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The role of phosphorus as a regulator of bloom-forming diazotrophic cyanobacteria in the Baltic Sea

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In science, "fact" can only mean "confirmed to such a degree that it would be perverse to withhold provisional assent." I suppose that apples might start to rise tomorrow, but the possibility does not merit equal time in physics classrooms.

Stephen Jay Gould

LIST OF ORIGINAL ARTICLES

This thesis is based on the following papers, which are referred to in the text by their Roman numerals:

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

II Vahtera, E., Autio, R., Kaartokallio, H. and Laamanen, M. Effect of pulsed nutrient supply on physiological nutrient limitation and community phosphorus dynamics during blooms of diazotrophic cyanobacteria in the Baltic Sea. – Submitted to Limnology and Oceanography.

III Vahtera, E., Laanemets, J., Pavelson, J., Huttunen, M. and 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.

IV Vahtera. E., Conley, D.J., Gustafsson, B.G., Kuosa, H., Pitkänen, H., Savchuk, O.P., Tamminen, T., Viitasalo, M., Voss, M., Wasmund, N. and Wulff, F. 2007: Internal ecosystem feedbacks enhance nitrogen-fixing cyanobacteria blooms and complicate management in the Baltic Sea. – Ambio 36: 186–194.

THE AUTHOR'S CONTRIBUTION TO THE ARTICLES

I Vahtera designed and executed the study together with Dr. Maria Laamanen. Vahtera analysed the data and wrote the manuscript. Dr. Maria Laamanen and M.Sc. Janne-Markus Rintala were responsible for comments.

II Vahtera designed the study together with Dr. Maria Laamanen and carried it out together with all the co-authors. Vahtera performed the radioactive tracer studies together with Dr. Hermanni Kaartokallio and analysed the data and wrote the manuscript. All co-authors contributed with comments.

III Vahtera participated in collecting the data on the research cruise. Vahtera performed light microscopy analyses of filamentous cyanobacteria and analysed the biological and chemical data. Vahtera wrote the manuscript together with Dr. Jaan Laanemets and Dr. Juss Pavelson.

IV Vahtera co-ordinated the writing of the manuscript that originated from a special workshop on Baltic Sea cyanobacterial blooms. Vahtera summarized the discussions, co-ordinated the comments made by the co-authors and wrote the manuscript together with the co-authors.

The role of phosphorus as a regulator of bloom-forming diazotrophic cyanobacteria in the Baltic Sea

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ABSTRACT

Eutrophication favours harmful algal blooms worldwide. Blooms caused by cyanobacteria are a prominent feature in eutrophied lakes. The blooms cause toxic outbreaks and deteriorated recreational and aesthetic values, causing both economic loss and illness or death of both humans and animals. The Baltic Sea is the world's only large brackish water habitat with recurrent blooms of toxic and diazotrophic (capable of nitrogen fixation) cyanobacteria. Phosphorus is assumed to be the main limiting factor, along with temperature and light, for diazotrophic organism growth. This thesis evaluated the role of phosphorus nutrition as a regulating factor for the occurrence of diazotrophic cyanobacteria blooms in the Baltic Sea utilising experimental laboratory and field studies and meso- (10–100 km) and large-scale (Baltic Proper and Gulfs of Finland and Riga) surveys.

The abilities of laboratory strains of the two main bloom forming species (*Aphanizomenon* sp. and *Nodularia spumigena*) for growth on cellular phosphorus stores and a synthetic phosphomonoester were evaluated. Cellular phosphorus sources were found to be able to support substantial growth of both species. However, under the experimental conditions *N. spumigena* growth seemed independent of phosphorus source, whereas, *Aphanizomenon* sp. grew best in a phosphate enriched medium. Apparent discrepancies with field observations and experiments are explained by the typical seasonal temperature dependent development of *Aphanizomenon* sp. and *N. spumigena* biomass allowing, the two species to store ambient pre-bloom excess phosphorus in different ways.

The competitive ability to utilise pulsed phosphate supply, along with indicators of community and species level phosphorus stress were assessed in field experiments. Cyanobacteria bloom communities were found to be predominantly phosphorus limited. Phosphate additions were found to increase the accumulation of phosphorus relatively most in the planktonic size fraction dominated by the diazotrophic cyanobacteria. *Aphanizomenon* sp. and *N. spumigena* populations showed phosphorus deficiency, expressed as species-specific alkaline phosphatase enzyme activity (APA). *Aphanizomenon* sp. responded to phosphate addition whereas *N. spumigena* APA seemed independent of phosphate addition. The seasonal development of phosphorus deficiency is different for the two species with *N. spumigena* showing phosphorus stress during a longer time period in pelagic regions.

The effect of coastal upwelling on extant diazotrophic cyanobacteria bloom populations was studied and the species specific ability of *Aphanizomenon* sp. and *N. spumigena* to utilise the predominant phosphate enrichment of the surface layer was clarified. Observed bloom time vertical distributions of biomass maxima renders *N. spumigena* populations more susceptible to advection by surface currents caused by coastal upwellings. *Aphanizomenon* sp. populations residing in the seasonal thermocline were observed to be able to utilise the phosphate enrichment caused by upwelling and a bloom was produced with a two to three weeks time lag subsequent to the relaxation of upwelling.

The role of external loading vs. internal sources of phosphorus was examined in order to determine the main large-scale phosphorus source for bloom formation. Consistent high concentrations of dissolved inorganic phosphorus, caused by persistent oxygen deficiency, were found to be the main source of phosphorus for large-scale pelagic blooms. External loads were estimated to contribute with only a fraction of available phosphorus. Remineralization of organic forms of phosphorus along with vertical mixing down to the permanent halocline during winter set the level of available phosphorus for the next growth season. Events such as upwelling are important in replenishing phosphate concentrations during the nutrient deplete growth season. The autecological characteristics of the two main bloom forming species favour *Aphanizomenon* sp. populations in utilising the abundant excess phosphate concentrations and phosphate pulses mediated through upwelling. Whilst, *N. spumigena* displays predominant phosphorus limited growth mode and relies on more scarce cellular phosphorus stores and presumably to some extent on dissolved organic phosphorus compounds for growth. The Baltic Sea is hypothesised to be in an inhibited state of recovery due to the extensive historical external nutrient loading and the substantial nitrogen load caused by cyanobacteria nitrogen fixation. This state of the sea is characterised as a “vicious circle”.

Key words: diazotrophic cyanobacteria, Baltic Sea, *Aphanizomenon* sp., *Nodularia spumigena,* eutrophication, phosphorus, internal phosphorus loading

1. INTRODUCTION

The Baltic Sea is a semi-enclosed, non-tidal, brackish and estuarine basin located in a depression in the Fennoscandian shield formed after the last glaciation, approximately 10 000 years ago. The basin has undergone several transformations between limnic, oceanic and brackish stages from the freshwater Baltic Ice Lake (13 000 to 10 000 B.P.) into finally taking shape as the present brackish water Baltic Sea, approximately 2 000 B.P. The Baltic Sea is geologically very young and the present state, forthright, infantile. Due to a relatively small volume and long mean residence time of water (30 years), a large drainage area including areas of 14 countries, and a human population of more than 80 million the sea is exceptionally vulnerable to anthropogenic influence.

The Baltic Sea is characterized by a distinct estuarine quality with a strong surface layer salinity gradient from north to southwest (< 3 to > 30 PSU), where the shallow and narrow Danish Straits form the oceanic connection to the North Sea and Atlantic Ocean via the Kattegat and Skagerak (Fig. 1). A large drainage area in relation to the basin volume, causing large freshwater input, and the restricted oceanic connection influence the typical horizontal and vertical characteristics of the waters in the basin. The Baltic Sea experiences, except for the northern sub-basins the Bothnian Sea and Bay of Bothnia, rather strong permanent vertical salinity stratification. The prevailing salinity stratification is governed by the balance of saltwater pulses through the Danish Straits and the fresh water runoff from land. The saltwater pulses entering the Baltic Sea are sporadic and have varying magnitude, depending on intricate hydrodynamic processes in the Skagerak, Kattegat and the southern Arkona Basin (Stigebrandt 2001). The permanent salinity- and the seasonal thermal stratification of the Baltic Sea determine large-scale physical and biogeochemical processes and they govern the dynamics of eutrophication related phenomena, such as increased offshore blooms of diazotrophic cyanobacteria.

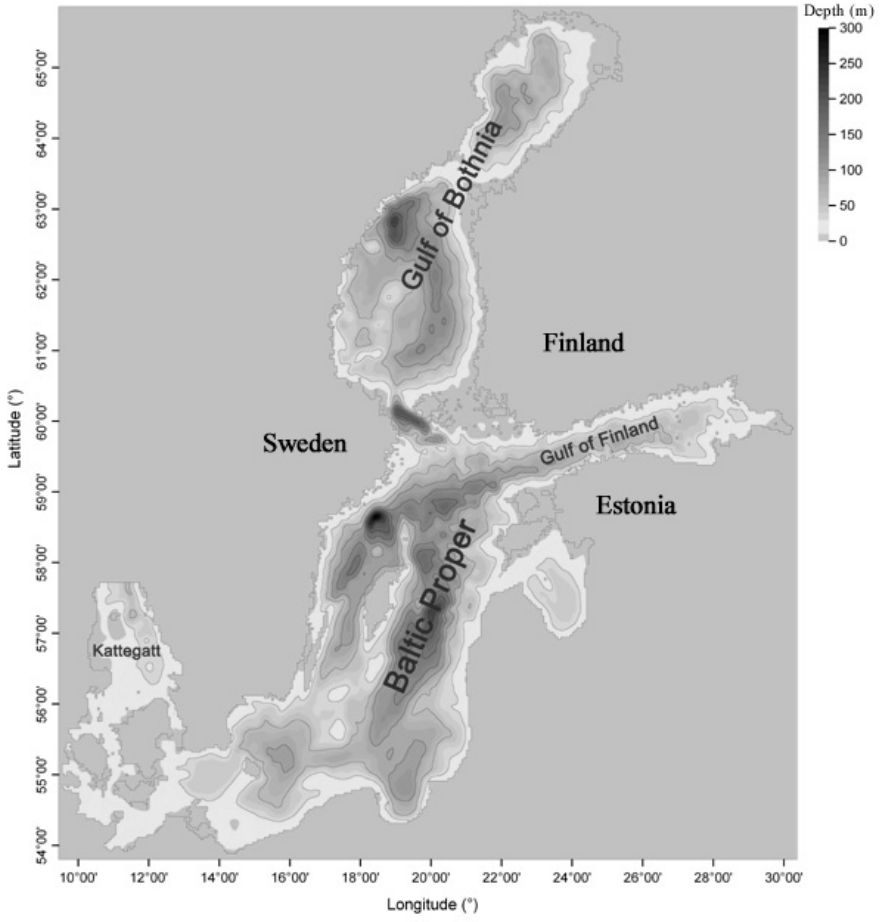

Fig. 1. Bathymetric map of the Baltic Sea.

Eutrophication of the Baltic Sea is a multiscale phenomenon, which is partly influenced by inherent features of the Baltic, along with the extensive anthropogenic loading. Eutrophication manifests it self as an interaction of physical, chemical and biological phenomenon on scales from single molecules and cells to scales encompassing the entire sea (Table 1). For example, the deep waters of the Baltic Sea are renewed by saltwater inflows through the Danish Straits (Stigebrandt 2001). Inflow frequency and magnitude is regulated by the sill depths in the Danish Straits, the density difference of inflowing and outflowing water, along with meteorological high-pressure systems above the northeast Atlantic. The physical exchange of deep waters influence internal removal of phosphate by either enhancing or reducing phosphate sequestration by sediments during oxic vs. hypoxic (oxygen concentration < 2 ml l^{-1}) / anoxic (absence of oxygen) deep waters and sediments, respectively. These processes work on long inter-annual scales (e.g. Conley & al. 2002). The transfer of deep nutrient rich water to the surface layer is influenced by the salt-water inflows by seasonal overturn of water masses and e.g. molecular diffusion through the pycnoclines. The depth to which primary producers again can effectively utilise the vertically transferred nutrient reserves is affected by solar irradiation, through light availability for photosynthesis and thermocline formation restricting vertical movement. Finally, nutrient uptake, trophic transfer and settling of particulate matter affects the available nutrients in the surface layer, where e.g. phosphate pool turnover times can be measured in minutes during summer (Grönlund & al. 1996).

Table 1. Characteristic scales of physical phenomena in the Baltic Sea and their relation to eutrophication related features and the papers of this thesis. Adapted from ICES – IOC GEOHAB Research plan for the Baltic Sea.

Spatial scales	Characteristic range	Description	Article:
Physical Microscales			
The Kolmogorov scale	0.1 cm	The length scale below which molecular viscosity is of importance. Below this scale random movements of molecules overrule the turbulent motion of water. The scale is of importance to movement of organisms smaller than this scale and nutrient acquisition of osmotrophs, which rely on diffusion and the diffusion limitation of osmotrophs is directly size dependent.	**I**, **II**
The Ozmidov scale	0.1–3.0 m	The length scale to describe turbulent flows under stable stratification. The length depends on the strength of stratification and it describes the vertical length scale at which the buoyancy force is of the same order of magnitude as the inertial forces. Important for description of vertical mixing of water masses and thus nutrient supply to osmotrophs during stratified conditions.	**II**, **III**
Scale of light penetration	1–10 m	Scale that defines the depth of the euphotic zone and the critical depth at which respiration and photosynthesis are balanced. It is affected by abiotic and biotic factors such as lithogenic particles, detritus, dissolved organic matter and phytoplankton.	**I**, **II**, **III**
Mixed layer depth			
Summer (thermocline)	10–20 m	Scale of vertical mixing of surface waters during thermally stratified conditions from April/May to September/October. Determines the depth to which neutrally buoyant particles are mixed and generally the boundary for depletion of dissolved inorganic nutrients. The strength of vertical stratification further determines the amount of external energy required for disrupting the stratification and therefore also the susceptibility of water masses to nutrient pulses.	**II**, **III**, **IV**
Winter (halocline)	60–70 m	Scale of vertical mixing of surface waters during the winter months when no thermal stratification exists in the upper 60 m layer. Separates the deep waters from surface waters and constitutes a boundary for phosphates originating from internal loading.	**IV**
Topographical scales			
Sill depths	20–60 m	Sill depths between basins regulate inflow events. The shallow Danish Straits have a strong influence on deepwater dynamics in the Baltic Sea.	**IV**

Spatial scales	Characteristic range	Description	Article:
Mesoscales			
The Rossby internal deformation scale	2–10 km	The width of the region of an interface of two water layers where the interface rises to the sea surface. I.e. the width of the region from where e.g. the thermocline rises to the sea surface from its present depth. The scale is highly variable in the Baltic, e.g. as density difference of the layers increases the length scale increases. The scale determines e.g. typical widths of coastal upwelling regions.	**III**
Temporal scales			
Instantaneous turnover time of nutrient pools	1 min – 10 h	Describes the demand of nutrients by the osmotrophic community.	**II**
Population growth rates	24 h – 10 days	Observed growth rates for diazotrophic cyanobacteria.	**I**, **II**
Time scale of weather patterns (wind forcing)	3–5 days	Typical time scale of external forcing creating hydrodynamic activity, such as coastal upwelling.	**III**
Time scale of thermal stratification	120–150 days	The time period when the surface layer is disconnected from deeper water masses by thermal stratification hampering transport of dissolved nutrients.	**IV**
Time scale of cyanobacteria blooms	60–120 days	*Nodularia spumigena* typically has a shorter time window during which blooms occur compared to *Aphanizomenon* sp.	**III**, **IV**
Residence time of total nutrient pools	87 years (P), 4.6 years (N)	The extremely long residence time of phosphorus in the Baltic Sea supplies phosphorus for bloom formation of diazotrophic cyanobacteria for several years.	**IV**
Residence time of water	30 years	Long residence times of water makes the Baltic a vulnerable sea to anthropogenic influence.	**IV**

The Baltic Sea has experienced progressing anthropogenic eutrophication since the beginning of the 20th century (Kauppila & al. 2005). Due to large anthropogenic influence the southern, central and eastern parts of the sea are more affected than the Bothnian Sea and Bay of Bothnia in the north. The increased anthropogenic nutrient input (Larsson & al. 1985, Granat 2001, Grimvall & Stålnacke 2001) has caused changes in phytoplankton species composition (Suikkanen & al. 2007) and increased production, which causes increased settling of organic matter (Struck & al. 2000, Voss & al. 2000). The increased amount of settling organic matter has increased the area of naturally occurring hypoxic and anoxic bottom areas, causing substantial internal loading of phosphate (Pitkänen & al. 2001, Conley & al. 2002). In the more eutrophied regions of the Baltic, primary production has been observed to be generally nitrogen limited (Granéli & al. 1990, Kivi & al. 1993, Tamminen & Andersen 2007). Thus, eutrophication hampering phosphorus sequestration by sediments is hypothesised to favour the occurrence of filamentous diazotrophic (capability to fix dissolved dinitrogen gas) cyanobacteria that gain competitive advantage over other osmotrophs during nitrogen limited conditions (Schindler 1977, Smith 1983). Diazotrophic cyanobacteria are likely a part of the natural biota of the present state of the Baltic Sea, as referred from sediment core analyses on alleged diazotroph pigments (Bianchi & al. 2000, Finni & al. 2001).

1.1 Diazotrophic cyanobacteria

Cyanobacteria are evolutionary one of the oldest organisms on earth, dating back more than 3 000 Ma (Schopf 2000). Cyanobacteria are oxygenic photosynthetic prokaryotes possessing the ability to synthesise chlorophyll *a* as their photosynthetic pigment. Species occurring in the Baltic Sea contain principally pelagic, benthic, epiphytic and epilithic species (Tikkanen & Willén 1992, Hällfors 2004). Many species are also diazotrophic, i.e. they possess the ability to fix dissolved atmospheric nitrogen gas. The most conspicuous groups in general are the pe-

lagic filamentous and unicellular pico-sized species (e.g. Stal & al. 2003), of which many of the filamentous species are diazotrophic. The filamentous and pico-sized species may form dense blooms causing toxic outbreaks and cause severe deterioration of recreational values.

Of the pelagic filamentous diazotrophic species occurring in the Baltic some are bloom-forming, specifically, *Nodularia spumigena* Mertens *ex* Bornet et Flahault 1886, species of the genus *Aphanizomenon* Morren *ex* Bornet et Flahault 1886 and to a lesser extent several species of the genus *Anabaena* Bory *ex* Bornet et Flahault 1886 (Rinne & al. 1978, Hübel & Hübel 1980, Niemistö & al. 1989). Previously the genus *Nodularia* has been argued to contain three main species present in the surface blooms, *N. spumigena*, *N. baltica* Komárek & al. 1993 and *N. litorea* (Kützing) Thuret *ex* Komárek & al. 1993. However, genetic studies (based on ITS1-S, PC-IGS and 16S rRNA sequences) support the presence of only one species, *N. spumigena* (Laamanen & al. 2001), the others being phenotypes. The only *Aphanizomenon* species always occurring in blooms has been suggested to be *Aphanizomenon flos-aquae* Ralfs ex Bornet et Flahault. The species has traditionally been divided in two varieties, var. *flos-aquae* and var. *klebahnii*. However, recent genetic studies (based on the ITS1-S sequence) do not support division of the species into to varieties (Laamanen & al. 2002), but a further revision of the genus is still under way (pers. comm. J. Komárek). Thus, *Aphanizomenon* sp. is generally used to refer to the bloom forming species in this thesis[1]. All three main bloom-forming taxa posses the ability to fix dissolved atmospheric nitrogen gas. They also may adjust their vertical positioning through buoyancy regulation by gas vesicles (e.g. Walsby & al. 1995). In the Baltic Sea *N. spumigena* and *Anabaena* spp. have been reported to produce toxins, nodularin and microcystin, respectively. (Sivonen & al. 1989a, Karlsson & al. 2005), whereas the *Aphanizomenon* sp. populations of the Baltic Sea have been found non-toxic (Sivonen & al. 1989a) as opposed to some fresh-water populations (Sawyer & al. 1968, Alam & al. 1973). Sedimentary and grazing losses of the filamentous species are generally small and most of the biomass is decomposed in the surface layer (Heiskanen & Kononen 1994, Sellner & al. 1994, Engström & al. 2001).

Specific bloom inocula of the main bloom forming species in the Baltic Sea are to date still unclear. Species of the genera *Nodularia*, *Aphanizomenon* and *Anabaena* all form resting cells called akinetes (e.g. Huber 1984, Komárek & Anagnostidis 1989). In shallow lakes the contribution of recruitment from sediments to the water column by *Aphanizomenon* sp. and *Anabaena* sp. has been observed to be small, ranging from 0.03 to 0.5 % of the total pelagic population (Karlsson-Elfgren & Brunberg 2004). In the pelagic Baltic Sea *Aphanizomenon* sp. populations are present in the water column throughout the year (Laamanen & Kuosa 2005) and they have been shown to be genetically identical (Laamanen & al. 2002), implying that blooms could mainly arise form over-wintering populations. *Anabaena* spp. akinetes are frequently found in plankton samples, and the sporadic occurrence of *Anabaena* spp. in pelagic blooms might indicate that recruitment from sediments may be more important for this species. In the Baltic Sea, *N. spumigena* akinetes are seldom encountered in sediment samples, and filaments are rarely found in wintertime water samples (e.g. Laamanen & Kuosa 2005). However, studies in an Australian estuary have revealed a significant contribution of akinetes to bloom formation with low continuous red light (620 to 655 nm), high temperature (22°C) and low phosphate concentrations facilitating germination (Huber 1984, 1985). However, the relative contribution of sediment recruitment or over-wintering water column populations for bloom formation of *N. spumigena* cannot reliably be assessed in the Baltic Sea at present.

Aphanizomenon sp. and *N. spumigena* co-exist and bloom during inorganic nutrient poor conditions in summer (Wasmund 1997, Laamanen & Kuosa 2005). *N. spumigena* is a better competitor for dissolved phosphate at lower concentrations due to a higher affinity for phosphate uptake (Wallström & al. 1992, Degerholm & al. 2006). *Aphanizomenon* sp. displays remarkable plasticity in C:P ratios with regards to growth and nitrogen fixation (Larsson & al. 2001) showing sustained growth for months relying only on intracellular phosphorus stores. Both species are known to benefit from low N:P ratios (Niemi 1979, Plinski & Józwiak 1999, Kangro & al. 2007). Despite general co-occurrence, both *N. spumigena* and *Aphanizomenon* sp. have typical temporal, vertical and horizontal distribution patterns that differ slightly, created by somewhat differing ecological niches of these two species. *Aphanizomenon* sp. pre-bloom biomasses are higher than for *N. spumigena* (Kononen & Leppänen 1997, Wasmund 1997, Laamanen & Kuosa 2005), creating larger bloom inoculum populations. Typically, the biomass of *Aphanizomenon* sp. also increases earlier than for *N. spumigena* but peak biomasses are lower

[1] In publication I included in this thesis *Aphanizomenon flos-aquae* is used to refer to the specific strain TR183 that has been characterised as *Aphanizomenon flos-aquae*. In Section 3.1.1. the name *Aphanizomenon* sp. is however used to avoid confusion with the rest of the thesis. Caution should be exercised when comparing the results of study I to natural conditions since it has been argued that the specific strain poorly represents the Baltic *Aphanizomenon* sp. populations (Laamanen & al. 2002). In publication III *Aphanizomenon flos-aquae* is used, in this publication the name refers to the yet uncharacterised Baltic Sea *Aphanizomenon* sp.

(Kononen 1992). *N. spumigena* is very scarce during winter when irradiance levels and temperatures are low (Laamanen & Kuosa 2005). A notable increase in biomass is preceded by an increase in global irradiation and water temperature (Wasmund 1997). Growth dependence on temperature of the two species show differences with *Aphanizomenon* sp. having a lower optimal growth temperature than *N. spumigena* (Lehtimäki & al. 1997). Temperature and irradiance seem to be strong regulating factors determining the bloom time species dominance patterns due to strong effects on realised growth rates. Both factors affect e.g. the size of the inoculum for summer blooms by allowing pre-bloom populations in spring and early summer to develop differently.

1.2 Eutrophication and blooms of diazotrophic cyanobacteria

Eutrophication is generally a result of excess availability of nitrogen and phosphorus that increase the biomass of algae and macrophytes. In oceanic areas characterised as high nutrient and low chlorophyll regimes, limiting trace elements such as iron can also induce a shift in the trophic state of the system by increasing primary productivity and biomass of phytoplankton (Boyd & al. 2000). Such areas include e.g. the eastern equatorial Pacific Ocean, the ice-free Southern Ocean and the open subarctic north Pacific (Chisholm & Morel 1991). The increased primary productivity alters biogeochemical cycles of nutrients and food web structure and may induce shifts of the community to altered stable states of differing community species compositions (Petraitis & Dudgeon 2004).

The Baltic Sea average (±SD) total nitrogen and total phosphorus loads are on an annual basis 752 ± 98 x 10^3 and 30 ± 3 x 10^3 t a^{-1}, respectively (Conley & al. 2002) and loads have shown an increasing trend from natural background loading levels (Cederwall & Elmgren 1990, Wulff & al. 1994). Estimates of nitrogen loads show up to 4-fold increase and phosphorus loads as much as 8-fold increase from the beginning of the 20th century (Larsson & al. 1985). The external load of nitrogen and phosphorus in the Baltic display an excess of nitrogen in relation to phytoplankton demand estimated as the traditional Redfield ratio of 16:1 (Redfield 1958). However, coastal surface waters show consistently nitrogen limitation of productivity and biomass (Tamminen & Andersen 2007) depicting to some extent the shorter residence times of nitrogen in the system (Wulff & al. 2001a) due to hypoxia and anoxia. Hypoxia and anoxia have, as diazotrophic cyanobacteria, been suggested to be inherent features of the brackish stages of the Baltic Sea, prevailing for more than 7000 years (Bianchi & al. 2000). However, eutrophication has lead to increasing chronic anoxic bottom areas and hypoxic water volumes in the Baltic Sea deep areas (Conley & al. 2002), facilitating the internal loading of phosphorus. Intermittent anoxic events have been observed to lead to increased susceptibility for further hypoxic events (Hagy & al. 2004) and prolonged hypoxia and anoxia to changes or elimination of benthic fauna (Diaz & Rosenberg 1995). Thus, changes in organic matter processing and further facilitation of persistent internal loading is observed, because intermittent oxic periods do not allow a re-establishment of benthic communities that would reoxygenate the sediments.

The Baltic Sea is recognized as a sea area heavily impacted by anthropogenic activities (Jansson & Dahlberg 1999, Wulff & al. 2001b) and eutrophication is deemed one of the most severe environmental threats to coastal marine and lake ecosystems globally (Schindler 2006). The Baltic Sea has a long history regarding eutrophication inferred from e.g. Secchi depth measurements (Sandén & Håkansson 1996, Laamanen & al. 2004) and paleoecological records (Clarke & al. 2006). Increased blooms of diazotrophic cyanobacteria (Kahru & al. 1994) may be attributed to ongoing eutrophication and subsequent ecosystem changes, including the spread of anoxic bottoms and hypoxic and anoxic water volumes caused by settling of the generally nitrogen limited spring bloom during deep water stagnation periods, which may last for years (e.g. Kivi & al. 1993, Heiskanen & Kononen 1994, Olli & Heiskanen 1999, Conley & al. 2002). Hypoxia and anoxia may also facilitate removal of nitrogen from the system through denitrification[2] and anammox[3] (Rönner & Sörensson 1985, Brettar & Rheinheimer 1991, Hannig & al. 2007).

Wide spread anoxic bottoms, subsequent internal loading of phosphorus and high winter-time surface layer phosphate concentrations are generally considered to translate into wide spread blooms of diazotrophic cyanobacteria (Fig. 2) (Jansen & al. 2004, Laanemets & al. 2006). However, the relationship is not always evident, especially on smaller spatial scales (Jaanus & Pellikka 2003). Since di-

[2] Denitrification is anaerobic reduction of nitrate to nitrogen gas through $NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$ by heterotrophic bacteria (Codispoti & Christensen 1985). Denitrification is an important nitrogen removal mechanism in the Baltic Sea (Stockenberg & Johnstone 1997, Tuominen & al. 1998, Gran & Pitkänen 1999).

[3] Anammox is an acronym for anaerobic ammonium oxidation. Anammox is a chemolithotrophic process whereby one mole of ammonium is oxidized by one mole of nitrite to produce nitrogen gas in the absence of oxygen ($NH_4^+ + NO_2^- \rightarrow N_2 + 2H_2O$) (Strous & al. 1999) Anammox has been observed to occurr in large scale in estuarine and marine sediments and in the water column (Thamdrup & Dalsgaard 2002, Kuypers & al. 2003, Trimmer & al. 2003, Myer & al. 2005, Hannig & al. 2007).

azotrophic cyanobacteria are dependent upon the availability of phosphorus, and generally gain competitive advantage from low DIN:DIP ratios (Smith 1983), the regulation of the size of the nitrogen pool is also of importance for questions pertaining to the nutrient dynamics of large scale pelagic blooms.

There has been extensive debate on whether phosphorus, nitrogen or reductions of both nutrients should be done from external emissions to curb adverse effects of eutrophication and diazotroph blooms in the Baltic Sea (e.g. Boesch & al. 2006). Phosphorus and nitrogen removal from wastewaters is as of now efficient in most surrounding countries and diffuse loading from agriculture and natural background losses are the most prominent external nutrient sources (HELCOM 2004). For nitrogen, nitrogen fixation constitutes a further substantial external source (180×10^3 to 790×10^3 t N a^{-1}) (Larsson & al. 2001, Wasmund & al. 2005). The internal source of phosphorus (release of phosphate from sediments during anoxic conditions) amounts approximately up to 14×10^3 to 100×10^3 t P a^{-1} (Emeis & al. 2000, Conley & al. 2002). Albeit large variation, the estimated ranges of nitrogen input through nitrogen fixation and internal load of phosphorus equals or even greatly exceeds the estimated amounts of external loads of nitrogen and total phosphorus of which only a part is bioavailable ($752 \pm 98 \times 10^3$ and $30 \pm 3 \times 10^3$ t a^{-1}, respectively).

Wintertime surface layer dissolved inorganic phosphorus (DIP) concentrations in the open northern Baltic Proper and western and central Gulf of Finland are generally high (approximately > 0.4 μM) (Suikkanen & al. 2007) and the dissolved inorganic nitrogen to DIP (DIN:DIP) ratio has been below the Redfield ratio of 16:1 at least since the mid 1960's (e.g. Niemi 1979, Suikkanen & al. 2007, V. Fleming-Lehtinen pers. comm.). This leads to faster depletion of nitrogen before phosphorus, due to uptake by the spring bloom (e.g. Larsson & al. 2001, Laanemets & al. 2006) and an excess of DIP is left over in the thermally stratified pelagic surface layer. Baltic Sea primary productivity and phytoplankton biomass (measured as chlorophyll *a*) has been generally observed to be nitrogen limited, except for the Gulf of Bothnia (Granéli & al. 1990, Kivi & al. 1993, Tamminen & Andersen 2007). In the post spring bloom-period, a further draw down of the spring bloom excess DIP, to levels below the detection limit of regular colorimetric methods (30 nM), continue (e.g. Larsson & al. 2001) along with a decrease in biologically available dissolved organic phosphorus (Nausch & Nausch 2006). Thus, a hypothesized link between general eutrophication and intensification of diazotrophic cyanobacteria blooms has emerged through the interlinked biogeochemical cycles of oxygen, nitrogen and phosphorus, an increased external load of nutrients and hydrographic characteristics of the Baltic Sea. The biomass of cyanobacteria blooms is to some extent governed by winter time surface layer phosphate concentrations (Jansen & al. 2004) presumably through late winter and pre-spring bloom luxury consumption (Larsson & al. 2001, Walve & Larsson 2007).

1.3 Upwelling as a transport mechanism of deep nutrient-rich waters in the Baltic Sea

In the Baltic upwelling can be considered one of the main hydrodynamic features replenishing surface layer nutrient reserves during thermally stratified conditions (Myrberg & Andrejev 2003, Zhurbas & al. in press). However, Baltic Sea upwelling events differ from oceanic scale upwelling events on several points. Oceanic scale upwelling events are persistent features bringing water to the surface occasionally from depths of more than 100 m (Mann & Lazier 1998) and of the large oceanic upwelling areas, e.g. the Peruvian upwelling centre can be active year round. Variation in upwelling intensity is detected on inter-annual scales and it is driven by the El Niño-Southern Oscillation phenomenon. Baltic Sea upwelling events are more sporadic and driven by passing high and low pressure systems with accompanying winds. The dominant wind direction in the Baltic area is from west to southwest, driving recurring upwelling events along e.g. the Swedish east coast and southern coast of Finland (Myrberg & Andrejev 2003). Even though upwelling events in the Baltic are driven more pronouncedly by smaller scale phenomena, the Baltic Sea upwelling events have also been shown to vary in frequency and magnitude with a large scale meteorological phenomenon, namely the North Atlantic Oscillation (Lehmann & al. 2002). The typical life span of upwelling events range from 0.5 to 10 days in the Baltic (Bychova & Viktorov 1986) and along with the shallow mean depth and intricate bottom topography, upwelling is a more dynamic feature in the Baltic Sea than in large oceanic upwelling centres.

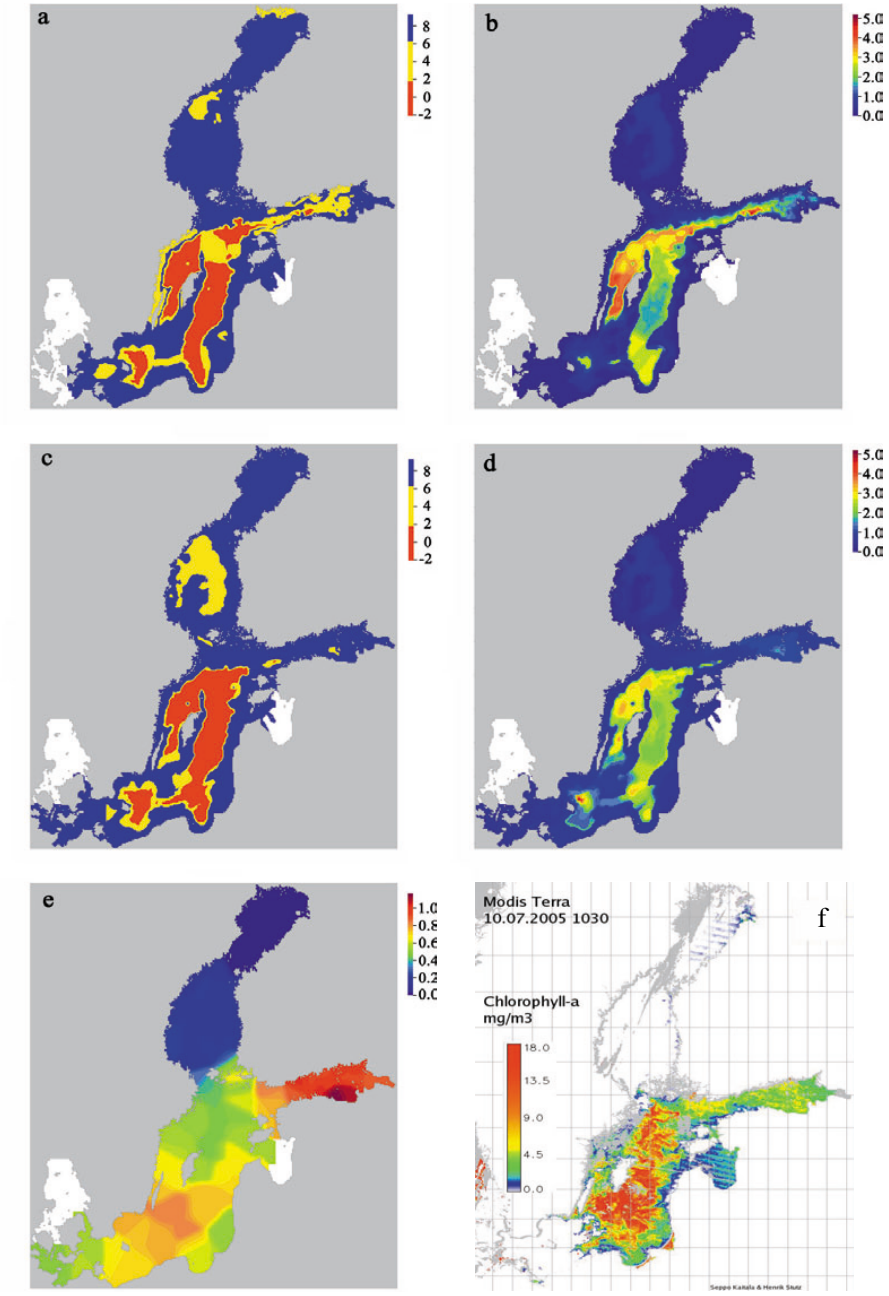

Fig. 2. Spatial and temporal development of bottom layer oxygen (ml O_2 l^{-1}) and phosphate (µmol l^{-1}) concentrations in summer 2004 and winter 2005 and wintertime (2005) surface layer phosphate concentration and a snapshot satellite image (Terra-MODIS) of surface blooms on 10 July 2005. Panels a and b: summertime bottom layer oxygen and phosphate concentrations. Panels c and d: wintertime bottom layer oxygen and phosphate concentrations. Panel e: wintertime surface layer phosphate concentration. Panel f: satellite image of surface blooms on 10 July 2005. Data on oxygen and phosphate concentrations from FIMR, satellite image of surface blooms interpreted by Seppo Kaitala and Henrik Stutz. Presence of hydrogen sulphide (H_2S) is indicated by negative oxygen concentrations in panels a and c.

Meso-scale coastal upwelling events, more precisely transversal Ekman upwelling strongly affect surface layer properties during the summer months as noted by field observations and modelling efforts (Nômmann & al. 1991, Haapala 1994, Myrberg & Andrejev 2003, Zhurbas & al. in press). Transversal Ekman upwelling is produced by external forcing, i.e. by winds blowing alongshore causing surface waters to be transported offshore, due to the deflection of currents from the wind direction caused by the Coriolis force. Surface layer temperatures may drop by up to 10°C and predominantly ammo-

nium and/or phosphate are transported to the surface layer (Haapala 1994). Cross-shore scales of developed coastal upwelling events are typically in the range of 5 to 20 km (Myrberg & Andrejev 2003) and distinct filaments form at the frontal zones (Zhurbas & al. in press). Formation of the filaments is strongly affected by bottom topography.

1.4 On the concept of nutrient limitation, inter-species competition and nutrient limitation of diazotrophic cyanobacteria

The main constituents of the cyanobacteria bloom community biomass are the diazotrophic filamentous cyanobacteria and unicellular colony forming and solitary picocyanobacteria (e.g. Kononen & al. 1993, Grönlund & al. 1996, Stal & Walsby 2000, Stal & al. 2003, Kangro & al. 2007). Nutrient limitation or deficiency in communities including organisms that are not constrained by the typical supply routes of nutrients (e.g. nitrogen fixers or mixotrophs) may experience multiple systemic nutrient limitation. Generally, in nitrogen-limited systems with diazotrophic organisms, the non-nitrogen fixing species experience nitrogen shortage and rely on leakage from the nitrogen fixing species. Nitrogen fixers are, on the other hand, partly limited by phosphorus (e.g. Kangro & al. 2007) or trace element availability (Stal & al. 1999). Thus, when communities are dominated by e.g. diazotrophic species in generally nitrogen limited environments, they will shift the limitation of the community towards phosphorus or trace elements (Karl & al. 2001, Lignell & al. 2003).

For the purpose of this thesis it is clarified here that the term "limitation" is used when a nutrient is short of demand in such extent that the actualised growth rate of the organisms is affected, the specific uptake affinity for nutrient uptake has been suggested to be a useful variable in distinguishing nutrient limitation (Tanaka & al. 2006 and references therein). The less stringent term "deficiency" is used when either single species or the entire community is observed to express a physiological state that indicates sub-optimal supply of the investigated nutrient (c.f. Thingstad & al. 1998).

The resource ratio theory as conveyed by Tilman (1980, 1982) describes the dynamic relationship of consumer species and limiting resources. The growth rates of individual species are functions of resource availability and the resource availability is a function of the resource consumption by the competing species and the environmental supply rates. A simple prediction derived from the resource ratio theory is that species that can reduce resource concentrations to low values and survive at these lower resource concentrations will outcompete other species when that specific resource is limiting. When two or more resources are limiting, tradeoffs in the use of these resources may allow coexistence of two competing species.

Sommer (1985) shows in his experiments that deviation from steady state conditions in resource supply, facilitates co-occurrence of several species. From these experiments a classification of phytoplankton into affinity-, velocity-, and storage-specialists was made. Many cyanobacteria species have been shown to be storage-specialists (e.g. Sommer 1985, Reinertsen & al. 1986, Sakshaug & Olsen 1986), being able to take up nutrients when supplied in pulses. However, unlike velocity-specialists, storage specialist diazotrophic cyanobacteria do not show immediate growth responses in relation to the pulses, but rather slow continuous growth of approximately $0.1 – 0.6\ \mu\ d^{-1}$ (Robarts & Zohary 1987, Lehtimäki & al. 1997, Stolte & al. 2002).

Nutrient limitation of growth is a "bottom-up" controlling mechanism, and occurs when the available nutrient supply falls short of organism demand. Nutrients may fall short of demand when the supply is very scarce or when the demand is very high, for autotrophs caused by e.g. high irradiance. Generally, physiological or systemic nutrient limitation can be identified (Thingstad & Rassoulzadegan 1995). Physiological nutrient limitation occurs when a single or multiple nutrients are short of demand reducing the maximal growth rate of an organism. Systemic nutrient limitation refers to the balance of nutrients in a given environment in relation to the requirement by the present organisms. The organisms may grow at maximal rates during these conditions but an addition of the least available nutrient will nevertheless increase biomass, e.g. due to increase in the number of supported trophic levels with increasing nutrient enrichment (Thingstad & Sakshaug 1990).

As stated above, nutrient limitation in natural communities is a dynamic phenomenon. The nature of nutrient control on phytoplankton growth is exemplified by a modelling effort of Thingstad & Sakshaug (1990). The authors show that nutrient limitation of phytoplankton growth is ultimately controlled by the combination of grazing and recycling, competition and level of nutrient enrichment of the system. The control is a property of the system since the rate of nutrient supply is not independent of any of the constituents in the food web, e.g. grazing on small fast growing algae allow proliferation of larger and slower growing algae when nutrient enrichment increases.

During conditions of sub-optimal nutrient supply, when uptake depletes extant external nutrient reserves, organisms with very efficient uptake systems and/or organisms relying on internal stores of nutrients prevail under starvation conditions. Disturbances of the balance by e.g. nutrient pulses might shift species dominance patterns (Sommer 1985).

The intermediate disturbance hypothesis (Connel 1978) predicts a relationship between species richness and disturbances depending on disturbance frequency, magnitude and elapsed time from the last major disturbance. Storage specialists (e.g. *Aphanizomenon* sp.) have been observed to benefit from pulsed nutrient supply (Sommer 1985), but the additional capability of alternative nutrient acquisition modes, i.e. nitrogen fixation or mixotrophy, further complicate competitive interactions. In the Baltic Sea where nitrogen is generally regarded as the limiting nutrient for primary productivity (e.g. Granéli & al. 1990) species not dependent on dissolved inorganic nitrogen are regulated by the supply of phosphorus (and possibly trace elements) and through grazing control on smaller algae detaching these species partly from competition with other osmotrophic organisms.

Physiological nutrient limitation is reduced by increased supply of the most limiting nutrient. However, increase of photosynthesis and carbon uptake, and thus growth, of algae is noted to be decoupled from nutrient uptake in non-steady state conditions (e.g. Lean & Pick 1981). During true steady state, the Monod equation (equation 1), which is derived from work describing enzyme kinetics at saturating substrate concentrations (Briggs & Haldane 1925), describes nutrient limited growth of phytoplankton or bacteria in relation to external nutrient supply in a satisfactory way, with some exceptions.

$$\mu = \mu'_m (S / K_S + S) \quad (1)$$

In equation 1 μ is the specific growth rate, μ'_m is an asymptotic maximal growth rate, S is the concentration of the limiting nutrient and K_S is the half saturation constant, i.e. the concentration of S where μ is one half of its maximum. The Monod equation is not useful as nutrient concentrations decline to values below analytical detection limits and it does not obviously work for the non-limiting nutrient (Kilham & Hecky 1988). Instead of external nutrient concentrations, the cellular nutrient content of phytoplankton can be used to describe growth of specific phytoplankton or bacteria. The cell quota (Q), defined as the quantity of nutrients in a cell, shows a relationship with growth rate (μ). This relationship was first introduced by (Droop 1978) who noticed that growth was linearly related to 1/Q.

$$\mu = \mu'_m (1 - Q_{min} / Q) \quad (2)$$

where Q_{min} is the minimum cellular quota of the limiting nutrient that sustains life functions. However, the Droop equation does not apply well to the non-limiting nutrient and when the cellular content of the limiting nutrient increases, i.e. luxury uptake of e.g. phosphorus occurs. It does also not describe growth in relation to external concentrations, thus growth in relation to supply is difficult to evaluate (Kilham & Hecky 1988). If the Droop model is applied with stoichiometric terms, relating the growth rate to the ratio of carbon and the limiting nutrient, we acquire a dynamic aspect to the measure of static elemental composition of organisms (Elser & Sterner 2002b). The authors showed that the relative growth rate decreases with increasing cellular C:P and C:N ratios for diatoms and green algae, either linearly or non-linearly. In order to exemplify the dynamic effect of nutrient availability through external supply and cellular content in an autotroph community consisting of several species, an analogy relating chemostat and batch culture conditions to situations in nature where populations are controlled heavily by grazing (top-down control) or when communities experience e.g. large pulses of nutrients (bottom-up control) was proposed.

In chemostats the nutrient ratios and contents of cells reflect the nutrient supply, and maximal growth rate is set accordingly. This situation might be expected during conditions in nature where regenerative pathways dominate nutrient supply and the phytoplankton community is controlled by grazing. The phytoplankton need to grow fast to keep up with grazing and cell quotas and growth rate is determined by the nutrient supply. Cellular C:P ratios have a linear relationship with relative growth rate. Whereas, during e.g. the spring bloom, which is not readily grazed due to lack of grazers, the phytoplankton community is provided with a ample supply of nutrients that is taken up and stored. This cellular quota produces a certain growth rate leading to the association between growth rate and cellular nutrient composition and a non-linear relationship of cellular C:P and relative growth rate due to use of nutrient stores in excess of physiological demand.

A situation where both views can be thought to be simultaneously true in nature can be found e.g. during blooms of diazotrophic cyanobacteria in the Baltic Sea. The community consists of a mix of grazable (e.g. fast growing nanoflagellates and bacteria) and less grazable (slow growing diazotrophic cyanobacteria) species. Assuming that energy, i.e. carbon supply does not limit growth of bacteria or heterotrophic flagellates a situation is created where relationships of nutrient content and growth rate are controlled differently for the different groups. The grazable fraction of the community behaves as in a chemostat with the cellular nutrient ratios and maximal growth rate set by the nutrient supply. The non-grazable fraction behaves more as in a batch culture with the cellular nutrient quota determining growth rates. This assumption works under the premises that the slow growing filamentous cyanobacteria do not effectively compete for scarce phosphorus sources but grow on cellular nutrient stores and respond to pulses of phosphorus that replenish the cellular stores and alleviate phosphorus defi-

ciency or limitation. Further, the non-nitrogen fixing community constituents rely on nitrogen introduced to the system by nitrogen fixation. Large phytoplankton species are typically storage specialists (Elser & Sterner 2002b), which also has been noted to be an important factor leading to the extensive blooms during the nutrient deficient period when blooms are most intense (e.g. Larsson & al. 2001, Nausch & al. 2004, Walve & Larsson 2007). Along with nutrient supply the structure of the food web and differential grazing pressures or other loss factors affecting the phytoplankton groups are of importance (Thingstad & Sakshaug 1990). The establishment of populations of large phytoplankton require a higher level of nutrient enrichment at which the growth rates of smaller phytoplankton are saturated and a linear food chain of grazers and predators is already established. The establishment of a trophic level including large phytoplankton with high nutrient demands does not occur until the nutrient concentrations are high enough to support a growth rate of large algae that balances both algal death rate and grazing. The generally slowly growing diazotrophic cyanobacteria are able to accumulate biomass due to the lack or relaxation of this balancing requirement because they are poorly grazed upon (Sellner & al. 1994, Koski & al. 1999) and experience small pre-bloom and bloom time loss-rates (Heiskanen & Kononen 1994).

Presently, we can account for three main phosphorus sources for the diazotrophs: 1. cellular nutrient stores stemming from an excess of phosphorus before, during and after the spring bloom (Larsson & al. 2001, Lignell & al. 2003, Walve & Larsson 2007), 2. regenerative pathways of phosphorus left in the upper mixed layer after establishment of the seasonal thermocline (Lignell & al. 2003) or 3. phosphorus brought to the surface layer during thermally stratified conditions (Kononen & al. 1996). The relative roles of these phosphorus sources for large scale pelagic bloom formation, sustenance and species selection are however to this date unclear.

2. OUTLINE OF THE THESIS

This thesis investigates the potential control of different phosphorus supply routes on the occurrence of diazotrophic cyanobacteria in the Baltic Sea. The aim was to investigate how anthropogenic nutrient inputs and internal eutrophication processes and nutrient supply modes (**III**, **IV**) in relation to phosphorus utilisation capabilities of the main bloom forming species (**I**, **II**) affect the potential control of the large-scale pelagic blooms occurring annually. Anthropogenic nutrient loading in the Baltic Sea has a long history, however external loads are small relative to changes in total pool sizes of both nitrogen and phosphorus, raising the question about whether internal or external nutrient sources are more important in controlling bloom formation and sustenance (**IV**). The assumption of phosphorus limitation of the diazotrophic cyanobacteria is investigated (**II**) along with a specification of differences of phosphorus deficiency characteristics of the main bloom forming species (**I**, **II**), *Aphanizomenon* sp. and *N. spumigena.* Further, the aim of the study was to elucidate if, and to what degree, and to which main bloom forming species phosphorus pulses mediated by hydrodynamic activity (such as upwelling) are channelled (**II**, **III**). The role of the form (inorganic vs. organic) of phosphorus as a factor influencing nutrient acquisition in bloom communities is investigated and the role of cellular phosphorus stores in controlling bloom formation is evaluated (**I**, **II**).

The questions have been studied by experimental and field studies. Species-specific differences in phosphorus utilization were examined in a laboratory experiment (**I**) that elucidated differences in growth of *Aphanizomenon sp.* and *N. spumigena* when the main phosphorus supply was either inorganic- or organic dissolved phosphate or the intracellular phosphorus stores of the species. The effects of pulsed phosphorus supply on cyanobacteria bloom communities were studied in field experiments (**II**) along with examinations of the severity of phosphorus deficiency of the communities and estimations of the role of pulsed nutrient supplies in relieving phosphorus deficiency of *Aphanizomenon* sp. and *N. spumigena*. The effect of coastal upwelling on species selection in blooms was studied in a field experiment (**III**). The capacity of diazotrophic cyanobacteria to utilize phosphorus brought to the surface layer during upwelling events was evaluated. Finally, a holistic approach linking biogeochemical cycles of oxygen, nitrogen and phosphorus was applied to try and explain the prevalence of cyanobacteria blooms in the Baltic Sea and to evaluate the main large-scale phosphorus sources for the blooms (**IV**). Results of these studies are discussed to increase knowledge, on scales from single cells to the whole Baltic, required for educated decisions on eutrophication abatement measures.

3. MATERIALS AND METHODS

3.1 Experimental studies

3.1.1 Experiment on species-specific differences in phosphorus utilization (I)

A batch culture experiment with the two main bloom-forming diazotrophic cyanobacteria species *Aphanizomenon* sp. and *N. spumigena* was conducted to reveal differences in the growth of these species when the main phosphorus source was either abundant external orthophosphate, an external supply of a synthetic organic phosphorus compound or intracellular stores of phosphorus. The experiment was carried out with a 2 x 3 factorial design (two different species and each one of them grown in three phosphorus conditions) and using the typical light regimes that the species experience during bloom periods.

Prior to the experiment, two axenic strains of cyanobacteria (TR183 *Aphanizomenon flos-aquae*) and (AV1 *N. spumigena*) isolated from blooms in the Baltic Sea (strains courtesy of Academy Professor K. Sivonen) were grown in liquid modified Z8 growth medium without nitrogen and with salt (Z8XS) (Sivonen & al. 1989b). The stock cultures were grown at 18°C in a climate-controlled room in low light (20 µmol q m^{-2} s^{-1}) produced by white-light fluorescent tubes (Osram L18 W/22 Lumilux White); the same light source was used during the experiment. Aliquots of each strain were transferred to duplicate acid-washed 1000 ml Erlenmeyer glass experimental units in a laminar flow cabinet to avoid contamination.

The experiment consisted of three treatments: (1) a control treatment with a replete external dissolved orthophosphate (PO_4^{3-}) supply (200 µM) in the Z8XS growth medium (P-replete treatment); (2) a treatment with a dissolved orthophosphate-deficient growth medium with an added dissolved organic phosphorus source (25 µM glycerol phosphate disodium salt hydrate [$C_3H_7Na_2O_6P$] added to Z8XS medium prepared without phosphorus) (DOP-enriched treatment) and (3) a treatment grown on Z8XS medium with phosphorus completely omitted (0 µM) (P-depleted treatment).

The experiment was run for 22 days at a temperature of 18°C in a climate-controlled room with a light dark cycle of 12:12 h. The units were sampled three times a week for concentrations of chlorophyll-*a*, phosphate phosphorus (PO_4^{3-}-phosphorus), nitrite and nitrate nitrogen (NO_2^-+NO_3^--nitrogen), particulate nitrogen (PN), particulate phosphorus (PP) and alkaline phosphatase activity (APA). On day 14 of the experiment, 300 ml of treatment-specific growth medium was added to the experimental units to dilute the cultures and to allow continuous growth. Transmission electron microscope (TEM) images and energy dispersive x-ray analyses to identify element composition of alleged polyphosphate granules were made at the end of the experiment from all the treatments.

Aphanizomenon sp. and *N. spumigena* display typical bloom time vertical distributions in the Baltic Sea (Niemistö & al. 1989, Hajdu & al. 2007). The vertical separation of biomass maxima during blooms leads to different light environments experienced by the species. Thus, different light levels (*Aphanizomenon* sp.: 40 µmol q m^{-2} s^{-1}, *N. spumigena*: 120 µmol q m^{-2} s^{-1}) were used in the experiment to establish any differences in phosphorus utilization occurring during these specific conditions. The light levels were derived from species-specific optimal light conditions determined by laboratory experiments (Lehtimäki & al. 1997) and light profiles (0–30 m) sampled from the northern Baltic Sea during July along with the typical vertical distributions of biomass maxima of the two species.

Mixed model repeated measures analyses (PROC MIXED) were used to test for mean differences in chlorophyll-*a* concentration, PN:PP ratio, SAPA, PP:chlorophyll-*a* ratio and PN:chlorophyll-*a* ratio between *Aphanizomenon* sp. and *Nodularia spumigena* in the three treatments. The main effects of species and treatment and the interactions of species-treatment, species-day and treatment-day on the measured parameters were tested. An autoregressive covariance structure was used and the experimental unit was used as the within subject factor and the treatment as the between subject factor. All analyses were conducted with the Statistical Analysis System (SAS V8.02 SAS Institute Inc., Cary, NC, USA). Appropriate transformations were applied where necessary to ensure normal distribution of model residuals.

3.1.2 Experiments on nutrient limitation characteristics of natural cyanobacteria bloom communities (II)

An experimental study was conducted along an east-northeast to west-southwest transect from the central Gulf of Finland to the northern Baltic Proper, encompassing a salinity gradient in the upper 5 m layer (from 5.4 to 6.6 PSU). Three geographically distinct experimental stations in the central Gulf of Finland, western Gulf of Finland and northern Baltic Proper were visited.

The experiments consisted of a control-, an ammonium (NH_4^+) addition- and a phosphate (PO_4^{3-}) addition-treatment incubated for 48 h in 14 l polyethylene containers placed in flow-through pools on the deck of R/V Aranda. Samples were acquired with a 30 l sampler from 2 m depth in triplicate casts, which were then pooled to a mixed sample. A 29 l sub-sample for each treatment was transferred into clean acid washed containers after, into which

nitrogen was added as ammonium (8000 nM) and phosphorus as phosphate (500 nM). From the nutrient added 29 l sub-samples a 14 l aliquot was transferred to duplicate experimental units (acid washed transparent polyethylene containers). Solar irradiation intensity in the on deck flow-through pools was reduced to levels equalling irradiation intensities at approximately 2–5 m depth (~100 µmol q m^{-2} s^{-1}) by covering the pools with fine black mesh. Samples for determination of particulate P, alkaline phosphatase activity (APA), chlorophyll *a* (chl *a*), soluble reactive phosphorus (SRP), dissolved inorganic nitrogen (DIN) concentrations, primary and bacterial productivity measurements and enzyme labelled fluorescence (ELF) counting (identifies species specific APA) were acquired on three occasions; 1–2, 24 and 48 h from the start of the experiment.

Parallel to the main experimental units, smaller experimental units for determination of phosphorus dynamics using ^{33}P as a tracer (added as carrier free ^{33}P labelled orthophosphate) were incubated under the same conditions. Samples for ^{33}P uptake experiments were acquired from the same nutrient added 29 l sub-samples as the sample water for the main 14 l experimental units. Single 500 ml samples were taken from the nutrient added 29 l samples for determination of ^{33}P accumulation into size fractions and transfer of ^{33}P between size fractions. The 500 ml sample was incubated on deck for 48 h in an acid washed 1 L polycarbonate flask and sampled at four occasions at 2, 8 25 and 46 h from the start of the experiments. Duplicate 250 ml samples from the 14 l main experimental units were taken at three occasions (2h, 24h and 46h) during the 48 h incubations and incubated on deck for 30 to 120 minutes in 500 ml acid washed glass flasks in order to determine the orthophosphate turnover-times (T_t).

Tanaka & al. (2006) suggested a set of indicators for studying phosphorus deficiency or limitation of the osmotroph community, including biomass specific APA (SAPA) and specific affinity for phosphate uptake. Levels of SAPA < 0.2 h^{-1}, were considered as indicators of phosphorus deficiency not limitation and α threshold values of 0.01 L nmol P^{-1} h^{-1} indicative of phosphorus limitation and 0.001 L nmol P^{-1} h^{-1} indicative of phosphorus deficiency. Increasing values of α indicate increasing deficiency or limitation. These indicators are used in assessing phosphorus deficiency or limitation in bloom communities but should be treated with caution and considered only as relative indicators.

Mixed model repeated measures analyses (PROC MIXED) of the Statistical Analysis System (SAS V8.02 SAS Institute Inc.) were used to test for mean differences in particulate P concentration, chl-*a* concentration, primary production and bacterial production. The main effect of treatment and time and the interaction of these terms on the measured variables were tested separately for each experiment. An autoregressive covariance structure was used and the experimental unit was used as the within subject factor and the treatment as the between subject factor. Appropriate transformations were applied where necessary to ensure normal distribution of model residuals. When the criteria for parametric tests could not be met by transformations a Kruskal-Wallis non-parametric test was used to test for difference between treatments separately for each time point. Differences in ELF labelling percentage between *Aphanizomenon* sp. *and N. spumigena* and treatments were examined separately with a X^2 –test.

3.2 Field studies

3.2.1 Study on mesoscale coastal upwelling (III)

The upwelling study was carried out in the period 19 July to 11 August 1999 on board R/V Aranda (Finnish Institute of Marine Research) in the northern entrance area to the Gulf of Finland (Fig. 1). The fieldwork consisted of 10 mesoscale surveys utilizing the ship's flow-through system. The surveys were made during $20^{th}/21^{st}$, 24^{th}, 25^{th} and $27^{th}/28^{th}$ July and on $2^{nd}/3^{rd}$, $3^{rd}/4^{th}$, $4^{th}/5^{th}$, $8^{th}/9^{th}$, $9^{th}/10^{th}$ and $10^{th}/11^{th}$ August. They consisted of 4 to 9 legs, about 20 km long and ~4.6 km to ~9.2 km apart. During the surveys the flow-through system recorded temperature, salinity and *in vivo* chlorophyll *a* fluorescence and collected water samples at a depth of 5 m for nutrient and chlorophyll *a* analyses and phytoplankton counts. Temperature, salinity and chlorophyll *a* fluorescence were recorded as 10-minute means of actual loggings at 10-second intervals. Samples for nutrient analyses were taken at 20 to 40-minute intervals, while phytoplankton and chlorophyll *a* samples were taken at 40-minute intervals with a refrigerated automatic sampler. For the three temporal sampling frequencies used, the corresponding spatial resolutions along the ship's track were approximately 2, 4 and 8 km.

In addition**,** three sampling periods at a fixed station (59°42.5'N, 23°37.8'E) were carried out on $22^{nd}/23^{rd}$ July, $25^{th}/27^{th}$ July and $28^{th}/29^{th}$ July to resolve the vertical distribution of temperature, nutrients, chlorophyll *a*, primary production and phytoplankton species abundances. A single sampling in the vicinity of the fixed station was performed on 4 August to measure temperature, nutrients and phytoplankton species abundances. The durations of the three fixed-station sampling periods were 41, 48 and 16 hours on $22^{nd}/23^{rd}$, $25^{th}/27^{th}$ and $28^{th}/29^{th}$ July, respectively. CTD/fluorometer casts at the fixed station were performed at 1 to 2-hour intervals. Water samples for nutrient analyses were collected with a Rosette sampler mainly from standard depths (0, 5, 10, 15, 20 m) at 2 to 4-hour intervals. Samples for the measurement of chlorophyll *a* were taken at 2 to 4-hour intervals, samples for

phytoplankton cell-counts at 4-hour intervals and samples for measurement of primary production were taken on two occasions (22nd and 27th July) with a vacuum sampler from depths ranging from 3–20 m so as to facilitate the calculation of a depth integrated primary productivity. The vacuum sampler used consists of a 35 l stainless steel cylindrical container with a pump inducing a negative pressure (~150 kPa). Water is sucked into the container through a hose (20 mm in diameter). The vacuum sampler was always rinsed once at the sampling depth, by filling and emptying it, before the actual sampling. During the field measurements wind data was acquired by the ship's weather station at 10-minute intervals.

To extend the interpretation of the effect of upwelling on the pelagic environment, additional data from meteorological and open sea environment monitoring programmes were used. For the interpretation of the development of upwellings from 1 July to 15 August, Utö weather station data recorded with 3-hour intervals (Finnish Meteorological Institute) were used to complement the R/V Aranda wind data series. With the aim of discovering the temporal course of the *Aphanizomenon* sp. and *N. spumigena* biomass and surface layer temperature, data collected within the Alg@line ship-of-opportunity (SOOP) monitoring programme (Leppänen & al. 1994) in the western Gulf of Finland were used. Surface layer temperature data from the Finnish coastal zone were acquired from the monitoring transect between Helsinki (Finland) and Travemünde (Germany) at 4-day intervals. Weekly phytoplankton species abundance data from eleven fixed locations between Helsinki and Tallinn were used for further interpretation of our own results.

3.2.2. Study on systemic scale biogeochemical cycles (IV)

The study examining the effect of large-scale biogeochemical cycles on bloom forming diazotrophic cyanobacteria employed several different approaches. The annual development of nitrogen and phosphorus pools and how they are related to hydrography and biogeochemistry were analyzed. The data was acquired mainly from the Swedish Meteorological and Hydrological Institute (SMHI), but contains also monitoring data from other nations. Data from the station BY15 in central Eastern Gotland basin for 1994–2005 is used for analysis of annual development of nutrient concentrations. The number of vertical profiles used ranges from 139 to 170 depending on the parameter. Vertical average concentrations were computed by first interpolating the measurements vertically to a 1 m resolution and then integrated using the hypsographic function for the Baltic Proper, excluding the Gulf of Finland, the Gulf of Riga and the Bornholm basin.

To examine the long term variation of water column pools of nitrogen a basin-wide approach as previously done for phosphorus (Conley & al. 2002) was applied. Nitrogen pools were summed up for three sub-basins where nitrogen fixation mainly occurs, the Baltic Proper and the Gulfs of Finland and Riga. Annually averaged pools of total nitrogen (TN) and dissolved inorganic nitrogen (DIN) as well as volumes of water confined by the oxygen isosurfaces of 0 and 1 ml l^{-1} were computed with the Data Assimilation System (Sokolov & al. 1997) on 3D fields reconstructed from observations found in the Baltic Environment Database (BED) (Stockholm University, Department of Systems Ecology, Marine Ecosystems Modelling Group, http://data.ecology.su.se/models/bed.htm) that is the best compilation of available data from monitoring programs and scientific cruises in the region. The time series of combined nitrogen input to the Baltic Proper from land and atmospheric sources were compiled from several sources, including (Stålnacke & al. 1999), unpublished data in BED, the periodic load compilations by HELCOM (e.g. HELCOM 2004), (Granat 2001), and published and unpublished data from the Co-operative Programme for Monitoring and Evaluation of the Long-range Transmission of Air pollutants in Europe (EMEP) (e.g. HELCOM 2005).

3.3 Measurements

Standard methods used in biological oceanography were employed in the studies. In the following an outline of the most important methods for this thesis performed by the author are presented.

3.3.1 Phosphorus turnover time, biologically available phosphate and specific phosphate affinity

The ratio between the concentration of naturally available orthophosphate (S_n; nmol-P l^{-1}) and the flux of phosphorus through this pool, measured as uptake rate by osmotrophs (V; nmol-P l^{-1} h^{-1}) is the turnover time of the biologically available free orthophosphate pool (T_t; h).

$$T_t = S_n / V \quad (3)$$

T_t in paper **II** was measured and calculated according to (Jones 1997). Samples for determination of T_t were allowed to acclimate for at least 30 min before addition of carrier free $^{33}PO_4$ (20 000–50 000 DPM $sample^{-1}$). T_t was measured by rapid filtration, using a 12-hole Micropore filtration manifold, onto 25 mm 0.2 µm pore size polycarbonate membrane filters (Whatman) at three time points. The filtration intervals ranged from 10–120 min, depending on expected turnover times estimated from measured

soluble reactive phosphorus (SRP) concentrations. The samples were filtered under low negative pressure (< 20 kPa) until the filters were completely dry, after which filters were transferred to liquid scintillation vials with immediate addition of scintillation liquid (Instagel). The incorporated ^{33}P was assayed with liquid scintillation techniques (Wallac Rack-Beta) and all results were corrected for decay time of ^{33}P. Formalin killed triplicate samples (500 µl 37 % formalin 10 ml^{-1} sample), treated similarly to live samples, were used to estimate the amount of abiotic adsorption of phosphate. T_t was calculated by subtracting killed blank values from counts retained on the filter then counting the percentage of ^{33}P in the filtrate and plotting the natural logarithm of the percent of ^{33}P in the filtrate against incubation time. The linear part of the slope was used to estimate the uptake rate constant (fraction of incorporated ^{33}P h^{-1}) and T_t is given as the reciprocal of the rate constant.

T_t, along with carbon primary productivity (PP: nM d^{-1}) and carbon bacterial productivity (BP: nM d^{-1}) estimates were used to calculate amounts of biologically available phosphate ($[PO_4]$) according to Moutin & al. (2002). An estimate of total phosphate uptake (V: nM d^{-1}) restricted to the Redfield ratio (C:P = 106) for algae and cyanobacteria and to a C:P ratio of 50 for bacteria (Vadstein 1998, Kirchman 2000) and assuming direct coupling of production and nutrient uptake was calculated according to equation (3)

$$V\ (nM\ d^{-1}) = PP/106 + BP/50 \quad (4)$$

The biologically available phosphate is then acquired using T_t according to equation (4)

$$[PO_4] = T_t\ (PP/106 + BP/50) \quad (5)$$

To account for a variable C:P of algae and bacteria and uncoupled uptake and growth, estimates of [PO4] range were made using a wide range of C:P ratios of 75–1 000 for algae and cyanobacteria (Hecky & Kilham 1988) and 35–178 (Vrede & al. 2002) for bacteria. The upper ends of these ranges are more probable due to a high light environment and a general phosphorus shortage of the organisms, increasing the C:P ratio (Elser & Sterner 2002b).

To describe phosphate uptake at low concentrations and to be able to compare the phosphorus nutritive status of communities the specific affinity for orthophosphate uptake (α) according to Thingstad & Rassoulzadegan (1999) was calculated. α is analogous to a clearance rate, i.e. how large a water parcel a certain biomass of osmotrophs can clear of a nutrient within a specified time. α approaches zero with increasing substrate concentrations as saturation of uptake sites of the substrate on the cell surface is approached. Increase in substrate concentrations is most efficient at reducing affinity at intermediate concentrations and the reduction of affinity due to saturation depends on the amount of free uptake sites and the residence time of the substrate at the uptake sites (Button 1998). Affinity, i.e. the efficiency of nutrient uptake and subsequent utilization in growth is also affected by the cell size due to a decrease in the surface to volume ratio of cells with increasing size according to $y = 3r^{-1}$, where r is the radius of the cell. Smaller cells thus have a larger surface to volume ratio and also higher affinities for nutrient uptake (Friebele & al. 1978, Smith & Kalff 1982, Jumars 1993). When substrate concentrations are sufficiently low the initial part of the hyperbolic uptake function can be approximated by a linear relationship, where uptake is proportional to substrate concentration.

Thus, as an osmotroph community consists of a group of organisms with the biomass B (measured as particulate phosphorus; nM-P) and these organisms take up orthophosphate proportional to the natural concentration orthophosphate in water (S_n) with a rate V, we have $\alpha S_n B = V$, where α is the specific affinity for orthophosphate uptake (l $nmol\text{-}P^{-1}$ h^{-1}). Since $T_t = S_n/V$, one obtains the total specific uptake rate of phosphorus (Thingstad & Rassoulzadegan 1999)

$$\alpha B = V/S_n = 1/T_t \quad (6)$$

The total specific uptake rate of the community can be normalized to the total biomass of osmotrophs and be defined as specific affinity for orthophosphate uptake by the osmotroph community (α):

$$\alpha = \alpha B/B = V/S_n B = 1/T_t B \quad (7)$$

A separation between bacterial and phytoplankton affinity was not made since limitation patterns were compared including the entire community. Thingstad & Rassoulzadegan (1999) have noted that bacterial and phytoplankton specific affinities are comparable in the Mediterranean, thus we assume that the affinities we measure describe the averaged uptake of bioavailable free orthophosphate at low concentrations.

3.3.3 Enzyme labelled fluorescence

Alkaline phosphatase activity (APA) has been widely used to infer phosphorus deficiency of phytoplankton in natural and laboratory conditions (review by Jansson & al. 1988) and it has recently been suggested to be a good indicator of phosphorus deficiency in natural communities (Tanaka & al. 2006). However, bulk alkaline phosphatase activity measurements do not discriminate between the source organisms of the enzyme. Therefore, species specific alkaline phosphatase activity of diazotrophic cyanobacteria was studied using the fluorogenic substrate Enzyme-labelled Fluorescence (ELF) substrate reagent (Molecular Probes Inc., OR, USA;

Larison & al. 1995). The soluble non-fluorescent substrate CPPCQ (2-(5'-chloro-2'-phosphoryloxypheny1)-6-chloro-4-(^{3}H)-quinazolinone) or more commonly known as ELF reacts with the alkaline phosphatase enzyme on the cell surface of organisms and liberates a phosphate molecule and precipitates rapidly as an insoluble yellow-green fluorescent product CHPQ [(2-(5'-chloro-2'-hydroxypheny1)-6-chloro-4-(3H)-quinazolinone] at the site of the enzymatic reaction. This ensures that sites of enzymatic activity on the cell surface are easily observed under fluorescence microscopy and species-specific alkaline phosphatase activity in mixed phytoplankton samples can be assessed (e.g. Rengefors & al. 2003).

The ELF 97 endogenous phosphatase detection kit (E-6601, Molecular Probes) was used on subsamples concentrated on 20-µm nylon mesh in order to asses the species specific alkaline phosphatase activity of *Aphanizomenon* sp. and *Nodularia spumigena* during our experiments presented in paper **II** and for monitoring samples presented in Fig. 11. The sample was transferred from the mesh with a pipette, using the filtrate to wash the mesh, into 1.5 ml centrifuge tubes. The samples were centrifuged at 1000 g for 5 minutes after which the supernatant was removed without disturbing the pellet. The pellet was incubated in dark in 500 µl 70 % ethanol for 30 minutes after which the centrifugation procedure was repeated and the supernatant removed. After which, a 70 µl mix of the ELF substrate and buffer solution (1:20) was added and the sample was incubated in dark for 30 minutes. The incubation was stopped by repeatedly washing the sample with filtered (0.2 µm) and autoclaved seawater. Samples were stored in 200–300 ml of filtered seawater in dark and cold (+5^{o}C). Samples were counted within a month of the preparation using epifluorescence microscopy (Leitz Aristoplan) using a DAPI filter. The total amount of filaments and amount of labelled filaments were counted and results are given as the percentage of labelled filaments in the total population. A total of at least 100 filaments per sample and species were counted.

4. RESULTS

4.1 Species specific differences in phosphorus utilization between *Aphanizomenon* sp. and *N. spumigena* in laboratory cultures (I)

N. spumigena grew equally well in all treatments, albeit slowly, whereas *Aphanizomenon* sp. seemed to require an ample supply of inorganic phosphate to be able to grow efficiently as shown by the slower growth rates in the DOP-enriched and P-depleted cultures (Fig. 3). The difference in chlorophyll *a* concentrations between species was significant (Table 2). The P-replete *Aphanizomenon* sp. treatment grew at a similar rate to the *N. spumigena* treatments. The relatively slower growth, regardless of treatment, of *Aphanizomenon* sp. is nevertheless mirrored by the significant Species and Day interaction term.

At the beginning of the study both species had low PN:PP ratios (<16:1) in all treatments and showed a similar temporal development of the ratio (Fig. 3, Table 2 non-significant Species and Treatment interaction term). The P-depleted treatments of both species showed the most notable increases in the PN:PP-ratios. *Aphanizomenon* sp. seemed to have a slightly more prominent increase of the PN:PP-ratio in the DOP-enriched (increase from 13:1 to 26:1) and in the P-replete (increase from 6:1 to 17:1) treatments than the *N. spumigena* DOP-enriched (increase from 13:1 to 22:1) and P-replete (increase from 7:1 to 11:1) treatments had. The general change in PN:PP ratios, from day to day, was different as indicated by the significant interaction term of Species and Day, with *Aphanizomenon* sp. showing a more rapid average increase in PN:PP ratios.

Both species displayed rather stable particulate phosphorus (PP) concentrations in the P-deplete treatments (Fig. 4), with *N. spumigena* concentrations being higher. *Aphanizomenon* sp. showed only modest increase of PP in both the DOP-enriched and P-replete treatments, whereas an increase for *N. spumigena* PP concentrations in these treatments was substantial. Treatments evoked clearly different effects on PP during the experiment and the day-to-day difference between species was significant (Table 2, species and day interaction term).

The treatments evoked clearly differing and significant effects in chlorophyll *a* normalized alkaline phosphatase enzyme activity (SAPA), with all but the P-replete treatments showing increasing specific enzyme activity (Table 2, Fig. 5). The species affected the response to treatment statistically significantly as shown by the Species and Treatment interaction term of mixed model repeated measures analyses (Table 2). *N. spumigena* SAPA in the DOP-enriched treatment showed a more pronounced increase than that of the *Aphanizomenon* sp. DOP-enriched treatment. APA in the dissolved fraction (< 0.2 µm) of the treatments for *Aphanizomenon* sp. varied from 3 to 35 % of total APA and for *N. spumigena* from 19 to 67 %.

DIP concentrations decreased for both species in the P-replete treatments and were on a constantly low level in the P-depleted treatments. However, in the DOP-enriched treatments phosphate concentrations increased initially for both species indicating

liberation of phosphate from the organic compound (Fig. 6). For *N. spumigena* the concentrations declined to levels matching the P-depleted treatment by day 11 of the experiment, concurrent with a rapid increase in SAPA (Fig. 5). Whereas for *Aphanizomenon* sp. phosphate concentrations stayed at a higher level of about 11 to 12 µM after day 9 of the study.

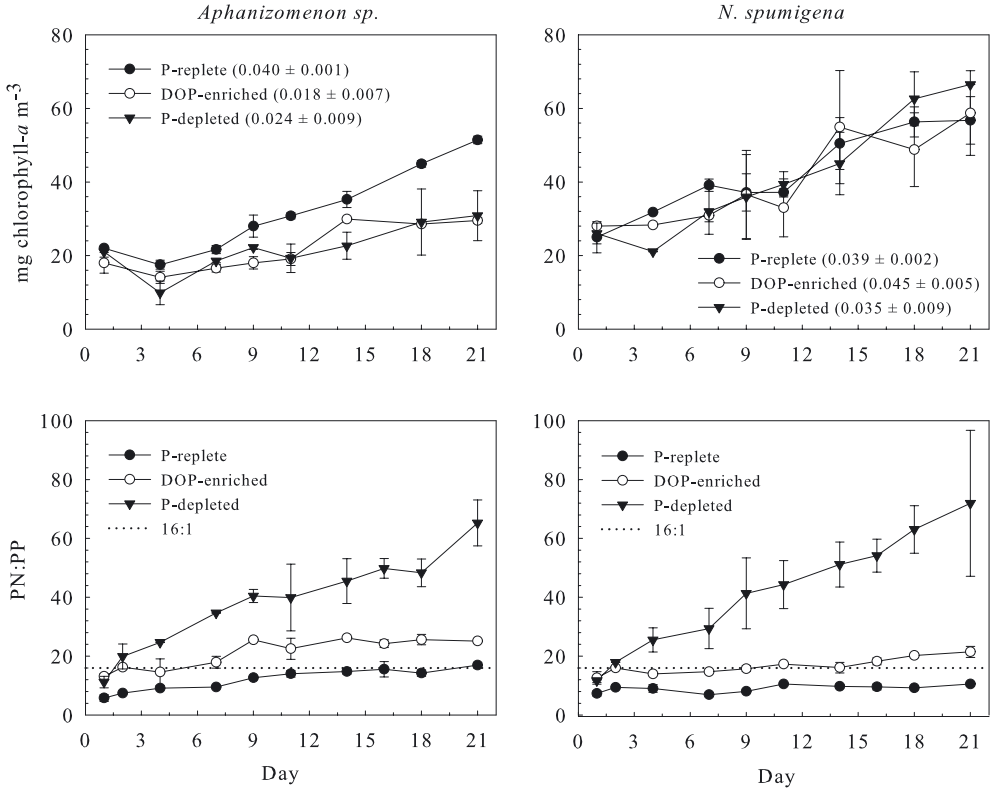

Fig. 3. Chlorophyll *a* concentrations (mg m^{-3}) (a and b) and particulate nitrogen to particulate phosphorus ratios (PN:PP) (c and d) during the experiment for *Aphanizomenon* sp. and *Nodularia spumigena* in phosphorus-replete (P-replete), phosphorus-deficient with an organic phosphorus addition (DOP-enriched) and phosphorus-depleted (P-depleted) treatments. Error bars indicate standard deviations. The average growth rates (µ: day^{-1}) and standard deviations for both species and all treatments are given by the values in the brackets in the figure legends. The dotted line shows the Redfield-ratio of 16:1 in panels c and d. Figure redrawn and modified from paper **I**.

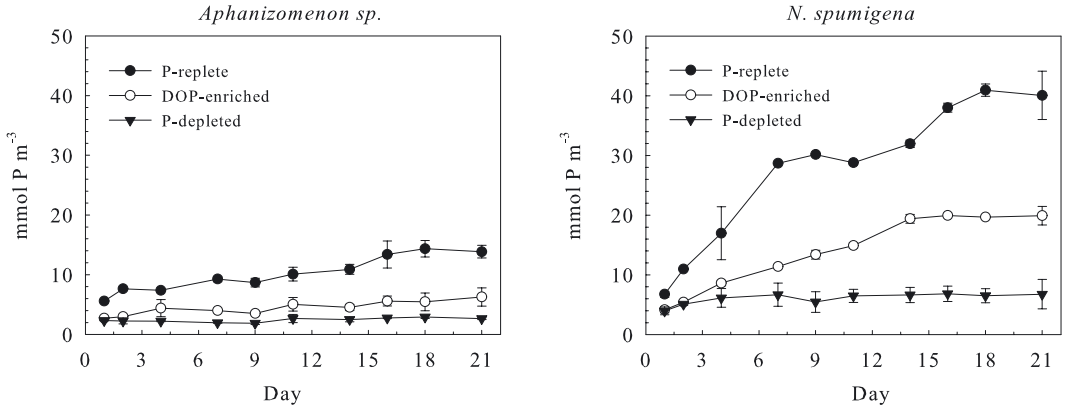

Fig. 4. Particulate phosphorus (PP) (µmol l^{-1}) concentrations during the experiment for *Aphanizomenon* sp. and *Nodularia spumigena* in phosphorus-replete (P-replete), phosphorus-deficient with an organic phosphorus addition (DOP-enriched) and phosphorus-depleted (P-depleted) treatments. Error bars indicate standard deviations.

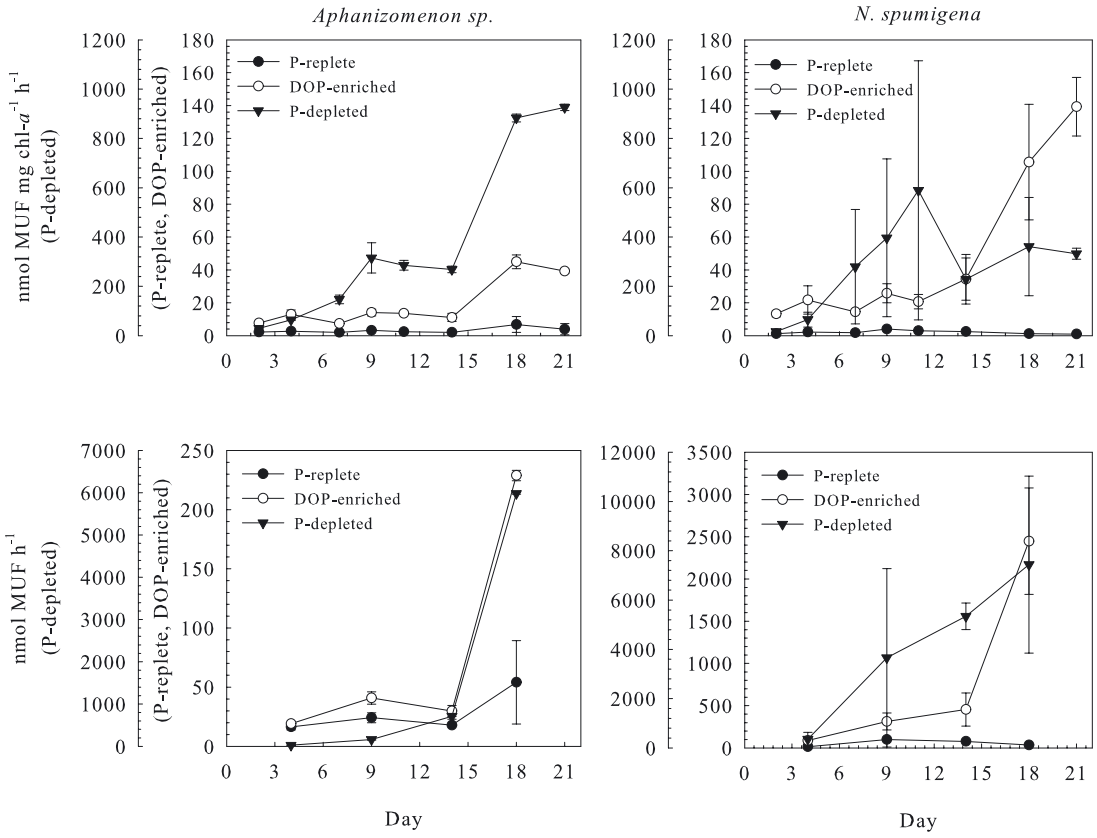

Fig. 5. Chlorophyll *a* specific alkaline phosphatase activity (SAPA) (nmol MUF mg chl a^{-1} h^{-1}) (a and b) and soluble alkaline phosphatase activity (APA) (nmol MUF h^{-1}) (c and d) during the experiment for *Aphanizomenon* sp. and *Nodularia spumigena* in phosphorus-replete (P-replete), phosphorus-deficient with an organic phosphorus addition (DOP-enriched) and phosphorus-depleted (P-depleted) treatments. Error bars indicate standard deviations. Figure redrawn from paper **I**.

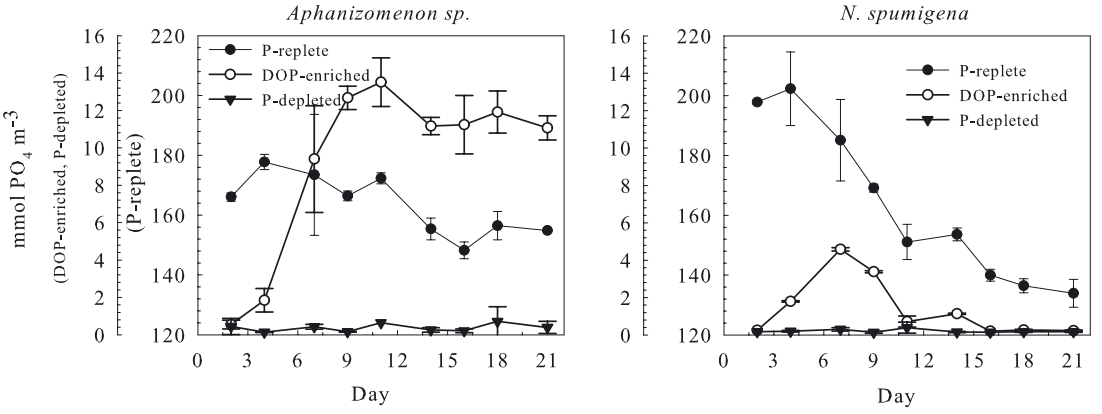

Fig. 6. Phosphate (PO_4) concentrations (mmol l^{-3}) during the experiment for *Aphanizomenon* sp. and *Nodularia spumigena* in phosphorus-replete (P-replete), phosphorus-deficient with an organic phosphorus addition (DOP-enriched) and phosphorus-depleted (P-depleted) treatments. Error bars indicate standard deviations. Figure redrawn from paper **I**.

Table 2. Results for type III tests for fixed effects and the least squares means and standard errors for the species and treatments in the mixed model repeated measures analysis. Differences in the dependent variables (chlorophyll-*a*, particulate nitrogen to particulate phosphorus ratio (PN:PP), particulate phosphorus (PP) and chlorophyll-*a* specific alkaline phosphatase activity (SAPA) between species and treatments and their interaction were tested. The interaction of treatment and day was also included in the model to discern different development of dependent variable values during the experiment. Table redrawn and modified from paper **I**.

Chlorophyll *a*

Effect	*Num DF*	*Den DF*	*F-value*	*Pr>F*	Least squares means and standard errors		
Species	1	6	87.26	< 0.0001	*Aphanizomenon* sp.		*N. spumigena*
Treatment	2	6	4.89	0.0550	4.66 ± 0.10		5.94 ± 0.10
Species*Treatments	2	6	4.11	0.0750	*P-replete*	*DOP-enriched*	*P-depleted*
Species*Day	8	64	2.42	0.0236	5.60 ± 0.12	5.17 ± 0.12	5.12 ± 0.12
Treatment*Day	16	64	3.12	0.0006			

PN:PP

Effect	*Num DF*	*Den DF*	*F-value*	*Pr>F*	Least squares means and standard errors		
Species	1	6	8.81	0.0250	*Aphanizomenon* sp.		*N. spumigena*
Treatment	2	6	236.20	< 0.0001	2.99 ± 0.03		2.82 ± 0.03
Species*Treatments	2	6	3.52	0.0973	*P-replete*	*DOP-enriched*	*P-depleted*
Species*Day	9	72	2.37	0.0210	2.31 ± 0.04	2.91 ± 0.04	3.55 ± 0.04
Treatment*Day	18	72	5.83	< 0.0001			

PP

Effect	*Num DF*	*Den DF*	*F-value*	*Pr>F*	Least squares means and standard errors		
Species	1	6	55.29	0.0003	*Aphanizomenon* sp.		*N. spumigena*
Treatment	2	6	39.85	0.0003	5.66 ± 0.95		15.67 ± 0.95
Species*Treatments	2	6	7.26	0.03	*P-replete*	*DOP-enriched*	*P-depleted*
Species*Day	9	72	7.72	<0.0001	18.72 ± 1.17	9.06 ± 1.17	4.24 ± 1.17
Treatment*Day	18	72	5.97	<0.0001			

SAPA

Effect	*Num DF*	*Den DF*	*F-value*	*Pr>F*	Least squares means and standard errors		
Species	1	6	0.06	0.8174	*Aphanizomenon* sp.		*N. spumigena*
Treatment	2	6	271.84	< 0.0001	1.03 ± 0.001		1.03 ± 0.001
Species*Treatments	2	6	8.00	0.0203	*P-replete*	*DOP-enriched*	*P-depleted*
Species*Day	7	56	3.87	0.0017	1.01 ± 0.001	1.03 ± 0.001	1.05 ± 0.001
Treatment*Day	14	56	8.89	< 0.0001			

4.2 Nutrient limitation characteristics of cyanobacteria bloom communities and species specific phosphorus deficiency of *Aphanizomenon* sp. and *N. spumigena* in natural communities (**II**)

Potential phosphorus deficiency or limitation of the plankton community was assessed by using particulate phosphorus specific APA (SAPA), orthophosphate turnover times (T_t) and calculated particulate phosphorus specific orthophosphate affinity (α) of the community as indicators. The specific orthophosphate affinity ranged between 0.0006 to 0.2010 L nmol P^{-1} h^{-1} for the Controls, between 0.0002 to 0.0209 L nmol P^{-1} h^{-1} for the phosphate addition treatments (samples with T_t > 10 h excluded, cf. Tanaka & al. 2006) and between 0.0025 to 0.1326 L nmol P^{-1} h^{-1} for the ammonium addition treatments. The specific orthophosphate affinity decreased with increasing estimated biologically available phosphate ($[PO_4]$) and was lower in the phosphate addition treatments than for the control and ammonium addition treatments, which clustered largely together within a wide range of variability (Fig. 7). The one exceptionally high α-value for the

phosphate added treatments is from the end of experiment C where added phosphate was efficiently assimilated into biomass, and T_t decreased to match the control treatments by the end of the study. In the Control treatments experiment A showed the lowest average (± SD) α of 0.0012 ± 0.0008 L nmol P^{-1} h^{-1}, indicative of phosphorus deficiency. In experiment B the Control treatment had an average (± SD) α of 0.0579 ± 0.0491 L nmol P^{-1} h^{-1}, experiment C and D had average (± SD) values of 0.0792 ± 0.1066 and 0.0127 ± N/A, L nmol P^{-1} h^{-1} respectively. The values for experiments B to D are largely indicative of phosphorus limitation of the community (c.f. Tanaka & al. 2006).

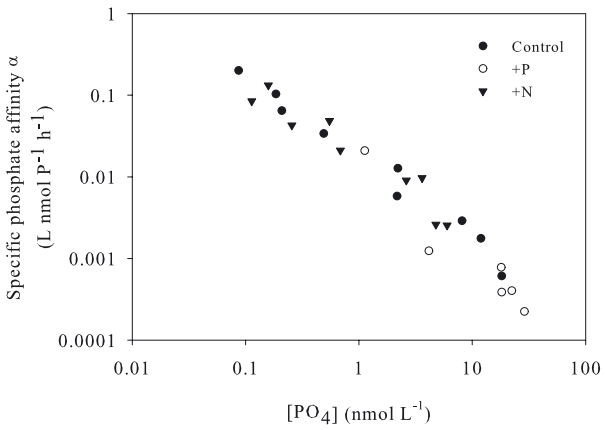

Fig. 7. Biomass specific phosphate affinity (l nmol-P^{-1} h^{-1}) plotted against the estimated biologically available phosphate concentration [PO_4] (nmol l^{-1}) in the experimental units. Figure redrawn from paper **II**.

During the alleged phosphorus limited conditions (experiments B to D) phosphate additions evoked clear and statistically significant increases in particulate phosphorus concentrations (Table 3, Fig. 8). However, during phosphorus deficient growth conditions (experiment A) no clear effect of phosphorus addition on particulate phosphorus concentrations was observed. Based on radionuclide (^{33}P) tracer experiments the phosphate additions accumulating into the particulate fraction generally accumulated most prominently in the 8–20 µm size fraction (**II**), albeit the largest relative increase with phosphate addition was consistently observed in the > 20 µm size fraction including most of the *Aphanizomenon* sp and *N. spumigena* biomass. The communities were dominated clearly by diazotrophic cyanobacteria (*Aphanizomenon* sp. and *N. spumigena* that comprised over 50 % of total phytoplankton community biomass in all experiments). The increase in accumulation into the > 20 µm size fraction in the phosphate added treatments in relation to the control treatments varied between 10 to 21 % and increased

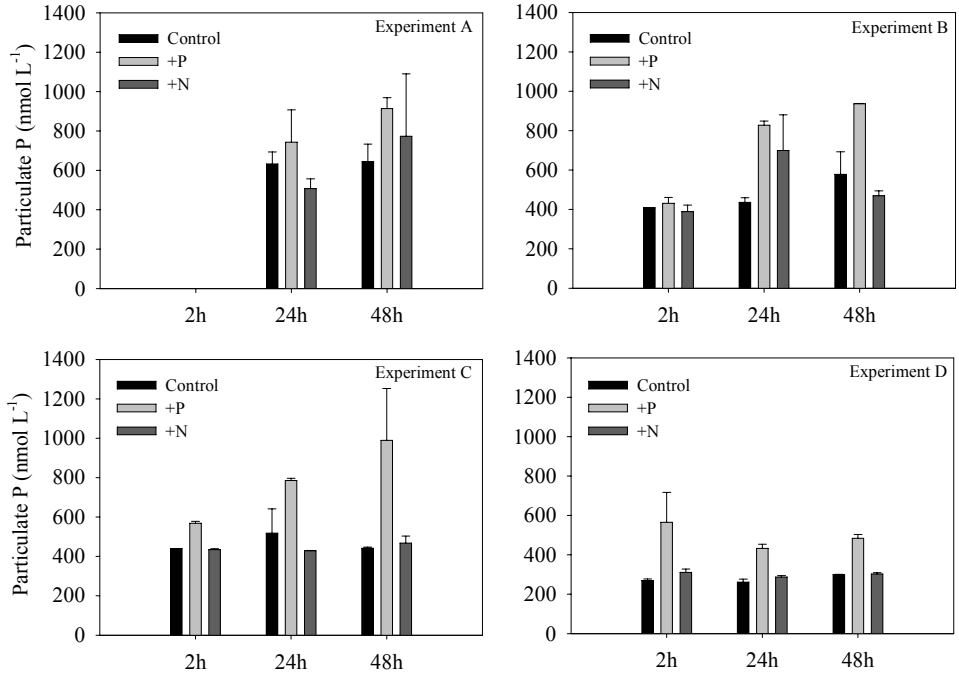

Fig. 8. Particulate phosphorus concentrations (nmol l^{-1}) in the experimental units in experiments A to D during the 48 h incubations. Error bars denote standard deviation. Measurements for time point 2 h in Experiment A are not available. Figure redrawn from paper **II**.

statistically significantly in relation to the controls (X^2-*test:* $X^2 = 11.5$, $df = 1$, $p > X^2$ 0.0007).

During supposed phosphorus limited growth conditions the community phosphorus stress, depicted as SAPA, was relieved by phosphate additions and steadily increased in the control and ammonium added treatments (Fig. 9). The treatment effects were found to be significant (Table 3) (the test for experiment C was conducted with a non-parametric test: $X^2 = 7.8$, $df = 2$, $p > X^2$ 0.02). The conclusions drawn from biomass specific phosphate affinity results are supported by measurements of SAPA showing experiment A having SAPA generally < 0.2 nmol MUF nmol P^{-1} h^{-1}, considered indicative of phosphorus deficiency, and experiments B to D showing SAPA generally > 0.2 nmol MUF nmol P^{-1} h^{-1}, indicative of more severe phosphorus deficiency. Experiments B and D are an exception where phosphate addition decreased SAPA to levels < 0.2 nmol MUF nmol P^{-1} h^{-1}, indicating transition away from a more severe community level phosphorus deficiency. ELF labelling studies of *N. spumigena* and *Aphanizomenon* sp. showed consistently higher and statistically significant labelling percentages for *N. spumigena* ($X^2 = 35.7$, $df = 1$, $p > X^2 <$ 0.0001), with phosphate additions having only slight or no effect on the labelling percentage (Fig. 10). *Aphanizomenon* sp. ELF labelling percentages showed response to phosphate additions as lowered labelling percentages during both phosphorus deficient or limiting conditions. In addition to species-specific differences in the response to phosphate pulses the temporal development of APA of *Aphanizomenon* sp. and *N. spumigena* pelagic populations differs. The *N. spumigena* species-specific APA (measured by ELF) increase earlier and to markedly higher labelling percentages than for *Aphanizomenon* sp. (Fig. 11).

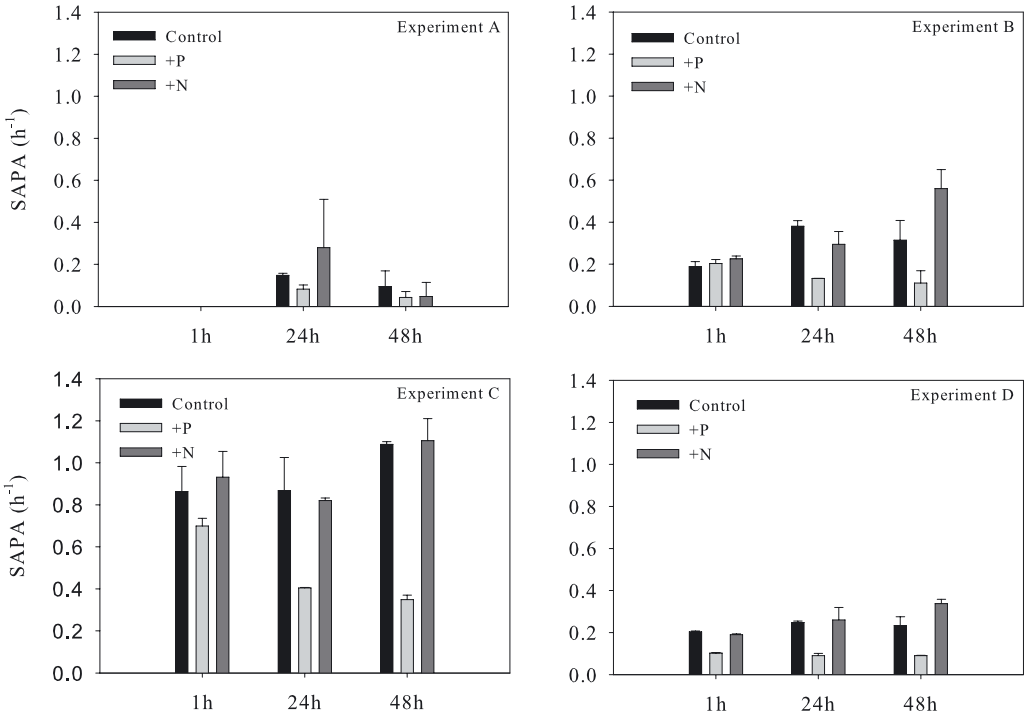

Fig. 9. Particulate phosphorus specific alkaline phosphatase activity (SAPA) as nmol MUF h^{-1} nmol P^{-1} in the experimental units in experiments A to D. Error bars denote standard deviation. Figure redrawn from paper **II**.

Table 3. Results for type III tests for fixed effects in the repeated measures mixed model analyses. Differences in the dependent variables (Particulate P (particulate phosphorus), SAPA (particulate phosphorus specific alkaline phosphatase activity)) were tested for treatment effects and the temporal evolution of variables and the interaction of treatment and time discerning possible opposite slopes of treatment effects. Particulate P results for Experiment C are unavailable due to non-normal distribution of model residuals and no suitable transformations. Therefore, a non-parametric Kruskal-Wallis test was used to test for treatment effects by the end of the experiment (results presented in the text). Table redrawn and modified from paper **II**.

	Experiment A				Experiment B			
	Num df	Den df	F-value	p > F	Num df	Den df	F-value	p > F
Particulate P								
Treatment	2	3	1.68	0.32	2	3	11.78	0.04
Day	1	3	3.34	0.16	2	6	37.02	<0.01
Interaction	2	3	0.81	0.52	4	6	9.69	<0.01
SAPA								
Treatment	2	3	3.31	0.17	2	3	17.67	0.02
Day	1	3	1.74	0.32	2	6	8.32	0.02
Interaction	2	3	0.79	0.56	4	6	12.90	<0.01
	Experiment C				Experiment D			
	Num df	Den df	F-value	p > F	Num df	Den df	F-value	p > F
Particulate P								
Treatment					2	3	179.87	<0.01
Day					2	6	2.42	0.17
Interaction					4	6	1.53	0.31
SAPA								
Treatment	2	3	33.66	<0.01	2	3	118.86	<0.01
Day	2	6	11.18	<0.01	2	6	8.44	0.02
Interaction	4	6	8.84	0.01	4	6	6.37	0.02

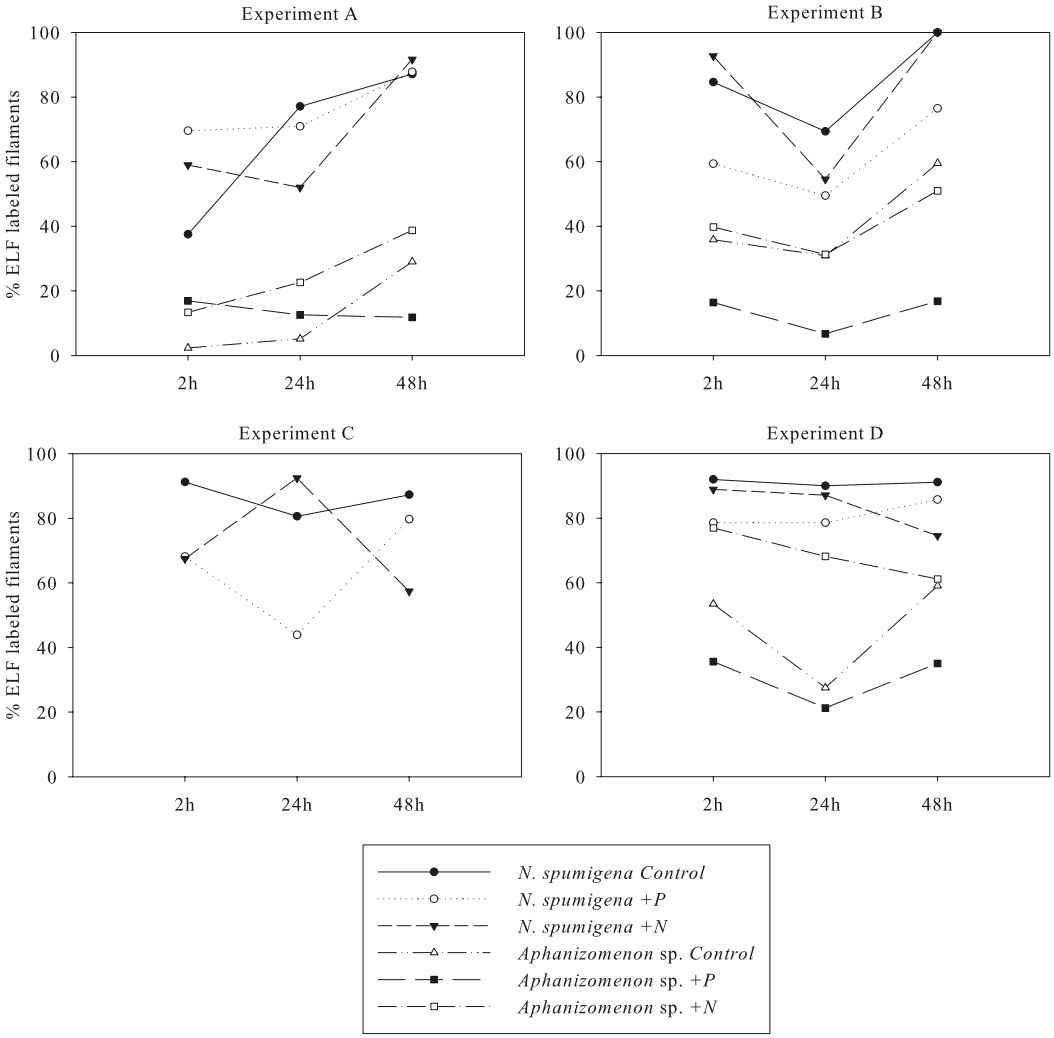

Fig. 10. Enzyme labelled fluorescence (ELF) labelling of *N. spumigena* and *Aphanizomenon* sp. in the experimental units during the 48 h incubations in experiments A to D as percentage of filaments labelled. Figure redrawn from paper **II**.

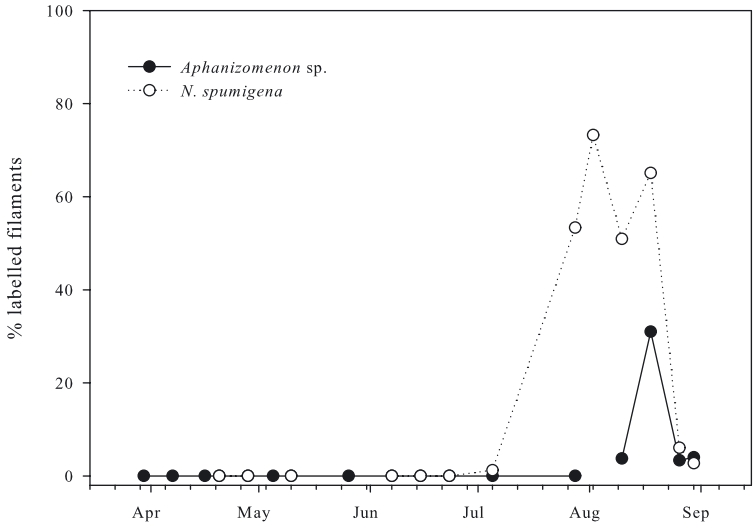

Fig. 11. ELF labelling percentage of the total population of *Aphanizomenon* sp, *N. spumigena* and *Anabaena* spp. in the pelagic western Gulf of Finland during summer 2004. Samples have been acquired from the Alg@line ship-of-opportunity (SOOP) transect from Helsinki to Travemünde by pooling five samples gathered between longitudes 22 to 25ºE at 5 m depth.

4.3 Effect of coastal upwelling on the occurrence of diazotrophic cyanobacteria (III)

The spatial distribution of the dominant filamentous and diazotrophic cyanobacteria (*Aphanizomenon* sp. and *N. spumigena*) was strongly affected by the upwelling event observed in this study. *N. spumigena* distribution was more pronouncedly affected through a more efficient southward advection of the surface dwelling populations along with the progressing upwelling front. The effect of water mass advection on *N. spumigena* was clearer due to the biomass maximum of the population residing at a more shallow depth than for *Aphanizomenon* sp. Surface dwelling *Aphanizomenon* sp. populations were advected southward with the progressing front as well. However, deeper populations were brought to the surface by the rising thermocline and remained in the upwelled water with high phosphate concentrations after the relaxation of the upwelling event.

Subsequently, surface water temperatures increased to levels comparable to the pre-upwelling period (approximately 18°C) in roughly 1 week (from July 27 to August 4). *N. spumigena* biomass in the area affected by the upwelling constantly decreased after the relaxation of the upwelling. *Aphanizomenon* sp. biomass showed a slightly lagging increase compared to surface water temperatures, displaying a biomass peak on August 15, 2 to 3 weeks after the relaxation of the upwelling (Fig. 12). *N. spumigena* biomass peaked simultaneously with the onset of the upwelling event in the open sea.

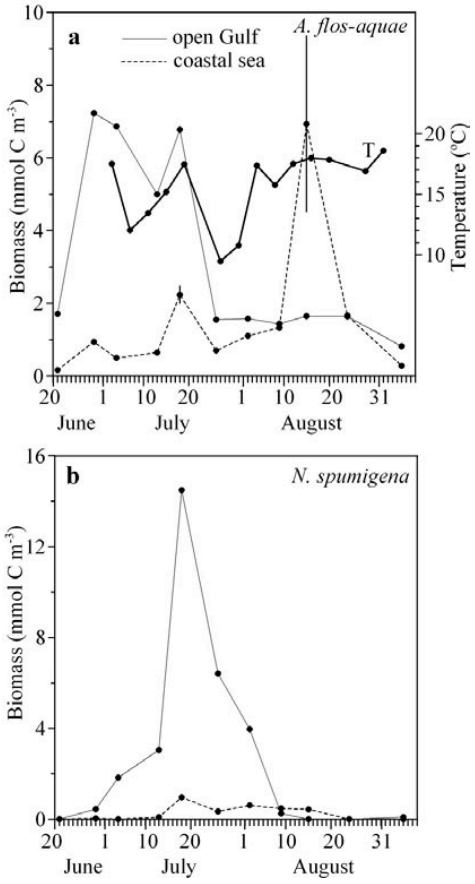

Fig. 12. Time series of mean *Aphanizomenon* sp. (a) and *N. spumigena* (b) biomass (mmol C m^{-3}) in the Finnish coastal sea affected by upwelling (dashed line) and in the open Gulf (solid line) at a depth of 5 m on a monitoring transect between Helsinki and Tallinn. The bold line marked with a T in panel a represents coastal seawater temperature at 5 m depth. The mean biomass was calculated from three sampling stations in the coastal area and two sampling stations in the open Gulf. Error bars on the two *Aphanizomenon* sp. biomass peaks indicate standard error. Figure redrawn from paper **III**.

4.4 The phosphorus sources of basin-scale pelagic blooms of diazotrophic cyanobacteria (IV)

Based on average time series (1994 to 2004) from a Baltic Proper open ocean monitoring station (BY15, eastern Gotland Basin) it is estimated that approximately 60 % of the seasonal build up of the inorganic phosphorus pool in the upper 60 m layer of the sea occurring from October to March stems from regeneration of organic phosphorus, external loads and possibly vertical diffusion from below the permanent halocline. The remaining 40 % originates from vertical mixing events occurring during January and February.

A more detailed examination of the integrated amount of nutrient s in the upper 60 m layer reveals that on average DIP concentrations increase from 0.25 to 0.35 µM from September to December, while total phosphorus (TP) remains constant (Fig. 13). This means that remineralized phosphorus builds up as a bioavailable DIP pool instead of being used or lost through burial or other processes. An

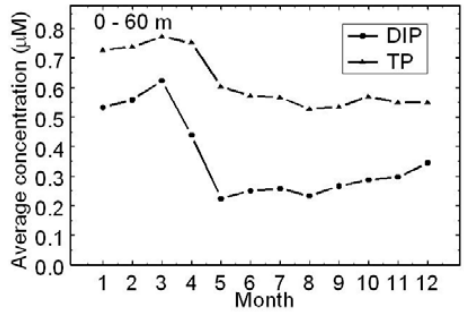

Fig. 13. Monthly average of the vertical mean (0 to 60 m) of dissolved inorganic phosphorus (DIP) and total phosphorus (TP) for the years 1994-2004 of observations from a central Baltic Sea monitoring station (BY15, eastern Gotland basin). The vertical mean is computed using the hypsographic function for the Baltic Proper, excluding the Gulfs of Finland and Riga, and Bornholm and Arkona basins. Figure redrawn and modified from paper IV.

increase of ca. 0.15 µM in both DIP and TP concentrations occurs from December to January with an additional increase of ca. 0.10 µM in March with DIP concentrations reaching 0.60 µM. The integrated average increase in DIP in the upper 60 m layer from September to March corresponds to approximately 0.35 µM, which equals to a monthly average increase of ca. 0.05 µM of which 60 % (corresponding to an increase of 0.03 µM month^{-1}) is estimated to be due to remineralization, calculated as the difference in increase between the increase in total phosphorus and dissolved inorganic phosphorus. Averaged over a water volume corresponding to the upper 60 m layer of the Baltic Proper, the phosphorus loads to the basin correspond to increases of approximately 0.005 µM month^{-1} phosphorus (18 300 tons year^{-1}), an order of magnitude smaller than the calculated average increase caused by internal sources assuming that external loads of phosphorus arrive in the surface layer of the sea. The monthly external load of TP is small relative to the internal sources and the total pool size of DIP in the Baltic Proper, which has varied between 322 000 to 486 000 tons during 1970 to 2000 (Conley & al. 2002).

The long-term annual (mean ± SD) load to the Baltic Proper and Gulfs of Finland and Riga for nitrogen is 752 000 ± 98 000 tons. The average year-to-year variation in nitrogen load is ca. 72 000 tons while the corresponding variation in the TN pool in the Gulfs of Finland and Riga and the Baltic Proper combined is about 225 000 tons. No clear correlation between loads and either the pool itself or its year-to-year changes were observed. The annual net exchange of TN between the Baltic Proper and adjacent basins is about 120 000 tons and varies between years by less than 30 000 tons. Subsequently, exchange processes cannot explain the variations in the TN pool. However, there is a significant negative relationship between the DIN pool and hypoxic water volume (Fig. 14). The relationship suggests that losses of the DIN pool through nitrogen removal processes may be higher during periods of more wide spread hypoxia.

The strong internal regulation of both phosphorus and nitrogen pools, the interconnection of oxygen, nitrogen and phosphorus biogeochemical cycles and the prevalence of diazotrophic cyanobacteria in the phytoplankton community during summer suggests the presence of a potentially self-sustaining vicious circle (Fig. 15), supporting an eutrophied state of the Baltic Sea and recurring large-scale diazotrophic cyanobacteria blooms.

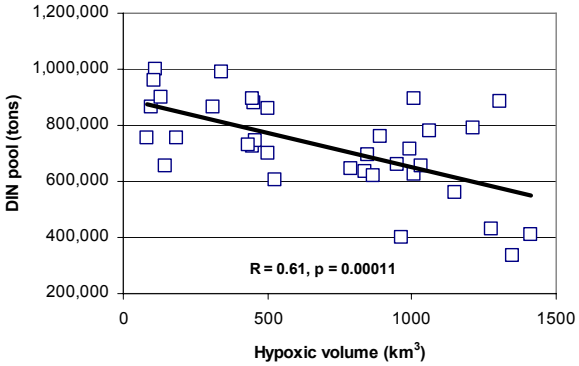

Fig. 14. Relationship between total amounts of dissolved inorganic nitrogen (DIN) in the Baltic Proper, Gulf of Finland and Gulf of Riga and hypoxic water volume confined by isosurfaces of 0 and 1 ml O_2 l^{-1} in the same basins. Figure redrawn from paper **IV**.

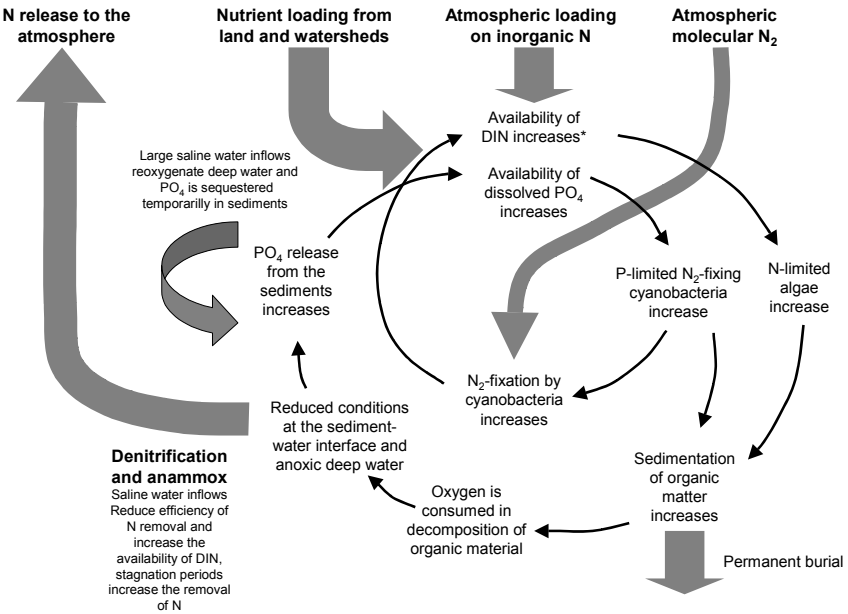

Fig. 15. A schematic presentation of main feedback processes that inhibit recovery from eutrophication and favour cyanobacteria blooms in the Baltic Sea. The 'Vicious Circle' is potentially sustained by nitrogen (N)-limited production and sedimentation of phytoplankton especially during the spring bloom, and subsequent oxygen depletion in bottom waters, causing internal loading of phosphorus (P). Physical transport of released phosphorus to surface layers would enhance N_2-fixation by diazotrophic cyanobacteria. These seasonal feedbacks between biogeochemical cycles of nitrogen, phosphorus and oxygen can effectively counteract reductions in the external phosphorus loading to the system if nitrogen loading is not reduced as well. Grey arrows depict material flows. Thin arrows depict causal relationships and successive events. Several potential feedback mechanisms and limiting factors are omitted for clarity. Figure redrawn and modified from paper **IV**.

5. DISCUSSION

5.1 Species-specific differences in phosphorus utilization of *Aphanizomenon* sp. and *N. spumigena* (**I** and **II**)

In study **I** the growth of *N. spumigena* seemed indifferent to the supplied form of phosphorus (phosphate, synthetic monoester or internal polyphosphate stores) and the growth of *Aphanizomenon* sp. was higher at elevated phosphate concentrations in relation to the other treatments. The above results are, at a glance, contradictory to a number of studies made in the Baltic Sea e.g. Larsson & al. (2001), Kangro & al. (2007) and Walve & Larsson (2007) who noted that *Aphanizomenon* sp. predominantly utilises cellular phosphorus stores and growth is more or less independent of post spring bloom external nutrient supply and that *N. spumigena* displays a phosphorus limited growth mode expected to be dependent on external phosphate supply. However, the different forms of available dissolved phosphorus (inorganic vs. organic) together with the capacity to withstand phosphorus starvation, the ability to replenish intracellular phosphorus stores and the difference in temperature dependence of growth might be important factors in the study of inter species competition for phosphorus between *Aphanizomenon* sp. and *N. spumigena*.

The results in paper **I** emphasise that *N. spumigena* has a remarkable capacity to grow on intracellular stores of phosphorus when they are available, which seems not always to be the case in natural environments. The typical seasonal biomass development of *Aphanizomenon* sp. allows large populations to store extensive amounts of phosphorus already during early summer and studies on heterocyst frequencies indicate onset of extensive nitrogen fixation already during the pre-bloom period (Laamanen & Kuosa 2005). Therefore, because nitrogen acquisition relies on nitrogen fixation (e.g. Kangro & al. 2007) and phosphorus stems from cellular stores, *Aphanizomenon* sp. might be able to display growth that seems independent of external nutrient supply and it might be expected not to respond to manipulations of external nutrient supply stoichiometry that aim to induce specific nutrient limitation. A later onset of APA as measured by ELF (Fig. 11) for natural *Aphanizomenon* sp. populations compared to *N. spumigena* populations also indicate extensive phosphorus storage capacity. However, the source of phosphorus might be composed of both the ambient excess phosphorus stored during the pre-bloom period in the inoculum population and pulses mediated through coastal upwelling (**III**) or frontal dynamics (Kononen & al. 1996) that are merely stored and used for subsequent slow growth. In the same vein, as *N. spumigena* growth is more temperature dependent (Lehtimäki & al. 1997, Wasmund 1997), the inoculum populations in natural conditions are smaller, and the total amounts of stored phosphorus are smaller, inducing a more severe phosphorus limitation when temperatures rise and rapid growth commences. Populations of *N. spumigena* need to compete more for available external phosphorus sources than *Aphanizomenon* sp.. Thus, the discrepancy between the laboratory study (**I**) and field observations and experiments (Larsson & al. 2001, Kangro & al. 2007) might be explained by the nutritional history of the populations. The intracellular stores of the *Aphanizomenon* sp. population might have been large enough to create similar biomasses as in the phosphorus-boosted treatments in the study made by Kangro & al. (2007).

In long term experiments (cf. Kangro & al. 2007) nutrient additions of inorganic form are bound to end up in the organic fraction, either in dissolved or particulate form. Phytoplankton acclimated to resource scarcity (e.g. of inorganic phosphorus) can shift primary uptake systems in favour of alternative sources (e.g. inorganic vs. organic phosphorus), by limiting transporter activity and increasing activity of nutrient sequestration enzymes (Button 1985). *N. spumigena* has been noted to have a higher affinity than *Aphanizomenon* sp. for dissolved organic phosphorus substrates and to grow better on an organic phosphorus source (**I**, Degerholm & al. 2006). Thus, if *N. spumigena* in the Baltic is adapted to low phosphate and high dissolved organic phosphorus conditions, as implicated by e.g. the frequently observed bloom time vertical distribution of the biomass peak (**III**, Niemistö & al. 1989, Hajdu & al. 2007) and typical vertical distributions of inorganic vs. organic phosphorus forms (Laanemets & al. 2004, Nausch & Nausch 2006)) and typical seasonal biomass development that shows strong dependence on temperature (e.g. Wasmund 1997), it might be predisposed to shift its uptake systems towards utilization of organic phosphorus compounds and thus not directly and efficiently compete for inorganic forms of phosphorus and not be able to utilise pulses of phosphate to replenish intracellular stores as efficiently as *Aphanizomenon* sp. (**II**, **III**).

The more pronounced APA as measured by ELF during the pre-bloom and bloom periods for pelagic *N. spumigena* populations also support the notion of a phosphorus limited growth mode as stated by Kangro & al. (2007) and implicate an acquisition strategy leaning towards organic compounds in natural conditions (Fig. 11). However, whether a shift in activity of uptake systems shows preference towards organic phosphorus forms in relation to inorganic forms for *N. spumigena* remains to be studied.

In study **I** the phosphate deficient treatments and the DOP-enrichment treatments both evoked clear responses in SAPA for both species, indicating a capacity for organic phosphorus utilisation with both

species. The increasing amount of free phosphate and high percentages of soluble APA indicate that APA for *Aphanizomenon* sp. and *N. spumigena* is not strictly membrane bound in the experimental conditions. The decreasing dissolved phosphate concentrations to very low levels in the DOP-enriched *N. spumigena* treatment depict efficient utilisation of phosphate liberated from the organic compound whereas *Aphanizomenon* sp. seemed not to be able to utilise the liberated phosphate below a certain threshold concentration, indicating an adaptation of this species to higher phosphate concentrations. The abnormally high phosphate concentrations (> 10 mmol l^{-3}) that *Aphanizomenon* sp. did not seem to be able to utilise probably reflects the long history in culture of this strain and only implies the lower phosphate affinity observed compared to *N. spumigena* (Wallström & al. 1992, Degerholm & al. 2006). Both strains used in the experiments have been in culture for years and presumably experienced very similar selective pressures.

Despite apparent capability of both species to utilise dissolved organic phosphorus sources a higher affinity for phosphate and organic phosphate substrates (Wallström & al. 1992, Degerholm & al. 2006) renders *N. spumigena* more predisposed to actually utilise such scarce sources for realised growth and therefore it shows phosphorus limited growth mode. The extensive capacity of *Aphanizomenon* sp. to store phosphorus intracellularly (Larsson & al. 2001, Kangro & al. 2007), renders it less susceptible to alterations in nutrient supply stoichiometry and it may display efficient growth seemingly independent of external nutrient supply for longer periods.

However, comparison of pure laboratory studies conducted on growth media acclimated strains with natural communities poses several difficulties, including loss of phenotypic and genotypic variation in the laboratory strains. Also, the strain of *Aphanizomenon* sp. used in study **I** has been argued to poorly represent the populations present in the Baltic Sea (Laamanen & al. 2002) due to it being different from the most abundantly occurring strain in the Baltic based on its ITS1-S sequence. Thus, the results should be considered with caution.

Study **II** of the thesis lets us consider a similar competitive setting for pulsed phosphate supply as study **I** set for different forms of phosphorus. The study sites in study **II** differ in their nutrient supply modes during the thermally stratified summer months. In the pelagic Baltic Proper the development of a strong thermocline restricts vertical movement of water and coastal processes do not strongly affect the system (Myrberg & Andrejev 2003). However, the formation of mesoscale eddies tend to cause uplifting of the halocline and possibly cause nutrient transport to the surface layer (Kononen & al. 1992, Kononen & al. 1999). In the Gulf of Finland coastal upwelling and frontal dynamics of waters of different salinity flowing in and out of the narrow Gulf play a crucial role in supplying the upper mixed layer with new nutrients, predominantly phosphate (Kononen & al. 1996, Laanemets & al. 1998, **III**).

In study **II**, the bloom time communities, dominated by *Aphanizomenon* sp. in the Gulf of Finland and *N. spumigena* in the Baltic Proper were found to be phosphorus deficient or limited to different degrees. The Gulf of Finland populations experienced less phosphorus deficiency than the more phosphorus deficient Baltic Proper bloom community, contrary to a previous observation (Moisander & al. 2003), which might indicate interannual variation. Bloom communities in the central Baltic Proper have been previously found to be phosphorus limited during the bloom peak (Nausch & al. 2004), and the period of phosphorus limitation has been noted to last for a few weeks in July coinciding generally with the dominance of diazotrophs in the community.

Phosphate additions to the communities improved the phosphorus nutritional status of the communities measured as increased particulate phosphorus concentrations, lowered SAPA and decreased specific orthophosphate uptake affinity. On occasions when the cyanobacteria were found to be phosphorus deficient, the phosphate addition increased accumulation relatively most in the > 20 µm size fraction (on average from 10 to 21 % in relation to the control) within 2 h after the phosphate addition. The > 20 µm size fraction includes the majority of the diazotrophic cyanobacteria biomass. The increase of accumulation of phosphorus into the > 20 µm size fraction is however only circumstantial evidence of pulses of phosphate favouring diazotrophic cyanobacteria and part of this increase might also be due to grazing on smaller phytoplankton and a sub sequential upward movement of the tracer in the food web to larger zooplankton, also included in the > 20 µm size fraction. However, the elevated level of phosphorus accumulation did not change markedly with time (± ~5 %) during the 48 h incubations in the > 20 mm size fraction (**II**). This indicates only small effects of grazing in transfer of the label to the largest size fraction or efficient regeneration of nutrients within the food web and no marked trophic accumulation to higher trophic levels. A lowered ELF labelling percentage of *Aphanizomenon* sp. populations also indicates relaxation of phosphorus stress for this species through uptake of added phosphate (**II**).

Enzyme labelled fluorescence (ELF) revealed a distinct difference between *Aphanizomenon* sp. and *N. spumigena* response to the phosphate additions (**II**). *Aphanizomenon* sp. had throughout the experiments significantly lower labelling percentages in the phosphate added treatments than in the other

treatments. *N. spumigena* populations did not seem to respond to phosphate additions directly, and ELF labelling percentages were at par with the control and ammonium addition treatments. These results indicate a constitutive APA of *N. spumigena* in natural conditions and a phosphorus acquisition strategy of this species leaning towards organic phosphorus compounds. The results further imply that *Aphanizomenon* sp. is able to replenish cellular stores of phosphorus through pulsed supply during the bloom period. Thus, a seeming independence of growth on nutrient supply (Kangro & al. 2007) might be masked by the slow continuous growth and storage of phosphorus, typical for storage specialist species (Sommer 1984, 1985).

The vertical distribution of *Aphanizomenon* sp., which is a storage-specialist, seems to suggest higher ambient phosphate concentrations requirements or internal nutrient stores than e.g. *N. spumigena* for efficient growth (Lehtimäki & al. 1997, **I**, **II**) because the biomass peak is often found at the seasonal thermocline (**III**, Niemistö & al. 1989, Hajdu & al. 2007) where also ambient DIP concentrations increase (Laanemets & al. 2004). However, these populations may experience light limited growth. Light attenuation in the Baltic Sea is rapid, the attenuation coefficient has been estimated to be 0.4 m^{-1} (Stal & Walsby 2000) and at 10 to 15 m depth only a small fraction (~1 %) of incident irradiation is left. Thus vertically displaced phosphorus sufficient populations may form blooms relatively rapidly in higher irradiances (**III**).

Whether the observed vertical distribution of *Aphanizomenon* sp. is a result of higher phosphate concentrations in the seasonal pycnocline or the lower irradiance levels (*Aphanizomenon* sp. optimal growth light levels have been estimated at 25 to 45 µmol q m^{-2} s^{-1}, Lehtimäki & al. 1997) is difficult to determine. Living at low irradiances infers relatively high-nutrient demands on *Aphanizomenon* sp. populations since they need to synthesize large amount of nitrogen rich proteins for photosynthesis in low light. If the nitrogen is sequestered through nitrogen fixation, which is an highly energy consuming process, requirements for phosphorus might be expected to be high due to high requirements of phosphorus in the energy metabolism of cells (Elser & Sterner 2002a). In the Baltic Sea *Aphanizomenon* sp. appears thus to occupy a growth strategy, if nitrogen is predominantly supplied through nitrogen fixation during bloom build-up, where cells have high phosphorus demands because of high rates of synthesis of nitrogen rich proteins (e.g. phycobiliproteins). These proteins are required for photosynthesis in the low light environment where phosphate is abundant. Thus, *Aphanizomenon* sp. growth might be considered light limited during most part of the year and phosphorus limitation would occur only during the bloom peak (cf. Fig. 11) (Nausch & al. 2004) along with possible iron limitation (Stolte & al. 2006, Stal & al. 1999).

The high energy demand for nitrogen fixation at depth might also be covered through vertical migration of populations, provided by the buoyancy regulation capacity, that have been observed for *Aphanizomenon* sp. and *N. spumigena* (Walsby & al. 1995, Walsby & al. 1997). *Aphanizomenon* sp. has been noted to posses a buoyancy regulation that is dependent on phosphorus nutritional status and energy limitation of cells (Konopka & al. 1987). An increased irradiance in phosphorus limited cultures induced settling of colonies and energy limitation induced synthesis of gas vesicles, however, colonies remained nonbuoyant as long as phosphorus was limiting. However, whether colonies replenish their cellular phosphorus stores through vertical migration has not been documented in natural conditions. The vertical migration of colonies could nevertheless be one mechanism through which cyanobacteria can effectively utilise deeper phosphate reserves. During a more shallow biomass distribution in a higher light environment they again could provide energy for nitrogen fixation in low-irradiance environments through formation of carbohydrate stores. Phosphorus sufficient natural populations of cyanobacteria have been observed to fix nitrogen during dark and at depth (8 m), albeit at lower rates than in higher irradiance (e.g. Evans & al. 2000, e.g. Moisander & al. 2007).

5.2 Phosphate uptake and potential size dependent phosphorus limitation of diazotrophic cyanobacteria (II)

The biomass specific orthophosphate uptake affinity is a good descriptor of uptake of phosphorus at low concentrations and is a sensitive variable in discerning phosphorus limitation or deficiency of populations or communities (Thingstad & Rassoulzadegan 1999, e.g. Moutin & al. 2002, Tanaka & al. 2006). Field studies show that specific affinities of functional groups of communities under phosphorus deficiency approach the theoretical maximum as predicted due to molecular diffusion being the rate limiting step (e.g. Moutin & al. 2002, Tanaka & al. 2003). Due to their relatively large size and small surface to volume ratio and a predominant notion of general phosphorus deficiency or limitation during blooms of diazotrophic cyanobacteria (**III**, Nausch & al. 2004) the phosphorus uptake of filamentous cyanobacteria might be expected to become rapidly diffusion limited even in eutrophic environments.

The specific affinity and diffusion limitation is dependent on cell size (Thingstad & Rassoulzadegan 1999) according to the following:

$$\alpha = 3D/\sigma r^2 \quad (8)$$

where D is the diffusion constant for the phosphate molecule (10^{-5} cm^2 s^{-1}) and σ is the volume specific content of phosphorus. The above relationship was formulated assuming diffusion limited spherical cells. If we make assumptions according to Thingstad & Rassoulzadegan (1999), with slight modifications regarding algal volume specific carbon content, that a phytoplankton cell has a density of 1.2 g cm^{-3}, that 50 % of wet weight is dry weight and that 20 % of dry weight is carbon and a molar C:P ratio of 106 in phytoplankton biomass we arrive at σ = 0.09 µmol-P cm^{-3}.

The typical cell diameter of *Aphanizomenon* sp. (*flos-aquae*) in the Baltic is approximately 5 to 6 µm and for *N. spumigena* 8 to 12 µm. Excluding the impact of non-spherical cell shape, inserting the above values and the estimated specific affinities for orthophosphate from our experiments in equation 8, and solving for r. The results indicate that in all but some of the phosphate added treatments the diazotrophic cyanobacteria cells would have been diffusion limited, i.e., the uptake systems were as efficient as they could be, absorbing all phosphate molecules that were encountered. In the control units the calculated average (± SD) spherical cell diameter at which theoretical diffusion limitation would occur was 9.2 (± 8.7) µm. In the phosphate addition treatments the calculated diffusion limited cell diameter was three times larger (27.6 (± 14.5) µm) than in the control treatments and in the ammonium addition treatments slightly smaller (6.4 (± 4.5) µm). This basically implies that during natural or nitrogen enriched conditions, observed during our experiments, diazotrophic cyanobacteria were hampered to compete for the phosphate reserves due to theoretical diffusion limitation of phosphate uptake. Phosphate pulses decrease the specific affinity for orthophosphate uptake of the community by saturating the efficient uptake systems of the smaller organisms (Button 1998) and thus allowing larger organisms to compete for the phosphate reserves (cf. also Thingstad & Sakshaug 1990). This mechanism would also allow coexistence of affinity-, velocity- and storage-specialists during pulsed nutrient supply. The composition of the community is then ultimately a product of pulse frequency and magnitude (Sommer 1995) along with e.g. reduced grazing on the filamentous cyanobacteria compared to smaller phytoplankton (Thingstad & Sakshaug 1990, Sellner & al. 1994). Affinity strategists dominate uptake in periods between pulses, velocity and storage strategists during the pulses. The velocity and affinity strategists are probably mainly nitrogen limited in the Baltic, as indicated for *Mesodinium rubrum* in paper **III**, or controlled by grazing, and therefore the slowly growing storage specialist diazotrophic cyanobacteria are able to utilize phosphate introduced to the surface layer by pulsed events. Based on the notions in section 5.1. *Aphanizomenon* sp. would be more prone to benefit from such pulses.

The above assumptions include the premise that the cells are perfectly absorbing spheres, which is not the case for living cells. Further, cyanobacteria cells are disc- or cylinder-shaped and arranged in filaments additionally reducing uptake efficiency by decreasing the surface to volume ratio. A single disc-shaped cell of *N. spumigena*, assuming a cell diameter of 10 µm and a height of 2.5 µm (Tikkanen & Willén 1992), has a larger surface to volume ratio than a sphere of the same diameter, 1.2 and 0.6, respectively. However, if the disk-shaped cell is part of a filament the surface to volume ratio decreases to 0.4, thus the calculations assuming sphere shaped cells overestimate the cell diameter at which theoretical diffusion limitation occurs with approximately 30 %. However, increasing the C:P ratio of the cells increases the diameter at which theoretical diffusion limitation occurs, a C:P ratio of 106 used in the calculations, is unrealistically low during bloom conditions for diazotrophic cyanobacteria. E.g. Larsson & al. (2001) observed C:P ratios of 420 in natural *Aphanizomenon* sp. populations. This would increase the cell diameter at which theoretical diffusion limitation occurs by approximately a factor of 2. The respective values in our experiments using a C:P ratio of 420 would yield estimates of cell diameters at which theoretical diffusion limitation occurs of 12.5 ± 11.9 for the controls, 37.5 ± 19.7 for the phosphate added treatments and 8.6 ± 6.2 for ammonium added treatments. Despite many factors influencing phosphate uptake the results indicate that during natural or nitrogen enriched conditions the diazotrophic cyanobacteria would be poor competitors for free orthophosphate solely due to their size and cellular arrangement into filaments. For example, Thingstad & Rassoulzadegan (1999) suggested that cells larger than 2–3 µm in Villefranche Bay summer waters in the Mediterranean would be diffusion limited and diffusion limitation of osmotrophs has been reported from the ultra-oligotrophic eastern Mediterranean as well (Fonnes Flaten & al. 2005). Our results suggest that diffusion limitation of phosphate uptake may occur in eutrophic environments, such as the Baltic Sea, during blooms of large filamentous diazotrophic cyanobacteria.

5.3 Hydrodynamic control of bloom formation and occurrence of blooms in relation to nutrient enrichment and physical transportation caused by upwelling (III)

Baltic Sea coastal upwelling events differ from oceanic scale upwelling both qualitatively (oceanic upwelling introduces high N:P deep waters, whereas

Baltic Sea upwelling introduces low N:P waters to the surface layer) and on temporal and spatial scales (MacIsaac & al. 1985, Bychova & Viktorov 1986, Mann & Lazier 1998, Myrberg & Andrejev 2003). Baltic Sea deep water and summer sub-thermocline water are characteristically of low N:P ratio due to the wide spread internal loading of phosphate (Pitkänen & al. 2001, Savchuk 2005, Carstensen & al. 2006) and more effective removal of nitrogen through denitrification (Rönner & Sörensson 1985, Brettar & Rheinheimer 1992) and anammox (Hannig & al. 2007) in hypoxic and anoxic conditions. Phosphate is typically coprecipitated as iron oxyhydroxides upon oxygenation. Two iron atoms per one phosphorus atom are needed in the oxidative hydrolysis of iron and the simultaneous coprecipitation of phosphorus, however anoxic Baltic waters have a P:Fe ratio < 2 leaving some phosphate in solution. The low P:Fe ratio is a result of enhanced iron sequestration by sulphides in anoxic marine coastal and brackish waters (Blomqvist & al. 2004). Different mechanisms are responsible of transport of deep waters containing free orthophosphate to the surface, transversal Ekman upwelling being among them. Coastal upwelling events thus generally enrich the surface layer with low N:P waters rich in free orthophosphate. The more sporadic upwellings in the Baltic introduce nutrients in pulses to the surface layer. The pulses are characteristically of low N:P ratio and thus favour nitrogen fixing storage or velocity specialist species, *sensu* Sommer (1985).

5.3.1 Effect of coastal upwelling on the occurrence of diazotrophic cyanobacteria

During bloom conditions *N. spumigena* biomass distribution is more pronouncedly affected by the upwelling events than *Aphanizomenon* sp. biomass. This is mainly due to the frequently observed bloom-time vertical distributions of the two species (Niemistö & al. 1989, **III**, Hajdu & al. 2007). During coastal upwelling events coastal surface water is transported offshore and replaced by deeper water from the seasonal pycnocline. The surface dwelling (from the surface down to 5 m) *N. spumigena* populations are effectively transported offshore along with the surface water and the upwelling waters are almost entirely free of *N. spumigena* filaments (**III**). *Aphanizomenon* sp. surface dwelling populations are also transported offshore, however, since the *Aphanizomenon* sp. biomass peak is often observed to be situated at 10 to 15 m depth or more evenly distributed in the water column (Niemistö & al. 1989, **III**, Hajdu & al. 2007), however variation also occurs (pers. comm. J. Walve). The population residing at deeper depth is displaced to the surface along with the upwelling water. *Aphanizomenon* sp. has thus an inoculum in the upwelled water and is predisposed to utilize the phosphate reserves present since other phytoplankton is limited by nitrogen availability due to the prominent phosphate enriching effect of upwelling events.

The upwelling observed in 1999 (**III**) has been estimated, by modelling, to have brought up to 387 tons of phosphate phosphorus to the upper 10 m layer, and only 36 tons of nitrate nitrogen (Zhurbas & al. In press). The N:P ratio of upwelled water would thus be <1, inducing a severe nitrogen shortage in relation to phytoplankton demand. The phosphate enrichment caused by the upwelling was initially observed to fuel a short-lived *Mesodinium rubrum* bloom that presumably relied on the small amount of nitrate transported to the surface layer by the upwelling. *M. rubrum* might be characterized as a velocity specialist and it is known for its high production capacity (e.g. Lindholm 1985). Blooms of *M. rubrum* have been shown to occur in upwelling regions elsewhere (Packard & al. 1978, Wilkerson & Grunseich 1990). Eventually the upwelling promoted the formation of an *Aphanizomenon* sp. bloom along the northern coast of the Gulf of Finland. The increase in biomass was found to be in accordance with estimated temperature constrained growth rates for *Aphanizomenon* sp. (**I**). Upwelling events have been previously noted to induce *Aphanizomenon* sp. blooms in frontal areas in the western Gulf of Finland (Kononen & al. 1996). The role of upwelling in bloom dynamics of diazotrophic cyanobacteria favours the occurrence of *Aphanizomenon* sp. on time scales of weeks. The effect of upwelling is most strongly dependent on the resident species inoculum, their temperature dependent growth rates and the nutrient demand of the inoculum.

Fig. 16 shows a schematic presentation of the effect of upwelling events on the diazotrophic cyanobacteria. During the pre-upwelling phase the community is assumed to be in equilibrium at steady state, with community growth (μ), loss rates (L) and nutrient regeneration (R) being equal. Biomass increase is zero and the nutrient pools are entirely recycled. New production of other than diazotrophs is based almost entirely on nitrogen fixation. As the upwelling commences, cold and phosphate enriched water from the thermocline reaches the surface and displacement of diazotroph populations takes place (**III**). The increased vertical mixing and shear stress reduces nitrogen fixation of *N. spumigena* and *Aphanizomenon* sp. (Moisander & al. 2002) and new production relies more on upwelled nutrients. The turnover times of nutrient pools decrease as the nutrient supply increases (**II**). Initial new production becomes however limited by the nitrogen supply due to low DIN:DIP ratios of deep water and thus upwelling events only produce sporadic blooms of other autotrophs than diazotrophs (**III**), unlike in oceanic upwelling regions (MacIsaac & al. 1985). The surface dwelling diazotroph populations coerce in frontal areas (**III**) and the inoculum of *Aphanizo*

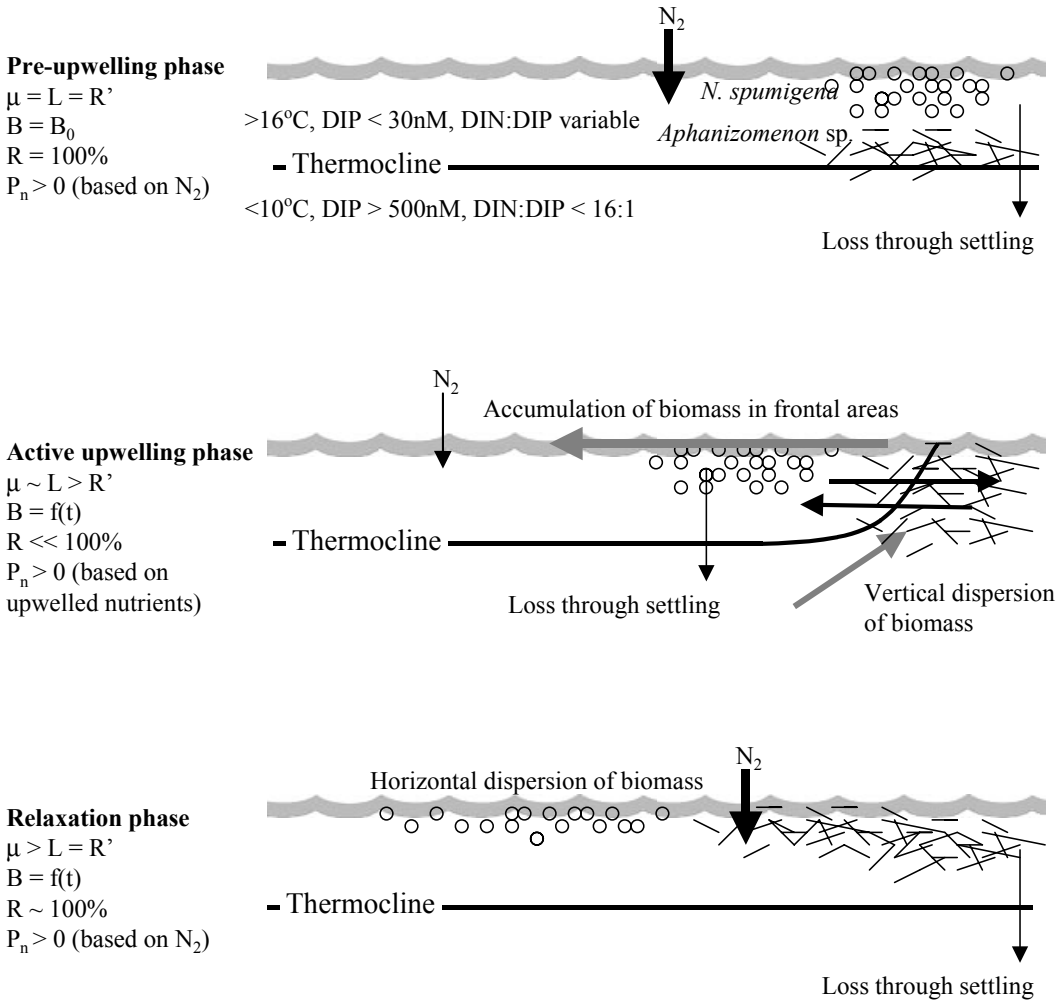

Fig. 16. A schematic presentation of the effect of transversal Ekman upwelling on diazotrophic cyanobacteria. μ=growth rate, L=loss rate through grazing and settling, R=nutrient regeneration, B=total biomass (autotrophs+heterotrophs), P_n=new production, N_2=nitrogen fixation. See text for details. The figure is adapted and modified from Kononen 1992.

menon sp. filaments in the upwelled water store the excess phosphates exhibiting temperature-limited growth. When the upwelling relaxes and the thermocline is re-established the horizontally transported diazotroph populations disperse due to slow phosphate limited growth. However, the growth rate of the inoculum population (consisting mainly of *Aphanizomenon* sp.) in the upwelled water increases with increasing water temperature with growth rates exceeding loss rates. Blooms are formed within a few weeks (**III**) and new production of other community constituents is again relying on nitrogen from nitrogen fixation and nutrient regeneration is becoming more important as a nutrient source.

5.4 The role of basin-scale nutrient dynamics in governing blooms of cyanobacteria (IV)

The Baltic Sea has inherent characteristics favouring diazotroph occurrence. Strong vertical stratification and limited water exchange induce hypoxia and anoxia that cause selective accumulation of phosphate and removal of nitrogen, which lowers the N:P ratio of deep waters. A low N:P ratio is a prerequisite for diazotroph dominance (e.g. Howarth & al. 1988) but is not necessary a driving force. For large-scale blooms to occur and dominance of diazotrophs to be established also a sufficient inoculum and a sufficient time window of suitable environmental conditions must prevail. In addition, despite generally slow growth the very low loss

rates of diazotrophic cyanobacteria populations through settling loss (Heiskanen & Kononen 1994) and grazing (Sellner & al. 1994) are of high importance in biomass accumulation. Taking into account physical regulation of water exchange that ventilates the hypoxic and anoxic deep waters of the Baltic and hence also regulates vertical phosphate flow, what are the main sources of phosphorus for the blooms?

5.4.1 Phosphorus sources of large scale pelagic blooms

The wintertime pools of nitrogen and phosphorus were found to be strongly influenced by hydrography (**IV**). Approximately 60 % of the annual replenishment of the dissolved inorganic phosphorus in the surface layer stems from remineralization of phosphorus originating from the previous growth season and 40 % originates from deep mixing events down to the halocline. Nutrient budget calculations show that the average residence time of total phosphorus in the Baltic Proper is in the range of 11 years and for nitrogen in the range of 5 years, indicating a stronger internal cycling of phosphorus (Wulff & al. 2001a). Modelling studies reveal a staggering residence time of 87 years for labile organic and inorganic phosphorus pools in comparison to approximately 5 years for the same nitrogen pools in the Baltic Proper (Savchuk & Wulff 2001). The differences in residence times are much smaller when comparing the pelagic and benthic systems separately (ranges approximately: 1.4 and 5.3 years, respectively).

These differences mirror the marked differences in internal sinks for nitrogen and phosphorus on an overall systemic scale, since hypoxia and anoxia seem to facilitate removal of nitrogen (**IV**) and retain phosphorus in the aquatic phase (Conley & al. 2002). The direct effect of phosphorus runoff is thus probably mediated through several cycles of uptake and remineralization, sedimentation and resuspension whereas effects of external nitrogen loads (runoff from land) affect more the coastal ecosystems, and pelagic areas rely to a larger extent on internal sources (remineralization) and external sources directly affecting the pelagic system (nitrogen fixation, atmospheric deposition, etc.). The external load of phosphorus does not cause detectable changes in concentration on seasonal time-scales. Thus, presumably remineralization of organic phosphorus compounds and mixing during the winter months are the main processes that replenishing DIP reserves in the upper 60 m layer in the Baltic Proper.

The observed relationship between the hypoxic volume and the DIN pool explains roughly 40 % of the variation in observations. Along with measurement inaccuracies, differences in nitrogen cycling between the basins included in the analysis (Baltic Proper, Gulf of Finland and Gulf of Riga) might be the most important factors explaining the residual variation. A smaller total nitrogen pool in relation to external loads in the Gulfs, compared to the Baltic Proper along with a larger fraction of bottom areas being at favourable depths for benthic denitrification might explain a more rapid removal of nitrogen from the smaller basins. The shallower mean depths of the Gulfs also cause more frequent reoxygenation of deep waters causing larger variation in the extent of hypoxic water volumes than in the Baltic Proper.

The pelagic pools of both total nitrogen and phosphorus in the Baltic Proper have been found to be largely unaffected by short term changes in external loading on a basin scale (**IV**), as shown by the much larger interannual variation in pool size compared to external loads (**IV**, Conley & al. 2002). Thus, the phosphate supply of large-scale offshore blooms of diazotrophic cyanobacteria is regulated to a large extent by internal processes, and the external loads from runoff affect the coastal ecosystems more directly. The pool sizes of nutrients in the pelagic are governed by physicochemical and biological transformations, sinks and sources related to the interconnected biogeochemical cycles of oxygen, nitrogen and phosphorus that are to a large extent governed by inflow events of saline water to the Baltic Sea via the Danish Straits (Conley & al. 2002). The oxygen conditions are thus in combination affected by the amount of sedimenting organic matter, stratification and saline water pulses through the Danish Straits. Due to the long residence time of phosphorus, which is a product of poor water exchange and high production, the Baltic Sea is in a state of inhibited recovery described as a vicious circle (Fig. 15) (**IV**, Fonselius 1969, Tamminen & Andersen 2007). The external load of nitrogen fuels the nitrogen limited spring bloom, which produces large amounts of settling organic material consuming deep-water oxygen that facilitates phosphorus recycling in the aquatic phase. The large quantity of biologically available phosphorus in relation to nitrogen facilitates the occurrence of diazotrophic cyanobacteria blooms, which seem to be self sustained at present external loads and frequency of oceanic water inflow events. The occurrence of diazotrophs fuels the effective internal cycling of phosphorus by introducing new nitrogen to the system. Nitrogen fixation in nitrogen deficient environments tends to stabilize and balance nutrient supply, driving the system towards phosphorus deficiency (Karl & al. 2001, **II**, Lignell & al. 2003), which basically is what the vicious circle describes. Based on long-term data, it is shown that, internal processes in the sea and the interlinked biogeochemical cycles of oxygen, nitrogen and phosphorus act to stabilize the nitrogen pool in the Baltic Sea (**IV**). The recovery of systems experiencing extensive internal loading depend on the historical external load (Jeppesen & al. 1999, Søndergaard & al. 2001) and sediment phosphorus saturation

(Moosmann & al. 2006). Model based estimates of the Baltic Sea recovery to a pristine state after cutting all external nutrient emissions span more than a century (Savchuk & Wulff 2007).

The actual functioning of the vicious circle is however dependent on several assumptions that cannot be directly proven at present. The calculations for the replenishment of upper 60 m layer phosphate concentrations rely on calculations done for the pelagic Baltic Proper only. Further, the estimates of the effect of fixed nitrogen on the system vary (Sommer & al. 2006). The seasonal increase in particulate nitrogen caused by nitrogen fixation (Larsson & al. 2001, Rolff & al. 2007) seems to be to a large part removed from the aquatic phase (**IV**). Sedimentation of the late summer blooms has been found to be small (Heiskanen & Kononen 1994, Heiskanen & Olli 1996), however opposite observations also exist (Tallberg & Heiskanen 1998). The amount of fixed nitrogen removed through trophic transfer is uncertain. However, nitrogen fixation has been found to compensate the lack of nitrogen in bloom communities, and up to 50 % of the nitrogen demand has been estimated to be supplied by leakage from diazotrophs (Stal & al. 2003, Kangro & al. 2007). The fixed nitrogen may consequently be transferred via the microbial loop to higher trophic levels (Kivi & al. 1993, Uitto & al. 1997) or settle from the euphotic zone via autumnal diatom or dinoflagellate blooms, faecal pellets etc. Struck & al. (2004) found that the largest downward particulate organic carbon, particulate organic nitrogen and particulate biogenic silica fluxes at 140 m depth in the Baltic Proper occurred during September coinciding with depleted PON $\delta^{15}N$ signals (0.5 ‰), this indicates that the source of the nitrogen in the PON to be nitrogen fixation and that a large part of the sedimenting material would be diatoms. Autumnal blooms of centric diatoms, such as *Cosinodiscus granii*, *Thalassiosira baltica* and *Actinocyclus octonarius* have been observed would occur in the western Gulf of Finland (Kononen & Niemi 1984). Sinking loss via autumnal diatom blooms and consequent burial might be one potential loss route for nitrogen fixed during diazotrophic cyanobacteria blooms elsewhere in the Baltic Sea as well.

5.5 A hypothesis for the patterns in biomass development and phosphorus utilisation by *Aphanizomenon* sp. and *N. spumigena* leading to bloom events (I, II, III, IV)

Based on the results presented in this thesis and previous work a hypothetical general frame for the typical evolution of *Aphanizomenon* sp. and *N. spumigena* biomasses is introduced. During winter and early spring phosphate concentrations are high and the relatively abundant *Aphanizomenon* sp. populations present presumably store phosphorus (e.g. Larsson & al. 2001, Laamanen & Kuosa 2005) and exhibit light and temperature limited slow growth (Wasmund 1997, Lehtimäki & al. 1997). *N. spumigena* filaments are very scarce in the water column during winter and early spring (Laamanen & Kuosa 2005). As light and temperature increases *Aphanizomenon* sp. growth increases and biomass accumulates while phosphate is still available for uptake. Relatively low temperatures hamper *N. spumigena* growth (Lehtimäki & al. 1997) but populations may nevertheless take up and store phosphorus, exhibiting luxury consumption common in planktonic algae and cyanobacteria (e.g. Droop 1978, Elser & Sterner 2002b). As temperatures further increase and phosphate concentrations decline, *Aphanizomenon* sp. growth relies mainly on intracellular stores (Larsson & al. 2001, Lignell & al. 2003, Kangro & al. 2007, Walve & Larsson 2007). Growth slows down as the cellular C:P ratio increases sufficiently. *N. spumigena* growth increases with increasing temperature and light and growth relies on cellular stores and on phosphorus hydrolysed from organic compounds (**I**, **II**, Degerholm & al. 2006, Nausch & Nausch 2006). However, since the available phosphorus pool for *N. spumigena* is smaller than for *Aphanizomenon* sp., due to a smaller inoculum and later biomass increase when phosphate concentrations have already declined markedly, the peak of the bloom is also typically shorter (e.g. Kononen 1992, Wasmund 1997, Laamanen & Kuosa 2005) and the bloom populations exhibit more pronounced phosphorus deficiency (Fig. 11) (**II**, Kangro & al. 2007).

Thus, both species are dependent on the excess DIP phenomenon, however, through different pathways. *Aphanizomenon* sp. acquires its phosphorus earlier in the season as phosphate and through pulsed supplies mediated by e.g. upwelling and *N. spumigena* relies on more scarce population level cellular stores and dissolved organic phosphorus compounds. ATP and nucleotides have been suggested to be preferential organic sources for cyanobacteria in the Baltic (Nausch & Nausch 2006).

6. CONCLUSIONS AND FUTURE PROSPECTS

Autecological traits of cyanobacteria explain their response to hydrodynamically mediated nutrient pulses (**I**, **II**, **III**). The supply mode of phosphorus during blooms of cyanobacteria can alter the species composition and function as a species selection mechanism along with physical forcing factors (**I**, **II**, **III**). These two selective processes work on different temporal scales, with physical forcing

having immediate effects and the phosphorus supply mode affecting community composition on longer time scales depending on the slow growth of the diazotrophic cyanobacteria and typical decoupling of production and nutrient uptake in storage specialist species. *Aphanizomenon* sp. growth is to a large extent reliant on cellular phosphorus stores, however *N. spumigena* also displays remarkable capacity to store phosphorus and utilise this source of nutrition for growth (**I**). However, the typical temperature and light optima of the two species drive them into separate ecological niches, accentuating the importance for dissolved organic phosphorus sources for *N. spumigena* growth. Nonetheless, the matter of interspecies phosphorus competition between diazotrophic cyanobacteria in the Baltic Sea still warrant further study. A careful characterisation of different forms of phosphorus along with the different supply routes in relation to the nutritional history of extant populations needs to be clarified.

The dominant diazotrophic cyanobacteria in the blooms can drive the entire plankton community into phosphorus deficiency (**II**, **IV**). In our study, the Baltic Proper bloom time community was observed to be more phosphorus deficient than the Gulf of Finland communities. Cyanobacteria bloom communities in the Gulf of Finland experience repeated replenishment of phosphate reserves mediated through coastal upwelling and frontal processes (**III**, Kononen & al. 1996). Inert stratified areas relying on recycling of phosphorus favour the occurrence of the hepatotoxic *N. spumigena* (Kononen & al. 1996), which dynamics are however not detached from the excess phosphorus phenomenon that strongly regulate potential *Aphanizomenon* sp. biomass (Larsson & al. 2001, Walve & Larsson 2007). Phosphorus supply of large-scale pelagic cyanobacteria blooms is governed to a large extent by internal processes of the sea (**IV**). The phosphorus supply modes act on different temporal scales, ranging from inter-annual variation in the amount of excess phosphorus to the frequency and magnitude of coastal and frontal upwelling events (**III**, **IV**). Our studies along with previous efforts by other authors show that *Aphanizomenon* sp. would rely mainly on cellular stores and phosphate inputs through hydrodynamic activity to cover their phosphorus demand and *N. spumigena* is more dependent on recycled inorganic and organic phosphorus along with internal phosphorus stores (**I**, **II**, **III**). The Baltic Sea is hypothesised to be in an inhibited state of recovery due to extensive historic nutrient loading and subsequent internal loading of phosphorus from the sediments and the prevalent diazotrophic cyanobacteria blooms causing substantial inputs of new nitrogen to the sea (**IV**, Fonselius 1969, Tamminen & Andersen 2007).

The reduction of different nutrients so as to induce more severe nutrient limitation of productivity in the Baltic may have several outcomes depending on to which degree and at what time of the year different osmotroph community constituents are limited by which nutrient. Some researchers advocate the idea that if nitrogen emissions are reduced, diazotrophic cyanobacteria blooms will be enhanced and the nitrogen fixation of the cyanobacteria will compensate for the external load reductions made. In order to make educated and cost effective decisions about eutrophication abatement measures we have to know which nutrient is causing the adverse effects that we want to rid. Phosphorus is likely the most important nutrient regulating diazotrophic cyanobacteria bloom development and modelling efforts show most drastic reductions in diazotrophic cyanobacteria biomass with phosphorus reductions (Wulff & al. 2007). On the smallest spatial and temporal scales (cf. Table 1) a reduction in phosphorus availability seems to be a key aspect in increasing nutrient limitatation of diazotrophic cyanobacteria and thus reducing bloom intensity.However, interconnected biogeochemical cycles of nitrogen, phosphorus and oxygen and food web interactions (e.g. trophic cascade effects) affect this conception. Reduction of only phosphorus loads might be ineffective since eutrophication related phenomena in the Baltic Sea show signs of self-sustenance due to extensive and prolonged anthropogenic eutrophication (**IV**).

On the largest scales reductions in both nitrogen and phosphorus external loads need to be done in order to improve the present eutrophied state of the Baltic Sea and to reduce the occurrence of toxic diazotrophic cyanobacteria blooms. If only phosphorus loads are reduced the nitrogen limited spring bloom will continue fuelling deep bottoms with organic material that consume oxygen upon bacterial degradation. Thus internal loading of phosphorus will persist and keep the levels of wintertime phosphate high because of the high residence time of phosphate in the aquatic phase in hypoxic and anoxic conditions. A concomitant substantial reduction in nitrogen and phosphorus might be expected to have more rapid effects due faster reductions in the amount of settling organic material by reducing the spring bloom due to general nitrogen limitation of Baltic Sea production. Therefore, also improvement of deep-water oxygen conditions would occur, which seems to be the key in restoring the Baltic Sea into a less eutrophied state.

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The world of microbes is cruel, as is the world of everyone else as well. Neal Stephenson has put it aptly: ”…let’s just stipulate that in *some* way, self-replicating organisms came into existence on this planet and immediately began trying to get rid of each other, either by spamming their environments with rough copies of themselves, or by more direct

means which hardly need to be belaboured. Most of them failed, and their genetic legacy was erased from the universe forever, but a few found some way to survive and to propagate". So basically, evolution has secured the proliferation of microbes through ages, they are simple yet deviously cunning in their ability to survive. If you didn't know better they could be considered a collective intelligence where the individual has no value but the survival of the kin is everything. We humans, we are good at surviving too, but it it's not enough. You have to remember why you are good, at surviving and other things too (I'm not explicitly stating that I'm good though). However, as microbes, we are good at taking each others lives as well (just have a look at the latest newspaper), but for us being "good" again is most often dependent on other people, so being a good microbe doesn't constitute being a good human, hmm… What I mean is that surviving in the scientific world isn't enough. The conscious acknowledgement of the cultural and scientific heritage we build our work on is essential. That's why I guess I'll have to state my thanks in the following lines of text. My work isn't my work at all. It is work done by a bunch of people (there are several pages of references listed in this thesis alone). Besides those authors listed in the references section, there are a lot of people that have helped me out more directly during the past years and produced this book of memetically transferred information on details of natural phenomena. But don't worry I do take full responsibility of all my writings even though I point you all out as culprits for the emergence of this work.

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