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Effect of salinity on the growth rate and nutrient stoichiometry of two Baltic Sea filamentous cyanobacterial species

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Abstract. Summer blooms of nitrogen-fixing filamentous cyanobacteria are recurrent phenomena in the Baltic Sea. Salinity, varying from 0 to10 PSU in the surface layer of the Baltic Sea, is among the major factors affecting the basin-scale distribution of various bloom-forming cyanobacterial species. The effects of salinity on the growth rate and cellular carbon, nitrogen, and phosphorus ratios of two major cyanobacterial species that form dense blooms in the Baltic, *Aphanizomenon* sp. (strain KAC 15) and *Nodularia spumigena* (strain HEM), were studied. Cells were grown under N_2-fixing conditions in a salinity gradient from 0 to 10 PSU. The growth rates of the species showed contrasting responses to salinity. For *Aphanizomenon* sp. the maximum growth rates (0.28–0.31 d^{-1}) were observed at salinities of 0–2 PSU, while for *N. spumigena* the maximum growth rate occurred at 8–10 PSU (0.14–0.16 d^{-1}). The latter species did not tolerate low salinities (<2 PSU). The observed differences in salinity tolerances constrain the distribution patterns of these two species during cyanobacterial blooms, *Aphanizomenon* sp. being more abundant in the coastal and less saline areas. The variations in growth rates were largely reflected in cellular N : P and C : P ratios, which varied two-fold, and in C : Chl*a* ratios with 5-fold variability. Cellular C : N ratios were rather constant at all salinities and close to the Redfield ratio for *Aphanizomenon* sp. (on average 5.9 g g^{-1}) and above the Redfield ratio for *N. spumigena* (on average 8.0 g g^{-1}). The relatively higher N : P and lower C : N ratio showed a higher need of N for *Aphanizomenon* sp. than for *N. spumigena*. This is partly explained by the higher abundance of N-rich phycobilin pigments in *Aphanizomenon* sp. as indicated by fluorescence measurements. The observed differences in pigmentation indicate species-specific strategies in light harvesting.

Key words: Baltic Sea, filamentous cyanobacteria, phytoplankton blooms, salinity, growth, nutrient stoichiometry.

INTRODUCTION

The Baltic Sea with a total area of almost 400 000 km^2 is one the largest brackish-water basins in the world with surface water salinity ranging from 10 practical salinity units (PSU) in the southernmost parts of the sea to below 1 PSU in its

eastern and northernmost parts. Summer blooms of N_2-fixing filamentous cyanobacteria are regular phenomena in the Baltic Sea, and especially *Nodularia spumigena* may form large (up to 100 000 km^2) persistent toxic blooms during late summer in the Gulf of Finland and Baltic Proper (Kahru et al., 1994). These blooms are as old as the brackish-water period (the Litorina Sea), which started about 7000 BP, but are reported to have increased in frequency, biomass, and duration in recent decades, presumably in response to anthropogenic eutrophication (Bianchi et al., 2000). The N_2-fixing cyanobacterial community of the Baltic Sea mainly comprises three taxonomic groups: *Nodularia spumigena*, *Aphanizomenon* sp., and *Dolichospermum* spp. (Stal et al., 2003). The last species is less abundant but has been reported to form a significant biomass in some years (Kanoshina et al., 2003).

Environmental factors controlling cyanobacterial blooms are numerous and complex. The occurrence of a bloom is a function of the different environmental factors and the resource requirements of the bloom-forming organism. Several factors have been considered to be responsible for these surface blooms in the Baltic Sea. In general, calm weather (Kanoshina et al., 2003), high temperature of surface water (>16 °C) (Kahru et al., 1994), and decreased inorganic nitrogen levels combined with excessive inorganic phosphorus (lower inorganic N : P ratio) (Niemi, 1979; Raateoja et al., 2011) have been suggested as principal factors favouring N_2-fixing cyanobacterial blooms in surface waters of the stratified Baltic Proper and the Gulf of Finland. Additionally, the growth of filamentous cyanobacteria is controlled by several physical and chemical factors like salinity (Lehtimäki et al., 1997; Wasmund, 1997) and trace metals (Howarth and Cole, 1985), and several adaptional and behavioural features like buoyancy regulation (Oliver, 1994), grazing resistance (Haney, 1987), effective competition for nitrogen via N_2 fixation (Smith, 1983), phosphorus and nitrogen storage capacity (Osgood, 1988; Pettersson et al., 1993), and ability to grow at low light (Smith, 1983).

Free-living heterocystous cyanobacteria are rare in the full-salinity marine systems, although the marine environment is generally considered as nitrogen limited. In the Baltic Sea bloom-forming diazotrophic cyanobacteria are encountered from the Bornholm Sea towards further east, but not towards the Kiel Bight, Kattegat, Skagerrak, or into the North Sea (Granéli et al., 1990). In fact, the borderline for cyanobacteria in the south seems to be at a salinity of 9–10 PSU, except when wind or water currents bring them across this border, but they do not seem to sustain at higher salinities (Kahru et al., 1994, 2007).

The horizontal distribution for two main bloom-forming cyanobacteria in the Baltic Sea, *N. spumigena* and *Aphanizomenon* sp., seems to be related to salinity. Roughly, the dominance of *N. spumigena* increases whereas the proportion of *Aphanizomenon* sp. decreases towards the more saline southern waters in the Baltic Sea. The former occurs generally in the open sea area, while the latter occurs in high abundance also in coastal areas (Niemistö et al., 1989).

Salinity may influence an organism directly by affecting its physiology or indirectly by changing the surrounding environment of the cell. Cyanobacteria are particularly susceptible to osmotic stress, unable to adjust by the production of

organic osmolytes, and show inhibition of both nitrogen and carbon fixation activity at increasing salt concentrations (Paerl, 2000; Vonshak and Tomaselli, 2000; Moisander et al., 2002).

Experiments with cultures indicate that the salinity for optimum growth of *N. spumigena* is in the range of 5–10 PSU, but growth has been observed over a salinity range of 0–35 PSU (Nordin and Stein, 1980; Lehtimäki et al., 1994; Blackburn et al., 1996; Hobson et al., 1999; Hobson and Fallowfield, 2001). For the Baltic Sea *N. spumigena* the growth at a certain salinity level might be variable for different strains. Lehtimäki et al. (1994) reported that the maximum growth for the Baltic Sea *N. spumigena* strain BY1 occurs at 5 PSU; however, the growth of strain HEM was found to be independent of salinity at 3–11 PSU. Additionally, the growth is strongly reduced in a salt-free medium and at salinities over 30 PSU. In the Baltic Sea *N. spumigena* forms blooms usually at salinities 4–8 PSU and is seldom seen at salinities >10 (Kattegat) or <2 PSU (Bothnian Bay) (Wulff et al., 1990).

The *Aphanizomenon* sp. (strain TR183) isolate from the Baltic Sea grew best at salinities from 0 to 5 PSU during an experiment with a salinity range of 0 to 30 PSU and with 5 PSU steps (Lehtimäki et al., 1997). Laamanen et al. (2002) found that *Aphanizomenon flos-aquae* strains occurring in the Baltic Sea and in fresh water differ from each other by genotype, and that – strain TR183 is genetically most similar to strain 202, an isolate from fresh water (Lake Vesijärvi). Therefore, strain TR183 is not a good representative of the major populations of the Baltic Sea. Laamanen et al. (2002) suggested that one of the genotypes found in the lakes (Baltic Sea genotype) is better adapted to the conditions of the Baltic Sea and that natural selection removes most of the other lake genotypes from the Baltic Sea *A. flos-aquae* populations. They assumed that salinity may be one restrictive factor for typical freshwater strains because they have a lower salinity tolerance.

Previous studies of salinity effects on *Nodularia spumigena* and *Aphanizomenon* sp. in the Baltic Sea have been carried out with a rather limited salinity gradient. Such data do not allow reliable parameterization of the growth rate as a function of salinity, which may be useful in the ecosystem models aiming to predict cyanobacterial blooms. The aim of this study is to provide a more detailed description of salinity tolerance of these species. A salinity gradient relevant for the Baltic Sea was used, from 0 to 10 PSU, with 1 PSU steps. Besides, the *Aphanizomenon* sp. strain that is typical for the Baltic Sea was selected for this study. In addition, responses of nutrient stoichiometry and pigmentation to salinity were studied.

MATERIAL AND METHODS

Organisms

Aphanizomenon sp. (strain KAC 15) was isolated in 1997 from the sound between Öland and the mainland of Sweden (Janson and Granéli, 2002). A toxic strain of *Nodularia spumigena* (strain HEM) was isolated in 1987 from hepatotoxic blooms

collected at the Gulf of Finland (Sivonen et al., 1989). Both cultures have been maintained in Tvärminne Zoological Station, University of Helsinki, Finland, for several years using liquid modified Z8 culture medium (Sivonen et al., 1989).

Experimental design

The strains of *Aphanizomenon* sp. and *N. spumigena* were grown in a salinity gradient from 0 to 10 PSU, with 1 PSU steps, although the actual salinities somewhat varied between the strains. Duplicate cultures were used at each salinity. Batch cultures were grown in 250 mL cell culture flasks (Greiner) with filter screw caps to allow gas exchange. The culture medium (Z8) contained 1.1 mg PO_4 L^{-1} and no inorganic nitrogen. Cultures were grown in 16 h light : 8 h dark cycle and illuminated from above with cool-white fluorescent tubes (Philips TLD 36W/965). Scalar photosynthetically active radiation (PAR) inside the bottles was 180 µmol q m^{-2} s^{-1} (measured with QSL-2100, Biospherical Instruments Inc.). The bottles were submerged into a water bath and kept at 20 °C. The initial chlorophyll *a* (Chl*a*) concentration was 9 µg L^{-1} for *Aphanizomenon* sp. and from 3 to 5 for *N. spumigena*. The experiment lasted for three weeks.

Sampling

The cultures were sampled daily for Chl*a* in vivo fluorescence (IVF). The samples for the extraction of Chl*a* and phosphate were taken at 2–4 day intervals. Samples for particulate C, N, and P were taken at the last day of the experiment (day 21). To prevent bacterial contamination of the cultures the samples were collected in a laminar flow hood. Sampling was always conducted at the same time of the day to prevent the effects of diel variability on cell physiology and on measured variables. The bottles were shaken gently by hand before sampling to make sure that the cells were evenly distributed in the sample. After sampling the bottles were relocated randomly.

Pigment analyses

For the determination of Chl*a* IVF subsamples of 1 mL were taken. To reduce variable fluorescence, 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU, Chem Service) was added to each subsample at final concentration of 20 µmol. The samples were illuminated 3–5 min with white light to saturate photosystem II and thus to reduce variable fluorescence (Johnsen and Sakshaug, 2007). Two replicates (0.4 mL) from each subsample were placed on a 96-well microwell plate and measured with a spectrofluorometer (Varian Cary Eclipse). To match the peak wavelengths of Chl*a* IVF, excitation and emission wavelengths were set at 440 and 682 nm, respectively (slit widths 10 nm). For the wells of each wellplate three

measurements were made to increase the reliability of measurements. Blank values measured daily with Milli-Q water were subtracted from each measurement. Additionally, day-to-day fluctuations of the instrument were corrected for by using daily measurements of pure Chl*a* (Sigma). In vivo fluorescence of phycocyanin was measured as above, but using excitation and emission wavelengths of 630 and 660 nm, respectively (Seppälä et al., 2007).

For the determination of Chl*a*, subsamples of 1–10 mL were filtered onto 13 mm Whatman GF/C filters, which were subsequently extracted in 2 mL Eppendorf vials with 1 mL of 96% ethanol for 24 h at room temperature (Jespersen and Christoffersen, 1987). In some cases when samples were not analysed after the 24 h extraction period, they were placed in a freezer (–20 °C). Samples were centrifuged for 10 min at a speed of 10 000 r min^{-1}, and the clear supernatant was pipetted onto a microwell plate.

The extracted Chl*a* was measured with a spectrofluorometer (Varian Cary Eclipse) using excitation at 430 nm and emission at 670 nm (slit widths 5 nm). Simultaneous calibrations with known concentrations of pure Chl*a* (Sigma) were performed.

Growth rate

During the exponential growth phase the cell number of the population at a given time is expressed as:

$$N = N_0 \exp^{\mu(t-t_0)}, \quad (1)$$

where N and N_0 are the number of cells at times t and t_0, μ is growth rate (d^{-1}), and $t–t_0$ is the time (d) between the measurements of N and N_0.

Instead of cell counts, which are elaborate and inaccurate to carry out for filamentous cyanobacteria, growth rates were estimated using Chl*a* IVF and Chl*a* concentration. As the growth rate was not stable over the whole experimental period, the growth rate for each culture was calculated for the exponential period only.

Nutrient analyses

Phosphate concentrations were analysed from samples filtered through acid-washed and precombusted Whatman GF/C filters and measured using standard methods with a Hitachi U-1100 spectrophotometer (Koroleff, 1983). To determine the elemental composition of cells, subsamples of 25–200 mL for particulate C, N, and P were filtered onto acid-washed and precombusted Whatman GF/C filters. Particulate C and N were measured from the same filters with a Roboprep/ Tracermass mass spectrometer (Europa Scientific, UK), and particulate P was measured according to Solorzano and Sharp (1980).

RESULTS

Growth

Nodularia spumigena showed unvarying growth rates during the whole experiment (Fig. 1a). In contrast, *Aphanizomenon* sp. had a clear lag phase in the growth, and the exponential growth phase started at experiment day 10 in all experimental units (Fig. 1b). Cultures remained long at the exponential growth phase, and there were no significant differences in growth rates whether measured using IVF or Chl*a* (Fig. 2). The correlation of growth rates between IVF and Chl*a* was stronger

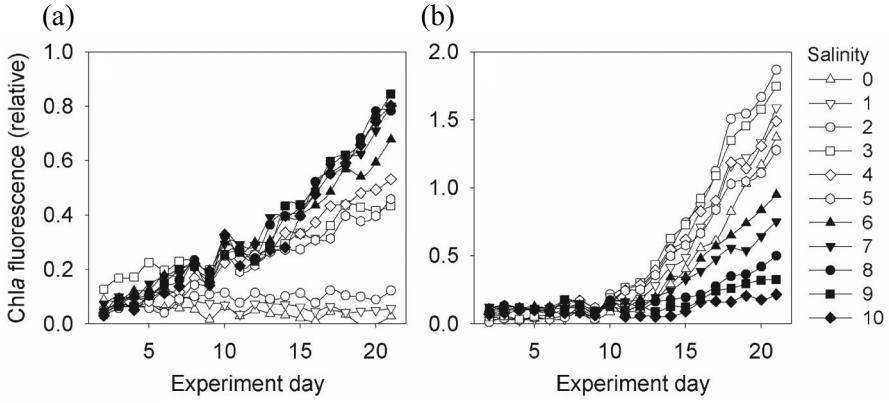

Fig. 1. Growth of *Nodularia spumigena* (a) and *Aphanizomenon* sp. (b) at different salinities, measured using Chl*a* in vivo fluorescence. Only one set of replicate cultures at each salinity is shown.

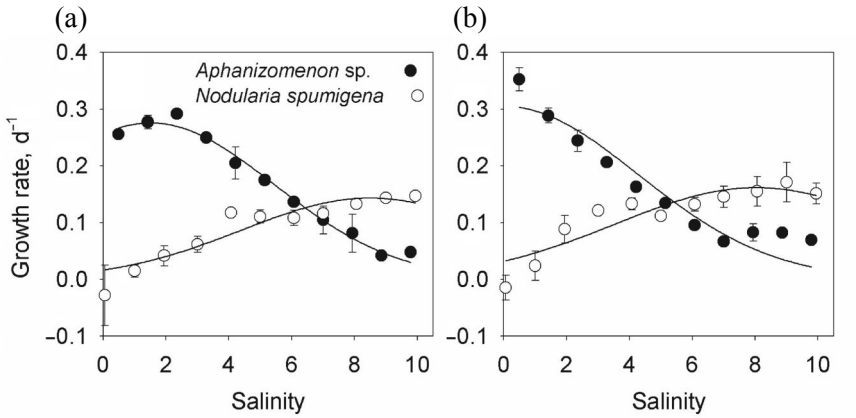

Fig. 2. Growth rates of *Aphanizomenon* sp. and *Nodularia spumigena* as a function of salinity in the growth medium. Growth rates were estimated from the increase of Chl*a* in vivo fluorescence (a) and Chl*a* content (b) at logarithmic growth stage (Eq. 1). Error bars indicate standard deviation in replicate cultures. The curves show the Gaussian fit (Eq. 2); see Table 1 for the parameters of the fit.

for *N. spumigena* ($r^2 = 0.95$) than for *Aphanizomenon* sp. ($r^2 = 0.88$). For the latter the correlation was affected by differences in the growth rates in the salinity range between 0 and 2 PSU.

The effect of salinity on growth rate was clear, but contrasting between species. For *Aphanizomenon* sp. the highest growth occurred at salinities of 0–2 PSU ($\mu = 0.24$–$0.35\ d^{-1}$). This species was able to grow also at higher salinities, although with reduced rates. Depending on the salinity, the growth rate *N. spumigena* varied from -0.02 to $0.17\ d^{-1}$, the highest growth rates were recorded at salinities of 8–10 PSU ($\mu = 0.13$–$0.17\ d^{-1}$), while at 0 PSU no growth was observed. *Aphanizomenon* sp. showed higher growth rates than *N. spumigena* at low salinities (<4 PSU), while *N. spumigena* grew faster at high salinities (>7 PSU) (Fig. 2). In the salinity range from 4 to 7 PSU, the species showed equal growth rates.

To describe the response of growth rate (μ) as a function of salinity (S), a Gaussian function was used:

$$\mu = \mu_{\max} e\left[-0.5\left(\frac{S - S_{\max}}{b}\right)^2\right], \tag{2}$$

where $\mu_{\max}$ is the maximum growth rate, $S_{\max}$ is the salinity where maximum growth rate occurs, and b is the half width of the Gaussian peak (here, with units of salinity). In analysing the growth rates for *Aphanizomenon* sp., estimated from Chl*a* concentrations, $S_{\max}$ was constrained to be equal or larger than zero. With this analysis, the optimal salinity for *Aphanizomenon* sp. was from 0 to 1.5 PSU, and from 8.0 to 8.5 PSU for *N. spumigena* (Table 1, Fig. 2). Salinity tolerance, expressed as the half width of the Gaussian peak, was equally wide for both species, around 4 PSU.

Table 1. Parameters of the Gaussian function (Eq. 1) describing the growth of *Aphanizomenon* sp. and *Nodularia spumigena* as a function of salinity. $S_{\max}$ is the salinity at which the maximum growth rate occurs, b is the half width of the Gaussian peak with units of salinity, $\mu_{\max}$ is the maximum growth rate, and r^2 is the coefficient of determination. Values in parentheses show the standard error of parameters. Growth was measured using Chl*a* in vivo fluorescence (IVF) and Chl*a* concentration (Chl*a*)

	Aphanizomenon sp.		*Nodularia spumigena*	
	IVF	Chl*a*	IVF	Chl*a*
$S_{\max}$, PSU	1.5 (0.3)	0.0 (1.7)	8.5 (1.0)	8.0 (1.0)
b, PSU	3.9 (0.3)	4.3 (1.1)	4.1 (0.9)	4.5 (1.0)
$\mu_{\max}$, d^{-1}	0.28 (0.01)	0.31 (0.04)	0.14 (0.01)	0.16 (0.01)
r^2	0.99	0.90	0.87	0.82

Elemental composition

In all cultures, PO_4 was in excess for the whole growth period while no inorganic nitrogen source was added and cells had to fix atmospheric N_2. For *Aphanizomenon* sp. the particulate N : P ratio varied from 5.3 to 8.6 g g^{-1} and for *N. spumigena* from 4.3 to 5.7 g g^{-1}. The N : P ratio was related to salinity for both species, but with contrasting trends (Fig. 3a). For *Aphanizomenon* sp. the particulate N : P ratios were above the Redfield ratio (7.2 g g^{-1}) at salinities 0–3 PSU and below at other salinities, while for *N. spumigena* the ratios were always below the Redfield ratio with a slight increasing trend with increasing salinity.

Similarly to the N : P ratio, the C : P ratio was clearly affected by salinity for both species, and the trends were also contrasting (Fig. 3c). For *Aphanizomenon* sp. the C : P ratio varied from 28 to 52 g g^{-1} and for *N. spumigena* from 34 to 45 g g^{-1}.

The carbon to nitrogen (C : N) ratio of cells was not influenced by salinity or growth rate (Fig. 3b), indicating that the ratio of nitrogen fixation rate to the

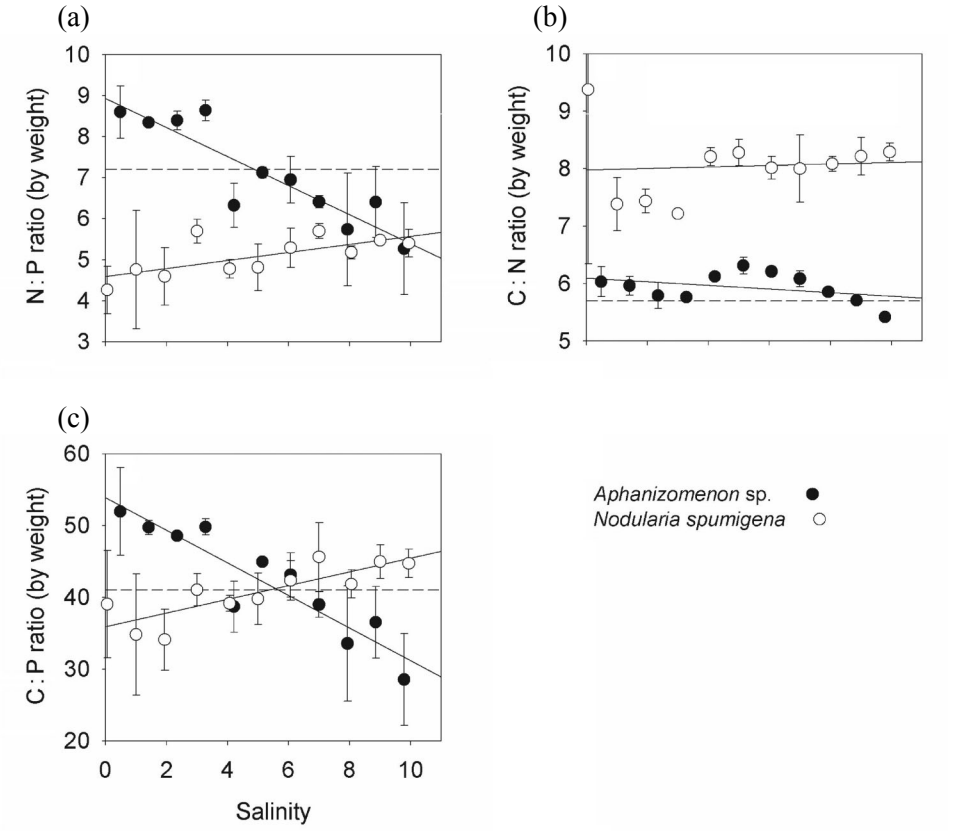

Fig. 3. Cellular N : P (a), C : N (b), and C : P (c) ratios of *Aphanizomenon* sp. and *Nodularia spumigena* as a function of salinity. Continuous lines indicate trends estimated using linear regression and dashed lines indicate the Redfield ratios by weight (N : P 7.2 g g^{-1}, C : N 5.7 g g^{-1}, and C : P 41 g g^{-1}). Error bars indicate standard deviations for replicate cultures.

rate of carbon incorporation into the cells remained constant in all conditions. Differences between species were, however, significant and the ratio was higher for *N. spumigena* (mean 8.0, range from 7.2 to 9.4 g g^{-1}) than for *Aphanizomenon* sp. (mean 5.9, range from 5.4 to 6.3 g g^{-1}) (ANOVA, $p < 0.001$), the latter being very close to the Redfield ratio (5.7 g g^{-1}; Redfield, 1958).

The difference in C : N ratios between the species and the low ratio found for *Aphanizomenon* sp. may be explained by the higher amount of N-containing phycobilin pigments in *Aphanizomenon* sp. Although there are no actual measurements of phycobilin concentrations, differences in the pigmentation were resolved by IVF measurements. Phycocyanin fluorescence, relative to Chl*a* fluorescence, was 2.8 times higher for *Aphanizomenon* sp. than for *N. spumigena* (Fig. 4). Within species the relation between elemental composition and pigmentation was not evident.

Growth rate and cellular N : P ratio were clearly linearly related ($r = 0.77$, $p < 0.001$) and the relationship was similar for both species (Fig. 5a). The correlation between the C : P ratio and growth rate was significant ($r = 0.68$, $p < 0.001$, data not shown). Because particulate nutrients were measured at the end of the experiment, the growth rates used in the above-mentioned comparisons were also estimated for the final days of the experiment.

The C : Chl*a* ratio varied widely for both species and was nonlinearly related to growth rate (Fig. 5b). For *Aphanizomenon* sp., the lowest C : Chl*a* ratios around 20 g g^{-1} were observed at low salinities with high growth rates. At the highest salinity with the lowest growth rates, C : Chl*a* values above 90 g g^{-1} were measured. The range of C : Chl*a* was even larger for *N. spumigena*, with the lowest values of 30 g g^{-1} in the cultures with the highest growth rates and the highest values clearly above 100 g g^{-1} in the cultures with very low or already negative growth rates.

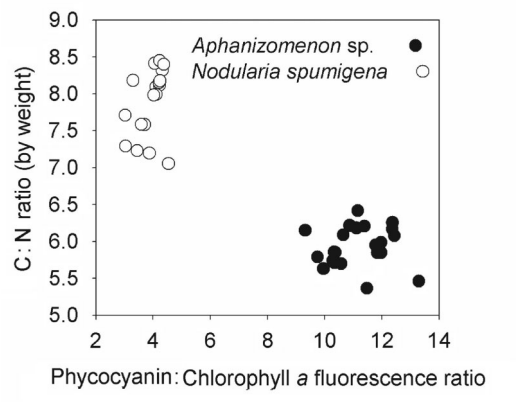

Fig. 4. Relationship between phycobilin content of *Aphanizomenon* sp. and *Nodularia spumigena* and their cellular C : N ratio. Phycobilin content is expressed as a ratio of in vivo phycocyanin fluorescence to Chl*a* in vivo fluorescence.

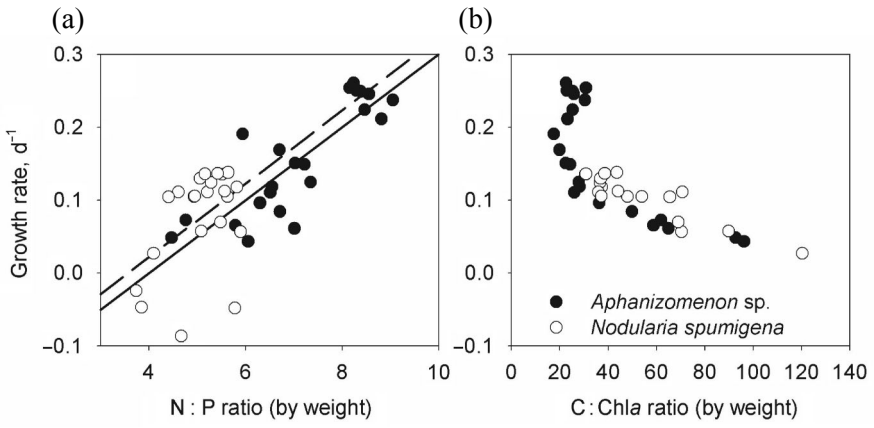

Fig. 5. Relationship between particulate N : P ratio and growth rate (a) (calculated from Chl*a* in vivo fluorescence data) and between C : Chl*a* ratio and growth rate (b) for *Aphanizomenon* sp. and *Nodularia spumigena*. Continuous and dashed lines in panel (a) show linear regression fits for *Aphanizomenon* sp. ($r^2 = 0.70$) and *N. spumigena* ($r^2 = 0.21$), respectively. The highest values for C : Chl*a* in *N. spumigena* (170–740 g g^{-1}), occurring in cultures with negative growth rates, are not shown.

DISCUSSION

Cyanobacterial blooms in the Baltic Sea

Filamentous cyanobacteria in the Baltic Sea show lower growth rates than other abundant phytoplankton groups (e.g. Lignell et al., 2003; Vahtera et al., 2005). Despite this, surface blooms of filamentous cyanobacteria are common as the species are not effectively grazed and they may utilize N_2 as a nitrogen source at times when other phytoplankton groups are limited by nitrogen availability. For the bloom to develop, also other environmental conditions must be favourable (Stal et al., 2003).

The effect of filamentous cyanobacteria on the flows of carbon and nutrients, especially nitrogen, in the Baltic Sea ecosystem are important (Larsson et al., 2001). Moreover, the blooms influence the recreational value of the sea. For these reasons, ecosystem and biogeochemical models for the Baltic Sea should include these species as a separate component (Stipa, 2002; Hense and Burchard, 2010). Numerical growth models of filamentous cyanobacteria should take into account their temperature dependence of nitrogen fixation, use of inorganic nutrients, light utilization, and include life cycle stages (Hense, 2007).

Typically the major filamentous species, *N. spumigena*, *Aphanizomenon* sp., and *Dolichospermum* spp., are considered as a single compartment in ecosystem models. The species differ, however, somewhat in their ecological niche, making unique parametrization possible for modelling purposes. This may be desired as *N. spumigena* may form toxic blooms. To exemplify differences between the species, Vahtera et al. (2007) showed that *N. spumigena* is able to use organic phosphorus while *Aphanizomenon* sp. is not. In addition, they noted that species

are often vertically separated, *N. spumigena* being more abundant in surface layers, suggesting that species have different light harvesting strategies. Salinity optima and tolerance seem to be different among species as well, making it possible to constrain the existence of blooms by different species in the Baltic Sea wide perspective (e.g. Lehtimäki et al., 1997).

Effect of salinity on the growth of filamentous cyanobacteria

The growth rates of *Aphanizomenon* sp. and *N. spumigena* were clearly affected by salinity, but with contrasting trends. The results follow closely the general patterns found already by Lehtimäki et al. (1994, 1997) using cultures and by Niemistö et al. (1989) in the field observations of the species distribution. *Aphanizomenon* sp. has a preference for low saline environments, but shows also persistent although low growth rates at salinities above 5 PSU ($\mu < 0.1$ d^{-1}). As reported by Laamanen et al. (2002), *Aphanizomenon* sp. strains found in the Baltic Sea are very closely related to the strains found in lakes in the Baltic Sea region.

Lehtimäki et al. (1997) reported that *Aphanizomenon flos-aquae* (strain TR183) tolerates salinities from fresh water to 10‰ salinity (test range 0–30‰) and found that the Chl*a* concentrations are similar at salinities from 0 to 5‰. The growth rates based on Chl*a* concentration for strain TR183 under N_2 fixation conditions at salinities 0, 5, and 10 PSU are 0.11, 0.09, and 0.04 d^{-1}, respectively (estimated from a figure in Lehtimäki et al., 1997). The corresponding values in the current experiment based on Chl*a* IVF were 0.26, 0.18, and 0.04 d^{-1}, respectively. As discussed by Laamanen et al. (2002), strain TR183 is genetically most similar to freshwater strains and therefore is not a good representative of the major populations of the Baltic Sea. However, further comparative studies are needed with different strains under different physical and chemical conditions to obtain more reliable data about the physiology of strains. This would help to ascertain whether freshwater strains are at all able to form massive blooms in the Baltic Sea.

In our experiment *N. spumigena* grew well at salinities above 3 PSU. The maximum growth rates by Chl*a* IVF were observed in the salinity range from 8 to 10 PSU ($\mu = 0.15$ d^{-1}). The growth rate was negative at a salinity of 0 PSU and was highly reduced at salinities of 1–2 PSU. Thus *N. spumigena* (strain HEM) is not able to grow in pure freshwater systems or in areas with intensive river inputs where the salinity is below 2 PSU (e.g. in the Bothnian Sea). In their study of different Baltic Sea *N. spumigena* strains Lehtimäki et al. (1994) showed that the biomass yield (dry weight) for strain HEM is the highest at salinities above 5‰ (test range 3–11‰) while for strain BY1 a higher biomass was observed at a salinity of 5‰. Afterwards, Lehtimäki et al. (1997) observed a very low growth rate for strain BY1 at a salinity of 0 PSU but at salinities of 5 and 10 PSU the growth rates were 0.15 and 0.14 d^{-1}, respectively (estimated on the basis of Chl*a* concentration from a figure), which is partly in line with the results of the current research, 0.11 and 0.14 d^{-1}, respectively. However, strain HEM tolerates higher salinities than strain BY1 and for the latter a positive growth was observed at a

salinity of 0‰ (Lehtimäki et al., 1997). Moisander et al. (2002) did not observe any significant differences in the growth rates for the Baltic Sea *N. spumigena* (strain FL2f) in the full range of salinities from 0 to 20 g L^{-1}. The *N. spumigena* (strain 001E) in Lake Alexandrina in Australia forms blooms at a salinity near 0‰, whereas the optimum growth stays at salinities 0.3–13 TDS (Hobson and Fallowfield, 2003). Lukatelich and McComb (1986) showed that the magnitude of the *N. spumigena* bloom in the Peel-Harvey estuary has a negative correlation with salinity and the blooms collapse at salinities over 30 PSU. Some studies have reported reduction in growth rates or increases in akinete numbers when salinity increases above 20–30 PSU (Jones et al., 1994; Lehtimäki et al., 1994). Therefore, it is apparent that *N. spumigena* may tolerate a wide range of salinities which is, however, highly strain-specific.

It is known that under nitrogen fixation conditions the energetic demands are greater and therefore the net growth might be lower. Our unpublished results show that if *N. spumigena* uses NO_3 instead of fixing N_2, the growth rate increases 1.5–2.3-fold (at 7 PSU, Seppälä and Rakko, unpublished). For many cyanobacteria nitrogen fixation is especially sensitive at higher salinities, which may partly be caused by the inadequate amount of enzymes responsible for N_2 fixation (Howarth and Cole, 1985).

Photosynthesis and thereby CO_2 fixation may also be affected by salinity. Several studies have reported that elevated salinity increases algal and cyanobacterial photoinhibition and reduces their maximum photosynthetic rate (P_{max}) (Vonshak and Tomaselli, 2000). Hobson and Fallowfield (2001) found that when irradiance increases, the maximum growth rate of *N. spumigena* occurs at a lower salinity. Moisander et al. (2002), on the contrary, did not observe any increase of photoinhibition under the salt stress in different cyanobacteria including *N. spumigena*.

With increasing salinity the energy that is spent on active transport of salts also increases, and therefore respiration will exceed production. In addition, energy is needed to provide N_2 fixation, and the more the cells use energy to cope at elevated salinities, the smaller the N_2 fixation rate is. The decrease of the growth rate with increasing salinity of both studied species may be related to molybdate–sulphate concentrations and osmoregulation (Howarth et al., 1988). In the experiments with Baltic Sea heterocystous cyanobacteria Stal et al. (1999) observed a dramatic increase of nitrogenase activity after the sulphates had been removed from the growth medium.

Effect of salinity on particulate nutrients

For either species, the C : N ratio was not influenced by salinity (Fig. 3b), which apparently indicates that CO_2 and N_2 fixation were balanced regardless of salinity. The higher cellular C : N ratio observed in *N. spumigena* than for *Aphanizomenon* sp. indicates a higher need for nitrogen in *Aphanizomenon* sp. Lehtimäki et al. (1997) reported a higher N_2 fixation rate in *Aphanizomenon flos-aquae* than in *N. spumigena*. They concluded that this may due to differences in the N_2 fixation potential in heterocysts.

Differences in the C : N ratio were reflected in the pigmentation as observed by fluorescence measurements. The results indicate that *Aphanizomenon* sp. has relatively more N-rich phycobilin pigments in the light-harvesting complex than *N. spumigena*. This partly explains why *Aphanizomenon* sp. filaments are equally distributed in the water column and may also inhabit deeper weakly illuminated water strata while *N. spumigena* is concentrated near the surface (Kononen and Nõmmann, 1992). It should be noted further that green light penetrates deepest in the Baltic Sea, and phycobilin pigments of cyanobacteria are well suited to harvest this light.

The N : P and C : P ratios were clearly related to salinity for both studied species but with contrasting trends (Fig. 3a, c). The higher cellular C : P ratio in fast-growing cells indicates a lower need for P than C and N. Nitrogen-limited cells accumulate carbohydrates that can be utilized as a source of energy and carbon skeletons for nitrogen assimilation (Turpin et al., 1985).

In *Aphanizomenon* sp. the cellular N : P ratio was above the Redfield ratio (7.2 : 1) at salinities of 0–5 PSU, which may indicate a reduced N_2 fixation rate at salinities higher than 5 PSU. In *N. spumigena* the cellular N : P ratio increased with increasing salinity, but differently from *Aphanizomenon* sp. these ratios were always below the Redfield ratio at all salinities tested. This observation indicates differences in the nitrogen fixation rates of the studied species. However, considering that the higher the cellular N : P ratio, the higher the growth rate will be, obviously the growth rate was controlled by the nitrogen fixation rate, which in turn was regulated by salinity.

Effect of salinity on the horizontal distribution of the species

The horizontal distribution patterns of *N. spumigena* and *Aphanizomenon* sp. in the Baltic Sea are different and partly explained by the salinity tolerance described earlier (e.g. Lehtimäki et al., 1997) or in more detail in this study. According to our results, the growth rate of both species was quite similar at salinities from 4 to 7 PSU. Therefore the distribution in this salinity range was expected to be equal. In this area, which forms the major part of the Baltic Sea, the species distribution is more determined by other factors. As a generalization, the salinity below 5 PSU is more favourable for *Aphanizomenon* sp. and the salinity above 5 PSU for *N. spumigena*.

The question is at what salinities *Aphanizomenon* sp. and *N. spumigena* are able to form blooms considering their specific growth rates at different salinities. Unfortunately, the term ‘bloom’ is poorly defined and therefore it is difficult to specify the biomass value from which there is a bloom. Nevertheless, let us assume that prior to the bloom the value of biomass for one species was 0.2 mg L^{-1}. In that case the value of 0.5 mg L^{-1} suggested in Kanoshina et al. (2003) would be obtained during one week when the growth rate was at least above 0.12 d^{-1}. As shown by Kanoshina et al. (2003), the biomass of *Aphanizomenon* sp. in the Gulf of Finland increased from 0.2 mg L^{-1} close to 0.8 mg L^{-1} during one week. To achieve that, the growth rate had to be at least 0.23 d^{-1}. Our experiment at a

salinity of 5 PSU showed a significantly lower specific growth rate, 0.13–0.17 d^{-1} (but in our salinity experiment cells had to fix all nitrogen). Therefore, the increase of biomass in the field conditions may have been partly due to upward migration of filaments from deepest zones, as shown by Gallon et al. (2002).

Considering growth rates at different salinities, the probability of mass occurrence for *Aphanizomenon* sp. is in the salinity range from 0 to 6 PSU and for *N. spumigena* in the range from 4 to 10 PSU. As discussed above, the initial biomass and upward drift form deeper zones and long-lasting favourable weather conditions should also be taken into account in predicting blooms as it is partly for these reasons the biomass increase may vary in situ.

To conclude, this study demonstrates how environmental resources are used by two ecologically similar cyanobacteria and how important complicated biochemical processes are in it. Concrete numerical physiological values of species allow us to understand and even predict water blooms in the Baltic Sea.

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