

HPLC analysis of phytoplankton pigments from Lake Kinneret with special reference to the bloom-forming dinoflagellate *Peridinium gatunense* (Dinophyceae) and chlorophyll degradation products

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Abstract. Photosynthetic pigments extracted from the particulate material of the water column of Lake Kinneret were studied throughout the periods of May 1988–June 1989, and November 1993–November 1994, by means of HPLC. The temporal and vertical variation of the pigment suite found agreed with the microscopically determined phytoplankton record. The regression calculations of taxon-specific biomass with the corresponding signature pigments suggest that pigment analysis may be a useful tool for the monitoring of bloom-forming species, e.g. the dinoflagellate *Peridinium gatunense* Nygaard. The HPLC pigment analysis permitted the identification and quantification of chlorophyll degradation products, providing for the first time information about their composition in Lake Kinneret. Chlorophyllide *a* was the major detectable degradation product of chlorophyll *a*, varying between 1 and 9% of the chlorophyll *a* concentration. Other chlorophyll *a* derivatives appeared mostly in minor quantities. Pheophytin *a* was virtually lacking in all the samples. Removal rates of pigments, measured by sedimentation traps, indicated that the degradation of chlorophyll *a* via chlorophyllide *a* is a dynamic process that continues during the sedimentation of the phytoplankton particles.

Introduction

The inventory of pigments is a key characteristic of phototrophic organisms which is used as a criterion in the classification of autotrophic bacteria (Trüper, 1987) and algae (Rowan, 1989). Since some pigments (or pigment combinations) are taxon unique, they have been used to monitor phytoplankton constituents. The analysis of pigments has been facilitated in recent years by the application of high-performance liquid chromatography (HPLC) (Abaychi and Riley, 1979; Mantoura and Llewellyn, 1983; Wright and Shearer, 1984; Korthals and Steenbergen, 1985) and it is now routinely used for the examination of pigments in aquatic samples. HPLC-analyzed pigment extracts have been used to determine the phytoplankton composition in marine and freshwater ecosystems, and to study algal bloom dynamics, by measuring the signature pigment(s) of the dominating species (Gieskes and Kraay, 1983a, 1986a; Ridout and Morris, 1985, 1988; Klein and Sournia, 1987; Fielding and Seiderer, 1991; Ondrusek *et al.*, 1991; Williams and Claustre, 1991; Bianchi *et al.*, 1993). Several attempts have been made to quantify dominant algal groups using pigment analysis (Gieskes and Kraay, 1986b; Gieskes *et al.*, 1988; Everitt *et al.*, 1990). In some cases, the pigment analysis was coupled with microscopic observation (Gieskes and Kraay, 1983a,b, 1984, 1986a,b; Ridout

and Morris, 1985, 1988; Klein and Sournia, 1987; Gieskes *et al.*, 1988; Steenbergen and Korthals, 1988; Volkman *et al.*, 1988; Wilhelm *et al.*, 1991; Soma *et al.*, 1993) or flow cytometric analysis (Vaulot *et al.*, 1990; Veldhuis and Kraay, 1990), thus verifying the correspondence of signature pigments with phytoplankton species composition.

In addition to the chemotaxonomic information provided, quantitative relationships between different pigments may be indicative of the physiological status of the phytoplankton community (Dubinsky and Polna, 1976; Ridout and Morris, 1985; Klein, 1988; Brunet *et al.*, 1992). Of particular importance is the ratio between chlorophyll *a* and its degradation products: chlorophyllide, pheophorbide and pheophytin. Prior to the advent of chromatographic methods, the presence of chlorophyll degradation products was deduced by observing the change obtained in a spectrophotometric reading or fluorometric reading of pigment extract following acidification. Chromatographic techniques, and HPLC in particular, permit the separation of different pigments, thereby allowing the direct measurement of the various chloropigments (Sartory, 1985; Murray *et al.*, 1986; Trees *et al.*, 1986).

The work presented here was carried out in Lake Kinneret, which has been regularly monitored for >20 years. Throughout this period, phytoplankton biomass, fluorometrically determined chlorophyll *a* and 'pheophytin' were recorded. The aim of this study was to measure the temporal and vertical distribution of the major photosynthetic pigments throughout a full annual cycle and to compare the microscopically determined phytoplankton biomass with the pigment record. Special attention was given to chlorophyll degradation products during the monitoring period, in order to determine signals that are related to degradation processes in the phytoplankton population.

The lake

Lake Kinneret is a warm monomictic lake with a surface area of 168 km². Its average and maximum depths are 23 and 43 m, respectively. The lake is stratified between May and December (thermocline at 15–17 m). The hypolimnion is devoid of O₂ and rich in H₂S. The Jordan River is the major inflow, while water pumped into the National Water Carrier constitutes the main outflow. Since the water provision and exploitation are not symmetrical, the lake level fluctuates between 209.0 and 212.5 m below sea level. For a more detailed description of the lake, see Serruya (1978).

The following features characterize the phototrophic populations of the lake (Pollinger, 1986). (i) A winter–spring bloom of the dinoflagellate *Peridinium gatunense* Nygaard, comprising >90% of the algal biomass during the bloom and 59–90% on an annual basis. (ii) A summer–autumn assemblage dominated by chlorophytes (e.g. *Scenedesmus* sp., *Pediastrum* sp., *Coelestrum* sp., *Chodatella* sp., *Tetraedron* sp., *Cosmarium* sp.) and accompanied by diatoms and nanoplanktonic cyanobacteria. (iii) Sporadic winter blooms of the large centric diatom, *Aulacoseira granulata* (Ehrbg.) Ralfs. This heavy filamentous alga reaches the photic layers of the lake from the sediments during periods of high turbulence, generated

by winds. (iv) The green sulfur bacterium, *Chlorobium phaeobacteroides* Pfennig, blooms in the metalimnion of Lake Kinneret in the summer–autumn period (Bergstein *et al.*, 1979).

Method

Water samples were collected at a fixed pelagic station located at the center of the lake with an Aberg–Rhode 5 l sampler twice a month between May 1988 and June 1989. Water samples were withdrawn from depths of 1, 5, 10, 20, 30 and 40 m on each date, transferred to plastic opaque containers, and taken to the laboratory within ~30 min. Duplicate subsamples of 500–2000 ml were filtered onto GF/C filters under subdued light and immediately processed or stored at -20°C in the dark. The latter procedure does not enhance pigment degradation, at least within the time span of 1 week (Gieskes and Kraay, 1983a) used in the current work.

A second sampling program was performed between December 1993 and November 1994. A composite sample taken from the depths of 1, 2, 3, 5, 7, 10 and 15 m was used as a representative of the euphotic zone. A sample taken from 40 m depth was used as a representative of the dark portion of the water column. During the stratification period, this sample always presented hypolimnetic water. Sediment traps were positioned at depths of 15 and 35 m at Station A, and sinking material was collected during 24 h. Applying this short time of particle collection reduced the chance for artifact formation, not occurring in the water column under natural circumstances. Sedimented subsamples of 250–300 ml were filtered onto GF/C filters and processed in the same way that water column samples were treated.

Frozen filters, and immediately processed filters, were sonicated in 10 ml of cold 90% acetone. The extraction lasted ~4 h, and the extracts were kept in the dark at $2-4^{\circ}\text{C}$. Comparison of extracts in 90% acetone and 100% methanol showed no measurable difference in the proportion of chlorophyll degradation products in these solvents, in our experiments. The acetone extract was filtered onto a GF/F filter, and a 500 μl aliquot was mixed with 150 μl of 1 M ammonium acetate to facilitate the separation of hydrophilic components of the extract, and to prevent coagulation/precipitation effects and therefore losses in pigments (Zapata *et al.*, 1987).

The reverse-phase HPLC system consisted of a multiple-solvent delivery pump (CM4000 LDC Milton-Roy), an injector (Rheodyne) equipped with a 100 μl loop and a C-18, 25×4.6 mm (Alltech) Spherisorb column. The pigments were detected with a variable-wavelength spectrophotometer (LDC Milton-Roy) set at 436 nm and a fluorometer (ex = 440 nm; em = 500–800 nm). Data were recorded with a CI-10 integrator (LDC Milton-Roy).

The experimental schedule of the HPLC system was a modification of those previously published by Klein and Sournia (1987) and Zapata *et al.* (1987). Two solvents were used in the system: solvent A consisted of 30% 1 M ammonium acetate (Sigma) in double-distilled deionized water and 70% methanol (HPLC grade, Bio Lab, Israel); solvent B consisted of 30% ethyl acetate (HPLC grade; Bio Lab, Israel) and 70% methanol. The solvent program showed a linear increase of

solvent B from 20 to 60% in 7 min, a hold at 60% for 5 min, a linear increase from 60 to 100% solvent B from 12 to 20 min, followed by a hold at 100% B for 20 min.

Peak identification was facilitated by comparison with (i) chromatograms from unialgal cultures of phytoplankton species isolated from Lake Kinneret, algae of typical known pigment composition were chosen, and (ii) purified pigments. Chromatograms obtained by the fluorometric detector were used to verify the identification of chlorophyllous pigments. We used the following cultures from our collection: (i) *A. granulata* (Bacillariophyceae); (ii) *P. gatunense* Nygaard (Dinophyceae); (iii) *Scenedesmus quadricauda* (Turp.) de Brebisson (Chlorophyceae); (iv) *Microcystis aeruginosa* Kutz. (Cyanophyceae); (v) an unidentified flagellate species (Chrysophyceae); (vi) *C. phaeobacteroides* (Chlorobiaceae). Purchased preparations of chlorophyll *a* and chlorophyll *b* (Sigma) were used to produce chlorophyllides, pheophorbides and pheophytins using methods described by Sartory (1985). Chlorophyll *c* and selected carotenoids were isolated from monoalgal culture extracts on-line the HPLC system. *Peridinium gatunense* was the source of purified chlorophyll *c*, peridinin, dinoxanthin and diadinoxanthin. *Aulacoseira granulata* was the source of fucoxanthin. Lutein was derived from *S. quadricauda*, violaxanthin from the unidentified chrysophyte, and zeaxanthin and β -carotene from *M. aeruginosa*. The spectral characteristics of the purified pigments in the HPLC eluent were examined using a spectrophotometer and the optical density determined at 440 nm. The peaks of our chlorophyll *c* preparation showed at 448–449 and 634–635 nm, suggesting a mixture of the c_1 and c_2 forms. The concentration of the pigment was calculated using published extinction coefficients (Davis, 1976; Mantoura and Llewellyn, 1983). The quantification of the chromatograms was facilitated by injection of known pigment concentrations into the HPLC system and calculation of the response factor based on the area under the peak. The average difference between the peak areas of the duplicated lake samples ranged between 8 and 15%.

Our chromatographic system did not separate zeaxanthin from lutein. The corresponding peak of those two pigments was quantified as if only lutein was present.

Concomitant with the study of pigment content and distribution in the lake, samples were routinely collected and monitored for phytoplankton assemblage characterization and algal biomass composition. The taxonomic composition of the phytoplankton and the biomass were determined on samples treated by Utermöhl's sedimentation method (1958). The determination of biomass wet weight standing stock per liter was based on microscopic counts of species, and measurements of the species dimensions and estimation of their volume. The total mass of a given algal species was calculated using the cell number, the average volume of the cells and their specific gravity (assuming that the specific gravity of the algae is 1.0). The areal density of phytobiomass was obtained by planimetry of the trophogenic layer.

Results

Typically, 10–20 peaks were detected on the 436 nm absorbance chromatograms of epilimnion samples (Figure 1a) and 3–10 peaks on chromatograms of

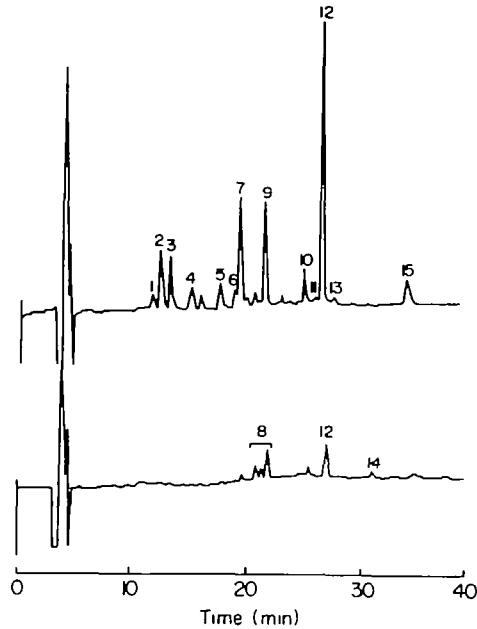


Fig. 1. Separation of pigment extracts from Lake Kinneret water samples. Eluted peaks were detected at 436 nm. **Upper:** Chromatogram of a sample collected on 12 June 1989 from 1 m depth. **Lower:** Chromatogram of a sample collected on 12 June 1989 from 40 m depth. Peak identification: s, solvent front; 1, chlorophyllide *a*; 2, chlorophyll *c*; 3, peridinin; 4, fucoxanthin; 5, violaxanthin; 6, dinoxanthin; 7, diadinoxanthin; 8, bacteriochlorophyll *c*; 9, lutein-zeaxanthin; 10, chlorophyll *b*; 11, allomerized chlorophyll *a*; 12, chlorophyll *a*; 13, epimeric chlorophyll *a*; 14, bacteriochlorophyll *a*; 15, β -carotene.

hypolimnion samples (Figure 1b). The signature pigments of photosynthetic bacteria could be identified on the hypolimnetic (and metalimnetic) samples, and have been described elsewhere (Yacobi *et al.*, 1990). During the mixing period, the number of peaks was similar to that of epilimnetic samples of the stratified lake.

The vertical profiles of different pigments showed diverse patterns of distribution (Figure 2). In the summer–autumn period, the proportions of the most abundant signature pigments did not point to an unequivocal domination by a single group (Figure 2a and b). When the lake was stratified, most of the pigments were detected above 20 m, but their concentrations rapidly declined below the thermocline and were below detection limit below the 30 m depth. Chlorophyll *a* was present in every sample taken from the lake (Figure 2). We found that the concentration of hypolimnetic chlorophyll ranged between 0.15 and 0.97 $\mu\text{g l}^{-1}$. Part of this chlorophyll derived from intact cells, because the carbon fixation of hypolimnetic samples brought to light was above the level of the dark control (data not shown). With the onset of the mixing period, in January, the vertical distribution of all the pigments was relatively uniform (Figure 2c). Fucoxanthin, a carotenoid found in several chlorophyll *c*-containing algal taxons (Bacillariophyceae, Chrysophyceae, Dinophyceae, Prymnesiophyceae), had the highest concentration in January 1989. Subsequently, a build-up of the *Peridinium*

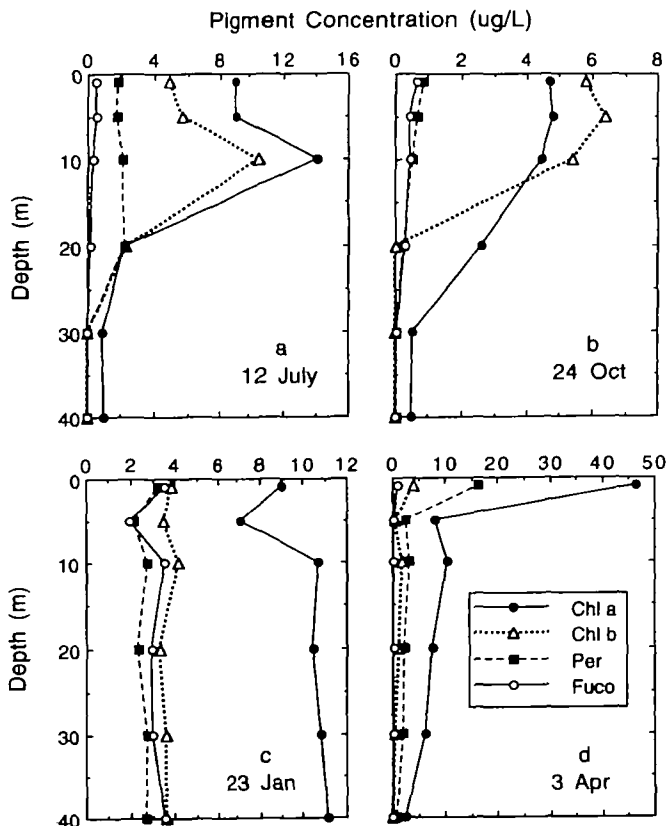


Fig. 2. Vertical distribution of selected pigments, integrated for the top 10 m water column, in water samples collected at different dates during the sampling season of 1988–1989 (the concentration of chlorophyll *b* was magnified 10 times).

population started, shown by a high concentration of peridinin (Figure 2d). The dinoflagellate *P. gatunense* dominates the lake annual bloom (Figure 3a), accounting for the resemblance of chlorophyll *a*, chlorophyll *c* and peridinin vertical and temporal distributions (Figure 3b).

From February to June, *Peridinium* dominates, and during parts of this period its abundance is so high that the lake may be considered practically as a unialgal ecosystem. Chlorophyll *a* content and pigment ratios were calculated for samples in which *P. gatunense* biomass contributed >90% of the phytoplankton standing stock (Table I). The chlorophyll *a* content in various *Peridinium*-dominated samples ranged between 1.4 and 3.8 mg mg⁻¹ wet weight biomass, averaged at 2.8 mg mg⁻¹ wet weight biomass. The average chlorophyll *a/c* ratio was 5.3, with a minimum value of 3.8 and a maximum value of 7.8. There was a tendency for the chlorophyll *a/c* ratio to increase towards the end of the *Peridinium* bloom. The chlorophyll *a*/peridinin ratio showed high stability throughout the *Peridinium* bloom period.

Fucoxanthin was relatively conspicuous (Figure 3c) after the *Peridinium* bloom of 1988 and before the annual bloom of 1989. In January and February 1989, a

Table 1. Chlorophyll *a* content and pigment ratios in Lake Kinneret samples dominated by *P.gatunense* (Dinophyceae)

Date	Depth (m)	Dinophyceae ^a	Chl <i>a</i> content ^b	Chl <i>a</i> /chl <i>c</i> ^c	Chl <i>a</i> /per ^d
15 May 1988	5	95	2.5	5.51	2.33
	10	92	2.6	5.08	2.30
7 March 1989	1	99	1.9	3.77	3.22
20 March 1989	1	99	1.8	4.00	2.98
	5	94	2.1	3.80	3.00
	20	93	1.8	4.91	3.21
	30	93	3.9	4.26	3.31
1 April 1989	1	98	1.6	4.16	2.86
	5	94	3.4	4.61	3.22
	10	92	1.7	4.60	3.16
	20	97	1.4	4.87	3.20
	30	97	3.5	5.02	3.01
	40	98	2.7	5.81	3.26
15 May 1989	1	97	1.4	5.70	2.52
	5	92	2.7	5.57	2.64
	10	93	1.7	5.49	2.54
Average			2.3	4.82	2.91
SD			0.8	0.66	0.34

^aPercentage of *P.gatunense* (Dinophyceae) out of total phytoplankton biomass.

^bChlorophyll *a* content, $\mu\text{g mg}^{-1}$ wet weight of *P.gatunense*.

^cChlorophyll *a* to chlorophyll *c* ratio.

^dChlorophyll *a* to peridinin ratio.

bloom of the diatom *A.granulata* occurred (Figure 3a), resulting in a fucoxanthin concentration of close to $30 \mu\text{g l}^{-1}$ (Figure 3c).

The temporal distribution of chlorophyll *b* did not show a defined period of particularly high or low concentrations (Figure 3c). Although lutein is a signature pigment of the Chlorophyceae, its concentration did not conform to the chlorophyll *b* distribution (Figure 3c).

The pigment data presented in Figure 3b and c were correlated with the biomass of the corresponding phytoplankton taxa shown in Figure 3a. A strong linear correlation was found between the biomass of Pyrrhophyceae and peridinin (Figure 4; correlation coefficient 0.91). The calculated correlation coefficient between the biomass of Chlorophyceae and chlorophyll *b* was 0.54, and that between the biomass of Bacillariophyceae and fucoxanthin was 0.68 (Figure 4). The three coefficients, based on >90 comparisons, were significant at the $P < 0.01$ level.

Several chlorophyll *a* derivatives were detected on the HPLC chromatograms in this study. Chlorophyllide *a* was particularly conspicuous at the onset of the *Peridinium* bloom, and apparently coincided with the decline in the chlorophyte and diatom populations in February 1988 (compare Figure 5 with Figure 3a). Integrated values of chlorophyllide *a* concentrations for the top 10 m of the water column did not exceed 9% of chlorophyll *a* (Figure 5). Pheophorbide *a* could not be detected on the 436 nm absorbance chromatograms as it was masked by peridinin. However, it was detected on fluorescence chromatograms in minute quantities. Pheophytin *a* was not found, even in a single sample.

In order to confirm these results, a second sampling program was carried out during the 1993–1994 season, with special attention given to chlorophyllide *a*

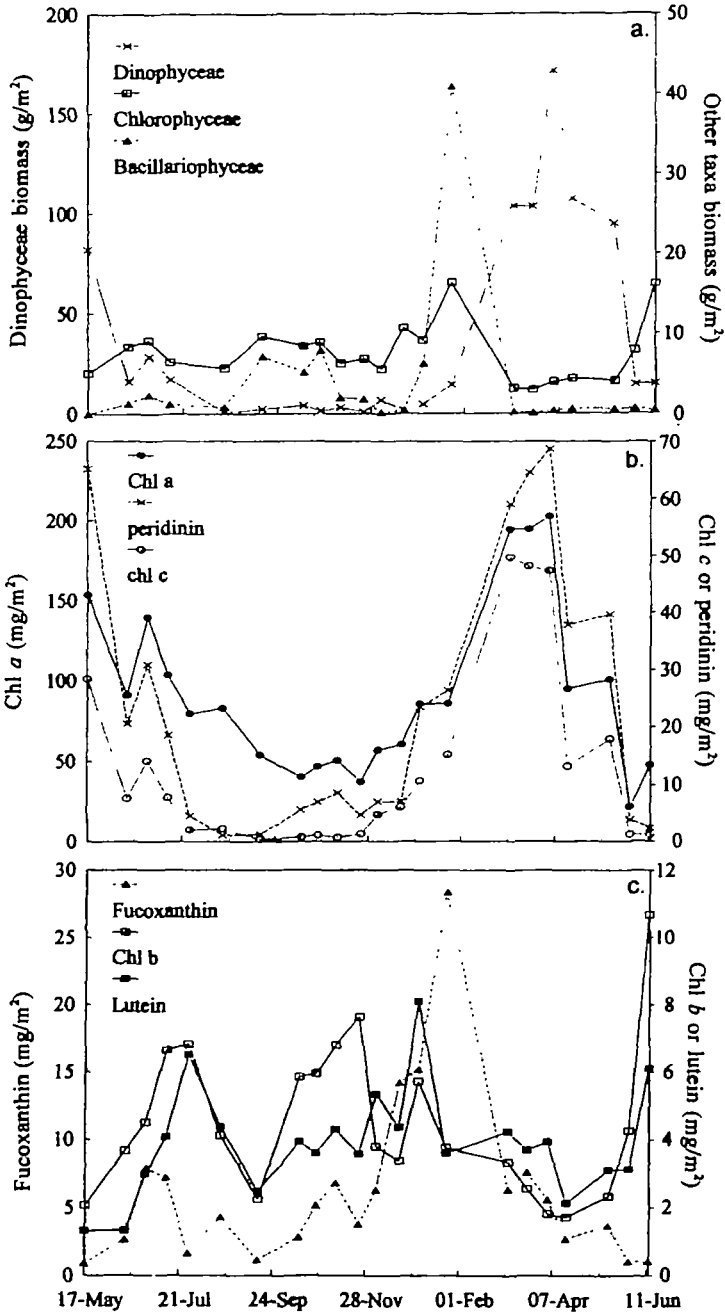


Fig. 3. Temporal variation of biomass and pigment concentrations of the major phytoplankton taxa in Lake Kinneret during the period between May 1988 and June 1989; integrated for the top 10 m water column. (a) Biomass density; (b) concentration of chlorophyll a, chlorophyll c and peridinin; (c) concentration of fucoxanthin, chlorophyll b and lutein.

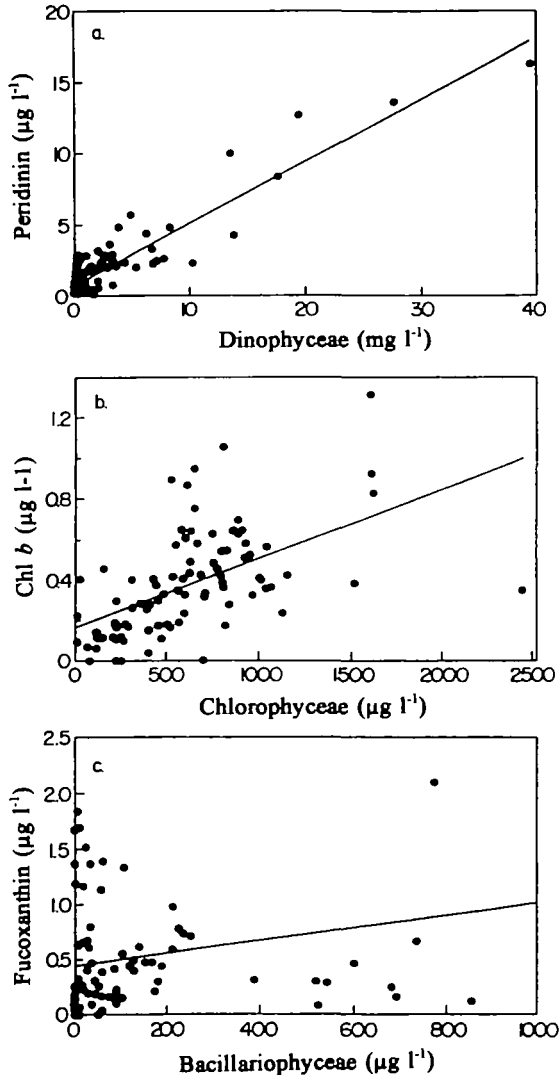


Fig. 4. Linear correlation between the biomass of specific phytoplankton taxa and their corresponding signature pigments.

temporal and spatial distribution. The season was characterized by an exceptionally high *Peridinium* bloom. The wet weight of this species exceeded 450 g m^{-2} during its maxima in April (Figure 6). Subsequently, an exceptional outbreak of the cyanophyte *Aphanizomenon ovalisporum* bloom occurred, which started in September and ended in late October. While the *Peridinium* bloom coincided with high values of chlorophyll *a* and peridinin (Figure 7a), relatively high chlorophyllide *a* concentrations in the water column were measured between January and April, a period that preceded the main peak of the 1994 annual bloom of

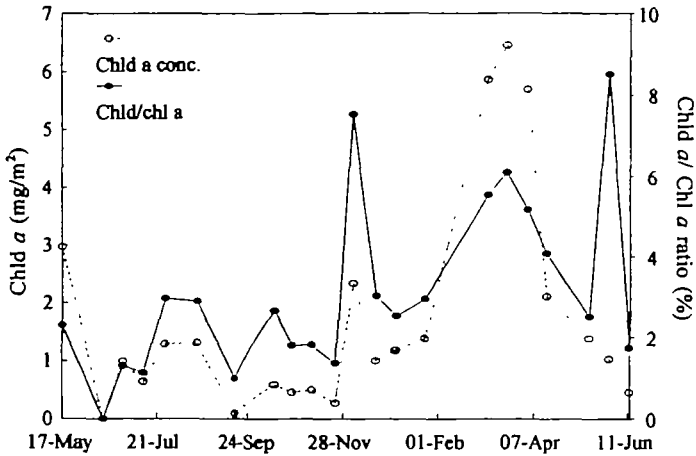


Fig. 5. Temporal distribution of chlorophyllide *a* in the integrated top 10 m water column in Lake Kinneret during the 1988–89 sampling season.

Peridinium (Figure 7b). The chlorophyllide *a* content in the water column during 1994 did not exceed 5% of the chlorophyll *a* content with three distinct temporal peaks. As in the earlier sampling that took place in 1989, only traces of pheophorbide *a* were detected, whereas pheophytin *a* was not detectable.

Pigment analysis of particles collected in sediment traps placed for 24 h in 35 m depth allowed a rough estimation of the daily sinking rate of pigments. The calculated sinking rate of chlorophyll *a* was relatively stable; the daily removal rate ranged between 10 and 30% of the chlorophyll *a* in the water column (Figure 8). The sinking rate of peridinin roughly followed the rate of chlorophyll *a* (data not shown). However, the calculated removal rate of chlorophyllide *a* was much higher than that of chlorophyll *a* (Figure 8) and in several cases exceeded 100%.

Discussion

Algae belonging to three classes, namely the Dinophyceae, Chlorophyceae and Bacillariophyceae, contribute >99% to Lake Kinneret phytoplanktonic biomass (Pollinger, 1986). The proportion of photosynthetic pigments to algal biomass, and the quantitative relationships between different pigments, are subjected to ambient and genetic differences (e.g. Klein, 1988; Roy, 1988; Buma *et al.*, 1991; Brown and Jeffrey, 1992). Hence, as the algal group studied is more uniform, both in spatial diversity and temporal occurrence, the link between algal biomass and signature pigment intensity is higher. Only a few attempts have been made to compare microscopically determined biomass and signature pigment abundance. Soma *et al.* (1993) found that the seasonal variation of the abundance of signature pigments reflected the observed trends in numbers of algae. A relatively high correlation coefficient ($r^2 = 0.76$) was found between diatom biomass and fucoxanthin concentration during a short-term study in the North Sea (Gieskes and Kraay, 1983b). A high correlation was also found between cell count of the

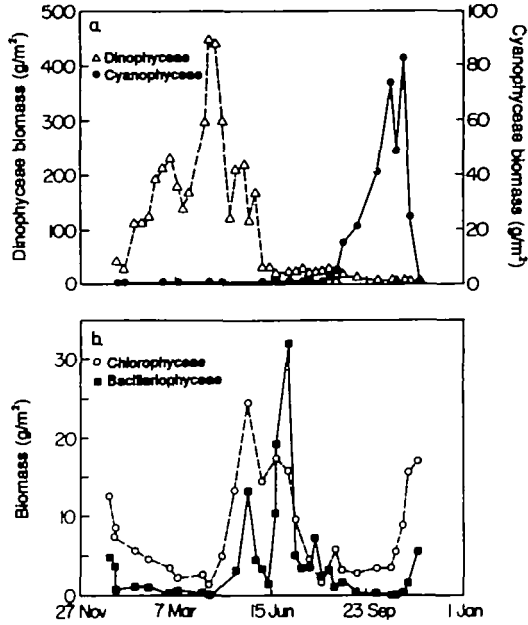


Fig. 6. Temporal variations in the biomass concentration of the major phytoplankton taxa in Lake Kinneret during the 1993–94 sampling season. Biomass of (a) Dinophyceae and Cyanophyceae, and (b) Chlorophyceae and Bacillariophyceae.

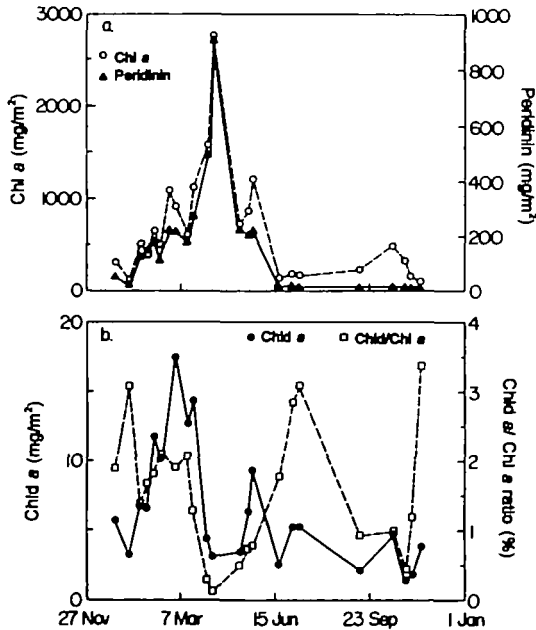


Fig. 7. Temporal distribution of (a) chlorophyll *a* and peridinin, and (b) chlorophyllide *a* and its ratio to chlorophyll *a* in the water column in Lake Kinneret during the 1993–94 sampling season.

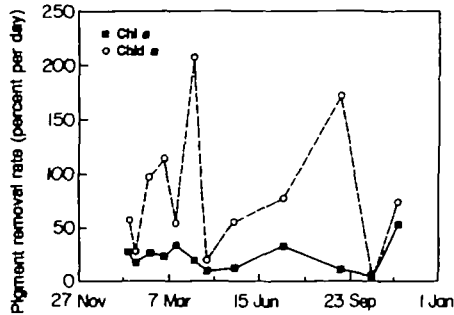


Fig. 8. Calculated rate of removal of chlorophyll *a* and chlorophyllide *a* in Lake Kinneret during the sampling season of 1994.

microflagellate *Corymbelus aureus* and the concentration of its signature pigment, 19-hexanoyl-oxy-fucoxanthin (Gieskes and Kraay, 1986b).

Our study clearly demonstrates that in Lake Kinneret there is a relatively strong correlation between the abundance of signature pigments of several phytoplankton divisions and their biomass distribution. Differences in the strength of the correlation between the biomass and signature pigments are attributed to variations in the nature of the three algal divisions considered. The biomass of Dinophyceae was composed mostly of a single species, *P.gatunense*, concentrated within a relatively short period of time. On the other hand, Chlorophyceae comprise many species, which occur throughout the year (Pollinger, 1986). Bacillariophyceae occupy a medium position between the other three divisions, as in Lake Kinneret it is a less diverse group than the Chlorophyceae. The Bacillariophyceae species *A.granulata* and *Cyclotella* sp. formed blooms in Lake Kinneret, but of shorter duration and lesser intensity than the Dinophyceae blooms. In addition, fucoxanthin is found in chlorophyll *c*-containing algal groups other than the Bacillariophyceae (Rowan, 1989). Relevant to the Kinneret case are Chrysophyceae flagellates. Algae of other fucoxanthin-containing classes do not form substantial populations in this lake (Pollinger, 1986) and the most important dinophyte in the lake, *P.gatunense*, does not harbor it. Therefore, it well may be that a relatively high concentration of fucoxanthin, found concomitantly with a low biomass of Bacillariophyceae (Figure 4), derived from algae of other groups, probably chrysophytes.

Dubinsky and Polna (1976) found that the chlorophyll *a/c* ratio in Lake Kinneret samples, collected during the *Peridinium* bloom, varied between 3 and 11. The ratio obtained in our study never exceeded 7.8, and is very compatible with ratios found for *Peridinium* and other cultured dinoflagellates (Jeffrey *et al.*, 1975). We found that the ratio of chlorophyll *a* to wet weight *Peridinium* biomass (mg g^{-1}) was 2.8, close to the value of 2.6 $\text{mg chlorophyll a g}^{-1}$ wet weight found in previous years (Wynne *et al.*, 1982). The ratio of chlorophyll *a* to peridinin in *Peridinium*-dominated samples was a relatively constant parameter; thus, an HPLC measurement of peridinin concentration may be used to estimate the *Peridinium* density. Monitoring of *Peridinium* concentration may be especially useful during

the build-up period of *Peridinium* biomass and when its bloom declines, periods when the algal assemblage cannot be considered a monoalgal ecosystem of *Peridinium*.

The distribution of chlorophyll *b* did not conform to the distribution of lutein. As noted earlier, zeaxanthin, a signature pigment of the Cyanophyceae, co-eluted with lutein. Consequently, part of the discrepancy of the chlorophyll *b*:lutein ratio could be attributed to this factor. However, it is more probable that the species diversity and environmental variation caused fluctuations in lutein:chlorophyll *b* ratios.

The dominant chlorophyll degradation product observed in Lake Kinneret was chlorophyllide *a*. This pigment is attributed to senescent cells (Barrett and Jeffrey, 1971; Suzuki and Fujita, 1986). The chlorophyll degradation products suite, found in this study, is not unique to Lake Kinneret. Chlorophyllide *a* was almost universally found in pigment extracts surveyed by HPLC, both in marine and freshwater samples, by different research groups. On the other hand, pheophytin *a* is less common and almost always forms a minute peak. Pheophytin *a* was present in most grazing experiments and in environmental pigment samples derived from grazed phytoplankton (Wright and Shearer, 1984; Gieskes and Elbrachter, 1986). Other types of samples which showed pheophytin were presumably loaded with a high concentration of detritus, i.e. estuary samples, sediment trap content (Hurley and Armstrong, 1990, 1991) and shallow lake samples (Korthals and Steenbergen, 1985; Steenbergen and Korthals, 1988). Examination of >300 samples in the current study did not yield even a single record of pheophytin, although it is normally observed in pigment extracts of sediments of Lake Kinneret (Yacobi *et al.*, 1991). The highest concentrations of chlorophyllide *a* were observed during the winter mixing period, preceding the annual bloom of *Peridinium*. The possibility that the observed chlorophyll degradation products during the mixing event originated from resuspension of the sediment is eliminated, since pheophytin, a chlorophyll degradation product typically found in the sediment (Yacobi *et al.*, 1991), was not found in the water column samples. The data collected in the 1988–89 season demonstrate that the increase in chlorophyllide *a* content in the water column is associated with a phytoplankton assemblage dominated by a mixture of chlorophytes and bacillariophytes, whereas only a low level of chlorophyllide *a* coincided with the main bloom of the dinoflagellate *Peridinium*. In the 1994 season, the major peak of chlorophyllide *a* coincided with a decline in the relatively small population of chlorophytes and an increase in the *Peridinium* biomass. An additional minor peak of chlorophyllide *a* concentration (Figure 7b) was observed upon the decline in the *Peridinium* population which coincided with a build-up and a decline of the chlorophyte population. These data suggest that chlorophyllide *a* found in Lake Kinneret water is more likely associated with non-pyrrhophyte populations. Therefore, a question may arise regarding the fate of the *Peridinium* chlorophyll which accumulates to an annual value of ~250 tons.

It has been reported that chlorophyll *a* is converted to chlorophyllide *a* in most bacillariophytes more readily than it is in algae of other taxa (Jeffrey and Hallegraeff, 1987). Conversion of chlorophyll *a* to chlorophyllide *a* is catalyzed by the

enzyme chlorophyllase. Preliminary measurements of chlorophyllase *in vitro* activity indicated a high activity in bacillariophytes and chlorophytes as compared with a relatively low activity in *Peridinium* (Y.Gonen, unpublished data). Accumulation of chlorophyllide *a* in the sediment trap reported in this study is not merely due to sinking of chlorophyll degradation products from the water column, but probably expresses a degradation process that continuously takes place during the sedimentation of senescent phytoplankton cells as well as during the short stay (up to 24 h) in the sediment trap itself.

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