Impact of temperature and species interaction on filamentous cyanobacteria may be more important than salinity and increased *p*CO2 levels

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Abstract

A future business as usual scenario (A1FI) was tested on two bloom-forming cyanobacteria of the Baltic Proper, *Nodularia spumigena* and *Aphanizomenon* sp., growing separately and together. The projected scenario was tested in two laboratory experiments where, A) interactive effects of increased temperature and decreased salinity and, B) interactive effects of increased temperature and elevated levels of pCO_2 were tested. Increased temperature, from 12 to 16°C had a positive effect on the biovolume and photosynthetic activity (F_v/F_m) of both species. Compared when growing separately, the biovolume of each species were lower when grown together. Decreased salinity, from 7 to 4, and elevated levels of pCO_2, from 380 to 960 ppm, had no effect on the biovolume, but on F_v/F_m of *N. spumigena* with higher F_v/F_m in salinity 7. Our results suggest that the projected A1FI scenario might be beneficial for the two species dominating the extensive summer blooms in the Baltic Proper. However, our results further stress the importance of studying interactions between species.

Introduction

Global climate change is a potential threat to all ecosystems (Fischlin et al. 2007). For the Baltic Proper, the major part of the Baltic Sea, annual precipitation, surface water temperature (HELCOM 2007) and atmospheric partial pressure of CO_2 (pCO_2) levels (Meehl et al. 2007) are expected to increase. Salinity may decrease from current 7 to 4, while mid-summer water temperature may increase from 12°C to 16°C, and pCO_2 levels may increase from 380 ppm to 960 ppm, by year 2100 (HELCOM 2007). In a recent review concerning effects of ocean acidification on Baltic ecosystems, it is concluded that it seems likely that the effects on the Baltic spring and summer blooms will be small, and perhaps positive (Havenhand, in press). However, available evidence for Baltic primary producers is scant and only three relevant studies are referred to. Thus, more studies are evidently needed.

In the Baltic Proper, the summer blooms are dominated by *Aphanizomenon* sp. (Morren ex Bornet et Flahault) and *Nodularia spumigena* (Mertens ex Bornet & Flahault) (Janson and Hayes 2006; Jonasson 2006). *Aphanizomenon* sp. initiates the blooms, while *N. spumigena* peaks later in the season. During calm weather, these species can create massive blooms (Kanoshina et al. 2003; Lehtimäki et al. 1997). *N. spumigena* produces the hepatotoxin nodularin, which can accumulate in the food web with as yet unknown consequences (Sipiä et al. 2002; Karjalainen et al. 2007). The concentration of nodularin varies under different environmental conditions, such as temperature, salinity, radiation and nutrient concentrations (Lehtimäki et al. 1994; Blackburn et al. 1996; Granéli et al. 1998; Hobson et al. 1999; Repka et al. 2001; Mazur-Marzec et al. 2005; Pattanaik et al. 2010).

Increasing surface water temperatures can increase stratification, leading to nutrient depletion and hence a competitive advantage for diazotrophic cyanobacteria in the euphotic zone. Moreover, a stagnant water column will expose the species in the euphotic zone to high intensities of ambient radiation. *N. spumigena* continues to grow under both nutrient deficiency and high ambient radiation intensities (Mohlin and Wulff 2009; Pattanaik et al. 2010; Mohlin et al. 2011). Furthermore, the stabilization of the water column in association with increasing temperatures may cause the spring bloom to begin earlier (Hagström and Larsson 1984) and potentially also the cyanobacteria bloom. Higher temperatures may favour cyanobacteria over diatoms, thereby affecting the composition of the phytoplankton community (Andersson et al. 1994; Wrona et al. 2006). Furthermore, if cyanobacteria growth increases, the amount of heterotrophic bacteria may also increase due to increasing

56 temperatures and increasing concentrations of dissolved organic carbon (Shiah and Ducklow
57 1994; Pomeroy and Wiebe 2001). This will result in complex feed-back effects on the
58 microbial community.

59 An increase of atmospheric pCO_2 (and a corresponding increase in seawater pCO_2) may
60 increase photosynthesis in phytoplankton (Raven et al. 2005; Hutchins et al. 2007), however,
61 the response differs between groups of microalgae and between cyanobacteria species. For
62 example, N_2 and CO_2 fixation rates of the diazotrophic filamentous cyanobacteria
63 *Trichodesmium* sp. increased with elevated pCO_2 (Hutchins et al. 2007), while the same pCO_2
64 level resulted in an increase of growth of the picocyanobacteria *Synechococcus* sp. but not
65 *Prochlorococcus* sp. (Fu et al. 2007). Benthic cyanobacteria seem unaffected by increased
66 pCO_2 levels (Johnson et al. 2011).

67 Diazotrophic bloom-forming cyanobacteria in the Baltic are cosmopolitan species existing in
68 fresh and brackish waters. A salinity decrease may therefore not have a negative effect on
69 these cyanobacteria. Laboratory studies on *N. spumigena* and *Aphanizomenon* sp. (Lehtimäki
70 et al. 1997; Pliński and Jóźwiak 1999) have shown highest growth rates of *N. spumigena* in
71 salinities ranging from 5 to 20, but this species can tolerate salinities up to 30. Highest growth
72 rates of *Aphanizomenon* sp. have been observed at salinities between 0 and 10. However, the
73 production rate of the hepatotoxin nodularin is highest in salinities of 5 to 15. Consequently,
74 a decrease in salinity may increase toxin levels (Blackburn et al. 1996).

75 Allelopathic interactions have been proposed to explain the temporal and spatial separation
76 between *N. spumigena* and *Aphanizomenon* sp. Previous studies have shown that the release
77 of nodularin and other secondary metabolites from *N. spumigena* have an allelopathic effect
78 on other organisms (Sellner 1997; Ibelings and Havens 2008), and Legrand et al. (2003)
79 reviewed its importance in phytoplankton competition. However, Pattanaik et al. (2010)
80 found nodularin accumulation and release were dependent on different environmental
81 conditions, but did not affect the co-existing species *Aphanizomenon* sp. Others have
82 suggested that the toxin stimulates the abundance of the same or other cyanobacterial species
83 in the community rather than inhibits the abundance of competitors (Suikkanen et al. 2004,
84 2005).

85 The aim of this study was to test impacts of the business as usual (A1FI) scenario (Mehl et al.
86 2007) on the two dominating filamentous cyanobacteria species during the summer bloom.

This scenario projects increased temperatures (by 4°C), increased atmospheric pCO_2 levels (from 380 to 960 ppm), and decreased salinity (by 3-4) for the Baltic Proper area in 2100.

Material and Methods

Two laboratory experiments, Expt A and Expt B, were performed with *Aphanizomenon* sp. and *N. spumigena,* growing separately and together. In the first experiment, Expt A, the interactive effects of salinity (7 and 4) and temperature (12 and 16°C) were tested (HELCOM 2007). In the second experiment, Expt B, the set-up was the same but the treatments were pCO_2 (380 and 960 ppm) in combination with temperature (12 and 16°C). In both experiments, the response variables were photosynthetic activity and total biovolume.

Experimental set-up

The two cyanobacteria strains used were *N. spumigena* (KAC 12) and *Aphanizomenon* sp. (KAC 15) from the Kalmar Algal Collection (KAC), Linnaeus University, isolated from the Baltic Proper. The two stock cultures were inoculated in f/2 medium (Guillard 1975) at salinity 7. The f/2 medium was prepared from filtered (GF/F, Whatman) natural seawater diluted to a salinity of 7. The cultures were kept in a temperature controlled room under ca.18°C, 16:8 h light:dark period with 75 µmol photons m^{-2} s^{-1} of photosynthetic active radiation (PAR, 400-700 nm).

At the start of the experiments the two inoculated species were distributed into 75 mL cell culture flasks (NUNC, Sarstedt) and mixed with water of salinity 4 or 7, and medium f/5 for Expt A, and f/5 with salinity 7 for Expt B. The species were either distributed into flasks alone or together with the other species. Initial biovolume concentrations in Expt A were 3.0 mm^3 L^{-1}, and 11.3 mm^3 L^{-1} for *Aphanizomenon* sp. and *N. spumigena* (respectively) in single species flasks, and 1.5 mm^3 L^{-1} and 5.3 mm^3 L^{-1}, respectively, in treatments where the two species grew together. In Expt B, initial concentrations were 5.6 mm^3 L^{-1} and 9.4 mm^3 L^{-1} for *Aphanizomenon* sp. and *N. spumigena* (respectively) in single species flasks, and 4.7 mm^3 L^{-1} and 8.1 mm^3 L^{-1}, respectively, in treatments where the two species grew together. Treatments were replicated three times in Expt A and four times in Expt B.

Temperatures were held constant using thermostatic water baths (Julabo F34-ED), and a HOBO temperature logger (HOBO UA-002-08) was placed in each bath. Flasks were distributed haphazardly in each bath. For Expt B, the two pCO_2 levels were accomplished by

bubbling with synthetic air (AGA Gas) containing 380 or 960 ppm pCO_2 with champagne size bubbles at a flow rate of approximately 4 mL min^{-1}. Optimal flow rate and bubble size were determined from Torstensson et al. (2012). A small hole in the caps of the flasks released excess pressure but allowed the head space to become saturated with the desired pCO_2 concentration. For Expt A flasks were not aerated but were shaken gently daily.

Measurements of photosynthetic activity and biovolumes were conducted initially, Day 0, and at the end of the experiment; Day 7 for Expt A, and Day 5 for Expt B.

Seawater carbonate system measurements and calculations

At the start and end of the experiment, three millilitres of sample was 0.2 µm filtered and adjusted to 15°C. pH of the sample was quickly determined spectrophotometrically (Diode Array, HP 8452) in a 1-cm quartz cell by the addition of the sulphonephtalein dye, *m*-cresol purple as indicator (Clayton and Byrne 1993). Temperature was measured using a thermistor with a precision of 0.1°C (Amadigit). Perturbation of the pH of the sample due to the addition of indicator solution was corrected according to Chierici et al. (1999). The pH_T of the indicator solution that was used for calculating the correction factor was determined at every sampling occasion using a 0.2-mm quartz cell. The analytical precision of the measurements was estimated to ±0.002 pH units by triplicate measurements of one sample. The remaining sample was adjusted to 15°C in darkness, and total alkalinity (A_T) was analysed within 24 h. A_T was determined by potentiometric titration (Metrohm Titrando and Aquatrode Plus with Pt1000) with 0.05 M HCl in an open cell, and the equivalence point was evaluated using a Gran function according to Haraldsson et al. (1997). At the beginning and at the end of each sampling day, the accuracy of the A_T measurements were controlled against a certified reference material (CRM) supplied by Andrew Dickson (Scripps Institution of Oceanography, San Diego, USA). Sample precision was estimated to ±3 µmol kg^{-1} during each sampling day by three replicated analyses of one sample. Together with data on pH at 15°C, A_T, salinity, *in situ* temperature, and inorganic phosphate, additional carbonate system variables (pCO_2 and $[CO_2]$) were calculated with the chemical speciation program CO2SYS (Pierrot et al. 2006). Calculations were performed on the total hydrogen ion scale (pH_T), using the KSO_4 constant as determined by Dickson (1990). The dissociation constants used for carbonic acid (K1 and K2) were determined by Mehrbach et al. (1973) and refitted by Dickson and Millero (1987).

149 ***Inorganic nutrients***

150 From two of the three replicate bottles in Expt A, a 12 mL sample was filtered through 0.2-
151 µm syringe filters for analysis of inorganic nitrite, nitrate, and phosphate using colorimetric
152 methods according to Grasshoff et al. (1999). The analyses were done by Swedish
153 Meteorological and Hydrological Institute, Oceanographic Laboratory (Göteborg, Sweden).

154 ***Photosynthetic activity***

155 To measure photosynthetic activity of PSII, the maximum photochemical yield (F_v/F_m) was
156 measured (Campbell et al. 1998) with a Pulse Amplitude Modulation (PAM) fluorometer
157 (WATER-PAM, Walz GmbH) optimized for cyanobacteria. Although the photosynthetic
158 physiology and fluorescence pattern of cyanobacteria differ from those of plants,
159 cyanobacterial F_v/F_m is a useful integrated measure of PSII activity (Campbell et al. 1998).
160 F_v/F_m was estimated by applying the cell suspension with a saturating light pulse that briefly
161 suppresses photochemical yield to zero and induces maximum fluorescence yield. The yield
162 was calculated according to $(F_m\text{-}F_0)/F_m = F_v/F_m$, where F_m is maximum fluorescent yield and
163 F_0 fluorescent yield before the light pulse in a dark-adapted state. The measurements were
164 done in the emitter-detector unit, of the CUVETTE version, with red LED light (650-730 nm)
165 optimized for cyanobacteria (WATER-ED 8, 487, Walz GmbH) equipped with a stirring
166 device (WATER-S, Walz GmbH) to homogenize the sample prior to measurement (Cosgrove
167 and Borowitzka 2006). A well-mixed 3 mL sample was transferred to the quartz cuvette, kept
168 dark for 3 minutes and after 10 seconds of stirring and 600 ms light pulse, the F_v/F_m was
169 measured. Because we could not separate treatment effects from species-specific differences,
170 photosynthetic activity was only measured in monocultures.

171 ***Biovolumes***

172 Because the photosynthetic process in many species can acclimate to variable stress
173 conditions, the key variable for ecological success in filamentous cyanobacteria under given
174 environmental conditions is growth. Growth integrates well the impacts of all positive and
175 negative abiotic factors. In this study, total cyanobacteria biovolume was considered an
176 estimate of growth. A well-mixed 4 mL sample from each experimental flask in Expt A, and
177 from three of the four replicates in Expt B, was preserved with Lugol´s solution, kept in the
178 dark and analysed within 2 months. Each Lugol sample was gently turned at least 10 times
179 before being analysed at 20 or 40x magnification (Zeiss Axiovert 40CFL, micrometerocular
180 44 42 32 E-Pl 10x/20) in a gridded Sedgewick rafter chamber (Wildlife Supply Company,
181 1801-G20). The length and width for each filament in 100 randomly selected squares, (100

182 µL each), was measured and the total biovolume (mm^3 L^{-1}) was calculated assuming a
183 cylindrical filament shape (filament length * π *radius^2). Total biovolume was used as an
184 estimate of growth because the species used in the experiments increase in filament number
185 and/or filament length.

186 ***Statistical analyses***

187 Data were analysed by 3-way ANOVA for biovolume using SPSS software (PASW Statistics
188 ver. 20, IBM) for each sampling day. For photosynthetic activity, 2-way ANOVA was
189 applied. Homogeneity was tested with Cochran's test and, where needed, data were
190 transformed according to Underwood (1997). Significant differences were set as $p<0.05$.

191

192 **Results**

193 ***Experiment A (Salinity + Temperature)***

194 Average temperatures differed 4.1°C (SE 0.01) between the treatments. Inorganic nutrient
195 concentrations between treatments at the end of the experiment were 260-325 µmol L^{-1} (SE
196 5.3) for NO_2+NO_3 1.6 and 9.5 µmol L^{-1} (SE 0.76) for PO_4. No interactive treatment effects
197 were found in any variables (Table 1).

198 For *N. spumigena* the total biovolume of the filaments was significantly higher at 16°C than
199 at 12°C (ANOVA, $F_{(1,16)} = 27.124$, P = 8.6E-6; Fig. 1; Table 1). This effect was also found
200 for *Aphanizomenon* sp. (ANOVA, $F_{(1,16)} = 12.340$, P = 0.003). Additionally, both species had
201 significantly higher biovolumes in treatments where they did not grow with the other species
202 (ANOVA, $F_{(1,16)} = 44.007$, P = 5.7E-6 and ANOVA, $F_{(1,16)} = 19.139$, P = 4.7E-4,
203 respectively; Fig. 1). For *Aphanizomenon* sp. assuming a cell volume of $8.9*10^{-8}$ mm^3
204 (Mohlin et al. 2011), initial cell numbers were $3.4*10^7$ for monocultures and $1.6*10^7$ cells L^{-1}
205 for mixed cultures. For *N. spumigena,* assuming a cell volume of $23.6*10^{-8}$ mm^3 (Mohlin et
206 al. 2011), initial cell numbers were $4.8*10^7$ and $2.3*10^7$ cells L^{-1}, for monocultures and mixed
207 cultures, respectively.

208 Initial F_v/F_m values were 0.08 and 0.41 for *Aphanizomenon* sp. and *N. spumigena*,
209 respectively. The low initial value for *Aphanizomenon* sp. was explained by stagnant pre-
210 culture conditions. During the experimental period, F_v/F_m increased for both species with
211 significantly higher values at higher temperature (ANOVA, $F_{(1,8)} = 30.916$, P = 0.001 and
212 ANOVA, $F_{(1,8)} = 44.308$, P = 1.6E-4, respectively; Fig. 2; Table 1). Lower salinity

significantly affected *N. spumigena*, with lower F_v/F_m values at salinity 4 compared to salinity 7 (ANOVA, $F_{(1,8)} = 32.855$, P = 4.4E-4).

Experiment B (CO_2 + Temperature)

Average temperatures differed 4.1°C (SE 0.002) between the treatments. Initially, mean pH was 7.82 and 7.80 in monocultures with *Aphanizomenon* sp. and *N. spumigena*, respectively. In mixed culture average initial pH was 7.86. Corresponding A_T values were 498, 487 and 489 μmol kg^{-1} (Table 2). Additional carbonate system parameters are shown in Table 2.

No interactive treatment effects were found in any variables (Table 1). For both *Aphanizomenon* sp. and *N. spumigena* the total biovolume was significantly larger at higher temperature (ANOVA, $F_{(1,16)} = 7.596$, P = 0.014 and ANOVA, $F_{(1,16)} = 7.829$, P = 0.013, respectively). Additionally, both species had significantly higher biovolumes in treatments where they did not grow with the other species (ANOVA, $F_{(1,16)} = 10.747$, P = 0.005 and ANOVA, $F_{(1,16)} = 17.621$, P = 6.8E-4, respectively; Fig. 1). For *Aphanizomenon* sp. assuming a cell volume of $8.9*10^{-8}$ mm^3 (Mohlin et al. 2011), initial cell numbers were $6.3*10^7$ for monocultures and $5.2*10^7$ cells L^{-1} for mixed cultures. For *N. spumigena,* assuming a cell volume of $23.6*10^{-8}$ mm^3 (Mohlin et al. 2011), initial cell numbers were $4.0*10^7$ and $3.4*10^7$ cells L^{-1}, for monocultures and mixed cultures, respectively.

Initial F_v/F_m values were 0.23 and 0.43 for *Aphanizomenon* sp. and *N. spumigena*, respectively. Temperature effects in *N. spumigena* and *Aphanizomenon* sp. showed similar tendencies as in Expt A; higher F_v/F_m values at higher temperatures (ANOVA, $F_{(1,12)} = 8.830$, P = 0.012 and ANOVA, $F_{(1,12)} = 43.639$, P = 2.5E-5, respectively; Fig. 2; Table 1). There was no statistically significant effect of *p*CO2 for either *Aphanizomenon* sp. or *N. spumigena* (Table 1).

Discussion

Temperature increases of 4°C in Expt A and Expt B resulted in higher total biovolume for both *Aphanizomenon* sp. and *N. spumigena*. Furthermore, both species had lower biovolumes in the presence of the other species (both experiments). For total biovolumes, no interactive effects were found in the variables studied and no statistically significant effects were observed for salinity or carbon dioxide treatments. For photosynthetic activity, measured as

F_v/F_m, increased temperature had positive effects for both species in both experiments. For *N. spumigena*, decreased salinity resulted in higher F_v/F_m.

The elevated temperature of 16°C was in the optimal range for both *Aphanizomenon* sp. and *N. spumigena* (Lehtimäki et al. 1997; Pliński and Jóźwiak 1999). *Aphanizomenon* sp. is the first species of the succession of bloom-forming cyanobacteria in the Baltic Proper, and has optimal growth rates at lower temperatures than *N. spumigena*. The cyanobacteria bloom in the Baltic Proper starts when the water temperature rises above 16-17°C (Edler 1979; Niemi 1979) and a future temperature increase could result in cyanobacteria blooms starting earlier but also lasting longer into the autumn. The spring bloom is presently dominated by diatoms and dinoflagellates, known to be favoured by colder waters (Andersson et al. 1994). An increase in temperature may inhibit cold-water diatoms and favour cyanobacteria species, thus ultimately changing the species composition of the Baltic Proper (Andersson et al. 1994). The positive effects of increased temperature in our experiments should be carefully interpreted (and not directly interpolated to what will happen in a future Baltic Proper). For example the strains used were pre-cultured in ca. 18°C.

In our experiments, nitrogen and phosphorus were added in surplus, and in high N:P ratio, a scenario not representative for Baltic summer conditions with low N:P ratios. However, the high N:P ratios in our experiments are not considered to favour cyanobacteria due to the nitrogen-fixing capacity of the diazotrophic cyanobacteria used in the experiment.

Under natural conditions, both *N. spumigena* and *Aphanizomenon* sp. grow in both of the salinities tested here (7 and 4). In our study decreased salinity did not affect the total biovolume of the tested species, but resulted in lower F_v/F_m of *N. spumigena*. In other laboratory studies, *N. spumigena* showed higher optimum growth rates at higher salinity levels than *Aphanizomenon* sp. (Lehtimäki et al. 1997; Pliński and Jóźwiak 1999). The response in photosynthetic activity (F_v/F_m) is rapid (minutes) and a treatment effect is detectable over much shorter time scales than e.g. growth. Thus, over a longer experimental period than in our experiment a reduction in photosynthetic activity could have been reflected in lower total biovolume for *N. spumigena*.

Both *Aphanizomenon* sp. and *N. spumigena* had higher biovolumes in treatments where they grew alone compared to when they grew in presence of the other species. Whether this was due to competition for resources (nutrients) or an allelopathic effect is yet to be elucidated. *N. spumigena* produces the hepatotoxin nodularin while *Aphanizomenon* sp. is not toxic in the

Baltic Sea although production of the neurotoxic amino acid BMAA has been detected in isolates of *A. flos-aquae* (Cox et al. 2005). Could the inhibition of filamentous growth be due to the release of extracellular compounds? Suikkanen et al. (2004) found allelopathic effects of *N. spumigena*, *Aphanizomenon flos-aque* and *Anabaena lemmermannii* on the phytoplankton species *Rhodomonas* sp. but not on *Prymnesium parvum*. However, their results further indicated that the release of cyanobacteria toxins could stimulate the abundance of cyanobacteria species in the community rather than inhibiting the abundance of competitors to cyanobacteria (Suikkanen et al. 2004, 2005, 2006). Moreover, from a series of experiments it was recently concluded that the presence of *N. spumigena* had no significant effect on the specific growth rate of *Aphanizomenon* sp. under different radiation and nutrient treatments (Pattanaik et al. 2010; Mohlin et al. 2011). These studies were performed under ambient radiation and nutrient stress conditions, where the environmental conditions overrode the potential allelopathic effects. In a study by Lehtimäki et al. (1994), an increase in temperature resulted in higher intracellular nodularin of *N. spumigena*. In the present study we used the same species as in the studies described above, but we used different strains. Intra-specific variation is the basis for acclimation (plasticity) and adaptation (gene-based) to changing environmental conditions but strain-specific differences are very seldom addressed. However, for *N. spumigena* intra-specific differences have been studied and results from these studies emphasize the need of including strain-specific differences in order to predict effects of climate change (Laamanen et al. 2001; Wulff et al. 2007; Schlüter et al. 2008). For the Baltic Sea population of Apha*nizomenon* sp., it has been shown that the genotype is homogenous (Barker et al. 2000; Laamanen et al. 2002) but for *N. spumigena* the genotype is more variable (Hayes et al. 2002). For future studies, and to be one step closer to predict effects of possible future scenarios, we recommend including several strains of each species, or preferably to study natural communities in out-door experiments.

In our experiment, A_T was generally low but increased slightly during the experiment. To reach salinity 4 and 7, seawater of higher salinity was mixed with Milli-Q water, thus reducing the buffering capacity of the experimental water. Consequently, the A_T of the experimental water differed from that of Baltic seawater of similar salinities. A possible reduction of pH caused by reduced buffering capacity was not expected to affect the cyanobacteria in our experiment. Under natural conditions, due to photosynthesis, autotrophs can be exposed to diurnal pH changes of several units (cf Ploug 2008). Addition of CO_2 does not affect A_T but the exudation of organic substances containing basic functional groups

could explain the observed pattern (cf Kim and Lee 2009). Thus, to better describe the carbonate system other parameters such as dissolved inorganic carbon might be preferred in similar experiments (Gattuso et al. 2010). Diurnal variability in photosynthesis (i.e. carbon uptake) results in large variations in pCO_2 levels. This is commonly observed as large diurnal pH changes in these types of experiments (Wulff et al. unpublished), despite the constant supply with CO_2-enriched air. Our measurements were performed in the middle of the light phase and provide a snapshot of the carbonate system. During the 8 hours darkness pCO_2 in the medium most likely reached similar levels as the commercially prepared air. Large variations of pCO_2 due to the diurnal cycle of primary production have also been observed in coastal surface waters (Borges and Frankignoulle 1999; Fransson et al. 2004). Despite the complexity, maintaining a constant pCO_2 in the medium lacks ecological relevance when performing CO_2 enrichment experiments on primary producers. Therefore, the type of set-up used in this experiment, i.e. bubbling with CO_2 enriched air is preferred (Gattuso et al. 2010; Torstensson et al. 2012).

Marine cyanobacteria show taxon-specific responses to increasing pCO_2 (Fu et al. 2007) but little information is available on Baltic diazotrophic cyanobacteria (for tropical diazotrophic cyanobacteria see Kranz et al. 2010 and references therein). Under natural conditions, pH can vary considerably due to photosynthetic activity, and in a study by Ploug (2008) pH in *N. spumigena* bundles varied between 7.4 and 9.0 between darkness and saturating light intensities. Many marine autotrophs have carbon-concentrating mechanisms (CCM) and under present day pCO_2 levels they do not appear carbon limited (Raven and Beardall 2003). On evolutionary time scales cyanobacteria have been exposed to changing gaseous environments and have evolved highly efficient CCM, concentrating CO_2 up to 1000 times around the carbon-fixing enzyme Rubisco (Badger and Price 2003). Nonetheless, Wulff et al. (unpublished) found that increased pCO_2 had a positive effect on growth when cell densities corresponded to Baltic bloom conditions (compared to lower cell densities), implying that under certain conditions cyanobacteria can be exposed to carbon limitation. In Expt A, *Aphanizomenon* sp. had initially very low F_v/F_m. Such low F_v/F_m values have earlier been observed for *Aphanizomenon* sp. (Vikström, unpublished) and were suggested to be caused by experimental stagnant water conditions. We do not know the direct effects of low dissolved inorganic carbon (DIC) levels on F_v/F_m but in a study by Poza-Carrion et al. (2001) CCM were induced when cells were grown at low or very low (standing cells) DIC concentrations. This led to an elevated photosynthetic affinity for extracellular DIC.

341 Furthermore, standing cells could have resulted in lower F_v/F_m as reported by Roleda et al.
342 (2008) for phosphorus-limited conditions. In several experimental studies using different
343 strains of *Aphanizomenon* sp. and *N. spumigena* (Wulff et al. unpublished), it has been
344 observed that *Aphanizomenon* sp. generally have lower F_v/F_m values compared to *N.*
345 *spumigena*. Similar to results by Johnson et al. (2011), we found no pCO_2 treatment effects
346 on either biovolume or F_v/F_m. Cell densities may have been too low to result in a positive
347 effect of additional CO_2.

348 In summary, our results indicate that the projected business as usual scenario may increase
349 the biovolume of the two species dominating the extensive summer blooms in the Baltic
350 proper. Both *Aphanizomenon* sp. and *N. spumigena* were inhibited by the presence of the
351 other species. Thus, our results further stress the importance of studying interactions between
352 cyanobacteria species, especially at the community level. Clearly, long-term studies together
353 with multifactorial and mesocosm/field experiments are needed to elucidate the future impact
354 of climate change effects on Baltic filamentous cyanobacteria.

355

356 **Acknowledgements**

357 This study was financed by Magnus Bergvall Foundation and Oscar and Lili Lamm
358 Foundation. We thank M. Appelgren, M. Mohlin, A. Torstensson and K. Vikström for
359 assistance in the laboratory. We are grateful to J. Havenhand and an anonymous reviewer for
360 constructive criticism, improving an earlier version of the manuscript.

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Figure legends

Fig. 1 Total biovolume at the end of Expt A for (a) *Aphanizomenon* sp. and (b) *Nodularia spumigena* as well as in Expt B for (c) *Aphanizomenon* sp. and (d) *Nodularia spumigena* in monoculture or in presence of the other species. Temperature treatments are T+ (16°C) and T- (12°C), salinities S+ (salinity 7) and S- (salinity 4), and CO_2 treatments are C+ (960 ppm) and C- (380 ppm). Error bars show standard error (n=3)

Fig. 2 Maximum photochemical yield (F_v/F_m) at the end of the experiments for *Aphanizomenon* sp. and *Nodularia spumigena* in (a) Expt A and (b) Expt B. Temperature treatments are T+ (16°C) and T- (12°C), salinities S+ (salinity 7) and S- (salinity 4), and CO_2 treatments are C+ (960 ppm) and C- (380 ppm). Error bars show standard error (n=3 in Expt A and n=4 in Expt B)