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## The occurrence of *Aphanizomenon flos-aquae* (Cyanophyceae) in a nutrient gradient in the Baltic

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### Abstract

The investigation area, Himmerfjärden Bay situated at the Swedish coast of the northern Baltic Proper, receives waste water from a sewage treatment plant. Phosphorus is efficiently reduced in the sewage treatment resulting in a high N : P ratio ( $\approx 65 : 1$ ) in the discharge. In the near future also the nitrogen discharge will be reduced, and it is feared that a lowered N : P ratio in the receiving waters may favour an undesired increase of nitrogen fixing blue-green algae. This study is focussed on the development of a common nitrogen fixing species in the area, *Aphanizomenon flos-aquae*, at varied nutrient loadings in 1983–85. The biomass as well as the number of heterocysts was low near the treatment plant and increased with increasing distance from the sewage discharge. The low biomass and number of heterocysts in Himmerfjärden Bay is interpreted as an effect of competition with other algae and of low level of phosphorus relative to nitrogen, i.e. unfavourable conditions for nitrogen fixation. The abundance of *Aphanizomenon* was highest in 1984 when the phosphorus load was higher than in the other years of investigation. As a tentative conclusion it is suggested that nitrogen fixing algae may increase as a result of reduction in the nitrogen discharge to Himmerfjärden Bay in the future. At the reference station outside the Bay, nutrient conditions favoured nitrogen fixing algae, as interpreted from an inorganic N:P ratio less than 10:1 during the summers of the investigation period. Here the variation in biomass between the years was mainly due to the variation in water temperature; *Aphanizomenon* was most abundant during the warm summer of 1984 and less abundant during the cold summer of 1985. A significant correlation was obtained between the heterocyst frequency and concentration of inorganic phosphorus in the trophogenic layer at the reference station.

### Introduction

Blue-green algae often dominate the phytoplankton community in areas of the Baltic which are influenced by high loading of nutrients (BAGGE and LEHMUSLUOTO 1971, NORDLING 1984, WIKTOR and PLINSKI 1975). Nitrogen fixing blue-green algae which are competitive in nitrogen deficient waters are often abundant in recipients receiving nutrient discharges with an excess of phosphorus relative to nitrogen (MELVASALO and VILJAMAA 1977, RINNE et al. 1981). Improvements in waste water treatment during the 1970's resulted in a reduction of the phosphorus load from sewage treatment plants along the Swedish coast. Especially in the Stockholm Archipelago the reduction of phosphorus caused a significant decrease in the abundance of nitrogen fixing algae due to the resulting increase of the N:P ratio in the area (BRATTBERG 1980).

Recent reports on increasing eutrophication in the Baltic have again brought into focus the load from the sewage treatment plants. The necessity of a reduction of nitrogen in addition to that of phosphorus has been discussed and nitrogen reduction will soon be introduced in the Himmerfjärdsverket sewage treatment plant situated at the Swedish coast of the northern Baltic Proper. In order to study future effects of a lowered N:P ratio in the receiving waters of Himmerfjärden Bay, a large-scale project was performed in 1984, where the phosphorus load from the sewage plant was increased (ELMGREN and LARSSON 1987).

It is feared that a reduction in the nitrogen outlets may favour an undesired increase in nitrogen fixing blue-green algae in the area. In this investigation *Aphanizomenon flos-aquae* (L.) Ralfs is studied as a representative of the nitrogen fixing population, consisting as well of *Anabaena* spp. and *Nodularia spumigena* Mert. in the coastal area outside the Bay (HOBRO 1979, LINDAHL et al. 1978). Biomass and heterocyst dynamics of *Aphanizomenon* are described in relation to different environmental conditions during the years 1983–85 along the nutrient gradient of Himmerfjärden Bay.

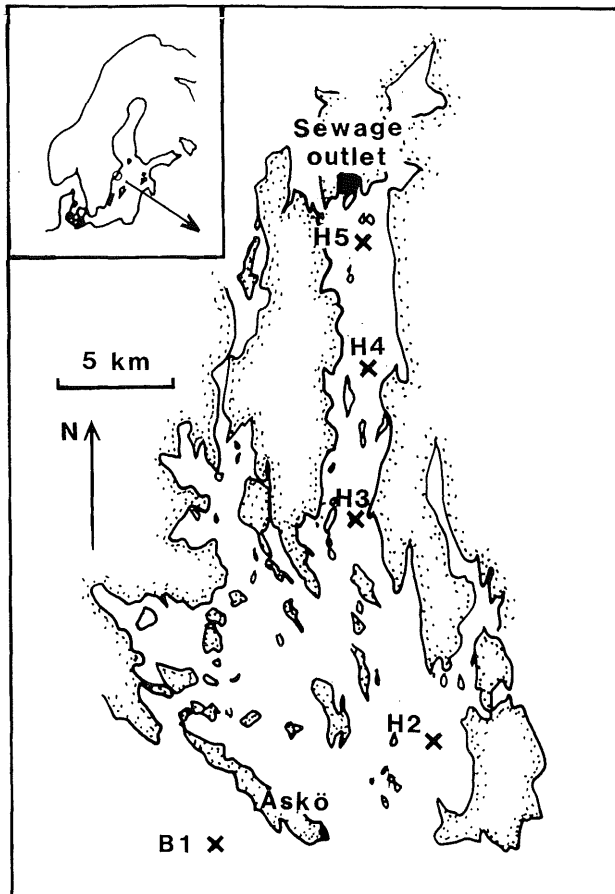


Figure 1

Himmerfjärden Bay with sampling stations H5-H2 and the reference station B1.

### Investigation Area

The Himmerfjärden investigation area consists of a 30 km long narrow bay bordering an open coastal area of the northern Baltic Proper (Fig. 1). In the inner part of the bay there is a sewage treatment plant (serving approximately 230 000 persons in 1985, ELMGREN and LARSSON 1987) with outlets into the area. The sampling sites H5-H2 represent basins with decreasing influence from the sewage outlet and station B1 represents a reference area.

During the years of study, 1983–85, the nutrient loading from the sewage treatment plant was different each year due to manipulations in the sewage treatment (Table 1). The efficient precipitation of phosphorus in the sewage treatment resulted in a nitrogen: phosphorus ratio of approximately 66:1 in the supplying waste water during 1983. In 1984 a large-scale experiment was performed whereby the phosphorus load increased about 3.5 times. The increased discharge of both nitrogen and phosphorus in 1985 was due to a higher supply of raw sewage into the sewage plant. The rainy summer of 1985 resulted in an increase of the diffuse load of nitrogen from land areas (LARSSON and JOHANSSON 1986). In this paper the years 1984 and 1985 are therefore called phosphorus and nitrogen years, respectively. The nutrient situation in the trophogenic layer during the investigated period is presented below.

**Table 1**

Annual load (tons/year) of total nitrogen and phosphorus from the Himmerfjärdsvverket sewage treatment plant, 1983–85. Data from ELMGREN and LARSSON (1987, and pers. comm.).

Year	Total-N	Total-P	N : P
1983	611	9	66 : 1
1984 P-year	717	31	23 : 1
1985 N-year	901	14	63 : 1

The freshwater influence in the Himmerfjärden Bay is rather weak, resulting in only 0.5–1 ‰ lower salinity compared to the control station B1 where the salinity is in the order of 6–7 ‰.

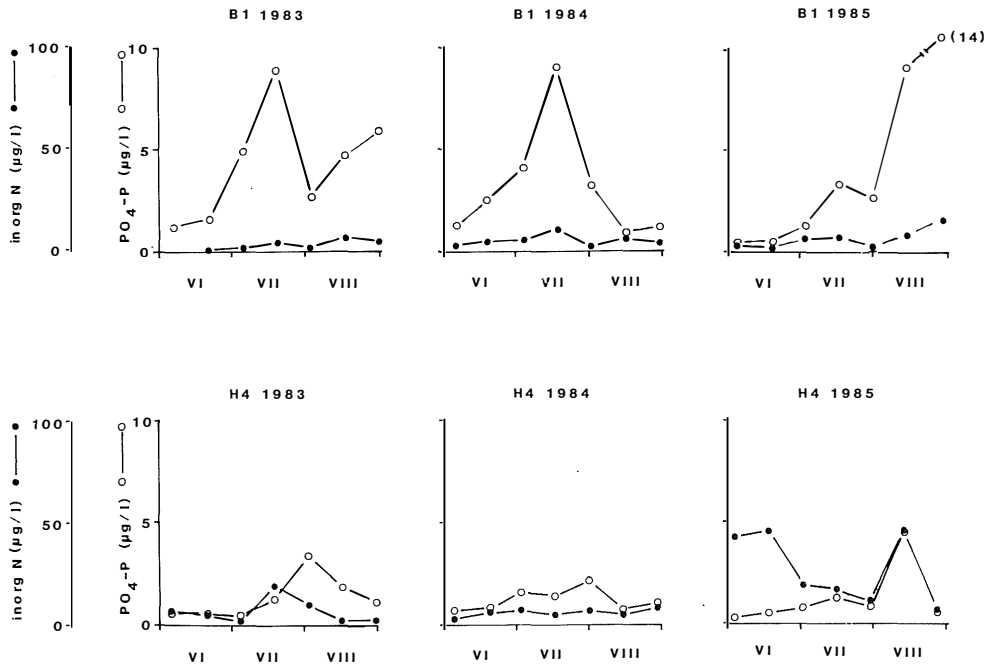
### Nitrogen, phosphorus and temperature during summer 1983–85

For comparison, the data as obtained by LARSSON and JOHANSSON (1985, 1986, 1987, pers. comm.) are given for the reference station B1 and for station H4 near the sewage outlet. The given concentrations of total as well as inorganic fractions of nitrogen and phosphorus represent mean values from 0–20m depth at station B1 and from 0–10m depth at station H4.

Both total nitrogen and phosphorus were higher in the inner area compared to the outer area. The concentration of total nitrogen at station H4 varied between 300 and 420 µg/l and total phosphorus between 14 and 30 µg/l. At station B1 the corresponding values were 240–310 µg/l and 13–25 µg/l, respectively. There were no clear differences between the years in the concentrations of total N and total P.

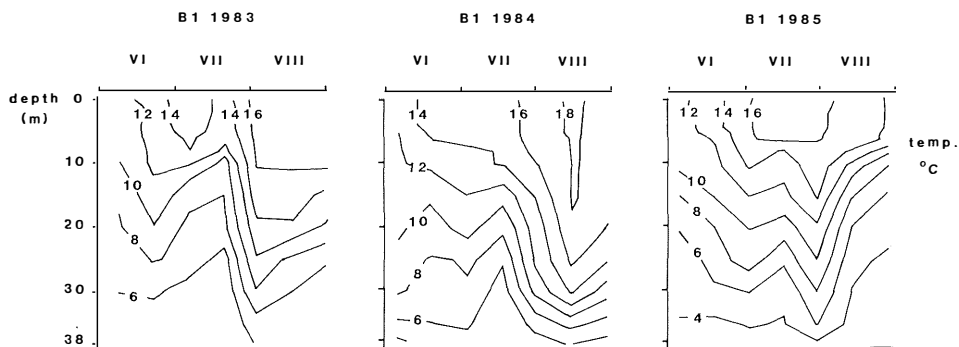
The seasonal variations of inorganic nitrogen and phosphorus at stations H4 and B1 are given in Figure 2. The concentrations of inorganic nitrogen were generally higher at H4 than at B1 whereas the concentration of inorganic phosphorus generally was lower at the inner station H4 than at station B1. The scales of the ordinates for the concentrations of

inorganic nitrogen and phosphorus in Figure 2 are drawn in the ratio 10:1 in order to indicate which nutrient was in excess for the algae (cf. REDFIELD ratio 7:1 by weight; REDFIELD et al. 1963). Phosphorus was in excess during all summers at station B1. At station H4 phosphorus was in excess at the end of the summer of 1983 and during the entire summer of 1984, however, not in 1985.



**Figure 2**

Concentrations of inorganic nitrogen and phosphorus at stations B1 and H4 during June – August (VI–VIII), 1983–85. (Based on original data from LARSSON and JOHANSSON 1985, 1986, 1987, pers. comm.)



**Figure 3**

Isotherms at station B1 during June – August (VI–VIII), 1983–85. (Based on original data from LARSSON and JOHANSSON 1985, pers. comm.)

At station B1 the upper water layer was warmest ( $> 16^{\circ}\text{C}$ ) in August 1983 and 1984 and in July 1985 (Fig. 3), reaching down to 6–10 m depth in 1983 and 1985 and down to 20–25 m in 1984. At station H4 the temperature was generally 1–2°C higher than at B1. A thermocline developed between 10 and 15 m depth in July and August.

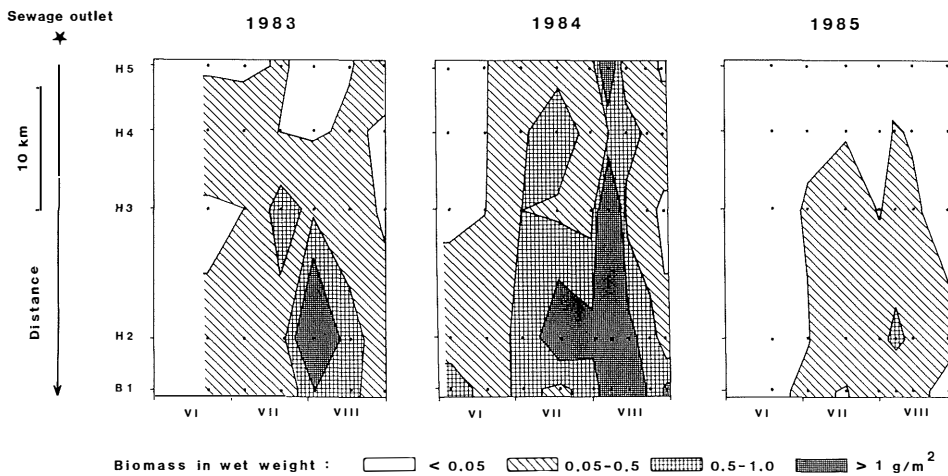
### Material and methods

Phytoplankton samples were usually taken at two-week intervals at stations H5 – B1. During the period of maximal occurrence of the blue-green algae samples were collected weekly. The samples were taken with a plastic tube ( $\varnothing$  26 mm), the length of which corresponded to the mean thickness of the trophogenic layer; i.e., 14m for stations H5 – H2 and 20m for station B1. The samples were immediately preserved in Lugol's solution supplemented with acetic acid.

Biomass, number of heterocysts and heterocyst frequency (number of heterocysts per mm filament) of *Aphanizomenon flos-aquae* were determined employing an inverted microscope according to EDLER (1979a). The number of heterocysts is an expression for the nitrogen fixation potential of the population and the heterocyst frequency expresses the environmental conditions for nitrogen fixation independent of the amount of filaments in the water (cf. LINDAHL et al. 1978, 1980).

### Results

The distribution of *Aphanizomenon flos-aquae* in the whole Himmerfjärden Bay area and at the reference station B1 during 1983–85 is shown in Fig. 4. It appears that *Aphanizomenon flos-aquae* was most common in 1984. Each year the highest biomass values generally developed in the outer areas and maximum abundance was attained in late July and in August.



**Figure 4**

Distribution of *Aphanizomenon flos-aquae* in Himmerfjärden during June – August (VI–VIII), 1983–85. The positions of stations H5 – B1 are given in Figure 1.

The highest biomass values in July and August coincided with the periods when the upper water layer was warmest in the area (cf. Fig. 3). A regression analysis of the biomass of *Aphanizomenon flos-aquae* at station B1 in 1983–85 versus the mean temperature of

the upper 20m water layer revealed a positive correlation between the parameters (Fig. 5). No such correlation was obtained for station H4.

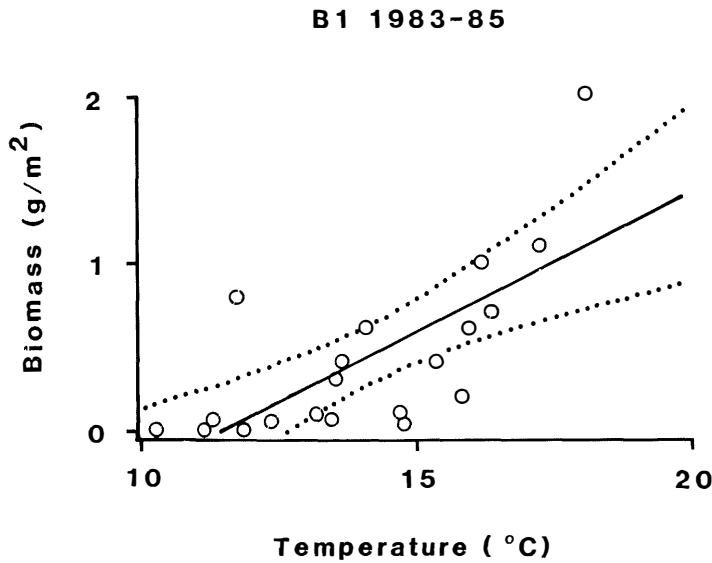


Figure 5

Linear regression of wet weight biomass of *Aphanizomenon flos-aquae* (0–20m) versus temperature of the 0–20m water layer at station B1 1983–85. Dotted curves: 95 % confidence limits.  
 $y = 1.1638x - 1.88$ ;  $n = 20$ ;  $r^2 = 0.47$ ;  $p = 0.0005$ .

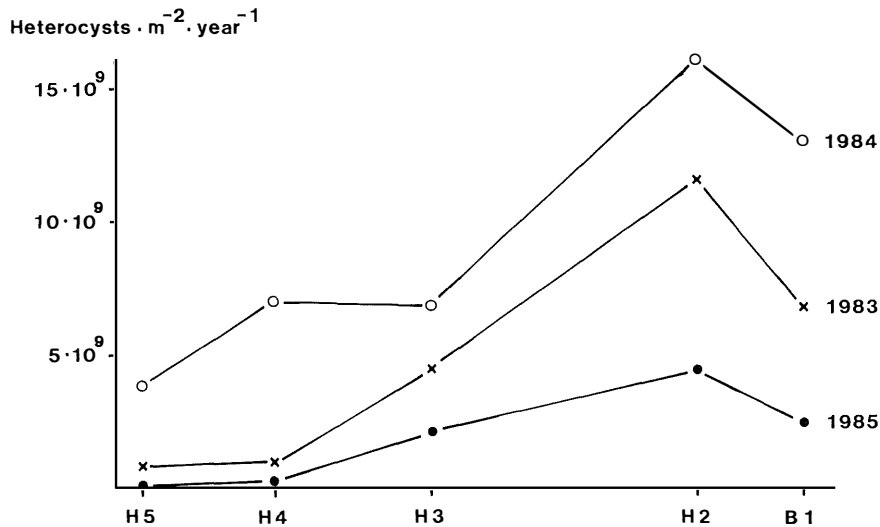
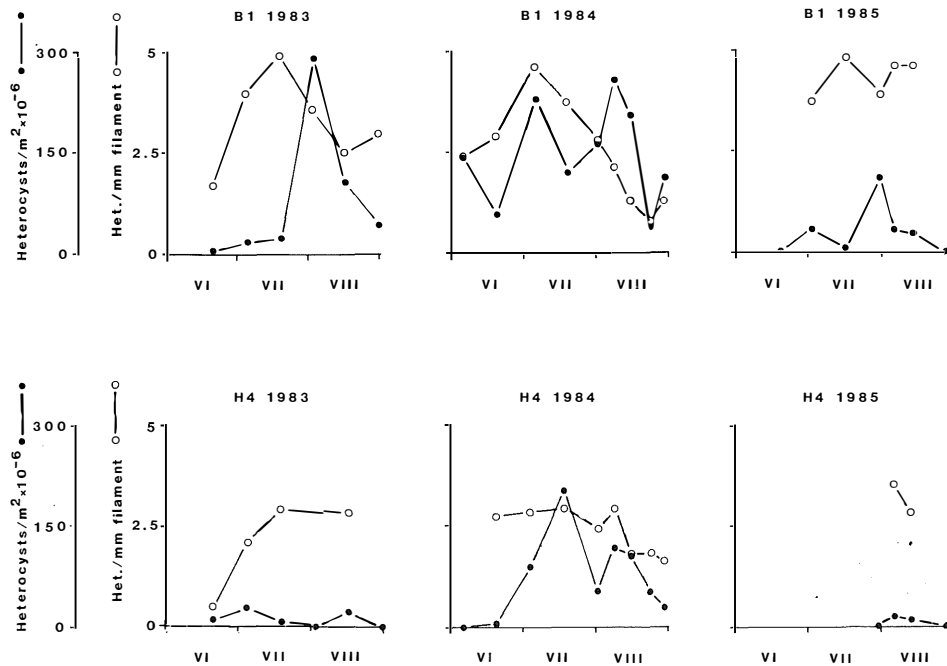


Figure 6

Integrated number of heterocysts of *Aphanizomenon flos-aquae* at each station H5–B1 during 1983–85.

Integrated values for the number of heterocysts at each station during the summers of 1983–85 are given in Figure 6. The number of heterocysts was always lower in the inner areas of Himmerfjärden Bay and increased with increasing distance from the sewage treatment plant. Further, there was a significant difference in the number of heterocysts between years; the heterocysts were most numerous in the phosphorus year (1984) and few in number in the nitrogen year (1985).

The variation of the number of heterocysts as well as the heterocyst frequency during the summer season is shown for stations B1 and H4 in Figure 7. As mentioned above, the number of heterocysts was lower at station H4 than at B1. This applies also for the magnitude of the heterocyst frequency with maximum values of 3.5 heterocysts/mm filament at H4 and 4.6 heterocysts/mm filament at B1. In 1984, when *Aphanizomenon* was most abundant, the heterocyst frequency varied less during the season at H4 (1.6–2.9 heterocysts/mm filament) than at B1 (0.8–4.6 heterocysts/mm filament).

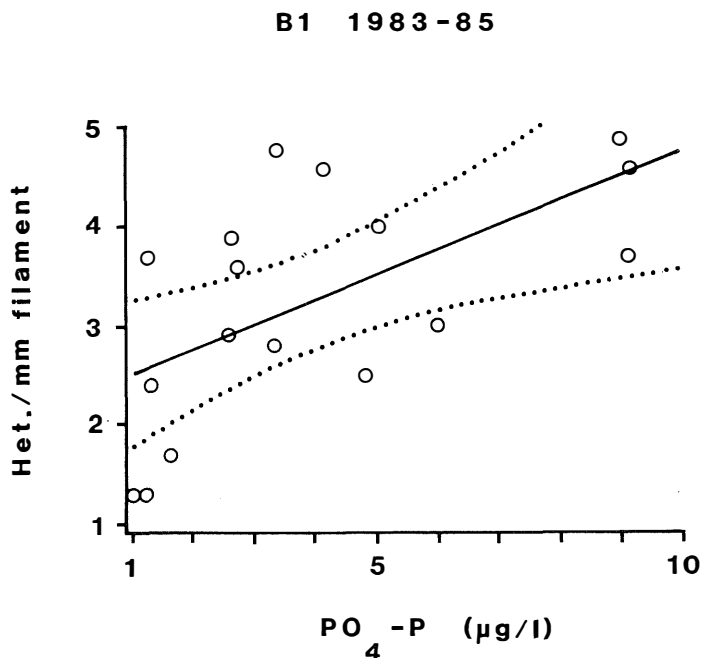


**Figure 7**

Number of heterocysts and heterocyst frequency (heterocysts/mm filament) of *Aphanizomenon flos-aquae* at stations B1 and H4 June – August (VI–VIII), 1983–85.

The variation of heterocyst frequency throughout the season at station B1 fluctuated in principle as did the concentration of inorganic phosphorus. A correlation test between these parameters in all years revealed a significant relation ( $\rho=0.011$ ) for station B1 (Fig. 8), however not for station H4.



**Figure 8**

Linear regression of heterocyst frequency (heterocysts/mm filament) of *Aphanizomenon flos-aquae* versus the mean concentration of phosphorus in the 0–20m water layer at station B1 in 1983–85.

Dotted curves: 95 % confidence limits.  $y = 0.2520x + 2.27$ ;  $n = 17$ ,  $r^2 = 0.36$ ;  $p = 0.011$ .

### Discussion

In order to interpret the occurrence of the *Aphanizomenon* population in Himmerfjärden Bay, its response to some environmental factors at reference station B1 will first be discussed. *Aphanizomenon flos-aquae* is a characteristic species of the summer phytoplankton community at station B1 (HOBRO 1979). Its abundance may vary widely between years, as in 1984 compared to 1985 in the present study, but even higher biomass and number of heterocysts than 1984 have been recorded (LINDAHL et al. 1978, and unpublished data).

The importance of a warm stable water column for the development of blue-green algae is often emphasized (e.g. REYNOLDS 1984). The results of the present investigation support this as well as EDLER's (1979b) suggestion that the maximum abundance of *Aphanizomenon* in the Baltic is correlated to the temperature increase of the water (cf. Fig. 5). As the increasing surface temperatures established a thermocline (Fig. 3), the high biomass at increasing temperatures might also be an effect of the stabilization of the water column.

Due to its nitrogen fixing ability, *Aphanizomenon flos-aquae* is particularly favoured in nitrogen deficient waters with an excess of available phosphorus. In this study a N:P ratio of 10:1 has been considered as an approximate value for the N:P need of phytoplankton whereby a lower ratio indicates phosphorus excess, i.e. favourable nutrient conditions for nitrogen fixing algae. Thus it appears that nutrient conditions were favourable for *Aphanizomenon* at station B1 as phosphorus was in excess relative to nitrogen in all summers of investigation (Fig. 2) and that, the variations in the abundance of *Aphanizomenon* between the years were mainly due to variations in the temperature conditions.

The nitrogen deficiency is accentuated for the algae when the concentration of available phosphorus increases. The formation of heterocysts which is induced by low concentrations of nitrogen in the water (KULASOORIYA et al. 1972, NEILSON et al. 1971) is therefore stimulated when phosphorus is added, resulting in an increase in heterocyst frequency as demonstrated in enrichment experiments (LINDAHL et al. 1980). A similar response of *Aphanizomenon* to nutrient conditions was obtained during the present study as the heterocyst frequency was positively correlated to the concentration of phosphorus in the water (Fig. 8).

In 1983 and 1984 there was a tendency for heterocyst frequency to decrease in time during the period of high biomass and high number of heterocysts at station B1 (Fig. 7), which is in accordance with results from other in situ investigations in the Baltic (LINDAHL et al. 1978, LINDAHL and WALLSTRÖM 1985). LINDAHL et al. (1978) suggest that the decrease of heterocyst frequency during the season was an effect of a suppression of heterocyst formation due to an increase of nitrogen attained by nitrogen fixation. This suggestion, which also applies to the present investigation, is supported by the results from 1985 (Fig. 7) as the heterocyst frequency remained on a high level throughout the summer when the number of heterocysts, and consequently the nitrogen fixation, was low.

When the number of heterocysts and biomass were high in August 1983 and 1984 the concentration of phosphorus decreased with time due to consumption by the algae. Thus, the decrease of phosphorus with a simultaneous increase in nitrogen supply due to nitrogen fixation accentuated a phosphorus deficiency for the algae whereby the heterocyst frequency decreased.

Compared to station B1, the heavy nitrogen load is an additional factor influencing the development of *Aphanizomenon flos-aquae* in the inner parts of Himmerfjärden Bay. Due to a higher concentration of nitrogen and a lower concentration of inorganic phosphorus at station H4 compared to station B1 (Fig. 2), the nutrient conditions were less favourable for nitrogen fixing species at H4. This was also evident from the fact that the number of heterocysts as well as the heterocyst frequency was lower at station H4 than at B1 (Figs. 6 and 7). Due to a more balanced nutrient situation for other phytoplankton than for nitrogen fixing species the blue-green algae were of no significance for the total phytoplankton biomass at station H4, which was dominated by monads and dinoflagellates during the time of investigation (LARSSON and JOHANSSON 1986, 1987). The low abundance of *Aphanizomenon* in the inner areas of Himmerfjärden Bay was most probably due to competition for phosphorus with the dominating algae. As the biomass of *Aphanizomenon* was highest in 1984 when there was an excess of phosphorus relative to nitrogen during the whole summer (Fig. 2), it seems that *Aphanizomenon* was competitive here mainly when nutrient conditions favoured nitrogen fixation. Under these conditions the development of *Aphanizomenon* was further stimulated by high water temperatures.

The occurrence of *Aphanizomenon* in Himmerfjärden Bay might sometimes also be due to transport of coastal surface waters into the Bay in connection with southerly winds. Such winds prevailed for instance in August 1984 (A. ENGQUIST, pers. comm), when a distinct increase of *Aphanizomenon* in the whole investigation area appeared (Fig. 4).

A future reduction of the nitrogen outlets will most likely favour a development of *Aphanizomenon flos-aquae* in the Himmerfjärden area.

For the avoidance of nitrogen fixing algal blooms in Himmerfjärden Bay it is therefore of crucial importance that the phosphorus discharges are kept low.

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