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Annual variability of biomass and heterocysts of the N_2-fixing cyanobacterium *Aphanizomenon flos-aquae* in the Baltic Sea with reference to *Anabaena* spp. and *Nodularia spumigena*

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The biomass, abundance of heterocysts and heterocyst frequencies of the N_2-fixing cyanobacterium *Aphanizomenon flos-aquae* and the corresponding environmental parameters were monitored during two separate years near the entrance of the Gulf of Finland. For reference, data on two other N_2-fixing cyanobacteria, *Anabaena* spp. and *Nodularia spumigena* were collected as well. *A. flos-aquae* was observed throughout the year, being most abundant in the summer, while *Anabaena* spp. and *N. spumigena* were found only during the warmest summer period. A biomass increase of *A. flos-aquae* was positively associated with temperature and negatively with the DIN:DIP ratio; the highest biomasses were found at temperatures above 10 °C and in molar DIN:DIP ratios less than 10. According to the changes in the heterocyst frequencies, *A. flos-aquae* had a seasonal N_2 fixation strategy in which the maximum frequencies of heterocysts preceded the annual biomass peak.

Introduction

Blooms of cyanobacteria are an annual phenomenon and an increasing nuisance in the open Baltic Sea (Kahru *et al.* 1994, Finni *et al.* 2001). The main factors enhancing the blooms are excessive amounts of nutrients, especially of phosphate, and high surface water temperatures combined with calm weather (Kononen *et al.* 1996).

The open sea blooms are mainly formed by three taxa of filamentous, N_2-fixing, heterocyst-containing cyanobacteria: *Aphanizomenon flos-aquae* (L.) Ralfs, *Nodularia spumigena* Mertens and *Anabaena* spp. Bory. *A. flos-aquae* can be found in the water column throughout the year, but it usually has its annual biomass peak in summer. *A. flos-aquae* is not known to produce toxins in the Baltic Sea but it is a neurotoxin producer in lakes (Sivonen *et al.* 1990). *N. spumigena* is a brackish water species that has a genetically diverse population in the Baltic Sea (Barker *et al.* 2000a, Laamanen *et al.* 2001). It occurs mainly during the warmest period of the year and makes up a large part of the annual blooms. A strong hepatotoxin, nodularin, is produced by *N. spumigena* (Sivonen *et al.* 1989). The third bloom-forming taxon consists of several species of the genus *Anabaena*. At least three morphologically-defined species are common in the blooms, *A. lemmermannii* P. Richter, *A. inaequalis* (Kützing) Bornet & Flahault and *A. cylindrica* Lemmermann. In lakes, *Anabaena* spp.

are noxious toxin-producers with the capability of producing both hepatotoxins and neurotoxins (Sivonen *et al*. 1990). In the Baltic Sea, *Anabaena* species are not known to produce toxins. In studies of cyanobacterial blooms, *Anabaena* spp. are a largely neglected component due to their minor contribution to the blooms, though they are occasionally abundant.

A. flos-aquae is a species having genetically a rather homogenous population in the Baltic Sea, whereas lake populations are genetically more diverse (Barker *et al*. 2000b, Laamanen *et al*. 2002). Based on the genetic (Barker *et al*. 2000b, Janson and Granéli 2002) as well as the ultrastructural (Janson *et al*. 1994) differences between *Aphanizomenon* sp. from the Baltic Sea and *A. flos-aquae* from lakes, the authors considered the two types to be two different species. The genetic differences established so far are very small (partial 16S rRNA gene similarities between the two types are from 99.14% to 99.9%), morphologically there are no discontinuities between the two types and the same 16S to 23S rRNA ITS genotype, which dominates the Baltic Sea population is found in the lakes of the Baltic Sea drainage area as well (Laamanen 2002). Furthermore, at least two varieties of *A. flos-aquae* occur in the Baltic Sea region, *A. flos-aquae* var. *flos-aquae* and *A. flos-aquae* var. *klebahnii* (Komárek and Kovácik 1989, Janson and Granéli 2002). Therefore, until further evidence by e.g. DNA–DNA hybridizations between *A. flos-aquae* and *Aphanizomenon* from the Baltic Sea is provided, we consider *Aphanizomenon* from the Baltic Sea to be *A. flos-aquae*.

Each of the bloom-forming cyanobacteria in the Baltic Sea has the ability to produce heterocysts. Heterocysts are differentiated cells that are sites for the fixation of atmospheric dinitrogen (N_2) into ammonium (NH_4^+). Heterocysts provide an anoxic atmosphere to the enzyme nitrogenase, which is responsible for converting N_2 to NH_4^+. The formation of the heterocysts is induced by the lack of combined nitrogen in the medium. The transformation of a vegetative cell into a proheterocyst and subsequently into a mature heterocyst is an irreversible process that has a complex regulation involving a high number of genes (Adams and Duggan 1999).

Cyanobacterial N_2 fixation has an ecological importance in aquatic environments (Paerl 1990, Karl *et al*. 2002). In nitrogen-deficient ocean regions, cyanobacterial N_2 fixation provides new combined nitrogen to the pelagic ecosystem and hence supports new planktonic production (Capone and Carpenter 1982). In the Baltic Sea, recent estimates based on biogeochemical calculations suggest that cyanobacterial N_2 fixation may provide an amount of nitrogen to the Baltic Sea larger than that from atmospheric deposition (Larsson *et al*. 2001). According to estimates by Larsson *et al*. (2001), cyanobacterial N_2 fixation supports from 30% to 90% of the net production by the summer plankton communities.

The number of heterocysts correlates with the N_2 fixation activity of the population (Lindahl *et al*. 1980, Riddolls 1985). Therefore the number and frequency (number of heterocysts per unit length of filament) of heterocysts can be considered as an indicator of the N_2 fixation capacity of the cyanobacterial population. *A. flos-aquae*, *N. spumigena* and *Anabaena* spp. have different distribution patterns of heterocysts within their filaments. In *A. flos-aquae* the heterocysts are few in number, often only 1 to 2 per filament, and are positioned subsymmetrically (Komárek and Kovácik 1989). Although summer populations of *A. flos-aquae* are found to contain heterocysts, winter populations are usually devoid of them (Laamanen 1996). Within *N. spumigena* filaments, the heterocysts are located symmetrically, and a new heterocyst always forms in between two mature heterocysts. This results in a higher frequency of heterocysts in *N. spumigena* than in *A. flos-aquae*. In *Anabaena* spp. the positioning of the heterocysts varies depending on the species, but the pattern is more similar to that of its close relative *A. flos-aquae* (Gugger *et al*. 2002).

Previous studies have assessed variations in the biomass, N_2 fixation and heterocyst frequencies of *A. flos-aquae* along horizontal transects in the Baltic Sea (Niemistö *et al*. 1989) and in the different sub-basins of the Baltic Sea (e.g. Rinne *et al*. 1981, Melvasalo *et al*. 1982). In addition, *A. flos-aquae* biomasses and heterocysts have been studied along a eutrophication gradient in the coastal region of Sweden (Wallström 1988). Detailed studies have also been made in summertime on shorter time-scales (e.g. Evans *et al*.

2000, Stal and Walsby 2000, Gallon *et al*. 2002). However, detailed investigations covering the full annual cycle have not been made. Our interest was to describe on an annual scale the interactions between environmental factors and the biomass and heterocyst development of *A. flos-aquae*. We wanted to determine the factors in the natural environment that affect biomass build-up and the development of heterocysts in the different species. The study focused on *A. flos-aquae*, which was the most abundant cyanobacterium during the two cool study years. Data on the less-abundant *Anabaena* spp. and toxic *N. spumigena* are presented because their annual cycles are not well-known; additionally, the species are of interest because of their toxic or potentially toxic nature.

Material and methods

Samples were collected in 1986 and 1993 at the entrance to the Gulf of Finland in the Baltic Sea. In 1986, the samples were collected at 59°51´N, 23°13´E, near the offshore station of Längden, while in 1993, they were collected at Längden (59°47´N, 23°16´E). In 1986, sampling was carried out 3 to 9 times per month from 5 February to 17 December. Upon each occasion, water samples were taken from depths of 0–5 m (by mixing the samples from depths of 0, 2 and 5 m), 10 m, and then 15 or 20 m. In 1993, samples were collected one to four times per month from 6 April to 14 December from depths of 0, 2, 6, 10, 20 and 40 m.

Temperature data were obtained with a mercury thermometer (±0.2 °C) or with a CTD sonde (CTDplus 100, SIS, Germany). Concentrations of NO_3^-, NO_2^-, NH_4^+, PO_4^{3-} and total nitrogen (TN) were analysed according to Koroleff (1979). For technical reasons NH_4^+ was only analysed from 29 May 1986 onwards. Total organic nitrogen (TON) was obtained by subtracting dissolved inorganic nitrogen (DIN) from TN. Water samples for microscope analyses were preserved with acid Lugol's solution. In 1986, filamentous cyanobacteria *A. flos-aquae*, *N. spumigena* and *Anabaena* spp. were enumerated with a Leitz Diavert inverted microscope with phase-contrast optics and a 10× Leitz PHACO objective; in 1993 a Leitz DM IL inverted microscope was used following the Utermöhl (Utermöhl 1958) method. Settling chambers of 50 ml volume were used, except when cyanobacteria were few 100 ml chambers were used. The filaments of cyanobacteria were counted in length units of 100 μm, while the numbers of heterocysts were counted in the same area of the chamber bottom as the filaments. As a result, the number of heterocysts per length of a cyanobacterial filament (heterocyst frequencies) were obtained. The biomass estimates of *A. flos-aquae*, *N. spumigena* and *Anabaena* spp. expressed as carbon were based on the conversion of counting units to volumes (for *A. flos-aquae* and *Anabaena* spp. 1960 μm^{-3} and for *N. spumigena* 9500 μm^{-3} per counting unit) and then obtained by multiplying this result by 0.11 pg C μm^{-3} (HELCOM 1988). Hence 1 μmol C of *A. flos-aquae* and *Anabaena* spp. corresponds to 55 705 counting units, and 1 μmol C of *N. spumigena* to 11 492 counting units.

The data were explored and analysed with the free statistical software package R (R Development Core Team, Vienna, Austria, http://www.R-project.org). The annual variability of hydrochemistry and cyanobacteria were plotted with the graphical software package Surfer version 6.01. To describe the associations between the variables, we used loess smoothing, a non-parametric regression technique, which estimates the local fit of a curve using a locally-weighted regression (Kafadar and Horn 2002). It is an exploratory method, which can be used to reveal the relationship between response and explanatory variables, and to suggest a functional model between them. The 95% confidence intervals were estimated on the basis of a standard error of ±2 of the curve estimation. In addition, non-parametric Spearman rank correlation was used to study associations between variables.

Results

The hydrochemical development of the two study years was highly similar. In 1986, the ice cover persisted until the beginning of April (Fig. 1A). In both years the temperature stratification started to develop at the beginning of

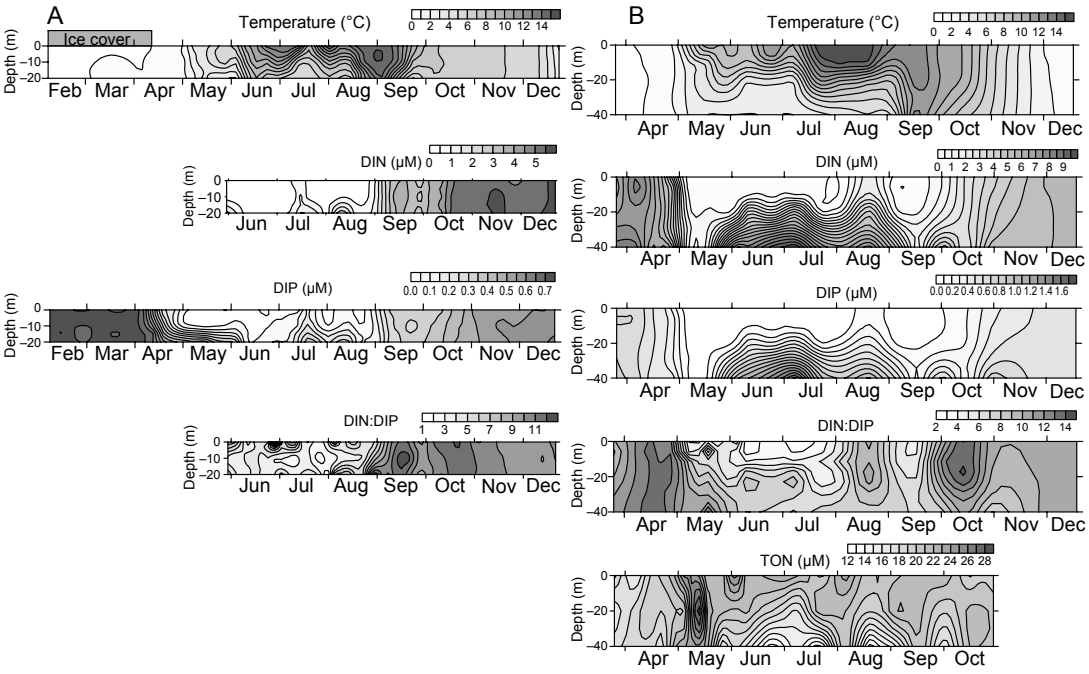

Fig. 1. Annual development of temperature (°C), dissolved inorganic nitrogen, consisting of NO_3^-, NO_2^- and NH_4^+ (DIN; µM), PO_4^{3-} (DIP; µM) and molar DIN:DIP ratio (**A**) between depths of 0 and 20 metres in 1986 and (**B**) between 0 and 40 m in 1993. For the year 1993, concentration of total organic nitrogen (TON; µM), calculated as total nitrogen minus DIN, is also shown.

May, and the phosphate (DIP; PO_4^{3-}) as well as the dissolved inorganic nitrogen (DIN; NO_3^-, NO_2^- and NH_4^+) were depleted from the water column almost simultaneously (Fig. 1). Both years were cool, with maximum surface water temperatures of slightly less than 16 °C. In 1986, surface water temperatures of over 14 °C were found from the end of June to mid-September, while in 1993 the highest surface water temperatures occurred in July and August (Fig. 1A and B). The temperature stratification was broken and new nutrients were brought into the mixed layer in July and August of 1986 (Fig. 1A) and in August 1993 (Fig. 1B). The development of the DIN:DIP ratio of dissolved inorganic nitrogen (DIN) to phosphorus (DIP) was parallel to the general development of the stratification and nutrient concentrations. The lowest surface water DIN:DIP ratios of approximately 1 were found in July 1986 (Fig. 1A) and during summer stratification in June and July 1993 (Fig. 1B).

In 1993, total organic nitrogen (TON) values ranged from 11.9 to 29.8 µM (Fig. 1B). The highest values were due to the spring bloom, and were found in the surface layer after the depletion of inorganic nutrients in May. In addition, high values were detected in the 0–10 m surface layer in July, August and September. Nevertheless, only a slight positive association between the total biomass of filamentous cyanobacteria and TON could be shown (Spearman rank correlation coefficient $r_S = 0.24$, $n = 98$). The mixing in August had a diluting effect on TON.

A. flos-aquae devoid of heterocysts were found in considerable amounts (2.1 µM C) under the ice in February 1986 (Fig. 2A). After winter, the biomass began to increase in May, but the highest biomasses of 6.1 and 7.3 µM C were not recorded until late August and early September, respectively. The population declined by mid-September. In 1993, *A. flos-aquae* were less abundant than in 1986 (Fig. 2B). In 1993, low amounts of cyanobacteria were found during the cooler June, and the proper biomass increase of the summer population did not start until July. The peak biomasses occurred in late July (2.8 µM C) and in September (2.7 µM C). In August and September of 1986 most of the *A. flos-aquae*

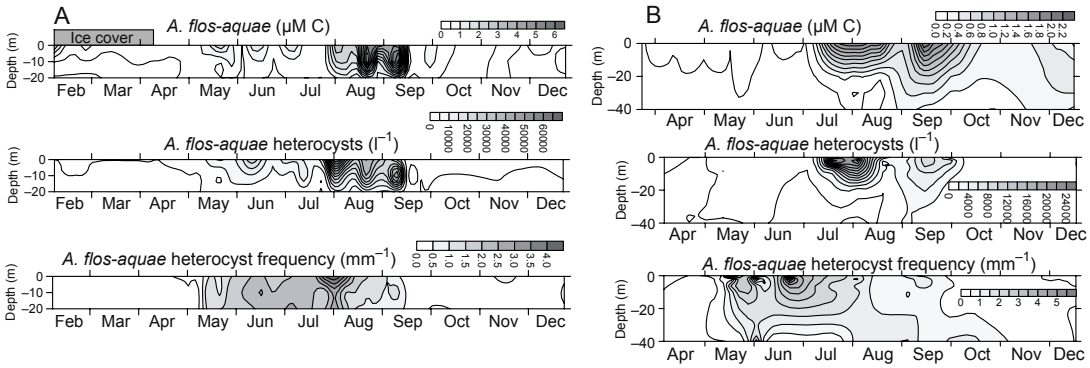

Fig. 2. Annual development of *A. flos-aquae* biomass (µM C), heterocyst abundance (l^{-1}) and frequency (mm^{-1}) (**A**) between depths of 0 and 20 metres in 1986 and (**B**) between 0 and 40 m in 1993.

biomass was found mixed in the top 20 metres with maximum densities at a depth of 5 to 10 m rather than at the surface (Fig. 2A). In 1993, the population densities were highest at the surface.

The abundance of *A. flos-aquae* heterocysts had a positive association with the biomass of *A. flos-aquae* (Spearman rank correlation coefficient $r_S = 0.73$, $n = 193$; Fig. 2). Nevertheless, the development of heterocysts was out of phase with the biomass development, as shown by the development of the heterocyst frequency. The *A. flos-aquae* heterocyst frequency displayed a developmental pattern that was associated mostly with temperature and nutrients. In winter and early spring the filaments were devoid of heterocysts. The first heterocysts were observed approximately a week after the depletion of dissolved inorganic nitrogen in spring (Figs. 1 and 2). The maximum frequencies were found prior to the biomass peaks: in 1986 in late July and in 1993 in June (Fig. 2). Hence the first biomass maximum lagged approximately one month behind the heterocyst frequency peak. The heterocyst frequency decreased to values lower than 1 heterocyst mm^{-1} coincidently with increasing nutrients in September (Fig. 2). The heterocyst frequency of *A. flos-aquae* varied from 0 to 5.7 heterocysts mm^{-1} (Table 1).

In 1986, *Anabaena* spp. consisted mainly of *A. inaequalis*, but in 1993, of *A. lemmermannii*, *A. cylindrica* and *A. inaequalis*. The biomass development of *Anabaena* spp. as well as that of *N. spumigena* had a different character from that of *A. flos-aquae* (Figs. 3 and 4). *Anabaena* spp. and *N. spumigena* only occurred during a limited time in summer. *Anabaena* spp. populations were restricted to early August in 1986 and to late July in 1993 (Figs. 3). In 1986, *N. spumigena* was only found in very low numbers (therefore no data shown). In 1993, the biomass distribution had a varying character as the species occurred from the end of July to mid-August in low biomasses (0.6 μM C) and then again at the beginning of September with a very high biomass of 21 μM C at the surface only (Fig. 4).

Similarly to the biomass development, the occurrence of heterocysts in *Anabaena* spp. and *N. spumigena* was different from that of *A. flos-aquae*. Both taxa had a higher average heterocyst frequency than *A. flos-aquae* (Table 1). *Anabaena* spp. had a frequency of up to 9.8 heterocysts mm^{-1} with a mean of 3.9 heterocysts mm^{-1}, while *N. spumigena* had up to 23.8 heterocysts mm^{-1} with a mean of 11.6 heterocysts mm^{-1}; *A. flos-aquae* had a maximum of 5.7 heterocysts mm^{-1} and in average 1.1 heterocysts mm^{-1}. In 1986, when only the *Anabaena inaequalis* species was studied, the heterocyst frequency was

Table 1. Variability (mean, minimum, maximum, coefficient of variation) of heterocyst frequency (heterocysts mm^{-1}) in *Aphanizomenon flos-aquae*, *Anabaena* spp. and *Nodularia spumigena* in the years 1986 and 1993 near the SW coast of Finland.

	Mean	Min	Max	CV (%)
A. flos-aquae ($n^1 = 196$)	1.1	0	5.7	109
Anabaena spp. ($n = 32$)	3.9	0	9.8	66
N. spumigena ($n = 19$)	11.6	0	23.8	74

[1] Number of observations (n).

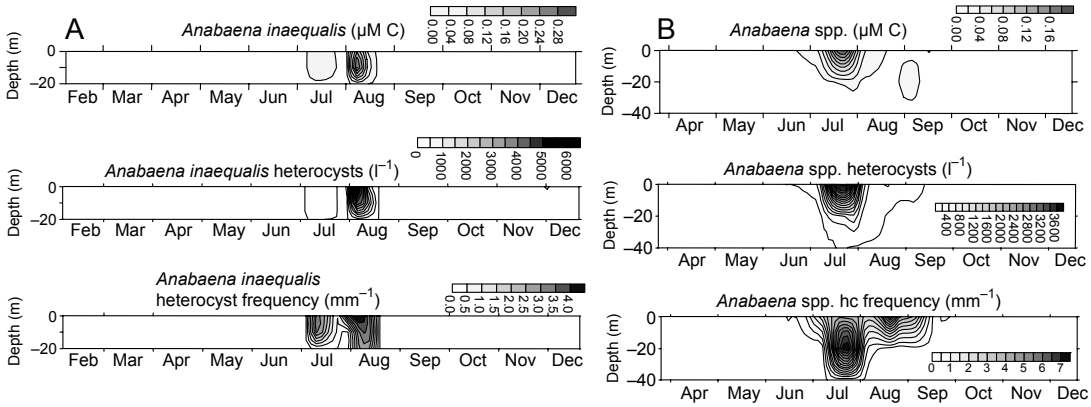

Fig. 3. Annual development of *Anabaena inaequalis* (in 1986) and *Anabaena* spp. (in 1993) biomass (µM C), heterocyst abundance (l^{-1}) and frequency (mm^{-1}) (**A**) in 1986 and (**B**) in 1993. The distributions are shown between depths of 0 and 20 metres in 1986 and between 0 and 40 m in 1993.

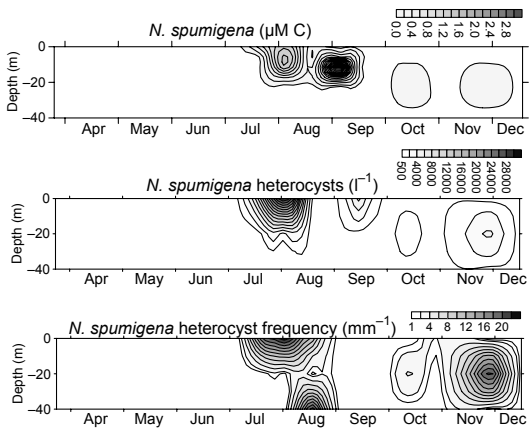

Fig. 4. Annual development of *N. spumigena* biomass (µM C), heterocyst abundance (l^{-1}) and frequency (mm^{-1}) between depths of 0 and 40 m in 1993. In order to display a higher resolution, the high biomass (21 µM C) and heterocyst abundance (7000 l^{-1}) of 1 September were excluded from the figure.

invariable at around 5 heterocysts mm^{-1}. In 1993, when three *Anabaena* species were included in *Anabaena* spp., a higher variability ranging between 0 and 9.8 heterocysts mm^{-1} was found. In *N. spumigena* the frequency was mostly from 14 to 22 heterocysts mm^{-1} except for the single observation during the very high biomass in September 1993, when the heterocyst frequency was < 1 heterocyst mm^{-1} (Fig. 4).

On the basis of loess smoothing, temperature and the DIN:DIP ratio were the factors that were clearly associated with changes in the *A. flos-aquae* and *Anabaena* spp. biomass, heterocyst number and heterocyst frequency. The biomass of *A. flos-aquae* started to increase at 7 °C and most of the biomass increase took place at temperatures > 10 °C (Fig. 5A). The biomass of *Anabaena* spp. was very low at < 6 °C, and similarly to *A. flos-aquae* the steepest biomass increase was recorded at temperatures > 10 °C (Fig. 6A). The *N. spumigena* biomass increase also coincided with the temperature increase; nevertheless, the highest biomass was recorded at 13 °C (Fig. 7A). The highest *A. flos-aquae* biomasses were found at DIN:DIP ratios ranging between 2 and 10 (Fig. 5B). Due to the limited material for *Anabaena* spp. (Fig. 6B) and *N. spumigena* (Fig. 7B), the relationship between the DIN:DIP ratio and biomass was less pronounced, but *Anabaena* spp. seemed to favour low DIN:DIP conditions.

The frequency of the heterocysts of *A. flos-aquae* had a different relationship with temperature than had the *A. flos-aquae* biomass. The increase in the heterocyst frequency was steepest between 0 and 10 °C after which it levelled out (Fig. 5C). The relationship between the *A. flos-aquae* heterocyst frequency and the DIN:DIP ratio was also clear: up to a DIN:DIP ratio of 12, the frequency of heterocysts decreased, and for DIN:DIP > 12 the frequency was mostly < 1 heterocyst mm^{-1} (Fig. 5D). The DIN or DIP alone did not have a clear relationship with the *A. flos-aquae* heterocyst frequency (data not shown). For the *Anabaena* spp. heterocyst frequencies, no meaningful relationship with any of the envi-

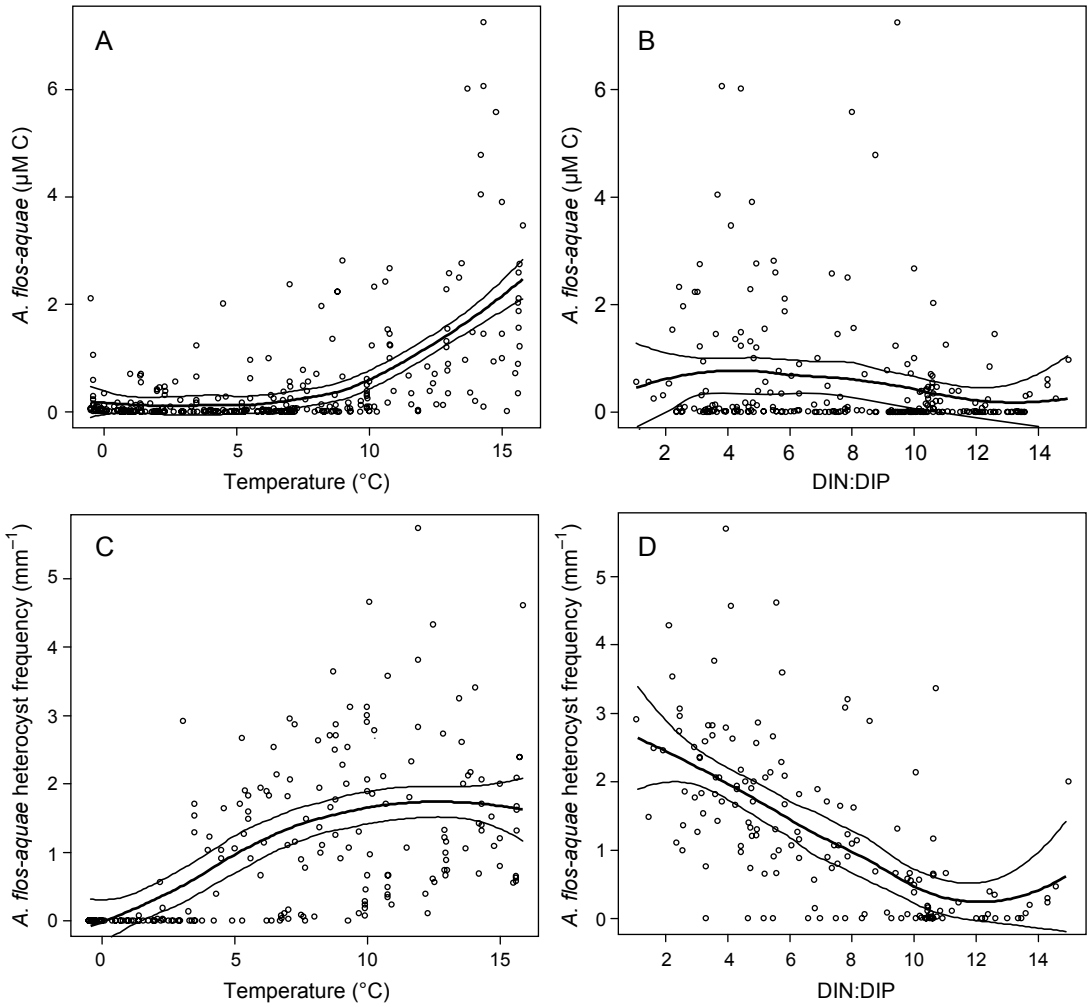

Fig. 5. Relationships of *A. flos-aquae* biomass (µM C) and heterocyst frequency (mm^{-1}) to temperature (°C) and molar ratio of dissolved inorganic nitrogen to phosphorus (DIN:DIP). The curves shown are loess smoothing curves with 95% confidence intervals, based on data pooled from years 1986 and 1993. — **A**: relationship of *A. flos-aquae* biomass (µM C) to temperature (°C); — **B**: relationship of *A. flos-aquae* biomass (µM C) to molar (DIN:DIP) ratio; — **C**: relationship of *A. flos-aquae* heterocyst frequency (mm^{-1}) to temperature (°C); — **D**: relationship of *A. flos-aquae* heterocyst frequency (mm^{-1}) to molar DIN:DIP ratio.

ronmental factors was found, probably due to the somewhat invariable frequencies. In *Nodularia*, the heterocyst frequencies were mainly between 14 and 23.8 heterocysts mm^{-1}, but at temperatures between ca. 10 °C and 14 °C the frequency was lower.

Discussion

The development of the *A. flos-aquae* population was strongly associated with water temperature. The starting point for the summer population increase coincided with the warming and stabilization of the water column, and in both years the highest biomasses were recorded at the times of the temperature maxima. Stabilization of the water column is critical because it reduces the mixing depth and increases light availability. Thus both light and temperature act at the same time, and in data from the nature their effects are difficult to differentiate. However, according to Stal and Walsby (2000), light, rather than temperature, is considered as the ultimate

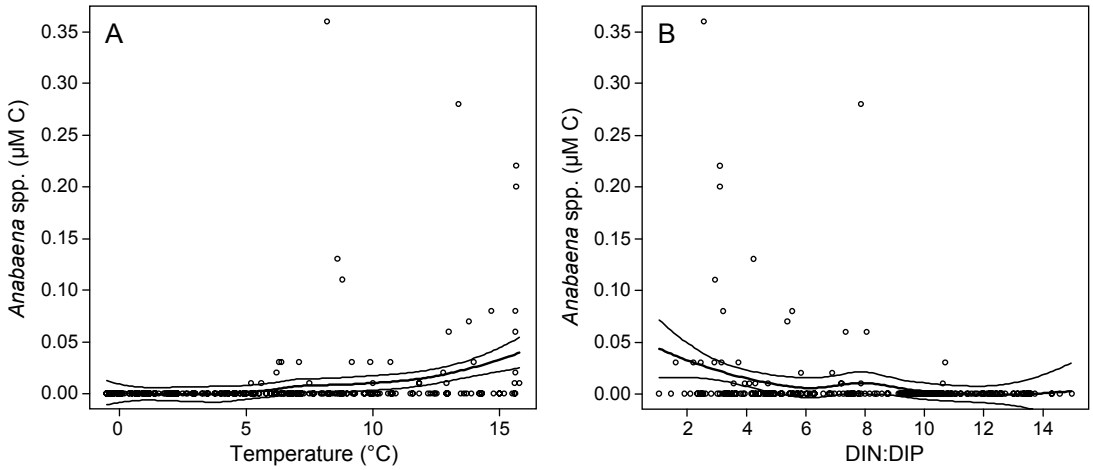

Fig. 6. Relationship of *Anabaena* spp. biomass (µM C) to (**A**) temperature (°C) and (**B**) molar ratio of inorganic nitrogen to phosphorus (DIN:DIP) with loess curves and 95% confidence limits based on data pooled from the years 1986 and 1993.

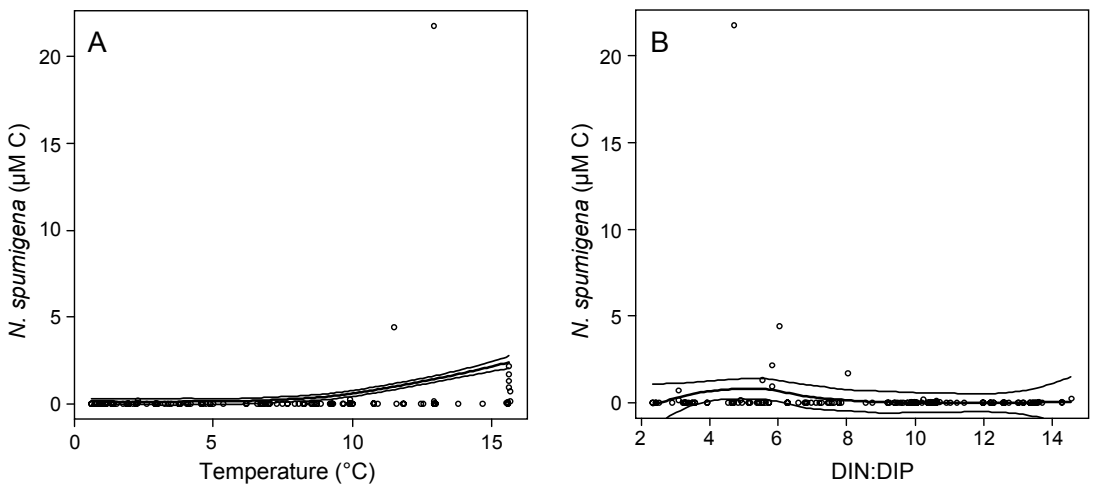

Fig. 7. Relationship of *N. spumigena* biomass (µM C) to (**A**) temperature (°C) and (**B**) ratio of inorganic nitrogen to phosphorus (DIN:DIP) with loess curves and 95% confidence limits based on data pooled from the years 1986 and 1993.

controlling factor for bloom formation, although temperature has a direct effect on growth rate. *A. flos-aquae* had both the highest biomass and the most intensive biomass increase at temperatures > 10 °C. These results are similar to the observations from the Swedish coast of the Baltic Sea proper (Wallström 1988, Larsson *et al*. 2001). In a data set covering the whole Baltic Sea, bloom biomasses of *A. flos-aquae* were only observed at temperatures exceeding 17 °C (Wasmund 1997). The results of culture experiments show the direct effect of temperature on growth: when test temperatures from 7 to 28 °C were used, poor growth of *A. flos-aquae* was observed at temperatures of 7 and 10 °C, with the most intensive growth occurring at temperatures of 16 and 22 °C (Lehtimäki *et al*. 1997).

The DIN:DIP ratio was another factor that had an association with the *A. flos-aquae* biomass development: the highest biomasses were observed at molar DIN:DIP ratios of < 10, well below the Redfield ratio (Redfield 1958). In general, the negative association between the abundance of heterocystous cyanobacteria and

the DIN:DIP ratio is due to the competitive advantage the N_2-fixing cyanobacteria gain in low DIN:DIP ratio conditions (Niemi 1979).

Anabaena spp. and *N. spumigena* had similar relationships with temperature and DIN:DIP ratio as did *A. flos-aquae*. However, high water temperatures are considered to especially favour *N. spumigena*, which explains its rarity in our material. In batch culture experiments *N. spumigena* had the most intensive growth and N_2 fixation activities at the highest test temperatures of 25 and 28 °C (range from 7 to 28 °C) (Lehtimäki *et al.* 1997). Field data, as well as experimental data, on Baltic Sea *Anabaena* spp. are scarce, but *Anabaena* strains isolated from Finnish lakes had a positive growth response to elevated temperatures (test range from 15 to 30 °C), light intensities (2 to 128 μmol m^{-2} s^{-1}) and PO_4^{3-} concentrations (0.05 to 5.5 mg l^{-1}) (Rapala *et al.* 1993). The differences between the species in their relationships with temperature may partly be explained by differences in the temperature regulation of N_2 fixation. Although the two taxa had similar variability in our data, *N. spumigena* and *Anabaena* sp. were shown to have different temperature dependencies of N_2 fixation, indicating a smaller limitation of N_2 fixation by increasing temperature in the case of of *N. spumigena* than in that of *Anabaena* sp. (Staal *et al.* 2003).

A. flos-aquae seems to be adapted to winter conditions. In 1986, we observed biomasses reaching 2.1 μM C beneath the ice cover. This biomass is equal to the maximum summer biomass of a cool summer, e.g., 1993. The filaments found under the ice were always devoid of heterocysts and hence they were likely to be new filaments, not remnants of the summer population. Since DIN tends to suppress new heterocyst formation (e.g., Adams and Duggan 1999), the original heterocysts had most probably diluted among the filaments after the autumn mixing and nutrient replenishment, implying growth after the autumn mixing. In addition, in our data from December 1993, a biomass increase without corresponding increase in heterocysts was observed, supporting the idea. There are a number of observations of *A. flos-aquae* occurring in winter (Laamanen 1996, Ikävalko and Thomsen 1997, Wasmund 1997, Laamanen 2002) but experimental information on its performance in those conditions is scarce. Indications of slow growth in temperatures < 10°C and in a low (2 μmol m^{-2} s^{-1}) light intensity (Lehtimäki *et al.* 1997) exist, but there is no proper knowledge on the potential growth in harsh winter conditions. Stal and Walsby (2000) argue that growth of cyanobacteria could only occur during summer months when the mixed layer is shallow and the critical depth of cyanobacteria species exceeds the mixed layer depth. In the shallow coastal areas of SW Finland, seaward-flowing river waters have a stabilizing effect on the wintertime water column under ice (Niemi 1973), which is likely to benefit *A. flos-aquae*. In addition, buoyancy provided by the gas vesicles of *A. flos-aquae* enables it to float to the surface to make the most out of the dim available light of the short winter days. *N. spumigena* and *Anabaena* spp., on the other hand, were not observed outside the summer period, and their mode of overwintering is largely unsolved.

The *A. flos-aquae* heterocyst abundance had a positive association (r_S = 0.73, n = 193) with biomass. A positive association on a seasonal time-scale has been shown between the number of heterocysts and the N_2 fixation activity (Rinne *et al.* 1978, Riddolls 1985), as well as between biomass and the N_2 fixation activity (Hübel and Hübel 1980). On temporal scales of hours and days, the N_2 fixation activity varies due to diurnal changes in light and due to day-to-day changes in the nitrogen status of the cells (Gallon *et al.* 2002). On short time-scales, heterocyst numbers or biomass are not reliable indicators of N_2 fixation, but on an annual scale they are more likely to give an indication of the N_2 fixation capacity of the population.

To estimate the amount of nitrogen the cyanobacteria could have theoretically introduced into the water, the difference between the summer maximum and the spring minimum cyanobacterial biomasses was used as an indicator of the introduction of new cyanobacterial biomass. It was assumed that the cyanobacteria fixed all the nitrogen they needed, and leakage of nitrogen from the cells was not accounted for; finally, the molar Redfield ratio of 106_C:16_N:1_P (Redfield 1958) was used to obtain an estimation of nitrogen introduced into the environment by cyanobacteria. Following this calculation, in 1986 and

1993, respectively, the cyanobacteria could have introduced 1.1 and 0.76 μM of new nitrogen into the water. The TON concentrations in the < 10 m surface water layer in summer 1993 varied from 18.1 to 29.0 μM, and although the TON maxima at the surface at the beginning of August and in September could be associated with biomass peaks of heterocystous cyanobacteria, there was no strong association between overall TON and cyanobacterial biomass. In our study area, other factors than cyanobacterial N_2 fixation cause most of the variation in surface water TON, at least during cool summers. In recent results from the western Baltic Sea proper, cyanobacteria were considered to contribute about 2 μM to the surface water layer during the summer time (Larsson *et al*. 2001), which is of the same magnitude as our theoretical calculation.

The annual pattern of development of *A. flos-aquae* heterocyst frequencies was similar to the pattern presented by Walve and Larsson (manuscript in Walve (2002)). Based on measurements of cell nutrient quota, they considered the cells to store PO_4^{3-} during the springtime, when inorganic nutrients are still abundant, and to consume this internal PO_4^{3-} storage during the biomass build-up, simultaneously filling the N needs primarily by N_2 fixation. The PO_4^{3-} availability seems to determine the frequency of heterocysts of *A. flos-aquae*, as was also shown along a nutrient gradient (Wallström 1988) and in experimental PO_4^{3-} addition (Lindahl *et al*. 1980). N_2 fixation activity reflects the nitrogen status of the cells (Gallon *et al*. 2002). When the PO_4^{3-} needs of the cells are satisfied and the cells are lacking in nitrogen, new heterocysts with nitrogenase and new N_2-reducing capacity are synthesized (Adams and Duggan 1999).

In terms of temperature, the annual pattern of *A. flos-aquae* heterocyst frequencies is interesting. With most of the heterocyst frequency increase taking place between temperatures of 5 and 10 °C and the steepest biomass increase occurring at > 10 °C, it seems that between 5 and 10 °C more N_2 is fixed than what is needed for growth. This assumption is valid only if the N_2 fixation efficiency of a heterocyst is approximately constant and not significantly reduced, for example, by low temperature or poor light availability. Data on the annual development of the cellular C:N ratios of *A. flos-aquae* presented by Walve and Larsson (manuscript in Walve (2002)) support the idea of increased N_2 fixation in relation to biomass and a subsequent increase of N storage at temperatures from 5 to 10 °C, because there is an abrupt drop in the C:N ratio in that temperature interval.

The development of *N. spumigena* and especially *Anabaena* spp. heterocyst frequencies was different from that of *A. flos-aquae*. The two former taxa either had a rather invariable frequency, indicating no specific seasonal strategy in regard to heterocyst frequency (*Anabaena* spp.), or a mostly invariable frequency with an abrupt decrease in autumn, as in the case of *N. spumigena*. Other reports of *N. spumigena* heterocyst frequencies, ca. 15 heterocysts mm^{-1} (Lindahl and Wallström 1985), 18 mm^{-1} (Niemistö *et al*. 1989) and 16–28 mm^{-1} (Wallström *et al*. 1992) are in line with our observations from the June–July population biomass maximum (ca. 22 heterocysts mm^{-1}). During the intense biomass peak of ca. 21 μM C in September, the heterocyst frequencies were very low (0–2 heterocysts mm^{-1}), which is not in line with previous studies. One explanation for the low values may be the mixing that occurred in August. It increased surface layer DIN concentration and resulted in an increased uptake of inorganic N by *N. spumigena* and may have subsequently led to decreased heterocyst frequencies.

In order to fully capture the ecological behaviour and the implications that the different bloom-forming cyanobacterial species have for the ecosystems, the different diazotrophies and seasonal N_2 fixation strategies of the taxa have to be acknowledged. Our study describes the occurrence in nature of three N_2-fixing species and shows the associations between them and the environmental factors. Although field data of this kind may be descriptive in nature, it is, however, the basis for an understanding of the functioning of single species as well as of entire ecosystems.

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