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Key role of phosphorus and nitrogen in regulating cyanobacterial community composition in the northern Baltic Sea

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ABSTRACT

Despite cyanobacteria being a key phytoplankton group in the Baltic Sea, the factors governing their community structure are still poorly understood. Here, we studied the occurrence of the orders Chroococcales, Oscillatoriales and Nostocales, and potentially explanatory variables at five locations in the northern Baltic Sea from June–September, 1998–2012. Cyanobacteria constituted 1–36% of the total phytoplankton biomass along the north–south gradient. In the Bothnian Bay, Chroococcales and Oscillatoriales dominated the cyanobacterial community, whereas in the Bothnian Sea and northern Baltic Proper, Nostocales was the dominant group. The dominance of Chroococcales was coupled to low salinity and low total phosphorus, whereas Oscillatoriales correlated with high total nitrogen and low salinity. Nostocales correlated to high total phosphorus, inorganic phosphorus and salinity. Chroococcales showed an increase over time in the offshore Bothnian Bay, whereas Nostocales increased in the coastal Bothnian Sea and coastal Baltic Proper. The increase of Nostocales in the coastal Bothnian Sea was explained by a rise in total phosphorus and decrease in dissolved inorganic nitrogen compared to an increase of total nitrogen and phosphorus in the coastal Baltic Proper. No significant trends were observed in the cyanobacterial community in the offshore Bothnian Sea and the offshore northern Baltic Proper. We concluded that Chroococcales may be a useful indicator for increased phosphorus levels in waters with low phosphorus concentrations, whereas Nostocales could be used as a quality indicator for increasing phosphorus concentrations in waters with low inorganic N/P ratios (<20), such as in the coastal Bothnian Sea and Baltic Proper.

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1. Introduction

Cyanobacteria are an important component of the phytoplankton community in aquatic systems, especially in nutrient rich lakes and brackish waters (Andersson et al., 1996; Pearl and Huisman, 2009; Pearl and Otten, 2013). Many cyanobacteria can produce toxins that are harmful to other aquatic organisms and also humans (Pearl and Huisman, 2009). They are also less edible and of lower food quality than many other phytoplankton owing to their poor lipid composition and toxicity (e.g., Nordbäck et al., 1998; Larsson et al., 2000; Elliott, 2012). Certain cyanobacterial species or groups are selectively promoted with increasing nutrient load

(Carstensen and Heiskanen, 2007; Jaanus et al., 2009), and thus cyanobacteria may serve as useful indicators of the ecological state of seawater (Gasiunaite et al., 2005; Carstensen and Heiskanen, 2007; Jaanus et al., 2009).

Cyanobacteria are divided into three main orders: Chroococcales, which includes coccoid unicellular or colony-forming cells, Nostocales, which comprises filamentous forms with ability to fix nitrogen as N₂ via heterocysts, and Oscillatoriales, which encompasses filamentous types which lack heterocysts (Tikkanen and Willén, 1992). These three orders show large size differences. The Chroococcales have the smallest cell sizes, ranging from ~1 to 5 µm, and thus have a competitive advantage at relatively low nutrient concentrations (Legendre and Rassoulzadegan, 1995). Nostocales, which consists of relatively large filaments (>100 µm), have the ability to utilize atmospheric nitrogen for growth and are thus selectively promoted by high phosphorus (P) – low nitrogen (N) conditions (Suikkanen et al., 2013). The Oscillatoriales species,

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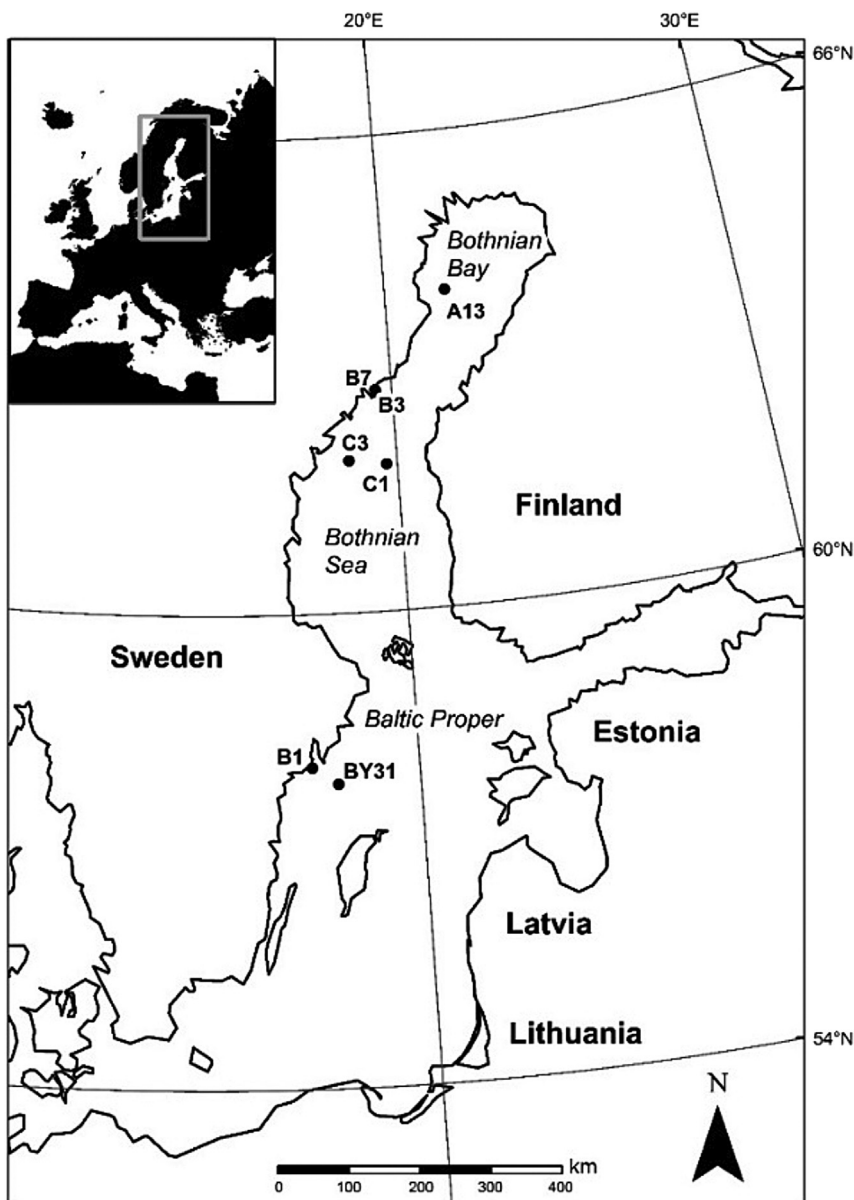


Fig. 1. Study sites in the northern Baltic Sea: A13 offshore Bothnian Bay, B3/B7 coastal Bothnian Sea, C1/C3 offshore Bothnian Sea, B1 coastal Baltic Proper and BY31 offshore northern Baltic Proper. Samples were taken from the photic zone during the summer (June–September) for the period 1998–2012.

occurring in the Baltic Sea are not known to perform nitrogen-fixation, and thus are favored by both high nitrogen and phosphorus concentrations.

Apart from the influence of nutrients, factors such as temperature, salinity, seawater stratification and upwelling events can have a direct or indirect influence on the occurrence of cyanobacteria and community composition (Kononen et al., 1996; Vahtera et al., 2005; Müren et al., 2005; Pearl and Huisman, 2009). Cyanobacteria have been shown to have higher temperature growth optima than many other phytoplankton groups, i.e., diatoms (Pearl and Huisman, 2009). High sulfate concentrations can inhibit nitrogenase activity in nitrogen-fixing cyanobacteria (Stal et al., 2003). As sulfate concentrations are high in seawater, high salinity has been hypothesized to decrease growth of nitrogen-fixing cyanobacteria. As a consequence, Nostocales may be promoted in brackish or low saline waters (Stal et al., 2003). Nutrient pulsing, for example induced by upwelling events or wind-induced vertical mixing, has been shown to induce blooms of filamentous

cyanobacteria (Kononen et al., 1996; Vahtera et al., 2005). On the other hand, stratification favors many cyanobacterial species because they employ gas vacuoles to regulate their buoyancy in the water mass (e.g., Walsby et al., 1997).

In the semi-enclosed and brackish Baltic Sea, summer blooms of cyanobacteria are a recurrent and frequent phenomenon which has been attributed to high nutrient load and eutrophication (Suikkanen et al., 2007). The nutrient load is partly an internal phenomenon, as the widely distributed anoxic bottoms in the Baltic Proper cause release of phosphorus from the benthos, which nurture nitrogen-fixing cyanobacteria (Vahtera et al., 2007). Due to both physical and biological processes, climate warming is expected to lead to larger volumes of anoxic water in the Baltic Proper, which in turn will cause further release of phosphorus and intensified cyanobacterial blooms (Funkey et al., 2014; Andersson et al., 2015).

The cyanobacterial biomass concentration during the summer has been shown to be lower in northernmost basin of the Baltic Sea, the Bothnian Bay, compared to that in the more southerly Gulf of Finland

(Jaanus et al., 2011). However, during the past 20 years, cyanobacterial biomass concentrations have been found to increase at several localities along a north–south gradient in the Baltic Sea (Jaanus et al., 2011). Correlative data indicate that the variation in the total number of cyanobacteria is mainly explained by variations in temperature, salinity and phosphorus (Jaanus et al., 2011). However, the composition of the cyanobacterial community in different areas of the Baltic has not yet been described and the factors governing the occurrence of different functional groups are still not fully understood.

The aim of the present study was to investigate the spatial variation of the cyanobacterial community structure at coastal and offshore areas in the northern Baltic Sea and to elucidate what factors may cause community variations. Furthermore, we wanted to identify possible upward or downward trends for different functional groups during the past 15 years as well as the mechanisms behind the changes. We included the three different orders of cyanobacteria in the study, i.e., Chroococcales, Oscillatoriales and Nostocales, as they possess different functional traits. This was important because environmental changes may affect the functional groups in different ways. Furthermore, it is not known whether environmental changes cause gradual community changes to occur or if threshold values exist. We hypothesized that Chroococcales and Oscillatoriales would be more abundant in areas with low nutrient concentrations owing to their small size and thus large surface to volume ratio, whereas Nostocales would be favored by high phosphorus concentrations and low concentrations of dissolved inorganic nitrogen owing to their relatively large size and ability to fix nitrogen from the atmosphere.

2. Materials and methods

2.1. Dataset

The study was based on 15 years of Swedish national monitoring data (1998–2012) collected at five different sites in the northern Baltic Sea (Fig. 1, Suppl. Table 1): Station A13 in the offshore Bothnian Bay, station B3 and B7 in the coastal Bothnian Sea (stations closely positioned, data pooled = B3/B7), station C1 and C3 in the offshore Bothnian Sea (stations relatively closely positioned, data pooled = C1/C3), station B1 in the coastal northern Baltic Proper, and station BY31 in the offshore northern Baltic Proper. Data were retrieved from the database dBotnia at Umeå Marine Sciences Centre and from the Marine Ecological laboratory at the Department of Ecology, Environment and Plant Sciences, Stockholm University. Since cyanobacteria occur most abundantly during summer, the seasonal period June–September was selected for the study.

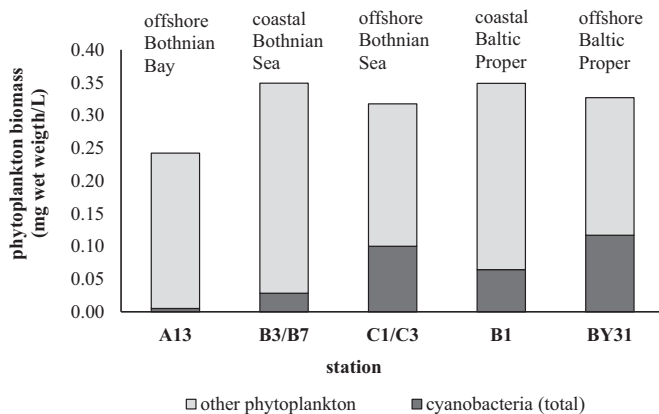


Fig. 2. Average phytoplankton and cyanobacterial biomasses at different study locations during the period 1998–2012.

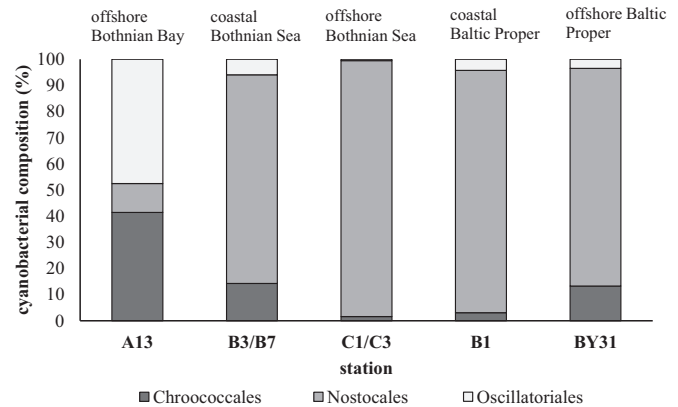


Fig. 3. Proportion of Nostocales, Oscillatoriales and Chroococcales in the total cyanobacterial biomass (wet weight) at different sites, mean for the entire study period (June–September, 1998–2012).

2.2. Sampling procedure and data handling

In the Gulf of Bothnia (Bothnian Bay + Bothnian Sea), 1–3 samplings per month were performed during at least 3 out of the 4 months of investigation (June–September). In the Baltic Proper, 1–3 (mostly 2) samplings per month were performed during June–September. The phytoplankton samples were collected using a plastic hose at depths of 0–10 m in the Gulf of Bothnia and 0–20 m in the Baltic Proper. Different depths were sampled since the photic zone is shallower in the Gulf of Bothnia than in the Baltic Proper.

Phytoplankton samples were preserved with acidic Lugol's solution and analyzed using the Utermöhl technique (HELCOM, 2014). Biovolume was calculated according to Olenina et al. (2006) and presented as wet weight (mg ww/L) (assuming a density of 1 g/cm³). Regarding cyanobacteria, the study included all Nostocales and Oscillatoriales and the colony-forming Chroococcales. However, unicellular picocyanobacteria were not included in the analysis as they cannot be identified and quantified by the Utermöhl technique.

Temperature and salinity (using practical salinity scale) were measured using CTD probes (Gulf of Bothnia; SeaBird, USA, Baltic Proper; Meerestechnik Elektronik GmbH, Germany). Samples for measurement of dissolved inorganic nitrogen (DIN), dissolved inorganic phosphorus (DIP), total nitrogen (Tot N) and total phosphorus (Tot P) were collected from surface water and at 5 m and 10 m depths. DIN and DIP were analyzed by flow injection analysis and segmented continuous flow analysis (Gulf of Bothnia: Traacs 800 system, Bran & Luebbe and Quattro system, Seal Analytical; Baltic Proper: QuikChem[®] 8000, Lachat Instruments and AlpkemFlow Solution IV, OI Analytical) following methods outlined in Grasshoff et al. (1983) and Helcom guidelines (HELCOM, 2014). Tot N and Tot P samples were oxidized using a modified method based on Koroleff (1983) and then analyzed using the same methods as for DIP and NO₂⁻+NO₃⁻. Most of the physicochemical variables were measured at all stations. However, DIN and DIP were analyzed only at the coastal stations in Bothnian Sea (B3 and B7) and the stations in the Baltic proper (B1 and BY31). Average values were calculated for depths of 0–10 m in the Gulf of Bothnia as well as in the northern Baltic Proper.

Monthly average values were calculated from the different sampling occasions and average annual summer values (June–September) were determined. Standard deviations for annual summer values were calculated from monthly averages.

2.3. Statistical analyses

Differences in summer mean values of physicochemical and cyanobacterial variables between the different study sites were

Table 1
Species/genera composition in the three different cyanobacterial orders at different stations in the northern Baltic Sea. Average % of cyanobacterial biomass for the period June–September 1998–2012 is presented.

Station	Chroococcales	%	Nostocales	%	Oscillatoriales	%
Offshore Bothnian Bay (A13)	<i>Microcystis</i> spp.	19	<i>Aphanizomenon</i> sp.	50	cf <i>Romeria</i> spp.	95
	<i>Cyanodictyon</i> spp.	32	<i>Dolichospermum</i> spp.	39	<i>Planktothrix agardhii</i>	5
	<i>Merismopedia</i> spp.	13	<i>Nodularia spumigena</i>	11		
	<i>Aphanothece</i> spp.	10				
	Chroococcales spp.	9				
	<i>Woronichinia</i> spp.	8				
	<i>Lemmermaniella</i> spp.	4				
	<i>Snowella</i> spp.	3				
	<i>Aphanocapsa</i> spp.	1				
	<i>Chroococcus</i> spp.	1				
Coastal Bothnian Sea (B3/B7)	Chroococcales spp.	26	<i>Aphanizomenon</i> spp.	94	cf <i>Romeria</i> spp.	94
	<i>Merismopedia tenuissima</i>	26	<i>Nodularia spumigena</i>	3	<i>Planktothrix agardhii</i>	5
	<i>Woronichinia</i> spp.	17	<i>Dolichospermum</i> spp.	3	<i>Pseudanabaena</i> spp.	1
	<i>Aphanothece</i> spp.	12				
	<i>Cyanodictyon</i> spp.	5				
	<i>Snowella</i> spp.	4				
	<i>Microcystis viridis</i>	3				
	<i>Lemmermaniella</i> spp.	3				
	<i>Chroococcus</i> spp.	2				
	<i>Aphanocapsa</i> spp.	2				
Offshore Bothnian Sea (C1/C3)	Chroococcales spp.	57	<i>Aphanizomenon</i> spp.	82	cf <i>Romeria</i> spp.	100
	<i>Cyanodictyon</i> spp.	13	<i>Nodularia spumigena</i>	16		
	<i>Microcystis</i> spp.	13	<i>Dolichospermum</i> spp.	2		
	<i>Woronichinia</i> spp.	6				
	<i>Merismopedia</i> spp.	3				
	<i>Aphanocapsa</i> spp.	3				
	<i>Aphanothece</i> spp.	3				
	<i>Snowella</i> spp.	2				
Coastal N Baltic Proper (B1)	cf <i>Cyanodictyon</i> spp.	84	<i>Aphanizomenon</i> sp.	82	cf <i>Pseudanabaena</i> spp.	100
	<i>Woronichinia</i> spp.	12	<i>Nodularia spumigena</i>	9		
	<i>Aphanothece paralleliformis</i>	3	<i>Dolichospermum</i> spp.	9		
Offshore N Baltic Proper (BY31)	cf <i>Cyanodictyon</i> spp.	88	<i>Aphanizomenon</i> sp.	78	cf <i>Pseudanabaena</i> spp.	100
	<i>Aphanothece paralleliformis</i>	8	<i>Nodularia spumigena</i>	15		
	<i>Woronichinia</i> spp.	4	<i>Dolichospermum</i> spp.	6		
	<i>Snowella</i> spp.	1	<i>Aphanizomenon gracile</i>	1		

tested on ln-transformed data using analysis of variance (1-way ANOVA, $n = 12–15$). Each variable was tested separately and the identity of the station was set as a fixed factor. Different years were treated as replicates. Tukey's HSD post hoc test (Tukey's honestly significant different post hoc test) was used to detect possible statistically significant differences between specific stations. If homogeneity of variances were not obtained (tested with Levene test of homogeneity of variances), the non-parametric Kruskal–Wallis and Mann Whitney U-test were used.

Large-scale relationships between cyanobacteria (total biomass, different classes and proportion of different classes) and physicochemical variables (Tot P, Tot N, salinity, and temperature) were analyzed using stepwise linear regression. Ln-transformed data from all stations were included in the analysis ($n = 65–68$). Linearity of the data was verified using Q–Q plots. The relationship between Tot P and salinity was analyzed using linear regression, including data from all sampling stations ($n = 75$).

Upward or downward time trends of different cyanobacterial groups and physicochemical variables at the different study sites were tested using linear regressions ($n = 12–15$), with year as

independent factor. The relationship between different cyanobacterial groups and physicochemical factors within a given station was analyzed using stepwise linear regression. Potential predictors were Tot N, Tot P, DIN, DIP, salinity and temperature for the coastal stations in the Bothnian Sea (B3/B7) and the coastal and offshore stations in the northern Baltic Proper (B1 and BY31), and Tot N, Tot P, salinity and temperature for the offshore stations in the Bothnian Bay and Bothnian Sea (A13 and C1/C3).

For all statistical analyses, IBM SPSS data statistics 22 was used and a significant level of 0.05 applied.

3. Results

3.1. Variation of cyanobacterial communities along the north–south gradient

Total phytoplankton biomasses were relatively similar at the different study sites (Fig. 2), with average biomasses ranging between 0.24 and 0.35 mg wet weight/L. However, cyanobacteria constituted only a minor part (~2%) of the phytoplankton biomass

Table 2
Average summer values (June–September) of physicochemical variables in the photic zone at the sampled stations in the northern Baltic Sea during the period 1998–2012. \pm values denote one standard deviation.

Station	Salinity	Temp (°C)	Tot N ($\mu\text{mol/L}$)	Tot P ($\mu\text{mol/L}$)	DIN ($\mu\text{mol/L}$)	DIP ($\mu\text{mol/L}$)	DIN/DIP (molar ratio)
OffshoreBothnian Bay (A13)	2.9 \pm 0.1	11.0 \pm 1.4	17.3 \pm 0.9	0.2 \pm 0.03			
Coastal Bothnian Sea (B3/B7)	3.6 \pm 0.2	13.2 \pm 1.4	17.0 \pm 0.7	0.3 \pm 0.05	1.2 \pm 0.6	0.05 \pm 0.03	28.3 \pm 15.0
Offshore Bothnian Sea (C1/C3)	4.9 \pm 0.2	12.9 \pm 1.4	16.3 \pm 0.6	0.3 \pm 0.04			
Coastal N Baltic Proper (B1)	6.1 \pm 0.1	14.3 \pm 1.0	20.9 \pm 0.8	0.6 \pm 0.07	0.2 \pm 0.1	0.1 \pm 0.06	2.2 \pm 1.5
Offshore N Baltic Proper (BY31)	6.2 \pm 0.2	14.6 \pm 1.3	20.3 \pm 0.7	0.5 \pm 0.06	0.1 \pm 0.02	0.05 \pm 0.03	2.6 \pm 1.4

Table 3

Large-scale relationship between the biomass of different cyanobacterial groups and physicochemical variables in the northern Baltic Sea, analyzed by stepwise linear regression. Ln transformed data from all stations were included in the analysis.

Dependent variable	Model Adj. R Square	Model significance	Predictor priority	Predictor	Beta/Slope	Predictor significance
Tot. Cyanob. biomass	0.70	<0.001	1	Salinity	+0.84	<0.001
	0.63		2	Tot N	-0.29	0.002
	0.67		3	Temperature	+0.24	0.008
Tot. Cyanob./Tot. Autotr.	0.75	<0.001	1	Salinity	+0.98	<0.001
	0.66		2	Tot N	-0.40	<0.001
	0.74		3	Temperature	+0.16	0.045
Nostocales biomass	0.78	<0.001	1	Salinity	+0.68	<0.001
	0.63		2	Tot N	-0.63	<0.001
	0.71		3	Tot P	+0.64	<0.001
Oscillatoriales biomass	0.05	0.043	1	Tot N	+0.25	0.043
	0.64		2	Salinity	+0.41	0.010
	0.43		3	Tot N	-0.75	<0.001
Nostocales/Tot. Cyanob.	0.51	<0.001	1	Salinity	+0.41	0.010
	0.64		2	Tot N	-0.75	<0.001
	0.35		3	Tot P	+0.90	<0.001
Chroococcales/Tot. Cyanob.	0.35	<0.001	1	Salinity	-0.77	<0.001
	0.31		2	Tot N	+0.30	0.033
	0.35		3	Salinity	-0.87	<0.001
Oscillatoriales/Tot. Cyanob.	0.37	<0.001	1	Salinity	-0.87	<0.001
	0.24		2	Tot N	+0.53	<0.001
	0.37		3	Tot N	+0.53	<0.001

in the Bothnian Bay (Fig. 2, Suppl. Table 1), whereas their contribution to the total phytoplankton biomass increased towards the south, reaching ~36% in the offshore northern Baltic Proper.

Total cyanobacterial biomass was lower in the Bothnian Bay

than in the areas further south (Mann–Whitney, all $p < 0.001$). The composition of the cyanobacterial community was also quite different in the Bothnian Bay compared to the more southerly study sites (Fig. 3, Suppl. Table 2). In the Bothnian Bay, Oscillatoriales and

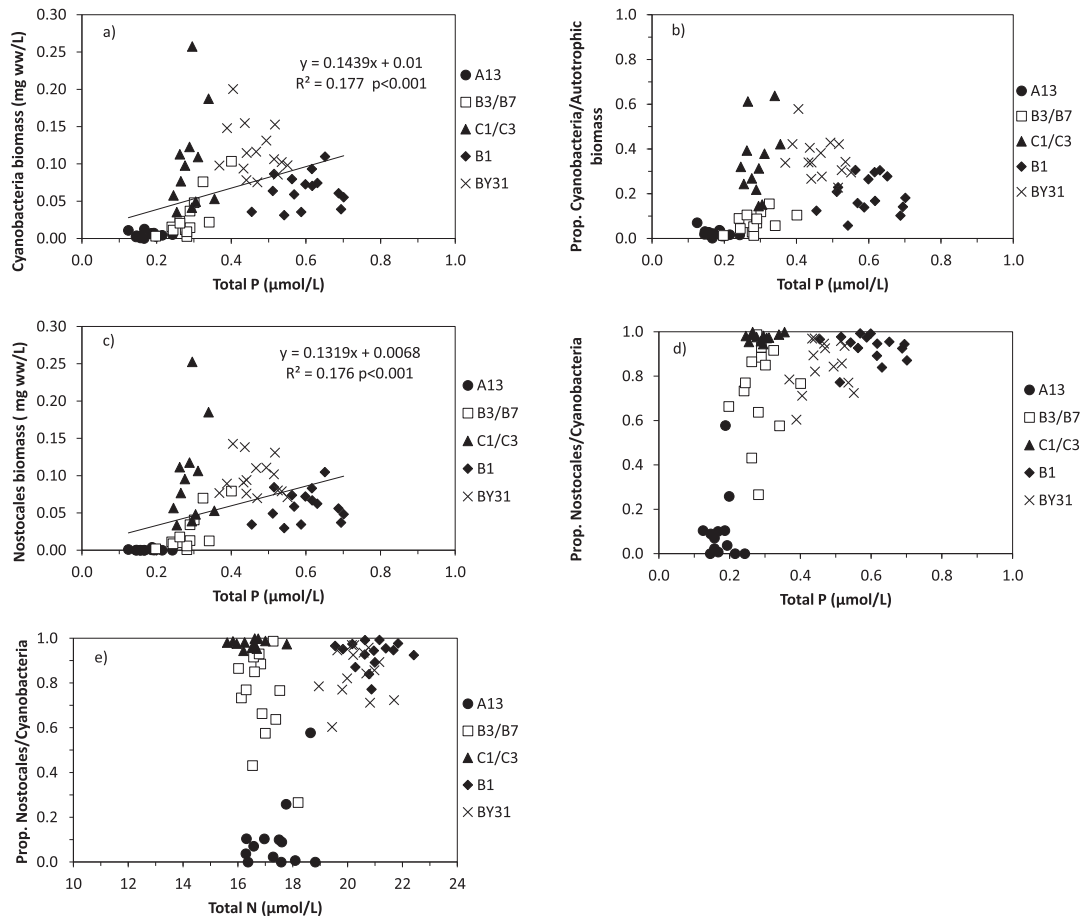


Fig. 4. Relationship between concentration of Tot P and a) cyanobacterial biomass, b) proportion of cyanobacteria in the phytoplankton community, c) Nostocales biomass, and d) proportion of Nostocales in the cyanobacterial community. e) Relationship between proportion of Nostocales in the cyanobacterial community and concentration of Tot N.

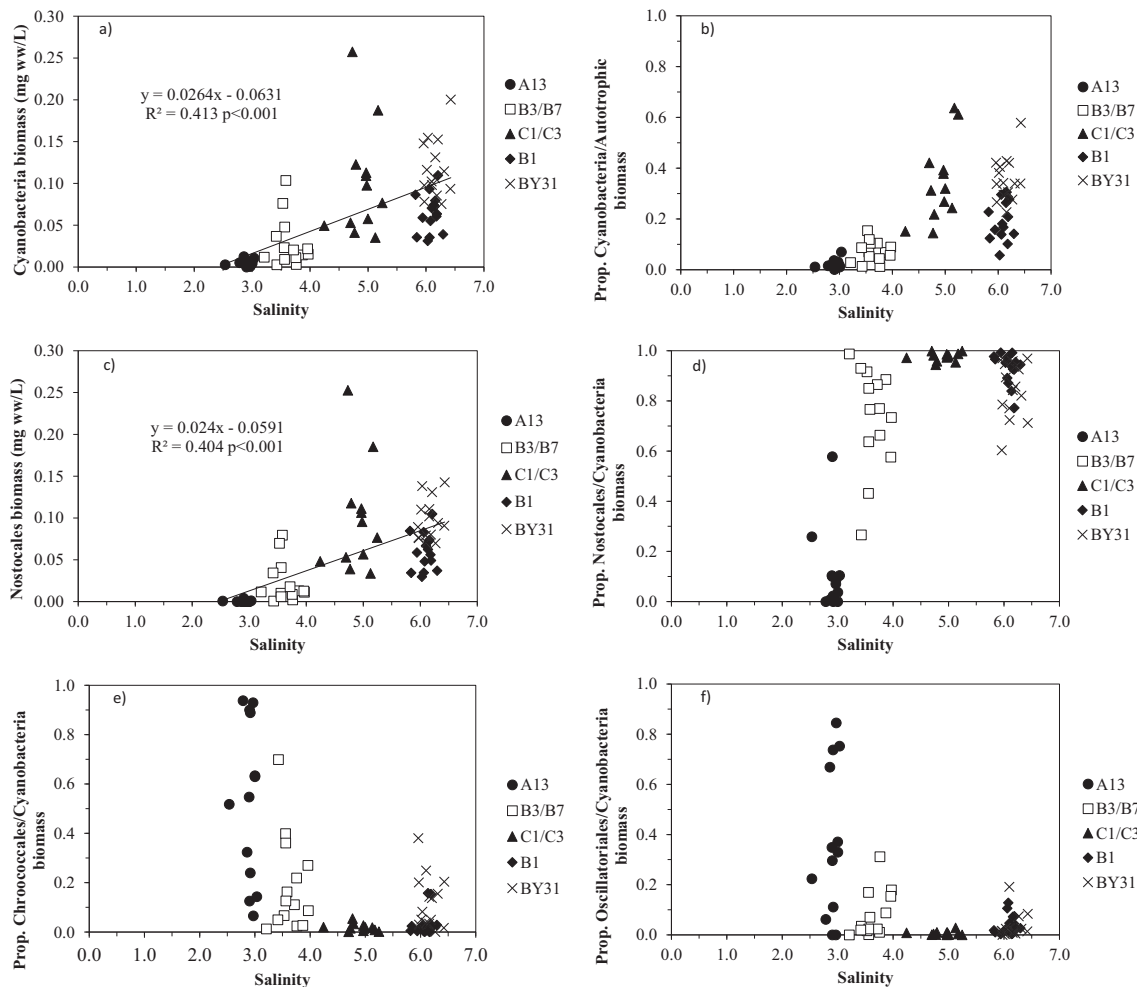


Fig. 5. Relationship between salinity and a) total cyanobacterial biomass, b) proportion of cyanobacteria in the phytoplankton community, c) Nostocales biomass, and d,e,f) proportion of Nostocales, Chroococcales and Oscillatoriales, respectively, in the cyanobacterial community.

Chroococcales constituted 37 and 53% of the cyanobacterial biomass, respectively, whereas Nostocales only made up 11%. Further south, Nostocales dominated the cyanobacterial communities, constituting on average >80% of the cyanobacteria biomass. In contrast, Oscillatoriales and Chroococcales contributed only a minor fraction of the cyanobacterial community biomass in the most southerly locations.

The biomass of Chroococcales was significantly higher in the offshore Baltic Proper compared to the other stations (Mann–Whitney, all $p = 0.001–0.018$). However, the proportion of Chroococcales in the total cyanobacterial biomass was highest in the Bothnian Bay (53%), intermediate at the coastal Bothnian Sea (19%) and the offshore Baltic Proper (11%) and lowest at the other areas (2–3%). In the Gulf of Bothnia, a wide variety of genera were common among the Chroococcales, e.g., *Microcystis*, *Cyanodictyon* and *Merismopedia* (Table 1). In the Baltic Proper, the genus *Cyanodictyon* was dominant, constituting on average 85–90% of the Chroococcales biomass (Table 1). Other common genera were *Woronichinia*, *Microcystis* and *Aphanothece*.

The biomass of Nostocales and the proportion of Nostocales in the cyanobacterial community were significantly lower in the Bothnian Bay and coastal Bothnian Sea compared to all other areas (Mann–Whitney, all $p < 0.001$, Suppl. Table 2). The genus *Aphanizomenon* constituted on average 80–90% of the Nostocales biomass at all stations except the Bothnian Bay station, where this genus

made up on average ~50% (Table 1). Other commonly occurring genera were *Nodularia* and *Dolichospermum*.

The biomass of Oscillatoriales was relatively similar at many of the studied stations. However, the station that differed most was the offshore Bothnian Sea, which showed a significantly lower biomass than the Bothnian Bay and Baltic Proper stations (Tukey HSD, $p = 0.02–0.04$, Suppl. Table 2). The proportion of Oscillatoriales in the cyanobacterial community was higher in the Bothnian Bay (37%) compared to all other stations (Tukey HSD, $p = 0.001–0.003$). The order Oscillatoriales was dominated by *Romeria* in the northern Gulf of Bothnia and by *Pseudanabaena* in the Baltic Proper (Table 1).

3.2. Variation of physicochemical variables along the north–south gradient

The physicochemical variables showed clear differences from north to south. Salinity increased two-fold along the gradient; the lowest salinity (2.9) was observed in the Bothnian Bay and the highest (6.2) in the offshore Baltic Proper (Table 2). In the Bothnian Bay, the temperature was on average ~11 °C, whereas further south, it was ~14 °C (Table 2). The relatively low temperature in the Bothnian Bay differed from the other stations (Tukey HSD; all $p < 0.002$).

The highest concentration of Tot N was recorded at the Baltic

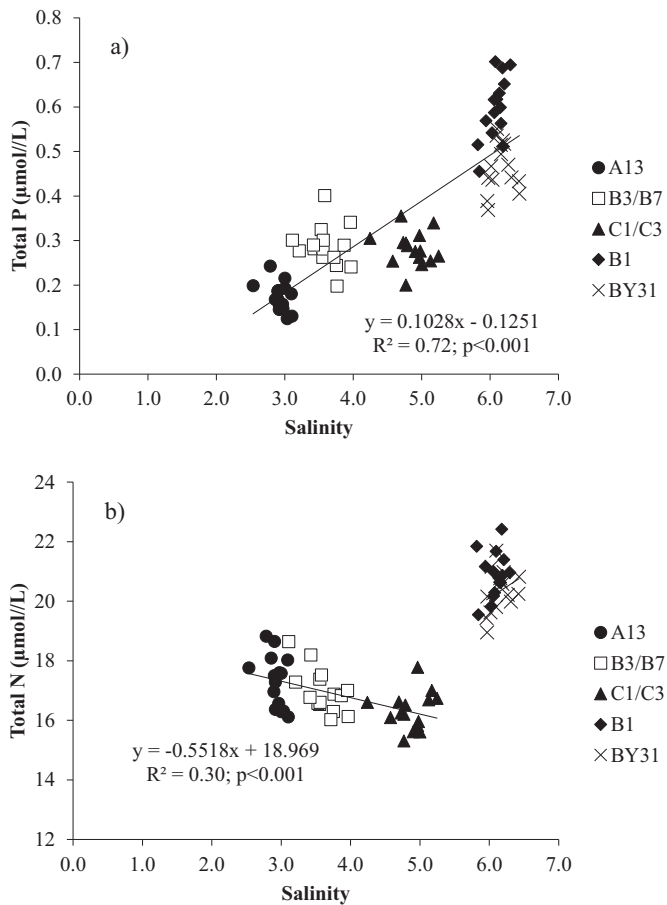


Fig. 6. Relationship between a) Tot P concentrations and salinity (including trend line for all stations), and b) Tot N and salinity (including trend line only for Gulf of Bothnia stations).

Proper stations and lowest in the offshore Bothnian Sea (Table 2). The Tot P concentration increased three fold from north to south (Table 2). The highest DIN concentration was observed in the coastal Bothnian Sea and the lowest in the offshore Baltic Proper (Table 2). The DIP concentration was higher at the coastal Baltic Proper station (0.2 µmol/L) than in the offshore Baltic Proper and coastal Bothnian Sea (Tukey HSD; both $p < 0.001$). The molar ratio between DIN and DIP was significantly higher in the Bothnian Sea coast than in the Baltic Proper (Tukey HSD; both $p < 0.001$), which can be explained by both a higher DIN concentration and a lower DIP concentration in the Bothnian Sea (Table 2).

3.3. Environmental factors shaping the cyanobacterial community

The “large-scale” analysis, including data from all stations, showed that salinity and Tot P concentrations were the major “positive” driving factors governing the biomass of Nostocales (Table 3, Figs. 4 and 5). From the data included in the study, it is difficult to ascertain which of these factors was most important in shaping the cyanobacterial community, since, for example, salinity and Tot P showed a high co-variance in the northern Baltic Sea (Fig. 6, linear regression, $r^2 = 0.72$, $p = 0.001$). In the Gulf of Bothnia, there was a negative relationship between Tot N concentration and salinity (Fig. 6, linear regression, $r^2 = 0.30$, $p < 0.001$), which was not found in the Baltic Proper. Negative relationships between Tot N and Nostocales and between Tot N and total cyanobacteria biomass were observed (Table 3) when using data from all stations.

The proportion of Oscillatoriales and Chroococcales in the cyanobacterial community showed a negative relationship with salinity (Table 3, Fig. 5). Oscillatoriales displayed a positive relationship with high Tot N concentrations. The proportion of Nostocales in the cyanobacterial community showed a positive relationship with salinity and Tot P concentration (Table 3) and they dominated the cyanobacteria community when Tot P concentrations exceeded 0.25 µmol/L (Fig. 4). The proportion of Nostocales had a negative relationship with Tot N when all stations were included in the analysis (Table 3). However this relationship was mainly found because of the conditions in the Gulf of Bothnia (Fig. 4).

3.4. Temporal trends of cyanobacteria and underlying causative factors

Temporal trends in the total cyanobacteria biomass were only found in the coastal Bothnian Sea, where the biomass significantly increased during the period 1998–2012 (linear regression, $r^2 = 0.36$, $p = 0.023$). However, temporal trends for the different cyanobacterial orders were found at three study sites (Fig. 7). The order Chroococcales in the Bothnian Bay and Nostocales in the coastal Bothnian Sea and coastal Baltic Proper all showed significant increases during the study period (linear regression, all $p < 0.05$) (Fig. 7). Increased cyanobacterial biomasses were thus observed in all three Baltic Sea basins, although no temporal trend could be found in the offshore Bothnian Sea and Baltic Proper.

In the Bothnian Bay, Chroococcales was observed to increase during the period 2002–2012 (linear regression, $r^2 = 0.42$, $p = 0.03$) (Fig. 7). During the same period, Tot P showed a slight increase (linear regression, $r^2 = 0.67$, $p = 0.002$) (Fig. 8a), but linear regression analysis did not reveal any significant relationship between the two factors.

In the coastal Bothnian Sea, the biomass concentration of the nitrogen-fixing cyanobacteria Nostocales increased during the period 1998–2012 (linear regression, $r^2 = 0.39$, $p = 0.016$) (Fig. 7). This was accompanied by a tendency for increased Tot P (linear regression, $r^2 = 0.23$, $p = 0.066$) (Fig. 8f), decreased DIN concentrations (linear regression, $r^2 = 0.70$, $p < 0.001$) (Fig. 8b) and decreased DIN/DIP ratio (linear regression, $r^2 = 0.40$, $p = 0.012$) (Fig. 8c). Linear regression analysis indicated that 65% of the Nostocales increase could be explained by decreasing DIN/DIP ratio (linear regression, $r^2 = 0.65$; slope = -0.80 ; $p = 0.001$).

No trends in the cyanobacteria community were found at the offshore Bothnian Sea station. However, there was a tendency for increased Tot P (linear regression, $r^2 = 0.21$, $p = 0.086$) (Fig. 8e).

In the coastal Baltic Proper, the Nostocales showed a significant increase from 1998 to 2012 (linear regression, $r^2 = 0.27$, $p = 0.046$) (Fig. 7), as did Tot P (linear regression, $r^2 = 0.29$, $p = 0.039$) and Tot N (linear regression, $r^2 = 0.34$, $p = 0.023$) (Fig. 8a and d), while DIP showed only a tendency to increase (linear regression, $r^2 = 0.22$, $p = 0.08$) (Fig. 8g). Linear regression showed that Nostocales and Tot N had a positive relation at this station ($r^2 = 0.32$, slope = $+0.57$; $p = 0.028$).

No trends in the cyanobacteria community were found at the offshore Baltic Proper station. However, the Tot N concentration at depths of 0–10 m increased significantly from 1998 to 2012 (linear regression, $r^2 = 0.32$, $p = 0.028$) (Fig. 8d) and there was a tendency to increase in Tot P (linear regression, $r^2 = 0.26$, $p = 0.054$) (Fig. 8e).

4. Discussion

The results from this study indicate that salinity, phosphorus, nitrogen and temperature are all key factors governing the occurrence of cyanobacteria and different cyanobacterial groups in the

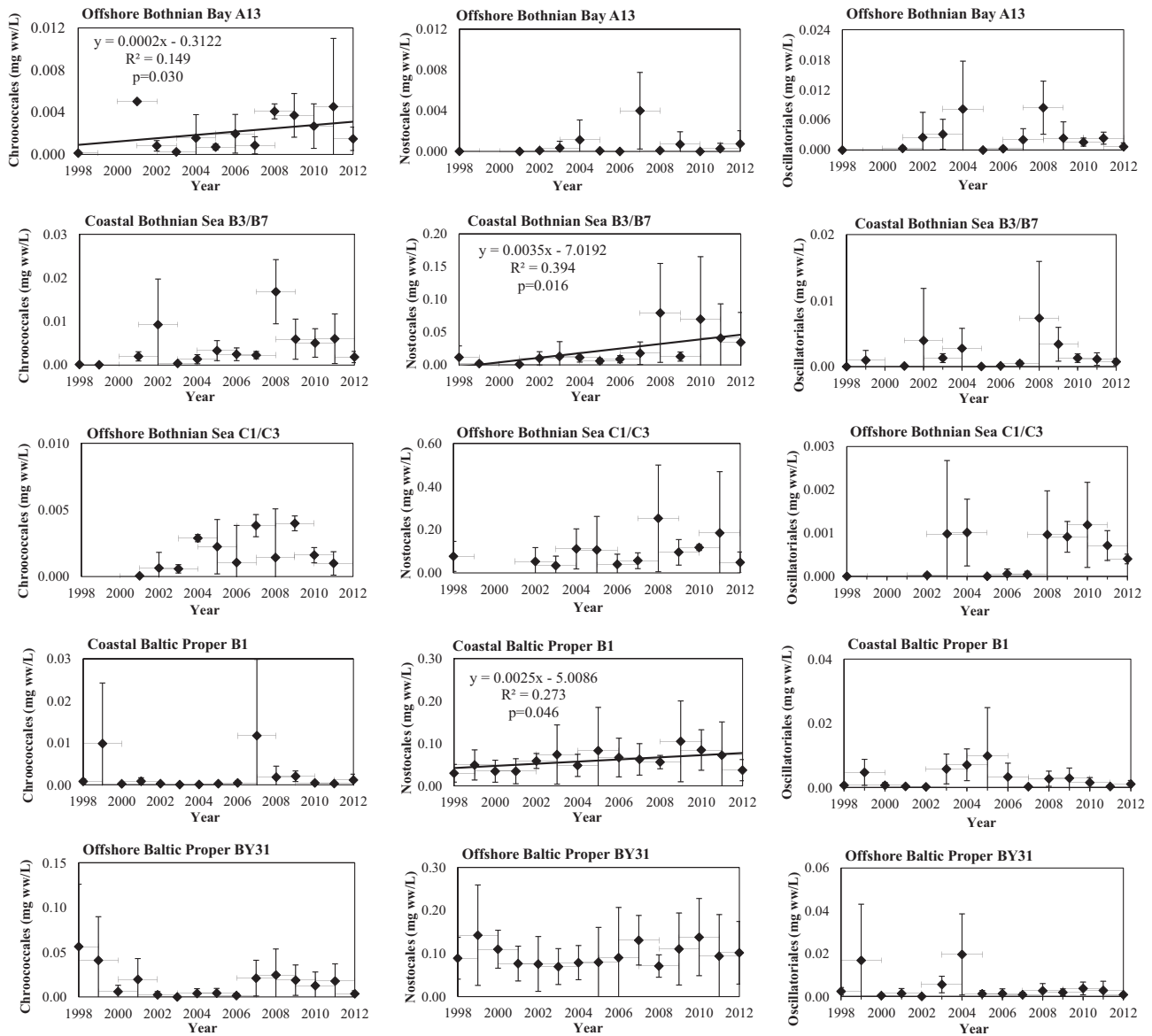


Fig. 7. Summer (June–September) biomasses of Nostocales, Oscillatoriales and Chroococcales (except picocyanobacteria) at different study locations during the period 1998–2012. Significant trends are denoted by trend lines and linear equations.

northern Baltic Sea. Tot N and Tot P were here used as a proxy for the carrying capacity of the system. The basis for this assumption was that in a nutrient constrained environment, like during the summer season in the northern Baltic Sea, inorganic nutrients are rapidly (within hours) assimilated by the organisms. Thus, assuming a significant turnover of N and P in the system, the Tot N and Tot P concentrations would reflect nutrients available to the planktonic organisms. The cyanobacterial biomass and Tot P concentrations increased 6 and 3 times, respectively, from the Bothnian Bay to the offshore Baltic Proper. However, salinity was found to explain most of the variation in the total cyanobacterial biomass. Both the total cyanobacteria biomass and the order Nostocales were positively related to higher temperature.

The observed north–south pattern in total cyanobacterial biomass are in agreement with a previous gradient study (Jaanus et al., 2011). However, we found that the community composition of the total cyanobacterial biomass was fairly different in the northernmost basin (Bothnian Bay) compared to the basins further

south. In the Bothnian Bay, colony-forming Chroococcales and the Oscillatoriales dominated, whereas the Nostocales constituted only a minor part. The order Chroococcales comprises species with small cell sizes and the genus *Cyanodictyon*, which consists of loosely aggregated cells with a cell size of $\sim 1 \mu\text{m}$, was commonly a significant contributor to the cyanobacterial biomass in the Bothnian Bay (Table 2). Due to the large surface to volume ratio of the Chroococcales, this group is likely to be dominant in environments with low nutrient concentrations (Raven, 1998), as also observed in this study. In the offshore Bothnian Bay, the DIP concentrations are very low and the molecular DIN to DIP ratios extremely high, >250 (Andersson et al., 1996), showing that this sea area is strongly limited by DIP. In the Baltic Proper, colony-forming Chroococcales co-occur with nitrogen-fixing Nostocales (Hajdu et al., 2007). It has been suggested that Chroococcales can utilize nitrogen released from the nitrogen-fixing species, which may explain their peak in the Baltic Proper even though the availability of inorganic nitrogen was low.

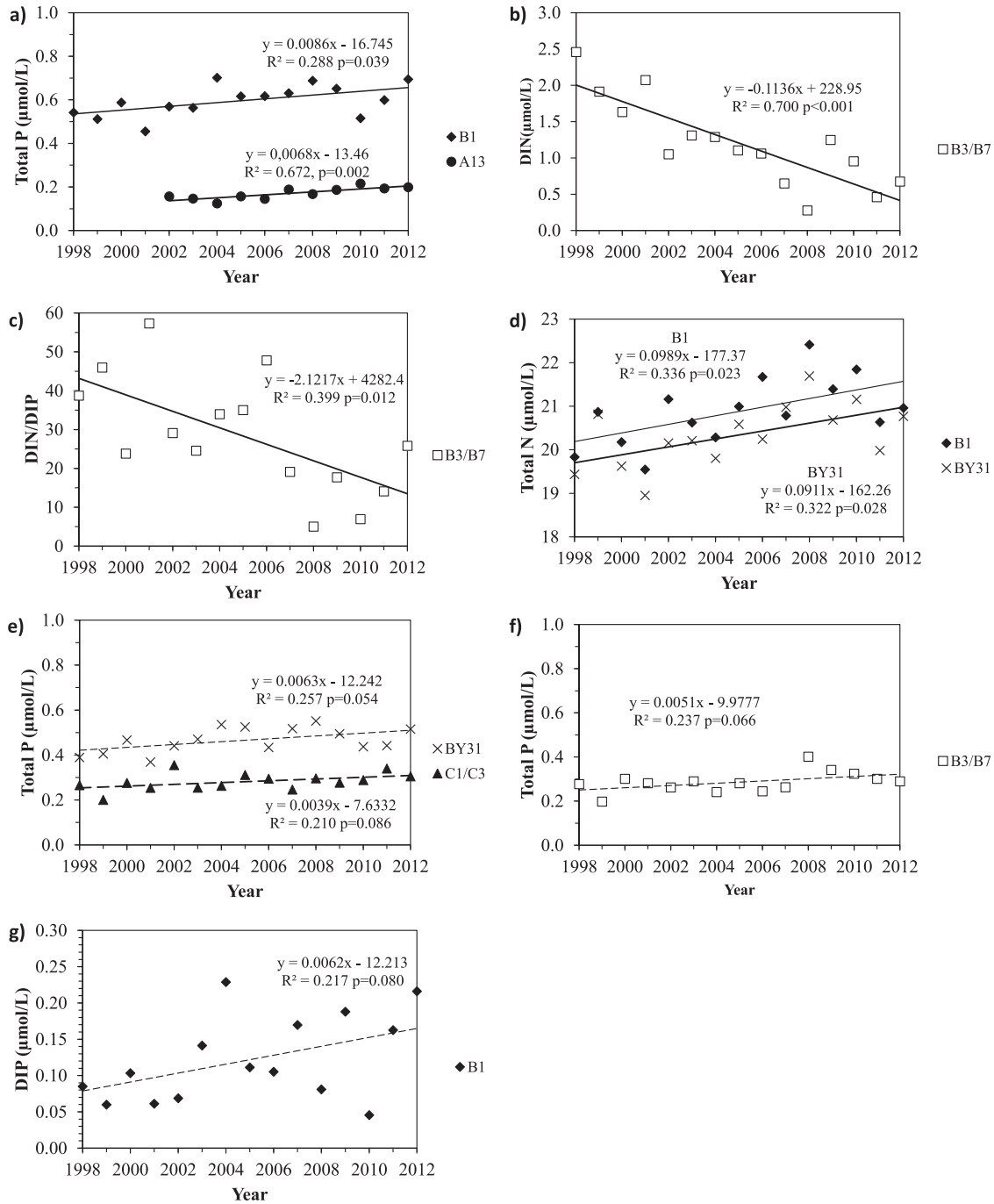


Fig. 8. Significant upward or downward trends in summer (June–September) nitrogen and phosphorus concentrations, a) Tot P, b) DIN concentration, c) DIN/DIP molar ratio, and d) Tot N at specific stations, during the period 1998–2012. Trends close to significance at the 95% level for Tot P are shown in e) and f) and for DIP in g).

The order Oscillatoriales, on the other hand, is composed of filamentous species with small and large cell sizes. Previous studies have shown that certain Oscillatorian species, e.g., *Planktothrix agardhii*, increases with increasing nutrient concentrations in semi-enclosed bays (Carstensen and Heiskanen, 2007; Jaanus et al., 2009). However, the present work did not include such habitats, neither did we observe significant abundances of *P. agardhii*. Instead, relatively small Oscillatorian genera were common, e.g., *Pseudanabaena* and *Romeria*. These small cyanobacteria would thus

be favored by low nutrient conditions (Raven, 1998; Stal et al., 2003). However, Jaanus et al. (2009) found that e.g., *Pseudanabaena* responded positively to increased phosphorus concentration and higher temperature. Since Oscillatorian species are not able to fix atmospheric nitrogen, they are expected to thrive in areas with higher nitrogen concentrations, as found in the Bothnian Bay.

Temporal trends in total cyanobacteria biomass were only found in the coastal Bothnian Sea, whereas trends for different cyanobacterial groups were found at three out of the five stations,

stressing the need for analysis of separate cyanobacteria groups instead of the total cyanobacteria biomass when searching for trends in the cyanobacteria community. Temporal trends showed that the Nostocales increased at coastal sites in the Bothnian Sea and the northern Baltic Proper. These results are in accordance with previous studies which have indicated that Nostocophyceae have increased in the Bothnian Bay, Bothnian Sea and Gulf of Finland, at least periodically from the 1990's until present (Jaanus et al., 2011). Our study indicated that the general underlying mechanism in the coastal Bothnian Sea was tendency for increasing total P and decreasing DIN concentrations (and therefore decreasing DIN/DIP ratio), while in the coastal Baltic Proper, the increase of Nostocales was accompanied by an increase of Tot N, Tot P and a tendency for increased DIP. The reason for the positive relation of Nostocales and Tot N at the coastal station in the Baltic Proper is likely the nitrogen fixation performed by the cyanobacteria (Larsson et al., 2001). However, when including data from all stations, Nostocales was found to be promoted by high Tot P and negatively related to Tot N. Tot N concentration was found to decrease with increasing salinity from the Bothnian Bay to the open Bothnian Sea. This may be due to the high humic content of the seawater in the Bothnian Bay (Skoog et al., 2010). Humic substances have high nitrogen content (Andersson et al., 2013), contributing to high Tot N concentration in the north. Our study showed that the Tot N concentration is slightly higher in the Bothnian Bay than in the Bothnian Sea, probably due to higher exposure to freshwater inflow in the northernmost basin of the Baltic (e.g., Elmgren, 1984).

The Chroococcales were observed to increase in the Bothnian Bay during the period of the study, 1998–2012. It is likely that small increases in Tot P, as those observed in the Bothnian Bay, will support small-celled cyanobacteria species, i.e., the Chroococcales. During the period 2002 to 2012, the concentration of Tot P increased from 0.15 to 0.2 $\mu\text{mol/L}$. Our analysis shows that the Nostocales start to be promoted at such phosphorus concentrations (Fig. 4), provided the nitrogen concentrations are at the same time low. If the nitrogen concentrations remain high, Chroococcales and Oscillatoriales seem to be dominant.

It has been shown that nutrient pulses, caused, e.g., by upwelling events or wind mixing, are very important for the occurrence of blooms of Nostocales (Kononen et al., 1996; Vahtera et al., 2005). It is reasonable to assume that phosphorus concentrations show drastic increases during such events. Our data also show that the Tot P concentration reached 0.25 $\mu\text{mol/L}$, before the nitrogen-fixing Nostocales became dominant in the cyanobacterial community (Fig. 4). At these phosphorus concentrations, the cyanobacterial community became dominated by the bloom forming species/genera *Aphanizomenon* sp., *Nodularia spumigena* and *Dolichospermum* spp. Unlike Jaanus et al. (2009), our study indicates that Nostocales can be used as indicator for eutrophication, especially concerning increased phosphorus concentrations, with a biweekly sampling frequency in the sampled region.

In conclusion, our findings suggest that Chroococcales could be used as indicator for increased phosphorus levels in waters with low phosphorus concentrations and high nitrogen concentrations, like the Bothnian Bay (Andersson et al., 1996), whereas Nostocales could be used in more productive waters with higher phosphorus and lower nitrogen concentrations, like the coastal Bothnian Sea and Baltic Proper (Samuelsson et al., 2006). This study also showed that the cyanobacterial community in brackish water responds strongly to changes in phosphorus and salinity. In the Baltic Sea, salinity and Tot P showed high co-variation, indicating that highly saline water contains more phosphorus than the more northerly regions that are strongly influenced by freshwater. We suggest that phosphorus and nitrogen are the governing factors, since the major part of the occurring cyanobacterial groups (orders) are adapted to

a wide salinity range (Hällfors, 2004). The increasing Tot P concentrations in the Gulf of Bothnia may be due to the recently extended suboxic or anoxic bottom water in the Baltic Proper (Funkey et al., 2014). Under such conditions, phosphorus is released from the benthic zone and ends up in the pelagic system. Since seawater from the Baltic Proper is transported northwards into the Gulf of Bothnia, increasing phosphorus concentrations in the Gulf of Bothnia are a likely consequence. The coastal Baltic Proper contains relatively high concentrations of phosphorus, due to upwelling and/or runoff from land. In addition, climate change, which is associated with higher land run-off and temperature, causing more stable stratification of the water, will likely enhance the release of phosphorus from the benthic zone in the open Baltic Proper (Funkey et al., 2014), thus driving phosphorus concentrations in the Gulf of Bothnia higher. Such increases could result in further alterations in the cyanobacterial community, with the possibility of permanent changes and subsequent ecological consequences.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.ecss.2015.07.013>.

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