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Phosphorus status of size-fractionated seston in Lake Erken

K. Pettersson, R. Bell, V. Istvánovics, J. Padisák and D. Pierson

Introduction

In order to penetrate the question of the phosphorus status of seston in Lake Erken a size fractionation into large (> 12 μm), medium (3–12 μm) and small (< 3 μm) seston was performed during the summer of 1989.

The effects of environmental factors on the chemical composition of phytoplankton and bacteria have been documented for algal and bacterial cultures as well for seston in natural systems (Fitzgerald & Nelson 1966, Healey 1978, Healey & Hendzel 1980). In accordance with this theory about nutrient deficiency indicators, studies were performed on seston in Lake Erken during the late 1970's and early 1980's (Pettersson 1980, 1985 and Eriksson & Pettersson 1984). However, separation of the planktonic community in order to establish the nutrient status of different size fractions were not done and has rarely been performed. Only very few reports are available within this field. Vadstein et al. (1989) as well as Jürgens & Güde (1989) separated phytoplankton and bacteria and investigated their phosphorus status in Lake Nesjövatn and Lake Constance, respectively.

Much earlier Rhee (1972) suggested that P storage of algae could compensate to some degree for their disadvantage in phosphate uptake in comparison to bacteria. The ability of algae to store P was also used by Fitzgerald & Nelson (1966) in their introduction of surplus P measurements as a tool to estimate the phosphorus status of algae in culture and phytoplankton. Occasional inputs of phosphate resulting in higher concentrations e.g. from the hypolimnion or epilimnetic sediments due to wind mixing can thus be taken up by phytoplankton and stored as surplus phosphorus, making them competitive to small seston. Transport of phosphorus rich algae from the sediments to the epilimnion is also possible.

In this paper the measurements of particulate phosphorus (PP), surplus phosphorus (SP) and alkaline phosphatase activity (APA) in combination with size fractionation of seston into large (> 12 μm), medium (3–12 μm) and small (< 3 μm) seston in order to determine their phosphorus status will be presented and discussed. Particulate carbon and chlorophyll *a* was used as biomass estimators. The species composition of the phytoplankton was determined in order to increase the informative value of the size fractionation.

Materials and methods

Lake Erken is a moderately eutrophic lake (surface area 24 km^2, average depth 9 m) situated in central Sweden. A summer stratification usually starts in May and lasts until late August or early September. Water samples were taken weekly with an electrical pump at a buoy situated 700 m offshore, above the deepest point of the lake. The water was sampled at about eight in the morning and transported to the laboratory at the shore in non-transparent 2 l bottles for immediate analysis. In this paper the analyses of water from 3 m depth is used as representative of the epilimnetic conditions and those from 15 m for the hypolimnion.

Soluble reactive phosphorus was determined as molybdate reactive phosphorus (Murphy & Riley 1962) and surplus phosphorus according to Fitzgerald & Nelson (1966) with minor modifications (Pettersson 1980). Particulate phosphorus was measured after persulfate oxidation in an autoclave (Menzel & Corwin 1965). The latter analyses were performed with 12 μm, 3 μm and 0.2 μm cellulose acetate filters (Schleicher and Schüll). The phosphorus analyses were run within 2–3 hours after sample collection.

Alkaline phosphatase activity was measured with a Turner filter fluorometer as described by Pettersson (1980).

Chlorophyll samples (whole water, 12 μm filtrate and 3 μm filtrate) were filtered onto Whatman GF/F glass-fiber filters. These were immediately extracted in 10 ml of 90 % acetone in the dark and at 4 °C. After an extraction time of 24–36 hours the chlorophyll *a* concentration was determined spectrophotometrically.

Particulate carbon (whole water, 12 μm filtrate and 3 μm filtrate) were determined with a CHN-analyzer (Carlo-Erba) after filtration onto precombusted (4 h, 550 °C) Whatman GF/F glass-fiber filters. Particulate carbon was also estimated from phytoplankton counts with a conversion factor of 0.17 pg C/μm^3 (Rocha & Duncan 1985).

Phytoplankton were fixed with acid Lugol's solution and counted with an inverted microscope (Utermöhl 1958).

Results

Physical and chemical conditions

The water temperature at 3 m depth increased from 8.5 °C on 8 May to a maximum of 19.5 °C on 11 July. The lake was stratified during the sampling period, however the hypolimnetic water was continuously warmed due to mixing events

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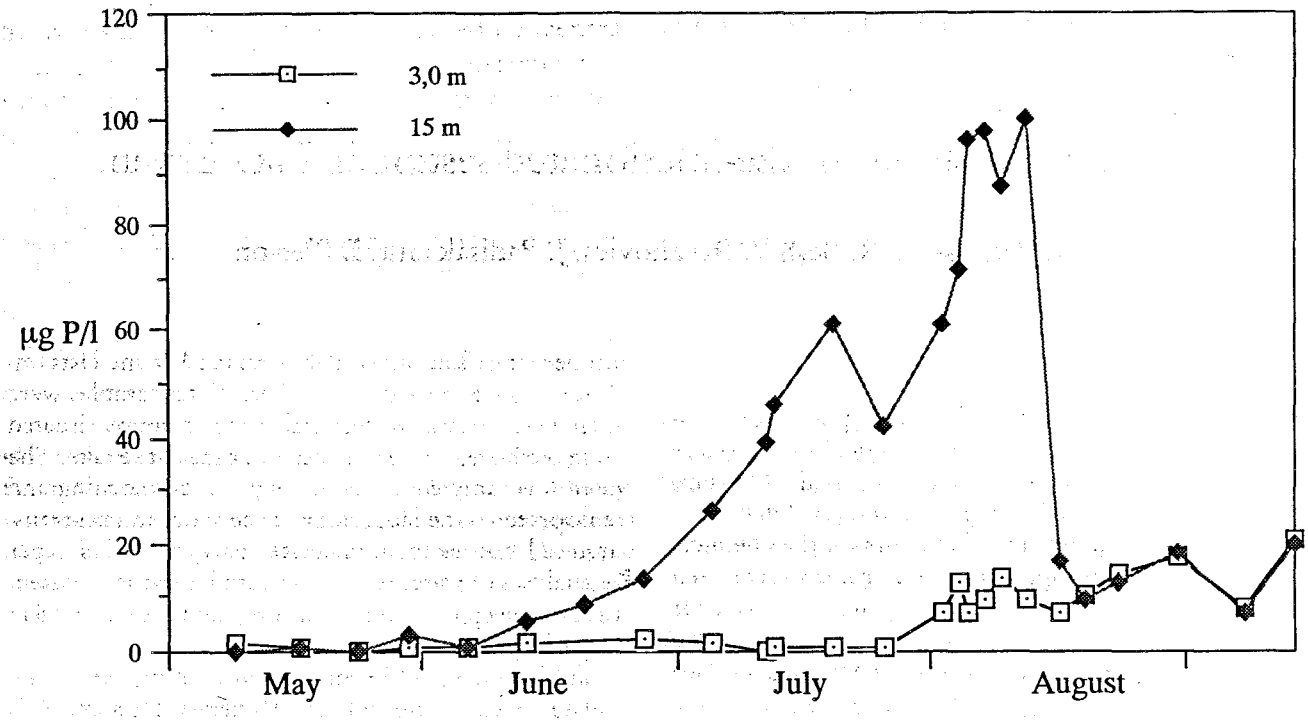

Fig. 1. The phosphate concentration (molybdate reactive P) at 3 m and 15 m depth in Lake Erken during the summer of 1989.

(Pierson et al. 1992). The lower part of the epilimnion was depressed from 7 to 12 m during the stratification.

The concentrations of SRP at 3 m and 15 m during the period of investigation are given in Fig. 1. In May the concentrations were very low, which was the case in the epilimnion until early August, when a level around 10 $\mu g P \cdot l^{-1}$ was reached due to mixing events, that transported nutrient rich hypolimnion water upwards. In the hypolimnion SRP started to rise in June and a dramatic increase took place during July and early August with a maximum concentration above 100 $\mu g P \cdot l^{-1}$ just before the turnover in the middle of August. The concentrations of nitrate and ammonium were low in the epilimnion until the beginning of August when ammonium concentrations around 30 $\mu g N \cdot l^{-1}$ were measured. The cause was the same as for the MRP increase, i.e. mixing with the hypolimnion. The inorganic N/P ratio (by weight) was fairly low in the epilimnion, most often in the range 10 to 15 and ammonium/SRP less than 5. In the hypolimnion (15 m) the ratio was even lower, mostly well below 5. The nutrient supply from the hypolimnion thus was in excess of phosphorus.

Phytoplankton biomass

The species composition of phytoplankton was dominated by diatoms in May and this algal group continued to be significant during the whole summer among the large seston (Fi+. 2). In this fraction dinoflagellates also played a dominant role until the bloom of cyanobacteria in July. During the period with low biomass in June, *Rhodomonas* became dominant and contributed with almost 50% of the biomass as a maximum. Among medium seston *Rhodomonas* was replaced by *Cryptomonas* as the dominant species in late July (Fig. 2). *Gloeotrichia echinulata* appeared in June but did not dominate the phytoplankton community until 19 July, when the first biomass peak with 18.9 μg chl $a \cdot l^{-1}$ was recorded. The variance of the biomass after this date until the *Melosira* bloom in late August with a maximum of 34.5 μg chl $a \cdot l^{-1}$ was mainly due to the appearance or non-appearance of *Gloeotrichia echinulata* as a result of mixing conditions and wind direction at the time of sampling. There was also an increase in *Gloeotrichia* colonies towards the water surface, especially on calm days, which was not taken into account in this presentation of the measurements at 3 m depth.

Separation of phytoplankton and bacteria

The separation of phytoplankton and bacteria by the 3 μm filtration was tested by determination of chlorophyll *a* in the fraction $< 3 \mu m$ and by measurement of the bacterial production in the frac-

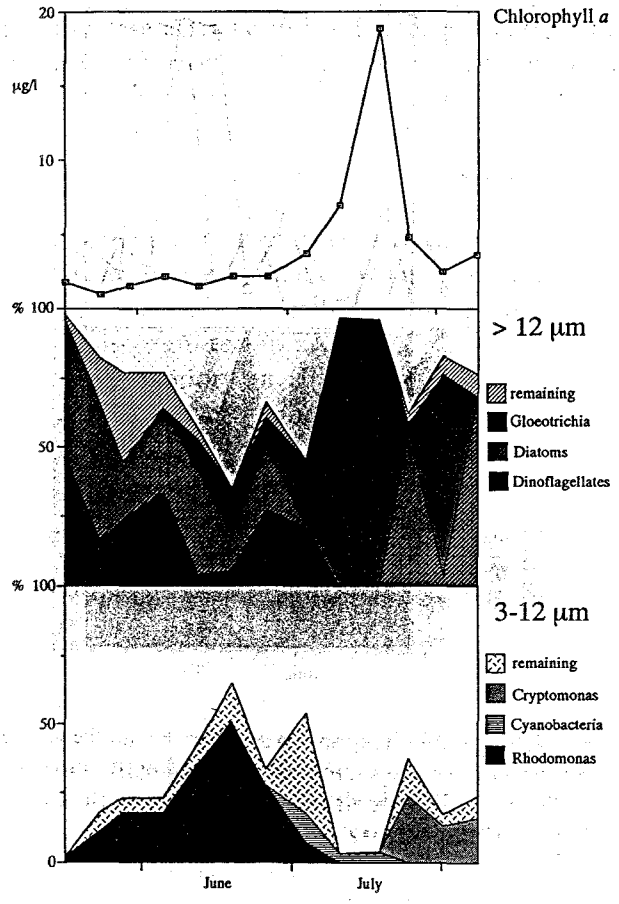

Fig. 2. The chlorophyll *a* concentration and the phytoplankton species composition in Lake Erken (3 m) during the summer of 1989. The phytoplankton are separated into two size groups, < 12 µm and 3–12 µm.

tions > 3 µm. The chlorophyll *a* concentrations in the bacterial fraction were converted to carbon by using the chl *a*/C ratio for the fraction 3–12 µm for each sampling occasion. The phytoplankton on average made up 5.7 % of the total particulate carbon in the small seston fraction and the maximum was 24 % on 23 May. The contribution was most significant in late May and early June. During that period the percentage of total chlorophyll *a* in the small seston was in the interval 5 to 12 %, mainly due to the very low phytoplankton biomass. At other times less than 1 % was found in this fraction and the summer average was 0.8 %.

Until the middle of June the bacterial production in size fractions > 3 µm was about 7 % of the total bacterial production as estimated by thymidine incorporation (Bell et al. submitted). In late June and July the percentage was somewhat higher, in the interval 10–19 %. In conclusion the bacteria and phytoplankton were fairly well separated by the 3 µm filter, but a complete separation was not possible to obtain during the entire sampling period.

Phosphorus

The particulate (PP) and surplus phosphorus (SP) concentrations at 3 m depth are presented in Fig. 3 (upper). PP maximized on 19 July with 22 µg P/l and the SP concentration on the same date with 5.0 µg P/l. High values were also registered dur-

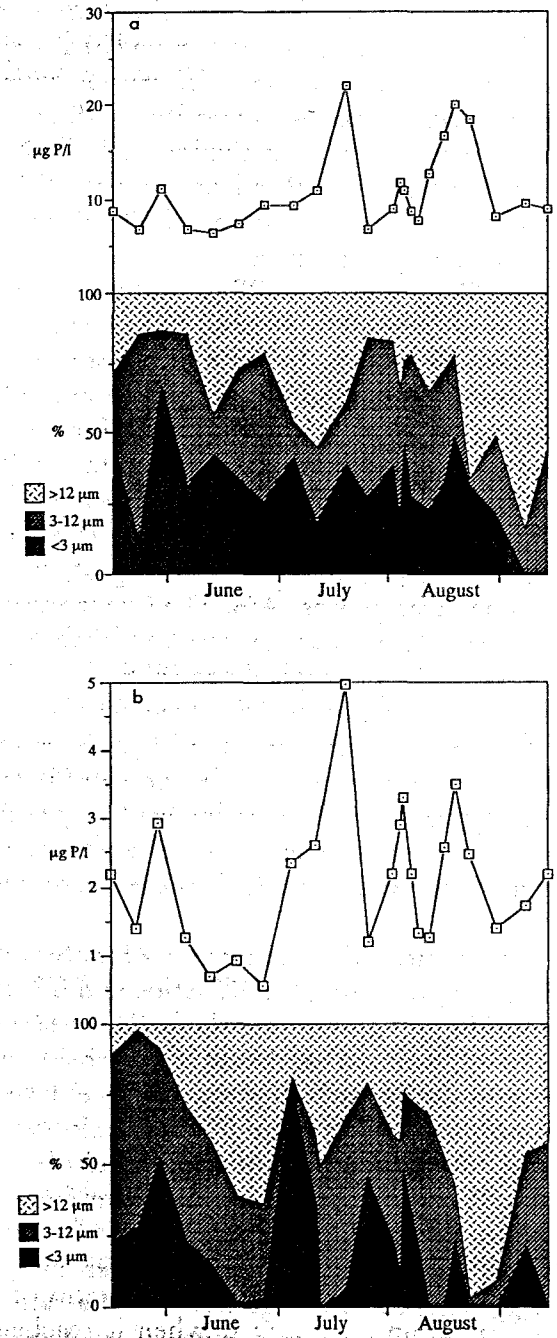

Fig. 3. Size fractionation of particulate (a) and surplus (b) P at 3 m depth in Lake Erken during the summer 1989. 3 µm = small seston; 3–12 µm = medium seston; > 12 µm = large seston.

ing August. SP was a significant proportion of PP and at most contributed with 30 % on 6 August. The fractional distribution of PP and SP is presented in Fi+. 3 (lower). On average 32 % of PP and 21 % of SP belonged to the fraction < 3 μm. The corresponding figures for medium seston were 33 and 38 %, while the large seston contributed with 35 and 41 % to the PP and SP, respectively. PP in the bacterial fraction was fairly stable, while SP varied considerably with marked peaks indicating a very dynamic system (Fig. 3). The large seston dominated the fractional distribution of PP and SP especially in late August and early September, when diatoms and *Gloeotrichia echinulata* dominated the species composition, while the medium seston were most significant in early summer, late June as well as late July–early August, coinciding with the maxima of *Rhodomonas* and *Cryptomonas*, respectively.

The SP percentage of PP in the bacteria fraction was very dynamic and varied from 1 to 50 % with an average of 16 %. In the medium seston the SP percentage decreased from a maximum of 66 % in May to a minimum of 4 % in the beginning of July and then it stabilized around 20 % until early September, resulting in a summer average of 24 %. The large seston showed an increase in SP percentage during July to 51 % on 6 August, while the summer average was 24 %. The size fractionation showed different seasonal patterns for the two size classes of the phytoplankton community as well as a significantly lower SP percentage in the bacteria fraction, which also showed the most drastic changes. The variation for the total plankton community was much smaller, but evidently there was a decrease in SP percentage until late June, when a higher level was established for the rest of the summer.

The P status of the different size classes can also be evaluated from PP : PC ratios and SP : PC ratios. The < 3 μm fraction showed no clear seasonal trend, with the exception of a decrease in early August and an establishment of a lower level afterwards, but the average PP : PC ratio was very high, 46 μg P/mg C. In the medium seston, 45 μg P/mg C was registered in the middle of May but later on the ratio stabilized on a level around 20 μg P/mg C generating a summer average of 18 μg P/mg C. The large seston had a ratio of < 10 μg P/mg C until mid June, when it reached a maximum of 24 μg P/mg C, whereafter the ratio decreased to a lower level until a second peak occurred on August 1 with 41 μg P/mg C and a third one later on in August with 67 μg P/mg C. The

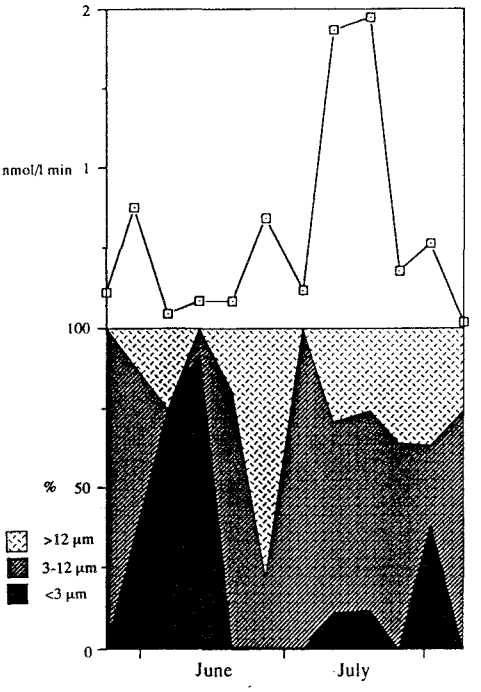

Fig. 4. Alkaline phosphatase activity in the different size fractions at 3 m depth in Lake Erken during the summer 1989. < 3 μm = small seston; 3–12 μm = medium seston; > 12 μm = large seston.

seasonal average was 19 μg P/mg C. Thus medium seston showed a sesonal trend with decreasing P content„ while P rich large seston occurred in August. The very dynamic bacterial fraction had the highest seasonal average, which was more than twice as high as that of the two phytoplankton fractions.

The SP : PC ratio in the bacterial fraction peaked on 29 May, when a value of 25 μg P/mg C was registered, but otherwise the SP : C ratio was low until a second peak in early August. The medium seston fraction decreased tenfold in surplus phosphorus content from May, when 13 μg P/mg C was measured, and onwards to a minimum on 4 July of 1.2 μg P/mg C, after which the ratio stabilized around 3 μg P/mg C. The large seston went from very low SP : PC ratios in May, June and July to a maximum of 13 μg P/mg C in early August. The summer averages for the large, medium and small seston were 1.4, 4.3 and 4.7 μg P/mg C, respectively.

The distribution of the particulate alkaline phosphatase activity is given in Fig. 4. The bacterial contribution was significant from late May until mid June, but otherwise the medium seston were dominant with a summer average of 55 % of the total activity to be compared to 15 % for bacteria and 30 % for large seston. The specific alkaline

phosphatase activity in the bacterial fraction was undetectable from 19 June to 4 July after peaks in late May and early June and maximized in mid July with 2.8 nmol/mg C min. The medium seston maximized at the same time with 5.6 nmol/mg C min to decrease drastically thereafter to 0.1 nmol/mg C min and less in August. The medium seston peak coincided with minimum levels of SP : PC, indicating phosphorus limitation, which also resulted in a drastic decrease in biomass and proportion (Fig. 2). The large seston showed low specific phosphatase activity during all summer.

Discussion

As pointed out by VADSTEIN et al. (1989) the application of biochemical measurements to evaluate the nutrient status of natural plankton must be made with great care. A size fractionation in combination with determination of species composition improves the possibilities for evaluation. The contribution of detritus to the measurements of PP, SP and APA as well as to the particulate carbon might interfere and must be taken into account. PETTERSSON (1980) estimated the living biomass of phytoplankton and bacteria in Lake Erken by ATP determinations to be about 50 % of the particulate carbon during summer. PIERSON (unpubl.) found through regression analysis of summer values (1987–1989) of chlorophyll *a* and particulate carbon that 200 μg C · l^{-1} was non-algal (detritus and bacteria) in the epilimnion of Lake Erken. In 1989 the summer average for particulate carbon in the bacterial fraction was 98 μg C · l^{-1}, which was 20 % of the total particulate carbon. Thus about 100 μg C · l^{-1} or 25 % of the particulate carbon was non-algal in the fractions > 3 μm. An estimate of bacterial biomass from bacteria counts using a cell volume of 0.17 μm^3 and a conversion factor to carbon of 0.3 pg C · μm^{-3} (BELL et al. submitted) gave 75 μg C · l^{-1} as living bacteria. In conclusion, as a summer average, between 50 and 75 % of the particulate carbon in the epilimnion of Lake Erken was to be found in living organisms. The PP : PC ratios of the different size fractions do indicate that the severest interference from detritus was in the large seston fraction. A comparison of the quotas for PP and SP obtained with particulate carbon and those resulting from phytoplankton counts and volume determinations using the factor 0.17 pg C · μm^{-3} resulted in very large discrepancies in PP/PC and SP/PC during May and early June in the medium seston fraction and was due to a very low biomass estimated from algal counts. The ratios obtained with the measured figures were much more realistic, but still higher than those reported by VADSTEIN et al. (1989) for Lake Nesjövatn, although the latter used phytoplankton counts and cell volumes converted to carbon. For the large seston major overestimations of specific PP and SP were made in late June with phytoplankton counts as basis for biomass. The appearance of *Gloeotrichia echinulata* in early July caused problems in biomass estimation due to severe difficulties in sampling and determination of colony- as well as cell-size. This very large algal colony thus affected all measurements and from phytoplankton counts and chlorophyll *a* concentrations it was evident that particulate carbon was underestimated by the carbon analysis and during this period the carbon content was estimated from the chlorophyll determinations. The summer average of PP/PC for the bacterial fraction, 46 μg P/mg C, was fairly well in agreement with the findings of VADSTEIN et al. (1989), especially if we consider the bacterial biomass to be 75 % of the particulate carbon (see above). SP/PC, however, was much lower with an average of 7.2 μg P/mg C and with very dynamic changes, to be compared with 33 μg P/mg C in Lake Nesjövatn. In this case the results of VADSTEIN et al. (1989) are somewhat contradictory, since they claim that bacteria do not store polyphosphates and thus surplus P in the case of bacteria estimates cytoplasmatic P_i. Conclusively, if using the criteria of VADSTEIN et al. (1989), the pelagic bacteria in Lake Erken seem to be phosphorus limited and thus tend to function as a sink for phosphorus and they also contribute significantly to the particulate phosphorus (32 %). GÜDE (pers. com.) reported that the bacterial P-pool represented more than half of PP in Lake Constance during summer. This percentage was however based on calculations from carbon content of small seston and bacterial C : P ratios. We consider the estimates from measurements of PP and PC to be more reliable, especially if the detritus fraction can be judged from bacterial counts. This means that the role of the bacteria might not be as dominant as have been postulated by others, which was also valid for the P-uptake (ISTVÁNOVICS et al. 1990).

The medium seston contained high amounts of phosphorus with an summer average of 19 μg P/mg C, which was significantly higher than the figures reported for phytoplankton in Lake Nesjövatn by VADSTEIN et al. (1989) with a median

of 5.1 μg P/mg C. The average SP/PC ratio, 4.3 μg P/mg C was also higher, but surplus P contents lower than the threshold value for phosphorus limitation of 2 μg P/mg C reported earlier for Lake Erken (Pettersson 1980) were measured during the declination of the *Rhodomonas* sp. bloom in June. The much lower PP/PC and SP/PC ratios registered for the large seston with e.g. values below 2 μg P/mg C for SP/PC on a majority of the sampling occasions indicates a severe phosphorus stress, although an influence of detritus might bias the results in this fraction. A comparison of the average percentage carbon in this fraction to the average percentage of chlorophyll showed that carbon was overrepresented with 10% (76% against 66%). Another explanation might be that large, colony-forming species contain more carbon, which would lower the ratio without a true lack of phosphorus. However, even if these facts are taken into account the phosphorus stress is obvious. This fact was, however, not detected in the APA measurements since the large seston showed a low activity. The low surface/volume ratio for the larger phytoplankton might be an explanation for this cell-surface bound enzyme activity to be lower. It was only during the dominance of *Gloeotrichia echinulata* that the PP : PC ratios were elevated. Ulén (1971) reported a PP/PC ratio of this alga in Lake Erken in early July to be 20 μg P/mg C and a similar value was registered at this time in 1989. Istvanovics et al. (accepted) measured over 30 μg P/mg C for the same alga in Lake Erken 1991. During the blooms of *Gloeotrichia* in 1969 (Ulén 1971) and 1989 the phosphorus content decreased to 6 and 4 μg P/mg C, respectively, in early August, while it was somewhat higher, around 8 μg P/mg C in the same period 1991.

The size fractionation of seston in Lake Erken performed in this study did reveal significant differences in the nutritional status and functional behaviour of the different size classes. The medium seston seems to have taken up phosphate in May to be stored as surplus P, which was consumed during their growth in June. In early July this process resulted in severe P deficiency for the medium seston as indicated by the high specific phosphatase activity. This development is in agreement with earlier findings concerning the spring–summer growth of phytoplankton in Lake Erken (Pettersson 1980, 1985). During this period the large seston have no chances to compete as shown by their phosphorus deficiency. In late July the inoculation of *Gloeotrichia* colonies from the sediments and phosphate from the hypolimnion changes the situation completely as to favour the large seston, now being rich in phosphorus. The role of the bacteria fraction as a significant P pool is obvious (Fig. 4) and a very dynamic system is indicated by the large fluctuations in especially surplus P. However, their contribution in P uptake (Istvánovics et al. 1990) was not as large as expected from other studies.

The results of the study are encouraging and do validate a further development of the separation of seston in combination with measurements of physiological indicators of nutritional status. A more close coupling to primary production estimations will then be essential in order to determine the role of nutrient availability.

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Authors' addresses:

K. PETTERSSON, Erken Laboratory, Institute of Limnology, Uppsala University Norr Malma 4200, S-76173 Norrtälje, Sweden.

R. BELL, Institute of Limnology, Uppsala University, Box 557, S-75 122 Uppsala.

V. ISTVÁNOVICS, Balaton Limnological Research Institute, H-8237 Tihany, Hungary.

J. PADISAK, Botanical Dept. of the Hungarian Natural History Museum, P.O. Box 222, H-1476 Budapest, Hungary.

D. PIERSON, Ben Nesn Lab., NYC Dept., Rt28A Shokun, N.Y. 12481, USA.