Faculty of Science

On the Ecophysiology of Baltic Cyanobacteria, Focusing on Bottom-up Factors

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Ph.D. thesis
Department of Marine Ecology
University of Gothenburg

UNIVERSITY OF GOTHENBURG

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Doctoral Thesis

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ABSTRACT

Cyanobacterial blooms in the Baltic Sea are dominated by diazotrophic cyanobacteria, i.e. *Aphanizomenon* sp. and *Nodularia spumigena*. The blooms coincide with a stable stratification and the organisms are concentrated to the surface water, exposed to high levels of both photosynthetically active radiation (PAR, 400–700 nm) and ultraviolet radiation (UVR, 280–400 nm), in combination with low ratios of dissolved inorganic nitrogen and inorganic phosphorus (DIN:DIP). The ability of nitrogen fixation, a high tolerance to phosphorus starvation and photo-protective strategies (production of mycosporine-like amino acids, MAAs) may explain their competitive advantage in the Baltic Sea. However, intraspecific variation in the response to environmental factors has been commonly overlooked.

The seasonal succession with peaks of *Aphanizomenon* sp. in early summer followed by peaks of *N. spumigena*, has been related to their interspecific preferences and response to abiotic conditions. *N. spumigena* dominates in late summer forming extensive toxic blooms, and its toxin nodularin, a hepatotoxin lethal to wild and domestic animals, may act as a tumour promotor. It has been suggested that the accumulation of nodularin within the *N. spumigena* cells and its release from the cells are affected by environmental factors. Hence, the seasonal succession may be explained by an allelopathic effect of nodularin on *Aphanizomenon* sp.

The aim of this thesis is to elucidate the factors controlling the cyanobacterial blooms, prevailing seasonal succession, intraspecific differences, toxin production and release. Moreover, to analyze the potential of future toxic blooms in a predicted climate change, e.g. increased UVR and stronger stratification due to increased precipitation and temperature. With a multi-factorial approach in the laboratory and in outdoor experiments, interactive effects of radiation (photosynthetic active radiation PAR and PAR + UV-A + UV-B), nutrients (nutrient replete, nitrogen limited, phosphorus limited) and species composition (monocultures of *N. spumigena* and *Aphanizomenon* sp. and mixed cultures with the respective species) were tested on these two species.

Although strain-specific differences in UV-B radiation tolerance were observed, *N. spumigena* is a species that is not generally negatively affected by UV-B radiation corresponding to ambient sea surface intensities/doses. *N. spumigena* tolerates high ambient UVR also under nutrient-limiting conditions and maintains positive growth rates even under severe phosphorus limitation. Interestingly, the specific growth rate of *N. spumigena* was stimulated by the presence of *Aphanizomenon* sp. and in contrast to our hypothesis, *Aphanizomenon* sp. was not negatively affected by the presence of *N. spumigena*. Nodularin accumulation and release were dependent on environmental conditions, but the released nodularin did not affect the co-existing species *Aphanizomenon* sp. The highest intra- and extracellular nodularin concentrations were observed under nitrogen limitation when shielded from UVR. In conclusion, I suggest that the seasonal succession, with peaks of *Aphanizomenon* sp. followed by peaks of *N. spumigena* is a result from species-specific preferences of environmental conditions and/or stimulation by *Aphanizomenon* sp., rather than an allelopathic effect of *N. spumigena*. Moreover, a possible increased toxin content of the *N. spumigena* should be considered when planning sewage treatment, since nitrogen removal may cause problems on a recreational level and increased accumulation of nodularin higher up in the food web. The results from this thesis, together with a predicted stronger stratification and increased UVR due to effects of climate change in the Baltic Sea, reflect a scenario with a continuing future dominance of the toxic *N. spumigena*.

Keywords: Allelopathy; *Aphanizomenon* sp.; Baltic Sea; Cyanobacteria; Diazotrophic; Multi-factorial; Nitrogen; Nodularin; *N. spumigena*; photosynthetic active radiation; Phosphorus; ultraviolet radiation; UV-A; UV-B.

POPULÄRVETENSKAPLIG SAMMANFATTNING

Under sommarmånaderna de senaste tio åren har man kunnat läsa i dagstidningarna om ”giftiga mördaralger” som omringar Gotland eller om ”mördaralger” som rör sig mot skånska kusten. Ofta tillsammans med bilder där badande människor flyr i panik mot stranden eller flygfoton på skepp som plöjer genom en grågrön sörja.

Det är inga alger som mördar, det är cyanobakterier som blommar.

Forskarna är idag oeniga om anledningen till att dessa blomningar har ökat. En del anser att det beror på övergödningen som vi människor orsakat genom utsläpp av kväve och fosfor i Östersjön. Andra har undersökt Östersjöns bottensediment och hävdar att det är ett naturligt fenomen som har pågått i över 7000 år och att det snarare beror på klimatförändringar. En viktig anledning till att cyanobakterierna trivs på sommaren är bl.a tillgången och förhållandet mellan näringsämnena fosfor och kväve. Dessa näringsämnen finns i riklig mängd under vintern men på våren blommar ett annat växtplankton, kiselalger, som förbrukar det mesta av kvävet och lite av fosfor. Det finns dock tillräckligt med fosfor kvar i ytvattnet för att cyanobakterier, som kan fixera kvävgas, ska kunna blomma senare under sommaren. Solljus är en annan viktig anledning som blivit förbisedd i forskarvärlden. En ökad mängd ultraviolett (UV) ljus, orsakad av uttunning av ozonskiktet, är inte bara ett problem på södra halvklotet utan även här på nordliga breddgrader. Ytblommande växtplankton skadas av UV-ljus men har olika strategier för att undvika denna skadliga del av solens ljus. Många arter har utvecklat ett solskydd i form av UV-absorberande ämnen. Eftersom dessa ämnen innehåller mycket kväve, gynnas kvävefixerande cyanobakterier vid kvävebrist. Därför kan ljus, i samverkan med de näringsämnen som finns i ytvattnet, påverka vilka arter som blommar i Östersjön. Den art som har bäst strategier är den som kommer att dominera. Olika arter av kvävefixerande cyanobakterier blommar vid olika tidpunkter. I maj-juni brukar *Aphanizomenon* blomma, men i juli-augusti dominerar i allmänhet den giftiga arten *Nodularia spumigena* (även kallad katthårsalg). Den fortsätter att dominera så länge ytvattnet är varmt och stilla. Giftet den producerar heter nodularin och är ett hepatotoxin, dvs. ett gift som angriper levern. Boskapsdjur och hundar kring Östersjön har dött efter att ha fått i sig stora mängder av det giftiga vattnet under blomningarna. Anledningen till att den giftiga arten dominerar under sensommaren anses bero på att dessa två arter föredrar olika temperaturer och näringsförhållanden.

Syftet med min avhandling är att förstå hur ljus och näringsämnen kontrollerar och framförallt samverkar till att cyanobakterierna är så framgångsrika. Ett annat syfte med avhandlingen är att se om samverkan av ljus och näringsämnen har olika effekt på de dominerande arterna eller om det är så att den giftiga *N. spumigena* använder sig av giftet nodularin för att vinna över *Aphanizomenon*. Resultaten från mina studier kan sammanfattas med att *N. spumigena* har en fortsatt god tillväxt trots ökad intensitet av UV-ljus, även vid fosforbrist. *Aphanizomenon* växer överlag mycket sämre än *N. spumigena*. Det verkar inte vara så att *N. spumigena* har någon som helst påverkan på *Aphanizomenon*, varken med sitt gift eller med sin närvaro. Vilken art som kommer att dominera styrs snarare av deras olika sätt att hantera situationer med ökad mängd UV-ljus och brist på näringsämnen, än av någon sorts kemisk krigföring. Något oväntat växer *N. spumigena* mycket bättre tillsammans med *Aphanizomenon* än ensam, vilket tyder på att *N. spumigena* gynnas av dess sällskap. *N. spumigena* producerar mest gift när det är lite kväve i vattnet men tillräckligt mycket fosfor. Man kan därför förvänta sig att *N. spumigena* innehåller mest gift i början av blomningen då dessa förhållanden råder. Om avloppsvatten renas från i huvudsak kväve, kan det få stora konsekvenser vad gäller mer gift i blomningarna. Om övergödning tillsammans med uttunning av ozonskiktet fortskrider, vilket forskarna förutspår, kommer cyanobakterierna och i synnerhet *N. spumigena* att gynnas.

There is a theory which states that if ever anybody discovers exactly what the Universe is for and why it is here, it will instantly disappear and be replaced by something even more bizarre and inexplicable. There is another theory which states that this has already happened.

Douglas Adams (1952 - 2001)
English humorist and science fiction novelist

Till Kent, Johannes och David

LIST OF PAPERS

This thesis is based on following publications/manuscripts. Publications will be referred to in the text by Roman numerals as follows:

I. Wulff, A., **Mohlin, M.** & Sundbäck K. (2007) Intraspecific variation in the response of the cyanobacterium *Nodularia spumigena* to moderate UV-B radiation, *Harmful Algae*, 6:388-399

II. **Mohlin, M.** & Wulff, A. (2009) Interaction effects of ambient UV-radiation and nutrient limitation on the toxic cyanobacterium *Nodularia spumigena*, *Microbial Ecology*, 57:675-686

III. Pattanaik, B., Roleda, M.Y., Garde, K., Wulff, A. & **Mohlin, M.** (2010) Production of the cyanotoxin nodularin – a multifactorial approach, *Harmful Algae*, 10:30-38

IV. **Mohlin, M.,** Pattanaik, B., Roleda, M.Y. & Wulff, A. (2010) Allelopathic and combined effects of radiation and nutrient limitation on Baltic cyanobacteria (Manuscript)

Related publications not included in the thesis:

Roleda, M.Y., **Mohlin, M.,** Pattanaik, B. & Wulff, A. (2008) Photosynthetic response of *Nodularia spumigena* to UV and photosynthetically active radiation depends on nutrient (N and P) availability, *FEMS Microbial Ecology*, 66: 230-242

Lindberg, V., **Mohlin, M**. & Wulff A. (2008) UV responses in three strains of the cyanobacterium *Nodularia spumigena*, Proceedings of the 12th International Conference on Harmful Algae. Ø. Moestrup et al. (Eds). ISSHA and IOC of UNESCO 2008, page 44

ABBREVIATIONS

AD	anno dato
APHA	*Aphanizomenon* sp.
BMAA	*β*-N-methylamino-L-alanine
BP	before present
BWF	biological weighting function
chl *a*	chlorophyll *a*
CIE	Commision Internationale de l´Eclairage
DIN	dissolved inorganic nitrogen
DIP	dissolved inorganic phosphorus
F_v/F_m	maximum quantum yield
HPLC	high-pressure liquid chromatography
MAAs	mycosporine-like aminoacids
MAPHA	*Aphanizomenon* sp. in mixed culture with *N. spumigena*
MNOD	*N. spumigena* in mixed culture with *Aphanizomenon* sp.
N	Nitrogen
-N	f/2 medium without nitrate
NO_2^-	Nitrite
NO_3^-	Nitrate
NH_4^+	Ammonium
NOD	*Nodularia spumigena*
NodApha	*N. spumigena* in mixed culture with *Aphanizomenon* sp.
NP	f/2 medium
P	phosphorus
-P	f/2 medium without phosphate
PAR	photosynthetic active radiation
PO_4^{3-}	phosphate
POC	particulate organic carbon
PON	particulate organic nitrogen
POP	particulate organic phosphorus
UV-A	ultraviolet-A radiation
UV-B	ultraviolet-B radiation
UVR	ultraviolet radiation

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1. INTRODUCTION

The Baltic Sea is situated between latitude 53°N to 66°N and between longitude 20°E to 26°E, and is one of the largest brackish-water bodies in the world. It consists of a number of basins, including the Gulf of Bothnia, Gulf of Finland and Gulf of Riga as well as the Baltic proper (Fig. 1). The anthropogenic stress to the Baltic Sea is high, as 16 million people live on the coast and a total of 85 million within the catchment area. The water exchange from the North Sea is restricted, due to the shallow sill and the narrow link situated between Denmark and Sweden. Together with a large river inflow those features give the Baltic Sea its brackish character.

1.1. *THE HISTORY OF THE BALTIC SEA AND CYANOBACTERIAL BLOOMS*

It has a very young history that have been influenced by the last glaciations of the Baltic basin, the Weichselian glaciations, and Baltic Sea as we know it today is a result of meltwater from the glaciations combined with saltwater from the North Sea when the straits between Sweden and Denmark opened. The postglacial history of the Baltic Sea is characterized by isostatic rebound, i.e. uplift of land masses that were depressed by the weight of ice together with eustatic changes in the volume of water in the world oceans caused by climate change (Winterhalter, 1992; reviewed by Björck, 1995). This resulted in a succession of freshwater and marine stages with either brackish condition with connections to the North Sea or isolated freshwater lake conditions (reviewed by Björck, 1995). Variations between oxic and hypoxic conditions in the deep water are recognizable in the sediment stratification. Under oxic conditions the sediments are homogenous because of the activity of the fauna in the sediments. Under hypoxic conditions the fauna that homogenize the sediments are eliminated and therefore sediments are laminated. By analyzing sediment cores and the stratigraphic succession including biogenic remains, researchers have been able to reconstruct the history of the Baltic Sea.

Fig. 1. Map made by John Malham in 1795. www.gracegalleries.com/images/S&B/S&B109.jpg

1.1.1. *STRATIGRAPHIC STUDIES*

The history of the Baltic Sea is traditionally divided into four main stages, i.e. the Baltic Ice Lake, the Yoldia Sea, the Ancylus Lake and the Litorina Sea (Fig. 2a- d). Deglaciation of the Baltic basin started around 17000–15000 before present (BP) and continued for at least 3000 years (Berglund, 1979; Andrén et al., 2000a and references therein). The ice-free freshwater stage, called the Baltic Ice Lake, began to form 16000 BP when large parts of the southern Baltic became ice-free relatively quickly (reviewed by Björck, 1995; Jensen, 1995). It existed until 10300 BP when the dam broke at Mount Billingen (arrow in Fig. 2b) and the melt water was flooding out in the North Sea (Andrén et al., 2002) resulting in a considerable fall of the Baltic lake-level (25 m), (Björck, 1995).

Fig. 2. Post-glacial stages of the Baltic basin. a) the Baltic Ice Lake, b) the Yoldia Sea, arrow indicate where the dam broke, c) the Ancylus Lake, arrow indicate where the connection to the North Sea closed d) the Litorina Sea, arrow indicate where the broadening of connection to the North Sea, Öresund (www.smf.se)

The Baltic Ice Lake was succeeded by the Yoldia Sea, 10300-9500 BP which consisted of two freshwater phases and a short brackish-water phase in between, caused by inflow of marine water from the North Sea (reviewed by Björck, 1995; Raukas, 1995). Sediment cores examined from this first freshwater phase do not contain siliceous microfossils or cyanobacteria, probably due to low primary production (Andrén et al., 2000a). The scenario would change when saline nutrient-rich water from the North Sea were brought into the Yoldia Sea, the upwelling of this nutrient-rich marine water caused high diatom abundances (Andrén et al., 2000a). The increase of diatom abundance has also been recorded in cores from the Bornholm Basin (Andrén et al. 2000b). According to a study by Andrén and Sohlenius (1995), the inflow of marine water during the brackish phase had probably the same periodicity as present in the Baltic Sea, approximately 15 years (Stigebrandt, 1987). The transition between the brackish and last freshwater phase in the Yoldia Sea is visible as a shift in the diatom composition to a freshwater flora dominated by a few taxa (Andrén et al., 2000a).

A new phase called the Ancylus Lake started around 9500 BP and ended around 8000 BP. The connections to the North Sea (at Mount Billingen, arrow in Fig. 2c) became shallow because of continuous land-uplift and forced the Baltic level to rise above sea level (Björck, 1995). This dammed-up freshwater lake is the most discussed of the many Baltic phases (summarized by Fredén, 1967). The land-uplift in the northern part of the basin was faster during this stage and this caused the Ancylus Lake to attain a link to the Kattegatt-Skagerack through the Great Belt region, 10 000 BP (Björck, 2008). This connection was broadened around 8500–7800 BP, and began to function as an important inlet of saltwater, the Sound (Öresund) (arrow in Fig. 2d), (Andrén et al., 2000a; Andrén et al., 2000b; Sohlenius et al., 2001). Until now there has been no particular sign of cyanobacterial blooms in the respective phases of the Baltic basin. The first indications were found during the transition phase between Ancylus Lake and the Litorina Sea. Sediment cores analysed from this transition phase showed a rapid increase in the organic carbon content (Sohlenius et al., 1996), thought to have been caused by cyanobacteria (Andrén et al., 2000b).

Finally we have reached the most interesting phase in the history of the Baltic Sea; the Litorina Sea 8000-4000 BP. I consider it most interesting, since it is in sediment layers from this phase that cyanobacteria have been recorded. The Litorina Sea was a marine stage (reviewed by Munthe, 1894) and the inflow of marine water created a halocline that most likely resulted in a decreased vertical mixing of the water column and may explain the extensive laminated sediments found from this stage (Fig. 3), (Bianchi et al., 2000 and references therein). It seems like anoxic conditions and dead bottoms existed in the Baltic basin already during the Litorina Sea.

In order to measure historical outbreaks of cyanobacterial bloom, researchers have measured the concentrations of the cyanobacteria-specific pigments echinenone, myxoxanthophyll (Poutanen and Nikkila**,** 2001) and zeaxanthin (Bianchi et al., 2000; Poutanen and Nikkila**,** 2001) in laminated sediments. Although common in cyanobacteria in general, it has been shown that *N. spumigena* contain no or very low concentrations of zeaxanthin (Paper I, II, IV; Henriksen, 2005; Schlüter et al., 2004), indicating that the zeaxanthin concentrations found in the laminated sediments originate from other cyanobacteria species or green algae (Jeffrey, 1997). Those findings have however been revised lately; Schlüter et al. (2008) found that zeaxanthin is a pigment found in *N.spumigena* from the Baltic Sea.

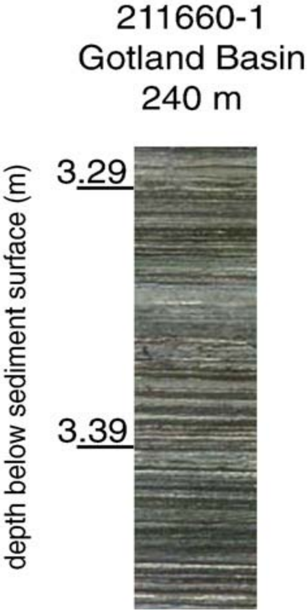

Fig. 3 Photo of laminated sediments dated to the Litorina Sea (from Zillén et al. 2008)

Bianchi et al. (2000) report the occurrence of cyanobacterial blooms already in the early stages of the Litorina Sea (7500-7000 BP), further supported by Poutanen and Nikkila (2001). By comparing sediments from early Litorina stage and modern sediments from the Gotland Basin, Struck et al. (2000), found that the productivity was nitrate based in spring and based on cyanobacterial nitrogen fixation in summer. Bianchi et al. (2000) suggest that the cyanobacterial blooms were initiated by increased availability of phosphorus, i.e. inflow of phosphorus-rich seawater and increased phosphorus-release from anoxic sediments. In addition, they detected the cyanobacterial pigment zeaxanthin in such high concentrations that the blooms during this phase were similar in magnitude to the cyanobacterial blooms that we experience today. They further suggest, based on the results and on the stability of this pigment (Bianchi et al. 1993), that the absence of zeaxanthin in sediments from the Ancylus Lake is likely to be a result of low virtual abundances rather than a degradation of the pigment. During the Late Litorina Sea (4000 BP – AD 1800) there was a succession between homogeneous sediments and laminated sediments due to oxic or hypoxic conditions, temperature increase or decrease, intrusion of saline water from expanded opening in the Danish Strait, increased or decreased population in the coastal area (reviewed by Zillén et al., 2008).

The studies on stratigraphic succession mentioned above have been, among others, the bases in a confusing public debate during the last decades concerning to what extent the occurrence of the cyanobacterial blooms reflects natural variability rather than anthropogenic impacts. There is no doubt that the human impact has increased dramatically from the medieval time to present time. During that time there have been major changes in agriculture, cutting of trees, population increase and an industrial revolution resulting in an increased nutrient load to the Baltic Sea drainage area (reviewed by Zillén et al., 2008). Pigment data from stratigraphic studies by Poutanen and Nikkila (2001) clearly show an increased intensity of algal blooms in the Baltic Sea since the early 1960s. Furthermore, from dated sedimentary records of organic compounds, Struck et al. (2000) show a clear history of eutrophication with enhanced nutrient supply after the start of using fertilizers in agriculture, with a subsequent increased deposition of organic matter in the Baltic from 1920s to 1980s (Jonsson and Carman, 1994). There is no doubt that this increased occurrence and intensity of cyanobacterial blooms is a result of increased nutrient load and nutrient concentrations in the Baltic Sea (HELCOM, 1996; HELCOM, 2001).

1.1.2. *PLANKTON STUDIES*

The history of quantitative plankton research in the Baltic Sea goes back to the 19th century. Victor Hensen (1835-1924), a German planktologist from Kiel, developed sampling nets, quantitative plankton analysis and conducted pioneer expeditions in the Baltic Sea in 1880 to 1890 (reviewed by Finni et al., 2001; Olenina et al., 2006). The first coordinated plankton research in the Baltic with a frequency of 4 times per year began after an inaugural meeting in Copenhagen 22 July, 1902, when The International Council for the Exploration of the Seas, ICES, was established (Finni et al., 2001).

Plankton sampling at the beginning of the 20th century onboard R/V Nautilus. Photo: Finnish Institute of Marine Research (from Finni et al., 2001)

It was mainly from the coastal area that most blooms of cyanobacteria were recorded and *Aphanizomenon* (nitrogen-fixing cyanobacterium) was the dominant genus (reviewed by Finni et al. 2001), also in waters near the large cities (Välikangas, 1926). Many researchers thought that this was the freshwater species *Aphanizomenon flos-aquae* (reviewed by Finni et al., 2001) but this idea was argued by Aurivillius (1896) among others, claiming that this was a brackish-water species. They should, however, have to wait until 1994 when Janson et al. (1994) demonstrated the difference in ultrastructure of the vegetative cells of the freshwater and brackish-water species, however they suggested that the Baltic form should be referred to as *Aphanizomenon* sp.. Häyrén (1921) also found that *Aphanizomenon flos-aquae, Nodularia spumigena* and *Anabaena baltica* were dominating the blooms outside Tvärminne, Finland, 1913. In september 1921 in the coastal area between the Island Ven and south of Sweden, Sjöstedt (1922) observed a bloom of *Nodularia spumigena*.

Records of open-sea blooms rarely occurred but in July 1854 Lindström et al (1855) were among the first to observe an intensive bloom in the Baltic proper. Local fishermen informed him that the layer of the bloom sometimes was so thick that the boats had difficulties to pass through. Thirty years later (14 August to 15 September in 1884), the Prince of Monaco made an expedition in the Baltic Sea with his yacht l´Hirondelle. Professor Pouchet and Baron Jules de Guerne (1885) observed a bloom that covered the area between Gotland, the entrance to Gulf of Finland and Prussia. They noticed that the colour of the bloom was olive-green. However, in none of these two studies did the researchers determine species composition of the bloom. As far as I know, we would have to wait until summer of 1925 when Hessle and Vallin (1934) started a three year study along the Swedish coast and around Gotland. During their expeditions, *Aphanizomenon* was the most common genus, being more abundant than *Nodularia*. The dominance of *Aphanizomenon* in relation to *Nodularia* was also reported in other studies between 1924 and 1930 (reviewed by Finni et al., 2001). The earliest record of a *Nodularia* dominated open-sea bloom as we know them today, was from the expedition by Rothe in August 1938 (Rothe, 1941). From this period and to date, records of *Nodularia spumigena* as the dominating species in the open-sea blooms became common (reviewed by Finni et al. 2001)

In summary, after a closer look into the history of cyanobacterial blooms, I can only agree that the abundance of *Nodularia spumigena* as well as *Aphanizomenon* sp. in the Baltic Sea have indeed increased since the beginning of the 20th century.

1.2. PRESENT SITUATION IN THE BALTIC SEA AND CONSPICUOUS BLOOMS OF CYANOBACTERIA

1.2.1. NUTRIENT SITUATION

There is an increased supply of nutrients and organic matter to the Baltic Sea, and the main sources are the industrial and agricultural activities of the human population, aquaculture, municipal sewage water, river run-off and erosion, atmospheric deposition and nitrogen fixation (Elmgren and Larsson, 2001; HELCOM, 2002; HELCOM, 2003). The nutrient supply is estimated to a four-fold increase in nitrogen and eight-fold in phosphorus during the 20th century (Larsson et al., 1985). It has been debated over the relative importance of dissolved inorganic nitrogen (DIN) and dissolved inorganic phosphorus (DIP) for the phytoplankton productivity and species composition in the Baltic Sea. The most limiting nutrient may vary between area and season (Moisander et al., 2003; Rahm and Danielsson, 2007). For example, periods with a molar DIN:DIP of 50,well above the Redfield ratio of 16, suggests phosphorus limitation, but occasional phosphorus release from oxygen depleted (hypoxic) sediments reduce this ratio and leads to nitrogen limitation (Pitkänen and Tamminen, 1995; Elmgren and Larsson, 2001). The Baltic Sea surface water is separated from the deep water with a permanent halocline at 60–70 m depth. In summer, when the temperature starts to increase in the surface water, a seasonal pycnocline is built up in the upper mixed layer at 10–20 m depth from the underlying nutrient rich winter water. During spring, a yearly diatom bloom removes DIN from the surface water but leaves high concentrations of DIP, resulting in a low DIN:DIP ratio in May. This changed ratio between the major nutrients will favour nitrogen fixing, i.e. diazotrophic, cyanobacteria due to their ability to fix atmospheric nitrogen (N_2) (Granéli et al., 1990; Larsson et al., 2001; Rydin, 2002; Kangro et al., 2007; Rolff et al., 2007; Sivonen et al., 2007). It is also considered that the availability of DIP is the most important factor determining spatial and temporal distribution and induction of diazotrophic cyanobacterial blooms (Niemi, 1979; Niemi, 1981; Panosso and Granéli, 2000; Paper II, IV).

1.2.2. HYPOXIA − CAUSES AND CONSEQUENCES

During the last decades, increased frequency and intensity of cyanobacterial blooms have been linked to the ongoing eutrophication of the Baltic Sea (Niemi, 1981; Larsson et al., 1985). Eutrophication has been defined as an increased input of nutrients or organic matter into an aquatic ecosystem, resulting in an increased primary production (Nixon, 1995). Following increased sedimentation in deep waters of the Baltic Sea will support an increased consumption of the bottom water oxygen by heterotrophic organisms. Nutrients will initially accumulate in the deep water as DIN and DIP, but in basins with limited water exchange like the Baltic Sea, the increased respiration leads to temporary or sometimes permanently hypoxic conditions in the near bottom area. During the 20th century, large areas of formerly oxic bottoms in the deep basins of the Baltic Sea have turned hypoxic (Fig. 4a) and dead zones have developed (Fig. 4b) (Karlson et al., 2002; Díaz and Rosenberg., 2008).

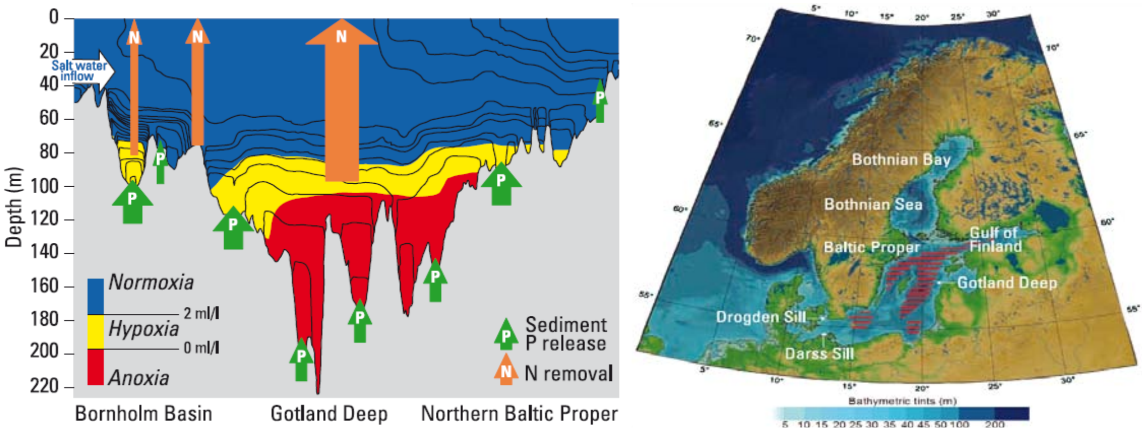

Fig. 4. a) Diagram of hypoxia in the Baltic Sea and the major basins. b) Map of the Baltic Sea identifying its major basins and sills governing the inflow of saltwater. The red lines designate the maximum hypoxic area that occurs in the Baltic Sea (from Conley et al. 2009)

When the deep water turns hypoxic, DIP increases in concentration due to phosphorus release from hypoxic sediments (HELCOM 1987; Vahtera et al. 2007a). Furthermore, a negative relationship between total amount of DIN in the Baltic Proper and hypoxic water volume indicate removal of nitrogen (Vahtera et al. 2007a). The processes behind this nitrogen removal are not yet understood but it seems like hypoxic conditions will alter the nutrient biogeochemical cycles (Conley et al., 2009). Of interest and very alarming is that the release of DIP from hypoxic sediments is approximately 1 order of magnitude greater than the anthropogenic total phosphorus loading to the Baltic Sea (Conley et al., 2002). The consequent decreasing DIN:DIP ratio will favour diazotrophic cyanobacterial blooms, and this will in turn lead to more sedimentation of organic matter and more hypoxia in the Baltic Sea. Vahtera et al. (2007a) have termed this internal acceleration of eutrophication the “vicious circle”.

In short, nutrient and nutrient ratios are important factors controlling and affecting cyanobacterial blooms. But when dealing with photosynthetic organisms, radiation (intensity and dose), and the spectral composition are all factors with the same magnitude of importance (Paper I, II, III, IV, Roleda et al., 2008).

1.2.3. *RADIATION SITUATION*

The Commision Internationale de l´Eclairage (CIE) has defined ultraviolet-B (UV-B) radiation as wavelengths of 280-315 nm. However, many aquatic scientists accept 320 nm as the upper limit and throughout this thesis I will use the definition of UV-B radiation as 280-320 nm.

An increased UV-B radiation caused by the ozone depletion is a well-known threat not only in the Antarctic region but also on Nordic latitudes (Aldhous, 2000; Shindell et al., 2001; Anonymous, 2002; Anonymous, 2006). It is difficult to assess the underwater UV radiation condition from space-based measurements (summarized by Vassilkov et al., 2001), but the long-term ultraviolet radiation (UV-B+UV-A, 280-400 nm) measurements on land with broadband radiometers show an overall increase towards late 1990s (Chubarova, 2005; Josefsson, 2006). At our latitudes, the UV-B radiation has increased by 6-14% during the last 20 years (Anonymous, 2002) and erythemal UV dose enhancements of 10–15% were observed in northern Norway during the in summer 2000 (Orsolini and Limpasuvan, 2001). This increase is due to the distribution and trends of atmospheric ozone in the Northern hemisphere, and is changing as a result of both natural and anthropogenic activities (Orsolini and Limpasuvan, 2001). Satellite ozone data have shown that since the late-1970, there has been a significant ozone decline over the northern mid-latitudes (Fig. 5) (Bojkov and Balis, 2001; Staehelin et al., 2001; Staehelin et al., 2002).

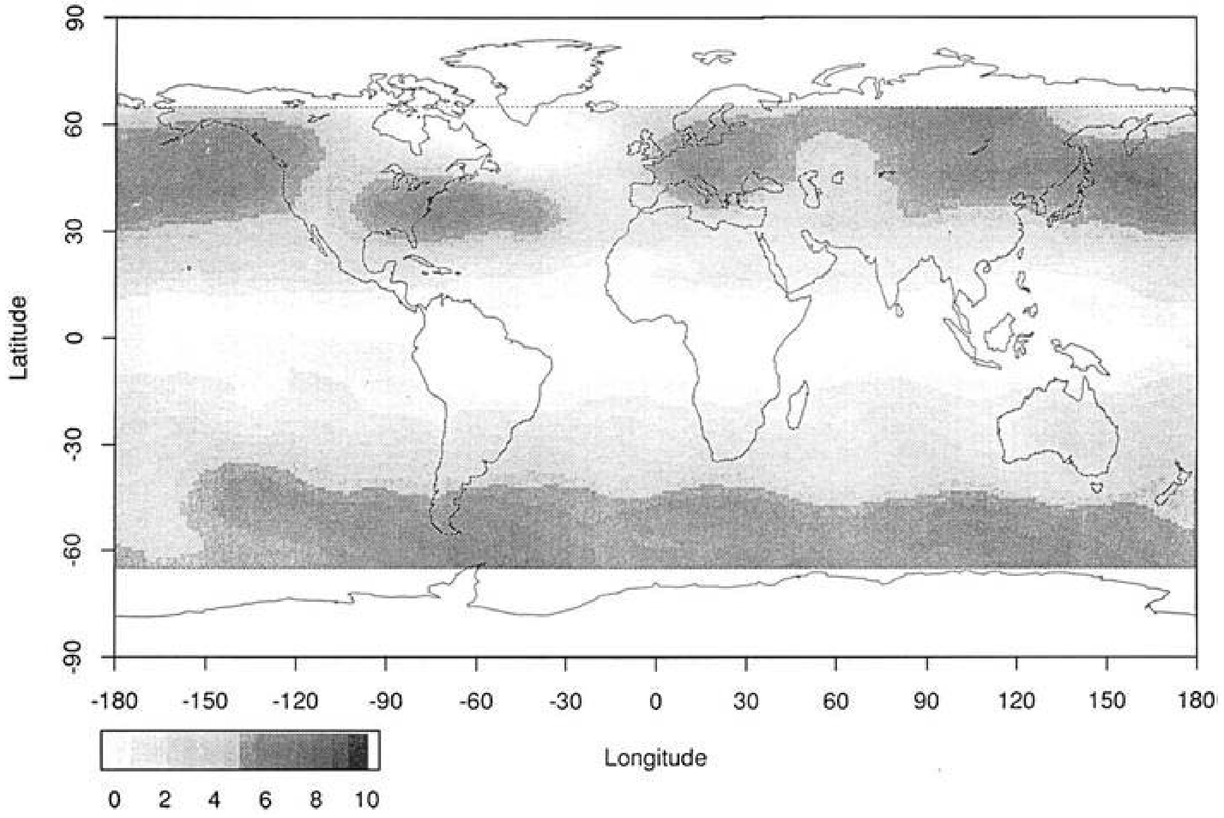

Fig. 5. Variation of ozone trends (percent change per decade) with longitude and latitude derived from Total Ozone Mapping Spectrometer (TOMS) satellite measurements for December–March, 1978–1991 (from Staehelin et al., 2001)

An additional threat to the ozone layer is the increasing level of greenhouse gases in the atmosphere (Shindell et al., 1998; Aldhous, 2000; Anonymous, 2002). The chemical reactions responsible for stratospheric ozone depletion are extremely sensitive to temperature. Greenhouse gases warm the Earth's surface but cool the stratosphere radiatively and therefore affect ozone depletion (Shindell et al., 1998 and references therein). Thus, climate change could prolong ozone depletion in the Arctic by many years despite the success of the Montreal Protocol of 1987 in reducing emissions of ozone-destroying chemicals (Shindell et al., 1998; Aldhous, 2000; Anonymous, 2006). The increase of longer wavelengths, i.e. UV-A radiation (320–400 nm), is a result of decreases in both aerosol optical thickness and effective cloud amount (Josefsson, 2006), and not related to the decreased ozone layer. Therefore, the role of clouds must be included in studies of long-term variations of UV, i.e. to be able to detect the recovery of the ozone layer in UV monitoring data, accurate measures of the effect of clouds is necessary (Josefsson, 2006).

High intensities and/or doses of both UV-B and UV-A could negatively affect phytoplankton and there are species-specific strategies to reduce UV-exposure and hence the amount of photo-damage (Karentz et al., 1991; Karentz, 1994, 2001). Cyanobacteria are among the oldest photosynthetic organisms (Summons et al., 1999) and they have evolved various strategies to limit the amount of photo-damage, including the production of mycosporine-like amino acids (MAAs) (Sinha et al., 2003; Sinha and Häder, 2008; Xue et al., 2005) and scytonemin (Garcia-Pichel and Castenholz, 1991). MAAs have absorbance peaks between 310 and 362 nm, while scytonemin absorbs the longer wavelengths in the UV range up to 382. Together they could effectively dissipate absorbed radiation without producing reactive oxygen species (Carreto et al., 1990; Conde et al., 2000). Although common in terrestrial and some benthic marine environments, scytonemin is not found in planktonic cyanobacteria. Due to their broad absorbance spectrum, MAAs can be an additional strategy to reduce negative effects of excessive photosynthetic active radiation (PAR, 400-700 nm). The exact location of MAAs in cyanobacteria is not known, but in *Nostoc commune*, MAAs are extracellular and bound to oligosaccharides in the sheath (Böhm et al., 1995).

Results from previous studies on plankton communities or cultures suggest that available inorganic nutrients are important to reduce negative effects of UVR (Neale et al., 1998; Wulff et al., 2000). Thus, since MAAs are nitrogen-containing compounds (Rozema et al. 2002), the prevailing nutrient situation during summer in the Baltic Sea might lead to a decreased production among the phytoplankton, giving N_2-fixing cyanobacteria an additional competitive advantage over other MAA-producing phytoplankton. Several laboratory studies have explored the effect of radiation on the ecology of Baltic cyanobacteria (Lehtimäki et al., 1994; Rapala et al., 1997), but the major weakness of these studies are the use of too low radiation intensities excluding UVR. Relevant radiation spectra and intensities are needed and are interesting from an ecological point of view (Roleda et al. 2008, Paper I, II, III, IV).

1.2.4. DOMINATING SPECIES

Cyanobacterial blooms in the open Baltic Sea consist of the N_2-fixing and filamentous genera *Nodularia*, *Aphanizomenon*, and A*nabaena* (Stal et al. 2003): all three belong to the *Nostocalean* taxa. Earlier morphological studies of phytoplankton samples from the Baltic Sea suggested three species of *Nodularia*: *N. spumigena, N. litorea*, and *N. baltica* (Komárek et al. 1993). However, detailed molecular studies of different strains from these species led to the conclusion that there was only one genetically distinct planktonic *Nodularia* species in the Baltic Sea, *N. spumigena* Mertens (Barker, 1999; Lehtimäki et al., 2000; Laamanen et al., 2001;). It has been suggested that genetic exchange may occur in the *Nodularia* populations (Barker et al. 2000). A large number of various *Nodularia*-infecting cyanophages have been found (Jenkins and Heyes, 2006) which putatively transfer genetic information between *Nodularia* strains and shape the structure of populations. The Baltic Sea *Aphanizomenon*, previously reported as *A. flos-aquae* (Linné) Ralfs, is suggested to be a genotype of the freshwater *A. flos-aquae* (Laamanen et al., 2002). Although Baltic Sea *Anabaena* show high morphological diversity (Hällfors, 2004), results from recent molecular studies have shown that there is only one genetically valid planktonic species (Barker et al. 2000; Halinen et al., 2008).

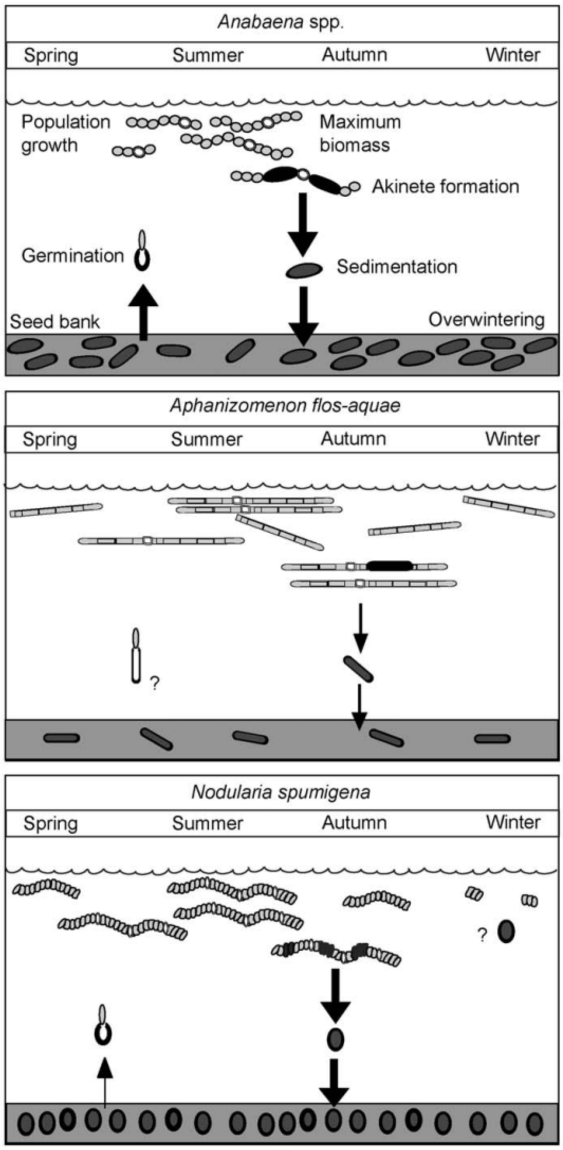

Fig. 6. The different lifecycles of the dominating cyanobacterial species in the Baltic Sea. Arrows denote the akinete flux to and germination from the sediment. Arrow size is proportional to the observed or assumed magnitude of respective fluxes (from Suikkanen et al. 2010).

The previously secret annual life cycles of the dominating species in the Baltic Sea has finally been revealed in very interesting studies in 2005 and 2006 (Suikkanen et al. 2010) (Fig. 6). Using pelagial monitoring, sedimentation traps and germination of akinetes, they found that the three co-occurring species have different overwintering strategies in the Baltic Sea. *N. spumigena* overwinters with a smaller fraction of vegetative filaments in the water column and a larger fraction as sedimented resting stages, i.e. akinetes. *A. flos-aquae* has the opposite strategy with vegetative filaments in the water column year-round and are considered holoplanctonic despite the fact that they form akinetes. *Anabaena* spp. has a meroplanktonic lifecycle, with the planktonic stage during summer and formation of akinetes in fall, germinating the following spring. Suikkanen et al. (2010) suggest that the akinetes formed by *N. spumigena* do not alone act as seeding population for cyanobacterial blooms, the contribution of vegetative filaments are more important. Nevertheless, the significance of the sediment akinetes in providing the inoculum for rapid onset of *Nodularia* blooms has been reported from Peel-Harvey Estuary (Western Australia) by Huber (1984). Huber (1985) also found that among several environmental conditions tested, light was shown to be the most important factor affecting germination.

In addition to different life cycle patterns, the three species have different vertical distribution pattern in the water column. One of the first studies in the open Baltic Sea was performed in the summer 1994 and 1997 (Hajdu et al. 2007). They reported that *N. spumigena* accumulated mainly in the top 5 m of the water mass, while *Aphanizomenon* sp. was found in the whole water column (0 - 20 m) and had bimodal vertical distributions (Fig. 7). *Anabaena* spp. was also found down to 20 m and had bimodal abundance depth distributions with the deeper peak somewhat shallower than *Aphanizomenon* sp..

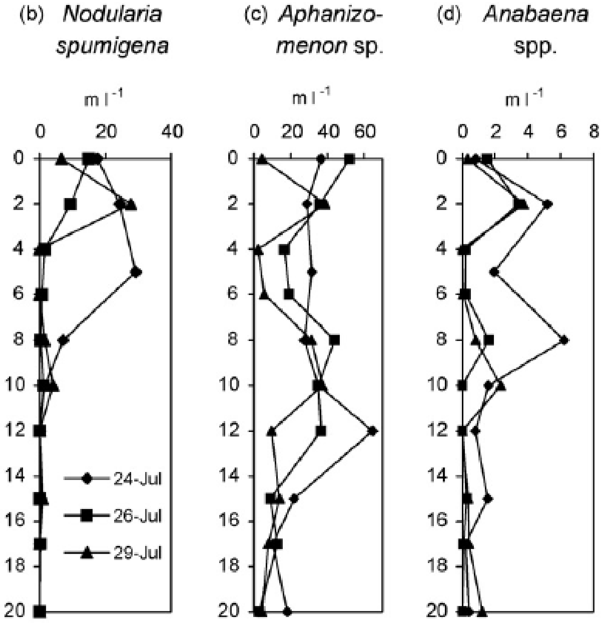

Fig. 7. Vertical distribution of dominating species in July 1994 in Hajdu et al. (2007)

1.3. *APHANIZOMENON SP. VERSUS NODULARIA SPUMIGENA*

1.3.1. *SEPARATED IN TIME AND SPACE*

In my thesis I have focused on *Aphanizomenon* sp. and *N. spumigena*. Over the year, the respective dominance of these two species is both temporally and spatially separated (Kononen et al., 1998, Sellner, 1997). It has been suggested that the temporal separation, e.g. seasonal succession, with peaks of *Aphanizomenon* sp. followed by peaks of *N. spumigena* are related to prevailing physical conditions (salinity, radiation and temperature) in the Baltic Sea during summer (Kononen, 1992; Lehtimäki et al., 1994, 1997; Mazur- Marzec et al., 2005, Paper III; Paper IV) and species-specific niches have been proposed for the two species (Niemistö et al., 1989; Kononen et al., 1996; Vahtera, 2007b).

In a field study, Andersson et al. (1996) observed that as DIP concentrations decreased during the summer, a peak of *Aphanizomenon* sp. was followed by a peak of *N. spumigena*.

Degerholm et al. (2006) reported that *Aphanizomenon* sp. was better adapted to environments with elevated concentrations of phosphorus or repeated intrusions of phosphorus-rich water. In contrast, *N. spumigena* seems to have an ecological advantage in stratified surface waters when phosphorus availability is low (Degerholm et al. 2006) due to a higher affinity for low phosphorus levels than *Aphanizomenon* sp. (Wallström et al. 1992). In addition, *N. spumigena* may utilize dissolved organic matter (DOM) as a nutrient source (Panosso and Granéli, 2000; Põder et al., 2003).

As shown in Fig. 7, *Aphanizomenon* sp. has a deeper biomass maximum than *N. spumigena* (Niemistö et al. 1989, Kononen et al. 1998; Vahtera et al. 2005; Hajdu et al., 2007). The vertical distribution pattern of these two species reoccurs in areas with similar environmental conditions, suggesting species-specific niche separation (Hajdu et al., 2007). It has been related to the variation in prevalent phosphorus source with depth (Vahtera et al., 2005, 2007b). Furthermore, stratification and temperature are two important factors with implications on the vertical pattern. Although having a wide temperature window (Lehtimäki et al., 1994, 1997; Mazur-Marzec et al., 2005), *N. spumigena* thrives in high temperatures in the surface water (Wasmund, 1997). Thus, living near the surface is advantageous for *N. spumigena*. *Aphanizomenon* sp. prefers lower temperature (Wasmund, 1997), and seems better adapted to areas and periods of hydrodynamic activity with weaker stratification (Niemistö et al., 1989; Vahtera et al., 2005).

Moreover, the vertical separation potentially reflects their different sensitivity to high radiation and their differences in photoprotective strategies. During a summer bloom, cells are often concentrated to the upper water layers where they are exposed to high radiation of both PAR, UV-A, and UV-B. In order to change the vertical positioning, both species can change their buoyancy using cellular gas vesicles (Paerl, 1988; Staal et al., 2003.). The deeper biomass of *Aphanizomenon* sp. could be a photoprotective strategy, while *N. spumigena*, often found in the surface water, produce MAAs. Despite the fact that *N. spumigena* is considered one of the most important phytoplankton species in the Baltic Sea, except from our work (Paper I, II, III, IV; Lindberg et al., 2008; Roleda et al., 2008) only one study has considered the impact of UV-B on this surface-blooming species (Sinha et al., 2003), and only in terms of the presence of MAAs.

1.3.2. *TOXIN PRODUCERS*

The two genera *Aphanizomenon* and *Anabaena* from the Baltic Sea have not been as intensively studied as *N. spumigena*, perhaps because of their assumed non-toxicity. The first scientific report of *N. spumigena* causing animal poisoning came from Australia (Francis, 1878). It took another 100 years until scientists from New Zealand identified the chemical structure of the toxin (Rinehart et al. 1988). At about the same time Sivonen et al. (1989) found that *N. spumigena* from the Baltic Sea produced this pentapeptide hepatotoxin and also that there must be non-toxic strains of *N. spumigena*. However, lately researchers have agreed that *N. spumigena* in the Baltic Sea is always nodularin-producing (Kononen, 1992; Chorus and Bartram, 1999; Laamanen et al. 2001).

Nodularin inhibit the protein phosphatase of eukaryotic cells; these enzymes play a major role in regulating cell division and influence the structure and function of cytoskeletal fibres (Runnegar et al., 1995; Annila et al., 1996 and references therein). The liver is the target organ of nodularin. Liver cells are damaged under acute intoxication and the toxin may also acts a tumour promotion under long-term exposition of small toxin doses (Runnegar et al., 1988; Nehring, 1993; Ohta et al., 1994; Humpage and Falconer, 1999; Song et al., 1999). Nodularin may cause toxic effects (Sellner, 1997; Ibelings and Havens, 2008) and lethal effects on wild and domestic animals have been reported (Edler et al., 1985; Nehring, 1993). Fishkills in Gulf of Finland in 1999 were suggested to be linked to the *N. spumigena* bloom that occurred during the same period (Kankaanpaa et al., 2002). The transfer of nodularin in the food web has been reported with accumulation in zooplankton (Karjalainen et al., 2006, 2008), blue mussels (*Mytilus edulis*) (Sipiä et al., 2002), tissues and liver of fish (Kankaanpaa et al., 2002; Sipiä et al., 2002; Persson et al. 2009) and in eiders (*Somateria mollissima*) (Sipiä et al., 2008).

Changes in nodularin production as a response to various environmental factors including temperature, salinity, radiation, and nutrient concentrations have been studied in laboratory experiments (Lehtimäki et al., 1994; Granéli et al., 1998; Repka et al., 2001; Mazur-Marzec et al., 2005; Hobson et al., 1999; Paper III). The increased production with radiation could imply that nodularin is produced as a photoprotective strategy. Gorokhova and Engström-Öst (2009) hypothesized a positive relation between intracellular nodularin concentration and grazing, but the grazing of the copepod *Eurytemora affinis* did not increase the concentration, on the contrary, a significant decrease was observed.

It appears that toxin production is highest under conditions that also favour growth of *N. spumigena*; high temperature and high irradiance, which correspond to conditions in late summer water column of the Baltic Sea (Lehtimäki et al., 1997). However, the effects of phosphorus concentration on nodularin production do not seem to be significant (Repka et al. 2001). It has been suggested that cyanobacterial toxins accumulate within the cells and are only passively released into the surrounding water due to cell lyses (Heresztyn and Nicholson, 1997). On the other hand, Hobson and Fallowfield (2003) suggest that high temperature and high irradiances could increase an active exudation of nodularin during natural blooms. The above cited studies on the effect of radiation on the toxin production of cyanobacteria used artificial radiation in the laboratory and the spectral composition differed from ambient solar radiation (e.g. Lehtimäki et al., 1994, 1997; Hobson et al., 1999). Once again I would like to stress the fact, that for ecologically relevant studies of radiation effects including UVR, it is crucial that the spectral composition should be realistic (Karentz, 1994; Neale et al., 1998).

To our knowledge, the interactive effect of nutrient limitation and radiation on nodularin production, is until now neglected in the literature.

1.3.3. *ALLELOPATHY*

The word allelopathy originates from the greek word ἀλλήλων (allelon) meaning mutually, and πάθος (pathos) meaning pathos. In a publication from 1937, Molisch coined the term allelopathy as the impact of a plant on another plant. According to Molisch (1937) allelo could mean either mutual impact, impact among each other, and pathos mean agony. The allelopathic inhibitory effects of secondary metabolites, e.g. nodularins, and its importance in phytoplankton competition have been reviewed by Legrand et al. (2003). Keating (1977) showed that allelopathy can affect the seasonal succession in a phytoplankton community. Furthermore, the release of cyanobacterial toxins have been suggested to play an ecological role in the interspecific competition via stimulating the abundance of the same or other cyanobacterial species in the community, rather than inhibiting the abundance of competitors to cyanobacteria (Suikkanen et al., 2004, 2005). Suikkanen et al. (2006) further suggest that nodularin is not the main allelopathic compound produced by *N. spumigena*. Interestinly, nodularin has been shown to inhibit growth of prokaryotic cells (Mazur-Marzec et al., 2009), but to my knowledge there are no studies made on the potential allelopathic effect of *N. spumigena* on the co-existing *Aphanizomenon* sp.. I find this surprising since evidence from field studies show clear seasonal succession between these two (Andersson et al., 1996), and that this pattern could be explained by allelopathy.

Hepatotoxin and neurotoxin are likely not expressed in the Baltic Sea bloom-forming *A. flos-aquae* (Sellner, 1997; Sivonen et al. 1989; Willén and Mattsson, 1997). However, several observations show that freshwater strains are producing compounds which may alter species composition and activity in at least some environments including the Baltic Sea (reviewed by Sellner, 1997). In a study by Suikkanen et al. (2006), cell-free filtrates of *A. flos-aquae* from the Baltic Sea significantly inhibited the growth and production of the cryptophyte *Rhodomonas* sp. In the same study, *A. flos-aquae* was observed to inhibit *Rhodomonas* sp. more strongly than *N. spumigena* was. These results imply that *A. flos-aquae* from the Baltic Sea may produce allelopathic compounds that affect the species composition. Even if earlier studies have shown that *Aphanizomenon* sp. from the Baltic Sea do not produce toxins, more recent studies on isolates of *A. flos-aquae* from the Baltic Sea have detected a neurotoxic amino acid, *β*-N-methylamino-L-alanine (BMAA) (Cox et al. 2005; reviewed by Jonasson et al., 2008). BMAA is a neurotoxic non-protein amino acid produced by most cyanobacteria, and has been proposed to cause neurodegenerative diseases (Cox et al. 2003; Murch et al. 2004). In a study from the Baltic Sea, Jonasson et al. (2010) suggested that BMAA is transferred from cyanobacteria and bioaccumulated via zooplankton to organisms at higher trophic levels (e.g. fish) in both pelagic and benthic ecosystems.

1.4. *BOTTOM-UP VERSUS TOP- DOWN*

In this project we have focused on bottom-up factors, but we are aware of other factors controlling primary production. It has been a key question in ecology whether primary production is controlled by nutrient and/or light (bottom-up), or by grazers (top-down). According to Tilman's resource competition theory (Tilman et al., 1982), those species which have either the lowest requirement for the limited resource (light or nutrients) or the highest ability to utilize it, will succeed in competition. Thus, a change in the resource will potentially change the species composition of the phytoplankton community (Tilman et al., 1982). Worm et al. (2002) conclude that bottom-up and top-down factors are greatly dependent on each other, but a eutrophic system such as the Baltic Sea, is supposed to be more strongly controlled by bottom-up factors (Lotze et al., 2001).

2. AIMS OF THE THESIS

This thesis aims at increasing the knowledge about factors controlling the occurrence and distribution of toxic cyanobacterial blooms, a knowledge crucial for predicting toxic blooms. The approach was to investigate the interactive effect of radiation (PAR and UVR) and nutrients (N, P) on the performance of bloom-forming cyanobacteria from the Baltic Sea.

The specific aims of this thesis are:

1. To detect and describe strain-specific differences of *Nodularia spumigena* in the response to UV-B radiation. (Paper I)

   In Paper I, the UV-B tolerance of four strains of *N. spumigena*, isolated from the Baltic Sea, was investigated in the laboratory. The working hypothesis was that there is an intra-specific variation in the response of *N. spumigena* to UV-B radiation. Parameters were chosen to elucidate UV-B treatment effects on photosynthesis and growth. Therefore, the variables measured included growth rate, photosynthetic capacity (fluorescence), photosynthetic pigments, and content of MAAs.

2. To improve our understanding of factors controlling bloom dynamics of *Nodularia spumigena*. (Paper II)

   In Paper II, we investigated if ambient solar radiation and nutrient limitation interact in their effects on the performance of *N. spumigena*. We hypothesized an additive negative effect of radiation when nutrient conditions are limiting. A two-factor outdoor experiment was designed with specific growth rate, cell concentrations of MAAs, and photosynthetic pigments as response variables. We also recorded heterocyst frequency, cell size, and particulate carbon (POC), particulate nitrogen (PON), and particulate phosphorus (POP). Radiation treatments were exposure to PAR and PAR+UV-A+UV-B (PAB), and nutrient treatments were NP (f/2 medium), –N (f/2 medium without nitrate) and –P (f/2 medium without phosphate).

3. To improve our understanding of factors controlling production and release of the toxin nodularin. (Paper III)

   In Paper III we investigated the interactive effects of radiation, nutrient limitation, and species composition on the accumulation and release of nodularin. We performed one laboratory experiment and two outdoor experiments. In addition, we investigated if the presence of *N. spumigena* would have an allelopathic effect on the specific growth rate of the co-existing *Aphanizomenon* sp. Radiation treatments were exposure to PAR and PAR+UV-A+UV-B (PAB), and nutrient treatments were NP (f/2 medium), –N (f/2 medium without nitrate) and –P (f/2 medium without phosphate). Species treatments were Nod (*N. spumigena*), Apha (*Aphanizomenon sp.*) and NodApha (*N. spumigena* in presence of *Aphanizomenon* sp.). Variables measured were intracellular nodularin, extracellular nodularin and specific growth rate of *Aphanizomenon* sp.

4. To improve our understanding of factors controlling the succession during the diazotrophic cyanobacterial blooms in the Baltic Sea. (Paper IV).

In Paper IV we tested the interactive effects between radiation and nutrient limitation, and how the presence (e.g. competition) of *N. spumigena* will affect *Aphanizomenon* sp. and vice versa. Radiation treatments were exposure to PAR and PAR+UV-A+UV-B (PAB), and nutrient treatments were –N (f/2 medium without nitrate) and –P (f/2 medium without phosphate). Species composition treatments for *Nodularia spumigena* are NOD (monoculture of *N. spumigena*), MNOD (*N. spumigena* in mixed culture with *Aphanizomenon* sp.). Species composition treatments for *Aphanizomenon sp.* are APHA (monocultures of *Aphanizomenon* sp.) and MAPHA (*Aphanizomenon* sp. in mixed cultures with *N. spumigena*). Response variables measured were specific growth rate, cell concentrations of MAAs, and photosynthetic pigments. We also recorded heterocyst frequency, particulate carbon (POC), particulate nitrogen (PON), and particulate phosphorus (POP).

3. COMMENTS ON THE METHODOLOGY

The difficulties that you encounter when dealing with radiation in the laboratory, are to obtain ecologically relevant radiation spectra, ratios and intensities. The ambient radiation intensities of UV-B, UV-A, and PAR that phytoplankton encounter in surface water is decreasing with depth (Table 1). Ambient solar spectra are of course preferable, but not always possible.

It could be argued that the UV-B levels we used in the laboratory were too low when compared with the levels that can be expected to occur at the water surface in the Baltic Sea, or even too high if we consider a bloom around 5 m depth. Despite our efforts to create an ecologically relevant light regime, our laboratory experiment (like all experiments using UV lamps) will remain more or less mechanistic due to technical difficulties in mimicking the solar spectrum over a surface large enough to allow for replicate samples. We used realistic ratios between PAR and UV-B, which is relevant for studies of UV-B effects, since DNA repair mechanisms after UV-B damage are dependent on these ratios (Karentz et al., 1991).

Table 1. The radiation intensities of UV-B, UV-A, and PAR measured, with broadband sensors in mid-summer 2008 at Askö Laboratory, Sweden.

Depth	**UV-B (Wm^{-2})**	**UV-A (Wm^{-2})**	**PAR (μmol photons m^{-2} s^{-1})**
In air	1.1	20	1470
Surface	0.7	12.4	720
0.5 m	0.06	5.2	
1 m	0.004	1.5	518

In Paper I we measured the chlorophyll fluorescence in order to assess the effect of radiation treatments on the photosynthetic capacity. Measurements of chlorophyll fluorescence give information about the efficiency of PSII and hence the photosynthetic capacity. Thus, any damage in PSII will result in a decrease of the maximum quantum yield of photosynthesis, F_v/F_m (Maxwell and Johnson, 2000). For the procedure and equipment see section 2.3 in Paper I.

In Paper III the results from the two outdoor studies were conflicting; the interactive effect on the nodularin production was significant in Experiment C but not in Experiment B. The major difference between the two outdoor experiments was the seasonal timing: Experiment B was conducted from 5 July with decreasing day-length (1 h) and radiation intensity during the experimental period. In Experiment C, on the contrary, the radiation intensity and day-length increased (1 h), during the experimental period. This was reflected in a higher daily average dose in PAR, UV-A and UV-B during the last 5 days of Experiment C compared to Experiment B.

Biological effects of UVR are generally a function of wavelength; therefore, they are best quantified with a spectral biological weighting function (BWFs). BWFs are sets of spectral weights that account for the wavelength dependency of the photoinhibition and properly scale the exposure spectra to the effective biological response and are usually obtained from polychromatic exposures (for reviews see Cullen and Neale, 1997; Neale, 2000). Photoinhibition is likely to be a function of both UVR and the ratio between UVR and PAR. A good model of spectral dependence is particularly important for studies of aquatic photosynthesis, because both UVR and the ratio UVR:PAR change with depth in the water column. To be able to understand to what extent the diminishing ozone layer on the Nordic hemisphere would have on *N. spumigena,* determination of BWFs were done in 2009 (Mohlin et al., unpublished). We studied the effects of P-limitation on carbon fixation in three strains of *N. spumigena* under two different radiation exposures; high PAR: 250 µmol photons m^{-2} s^{-1} and low PAR: 50 µmol photons m^{-2} s^{-1}. Vulnerability of photosynthesis to UVR was estimated using BWFs for the inhibition of photosynthesis and a model that predicts primary productivity under PAR and UVR exposures. As appropriate for predicting the response to in situ irradiance, BWFs were derived from polychromatic experimental treatments in which UVR and PAR from a solar simulator (xenon) lamp was varied using long-pass cut-off filters. The results show a strain-specific difference in sensitivity in the UV-spectrum and the sensitivity increased in P-deplete growth conditions.

4. SUMMARY OF RESULTS

4.1. *PAPER I: INTRASPECIFIC VARIATION*

Intraspecific differences were observed both regardless of treatment and as a result of UV-B exposure. The only consistent UV-B effect on all strains was significantly higher MAAs content at the end of the experiment. Most negative effects of UV-B were found for the maximum quantum yield (F_v/F_m), mainly in the beginning of the experiment. One out of four strains showed a lower growth rate at UV-B exposure. For photosynthetic pigments, either no UV-B effects were found, or the concentration of pigments increased.

Table 2. Summary of significant treatment effects (PAR + UV-A vs. PAR + UV-A + UV-B) on measured variables during the experiment. Numbers denote days when significant ($p < 0.05$) treatment effects were observed (ANOVA, $p < 0.05$). (+) denotes a higher value and (−) a lower value in the UV-B treatment.

Strain	**F_v/F_m**	**Cells**	**Growth**	**Chl *a***	**Carotenoids**	**MAAs**
KAC 11	2 (−)			8 (+)	8 (+)	8 (+)
KAC 64	4 (+)			8 (+)	8 (+)	8 (+)
KAC 7	2 (−), 4 (−), 8 (−)					8 (+)
KAC 66	2 (−)	4 (−), 8 (−)	0–8 (+), 4–8 (+)	8 (+)	8 (+)	8 (+)

4.2. *PAPER II: INTERACTION OF RADIATION AND NUTRIENT LIMITATION*

The specific growth rate of *N. spumigena* was surprisingly positive despite changes in solar radiation and nutrient conditions. Of interest, but not of surprise, was the lack of effect of nitrogen limitation on the specific growth rate, but there was an overall effect of phosphorus limitation on variables measured. Effects on variables measured are summarized in Table 2. The table does not show the individual concentrations of photosynthetic pigments, POC, PON, or POP. Those variables can be found in Paper II.

Table 3. Summary of most interesting results from Paper II. Radiation treatments are exposure to PAR and PAR+UV-A+UV-B (PAB), respectively, and nutrient treatments are NP (f/2 medium), -N (f/2 medium without nitrate) and -P (f/2 medium without phosphate). Particulate organic carbon (POC), particulate organic nitrogen (PON), particulate organic phosphorus (POP), chlorophyll *a* (chl *a*), mycosporine-like amino acids (MAAs).

Variable	**General results**
Specific growth rate	Although overall positive regardless of treatment, there was a nutrient effect; lowest in –P
Heterocyst cell^{-1}	Nutrient effect; more heterocysts in –N
Cell size	Interactive effect ; under PAB cells are smallest in –N, under PAR cells are small in both –N and –P
POC:PON	Interactive effect; in −P, the ratio was significantly higher in PAR compared to PAB, but in −N and NP, no effect of radiation was found. Under PAR, the ratio was higher for −P compared to NP.
POC:POP	Nutrient effect; the ratio was higher in −P compared to −N and NP.
PON:POP	Interactive effect; the ratio was higher in −P compared to −N and NP, irrespective of radiation treatment. In PAB the ratio was significantly lower in −N compared to NP.
MAAs (abs units cell^{-1})	For total MAAs, no significant interaction effect between radiation and nutrient limitation was observed. Cells exposed to PAB had significantly higher MAAs concentrations in −P compared to −N (nutrient effect). Under −P conditions, significantly more MAAs were found in PAB compared to PAR (radiation effect).
Chl *a* (ng cell^{-1})	Under PAR, significantly less chl *a* was found in −P compared with −N, and a radiation effect was observed in the NP and −N treatments where PAB-treated cells showed lower concentrations compared to PAR-treated cells.
Photosynthetic pigments (ng cell^{-1})	Lowest concentrations were observed under −P conditions, higher concentrations in cells exposed to PAR compared to PAB-treated cells. Individual pigments showed some differences with respect to the different radiation and nutrient treatments.

4.3. *PAPER III: A MULTIFACTORIAL APPROACH ON THE PRODUCTION AND RELEASE OF NODULARIN*

Highest intra- and extracellular nodularin concentrations were observed in –N, when shielded from UVR. Conversely, the lowest intracellular nodularin concentrations were found under phosphorus limitation under UVR. The presence of *Aphanizomenon* sp. did neither increase the intracellular nodularin production, nor the release of nodularin. The presence of *N. spumigena* had no impact on the specific growth rate of *Aphanizomenon* sp., irrespective of radiation and nutrient treatments. A significant correlation between intracellular nodularin and extracellular nodularin ($r^2 = 0.4132$, Pearson correlation=0.643) indicated that accumulation and release of nodularin were showed similar response, increased intracellular nodularin resulted in increased extracellular nodularin.

Table 4. Summary of results from Paper III. Radiation treatments are exposure to PAR and PAR+UV-A+UV-B (PAB), respectively, and nutrient treatments are NP (f/2 medium), -N (f/2 medium without nitrate) and -P (f/2 medium without phosphate). Species treatments were Nod (*N. spumigena*), Apha (*Aphanizomenon sp.*) and NodApha (*N. spumigena* in presence of *Aphanizomenon* sp.).

Experiment	**Treatment factors**	**Variable**	**General results**
A Laboratory	Radiation Nutrients	Intracellular nodularin	Nutrient effect: highest intracellular nodularin concentrations in –N and NP compared to –P, (–N=NP > –P)
B Outdoor	Radiation Nutrients	Intracellular nodularin	Nutrient effect: highest intracellular nodularin concentrations in –N and NP compared to –P, (–N=NP > –P)
C Outdoor	Radiation Nutrients Species	Intracellular nodularin	Interactive effect, radiation and nutrient: Higher concentration in –NPAR compared to –NPAB, –P PAR and –PPAB (–NPAR > –N PAB > –P PAB=–PPAR) Interaction effect, radiation and presence of *Aphanizomenon* sp.: Higher concentration in NodPAR compared to NodPAB, NodAphaPAR and NodAphaPAB (NodPAR > NodPAB=NodAphaPAR=NodApha PAB)
		Extracellular nodularin	Interactive effect, nutrient and presence of *Aphanizomenon* sp. Highest concentration (e.g. release of nodularin) was found in Nod –N PAR compared to all other treatments.

	Specific growth rate of *Aphanizomenon* sp.	There was no effect of treatment

4.4. *PAPER IV: FACTORS CONTROLLING THE SEASONAL SUCCESSION*

Our results indicate an interspecific difference regarding nitrogen versus phosphorus limitation. *N. spumigena* had a lower specific growth rate in -P compared to –N, and for *Aphanizomenon* sp. the results were opposed. The presence of did not affect *Aphanizomenon* sp. negatively however, the growth rate of *N. spumigena* was stimulated by the presence of *Aphanizomenon* sp.. Furthermore, *N. spumigena* had a higher specific growth rate compared to *Aphanizomenon* sp., regardless of treatment. In the variables measured *N. spumigena* seemed better adapted to both -N and –P in interaction with UVR than *Aphanizomenon* sp.

Table 5. Summary of results from Paper IV. Radiation treatments were exposure to PAR and PAR+UV-A+UV-B (PAB), and nutrient treatments were –N (f/2 medium without nitrate) and –P (f/2 medium without phosphate). Species composition treatments for *Nodularia spumigena* are NOD (monoculture of *N. spumigena*), MNOD (*N. spumigena* in mixed culture with *Aphanizomenon* sp.). Species composition treatments for *Aphanizomenon sp.* are APHA (monocultures of *Aphanizomenon* sp.) and MAPHA (*Aphanizomenon* sp. in mixed cultures with *N. spumigena*). Particulate organic carbon (POC), particulate organic nitrogen (PON), particulate organic phosphorus (POP), chlorophyll *a* (chl *a*), and mycosporine-like amino acids (MAAs).

Variable	**General results** ***Aphanizomenon* sp.**	***Nodularia spumigena***
Specific growth rate	Interaction effect; radiation and nutrient. Highest in –P, regardless of radiation treatment (PAB–P=PAR–P). In –N, lower in PAB compared to PAR (PAR–N>PAB–N). In PAB, lower in –N compared to –P (PAB–N<PAB–P).	Interaction effect; nutrient and competition. Higher in MNOD–N compared to MNOD –P. In –N, higher in MNOD compared to NOD In –P, no difference between MNOD and NOD
Heterocyst cell^{-1}	Nutrients effect Higher frequency in –N compared to –P	Nutrient effect Higher frequency in –N compared to –P Species composition effect Lower in MNOD compared to NOD.
POC:PON	Nutrient effect Higher ratio in –P compared to –N	Nutrient effect Higher ratio in –P compared to –N Radiation effect Higher ratio in PAB compared to PAR
POC:POP	Interactive effect; radiation and nutrient In –N, irrespective radiation treatment In –P, there was an additional effect of radiation; higher in PAB –P compared to PAR-P.	Nutrient effect Higher ratio in –P

PON:POP	Nutrient effect Higher ratio in –N compared to –P	Nutrient effect Higher ratio in –P compared to –N
MAAs (abs units cell^{-1})	No treatment effects	Interactive effect; radiation and nutrient In PAR, higher in -P compared to –N In PAB, no difference between –N and -P. Furthermore, In –P, higher in PAR compared to PAB In –N, no difference between PAR and PAB
Chl *a* (ng cell^{-1})	No treatment effects	Radiation effect Higher in PAR compared to PAB Nutrient effect Higher in –N compared to -P
Photosynthetic pigments (ng cell^{-1})	No treatment effects	Nutrient effect Higher 4-ketomyxoxanthophyll concentration –N compared to –P The same pattern for carotenoids
Phycocyanin (pg cell^{-1}),	No treatment effects	No treatment effects

5. DISCUSSION

5.1. *EFFECTS OF UV-B RADIATION AND INTRASPECIFIC VARIATIONS*

The first paper (Paper I) revealed the strain specific differences in the response of *N. spumigena* to UV-B radiation.

We hypothesised:

No negative effect of UV-B on the specific growth rate of *N. spumigena*.
There would be species-specific differences in response to UV-B radiation.

5.1.1. *UV-B, THEY DON´T CARE!*

The results in Paper I show that *N. spumigena* is a species that is not generally negatively affected by moderate levels of UV-B radiation. Despite the fact that *N. spumigena* is considered one of the most important phytoplankton species in the Baltic Sea, only one study has considered the impact of UV-B on this surface-blooming species (Sinha et al., 2003), and only in terms of the presence of mycosporine-like amino acids (MAAs).

The maximum quantum yield, (F_v/F_m) has been shown to be a sensitive non-specific marker of UV-B exposure (Cordi et al., 1997). For all strains, this value was low in the beginning of the experiment; however, the photosynthetic activity was high enough to sustain a positive growth rate among the strains. A decrease in the F_v/F_m ratio could reflect either dynamic or chronic photoinhibition (Hanelt et al., 2003). Dynamic photoinhibition is a regulative mechanism for protection against excessive radiation (Hanelt et al., 2003, and references therein), a mechanism that most certainly caused the lowering of F_v/F_m in our study, since no negative impact on growth was observed. For the genus *Anabaena*, Kumar et al. (2003), observed that although growth was inhibited at UV-B intensities as low as 0.2 W m^{-2}, in the presence of white light, a positive growth was found up to 2.4 W m^{-2}. The positive role of photoreactivation by PAR and UV-A in UV-B-damaged cyanobacterial cells has also been shown by Blakefield and Harris (1994) and Ivanov et al. (2000). Although we never tested the impact of photoreactivation, the PAR and UV-A levels used in our study were high enough to repair or reduce the damage of the UV-B-damaged cells (cf. Kumar et al., 2003).

UV-B-treated cells, in all strains, showed a significant accumulation of total MAAs, indicating that UV-B had an important role in the induction or accumulation of MAAs. Sinha et al. (2001, 2003) found that UV-B played a vital role in the accumulation of MAAs in cyanobacteria; UV-B was shown to be most efficient in the induction of shinorine (Portwich and Garcia-Pichel, 2000). Based on spectra and retention time and other studies (Sinha et al.,

2003) we concluded that the different MAAs present in our *Nodularia* strains were shinorine and porphyra-334 (Fig. 8).

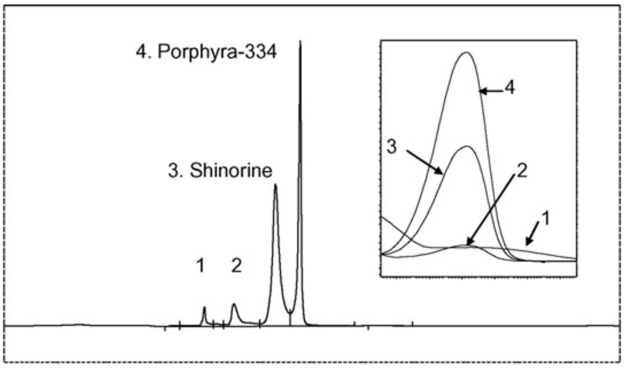

Fig. 8. High-pressure liquid chromatography (HPLC) chromatogram showing the different mycosporine-like amino acids found in *Nodularia spumigena*, together with their normalized absorbance spectra.

On a cell basis, the MAAs concentrations provide a sunscreen factor of 0.11–0.26 at 320 nm (Garcia-Pichel and Castenholz, 1993), implying that at least one out of ten photons are prevented from hitting their target. However, as the authors point out, a colonial growth form (like in our case) will provide a substantially higher sunscreen effect. In our study we cannot conclude that the accumulation of MAAs was the sole factor enabling the cells to retain (or increase) their photosynthetic activity and growth in the presence of UV-B. The significantly lower F_v/F_m in KAC7 does not support the theory that MAAs provide a protection against UV-B. On the other hand, support is found in the F_v/F_m data for the other three strains.

Although MAAs have been described as giving protection against UV-B damage through their sunscreen properties (Garcia-Pichel and Castenholz, 1993; Shick and Dunlap, 2002), there have been suggestions that this is not their only role. Dunlap and Yamamoto (1995) proposed that some MAAs may act as antioxidants, thereby preventing UV-induced cellular damage from active oxygen species. UV-B has been shown to increase the production of reactive oxygen species in *Anabaena* sp. (He and Häder, 2002). In addition, MAAs have been proposed to have a role as osmolytes (Shick and Dunlap, 2002 and references therein). In *Chlorogloeopsis* the steady-state concentration of MAAs was positively related to environmental salinity: the osmotically induced mycosporine-glycine was enhanced by UV-B, and shinorine accumulated due to UV-B only (Portwich and Garcia-Pichel, 1999). Although we did not test any variables other than light on the induction and accumulation of MAAs, our study also showed that shinorine accumulated due to UV-B.

5.1.2. *STRAIN SPECIFIC DIFFERENCES, SHOULD WE CARE?*

The results from Paper I suggest that *N. spumigena* is a species that is not negatively affected by moderate UV-B intensities, since no lasting overall negative effects of the UV-B treatment were evident. However, assuming this study was performed on one of the strains, for example KAC66, the conclusion would have been different. For this strain, significant negative UV-B effects were observed for cell numbers and growth rate and although not statistically significant, also the F_v/F_m was lower by Day 8. For photosynthetic pigments the conclusion would have been that the pigments myxoxanthophyll and canthaxanthin are not affected by UV-B. On the other hand, conclusions drawn from KAC64 points in a different direction. No or positive UV-B effects were found for cell numbers, growth rate and F_v/F_m. In this strain, the pigments myxoxanthophyll and canthaxanthin increased in the UV-B treatment but 4-keto- myxoxanthophyll-like pigment, now defined as 4-keto-myxoxanthophyll (Schlüter et al., 2008), and b-cryptoxanthin-like remained unaffected. Interestingly, 4-keto-myxoxanthophyll showed the largest variation among strains. Considering the fact that this pigment has been suggested as a biomarker for both *N. spumigena* and nodularin in the Baltic Sea (Schlüter et al., 2004, 2008), further studies are needed to elucidate how its content varies between strains and with environmental conditions. An intraspecific variation in the requirement of nutrients for optimal growth was shown for the dinoflagellate *Gymnodinium catenatum* (Bolch et al., 1999). It is possible that also our variation in growth rate was a consequence of strain-specific requirements for nutrients.

To answer the question I would say, yes we should care!

Thus, we conclude that studying one strain is not enough to draw conclusions about a species response to environmental stress. Burkholder and Glibert (2004) found that ‘‘different and often opposite interpretations at the species level, would have resulted from consideration of individual strains’’. In a 5-year literature survey of the harmful algal literature they conclude that intraspecific variation is commonly overlooked in characterizations of harmful algal species and they stress the importance of including multiple strains in research studies.

5.2. *NUTRIENT LIMITATION + UVR, THEY DON´T CARE!*

In Paper II, we investigated if ambient solar radiation and nutrient limitation have interactive effects on the performance of *N. spumigena*

We hypothesized:

Ambient UVR would have an additive negative effect when nutrients are limiting

N. spumigena had surprisingly a positive growth rate, despite changes in solar radiation and nutrient conditions (Paper II). We expected that ambient UVR used in this outdoor experiment, compared to artificial UVR used by Wulff et al. (Paper I), would have a significant impact on the growth rate of *N. spumigena*. In addition, we expected a additive effect of radiation when the nutrient conditions were limiting (Wulff et al. 2000). Although strain-specific differences in UV-B tolerance have been observed, we conclude that *N. spumigena* is a species that is not negatively affected by moderate levels of UV-B (Paper I). Another explanation might be that they are already stressed by the high PAR. In other studies (Lehtimäki et al., 1994), where growth rate was stimulated by increased radiation, the highest radiation intensities used were less than 20% of the radiation intensities in Paper II. However, despite an overall positive growth rate regardless treatment, there were interactive effects of radiation and nutrient limitation on important functions for fitness of *N. spumigena*.

P-limitation had a negative impact on growth rate in this study. According to Goldman et al. (1979), phytoplankton deprived of phosphorus during growth have PON/POP ratios greater than 30:1, whereas phytoplankton deprived of nitrogen during growth have PON/POP ratios less than 10:1. In Paper II, the POP/PON ratios found in –P treatment indicate severe phosphorus limitation (Healy, 1975; Paasche and Erga, 1988). The low PON/POP (−N and NP treatments) rather indicated internal storage of P than an indication of nitrogen limitation since nitrogen limitation is not expected in the diazotrophic *N. spumigena*. This is supported by the fact that the POC/PON ratio did not indicate nitrogen limitation.

When light energy is the most limiting factor, cells adjust chemically and physically to capture more light, but when nutrient deficiency is the growth-limiting factor, pigment concentrations depend upon precursors for pigment synthesis (Rosen and Lowe, 1984). In Paper II, pigment concentrations were generally lower under phosphorus limited conditions. Latasa and Berdalet (1994) studied phosphorus starvation on the dinoflagellate, *Heterocapsa* sp. The authors hypothesize that when phosphorus is depleted the cells can no longer synthesize RNA, and thus, pigment synthesis stops (no transcription of DNA into RNA). It has been shown that concentrations of nitrogen-containing pigments like chl *a* decrease more than carotenoids under low nitrogen availability (e.g., Latasa and Berdalet, 1994; Staehr et al.

2002). No such decrease was observed in Paper II, that is, no difference between the NP and –N treatments was found. This suggests that *N. spumigena* was not N-limited due to their ability to fix atmospheric nitrogen.

Photosynthesizing diazotrophic cyanobacteria encounter ecophysiological problems because oxygen (O_2) evolved during photosynthesis inhibits nitrogenase, the enzyme responsible for N_2-fixation (Bothe, 1982). To solve the problem some genera form O_2 deplete heterocysts where nitrogenase is located (Paerl, 2000). The frequency of these specialized cells is usually indicative of the N_2-fixation activity level in cyanobacterial filaments. N_2-fixation activity was not measured in our studies, but it has previously been shown that changes in nitrate concentrations do not affect nitrogenase activity in *Nodularia* spp. (Sanz-Alferez and del Campo, 1994, and references therein). Further, Vintila and El-Shehawy (2007) showed that the genes involved in heterocyst differentiation and N_2-fixation in *N. spumigena* strain AV1 are continuously expressed and not affected by changed concentrations of the nitrogen source. Others have reported that the formation of these specialized cells is enhanced under nitrogen limited conditions (Paerl, 2000), explaining the higher heterocyst frequency in −N compared to –P (Paper II). Additionally, it has been shown that low phosphorus availability may suppress heterocyst formation, with a subsequent reduction in N_2-fixation activity and hence, cellular nitrogen content (Healey, 1973; Layzell et al., 1985). In our study, there was a lower heterocyst frequency and lower PON in −P PAR compared to −N PAR. The results from Paper II show that heterocyst frequency is not a good indicator of nitrogen limitation in the surrounding water since the frequency vary depending on environmental conditions.

Despite the negative effect of −P, the growth rate was still positive (Paper II); the reason might be a capability of storing phosphorus for future phosphorus scarcity. Similar results was found by Degerholm et al. (2006) and are further supported by a study by Vahtera et al. (2007b), where a phosphorus limitation (PO_4^{3-}, <0.5 μM; NO_3^- + NO_2^-, 0.5–2.0 μM) showed the most distinct increase in PON/POP caused by decrease in cellular phosphorus stores (TEM images). The authors concluded that PON/ POP of *N. spumigena* has high plasticity, and efficient growth can occur at DIN/DIP, far exceeding the Redfield ratio of 16:1 (up to 80:1). The high POC/POP value in −P treatment compared to −N and NP treatments (paper II) indicate that internal phosphorus storages are used to maintain a low and positive growth rate (Walve and Larsson, 2007). DIP was quickly vanished within 1 day after addition of new medium in the nutrient treatments. Also Vathera et al. (2007b) and Roleda et al. (2008) noticed a rapid decline in DIP concentrations in phosphorus limited treatments.

As we observed in Paper I, UV-B is responsible for the induction and/or accumulation of MAAs in *N. spumigena*. In Paper II the exposure to UVR did not increase MAAs except in −P. Thus, apparently ambient PAR intensities in our study were so high that additional UVR did not have any further impact on the MAAs concentrations. Compared to radiation conditions in our laboratory study (Paper I) and others (Portwich and Garcia-Pichel 2000; Sinha et al., 2003), the ambient PAR intensity was 10 to 20 times higher. Our results stress the fact that ecologically relevant UVR studies should be performed under ambient solar radiation. We expected lower MAAs concentrations in –N conditions because those compounds contain nitrogen (Karentz, 2001). This was not the case, obviously the cells fix enough atmospheric nitrogen (or use a different nitrogen source) to account for the MAAs production. Despite the cost of N_2-fixation and MAAs production, the overall fitness reflected as specific growth rate was not lower in the –N treatment compared with the NP treatment. Interestingly and unexpectedly, significantly higher MAAs concentrations were found in UV-exposed cells under phosphorus limitation, although the cell density was higher compared with the PAR treatment. Possibly, the higher MAAs concentration was due to the larger cell volume (25% larger cells in PAB compared to PAR).

5.3. COMBINED EFFECTS ON ACCUMULATION AND RELEASE OF NODULARIN, SHOULD ANYONE CARE?

In Paper III we investigated if ambient radiation, nutrient limitation and the presence of *Aphanizomenon* would have interactive effects on the production and/or release of the toxin nodularin. We investigated if the presence of *N. spumigena* would have an allelopathic effect on the specific growth rate of the co-existing *Aphanizomenon* sp.

We hypothesized:

Nodularin production would be negatively affected in –N and UVR would have additive negative effects.

Extracellular nodularin would increase in presence of *Aphanizomenon* sp.

N. spumigena would have a negative effect on the specific growth rate of the co-existing *Aphanizomenon* sp.

We expected lower intracellular nodularin concentration in –N, because nodularin is a nitrogen-rich compound, 8 mol N (mol nodularin)$^{-1}$ (Stolte et al., 2002). Surprisingly, no such correlation was found: highest concentrations were observed in –N. Stolte et al.(2002) did not find any significant differences in nodularin production rate due to different nitrogen source (NO_3^-, N_2 and NH_4^+), implying that *N. spumigena* is able to fix enough atmospheric N_2 to account for the nodularin production. This is further supported by Paper II where POC:PON did not indicate that *N. spumigena* was nitrogen limited even under severe N-limited growth conditions.

Earlier results from single-factor experiments are contradictory regarding intracellular nodularin with respect to phosphorus conditions. There are observations of increased nodularin in phosphorus limited conditions (Granéli et al. 1998) while others report increased nodularin in phosphorus replete conditions (Lehtimäki et al. 1997) and finally no impact of phosphorus on nodularin accumulation (Repka et al. 2001; Stolte et al. 2002; Vuorio et al., 2005; Lagus et al., 2007). Our study show that the intracellular nodularin concentration was lowest in -P and that UVR had an additional reducing effect. The results suggest that ambient radiation and nutrient conditions do have significant interactive effects on the accumulation of nodularin and stress the importance of multi-factorial approaches. Further support for multi-factorial approaches were provided by Hobson et al. (1999) and Hobson and Fallowfield (2003); whereby combined effects of radiation and salinity (Hobson et al., 1999) and of temperature and radiation (Hobson and Fallowfield, 2003) on the nodularin production were suggested.

It has been suggested that most of the nodularin produced remain within the cells (Repka et al. 2004) and is only released upon cell lyses (Park et al., 1998; Sivonen and Jones, 1999; Suikkanen et al., 2004). Others have reported that environmental conditions seem to affect the release of nodularin and other cyanobacterial toxins. In single-factor experiments the release has been reported to increase with increased phosphate concentrations (Lehtimäki et al. 1997), increased radiation (Sivonen, 1990; Lehtimäki et al. 1997) and increased temperature (Sivonen, 1990). Only a few studies have investigated the effect of combined environmental conditions. Hobson and Fallowfield (2003) showed that interaction of temperature and high radiation increased the release of nodularin into the surrounding water and they also observed that the release originated from intact cells, not from degraded cells. Our results suggest that ambient radiation and nutrient conditions have significant interactive effects on the release of nodularin. To our knowledge, few have studied bloom dynamics and nodularin concentrations of *N. spumigena* in the field. In filtered water samples collected three days after the peak of the bloom, a dramatic decrease of nodularin due to water mixing or degradation by the natural microbial community was observed (Mazur-Marzec et al. 2006a). The maximum nodularin concentration where found in *N. spumigena* blooms of moderate intensity.

The correlation between intracellular nodularin and extracellular nodularin (Paper III) indicated that accumulation and release of nodularin were dependent on each other. Jonasson (2006) suggested that when intracellular nodularin concentration reaches a certain threshold, the excess nodularin will either be released or degraded within the cell. We could speculate that those threshold intracellular concentrations vary with respect to environmental conditions. Thus the release of extracellular nodularin is only related to those threshold values and not to environmental conditions.

The presence of *Aphanizomenon* sp. did not increase the release of nodularin and the growth rate of *Aphanizomenon* sp. was not affected by the presence of *N. spumigena*. That was unexpected since we hypothesized that the presence of *N. spumigena* (e.g. nodularin) would have an allelopathic effect on *Aphanizomenon* sp.. On the contrary, highest extracellular nodularin concentration found was in absence of *Aphanizomenon* sp. Thus, we suggest that the allelopathic effect of nodularin on *Aphanizomenon* sp. could be neglected. This is further supported in a mesocosm study by Engström-Öst et al. (2002). They created an artificial *N. spumigena* bloom in mesocosms and investigated the development of a natural plankton community; the results did not reveal harmful effects of nodularin on the organisms studied. Our results support the suggestion that *N. spumigena* and *Aphanizomenon* sp. occupy different niches (Kononen et al., 1996; Lehtimäki et al., 1997; Vahtera et al., 2007b) rather than perform a chemical warfare. In contrast and further discussed in Paper IV and in Section 5.4., cyanobacterial allelochemicals have been proposed to have an ecological role in maintaining Baltic cyanobacterial dominance by stimulating the abundance of the same or other species of cyanobacteria, rather than inhibiting the abundance of competitors to cyanobacteria (Suikkanen et al., 2004, 2005, and 2006).

We believe that the photoprotective role of nodularin is negligible; we observed lower nodularin concentrations under UVR compared to PAR. The absorbance spectrum of nodularin peaking at 238 nm (Harada, 1995), could imply a possible photoprotective role of this compound. This is relevant from an evolutionary perspective when UVC still reached the Earth's surface; at present day radiation condition this is no longer of ecological relevance. The lower concentration under UVR could be explained by a degradation of nodularin. It has been shown that both intracellular and extracellular nodularin are degraded by UVR (Twist and Codd, 1997; Mazur-Marzec et al., 2006b).

To answer the question, two answers are needed:

No, *Aphanizomenon* sp. should not care since its presence did not increase the release of nodularin. Thus the potential target species of chemical defence or attach of *N. spumigena* is probably found somewhere else.

Yes, we should definitely care since the nodularin concentration was highly variable depending on treatment. Discussions concerning further increase of nitrogen removal in sewage treatment, should take into account that this may lead to increased toxicity during the *N. spumigena* blooms. The results further suggest that *N. spumigena* may be expected to produce less nodularin in the end of the bloom when phosphate concentrations are very low in the surface water.

5.4. *SEASONAL SUCCESSION; PREFERENCES OR ALLELOPATHY?*

To improve our understanding of factors controlling the succession during the diazotrophic cyanobacterial blooms in the Baltic Sea.

We hypothesized:

Nutrient limitation in interaction with UVR would negatively affect *Aphanizomenon* sp.

The presence of *N. spumigena* would have additive negative effects on the performance of *Aphanizomenon* sp.

The presence of *Aphanizomenon* sp. would not have additive negative effects on the performance of *N. spumigena*.

Even if *N. spumigena* had an overall higher specific growth rate compared to *Aphanizomenon* sp., our results indicate an interspecific difference regarding nitrogen versus phosphorus limitation. *N. spumigena* had a lower specific growth rate in –P compared to -N and for *Aphanizomenon* sp. the results were opposite. This is consistent with a study by Lehtimäki et al. (1997), who suggested that *A. flos-aquae* has lower phosphorus demand compared to *N. spumigena*. Kononen et al. (1998) suggested that this species difference in utilizing surplus phosphorus could be explained by different nutrient uptake kinetics. It has been shown that *N. spumigena* has higher affinity for phosphorus than *A. flos-aquae* (K_s= 0.016 and 0.048 pM, respectively) (Wallström et al. 1992), but of explicable reasons this was not obvious in –P treatment in our study. We observed that *N. spumigena* efficiently absorbed all phosphorus after addition of new –N media while minor amounts were absorbed by *Aphanizomenon* sp., the observations indicate their different affinity for phosphorus. Despite the fact that *N. spumigena* absorbed phosphorus from the media, POC:POP remained high, most probably because of the high growth rate and the consumption of the internal storage. The opposite situation was found in the slow growing *Aphanizomenon* sp.; excess phosphorus in –N treatment was not absorbed from the media and a low POC:POP indicated that the internal storage of phosphorus was not consumed. The POC:POP ratio further indicates that both species are under severe phosphorus limitation, however, *Aphanizomenon* sp. have a lower ratio and more phosphorus stored per cell than *N. spumigena*. We suggest that these results, together with the lower specific growth rate in –N compared to –P for *Aphanizomenon* sp., indicate a lower efficiency in N_2 fixation of *Aphanizomenon* sp. rather than having a lower affinity for phosphorus compared to *N. spumigena*. This is consistent with the higher growth rate in –P with surplus of N.

We did not expect *N. spumigena* to be nitrogen limited based on previous results (Paper II) and this was the case (POC:PON <7). The opposite was found for *Aphanizomenon* sp. (POC:PON ratio >7) and their contrasting results could be explained by a previous study where Stal et al. (2003) reported a higher efficiency of N_2 fixation of *N. spumigena* than *Aphanizomenon* sp. The PON:POP found in the –P treatment (our study), indicated severe phosphorus limitation (Healey, 1975; Paasche and Erga, 1988): 40 for *N. spumigena* and 20 for *Aphanizomenon* sp.. The negative impact of –P was reflected in the specific growth rate of *N. spumigena* and our results corroborate with previous studies (Lehtimäki et al., 1997; Paper II). Vahtera et al. (2007b) found that an increase in PON:POP is caused by decrease in cellular phosphorus stores (TEM images). Furthermore, Walve and Larsson, (2007) found that a high POC:POP indicated that internal phosphorus storages were used for growth. According to the POC:POP ratio in our study *N. spumigena* was phosphorus limited even in –N where DIP was added in surplus (10 µM), but not reflected in the specific growth rate. We noticed that DIP declined from ~5µM to 0.3-1 µM within one day after addition of new medium in the nutrient treatments. Also, Vahtera et al. (2007b) and Roleda et al. (2008) noticed a rapid decline in phosphate concentrations in DIP-replete treatments. The rapid decline must be due to the high specific growth rate in –N treatment and a high consumption of DIP. Vahtera et al. (2007b) concluded that PON:POP of *N. spumigena* has high plasticity and efficient growth can occur at DIN:DIP far exceeding the Redfield ratio of 16:1 (up to 80:1).

As in Paper II, the heterocyst frequency was high in –N, but this time for both *N. spumigena* and *Aphanizomenon* sp. Furthermore, *N. spumigena* and *Aphanizomenon* sp. had lowest heterocyst frequency in –P conditions with excess of DIN. It is known that DIN tends to suppress heterocyst formation (Adams and Duggan, 1999). In a field study, Laamanen et al. (2005) observed that the annual pattern of development of *N. spumigena* had a rather invariable frequency with an abrupt decrease in autumn when DIN increased in the surface layer, these findings corroborate our results. Laamanen et al. (2005) also found that *A. flos-aquae* displayed a more variable annual development pattern that was associated with changes in temperature, increasing biomass and nutrients. They observed that *A. flos-aquae* had lower average heterocyst frequency than *N. spumigena* and corroborate our results with an overall threefold higher heterocyst frequency for *N. spumigena*. Surprisingly, we found that *N. spumigena* had lower heterocyst frequency in presence of *Aphanizomenon* sp. compared to monocultures.

The additional negative effect of UVR under nutrient limitation was obvious in the specific growth rate of *Aphanizomenon* sp. The specific growth rate was significantly lower in –N; however the effect of UVR was not reflected in the MAAs concentration. Previous observations *in situ* have revealed that *Aphanizomenon* filaments are usually evenly distributed down to 10 m, but *N. spumigena* is concentrated near the surface (Niemistö et al., 1989; Kononen et al., 1998). In our study *Aphanizomenon* sp. was not able to avoid radiation by vertical migration in the water column, and the overall high concentration of MAAs per cell indicates that the radiation exposure was high in the PAR treatment. This was reflected in the lack of treatment effect and an increased concentration per cell from initial conditions. Our results, together with previous studies (Niemistö et al., 1989; Kononen et al., 1998) indicate that the vertical positioning in the water column can be a photoprotective strategy of *Aphanizomenon* sp. We observed that there was a difference of vertical positioning in the aquaria when bubbling was interrupted, *N. spumigena* had positive and *Aphanizomenon* sp. had negative buoyancy. The buoyancy of cyanobacteria in general is affected by environmental factors and has markedly different behavior in different species, explaining the occurrence of blooms under various conditions (reviewed by Oliver, 1994). *A. flos-aquae* seem to lose buoyancy when radiation intensities increase (Kromkamp et al. 1986; Oliver and Walsby, 1984).

N. spumigena was not negatively affected by UVR and this is consistent with our previous studies (Paper II). In laboratory studies, the importance of UV-B in the induction or accumulation of MAAs in cyanobacteria has been observed (Portwich and Garcia-Pichel, 2000, Sinha et al. 2003, Paper I, Paper II). In the present study the highest MAAs concentration in *N. spumigena*, was found under PAR and –P in contrast to Paper II, where the highest concentration was found under PAB and phosphorus limitation. Results from both studies confirm our hypothesis that under high ambient PAR additional UVR do not have any further impact on the MAAs concentrations. The lowest MAAs concentration was not found in –N, despite the fact that MAAs is a nitrogen containing compound (Karentz 2001). In accordance with previous studies (Paper I and Paper II), *N. spumigena* fix enough N_2 (or use a different N-source) to account for the MAAs production.

For *N. spumigena* cells in this study, radiation and nutrient effects, respectively, were found for both chl *a* and carotenoids. The higher concentrations in PAR compared to PAB is probably due to a down regulation of the rate of light absorption through a decrease in the concentration of chl *a* and light harvesting pigments (cf. Miskiewicz et al 2000). However, myxoxanthophyll has been suggested to have a photoprotective role (Miskiewicz et al 2000) but in our study, myxoxanthophyll did not increase in the PAB treatment. Although not statistically significant, the phycocyanin concentrations in *N. spumigena* followed the same pattern as chl *a* and carotenoids. Consistent with our results, Collier and Grossman (1992) showed decreased phycobilisome concentrations under phosphorus depleted conditions. Due to fixation of N_2, it is of no surprise that the pigment composition was not affected by the –N treatment. Due to severe phosphorus limitation in the –P treatment, the low pigment concentrations could be explained by a disrupted pigment synthesis (Latasa and Berdalet, 1994). The pigment 4-keto-myxoxanthophyll has been suggested as a biomarker for both *N. spumigena* and nodularin in the Baltic Sea (Schlüter et al., 2004, 2008). In Paper I, this pigment showed the largest variation between different strains of *N. spumigena*. In the present study, 4-keto-myxoxanthophyll was the only pigment being affected by the presence of *Aphanizomenon* sp. why its variation with environmental conditions as well as with co-existing species should be further investigated.

The growth rate of *Aphanizomenon* sp. was unaffected by the presence of *N. spumigena*. The results are consistent with previous observations in a parallel study (Paper III); no correlation between increased nodularin concentration and the specific growth rate of *Aphanizomenon* sp. was found. The temporal succession seen in the Baltic Sea is better explained by the species-specific niches that have been proposed for the two species (Niemistö et al., 1989; Kononen et al., 1996; Vahtera et al., 2007b). *N. spumigena* had higher specific growth rate compared to *Aphanizomenon* sp., regardless of treatment, and most surprisingly the growth rate of *N. spumigena* was stimulated in presence of *Aphanizomenon* sp. As I discussed earlier, it has been suggested that the release of cyanobacterial toxins may play an ecological role in the interspecific competition via stimulating the abundance of the same or other cyanobacterial species in the community, rather than inhibiting the abundance of competitors to cyanobacteria (Suikkanen et al., 2004, 2005). Even if earlier studies have shown that *Aphanizomenon* sp. from the Baltic Sea do not produce toxins (Willén and Mattsson, 1997) more recent studies on isolates of *A. flos-aquae* from the Baltic Sea have detected a neurotoxic amino acid, *β*-N-methylamino-L-alanine (BMAA) (Cox et al. 2005); Jonasson et al (2008). BMAA is a neurotoxic non-protein amino acid produced by most cyanobacteria, has been proposed to cause neurodegenerative diseases on the island of Guam in the Pacific Ocean. BMAA has been related to neurodegenerative diseases among Chamorroan and Canadian patients (Cox et al. 2003; Murch et al. 2004). It may not be this compound that is responsible for the stimulated specific growth rate of *N. spumigena*, but it shows that *Aphanizomenon* spp. produce compounds that can affect other organisms.

To answer the question:

The seasonal succession, with peaks of *Aphanizomenon* sp. followed by peaks of *N. spumigena* is a result from species-specific preferences and/or stimulation by *Aphanizomenon* sp., rather than the allelopathic effect of *N. spumigena*.

CONCLUSIONS AND FUTURE PROSPECTS

How will the future appear with respect to environmental conditions, cyanobacterial blooms, dominating species and toxin production?

Global models, scenarios, and data sets are tools that we have at hand to make predictions of future climate changes. Although difficult to predict with certainty, it has been projected that the surface water temperature will increase about 3ºC to 5ºC during the 21st century in the Baltic area. This scenario will result in a decreasing ice cover period and a consequent prolongation of the growing season (1 to 3 months) (HELCOM 2007; Neumann, 2010). The predicted increase in temperature together with a general increase in annual precipitation will promote a stronger stratification, beneficial conditions for bloom forming cyanobacteria. The climate change is further predicted to increase the UV-B radiation, due to prolonged reductions of the ozone layer (Shindell et al.,1998; Aldhous, 2000; Anonymous 2002; Anonymous, 2006), despite the success of the Montreal Protocol of 1987 in reducing emissions of ozone-destroying chemicals.

Sweden and other countries around the Baltic Sea, through the Helsinki Commission (HELCOM), have agreed to reduce the emissions of nutrients to sustainable levels (HELCOM, 2007). From 1990 to 2000, inputs of phosphorus and nitrogen decreased by 68% and 60%, respectively (HELCOM, 2010). Despite the success in reducing the inputs of nitrogen and especially phosphorus to the Baltic Sea, almost all open basins and coastal waters are still classified as areas affected by eutrophication. The only coastal areas not affected by eutrophication are found in the Gulf of Bothnia (HELCOM, 2010). If the eutrophicated nutrient conditions will continue, where diatoms are promoted to form spring blooms and remove DIN, and the subsequent hypoxic bottom-water will increase DIP, the situation will most certainly continue to supply an advantageous situation for N_2-fixing cyanobacteria during late summer.

After studying the historical and predicted environmental conditions in the Baltic Sea together with our results, I get more and more convinced that the ratio between the major nutrients will decide whether there will be extensive cyanobacterial blooms or a more diverse phytoplankton composition. As long as the N/P ratio can be reduced by a diatom spring bloom to a threshold that counteracts a diverse phytoplankton composition, the N_2-fixing cyanobacteria will remain the “Lords of the Baltic” in the future.

I am also convinced that the ultimate “Lord of Summer blooms”, either *Aphanizomenon* sp. or *N. spumigena*, without hesitation is a question of their respective response to the future scenario of UVR in combination with nutrient conditions. Our results together with previous research stress the fact that under predicted environmental conditions with stronger stratification, low N:P ratios and increased UV-B radiation, *N. spumigena* is the most qualified to win this title. We have shown that this domination over *Aphanizomenon* sp. is not a question of chemical arms (e.g. allelopathy), just a question of a successful adaptation to UV-B and nutrient limitations.

The environmental (e.g. radiation and phosphorus concentrations) conditions on nodularin production of *N. spumigena* should nevertheless be taken into account. Discussions concerning further increase of nitrogen removal in sewage treatment, should take into account that this may lead to increased toxicity during the *N. spumigena* blooms. Our results suggest that the highest nodularin concentrations during a *N. spumigena* bloom may occur during the initiation of the bloom when phosphorus is still present in excess while nitrogen is limited and when filaments are found deeper in the water column not exposed to UVR.

For the future

From our study (Paper I) it is obvious that culture experiments that aim at testing the response of a phytoplankton species to external stressors should be implemented using several strains.

Nodularin production and release from *N. spumigena* is not yet clearly understood. Interactive effects of several different environmental factors on more strains should be included in future outdoor studies to answer these questions. In situ monitoring of intra- and extracellular nodularin during *N. spumigena* bloom relative to ambient nutrient concentration and other environmental factors is needed to support the result of our study.

Furthermore, future studies should include the effect of increased CO_2 and the predicted acidification on the performance and nodularin production of *N. spumigena*. The response of increased CO_2 on the phytoplankton composition in the Baltic Sea should also be emphasized in future studies. The overriding impression of cyanobacteria is their diverse ways of adapting to a wide spectrum of environmental variability (Reynolds, 1987), but will their reputation as a prominent and successful group of organisms remain even in an acidified Baltic Sea?

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REFERENCES

Adams DG, Duggan PS (1999) Heterocyst and akinete differentiation in cyanobacteria. New Phytologist 144:3-33

Aldhous P (2000) Global warming could be bad news for Arctic ozone layer. Nature 404:531-531

Andersson A, Hajdu S, Haecky P, Kuparinen J, Wikner J (1996) Succession and growth limitation of phytoplankton in the Gulf of Bothnia (Baltic Sea). Marine Biology 126:791-801

Andrén T, Sohlenius G (1995) Late Quaternary development of the north-western Baltic Proper -- Results from the clay-varve investigation. Quaternary International 27:5-10

Andrén E, Andrén T, Kunzendorf H (2000a) Holocene history of the Baltic Sea as a background for assessing records of human impact in the sediments of the Gotland Basin. The Holocene 10:687-702

Andrén E, Andrén T, Sohlenius G (2000b) The Holocene history of the southwestern Baltic Sea as reflected in a sediment core from the Bornholm Basin. Boreas 29:233-250

Andrén T, Lindeberg G, Andrén E (2002) Evidence of the final drainage of the Baltic Ice Lake and the brackish phase of the Yoldia Sea in glacial varves from the Baltic Sea. Boreas 31:226-238

Anonymous (2002) Executive summary. UNEP/WMO. Scientific assessment of ozone depletion.

Anonymous (2006) Environmental Effects of Ozone Depletion: 2006 Assessment, United Nations Environment Programme, ISBN: 978-92-807-2821-7

Annila A, Lehtimaki J, Mattila K, Eriksson JE, Sivonen K, Rantala TT, Drakenberg T (1996) Solution structure of nodularin - An inhibitor of serine/threonine-specific protein phosphatases. Journal of Biological Chemistry 271:16695-16702

Aurivillius CWS (1896) Das Plankton des baltischen Meeres. Kungl Sv Vet-Akad Handl Bd1, Afd IV 8:1-30 (In German)

Barker GLA, Hayes PK, O'Mahony SL, Vacharapiyasophon P, Walsby AE (1999) A molecular and phenotypic analysis of *Nodularia* (cyanobacteria) from the Baltic Sea. Journal Of Phycology 35:931-937

Barker GLA, Handley BA, Vacharapiyasophon P, Stevens JR, Hayes PK (2000) Allele-specific PCR shows that genetic exchange occurs among genetically diverse *Nodularia* (Cyanobacteria) filaments in the Baltic Sea. Microbiology-Sgm 146:2865-2875

Berglund BE (1979) The deglaciation of southern Sweden 13,500-10,000 B.P. Boreas 8:89-117

Bianchi TS, Dibb JE, Findlay S (1993) Early Diagenesis of Plant Pigments in Hudson River Sediments. Estuarine Coastal and Shelf Science 36:517-527

Bianchi TS, Engelhaupt E, Westman P, Andren T, Rolff C, Elmgren R (2000) Cyanobacterial blooms in the Baltic Sea: Natural or human-induced? Limnology And Oceanography 45:716-726

Björck S (1995) A review of the history of the Baltic Sea, 13.0-8.0 ka BP. Quaternary International 27:19-40

Björck S (2008) The late Quaternary development of the Baltic Sea basin. In: The BACC author Team (ed) Assessment of climate change for the Baltic Sea Basin. Springer- Verlag, Berlin, Heidelberg, p 398-407

Blakefield MK, Harris DO (1994) Delay of cell differentiation in *Anabaena aequalis* caused by UV-B radiation and the role of photoreactivation and excision repair. Photochemistry and Photobiology 59:204-208

Bojkov RD, Balis DS (2001) Characteristics of episodes with extremely low ozone values in the northern middle latitudes 1957-2000. Annales Geophysiceae 19:797-807

Bolch CJS, Blackburn SI, Hallegraeff GM, Vaillancourt RE (1999) Genetic variation among strains of the toxic dinoflagellate *Gymnodinium catenatum* (Dinophyceae). Journal Of Phycology 35:356-367

Bothe H (1982) Nitrogen fixation. In: Carr NG, Whitton BA (eds) The Biology of Cyanobacteria. Blackwell Scientific Publications, Oxford, p 87-104

Burkholder JAM, Glibert PM (2004) Intraspecific variability in toxicity, behaviour and nutrition in toxigenic microalgae. HAB Capetown 2004

Böhm GA, Pfleiderer W, Boger P, Scherer S (1995) Structure of a novel oligosaccharide-mycosporine-amino acids ultraviolet A/B sunscreen pigment from terrestrial cyanobacteria *Nostoc commune*. Journal of Biological Chemistry 270:8536-8539

Carreto JI, Carignan MO, Daleo G, De Marco SG (1990) Occurrence of mycosporine-like amino acids in the red-tide dinoflagellate *Alexandrium excavatum*: UV-photoprotective compounds? Journal of Plankton Researche 12:909-922

Chorus I, Bartam J (1999) Toxic cyanobacteria in water: a guide to their public health consequences, monitoring and management. E & FN Spon, London and New York, p 78

Chubarova N, Nezval Y, Verdebout J, Krotkov N, Herman J (2005) Long-term UV irradiance changes over Moscow and comparisons with UV estimates from TOMS and METEOSAT. In: Bernhard G, Slusser J, Herman J, Gao W (eds) Ultraviolet Ground- and Space-based Measurements, Models and Effects SPIE, San Diego

Collier J, Grossmann A (1992) Chlorosis induced by nutrient deprivation in *Synechococcus* sp. strain PCC 7942: Not all bleaching is the same. Journal of Bacteriology 174: 4718 – 4726

Conde FR, Churio MS, Previtali CM (2000) The photoprotector mechanism of mycosporine-like amino acids. Excited-state properties and photostability of porphyra-334 in aqueous solution. Journal of Photochemistry and Photobiology B: Biology 56:139-144

Conley DJ, Humborg C, Rahm L, Savchuk OP, Wulff F (2002) Hypoxia in the Baltic Sea and basin-scale changes in phosphorus biogeochemistry. Environmental Science & Technology 36:5315-5320

Conley DJ, Bonsdorff E, Carstensen J, Destouni G, Gustafsson BG, Hansson L-A, Rabalais NN, Voss M, Zillén L (2009) Tackling Hypoxia in the Baltic Sea: Is Engineering a Solution? Environmental Science & Technology 43:3407-3411

Cordi B, Depledge MH, Price DN, Salter LF, Donkin ME (1997) Evaluation of chlorophyll fluorescence, in vivo spectrophotometric pigment absorption and ion leakage as biomarkers of UV-B exposure in marine macroalgae. Marine Biology 130:41-49

Cox PA, Banack SA, Murch SJ (2003) Biomagnification of cyanobacterial neurotoxins and neurodegenerative disease among the Chamorro people of Guam. Proceedings of the National Academy of Sciences of the United States of America 100:13380-13383

Cox PA, Banack SA, Murch SJ, Rasmussen U, Tien G, Bidigare RR, Metcalf JS, Morrison LF, Codd GA, Bergman B (2005) Diverse taxa of cyanobacteria produce beta-N-methylamino-L-alanine, a neurotoxic amino acid. Proceedings of the National Academy of Sciences of the United States of America 102:5074-5078

Cullen JJ, Neale PJ (1997) Biological weighting functions for describing the effects of ultraviolet radiation on aquatic systems. In: Häder DP (ed) The effects of ozone depletion on aquatic ecosystems, R.G. Landes, p 97-118

Degerholm J, Gundersen K, Bergman B, Söderbäck E (2006) Phosphorus-limited growth dynamics in two Baltic Sea cyanobacteria, *Nodularia* sp. and *Aphanizomenon* sp. FEMS Microbiology Ecology 58:323-332

Diaz RJ, Rosenberg R (2008) Spreading Dead Zones and Consequences for Marine Ecosystems. Science 321:926-929

Dunlap WC, Yamamoto Y (1995) Small-molecule antioxidants in marine organisms: Antioxidant activity of mycosporine-glycine. Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology 112:105-114

Edler L, Ferno S, Lind MG, Lundberg R, Nilsson PO (1985) Mortality of dogs associated with a bloom of the cyanobacterium *Nodularia spumigena* in the Baltic Sea. Ophelia 24:103-109

Elmgren R, Larsson U (2001) Eutrophication in the Baltic Sea Area: Integrated coastal management issues. In: von Bodungen B, Turner RK (eds) Science and integrated coastal management Dahlem University Press, p 15-35

Engström-Öst J, Koski M, Schmidt K, MarkkuViitasalo, Jónasdóttir SH, Kokkonen M, Repka S, Sivonen K (2002) Effects of toxic cyanobacteria on a plankton assemblage: community development during decay of *Nodularia spumigena*. Marine Ecology Progress Series 232:1-14

Finni T, Kononen K, Olsonen R, Wallstrom K (2001) The history of cyanobacterial blooms in the Baltic Sea. Ambio 30:172-178

Francis G (1878) Poisonous Australian lake. Nature 18:11

Fredén C (1967) A historical review of the Ancylus Lake and the Svea River. Geologiska Föreningens i Stockholm Förhandlingar, p 239-267

Garcia-Pichel F, Castenholz RW (1991) Characterization and biological implications of scytonemin, a cyanobacterial sheath pigment. Journal of Phycolology 27:395-409

Garcia-Pichel F, Castenholz RW (1993) Occurrence of UV-absorbing, mycosporine-like compounds among cyanobacterial isolates and an estimate of their screening capacity. Applied Environmental Microbiology 59:163-169

Goldman JC, McCarthy JJ, Peavey DG (1979) Growth rate influence on the chemical composition of phytoplankton in oceanic waters. Nature 279:210-215

Graneli E, Wallstrom K, Larsson U, Graneli W, Elmgren R (1990) Nutrient Limitation of Primary Production in the Baltic Sea Area. Ambio 19:142-151

Granéli E, Johansson N, Panosso R (1998) Cellular toxin contents in relation to nutrient conditions for different groups of phycotoxins. In: Reguera B, Blanco J, Fernéndez ML, Wyatt T (eds) Harmful algae. Xunta de Galicia and Intergovermental Oceanographic Commission of UNESCO, Paris, p 321-324

Gorokhova E, Engström-Öst J (2009) Toxin concentration in *Nodularia spumigena* is modulated by mesozooplankton grazers. Journal Of Plankton Research 31:1235-1247

Hajdu S, Höglander H, Larsson U (2007) Phytoplankton vertical distributions and composition in Baltic Sea cyanobacterial blooms. Harmful Algae 6:189-205

Halinen K, Fewer DP, Sihvonen LM, Lyra C, Eronen E, Sivonen K (2008) Genetic diversity in strains of the genus *Anabaena* isolated from planktonic and benthic habitats of the Gulf of Finland (Baltic Sea). FEMS Microbiology Ecology 64:199-208

Hanelt D, Wiencke C, Bischof K (2003) Photosynthesis in marine macroalgae. In: Larkum A, Douglas S, Raven JA (eds) Photosynthesis in Algae. Kluwer Academic Publishers p413-435

Harada K (1995) Chemistry and detection of microcystins. In: M.F Watanabe, K.I Harada, W.W Carmichael and Fujiki, (eds), Toxic Microcystis, CRC Press, Boca Raton, FL, USA (1995), pp. 103–148.

He YY, Häder DP (2002) Involvement of reactive oxygen species in the UV-B damage to the cyanobacterium Anabaena sp. Journal of Photochemistry and Photobiology B: Biology 66:73-80

Healey FP (1973) Characteristics of phosphorus deficiency in *Anabaena*. Journal of Phycology 9:383-394

Healey FP (1975) Physiological indicators of nutrient deficiency in algae. Fish Mar Serv Tech Rep 585:30

HELCOM (1987) First Periodic Assessment of the State of the Marine Environment of the Baltic Sea, 1980-1985. Background document. Baltic Sea Environmental Proceedings No.17B

HELCOM (1996) Third Periodic Assessment of the State of the Marine Environment of the Baltic Sea, 1989-1993. Executive Summary, Baltic Sea Environmental Proceedings No.64 A

HELCOM (2001) The Fourth Periodic Assessment of the State of the Marine Environment of the Baltic Sea, 1994-1998. Executive Summary, Baltic Sea Environmental Proceedings No.82 A

HELCOM (2002) Fourth Periodic Assessment of the State of the Marine Environment of the Baltic Sea Area, 1994-1998 Baltic Sea Environmental Proceedings No 82B

HELCOM (2003) The Baltic Marine Environment 1999-2002 Baltic Sea Environmental Proceedings No 87

HELCOM (2007) Climate Change in the Baltic Sea Area - HELCOM Thematic Assessment 2007, Baltic Sea Environmental Proceedings No.111

HELCOM (2010) Ecosystem Health of the Baltic Sea 2003–2007: HELCOM Initial Holistic Assessment. Baltic Sea Environmental Proceedings No. 122.

Henriksen P (2005) Estimating nodularin content of cyanobacterial blooms from abundance of Nodularia spumigena and its characteristic pigments - a case study from the Baltic entrance area. Harmful Algae 4:167-178

Heresztyn T, Nicholson BC (1997) Nodularin concentrations in Lakes Alexandrina and Albert, South Australia, during a bloom of the cyanobacterium (blue-green alga) *Nodularia spumigena* and degradation of the toxin. Environmental Toxicology and Water Quality 12:273-282

Hessle C, Vallin S (1934) Undersökningar över plankton och dess växlingar i Östersjön under åren 1925-1927. (Investigations of plankton and its fluctuations in the Baltic during the years 1925-1927). Svenska Hydr-Biol Komm Skr Ny Serie: Biologi Band 1, No 5, 1-132 (In Swedish, English Summary).

Hobson P, Burch M, Fallowfield HJ (1999) Effect of total dissolved solids and irradiance on growth and toxin production by *Nodularia spumigena.* Journal of Applied Phycology 11:551-558

Hobson P, Fallowfield HJ (2003) Effect of irradiance, temperature and salinity on growth and toxin production by *Nodularia spumigena*. Hydrobiologia 493:7-15

Huber AL (1984) *Nodularia* (Cyanobacteriaceae) Akinetes in the Sediments of the Peel-Harvey Estuary, Western Australia: Potential Inoculum Source for *Nodularia* Blooms. Applied and Environmental Microbiology 47:234-238

Huber AL (1985) Factors affecting the germination of akinetes of *Nodularia spumigena* (Cyanobacteriaceae). Applied and Environmental Microbiology 49:73-78

Humpage AR, Falconer IR (1999) Microcystin-LR and liver tumor promotion: Effects on cytokinesis, ploidy, and apoptosis in cultured hepatocytes. Environmental Toxicology 14:61-75

Hällfors G (2004) Checklist of Baltic Sea phytoplankton species. Baltic Sea Environment Proceedings, No. 95. Helsinki Commission, Baltic Marine Environment Protection Commission.

Häyrén E (1921) Meddelande om vegetationsfärgningar. Flora Fennica 47:82-89 (In Swedish)

Ibelings BW, Havens KE (2008) Cyanobacterial toxins: a qualitative meta-analysis of concentrations, dosage and effects in freshwater, estuarine and marine biota. In: Hudnell HK (ed) Cyanobacterial Harmful Algal Blooms: State of the Science and Research Needs, Vol 619. Springer-Verlag Berlin, Berlin, p 675-732

Ivanov AG, Miskiewicz E, Clarke AK, Greenberg BM, Huner NPA (2000) Protection of photosystem II against UV-A and UV-B radiation in the cyanobacterium *Plectonema boryanum*: The role of growth temperature and growth irradiance. Photochemistry and Photobiology 72:772-779

Janson S, Carpenter EJ, Bergman B (1994) Fine structure and immunolocalisation of proteins in *Aphanizomenon* sp. from the Baltic Sea. European Journal of Phycology 29:203-211

Jeffrey SW (1997) Application of pigment methods to oceanography. In: Jeffrey S, Mantoura R, Wright S (eds) Phytoplankton pigments in oceanography. UNESCO, Paris, pp 127-166

Jenkins CA, Hayes PK (2006) Diversity of cyanophages infecting the heterocystous filamentous cyanobacterium *Nodularia* isolated from the brackish Baltic Sea. Journal of the Marine Biological Association of the UK 86:529-536

Jensen JB (1995) A Baltic ice lake transgression in the southwestern Baltic: evidence from Fakse Bugt, Denmark. Quaternary International 27:59-68

Jonasson, S., 2006. Monitoring the cellular phosphate status in bloom-forming cyanobacteria of the Baltic Sea. Doctoral Thesis, Stockholm University.

Jonasson S, Eriksson J, Berntzon L, Rasmussen U, Bergman B (2008) A novel cyanobacterial toxin (BMAA) with potential neurodegenerative effects. Plant Biotechnology 25:227-232

Jonasson S, Eriksson J, Berntzon L, Spacil Z, Ilag LL, Ronnevi LO, Rasmussen U, Bergman B (2010) Transfer of a cyanobacterial neurotoxin within a temperate aquatic ecosystem suggests pathways for human exposure. Proceedings of the National Academy of Sciences of the United States of America 107:9252-9257

Jonsson P, Carman R (1994) Changes in sediment deposition of organic matter and nutrients in the Baltic Sea during the twentieth century. Marine Pollution Bulletin 28:417-426

Josefsson W (2006) UV-radiation 1983-2003 measured at Norrköping, Sweden. Theoretical and Applied Climatology 83:59-76

Kangro K, Olli K, Tamminen T, Lignell R (2007) Species-specific responses of a cyanobacteria-dominated phytoplankton community to artificial nutrient limitation in the Baltic Sea. Marine Ecology Progress Series 336:15-27

Kankaanpaa H, Vuorinen PJ, Sipiä V, Keinanen M (2002) Acute effects and bioaccumulation of nodularin in sea trout (*Salmo trutta* m. *trutta* L.) exposed orally to *Nodularia spumigena* under laboratory conditions. Aquatic Toxicology 61:155-168

Karentz D, Cleaver JE, Mitchell DL (1991) Cell survival characteristics and molecular responses of Antarctic phytoplankton to ultraviolet-B radiation. Journal of Phycology 27:326-341

Karentz D (1994) Ultraviolet tolerance mechanisms in Antarctic marine organisms. Antarctic Research Series 62:93-110

Karentz D (2001) Chemical defenses of marine organisms against solar radiation exposure: UV-absorbing mycosporine-like amino acids. In: Marine Chemical Ecology. CRC Press, p 481-520

Karjalainen M, Kozlowsky-Suzuki B, Lehtiniemi M, Engström-Öst J, Kankaanpaa H, Viitasalo M (2006) Nodularin accumulation during cyanobacterial blooms and experimental depuration in zooplankton. Marine Biology 148:683-691

Karjalainen M, Paakkonen JP, Peltonen H, Sipiä V, Valtonen T, Viitasalo M (2008) Nodularin concentrations in Baltic Sea zooplankton and fish during a cyanobacterial bloom. Marine Biology 155:483-491

Karlson K, Rosenberg R, Bonsdorff E (2002) Temporal and spatial large-scale effects of eutrophication and oxygen deficiency on benthic fauna in Scandinavian and Baltic waters - A review. Oceanography and Marine Biology, An Annual Review 40:427-489.

Keating K (1977) Allelopathic Influence on Blue-Green Bloom Sequence in a Eutrophic Lake. Science 196:885-887

Kondo T, Mori T, Lebedeva NV, Aoki S, Ishiura M, Golden SS (1997) Circadian Rhythms in Rapidly Dividing Cyanobacteria. Science 275:224-227

Komárek J, Hübel M, Hübel H, Šmarda J (1993) The *Nodularia* studies 2. Taxonomy. Archiv für Hydrobiologie/Supplementband 96 Algological Studies 68:1–25

Kononen K (1992) Dynamic of the toxic cyanobacterial blooms in the Baltic Sea. Finn Mar Res 261:3–36

Kononen K, Kuparinen J, Mäkela K, Laanemets J, Pavelson J, Nommann S (1996) Initiation of cyanobacterial blooms in a frontal region at the entrance to the Gulf of Finland, Baltic Sea. Limnology and Oceanography 41:98-112

Kononen K, Hällfors S, Kokkonen M, Kuosa H, Laanemets J, Pavelson J, Autio R (1998) Development of a subsurface chlorophyll maximum at the entrance to the Gulf of Finland, Baltic Sea. Limnology and Oceanography 43:1089-1106

Kromkamp JP, Konopka A, Mur LR (1986) Buoyancy regulation in a strain of *Aphanizomenon flos-aquae* (Cyanophyceae): the importance of carbohydrate accumulation and gas vesicle collapse. Journal of General Microbiology 132:2113-2121

Kumar A, Tyagi MB, Singh N, Tyagi R, Jha PN, Sinha RP, Häder D-P (2003) Role of white light in reversing UV-B-mediated effects in the N2-fixing cyanobacterium *Anabaena* BT2. Journal of Photochemistry and Photobiology B: Biology 71:35-42

Laamanen MJ, Gugger MF, Lehtimaki JM, Haukka K, Sivonen K (2001) Diversity of toxic and nontoxic *Nodularia* isolates (Cyanobacteria) and filaments from the Baltic Sea. Applied And Environmental Microbiology 67:4638-4647

Laamanen MJ, Forsstrom L, Sivonen K (2002) Diversity of *Aphanizomenon flos-aquae* (cyanobacterium) populations along a Baltic Sea salinity gradient. Applied and Environmental Microbiology 68:5296-5303

Laamanen M, Kuosa H, Maximum S (2005) Annual variability of biomass and heterocysts of the N-2-fixing cyanobacterium *Aphanizomenon flos-aquae* in the Baltic Sea with reference to *Anabaena* spp. and *Nodularia spumigena*. Boreal Environ Res 10:19-30

Lagus A, Suomela J, Helminen H, Lehtimäki JM, Sipura J, Sivonen K, Suominen L (2007) Interaction effects of N:P ratios and frequency of nutrient supply on the plankton community in the northern Baltic Sea. Marine Ecology Progress Series 332:77-92

Larsson U, Elmgren R, Wulff F (1985) Eutrophication and the baltic sea: Causes and consequences. Ambio 14: 9-14.

Larsson U, Hajdu S, Walve J, Elmgren R (2001) Baltic Sea nitrogen fixation estimated from the summer increase in upper mixed layer total nitrogen. Limnology And Oceanography 46:811-820

Latasa M, Berdalet E (1994) Effect of nitrogen or phosphorus starvation on pigment composition of cultured *Heterocapsa* sp. Journal Of Plankton Research 16:83-94

Layzell DB, Turpin DH, Elrifi IR (1985) Effects of N-source on the steady-state growth and assimilation of P-limited *Anabaena flos-aquae*. Plant Physiology 78:739-745

Legrand C, Rengefors K, Fistarol GO, Granéli E (2003) Allelopathy in phytoplankton - biochemical, ecological and evolutionary aspects. Phycologia 42:406-419

Lehtimäki J, Sivonen K, Luukainen R, Niemela SI (1994) The effects of incubation time, temperature, light, salinity, and phosphorus on growth and hepatotoxin production by *Nodularia* strains. Archiv für Hydrobiologie 130:269-282

Lehtimäki J, Moisander P, Sivonen K, Kononen K (1997) Growth, nitrogen fixation, and nodularin production by two Baltic sea cyanobacteria. Applied and Environmental Microbiology 63:1647-1656

Lehtimäki J, Lyra C, Suomalainen S, Sundman P, Rouhiainen L, Paulin L, Salkinoja-Salonen M, Sivonen K (2000) Characterization of *Nodularia* strains, cyanobacteria from brackish waters, by genotypic and phenotypic methods. International Journal of Systematic and Evolutionary Microbiology 50:1043-1053

Lindberg V, Mohlin M, Wulff A (2008), UV responses in three strains of the cyanobacterium *Nodularia spumigena*, Proceedings of the 12th International Conference on Harmful Algae. Ø. Moestrup et al (ed). ISSHA and IOC of UNESCO 2008, page 44

Lindström G (1855) Bidrag till kännedom om Östersjöns invertebratfauna. Öfversigt KVA:s Förh Årg 12. Stockholm, 49-73 (In Swedish)

Lotze HK, Worm B, Sommer U (2001) Strong bottom-up and top-down control of early life stages of macroalgae. Limnology And Oceanography 46:749-757

Maxwell K, Johnson GN (2000) Chlorophyll fluorescence - a practical guide. J Exp Bot 51:659-668

Mazur-Marzec H, Zeglinska L, Plinski M (2005) The effect of salinity on the growth, toxin production, and morphology of *Nodularia spumigena* isolated from the Gulf of Gdansk, southern Baltic Sea. Journal of Applied Phycology 17:171-179

Mazur-Marzec H, Krezel A, Kobos J, Plinski M (2006a) *Toxic Nodularia spumigena* blooms in the coastal waters of the Gulf of Gdansk: a ten-year survey. Oceanologia 48:255-273

Mazur-Marzec H, Meriluoto J, Plinski M (2006b) The degradation of the cyanobacterial hepatotoxin nodularin (NOD) by UV radiation. Chemosphere 65:1388-1395

Mazur-Marzec H, Torunska A, Blonska MJ, Moskot M, Plinski M, Jakóbkiewicz-Banecka J, Wegrzyn G (2009) Biodegradation of nodularin and effects of the toxin on bacterial isolates from the Gulf of Gdansk. Water Research 43:2801-2810

Miśkiewicz E, Ivanov AG, Williams JP, Khan MU, Falk S, Huner NP (2000) Photosynthetic acclimation of the filamentous cyanobacterium, *Plectonema boryanum* UTEX 485, to temperature and light. Plant Cell Physiology 41:767-775

Mohlin M, Roleda MY, Neale PJ, Wulff A (In prep) Biological weighting functions, BWFs on three strains of *Nodularia spumigena* from the Baltic Sea.

Moisander PH, Steppe TF, Hall NS, Kuparinen J, Paerl HW (2003) Variability in nitrogen and phosphorus limitation for Baltic Sea phytoplankton during nitrogen-fixing cyanobacterial blooms. Marine Ecology Progress Series 262:81-95

Molisch H (1937) Der Einfluss einer Pflanze auf die andere: Allelopathie. Fischer Verlag

Munthe H (1894) Preliminary report on the physical geography of the Litorina-Sea. Bulletin of the Geological Institutions of the University of Uppsala 2:1-38

Murch SJ, Cox PA, Banack SA, Steele JC, Sacks OW (2004) Occurrence of beta-methylamino-L-alanine (BMAA) in ALS/PDC patients from Guam. Acta Neurologica Scandinavica 110:267-269

Neale PJ, Davis RF, Cullen JJ (1998) Interactive effects of ozone depletion and vertical mixing on photosynthesis of Antarctic phytoplankton. Nature 392:585-589

Neale PJ (2000) Spectral weighting functions for quantifying the effects of ultraviolet radiation in marine ecosystems. In: de Mora SJ, Demers S, Vernet M (eds) The effects of UV radiation on marine ecosystems. Cambridge Univ. Press, Cambridge, p 73-100

Nehring S (1993) Mortallity of dogs associated with a mass development of *Nodularia spumigena* (Cyanophyceae) in a brackish lake at the German North Sea coast. Journal of Plankton Researche 15:867-872

Neumann T (2010) Climate-change effects on the Baltic Sea ecosystem: A model study. Journal of Marine Systems 81:213-224

Niemi Å (1979) Blue-green algal blooms and N:P ratio in the Baltic Sea. Acta Botanica Fennica 11:57-61

Niemi Å (1981) Har produktionsnivån i Östersjön ökat? Societas Scientiarum Fennica 59:115-137 (In Swedish)

Niemistö L, Rinne I, Melvasalo T, Niemi Å (1989) Blue-green algae and their nitrogen fixation in the Baltic Sea in 1980, 1982 and 1984. MERI, Finnish Institute of Marine Research 17:3-20

Nixon SW (1995) Coastal marine eutrophication: A definition, social causes, and future concerns. Ophelia 41:199-219

Ohta T, Sueoka E, Iida N, Komori A, Suganuma M, Nishiwaki R, Tatematsu M, Kim SJ, Carmichael WW, Fujiki H (1994) Nodularin, a potent inhibitor of protein phosphatase-1 and phosphatase-2A, is a new environmentalcarcinogen in male F344 rat –liver. Cancer Research 54:6402-6406

Olenina I, Hajdu S, Edler L, Andersson A, Wasmund N, Busch S, Göbel J, Gromisz S, Huseby S, Huttunen M, Jaanus A, Kokkonen P, Ledaine I, Niemkiewicz E (2006) Biovolumes and size-classes of phytoplankton in the Baltic Sea. HELCOM, Baltic Sea Environmental Proceedings No.106

Oliver R (1994) Floating and sinking in gas-vacuolate cyanobacteria. Journal of Phycology 30:161-173

Oliver R, Walsby A (1984) Direct evidence for the role of light-mediated gas vesicle collapse in the buoyancy regulation of *Anabaena flos-aquae* (cyanobacteria). Limnology and Oceanography 29:879-886

Orsolini YJ, Limpasuvan V (2001) The North Atlantic Oscillation and the occurrences of ozone miniholes. Geophysical Research Letters 28:4099-4102

Paasche E, Erga SR (1988) Phosphorus and nitrogen limitation of phytoplankton in the inner Oslofjord (Norway). Sarsia 73:229-243

Paerl HW (1988) Nuisance Phytoplankton Blooms in Coastal, Estuarine, and Inland Waters. Limnology and Oceanography 33:823-847

Paerl HW (2000) Marine plankton. In: Whitton BA, Potts M (eds) The ecology of cyanobacteria Their diversity in time and space. Kluwer, Dordrecht, p 121-148

Panosso R, Graneli E (2000) Effects of dissolved organic matter on the growth of Nodularia spumigena (Cyanophyceae) cultivated under N or P deficiency. Marine Biology 136:331-336

Park HD, Iwami C, Watanabe MF, Harada KI, Okino T, Hayashi H (1998) Temporal variabilities of the concentrations of intra- and extracellular microcystin and toxic *Microcystis* species in a hypertrophic lake, Lake Suwa, Japan (1991–1994). Environmental Toxicology and Water Quality 13:61-72

Persson KJ, Legrand C, Olsson T (2009) Detection of nodularin in European flounder (*Platichthys flesus*) in the west coast of Sweden: Evidence of nodularin mediated oxidative stress. Harmful Algae 8:832-838

Pitkänen H, Tamminen T (1995) Nitrogen and phosphorus as production limiting factors in the estuarine waters of the eastern Gulf of Finland. Marine Ecology Progress Series 129:283-294

Põder T, Maestrini SY, Balode M, Lips U, Béchemin C, Andrushaitis A, Purina I (2003) The role of inorganic and organic nutrients on the development of phytoplankton along a transect from the Daugava River mouth to the open Baltic, in spring and summer 1999. ICES Journal of Marine Science 60:827-835

Portwich A, Garcia-Pichel F (1999) Ultraviolet and osmotic stresses induce and regulate the synthesis of mycosporines in the cyanobacterium *Chlorogloeopsis* PCC 6912. Arch Microbiol 172:187-192

Portwich A, Garcia-Pichel F (2000) A novel prokaryotic UVB photoreceptor in the cyanobacterium *Chlorogloeopsis* PCC 6912. Photochemistry and Photobiology 71:493-498

Pouchet G, de Guerne J (1885) Sur le faune pelagique de la mer Baltique et du Golfe de Finlande. Comptes Rendus des Seances de l’Academie des Sciences 100: 919–921 (In French)

Poutanen EL, Nikkila K (2001) Carotenoid pigments as tracers of cyanobacterial blooms in recent and post-glacial sediments of the Baltic Sea. Ambio 30:179-183

Rahm L, Danielsson A (2007) Spatial heterogeneity of nutrients in the Baltic Proper, Baltic Sea. Estuarine, Coastal and Shelf Science 73:268-278

Rapala J, Sivonen K, Lyra C, Niemela SI (1997) Variation of microcystins, cyanobacterial hepatotoxins, in *Anabaena* spp. as a function of growth stimuli. Applied and Environmental Microbiology 63:2206-2212

Raukas A (1995) Evolution of the Yoldia Sea in the Eastern Baltic. Quaternary International 27:99-102

Repka S, Mehtonen J, Vaitomaa J, Saari L, Sivonen K (2001) Effects of nutrients on growth and nodularin production of *Nodularia* strain GR8b. Microbial Ecology 42:606-613

Repka S, Meyerhöfer M, Von Bröckel K, Sivonen K (2004) Associations of cyanobacterial toxin, nodularin, with environmental factors and zooplankton in the Baltic Sea. Microbial Ecology 47:350-358

Reynolds,CS (1987) Cyanobacterial water blooms. In: Callow JA (ed), Advances in Botanical Research. Academic Press, London, pp. 68-143

Rinehart KL, Harada K, Namikoshi M, Chen C, Harvis CA, Munro MHG, Blunt JW, Mulligan PE, Beasley VR, Dahlem AM, Carmichael WW (1988) Nodularin, microcystin, and the configuration of ADDA. Journal of American Chemical Society 110:8557-8558

Roleda MY, Mohlin M, Pattanaik B, Wulff A (2008) Photosynthetic response of *Nodularia spumigena* to UV and photosynthetically active radiation depends on nutrient (N and P) availability. FEMS Microbiology Ecology 66:230-242

Rolff C, Almesjö L, Elmgren R (2007) Nitrogen fixation and abundance of the diazotrophic cyanobacterium *Aphanizomenon* sp in the Baltic Proper. Marine Ecology Progress Series 332:107-118

Rosen BH, Lowe RC (1984) Physiological and ultrastructural responses of *Cyclotella meneghiniania* (Bacillariophyta) to light intensity and nutrient limitation. Journal of Phycology 20:173-180

Rothe F (1941) Quantitative Untersuchungen über die Planktonverteilung in der östlichen Ostsee. Ber Deutsch Wissenschaft Kommission Meeresforsch NF Bd103, 291-368 (In German)

Rozema J, Bjorn LO, Bornman JF, Gaberscik A, Hader DP, Trost T, Germ M, Klisch M, Groniger A, Sinha RP, Lebert M, He YY, Buffoni-Hall R, de Bakker NVJ, van de Staaij J, Meijkamp BB (2002) The role of UV-B radiation in aquatic and terrestrial ecosystems - an experimental and functional analysis of the evolution of UV-absorbing compounds. Journal of Photochemistry and Photobiology B: Biology 66:2-12

Runnegar MTC, Jackson ARB, Falconer IR (1988) Toxicity of the Cyanobacterium *Nodularia spumigena* Mertens. Toxicon 26:143-151

Runnegar M, Berndt N, Kong SM, Lee EYC, Zhang LF (1995) In vivo and in vitro binding of microcystins to protein phosphatases 1 and 2A. Biochemical and Biophysical Research Communications 216:162-169

Rydin E, Hyenstrand P, Gunnerhed M, Blomqvist P (2002) Nutrient limitation of cyanobacterial blooms: an enclosure experiment from the coastal zone of the NW Baltic proper. Marine Ecology Progress Series 239:31-36

Sanz-Alferez S, del Campo FF (1994) Relationship between nitrogen fixation and nitrate metabolism in the Nodularia strains M1 and M2. Planta 194:339-345

Schlüter L, Garde K, Kaas H (2004) Detection of the toxic cyanobacteria *Nodularia spumigena* by means of a 4-keto-myxoxanthophyll-like pigment in the Baltic Sea. Marine Ecology Progress Series 275:69-78

Schlüter L, Lutnaes BF, Liaaen-Jensen S, Garde K, Kaas H, Jameson I, Blackburn S (2008) Correlation of the content of hepatotoxin nodularin and glycosidic carotenoids, 4-ketomyxol-2 '-fucoside and novel 1 '-O-methyl-4-ketomyxol-2 '-fucoside, in 20 strains of the cyanobacterium *Nodularia spumigena*. Biochemical Systematics and Ecology 36:749-757

Sellner KG (1997) Physiology, ecology, and toxic properties of marine cyanobacteria blooms. Limnology And Oceanography 42:1089-1104

Shick JM, Dunlap WC (2002) Mycosporine-like amino acids and related gadusols: Biosynthesis, accumulation, and UV-protective functions in aquatic organisms. Annual Review of Physiology 64:223-262

Shindell DT, Rind D, Lonergan P (1998) Increased polar stratospheric ozone losses and delayed eventual recovery owing to increasing greenhouse-gas concentrations. Nature 392:589-592

Shindell DT, Schmidt GA, Miller RL, Rind D (2001) Northern Hemisphere winter climate response to greenhouse gas, ozone, solar, and volcanic forcing. Journal of Geophysical Research-Atmospheres 106:7193-7210

Sinha RP, Klisch M, Helbling EW, Häder DP (2001) Induction of mycosporine-like amino acids (MAAS) in cyanobacteria by solar ultraviolet-B radiation. Journal of Photochemistry and Photobiology B: Biology 60:129-135

Sinha RP, Ambasht NK, Sinha JP, Klisch M, Häder DP (2003) UV-B-induced synthesis of mycosporine-like amino acids in three strains of *Nodularia* (cyanobacteria). Journal of Photochemistry and Photobiology B: Biology 71:51-58

Sinha RP, Häder DP (2008) UV protectants in cyanobacteria. Plant Science 174:278-289

Sipiä VO, Kankaanpaa HT, Pflugmacher S, Flinkman J, Furey A, James KJ (2002) Bioaccumulation and detoxication of nodularin in tissues of flounder (*Platichthys flesus*), mussels (*Mytilus edulis, Dreissena polymorpha*), and clams (*Macoma balthica*) from the northern Baltic Sea. Ecotoxicology and Environmental Safety 53:305-311

Sipiä VO, Neffling MR, Metcalf JS, Nybom SMK, Meriluoto JAO, Codd GA (2008) Nodularin in feathers and liver of eiders (*Somateria mollissima*) caught from the western Gulf of Finland in June-September 2005. Harmful Algae 7:99-105

Sivonen K (1990) Effects of Light, Temperature, Nitrate, Orthophosphate, and Bacteria on Growth of and Hepatotoxin Production by *Oscillatoria-Agardhii* Strains. Applied and Environmental Microbiology 56:2658-2666

Sivonen K, Kononen K, Carmichael WW, Dahlem AM, Rinehart KL, Kiviranta J, Niemela SI (1989) Occurrence of the hepatotoxic cyanobacterium *Nodularia spumigena* in the Baltic Sea and the structure of the toxin. Applied and Environmental Microbiology 55:1990-1995

Sivonen K, Jones G (1999) Cyanobacterial toxins. In: Chorus I, Bartram J (eds) Toxic cyanobacteria in water, A guide to public health consequences, monitoring and management. WHO Series in Environmental Management Routledge, London, p 41-111

Sivonen K, Halinen K, Sihvonen LM, Koskenniemi K, Sinkko H, Rantasarkka K, Moisander PH, Lyra C (2007) Bacterial diversity and function in the Baltic Sea with an emphasis on cyanobacteria. Ambio 36:180-185

Sjöstedt G (1922) Biologisk-Botaniska undersökningar av Öresund II. En vegetationsfärgning av *Nodularia spumigena* i Öresund jämte biologisk översikt över övriga marina baltiska planktonfärgningar. Lunds Univ. Årsskrift N.F. Avd. 2. Bd 18. Nr. 2, pp1-25 (In Swedish)

Sohlenius G, Sternbeck J, Andrén E, Westman P (1996) Holocene history of the Baltic Sea as recorded in a sediment core from the Gotland Deep. Marine Geology 134:183-201

Sohlenius G, Emeis KC, Andrén E, Andrén T, Kohly A (2001) Development of anoxia during the Holocene fresh-brackish water transition in the Baltic Sea. Marine Geology 177:221-242

Song KY, Lim IK, Park SC, Lee SO, Park HS, Choi YK, Hyun BH (1999) Effect of nodularin on the expression of glutathione S-transferase placental form and proliferating cell nuclear antigen in N-nitrosodiethylamine initiated hepatocarcinogenesis in the male Fischer 344 rat. Carcinogenesis 20:1541-1548

Staal M, Stal LJ, Hekkert ST, Harren FJM (2003) Light action spectra of N-2 fixation by heterocystous cyanobacteria from the Baltic Sea. Journal of Phycology 39:668-677

Staehelin J, Harris NRP, Appenzeller C, Eberhard J (2001) Ozone trends: A review. Reviews of Geophysics 39:231-290

Staehelin J, Mader J, Weiss AK, Appenzeller C (2002) Long-term ozone trends in Northern mid-latitudes with special emphasis on the contribution of changes in dynamics. Physics and Chemistry of the Earth 27:461-469

Staehr PA, Henriksen P, Markager S (2002) Photoacclimation of four marine phytoplankton species to irradiance and nutrient availability. Marine Ecology Progress Series 238:47-59

Stal LJ, Albertano P, Bergman B, von Brockel K, Gallon JR, Hayes PK, Sivonen K, Walsby AE (2003) BASIC: Baltic Sea cyanobacteria. An investigation of the structure and dynamics of water blooms of cyanobacteria in the Baltic Sea - responses to a changing environment. Continental Shelf Research 23:1695-1714

Stigebrandt A (1987) A model for the vertical circulation of the Baltic deep water. Journal of Physical Oceanography 17:1772-1785

Stolte W, Karlsson C, Carlsson P, Granéli E (2002) Modeling the increase of nodularin content in Baltic Sea *Nodularia spumigena* during stationary phase in phosphorus-limited batch cultures. FEMS Microbiology Ecology 41:211-220

Struck U, Emeis KC, Voss M, Christiansen C, Kunzendorf H (2000) Records of southern and central Baltic Sea eutrophication in delta C-13 and delta N-15 of sedimentary organic matter. Marine Geology 164:157-171

Suikkanen S, Fistarol GO, Graneli E (2004) Allelopathic effects of the Baltic cyanobacteria *Nodularia spumigena*, *Aphanizomenon flos-aquae* and *Anabaena lemmermannii* on algal monocultures. Journal Of Experimental Marine Biology And Ecology 308:85-101

Suikkanen S, Fistarol GO, Granéli E (2005) Effects of cyanobacterial allelochemicals on a natural plankton community. Marine Ecology Progress Series 287:1-9

Suikkanen S, Engström-Öst J, Jokela J, Sivonen K, Viitasalo M (2006) Allelopathy of Baltic Sea cyanobacteria: no evidence for the role of nodularin. Journal of Plankton Research 28:543-550

Suikkanen S, Kaartokallio H, Hällfors S, Huttunen M, Laamanen M (2010) Life cycle strategies of bloom-forming, filamentous cyanobacteria in the Baltic Sea. Deep-Sea Research Part II-Topical Studies In Oceanography 57:199-209

Summons RE, Jahnke LL, Hope JM, Logan GA (1999) 2-Methylhopanoids as biomarkers for cyanobacterial oxygenic photosynthesis. Nature 400:554-557

Tilman D, Kilham SS, Kilham P (1982) Phytoplankton community ecology- The role of limiting nutrients. Annual Review of Ecology and Systematics 13:349-372

Twist H, Codd GA (1997) Degradation of the cyanobacterial hepatotoxin, nodularin, under light and dark conditions. FEMS Microbiology Letters 151:83-88

Vahtera E, Laanemets J, Pavelson J, Huttunen M, Kononen K (2005) Effect of upwelling on the pelagic environment and bloom-forming cyanobacteria in the western Gulf of Finland, Baltic Sea. Journal of Marine Systems 58:67-82

Vahtera E, Conley DJ, Gustafsson BG, Kuosa H, Pitkanen H, Savchuk OP, Tamminen T, Viitasalo M, Voss M, Wasmund N, Wulff F (2007a) Internal ecosystem feedbacks enhance nitrogen-fixing cyanobacteria blooms and complicate management in the Baltic Sea. Ambio 36:186-194

Vahtera E, Laamanen M, Rintala JM (2007b) Use of different phosphorus sources by the bloom-forming cyanobacteria *Aphanizomenon flos-aquae* and *Nodularia spumigena*. Aquatic Microbial Ecology 46:225-237

Vassilkov A, Herman J, Krotov N, Mitchell G, Kahru M (2001) Problems in assessment of the UV penetration into natural waters from space-based measurements, In: Slusser JR, Herman JR, Gao W (eds) Ultraviolet Ground- and Space-based Measurements, Models and Effects Proceedings SPIE, San Diego, pp142

Vintila S, El-Shehawy R (2007) Ammonium ions inhibit nitrogen fixation but do not affect heterocyst frequency in the bloom-forming cyanobacterium *Nodularia spumigena* strain AV1. Microbiology UK 153:3704-3712

Vuorio K, Lagus A, Lehtimäki JM, Suomela J, Helminen H (2005) Phytoplankton community responses to nutrient and iron enrichment under different nitrogen to phosphorus ratios in the northern Baltic Sea. Journal of Experimental Marine Biology and Ecology 322:39-52

Välikangas I (1926) Planktologische Untersuchungen im Hafengebiet von Helsingfors. I. Über das Plankton, inbesondere das Netz-Zooplankton, des Sommerhalbjahres. Acta Zoologica Fennica. 1:1-298 (In German)

Wallström K, Johansson S, Larsson U (1992) Effects of nutrient enrichment on planktic blue-green algae in the Baltic Sea. Acta Phytogeographica Suecia 78:25-31

Walve J, Larsson U (2007) Blooms of Baltic Sea *Aphanizomenon* sp. (Cyanobacteria) collapse after internal phosphorus depletion. Aquatic Microbial Ecology 49:57-69

Wasmund N (1997) Occurrence of cyanobacterial blooms in the Baltic Sea in relation to environmental conditions. Internationale Revue der gesamten Hydrobiologie und Hydrographie 82:169-184

Willén T, Mattsson R (1997) Water-blooming and toxin-producing cyanobacteria in Swedish fresh and brackish waters, 1981-1995. Hydrobiologia 353:181-192

Winterhalter B (1992) Late-Quartenary stratigraphy of Baltic Sea basins-a review. Bulletin of the Geological Society of Finland 64:471-487

Worm B, Lotze HK, Hillebrand H, Sommer U (2002) Consumer versus resource control of species diversity and ecosystem functioning. Nature 417:848-851

Wulff A, Wängberg SA, Sundbäck K, Nilsson C, Underwood GJC (2000) Effects of UVB radiation on a marine microphytobenthic community growing on a sand-substratum under different nutrient conditions. Limnology and Oceanography 45:1144-1152

Xue LG, Zhang Y, Zhang TG, An LZ, Wang XL (2005) Effects of enhanced ultraviolet-B radiation on algae and cyanobacteria. Critical Reviews in Microbiology 31:79-89

Zillén L, Conley DJ, Andrén T, Andrén E, Björck S (2008) Past occurrences of hypoxia in the Baltic Sea and the role of climate variability, environmental change and human impact. Earth-Science Reviews 91:77-92