinenone content to values below 0.20 µg/mm^3. These remarkable differences lead either to an over- or an underestimation of the cyanophyceaen biovolume (see Fig. 4 B) or to a miscalculation of the contribution of this algal class to total chlorophyll-a (see Fig. 4 C), respectively. Similar variations of marker pigment/biovolume ratios, due to changing species composition, were found for the other investigated algal classes. A further examination of the pigment content or the pigment/chlorophyll-a ratio of various species within the same algal class, using laboratory grown unialgal cultures, would clarify their influence on the estimation of phytoplankton biovolume or composition under field conditions.

On the other hand, different physiological states (for example, nutrient and/or light limitation or saturation) caused by changing environmental conditions can effect varying pigment contents due to adaptation of the photosynthetic apparatus. HPLC-aided pigment analysis should be useful to detect such adaptations in combination with cell counting, and would offer the possibility of monitoring phytoplankton dynamics together with an insight into the ecophysiology of algae. Taking into account different pigment patterns of various species within the same class, this may only be possible during time periods with dominance of a single species. Therefore, the data sets were checked for time periods, in which single species or groups amounted to more than 80 % biovolume of the whole class. This does not necessarily imply an overall dominance within the whole phytoplankton. Pooled data of these time periods were chosen for testing the influence of selected environmental parameters on marker pigment/biovolume ratios. In addition to the parameters listed in Table 2, the following data were used for a correlation analysis of the marker pigment content against selected environmental variables: PAR, TN/PP (total nitrogen in the samples related to total phytoplankton biovolume as an estimate for phytoplankton nitrogen supply, [µmol/mm^3]) and TP/PP (total phosphorus in the samples related to total phytoplankton biovolume as an estimate for phytoplankton phosphorus supply, [µmol/mm^3]). The results of this correlation analysis are listed in Table 7. Although all data were reduced to time periods with single species dominance within a regarded algal class, there are only two parameters which are significantly correlated with the marker pigment content. During time periods with dominating *Aulacoseira* spec., relations between their fucoxanthin content and light climate, silicate, nitrogen or phosphorus supply could not be found (Table 7), but a strong positive correlation could be detected for *Microcystis* spec. between their echinenone content and the concentration of dissolved inorganic nitrogen (DIN). This indicates an increased synthesis of this xanthophyll under saturating nitrogen conditions or a

Table 7. Correlation coefficients of marker pigment/biovolume ratios against measured environmental variables (bold typed values are above the 95 % significance level) for time periods with single species dominance within their algal class (more than 80 % of algal class biovolume).

Parameter*	dominating species within the appropriate algal class		
	Aulacoseira spec. (Bacillariophyceae) fucoxanthin content	*Planktothrix agardhii* (Cyanophyceae) echinenone content	*Microcystis* spec. (Cyanophyceae) echinenone content
silicate	0.041	−0.332	−0.050
DIN	−0.044	0.364	**0.920**
phosphate	0.104	−0.308	−0.332
TP	0.070	−0.315	−0.298
TN	0.064	−0.165	−0.112
PAR	−0.139	−0.009	−0.172
TN/PP	0.038	**0.712**	**0.850**
TP/PP	0.016	0.398	0.575

* For abbreviations see Table 2.

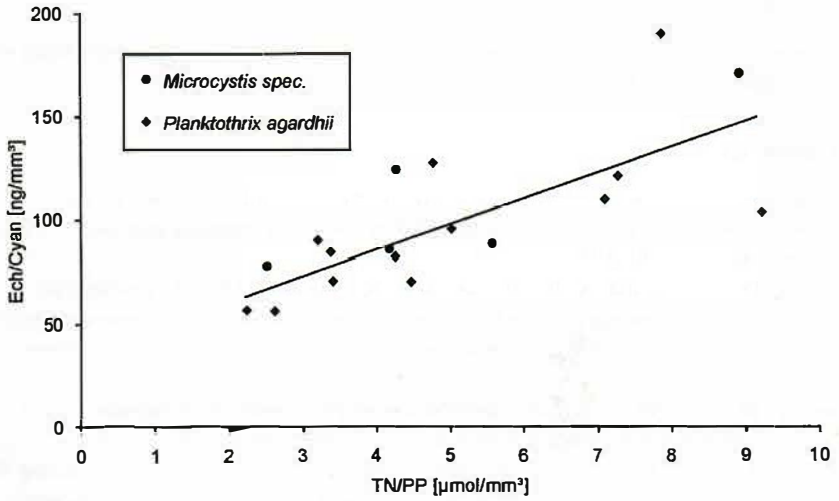

Fig. 6. Relation between the echinenone content of cyanophytes (Ech/Cyan) and nitrogen availability (calculated as the quotient of total nitrogen and total phytoplankton – TN/PP) during dominance (>80 % of the cyanophycean biovolume) of either *Planktothrix agardhii* (Lake Langer See) or *Microcystis* spec. (Lake Müggelsee); (regression function: $y = 12.4 * x + 36.7$, $r^2 = 0.5829$, d.f. = 15; tabulated correlation coefficient: $r^2 = 0.3672$, $P < 0.01$).

reduction of the echinenone content under nitrogen limitation, respectively. The correlation between the echinenone content and the nitrogen availability can be better demonstrated using the TN/PP parameter, a value corresponding to the nitrogen limitation status. Fig. 6 shows an increasing echinenone con-

tent of *Microcystis* spec. and of *Planktothrix agardhii* with increasing values of TN/PP, indicating a decreasing content of this xanthophyll during time periods of reduced nitrogen availability. A decreased echinenone content was also found by RÜCKER et al. (1995) in laboratory grown *Planktothrix agardhii* cultures under nitrogen as well as under phosphorus limitation. Therefore, the echinenone/biovolume ratio may be usable as an indicator for the nutrient status of *Microcystis* spec. or of *Planktothrix agardhii*. In combination with cell counting it demonstrates the applicability of chromatographically determined pigment patterns of field samples for obtaining more pronounced information about the ecophysiology of lake phytoplankton. Further investigations using laboratory grown algal cultures, as well as field observations are necessary to get a better insight into the question if and how changing environmental parameters influence the pigment pattern of phytoplankton and if pigment determinations are a helpful tool to characterize physiological states of algae under field conditions.

Acknowledgement

This work is part of a project supported by the Bundesministerium für Forschung und Technologie (BEO 339400 A).

References

BERKALOFF, C., CARON, L. & ROUSSEAU, B. (1990): Subunit organization of PSI particles from brown algae and diatoms: polypeptide and pigment analysis. – Photosynth. Res. **23:** 181–193.

EVERITT, D. A., WRIGHT, S. W., VOLKMAN, J. K., THOMAS, D. P. & LINDSTROM, E. J. (1990): Phytoplankton community compositions in the western equatorial Pacific determined from chlorophyll and carotenoid pigment distribution. – Deep-Sea Res. **37:** 975–997.

FAWLEY, M. W. (1991): Disjunct distribution of the xanthophyll loroxanthin in the green algae (Chlorophyta). – J. Phycol. **27:** 544–548.

GIESKES, W. W. C. & KRAAY, G. W. (1983): Dominance of Cryptophyceae during the phytoplankton spring bloom in the central North Sea detected by HPLC analysis of pigments. – Mar. Biol. **75:** 179–185.

GIESKES, W. W. C., KRAAY, G. W., NONTJI, A., SETIAPERMANA, D. & SUTOMO (1988): Monsoonal alteration of a mixed and a layered structure in the phytoplankton of the euphotic zone of the Banda Sea (Indonesia): a mathematical analysis of algal pigment fingerprints. – Neth. J. Sea Res. **22:** 123–137.

GILSTAD, M., JOHNSON, G. & SAKSHAUG, E. (1993): Photosynthetic parameters, pigment composition and respiration rates of the marine diatom *Skeletonema costatum* grown in continuous light and a 12 : 12 h light-dark cycle. – J. Plankton Res. **15:** 939–951.

GOODWIN, T. W. (1988): Plant Pigments. – Academic Press, London.

– (1976): Chemistry and biochemistry of plant pigments. Vol. 2. – Academic Press, London.

GROTJOHANN, R., RHO, M.-S. & KOWALLIK, W. (1992): Influences of blue and red light on the photosynthetic apparatus of *Chlorella kessleri*. – Bot. Acta **105:** 168–173.

HALLEGRAEF, G. M., NICHOLS, P. D., VOLKMAN, J. K., BLACKBURN, S. I. & EVERITT, D. A. (1991): Pigments, fatty acids, and sterols of the toxic Dinoflagellate *Gymnodium catenatum*. – J. Phycol. **27:** 591–599.

HURLEY, J. P. & ARMSTRONG, D. E. (1990): Fluxes and transformation of aquatic pigments in Lake Mendota, Wisconsin. – Limnol. Oceanogr. **35:** 384–398.

JEFFREY, S. W. & VESK, M. (1977): Effect of blue-green light on photosynthetic pigments and chloroplast structure in the marine diatom *Stephanopyxis turris*. – J. Phycol. **13:** 271–279.

KOHATA, K. & WATANABE, M. (1988): Diel changes in the composition of photosynthetic pigments and cellular carbon and nitrogen in *Chattonella antiqua*. – J. Phycol. **24:** 58–66.

KOHL, J.-G., DUDEL, G., HENNING, M., HENZE, R., NICKLISCH, A., TEUBNER, K. & TIPPMANN, P. (1991): Langzeittrends und multistabile Zustände im Phytoplankton eines polymiktischen Fluß-Sees (Großer Müggelsee, Berlin). – In: Deutsche Gesellschaft für Limnologie, Erweiterte Zusammenfassung der Jahrestagung 1991 (30.9.–6.10.1991), Mondsee, pp. 282–232.

KOHL, J.-G., DUDEL, G., SCHLANGSTEDT, M. & KÜHL, H. (1985): On the morphological and ecological distinction of *Aphanizomenon flos-aquae* RALFS ex BORN. et FLAH. and *A. gracile* (LEMM.) LEMM. – Arch. Protistenk. **130:** 119–131.

KOHL, J.-G. & NICKLISCH, A. (1988): Ökophysiologie der Algen. – Akademie-Verlag, Berlin.

LAWS, E. A. & BANNISTER, T. T. (1980): Nutrient- and light-limited growth of *Thalassiosira fluviatilis* in continuous culture, with implications for phytoplankton growth in the ocean. – Limnol. Oceanogr. **25:** 457–473.

LEAVITT, P. R., CARPENTER, S. R. & KITCHELL, J. F. (1989): Whole-lake experiments: The annual record of fossil pigments and zooplankton. – Limnol. Oceanogr. **34:** 700–717.

MANTOURA, R. F. C. & LLEWELLYN, C. A. (1983): The rapid determination of algal chlorophyll and carotenoid pigments and their breakdown products in natural waters by reverse-phase high-performance liquid chromatography. – Anal. Chim. Acta **151:** 297–314.

MILLIE, D. F., INGRAM, D. A. & DIONIGI, C. P. (1990): Pigment and photosynthetic response of *Oscillatoria agardhii* (Cyanophyta) to photon flux density and spectral quality. – J. Phycol. **26:** 660–666.

NIXDORF, B. & HOEG, S. (1993): Phytoplankton – Community Structure, Succession and Chlorophyll Content in Lake Müggelsee from 1979 to 1990. – Int. Rev. ges. Hydrobiol. **78:** 359–377.

PALERMO, J. A., GROS, E. G. & SELDES, A. M. (1991): Carotenoids from three red algae of the Corallinaceae. – Phytochem. **30:** 2983–2986.

RÜCKER, J. & KOHL, J.-G. (1994): Indication of growth-limiting factors of planktonic cyanobacteria by application of microscope-photometry. – Verh. Int. Verein. Limnol. **25:** 2218–2220.

RÜCKER, J., KOHL, J.-G. & KAISER, K. (1995): Responses of carotenoids and chlorophylls to variations of growth-limiting factors in three filamentous blue-green algae. – Algological Studies **77:** 51–65.

SENGE, M. & SENGER, H. (1990 a): Response of the photosynthetic apparatus during adaptation of *Chlorella* and *Ankistrodesmus* to irradiance changes. – J. Plant Physiol. **136:** 675–679.

– – (1990 b): Adaptation of the Photosynthetic Apparatus of *Chlorella* and *Ankistrodesmus* to Blue and Red Light. – Bot. Acta **104:** 139–143.

STRANSKY, H. & HAGER, A. (1970): Das Carotinoidmuster und die Verbreitung des lichtinduzierten Xanthophyllcyclus in verschiedenen Algenklassen. IV. Cyanophyceae und Rhodophyceae. – Arch. Mikrobiol. **72:** 84–96.

TÄUSCHER, L. (1980): Untersuchungen zur Art- und Biozönosestruktur des Phytoplanktons im Großen Müggelsee (Berlin) unter Berücksichtigung produktions- und sapro-biologischer Aspekte. – Ph. D. Thesis, Humboldt-University, Berlin.

TOLSTOY, A. (1979): Chlorophyll-a in relation to phytoplankton volume in some Swedish lakes. – Arch. Hydrobiol. **85:** 133–151.

UTERMÖHL, H. (1958): Zur Vervollkommnung der quantitativen Phytoplankton-Methodik. – Mitt. Int. Verein. Limnol. **9:** 1–38.

VESK, M. & JEFFREY, S. W. (1977): Effect of blue-green light on photosynthetic pigments and chloroplast structure in unicellular marine algae from six classes. – J. Phycol. **13:** 280–288.

WILHELM, C. (1990): The biochemistry and physiology of light-harvesting processes in chlorophyll-b- and chlorophyll-c-containing algae. – Plant Physiol. Biochem. **28:** 293–306.

WILHELM, C. & MANNS, L. (1991): Changes in pigmentation of phytoplankton species during growth and stationary phase – consequences for reliability of pigment-based methods of biomass determination. – J. Appl. Phycol. **3:** 305–310.

WILHELM, C., RUDOLPH, I. & RENNER, W. (1991): A quantitative method based on HPLC-aided pigment analysis to monitor structure and dynamics of the phytoplankton assemblage – A study from Lake Meerfelder Maar (Eifel, Germany). – Arch. Hydrobiol. **123:** 21–35.

WOITKE, P., MARTIN, C.-D., NICKLISCH, S. & KOHL, J.-G. (1994): HPLC-determination of lipophilic photosynthetic pigments in algal cultures and lake water samples using a non-endcapped C18-RP-column. – Fresh. J. Anal. Chem. **348:** 762–768.

Submitted: 18 July 1995; accepted: 25 February 1996.