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Blooms of Baltic Sea *Aphanizomenon* sp. (Cyanobacteria) collapse after internal phosphorus depletion

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ABSTRACT: We studied C:N:P stoichiometry, heterocyst frequency, and biomass of the N_2-fixing cyanobacteria *Aphanizomenon* sp. and *Nodularia spumigena* from May to September 1999 and 2000 at an offshore station in the NW Baltic Proper. In 2000, we included iron (Fe) and molybdenum (Mo) contents and biomass-specific ^{14}C uptake. We also complemented published C:N:P stoichiometry data from 1998 with heterocyst frequency. We found a drastic increase in C:P and N:P ratios in *Aphanizomenon* sp., indicating severe P deficiency at the biomass maximum. *N. spumigena* also had high C:P and N:P ratios at high abundances. In 2000, and 1998, the total amount of P stored in *Aphanizomenon* sp. biomass in early summer equalled that at the bloom peak. Only a small part of the DIP surplus remaining after the spring bloom of diatoms and dinoflagellates ended up in the peak biomass of the subsequent cyanobacterial bloom. *Aphanizomenon* sp. heterocyst frequency peaked in early summer when the C:P ratio was near the Redfield value, and then decreased with increasing C:P ratio, initially perhaps due to increased temperature, and later to P limitation. This decrease occurred parallel to a decrease in *Aphanizomenon* sp. Mo content. We found no indication of Fe limitation since there was no clear decrease in *Aphanizomenon* sp. Fe content as the bloom progressed. *N. spumigena* had considerably higher Fe content, but a high Fe:Mo ratio suggests that Fe adsorbed to cell surfaces biased the measurements. An expected reduction in growth rate due to the high C:P ratio was not mirrored in a decreased specific C uptake of *Aphanizomenon* sp. as measured in short-term ^{14}C incubations. This may indicate short-term C storage with subsequent respiration or excretion of excess carbon when growth is P limited.

KEY WORDS: Cyanobacteria · Stoichiometry · Cell quota · Nitrogen · Phosphorus · Iron · Molybdenum · Heterocysts · Baltic Sea

INTRODUCTION

Temperate estuaries generally lack dinitrogen (N_2)-fixing planktonic cyanobacteria, despite prevailing nitrogen limitation (Howarth et al. 1988). High turbulence, short water residence time, grazing, and limitation by molybdenum (Mo) or iron (Fe) are factors hypothesized to be responsible, either alone or in combination. Mo, which is a necessary component of the N_2-fixing enzyme-complex nitrogenase (Bothe 1982), occurs at high concentrations in seawater in the form of molybdate, but interference with sulfate may lower uptake rates, leading to slow growth rate and high susceptibility to grazing (Marino et al. 2002). The high Fe requirement in nitrogenase (Bothe 1982, Sañudo-Wilhelmy et al. 2001), and the high ratio of Fe-rich Photosystem I (PS I) to PS II reaction centers in cyanobacteria (Falkowski & Raven 1997), combined with the low solubility of Fe in oxic seawater, may lead to Fe limitation. It has been suggested that cyanobacteria of the genus *Trichodesmium*, found in oligotrophic tropical oceans, are limited by Fe (Berman-Frank et al. 2001) or phosphorus (P) (Sañudo-Wilhelmy et al. 2001, Kustka et al. 2003) or co-limited by these nutrients (Mills et al. 2004). Fe limitation has also been indicated for lake cyanobacteria (Hyenstrand et al. 1999).

The large, brackish and semi-enclosed Baltic Sea is an exception to the general scarcity of planktonic N_2 fixers

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in estuaries. The Baltic Proper, the main basin of the Baltic Sea, commonly has high abundances of the heterocystous N_2-fixing species *Nodularia spumigena* and *Aphanizomenon* sp. (hereafter referred to by their genus names) in the stratified summer period (Granéli et al. 1990, Larsson et al. 2001). New estimates indicate N_2 fixation in the Baltic Proper to be a larger source of nitrogen than previously thought, almost as large as that from river discharges or up to twice that from atmospheric inputs (Larsson et al. 2001, Wasmund et al. 2005). Thus, N_2 fixation may contribute 30 to 90% of the nitrogen needed to sustain the phytoplankton net community production during the summer (Larsson et al. 2001).

The severely N-limited spring bloom of diatoms and dinoflagellates in the Baltic Proper leaves a substantial pool of dissolved inorganic P (DIP) (Larsson et al. 2001), which is generally assumed to support the succeeding large N_2-fixing cyanobacterial blooms (e.g. Rahm et al. 2000). The DIP pool is more or less depleted in summer and the cyanobacterial blooms have generally been considered P-limited (Granéli et al. 1990), which is supported by high C:P ratios of *Aphanizomenon* cells (Larsson et al. 2001). It has been suggested that the high concentration of dissolved organic carbon increases the availability of Fe by complexation (Howarth et al. 1988). Despite this, *Nodularia* growth was recently indicated to be Fe-limited (Stal et al. 1999). It is unclear whether Mo can be limiting in the Baltic or if the low salinity (6 to 8), and hence low sulfate concentration, alleviates any Mo deficiency. Grazing on Baltic Sea cyanobacteria has generally been considered negligible (Sellner 1997).

Our aim was to study elemental constraints on cyanobacterial bloom development in the Baltic by measuring seasonal changes in cyanobacterial Fe and Mo contents, heterocyst frequency as an index of N_2 fixation activity, and specific C fixation as an index of physiological status, in relation to changes in cellular P content. A decreased cell content of Fe and Mo with increasing biomass, as already demonstrated for P, could indicate that these elements are involved in the growth regulation of Baltic Sea cyanobacteria. We present data on seasonal changes in C:N:P:Fe:Mo stoichiometry of filamentous cyanobacteria at an offshore station in the NW Baltic Proper in 1999 and 2000. We also complement C:N:P stoichiometry data from 1998 (Larsson et al 2001) with heterocyst frequency.

MATERIALS AND METHODS

Study area and sampling. We visited the Landsort Deep (Stn BY31, 58° 35′ N, 18° 14′ E, depth 459 m, Fig. 1) in the NW Baltic Proper every second week from April through September 1999 and 2000.

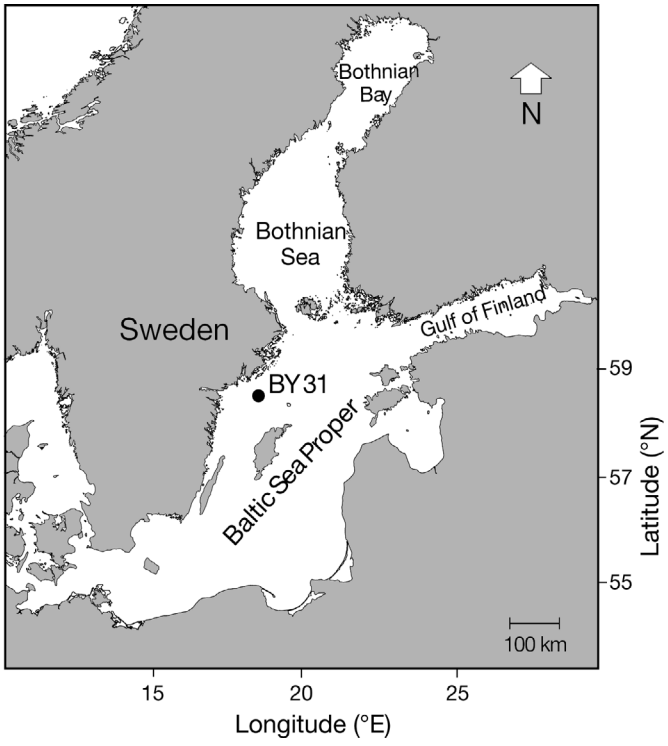

Fig. 1. Baltic Sea Proper, showing sub-basins and Stn BY31 in the Landsort Deep

Water chemistry. Water temperature and conductivity were measured with a CTD (Meerestechnik Elektronik). The top and bottom depths of the seasonal pycnocline were determined by eye from the CTD data, and the density change (ΔSigma-t, m^{-1}) was calculated as a measure of pycnocline stability. Water for inorganic nutrient analysis was taken from 0, 5, 10, 15, 20, 25, 30, 40, 50, 60 and 80 m depth with serial 5 l water bottles (Hydrobios). Samples were filtered (0.45 µm membrane filter, Millipore Millex-HA, from August 1999 Sarstedt Filtropur S) into 12 ml plastic vials, and kept refrigerated until analysed within 24 h of sampling. DIP and DIN (NH_4^++NO_2^-+NO_3^-) were determined by standard flow injection analysis (FIA, slightly modified QuikChem® 8000 methods: 31-115-01-3-A, 31-107-04-1-A and 31-107-06-1-A, Lachat Instruments, reporting limits: PO_4^{3-}, 16 nmol l^{-1}, NH_4^+, 36 nmol l^{-1}, and NO_2^-+NO_3^-, 14 nmol l^{-1}).

Cyanobacterial abundance and heterocyst frequency. An integrated phytoplankton sample (0 to 20 m) was collected with a 25 m long plastic tube (inner diameter 2.5 cm). One end, equipped with a weight, was gently lowered to 20 m depth, after which the tube was closed at the upper end, retrieved, and emptied in a bucket. A 200 ml sub-sample, siphoned from the bucket while stirring, was preserved with 0.8 ml of Lugol's iodine (I_2KI) solution supplemented with acetic

acid. Microplankton were counted in a settling chamber (10 to 50 ml) using a Nikon inverted microscope with phase contrast, according to the HELCOM (1988) guidelines. Microplankton were counted in diagonals or the half or whole chamber bottom with 10× objective (150× total magnification). Biomass was estimated by multiplying the cell numbers with species-specific mean cell volumes, determined from measurements. A factor of 0.11 pg C μm^{-3} was used to convert volume to carbon (HELCOM 1988).

We used a membrane pump (Sandpiper PB1½-A, water flow 50 l min^{-1}) to sample vertical profiles (0, 2.5, 5, 7.5, 10, 12.5, 15, 17.5, 20, 25 m) of cyanobacteria in 1999, and water bottles (see above) in 2000 (0, 2, 4, 6, 8, 10, 12.5, 15, 20, 25 m). A sub-sample of 200 ml from each depth was preserved as described above. Linear interpolation over depth was used to calculate integrated biomass (0 to 20 m).

Counts of *Aphanizomenon* heterocyst frequency (number of heterocysts per length of filament) from individual depths down to 20 m were averaged (n = 7 to 10, at least 10 mm filament counted giving 10 to 40 heterocysts per sample). When discrete water samples were lacking (see Fig. 2a,b), heterocyst frequency was determined in 3 to 4 sub-samples from the tube sample. At least 100 heterocysts were counted in May to September. In April, when few heterocysts were found, 10 mm of filaments were examined, resulting in 2 to 12 heterocysts per sub-sample. Heterocyst frequencies for 1998 were determined from concentrated samples (>90 µm) from 5 m depth, treated in the same way as the tube samples.

Elemental composition of filamentous cyanobacteria. Cyanobacteria were collected with a 90 µm net (50 cm diameter, 1 m long) with a plastic-covered lead weight, hauled vertically from 10 m depth to the surface with a polyester rope. Sometimes additional hauls were done from 20 m and also from 0 to 1 m depth, to collect surface accumulations of *Nodularia*. Zooplankton were removed in a light trap and cyanobacteria concentrated on a plankton net in amounts sufficient for determination of C, N and P contents per dry weight and trace elements. However, since at times this resulted in a mixture of *Aphanizomenon* and *Nodularia*, cyanobacterial colonies were also hand-picked for N and P analysis (see below). The light trap is a 1 l Imhoff funnel partly covered with black plastic so that only the bottom (corresponding to 15 ml of the 1 l volume) is exposed to direct light (from ceiling lighting). Zooplankton were attracted to the light and removed through a bottom tap, while cyanobacteria remained buoyant or floated to the surface. Cyanobacteria were then pipetted or decanted onto a plankton net and a sub-sample suspended in a small Petri dish. Any remaining zooplankton were anaesthetised by adding carbonated water and removed with a pipette under a dissecting microscope. Cyanobacteria were rinsed in a 0.7 % NaCl solution on a 10 µm net and most residual water removed from underneath the net with paper tissue. Sub-samples of cyanobacteria (0.3 to 1.3 mg dry weight) were transferred to pre-weighed (Sartorius M3P microbalance, precision ± 1 µg) tin capsules for C and N analyses and to pre-weighed, acid-washed cover glasses for P analyses, and stored frozen (–20°C). Before analysis, samples were dried to constant weight at 60°C (~3 d). The C and N contents were determined on a Leco CHN-900 Analyzer (samples from July to August 1999), or a Leco CHNS-932 Analyzer (remaining samples), with EDTA as a standard. The P content was determined by combustion for 2 h at 500°C in 15 ml glass tubes followed by digestion in 6 ml persulfate solution (50 g l^{-1} + 30 ml l^{-1} H_2SO_4, 1 h at 120°C). Four ml of 1.6 mol l^{-1} NaOH were added and molybdate-reactive orthophosphate determined with a flow injection system, see above. The same procedure was used for blanks and standards, prepared by adding stock phosphate solution to vials before digestion.

To determine the species-specific N:P ratio for *Aphanizomenon* and *Nodularia*, we used a method that required smaller sample sizes than the previous procedure. We hand-picked colonies with a needle to a N- and P-free 0.7 % NaCl solution in a Petri dish for rinsing and finally to Eppendorf tubes with 2 ml of NaCl solution (minimum 5 replicates). Blanks were made by repeated dipping of the needle into the cleaning solution and then into Eppendorf tubes. The samples (containing 20 to 100 *Aphanizomenon* bundles or *Nodularia* filaments, ca. 60 µg dry weight) were transferred to 15 ml glass tubes and stored in the dark at 4°C until further treatment in the laboratory (within 24 h). Tubes were shaken to separate filaments, and N and P were simultaneously oxidized using a modification of the Koroleff (1983) method, yielding a lower final pH. Three ml of borax-buffered persulfate solution (50 g l^{-1}) was added and samples were incubated for 30 min at 120°C. After addition of 5 ml NaCl solution, FIA (see 'Water chemistry' above) was used to measure N and P. This method has been extensively tested for total N and P determinations of Baltic Sea waters. The N:P ratio for cyanobacteria obtained by this method was 97 ± 6 % (average ± SD, n = 13, N:P ratios ranging from 6 to 100) of the ratio obtained with the methods involving combustion described above.

In 2000, samples from the net hauls were also analysed for micro-elements. To prevent contamination, only acid-washed equipment was used (soaked in 1.2 M HCl for 1 d and rinsed in Milli-Q water). Cyanobacteria concentrated in a light trap (see above) were directly filtered (a few ml) onto ultra-clean filters

(Millipore 0.45 µm HA, 47 mm) placed on a filter holder without a top funnel. Two blank filters were prepared on each occasion using the same filter holder and allowing a similar airflow through the filter. Filters were stored frozen (–20°C) in small, sealed Petri dishes. Duplicate filters were analysed for trace metals and P, using Analytica AB at Luleå University of Technology, Sweden, by sector-field inductively coupled plasma mass spectrometry (ICP-SMS) after microwave digestion in nitric acid (Rodushkin et al. 1999). The resulting P:trace element ratios were related to C using our own measurements of C and P. Blank values were generally <5% of samples, which contained approximately 40 nmol Fe filter^{-1} (median). On 2 occasions (May and late June), one of the blanks was obviously contaminated (up to 25% of the sample values, the other blank low) and discarded.

^{14}C incubations. In 2000, cyanobacteria were collected with a separate net haul between 0 and 10 m, and most zooplankters were removed in a light trap (see above). A suspension of cyanobacteria with enough biomass for later analysis (ca. 15 µmol C l^{-1} or 7 times the observed maximum biomass) was transferred to 75 ml acid-washed polycarbonate bottles. Triplicate bottles with an addition of 5.78 MBq ^{14}C bottle^{-1} were incubated *in situ* at 0.3, 5, 10, and 15 m depth. Single dark bottles were incubated at 0.3 and 10 m depth. After incubation for 3 to 4 h around noon, retrieved bottles were stored in the dark at 4°C, and processed in random order within 1 h. To remove exess ^{14}C, the cyanobacterial colonies were collected on a sieve (60 µm) and rinsed 4 times with 4°C 0.7% NaCl solution. Rinsed cyanobacteria were placed in 10 ml 0.7% NaCl solution and stored on ice in the dark. Within 2 h, remaining zooplankton were anaesthetised with a few drops of carbonated water and removed from the samples using a Pasteur pipette. A cyanobacterial fraction dominated by *Aphanizomenon* (confirmed by microscopic analysis of preserved samples) was collected and rinsed on a 60 µm plankton net. Sub-samples (ca. 0.2 ml) were transferred to 2 Eppendorf vials with 2 ml of 0.7% NaCl solution and stored on ice in the dark. Blanks were prepared by rinsing a plankton net without cyanobacteria but transferring the same amount of water to Eppendorf vials. After shaking the vials to obtain a homogenous solution of single filaments, 1 ml from each vial was taken for total N analysis and 1 ml for determination of ^{14}C uptake. For total N analysis, 1 ml 0.7% NaCl solution was added, and the analysis was performed as described above. For analysis of ^{14}C uptake, 9 ml 0.7% NaCl solution and 10 ml of Lumagel Safe scintillation cocktail was added (Lumac LSC) and samples counted in a Tri-Carb 1600 TR (Packard).

Carbon uptake was calculated according to Parsons et al. (1984) without correction for respiration during incubation. Specific carbon uptake (h^{-1}) was calculated from measured total N and C:N ratio on each occasion. Dark fixation was low (average 6, 5, 13, and 45% of light fixation at 0, 5, 10 and 15 m depth, respectively) and the variability small between dark bottles at 0 and 10 m. The uncertainty from subtraction of the dark fixation value was estimated to be <5% at 0, 5 and 10 m and <14% at 15 m depth. Dissolved inorganic C was calculated from pH, salinity, and temperature, and ranged between 1.23 and 1.39 mmol l^{-1}. The average light intensity (µmol quanta m^{-2} s^{-1} as photosynthetically active radiation, PAR) during the incubation was calculated for the incubation depths from the extinction coefficient obtained from light profiles measured on each occasion (at 2, 4, 6, 8, 10, 12.5, 15 m with a LI-COR LI-1000), together with insolation measurements from Visby on the island of Gotland, 100 km south of the sampling station. The data from Visby were available as hourly integrated continuous measurements of total irradiation (290 to 4000 nm) on a horizontal surface. PAR was estimated as 50% of total irradiation (Kvifte et al. 1983).

RESULTS

Biomass

Cyanobacterial biomass estimates from tube samples and vertical profiles were generally in good agreement for *Aphanizomenon*. Abundance estimates deviated considerably for *Nodularia* (Fig. 2a,b), which may be a consequence of a more patchy distribution of *Nodularia* near the surface.

After the formation of a shallow pycnocline in late May 1999, surface water temperature increased continuously until mid-July, and *Aphanizomenon* increased rapidly to a high biomass in early July (Fig. 2a,c). In contrast, the following year (2000) saw a pronounced increase in temperature in May, followed by a moderate further increase in summer and a markedly lower peak biomass of *Aphanizomenon* (Fig. 2b). The upper mixed layer was much shallower in 1999, and DIP was depleted a month earlier than in 2000 (Fig. 2c–h, Table 1). In both years, the decrease in biomass in July was associated with a stable pycnocline and an increase in surface water temperature.

The biomass peaks of *Nodularia* (integrated discrete samples, Fig. 2a,b) lagged the *Aphanizomenon* peaks by 2 to 4 wk and were associated with shallow secondary pycnoclines (at 3 to 4 m in 1999, and 8 to 10 m in 2000, Fig. 2c,d). In 1999, there was no salinity difference, and the shallow thermocline may have been present tem-

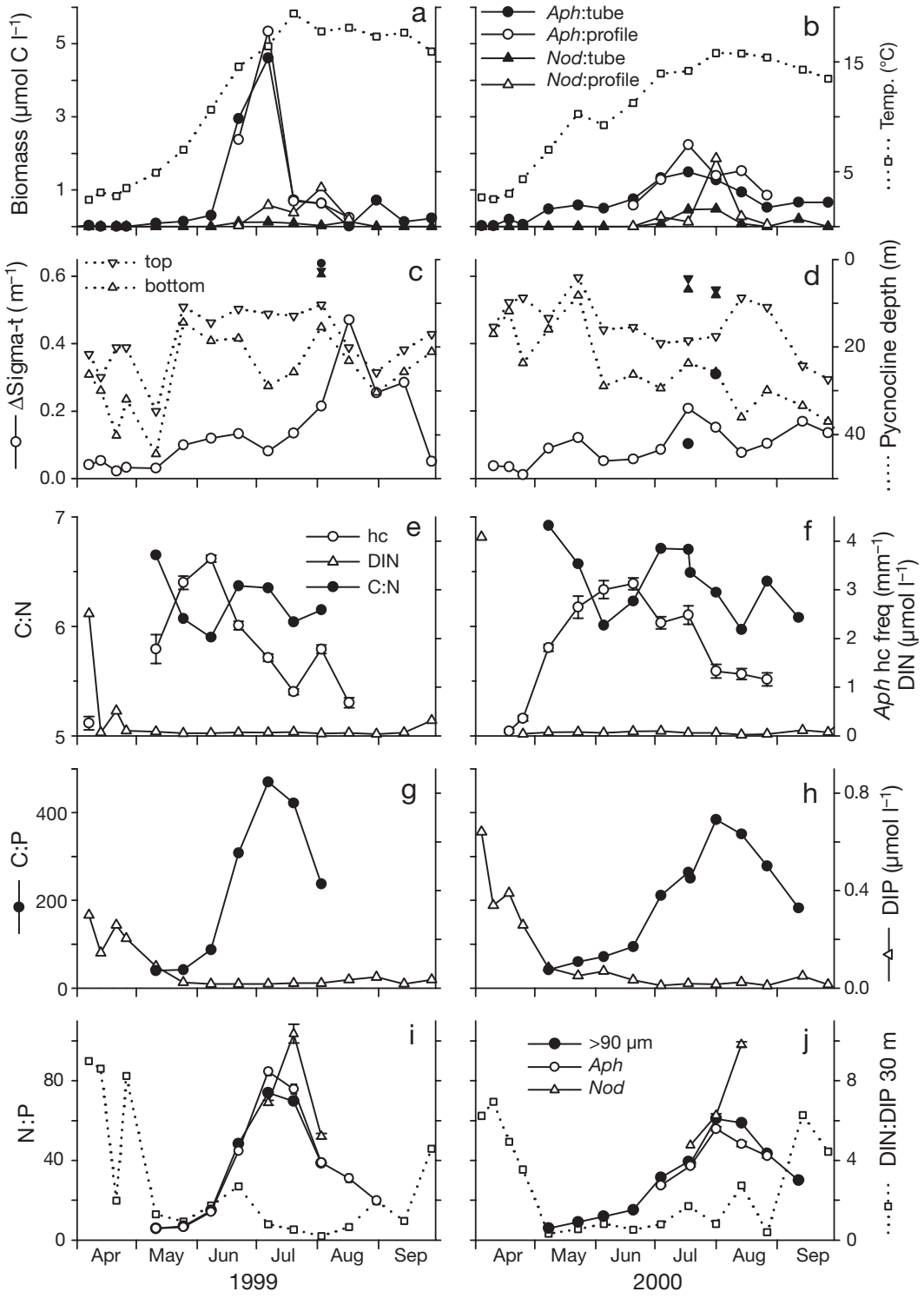

Fig. 2. Results from the Landsort Deep Stn BY31 in 1999 and 2000. (a,b) *Aphanizomenon* sp. (*Aph*) and *Nodularia spumigena* (*Nod*) biomass at 0 to 20 m depth, and temperature (average 0 to 10 m). (c,d) Density change across seasonal pycnocline and top and bottom depths (note: filled symbols show occasional secondary shallow pycnocline). No pycnocline was observed 4 April 2000. (e,f) C:N ratio of filamentous cyanobacteria from net (90 µm) haul at 0 to 10 m (0 to 20 m on 11 May to 8 June 1999), heterocyst frequency (hc, 0 to 20 m) of *Aphanizomenon* sp. (±SE), and DIN concentration at 0 to 10 m. (g,h) C:P ratio of cyanobacteria >90 µm, and DIP concentration at 0 to 10 m. (i,j) N:P ratio in cyanobacteria >90 µm (0 to 10 m), picked *Aphanizomenon* sp. (±SE, 0 to 10 m), *N. spumigena* (±SE, 0 to 1 m), and DIN:DIP ratio at 30 m depth (potentially upwelling water)

Table 1. Vertical extension of the summer pycnocline (top and bottom depth, secondary shallow pycnocline not shown, cf. Fig. 2c,d). Vertical distribution of *Aphanizomenon* sp. biomass as depth of maximum (depths with >70% of the maximum biomass are included), and median depth (above which half the total biomass 0–25 m is found). Biomass distribution is shown as integrated biomass concentrations (µmol C l^{-1}) above pycnocline, between 0–10, 10–20, and 20–25 m depth, and as percentage (values in parentheses for each depth interval) of total biomass 0–25 m

Date	Pycnocline depth, top–bottom (m)	Depth of biomass max. (m)	Median depth of biomass (m)	Biomass			
				Above pycnoline	0–10 m	10–20 m	20–25 m
1999							
22 Jun	11.4–18.6	2.5–5	5.1	3.84 (92)	4.05 (85)	0.69 (15)	0.00 (0)
7 Jul	12.4–27.9	5–10	7.2	7.49 (86)	7.72 (72)	2.97 (27)	0.20 (1)
20 Jul	12.9–25.7	10–12.5	10.8	0.77 (67)	0.64 (43)	0.74 (49)	0.22 (8)
3 Aug	10.3–15.5	7.5	8.5	0.91 (71)	0.92 (69)	0.34 (25)	0.14 (5)
17 Aug	20.1–23.2	12.5	11.1	0.24 (87)	0.24 (43)	0.24 (44)	0.15 (13)
2000							
23 May	4.7– 7.7	≤2[a]	–[a]	–[a]	–[a]	0.31	0.02
5 Jun	17.0–23.8	–[b]	≤8[b]	0.33 (98)[b]	0.45 (77)[b]	0.13 (23)	0.00 (0)
20 Jun	15.5–23.8	1	7.9	0.64 (85)	0.70 (61)	0.45 (39)	0.00 (0)
04 Jul	19.1–24.8	1	5.8	1.33 (99)	2.05 (80)	0.52 (20)	0.01 (0)
18 Jul	18.6–23.2	6–8	6.0	2.35 (97)	3.73 (83)	0.74 (16)	0.03 (0)
01 Aug	17.5–24.7	1–2; 8	6.0	1.54 (95)	2.30 (81)	0.49 (17)	0.08 (1)
14 Aug	8.3–21.2	0; 8	7.5	2.15 (57)	2.25 (73)	0.79 (26)	0.11 (2)
27 Aug	10.9–19.1	8	8.1	1.22 (71)	1.29 (69)	0.42 (22)	0.32 (9)

[a]Samples at 0 and 1 m missing. Biomass integration excluded for surface layer
[b]Sample at 1 m missing, sample depths not noted for samples 0–8 m. Biomass integration made for surface layer, but is more uncertain

porarily in the daytime (Fig. 2c). In 2000, the pycnocline (both temperature and salinity differences) persisted for at least 2 wk (Fig. 2d). At the *Nodularia* biomass peaks, most of the biomass was found in the upper mixed layer (67% in 1999, 65% in 2000), with only small amounts (<4% both years) below the deep pycnocline.

Most of the *Aphanizomenon* biomass was generally found above the pycnocline (Table 1), often with a biomass maximum at 5 to 10 m depth. The highest proportion of biomass below the top of the pycnocline was found after the peak in C:P ratio in both years. In 2000, this coincided with a shallower pycnocline. There was little biomass below 20 m, except in August 2000, when it increased slightly.

C, N and P contents and ratios

To prevent cell lysis, samples were rinsed in a NaCl solution. Due to the presence of salt this gave a slight underestimation of C, N and P content per dry weight when compared to rinsing in deionized water (average C, N, and P contents 3.3 ± 1.4% lower on 5 occasions in 2000, n = 15). Ratios of C:N, C:P and N:P were unaffected (–0.1 ± 1.2%).

The observed C and N contents and molar C:N ratios varied little (average ± SD in 1999: 45 ± 2% C of dry weight [dry wt], 8.4 ± 0.4% N, C:N = 6.2 ± 0.3, n = 7; in 2000: 46 ± 1% C, 8.4 ± 0.5% N, C:N = 6.4 ± 0.3, n = 11). In both years, the highest C:N ratio (Fig. 2e,f) was in early May due to a low N content (7.7% dry wt in 1999 and 7.2% in 2000). The C:N ratio decreased in late May–early June due to an increasing N content (maximum 8.7% dry wt in 1999 and 8.9% in 2000, *Aphanizomenon* dominant) and returned to higher values at the biomass peak of *Aphanizomenon* (Fig. 2e,f). The same pattern was found for cyanobacteria collected from the depth interval 0 to 20 m from 8 May to 4 July 2000 (data not shown). The C:N ratio decreased after the *Aphanizomenon* biomass peak in both years, possibly due to a greater proportion of *Nodularia* in these samples (see Fig. 2a,b). After the biomass peak in 2000 (1 and 27 August), sinking *Aphanizomenon* bundles collected separately had high C:N ratios (7.3 and 7.0), due to low N content (7.3 and 7.5% dry wt).

The P content was highest in early May (2.9 and 2.7% dry wt in 1999 and 2000), giving a C:P ratio of ~40 (mol:mol), far below the Redfield value (106) (Fig. 2g,h). The P content decreased as biomass increased, and in 1999 the C:P ratio reached its maximum (470) at the biomass peak (P content 0.25% dry wt) and then stayed high as the biomass declined rapidly (Fig. 2g). In 2000, the highest C:P ratio (380, P content 0.31% dry wt) was found 2 wk after the biomass peak (Fig. 2h). Due to the small variability in C and N content, changes in N:P and C:P ratios were mainly a function of P content (Fig. 2g–j). In 1999, the N:P ratio peaked at a high value (85) in hand-picked colonies of *Aphanizomenon*, corresponding to a C:P of 540 and a P content of 0.22% dry wt (assuming C:N = 6.5). The N:P ratio of *Nodularia* was similar or higher than that of *Aphanizomenon* (Fig. 2i,j). *Nodularia* was never abundant enough to

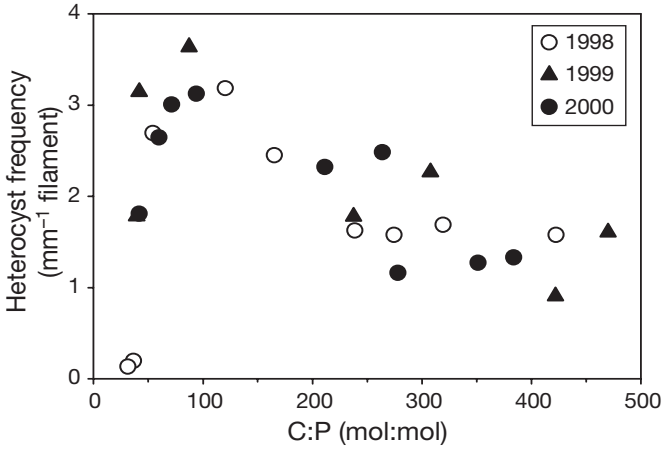

Fig. 3. Relationship between *Aphanizomenon* sp. heterocyst frequency and cellular C:P ratio. Data on C:P ratio for 1998 are from Larsson et al. (2001)

permit separate analyses early in the season, and C:N and C:P ratios were measured only twice in 2000 (19 July: C:N = 6.7, C:P = 319; 1 August: C:N = 6.3, C:P = 450; not in Fig. 2). The highest recorded N:P ratios in *Nodularia* (N:P = 104 in late July 1999 and N:P = 98 on 14 August 2000), correspond to C:P ratios of 590 to 630 or P contents of 0.19 to 0.20% dry wt. Cell decay may have affected these results since microscopic examination showed that some cells were empty (1999: 10% in early July and August, unknown in late July; 2000: <2% in July, ca. 10% on 1 August, and ca. 25% on 14 August). *Aphanizomenon* filaments always had a low proportion of empty cells (<1%).

Heterocyst frequency in *Aphanizomenon*

In both 1999 and 2000, the heterocyst frequency in *Aphanizomenon* was low in April but increased after DIN was depleted in the mixed layer (Fig. 2e,f), reaching a maximum (3.2 to 3.6 mm^{-1} filament) in May to June, coinciding with the minimum C:N ratio. The heterocyst frequency then declined with increasing biomass and C:P ratio. Plotted against the C:P ratio (Fig. 3, data from 1998 included), the heterocyst frequency shows a maximum close to the Redfield ratio (C:P = 106) and a decrease with increasing C:P ratio, a pattern found in all 3 years. Excluding the data before the maximum heterocyst frequency (<2 heterocysts mm^{-1} and C:P < 50, n = 4), the C:P ratio and heterocyst frequency were negatively correlated (r^2 = 0.72, p < 0.000001, n = 21).

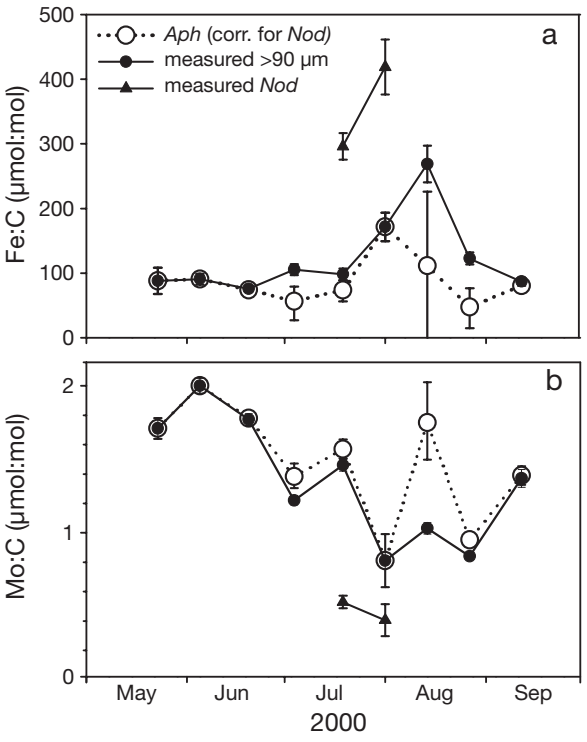

Fig. 4. (a) Fe:C and (b) Mo:C ratios measured in cyanobacteria < 90 µm and in *Nodularia spumigena* (filled symbols, with error bars indicating range of 2 replicates), and ratios estimated for *Aphanizomenon* sp. by correction for contribution of *Nodularia spumigena* (open symbols, with error bars indicating estimated range of uncertainty from measurement and counts)

Trace metal contents

Samples of *Nodularia* had higher Fe content and lower Mo content than samples dominated by *Aphanizomenon* (Fig. 4). Since both species are present in some net samples from July to August 2000 (Table 2), this confounds the interpretation of the Fe and Mo content of *Aphanizomenon* (Fig. 4a,b), with greatest effect on Fe, due to a larger difference. The decrease in Mo content was correlated to the decrease in heterocyst frequency in *Aphanizomenon*, keeping the Mo per heterocyst fairly constant (Fig. 5).

Table 2. *Nodularia spumigena* as percent of the total biomass in samples analysed for trace metals in 2000 (average ± SD for 2–3 replicates)

	5 Jun	20 Jun	4 Jul	19 Jul	1 Aug	14 Aug	27 Aug	12 Sep
% *N. spumigena*	0	1 ± 0	21 ± 3	11 ± 3	0	54 ± 2	21 ± 2	2 ± 0

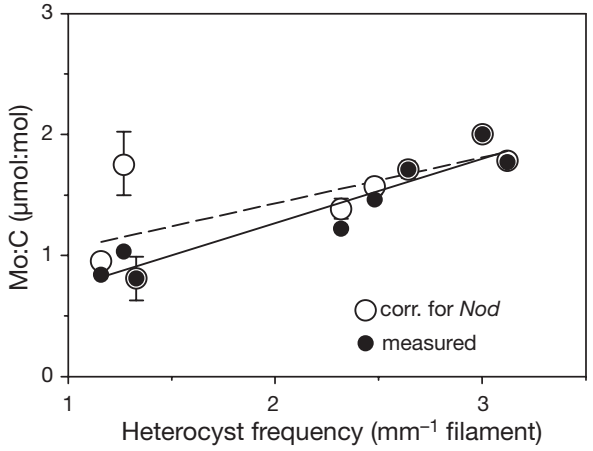

Fig. 5. Relationship between Mo:C ratio and heterocyst frequency, both for the measured Mo:C in cyanobacteria > 90 µm (solid line: $y = 0.53x + 0.20$, $r^2 = 0.90$, $p < 0.001$, $n = 8$) and estimated *Aphanizomenon* sp. Mo:C ratio (corrected for *Nodularia spumigena*) (dashed line: $y = 0.38x + 0.67$, $r^2 = 0.52$, $p < 0.05$, $n = 8$)

On 1 August 2000 the high *Nodularia* abundance (Fig. 2) required *Aphanizomenon* to be collected from the sinking fraction in the light traps. This resulted in *Nodularia*-free samples (Table 2), but the high Fe content may be due to contamination from detritus as indicated by microscopic examination.

Carbon uptake

The specific C uptake rate (h^{-1}) was generally highest at 5 m depth, indicating photoinhibition at the surface (Fig. 6a). Only on 19 July, a cloudy day with low light intensity (Fig. 6b), was the surface rate the highest. All other sampling days were calm and sunny. Despite high light intensities, good light penetration, and sufficient P supply in early summer, specific C uptake rates were lower than in late summer. Photosynthetic affinity thus increased during summer (Fig. 6c). Carbon uptake at light inhibition (0 m, except 19 July) and light 'optimum' (5 m, except 19 July) was positively correlated with temperature (Fig. 6d).

DISCUSSION

Growth limitation

Phosphorus

The filamentous diazotrophic cyanobacterium *Aphanizomenon* sp. in the Baltic Proper stores significant quantities of P early in the season to sustain later

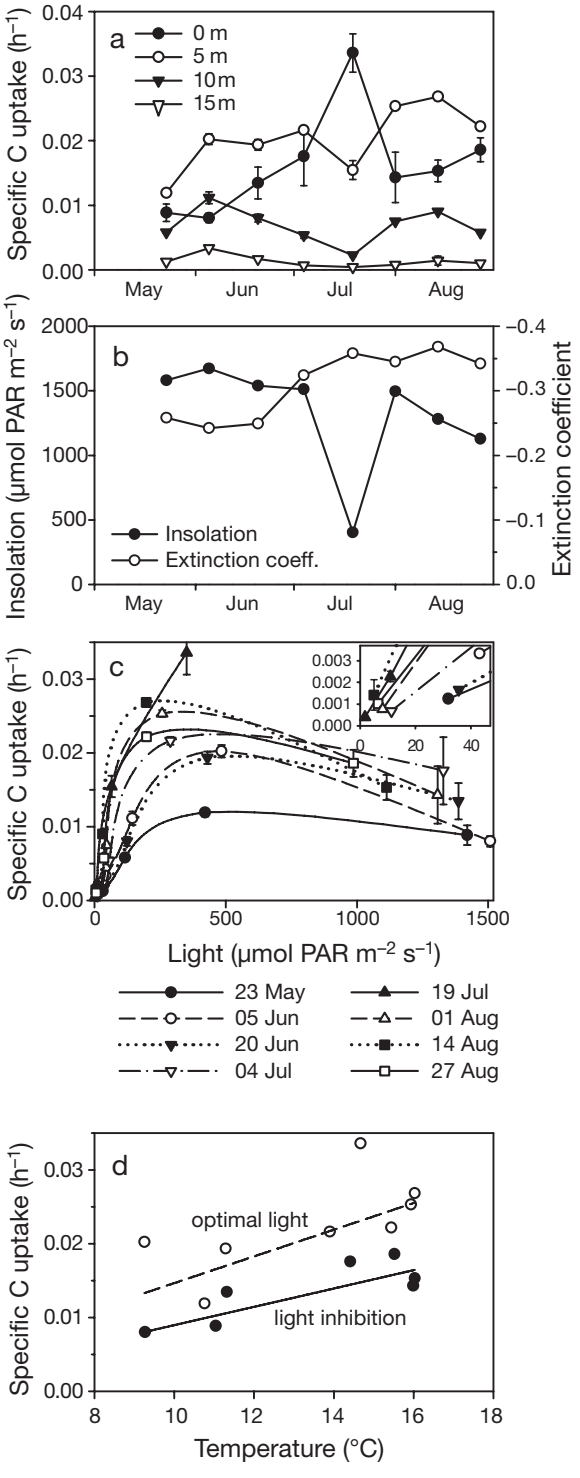

Fig. 6. (a) C uptake at different incubation depths. (b) Surface insolation and calculated light extinction coefficient in water during incubations. (c) *In situ* C uptake versus light intensity at incubation depths for each occasion (inset shows C uptake at low light intensity). (d) Relationship between C uptake and temperature in the light-inhibited surface layer (0 m, 19 July excluded, $r^2 = 0.77$, $p < 0.01$, $n = 7$) and at depth of highest measured production (5 m, but 0 m for 19 July, $r^2 = 0.49$, $p = 0.053$, $n = 8$ [$r^2 = 0.64$, $p < 0.05$ excluding 19 July])

growth in a P-depleted mixed layer (Larsson et al. 2001). We confirm a recurrent seasonal pattern in cell C:P and N:P ratios that spans more than an order of magnitude, and we present the first observations of a similar pattern in another dominant Baltic Sea diazotroph, *Nodularia spumigena*. Cellular C:N ratios vary much less, but also show a recurrent seasonal pattern in *Aphanizomenon* sp.

The high P content in *Aphanizomenon* in early May (2.7 to 2.9% dry wt) is accumulated from the large winter pool of DIP, when growth is limited by low temperature and light (Lehtimäki et al. 1997). As the biomass slowly increases in May and early June, the heterocyst frequency and photosynthetic rate rises, as does the C:P ratio, indicating that P stores are used for growth. In 1998 and 2000, P stored in *Aphanizomenon* before the start of the intensive growth period almost equalled that found in its peak biomass, while in 1999 it was only a third (Table 3). This suggests that P storage is an important adaptation for *Aphanizomenon* success.

The amount of P stored in *Aphanizomenon* biomass (0.002 to 0.012 µmol P l^{-1}) in May was small compared to the DIP pool (0.048 to 0.153 µmol P l^{-1}, Table 3). This indicates that only a small fraction of the DIP surplus remaining after the spring bloom can be taken up by *Aphanizomenon*, unless net (apparent) population growth in May is much smaller than the gross growth rate, which would imply an unrealistically rapid biomass turnover. This substantiates the conclusion of Larsson et al. (2001) and Jaanus & Pellikka (2003) that the DIP left over in the mixed layer from the N-limited spring bloom may influence the summer bloom magnitude less than usually assumed (e.g. Rahm et al. 2000). This would be particularly true for *Nodularia* and also *Anabaena*, a less common diazotroph, since they usually occur in abundances too low to use much of the early DIP surplus (Larsson et al. 2001). Thus, much of the early DIP decrease must be explained by factors other than uptake by N-fixing cyanobacteria, e.g. uptake by non-N fixers. These observations do not, however, rule out a significant P uptake during the bloom, e.g. P from mineralization as well as mixing.

Table 3. *Aphanizomenon* sp. C and P in biomass at 0 to 20 m depth and as percent of the C and P at the biomass peak (values in parentheses), and dissolved inorganic phosphorus (DIP) at 0 to 20 and 0 to 10 m depths, at Stn BY31, 1998 to 2000. For C:P ratios, see Fig. 2g,h

Date	C in biomass (µmol l^{-1})	P in biomass (µmol l^{-1})	C:P	DIP (µmol l^{-1}) 0–20 m	0–10 m
1998					
2 Jun	0.28 (14)	0.0051 (107)	54	0.031	0.026
16 Jun	0.38 (19)	0.0032 (66)	120	0.014[c]	0.015[c]
30 Jun	0.45 (23)			0.051	0.008[c]
17 Jul	0.50 (25)			0.050	0.029
28 Jul	0.37 (18)	0.0023 (47)	165	0.113	0.027
11 Aug	1.15 (57)	0.0037 (77)	310	0.034	0.013[c]
25 Aug	0.39 (20)	0.0017 (35)	240	0.031	0.013[c]
8 Sep	2.02 (100)	0.0048 (100)	420	0.022	0.018[c]
1999					
11 May	0.077 (1.4)	0.0019 (17)	40	0.093	0.089
25 May	0.068 (1.3)	0.0016 (14)	42	0.048	0.023
8 Jun	0.33 (6.1)	0.0038 (33)	87	0.025	0.016[c]
22 Jun	2.37[a] (44)	0.0077 (68)	310	0.033	0.016[c]
07 Jul	5.34[a] (100)	0.011[b] (100)	470	0.018[c]	0.017[c]
2000					
8 May	0.47 (21)	0.012 (150)	37	0.153	0.083
23 May	0.60 (26)	0.011 (126)	55	0.068	0.051
5 Jun	0.49 (22)	0.0070 (83)	71	0.085	0.069
20 Jun	0.60 (26)	0.0071 (85)	81	0.041	0.033
4 Jul	1.28 (58)	0.0065 (77)	200	0.014[c]	0.011[c]
18 Jul	2.23[a] (100)	0.0084[b] (100)	260	0.028	0.018[c]

[a]Biomass integrated from vertical profile (see Fig. 2)
[b]C:P from 0 to 10 m used. Available data on vertically resolved N:P ratios and biomasses indicated that this caused no or small overestimation of the importance of stored P
[c]Value lower than or close to analytical reporting limit (0.016 µmol l^{-1})

Phytoplankton with a nutrient-limited growth rate generally show a decreased cellular content of the limiting nutrient (Droop 1974). Cyanobacteria growing slowly in P-limited cultures display high C:P and N:P ratios (Healey 1982), suggesting P deficiency and low growth rate in *Nodularia* and *Aphanizomenon* at the peak C:P and N:P ratios. Healey & Hendzel (1976) found similarly low P content (~0.2% P dry wt^{-1}, corresponding to a C:P ca. 580) at the biomass peak of field populations of the freshwater species *Aphanizomenon flos-aquae*. Sañudo-Wilhelmy et al. (2001) found high C:P and N:P ratios (up to nearly 400 and 60, respectively) in *Trichodesmium* from the tropical Atlantic, and N_2 fixation rate was related to cell content of P rather than Fe. Kustka et al. (2003) suggested a critical cellular N:P ratio of 40 to 50 for the onset of P limitation for *Trichodesmium*. The synchronization in time of the maxima in C:P ratio and biomass peak in 3 consecutive years (1998 in Larsson et al. [2001], 1999 and 2000 this study) strongly suggests that P availability limits the biomass build-up of *Aphanizomenon*.

Upwelling events can, in the short term, result in drastic decreases in temperature and biomass. In both 1999 and 2000, however, we found an increase in temperature at the collapse of the bloom and the pycnocline remained stable (Fig. 2). This suggests that upwelling events were not responsible for the observed decrease in *Aphanizomenon* biomass.

Mesozooplankton, dominated by the copepods *Acartia* spp., *Eurytemora* and *Temora*, generally increase from May to maximum abundances in July and August (Johansson et al. 2004) often in parallel with the increase in diazotrophs. Grazers do not seem to exercise strong control of the Baltic Sea bloom-forming cyanobacteria *Aphanizomenon* and *Nodularia* (Sellner 1997). *Aphanizomenon* occurs in large filament bundles which discourage grazing (Lehtiniemi et al. 2002), and grazers seem to selectively avoid the toxic single filaments of *Nodularia* (Engström et al. 2000).

Iron

Stal et al. (1999) reported that Fe stimulates Baltic Sea *Nodularia* growth. However, our data on cellular Fe in *Aphanizomenon* (Fig. 4a) do not indicate a shortage of Fe in the NW Baltic Sea Proper. Despite the decrease in heterocyst frequency in *Aphanizomenon*, there was no clear decrease in Fe:C ratio as the bloom progressed. This suggests Fe-sufficient conditions since a decrease should be expected if Fe was limiting growth rates (Berman-Frank et al. 2001). P rather than Fe limitation is in accordance with recent nutrient addition experiments during Baltic cyanobacterial blooms (Moisander et al. 2003).

Availability of Fe in the Baltic should generally be lower than in freshwater due to faster coagulation and deposition of Fe-rich colloids at higher salinity (Gunnars et al. 2002) and more efficient binding in sediments as sulfides (Blomqvist et al. 2004). However, compared to oceanic waters, dissolved Fe concentrations in the Baltic seem to be relatively high. Kremling & Petersen (1984) found ca. 13 nmol l^{-1} of filterable Fe (passing through a 0.4 µm pore filter) during August to September in the Baltic Proper. In the subtropical North Pacific, Landing & Bruland (1987) reported levels of 0.2 to 0.5 nmol l^{-1}, while Sañudo-Wilhelmy et al. (2001) found 0.5 to 1.8 nmol l^{-1} in the Atlantic.

Our estimate of *Aphanizomenon* Fe content (average ± SD: 95 ± 15 µmol mol^{-1} C in *Aphanizomenon*-dominated samples, 73 ± 16 after correction for *Nodularia*, n = 7) is in the lower range of Fe content measured in oceanic *Trichodesmium* populations (from 20 to >500 µmol Fe mol^{-1} C, Kustka et al. 2002), while *Nodularia* Fe content is in the upper range (350 ± 90 µmol mol^{-1} C, n = 2). The higher heterocyst frequency in *Nodularia* compared to *Aphanizomenon* (Laamanen & Kuosa 2005) is a possible reason for the higher Fe content in *Nodularia*. However, assuming that all Mo is used in nitrogenase with a Fe:Mo ratio of 19:1 to 25:1 (Tuit et al. 2004), 41 to 54% of the total Fe content in *Aphanizomenon* is in nitrogenase when the heterocyst frequency and Mo content is maximal in early June. In *Nodularia*, with lower Mo and higher Fe contents, the corresponding proportion is only ca. 3%. The high Fe:Mo ratio in *Nodularia* indicates that a large proportion of the Fe is not in nitrogenase, and we suspect there is a large proportion of cell-surface-bound Fe that confounds these data.

Molybdenum

The absence of N_2 fixation in N-limited estuaries may be due to an interaction of bottom-up and top-down controls involving Mo deficiency and zooplankton grazing (Marino et al. 2002). Mo may control estuarine N_2-fixers since dissolved sulfate, an important component of sea salt, competitively inhibits molybdate uptake (Howarth et al. 1988). A decrease in cellular Mo content suggests Mo deficiency (Ter Steeg et al. 1986). However, the decrease in *Aphanizomenon* Mo content could largely be an effect of the decreased heterocyst frequency, as suggested by the linear relationship between Mo content and heterocyst frequency (Fig. 5). Furthermore, Mo sufficiency is supported by studies showing that diazotrophs respond to Mo shortage by increasing, not decreasing, the heterocyst frequency (Fay & de Vasconcelos 1974, Ter Steeg et al. 1986). In the study by Fay & de Vasconcelos (1974) on Mo-starved *Anabaena cylindrica*, nitrogenase activity did not increase with increased heterocyst frequency, suggesting that Mo-deficient cyanobacteria experience N limitation and therefore produce new heterocysts that contain little functioning nitrogenase. Increased temperature and P limitation may be the prime factors for the decrease in *Aphanizomenon* heterocyst frequency (see 'Heterocyst frequency in *Aphanizomenon*' below).

We have found few published data on Mo content in cyanobacteria. Our values (Fig. 4) fall within the range (0.1 to 4 µmol Mo mol^{-1} C assuming 45% C per dry wt) found by Ter Steeg et al. (1986) for *Anabaena oscillaroides* cultured under 3 different Mo concentrations and were close to that in cultured *Trichodesmium* sp. (2.56 ± 2.02 µmol mol^{-1} C, average ± SD), but clearly lower than for *Trichodesmium* sp. collected in the field (22.5 ± 15.1 µmol mol^{-1} C) (Tuit et al. 2004).

Although Mo uptake rates may generally be lower in the Baltic Sea than in fresh water due to interference from sulfate, limitation of bloom biomass by Mo seems

unlikely considering the high Mo concentration in the water. Prange & Kremling (1985) found the concentration of dissolved Mo in Baltic surface water (~20 nmol l^{-1} at salinity of 7) to be determined by conservative mixing of Mo-rich Atlantic water (~110 nmol l^{-1}) with Baltic river waters, giving a linear relationship with salinity. The salinity in the present study (0 to 10 m depth) was 5.5 to 6.7. Our data also suggest Mo-sufficient conditions for the Baltic Sea *Aphanizomenon* near the biomass maximum, when heterocyst frequency decreases. However, low Mo availability due to high sulfate concentration may be the most important factor inhibiting growth at the higher salinity of the Kattegatt (west coast of Sweden) (Stal et al. 1999).

Heterocyst frequency in *Aphanizomenon*

In March 1998, before the spring bloom had depleted DIN, *Aphanizomenon* N content was high (about 10.6% N of dry wt, C:N ca. 4.6, Larsson et al. 2001) and heterocyst frequency very low (Fig. 3). After DIN was depleted, the C:N ratio in *Aphanizomenon* increased, which seems to signal shortage of N and initiates heterocyst formation. As the biomass of *Aphanizomenon* increased slowly in May, there was a rapid increase in heterocyst frequency (Fig. 2e,f). A concomitant increase in N content indicates that N needed for growth comes from N_2 fixation, as also shown by low $\delta^{15}N$ (<–1) in Baltic *Aphanizomenon* filaments (Höglander 2005).

The maximum in heterocyst frequency was reached by June, when biomass was still low, at a C:P ratio close to the Redfield ratio (106). The heterocyst frequency then declined as biomass and C:P ratio increased (Fig. 3). At a nearby coastal station, Wallström (1988) also found an initial increase in heterocyst frequency from June to July (from 1.7 to 4.9 mm^{-1} in 1983, from 2.4 to 4.6 mm^{-1} in 1984) and a decrease in July and August (to 2.5 in 1983 and to 0.8 mm^{-1} in 1984). Similarly, Laamanen & Kuosa (2005) found heterocyst frequency to peak before the biomass maximum, and Lindahl et al. (1978) found a decrease in heterocyst frequency from early July (3 to 4 mm^{-1}) to late August (1 to 2 mm^{-1}). Wallström (1988) found a significant positive relationship ($r^2 = 0.36$) between DIP concentration and heterocyst frequency. Our data suggest, however, that cell P content may be what really determines heterocyst frequency, once DIN is depleted. The generally low heterocyst frequency in *Aphanizomenon* (<5% of the biomass as heterocysts) and the inevitable need for P in, e.g., DNA and RNA in all cells suggest that only a small portion of the cellular P is found in the heterocysts. Thus, the variations in heterocyst frequency should have only a small effect on the P content.

Despite high heterocyst frequency and a C:P ratio close to the Redfield ratio in early summer, biomass increased slowly, particularly in 2000, and rapid biomass increase required temperatures above 10°C. This suggests temperature control of growth and N_2 fixation in early summer, and the initial decrease in heterocyst frequency in June may be due to increased N_2 fixation per heterocyst at higher temperatures. Phosphorus limitation, as suggested by the high C:P ratios, could also suppress heterocyst formation, since each heterocyst could supply more cells with N at a slower growth rate as indicated in experimental studies on P-limited chemostat cultures of cyanobacteria (e.g. Lee & Rhee 1997).

Specific carbon uptake rate

Specific carbon uptake rates were positively correlated with temperature and highest in late summer, despite lower light intensities and higher light extinction coefficients than in early summer. This is likely a temperature effect, but adaptation to low light may also be involved. If the cyanobacteria are adapted to the prevailing light conditions at the depths at which they occur, the use of a mixture of cyanobacteria from 0 to 10 m in the incubations may underestimate the true specific photosynthesis at the incubation depths. However, since the vertical distribution of the cyanobacteria in 2000 was fairly similar over the season (Table 1), it is reasonable to assume a similar relative error.

There is an apparent contradiction between the seasonal increase in specific C uptake rates in *Aphanizomenon* and the assumed growth rate decline due to lowered cell P quota. Gallon et al. (2002) found a high ^{14}C fixation in diazotrophic cyanobacteria relative to $^{15}N_2$ fixation (0 to 7 m depth), suggesting an accumulation of C reserves that could be used for N_2 fixation during night or mobilized in deeper waters after a mixing event. This indicates that short-term ^{14}C incubations may give results closer to gross rather than net primary production. A low growth rate could be compatible with a high short-term C uptake if there is a seasonal increase in respiration, especially if nighttime respiration is important, or if there is a delayed release of dissolved organic carbon not accounted for in the short-term ^{14}C uptake experiments. Excretion of excess C is a common phenomenon among phytoplankton, and usually increases at nutrient limitation (Dubinsky & Berman-Frank 2001). A large release of N is suggested by Larsson et al. (2001) to explain the large total N increase during the Baltic blooms compared to the biomass increase. This indicates a large C respiration to sustain N_2 fixation and, if N is released in the form of amino acids, a simultaneous release of C.

The present study corroborates the earlier findings (Larsson et al. 2001) of a drastically increased C:P ratio in *Aphanizomenon*, culminating at the biomass peak. We also demonstrate a similar development for *Nodularia* with C:P and N:P ratios far above Redfield values at the biomass peak. The dynamics of C:P and N:P ratios in cyanobacteria need to be considered in models and calculations to estimate potential N_2 fixation. The study underscores the potential importance of early season P storage in *Aphanizomenon* for later biomass build-up, but our data do not support the use of the P left over from the spring bloom as a proxy to estimate summer N_2 fixation (e.g. Rahm et al. 2000), since only a fraction of that P ended up in diazotroph biomass at the biomass maximum. Fe content in *Aphanizomenon* was in the lower range of published values for the marine cyanobacteria genus *Trichodesmium*, and the small seasonal changes imply Fe sufficiency. In the study area (NW Baltic Proper), blooms appear to collapse due to P shortage, but conditions could differ in other parts of the Baltic Proper. In *Aphanizomenon*, both the P and Mo contents are related to the heterocyst frequency. The results suggest that the variation in Mo content is an effect of the changes in heterocyst frequency, and P limitation may contribute to these changes.

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