RESPONSES OF FILAMENTOUS CYANOBACTERIA TO NATURAL AND ANTHROPOGENIC CHANGES IN THE BALTIC SEA

Dissertation

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SUMMARY

This PhD thesis aimed to contribute to the existing knowledge about filamentous cyanobacteria, common in the Baltic Sea. In detail the studies concentrated on two diazotrophic cyanobacteria species, *Nodularia spumigena* and *Aphanizomenon* sp., which form wide spread surface blooms in the Baltic Sea. The thesis includes diurnal observations, laboratory and field studies. The experiments were conducted to examine variations in autotrophic activity with respect to a natural day/night rhythm or anthropogenic induced changes of pCO_2 levels, and how this further affects the accompanied heterotrophic bacterioplankton.

The investigations presented herein were funded by the BMBF (Bundesminesterium für Bildung und Forschung). The studies were part of the project "SOPRAN" (surface ocean processes in the anthropocene). Within the framework of TP 2.2 this thesis focussed on the effect of anthropocene CO_2 levels on marine ecosystems and sea-to-air gas fluxes.

The first study showed large diurnal variations (two and four fold) within the single nutrient incorporation rates (dissolved inorganic carbon, dinitrogen, and dissolved inorganic phosphorus). Different stages within the development and the dominance of different filamentous species (*Aphanizomenon* sp. or *Nodularia spumigena*) affected the course of the single fixation rates. Once the bloom reached a senescent stage diurnal patterns disappeared. It was examined that DIP availability alters diurnal and absolute values of autotrophic growth. Carbon (C) and dinitrogen (N_2) fixation are reduced at low DIP concentrations. Increasing POC:POP ratios indicated reduced DIP supply whereas the dissolved inorganic carbon (DIC) supply was not limiting for autotrophic growth.

Secondly a laboratory experiment was conducted to obtain whether the hypothesis that filamentous cyanobacteria growth increases with elevated pCO_2 is true. The results show that autotrophic C fixation was stimulated by increasing CO_2 concentrations. Reduced or depleted DIP concentrations debate this effect. N_2

fixation seems to depend to a higher degree on sufficient DIP availability. A restrained POP build up probably inhibits N_2 fixation and suppresses stimulated N_2 fixation at high pCO_2 values. It can be assumed, that repressing effects due to constrained P sources for diazotrophic growth preponderate the measured stimulating effects of elevated CO_2 concentrations. Therefore CO_2 stimulation and DIP limitations probably interfere with each other.

During a field experiment with diazotrophic cyanobacteria accompanied by heterotrophic bacteria these effects were approved. A stimulation effect of enhanced pCO_2 levels on autotrophic diazotrophic growth is the combined effect of higher pCO_2 levels and new input DIP. During this study it was also shown, that heterotrophic bacteria enhance activity at high CO_2 concentrations as long as filamentous cyanobacteria are not DIP limited. Thus heterotrophic bacteria are indirectly positively affected by increasing CO_2 values.

In general, the stimulation by elevated pCO_2 levels along the gradient of 280 to 780 µatm was measured with a factor of two to four which approximates the same range like daily variations. Due to it is indispensable to sample at similar time points during the day to avoid misinterpretation and to allow the discrimination between present and future variations in autotrophic growth.

The here presented results also indicate that impacts of enhanced CO_2 concentrations might not be visible during certain periods in the summer or appear in areas with high nutrient concentrations only, like upwelling areas.

The obtained data emphasize on the importance of nutrients concentrations combined with changing CO_2 levels. Presumably future metabolic reactions within the phytoplankton are subjected to big variations and these might also result in shifts in the species composition in the Baltic Sea.

ZUSAMMENFASSUNG

Im Rahmen dieser Dissertation galt es, das Wissen über filamentöse Cyanobakterien zu erweitern. Die Untersuchungen konzentrierten sich im Wesentlichen auf zwei diazotrophe Arten, *Nodularia spumigena* und *Aphanizomenon* sp.. Im Verlauf eines Sommers können diese Arten große Oberflächenblüten in der zentralen Ostsee ausbilden. Die Arbeit basiert auf umfassenden Untersuchungen von Tageszyklen, Labor- und Feldexperimenten. Ziel war es dabei, Variationen autotropher Aktivitäten bezüglich der natürlichen Tag/Nacht Rhytmik oder anthropogener Veränderungen, die durch erhöhte pCO_2 Niveaus verursacht werden, zu verfolgen. Im Zuge dessen wurden zudem die Einflüsse auf das umgebende Bakterioplankton untersucht.

Die im Nachfolgenden präsentierten Experimente wurden durch das BMBF (Bundesminesterium für Bildung und Forschung) im Rahmen des Verbundprojektes "SOPRAN" (surface ocean processes in the anthropocene) gefördert. Die Untersuchungen erfolgten innerhalb des Teilprojektes mit der Zielstellung die Effekte anthropogen verursachter CO_2 Level auf marine Ökosysteme und den Gasaustausch zwischen Meer und Atmosphäre zu verfolgen.

Im Verlauf der ersten Kampagne wurden beträchtliche Variationen (zwei bis vierfach) der einzelnen Nährstoffaufnahmen (gelöster anorganischer Kohlenstoff – DIC, molekularer Stickstoff – N_2 und gelöster anorganischer Phosphor – DIP) in Abhängigkeit von der Tageszeit nachgewiesen. Unterschiedliche Entwicklungsstadien, sowie die Dominanz verschiedener Arten (*Aphanizomenon* sp. oder *N. spumigena*) wirkten sich auf den Verlauf der einzelnen Fixierungsraten aus. Beobachtungen an einer alternden Blüte, zeigten eine Reduzierung der tageszeitlichen Schwankungen. Ferner wurde demonstriert, dass die DIP-Verfügbarkeit tageszeitliche und absolute Größen autotrophen Wachstums beeinflusst. Kohlenstoff- und Stickstofffixierung verringerten sich bei niedrigen DIP Konzentrationen. Steigende POC:POP

Verhältnisse innerhalb der filamentösen Cyanobakterien lassen eine reduzierte DIP aber ausreichende DIC Zufuhr vermuten.

Im Zuge eines Laborexperimentes, galt es, die Hypothese, ob filamentöse Cyanobakterien durch erhöhte CO_2 Konzentrationen stimuliert werden, zu überprüfen. Offensichtlich wird die autotrophe C Fixierung durch erhöhte CO_2 Koncentrationen stimuliert. Reduzierte oder erschöpfte DIP Konzentrationen wirken dieser positiven Entwicklung entgegen. Insbesondere die Stickstofffixierung scheint sensitiv gegenüber einer ausreichenden Phosphorversorgung zu reagieren. Vermutlich hemmt ein reduzierter POP-Aufbau die Stickstofffixierung und unterdrückt gleichzeitig die Stimulierung der Stickstofffixierung unter erhöhten CO_2 Bedingungen. Es kann davon ausgegangen werden, dass die hemmenden Effekte geringer DIP Konzentrationen der Stimulation erhöhter CO_2 Konzentrationen entgegenwirken. CO_2 Stimulation und DIP Limitation sind sich überlagernde Vorgänge.

Das Ziel der durchgeführten Feldstudie war die Untersuchung diazotropher Cyanobakterien und des umgebenden heterotrophen Bakterioplankton. Die erzielten Resulte bestätigten hierbei die zuvor dokumentierten Ergebnisse der Laborversuche. Eine Steigerung des autotrophen Wachstums auf Grund erhöhter pCO_2 Niveaus wurde jedoch nur in Kombination mit ausreichender DIP Zufuhr nachgewiesen. Zudem zeiget auch die heterotrophe bakterielle Aktivität erhöhte Werte, sofern filamentöse Cyanobakterien nicht DIP limitiert sind. Somit werden heterotrophe Bakterien nur indirekt von steigenenden CO_2-Werten beeinflusst.

Entlang des Gradienten von 280 bis 780 µatm pCO_2 wurden die autotrophen Nährstoffaufnahmen um das zwei- bis vierfache erhöht, was der zuvor demonstrierten täglichen Variation der einzelnen Parameter entspricht. Daraus ergibt sich, dass die Zeitpunkte der Probenahmen von extremer Bedeutung sind. Identische Probenahmezeitpunkte an unterschiedlichen Tagen ermöglichen die eindeutige Diskreminierung heute natürlich vorkommender und anthropogen verursachter Variationen.

Die vorgestellten Experimente lassen zudem vermuten, dass die stimulierenden Effekte erhöhter CO_2 Konzentrationen nur zeitweise (Zeitperioden mit ausreichender DIP Versorgung) oder lediglich in Auftriebsgebieten vorzufinden sind.

Die erzielten Ergebnisse unterstreichen die Bedeutung hinreichender Nährstoffkonzentrationen in Kombination mit veränderten pCO_2 Niveaus. Die zukünftigen abiotischen Veränderungen in der Ostsee können desweiteren zu Verschiebungen innerhalb des Artengefüges führen.

1. INTRODUCTION

1.1. IMPORTANCE OF DIAZOTROPHIC CYANOBACTERIA WITH RESPECT TO ATMOSPHERIC pCO_2 CHANGES

Present and future environmental conditions are affected by anthropogenic influences. During the past century enhanced industrial and human energy demands caused increasing fossil fuel burning. Measured atmospheric CO_2 concentrations today are double those recorded in preindustrial times. Until the beginning of the 21th century this change was widely ignored. During the past 20 years politics and science paid more attention onto anthropogenic impacts on terrestrial, limnic and marine ecosystems. To broader the knowledge about ecosystem and species specific feedbacks on changing CO_2 conditions, worldwide monitoring programs and projects were funded. In 2001 and 2007 international reports on climate change summarized the results acquired by these programs and directed the focus of politics to the issue of increasing CO_2 concentrations as a driver of climate change (e.g. Houghton et al. 2001, Solomon et al. 2007). As a result, the human population recognized and accepted that continuing with the so-called "business as usual" scenario would lead to the destruction of fauna and flora. To avoid an ongoing alteration of environmental conditions, it is indispensible to obtain new strategies for the utilization of fossil resources, and to realize benefits of renewable energy technologies. Recently, leading industrial companies and politicians are reacting and investing in future research projects, especially focussing on these topics in the ocean.

The ocean is the largest global carbon reservoir, which has the potential to counteract atmospheric CO_2 elevation to some degree via primary production. One group of phytoplankton (autotrophic component of the plankton), which is probably responsible for a net removal of $CO_{2(aq)}$ from the water column, are diazotrophic (dinitrogen fixing) cyanobacteria. Cyanobacteria are also known as blue-green algae, blue-green bacteria or Cyanophyta. Due to their ability to convert dinitrogen into

ammonia, primary production of this taxon does not rely on dissolved inorganic nitrogen (DIN), a fact that may have led to their evolutionary and ecological success. Therefore, it can be assumed that cyanobacteria also react sensitively to atmospheric alteration and further influence the carbon budget in the atmosphere and water column.

Fossil records attested cyanobacteria to be one of the oldest organisms on earth (3000 Ma) (Schopf 2000, Brasier et al. 2002). Cyanobacteria are believed to have created our present day oxygen-enriched atmosphere; they originated oxygenic photosynthesis with the help of the pigment Chl*a* (Kasting & Siefert 2002, Williams 2006). Since their first occurrence in the Archaen time period cyanobacteria were exposed to a wide range of conditions, subjected to natural and anthropogenic changes, including varying CO_2 concentrations. Therefore, their successful growth, evolution, the survival in varying conditions despite their special nutrient demands make cyanobacteria a suitable group of organisms, studying changing mechanisms of nutrient acquisition, growth and species composition in a high CO_2 world.

1.2. STUDY AREA – THE BALTIC SEA

The Baltic Sea is a semi-enclosed, non tidal, brackish, estuarine basin. The surface area is approximately 412560 km². Maximum depth, located in the Landsort Deep (northwest Baltic Sea), is 459 m. Meanwhile the average depth is only 52 m. From north to south the Baltic Sea spans about 1300 km and 1000 km from west to east. The Baltic Sea was formed after the last glaciation approximately 10000 years ago. Today it is located in a depression of the Fennoscandian shield. Since that time it has undergone several conversions between limnic, oceanic and brackish conditions. The present brackish situation prevails since 2000 BP. The Baltic Sea is characterized by a small volume (21631 km³), a long mean residence time of water (30 years), and is highly influenced by the large drainage area including 14 countries with a total human population of approximately 80 million inhabitants (Bergström & Carlsson 1994). Hence the Baltic Sea is very vulnerable to anthropogenic impacts. Due to its typical estuarine nature, a strong surface layer salinity gradient from the north to the

southwest, 3 to 30 PSU, occurs. The typical horizontal and vertical stratification is caused by high freshwater inputs from the large drainage area, as well as by the restricted oceanic connection (Matthäus & Franck 1992). The Baltic Sea is characterized by a permanent pycnocline and seasonal thermocline (Malmberg & Svansson 1982), determining large-scale physical and biogeochemical processes. Importantly, the extensive layering results in limited convection of oxygen, which results in regulated dynamics of eutrophication and can be directly related to phenomena like offshore blooms of diazotrophic cyanobacteria (Niemi 1979).

1.3. FILAMENTOUS DIAZOTROPHIC CYANOBACTERIA

1.3.1. Occurrence of filamentous diazotrophic cyanobacteria in marine environments

In the Baltic Sea cyanobacteria occur in different habitats. Descriptions of pelagic, benthic, epiphytic and epilithic species exist (Hällfors 2004). Pelagic cyanobacteria are the most conspicuous group, comprising multicellular filamentous and unicellular pico-sized taxa. Many of these species developed a diazotrophic life strategy. On a global scale cyanobacterial dinitrogen (N_2) fixation contributes significantly to new production in aquatic ecosystems (Capone et al. 1997, Montoya et al. 2004). Especially in tropical and subtropical oceans, diazotrophic cyanobacteria are known to be highly important as providers of new nitrogen (N) (Capone et al. 1997, Karl & Yanagi 1997, Karl et al. 2002). In the contemporary ocean, a large fraction of N_2 fixation is attributed to the filamentous, nonheterocystous cyanobacteria belonging to the genus *Trichodesmium*. These planktonic microorganisms are cosmopolitan. *Trichodesmium* sp. forms massive near-surface blooms in the low nutrient tropical and subtropical seas (Carpenter & Capone 1992). Recently it came to light that N_2 fixation by *Trichodesmium* is much more important than previously suspected, and most likely many other presently unknown diazotrophic microorganisms also contribute to the global ocean N budget. In general heterocystous cyanobacteria are quite rare in the marine environment and most estuaries, while on the other hand they are common in freshwater environments.

Certainly the Baltic Sea is one of the few brackish water areas where planktonic diazotrophic cyanobacteria are an important component of the phytoplankton community (Howarth et al. 1988).

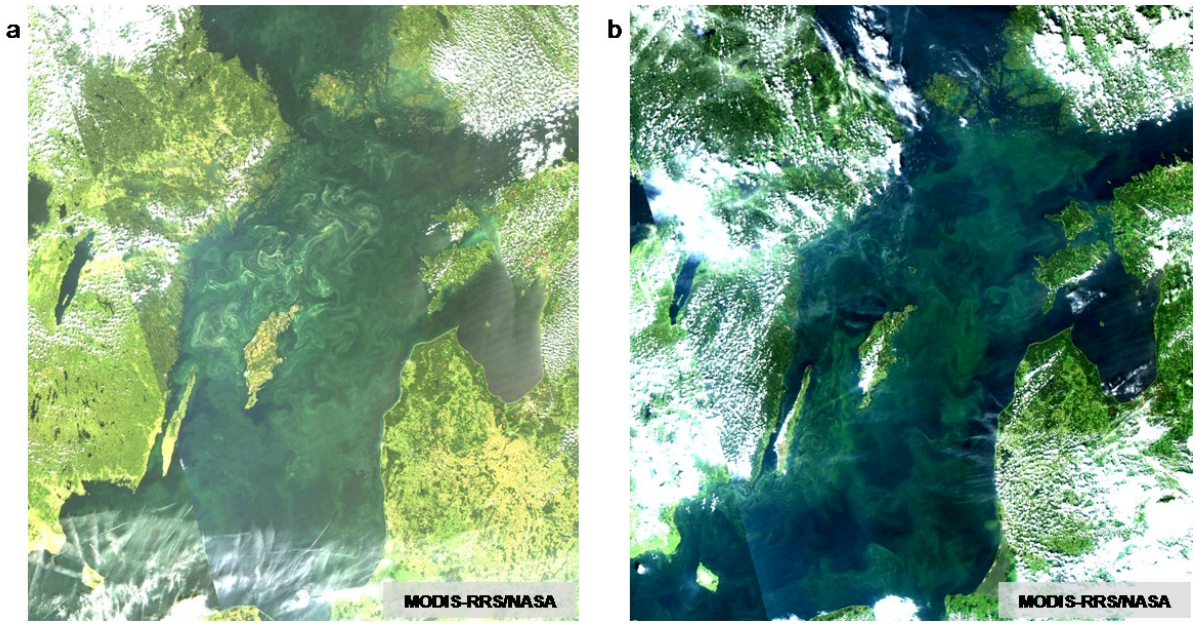


Fig. 1. Satellite pictures of the central Baltic Sea; a) July 2008; b) July 2009.

In the Baltic Sea diazotrophic filamentous cyanobacteria have occured for at least 7000 years, when the Ancylus Lake transformed into the saltwater Litorina Sea (Bianchi et al. 2000). Direct observations of cyanobacterial blooms have been reported since phytoplankton records were first conducted in the early 19th century. Long-term monitoring as well as satellite observations indicate an increase in the frequency of blooms during the past two decades (Kahru et al. 1994). These wide spread cyanobacterial blooms arise periodically at optimal temperature, nutrient and wind conditions (defined below). The initiation of cyanobacteria blooms in the Baltic Sea has been related to a low N to phosphorus (P) ratio (Niemi 1979), resulting from the high concentrations of P in the low-oxygen or anoxic bottom layers (Niemistö et al. 1989). It is tempting to blame increased eutrophication, in particular discharge of municipal effluents and industrial waste water via riverine input, for increased occurrence of cyanobacterial blooms. However, this theory was weakened because

the enhanced bloom formation continued even though the nutrient input decreased(Howarth et al. 1988, Marino et al. 2002). Observations and monitoring of cyanobacterial blooms were also part of the field campaigns with investigations presented in this dissertation (Fig. 1)

Recent studies of N_2 fixation estimated that diazotrophic cyanobacteria are a source for N input up to $4 – 8 \times 10^6$ t yr^{-1} in the Baltic Sea, which equals or even exceeds the annual riverine input (3.63×10^6 t yr^{-1}) (Wasmund et al. 2005). Consequently diazotrophs are responsible for 20 – 40 % of the total annual N loading of the Baltic Sea (Larsson et al. 2001), even though growth is mainly restricted to a three to four month season. This highlights that N_2 fixation by diazotrophic cyanobacteria is an important factor determining subsequent autotrophic biomass production of non N_2 fixing plankton. Accordingly, changes in extent and duration of diazotrophic blooms affect biochemical and food web interactions in the Baltic Sea. Hence, it is important to understand the conditions that lead to the formation of blooms and pontial influences on spatial and temporal distribution (Kononen & Leppänen 1996), considering the changes expected due to climate change and ocean acidification.

Three pelagic cyanobacterial taxa are commonly considered to actively fix N_2 in the Baltic Sea: *Nodularia spumigena* (one specie in the Baltic Sea) (Wasmund 1997), *Aphanizomenon* sp. (probably one species in the Baltic Sea) (Janson et al. 1994) and *Anabaena* sp. (several bloom forming species) (Niemistö et al. 1989) (Fig. 2).

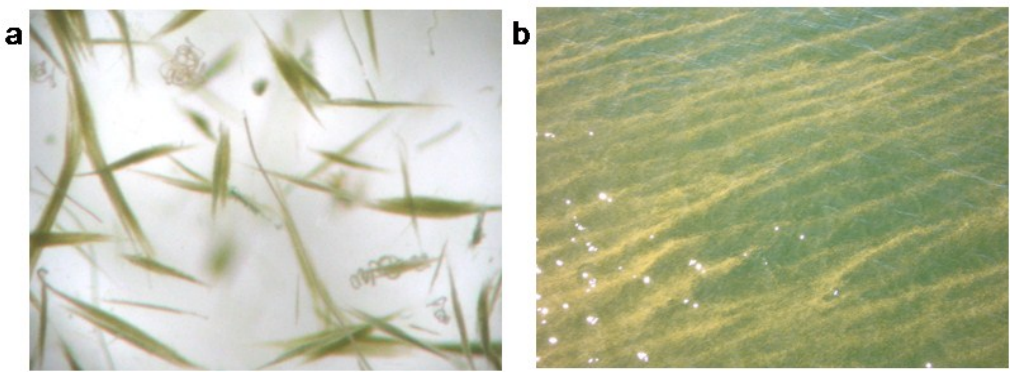


Fig. 2. Photos of bloom forming diazotrophic cyanobacteria, including *Aphanizomenon* sp. and *N. spumigena* during July 2009; a) photo via a binocular microscope, b) photo of the seawater surface.

Direct relationships between spatial and temporal variability in their occurrence and N_2 fixating rates during the productive summer season, demonstrate that these three taxa are mainly responsible for N_2 fixation in the Baltic Sea (Ohlendieck et al. 2000, Wasmund et al. 2001).

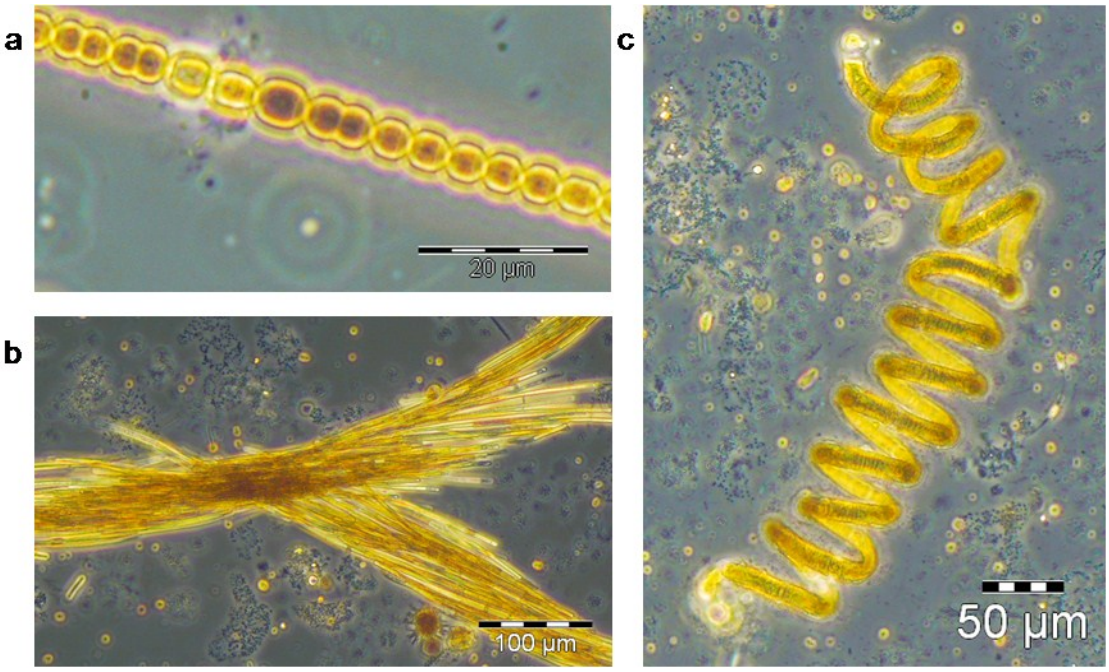


Fig. 3. Microscopic pictures of filamentous cyanobacteria, typical for the Baltic Sea; a) *Anabaena* sp., b) *Aphanizomenon* sp., c) *N. spumigena*.

1.3.2. Regulation by physical conditions

As mentioned before, blooms of filamentous cyanobacteria occur in distinct temporal, vertical, and horizontal distribution patterns, related to the physical variability of the water column (Kononen & Leppänen 1996). In general, *N. spumigen*a and *Aphanizomenon* sp. occur at the same time in the same areas. According to several studies salinity is one of the important abiotic factors, determining the structure and development of cyanobacterial blooms. It has been demonstrated that the optimal salinity level for growth and N_2 fixation is between 5 – 13 PSU (Sivonen et al. 1989). Growth of *Aphanizomenon* sp. seems to be restricted to salinity levels below 10 PSU.

Optimal growth conditions were revealed between 0 – 5 PSU (Lehtimäki et al. 1997). High levels of salinity decrease the nitrogenase activity. Besides salinity, temperature and light regulate diazotrophic growth (Lehtimäki et al. 1997). The *N. spumigena* cell concentration increases with rising temperatures and irradiance (Wasmund 1997), while high *Aphanizomenon* sp. biomass values are measured during the pre-bloom stage at temperatures and irradiance levels below maximum values, too (Kononen & Leppänen 1996, Wasmund 1997, Laamanen et al. 2005). More detailed studies ascertained that *N. spumigena* dominance exhibited stronger correlations to elevated temperatures than *Aphanizomenon* sp. (Kanoshina et al. 2003, Lips & Lips 2008). But factors like wind, light conditions and the mixed layer depth also have to suit cyanobacterial requirements. *N. sp*umigena and *Aphanizomenon* sp. are able to form gas vesicles, the so called buoyancy. This feature allows them to change their specific gravity and to return to the illuminated surface waters after mixing events. After an extended period of warm and sunny weather, inside a stable water column gas-vacuolate taxa become dominant and can form extensive surface scums at the water surface. Constant wind speeds higher than 5 m s^{-1} seem to supress bloom development, probably by interfering with this ability (Walsby 1994).

Light intensities for optimal growth differ for *N. spumigena* and *Aphanizomenon* sp., 105 – 155 and 25 – 45 µmol photons m^{-2} s^{-1} respectively (Kanoshina et al. 2003). Therefore, light influences the species specific distribution in the water column. Not to forget, that daily differences within light intensities determine diurnal pattern of autotrophic processes inside the cyanobacterial bloom. Especially primary production and N_2 fixation are assumed to be primarily light driven (Evans et al. 2000, Gallon et al. 2002).

1.3.3. Adaptation to DIP availability

Concentrations of DIN below the detection limit and dissolved inorganic phosphosrus (DIP) concentrations of 0.1 µmol l^{-1} are presumed to be triggering factors for cyanobacterial development in the Baltic Sea. After the spring bloom of diatoms intense denitrification processes and high winter DIP concentrations as a result of a high NAO (North Atlantic Oscillation) index cause DIN:DIP ratios below

the Redfield ratio (7 – 8) (Nausch et al. 2008, Redfield 1958). Both a high NAO index, which is characterized by high wind stress, low ice cover, high mixed-layer depth, and strong upwelling are assumed to be a potential prerequisite for extensive cyanobacterial blooms (Janssen et al. 2004). Most diazotrophic cyanobacteria seem to rely on a P reserve, synthesized during this initial growth phase after the diatom bloom (Janssen et al. 2004). Indeed, many cyanobacteria species have been shown to be real storage-specialists. Polyphosphate accumulations can be easily detected in filamentous cyanobacterial cells grown under phosphorus replete conditions (Fig. 4) (Sommer 1985, Sakshaug & Olsen 1986). It is indicated that growth and N_2 fixation rates are regulated by the availability of P (Wu et al. 2000, Sanudo-Wilhelmy et al. 2001).

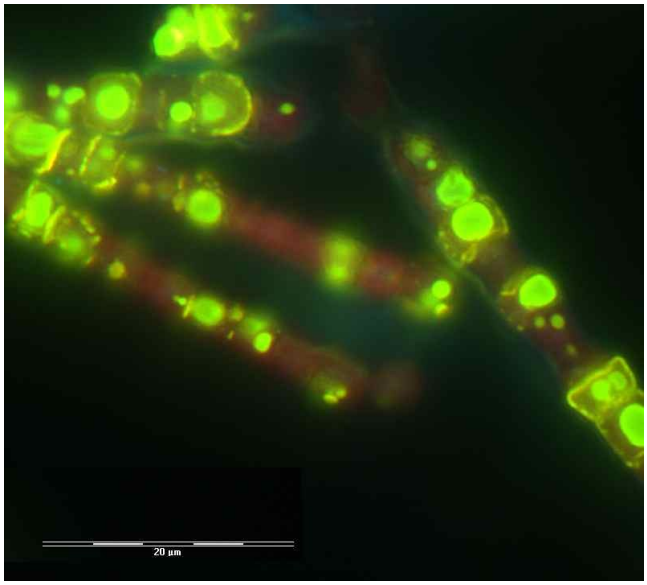


Fig. 4. Filaments of *N. spumigena* stained with DAPI. Yellow vesicles represent stored polyphosphates.

However cyanobacteria developed various strategies to overcome their inferiority in the DIP uptake compared to the picoplankton during DIP deficiency in surface waters (Cotner & Wetzel 1992, Thingstad et al. 1993, Tanaka et al. 2004): a) utilization of regenerated DIP and DOP, b) internal polyphosphate storage (Fig. 4),

variable POC:POP ratios, and c) fast utilization of additional DIP supply via upwelling (Vahtera et al. 2007a, Nausch et al. 2009).

The internal cycling of phosphorus within the bloom was shown by previous investigations (a). For example, while measurements inside the water column without bloom obtained depleted DIP concentrations, inside the bloom values of 0.02 µmol l^{-1} for DIP and dissolved organic P (DOP) concentrations of 0.2 µmol l^{-1} or even higher were detected (Nausch et al. 2004). This supports the theory of regenerated phosphorus supply, due to remineralization and breakdown of POM by heterotrophic bacteria (Nausch et al. 2004). It is reported, that filamentous cyanobacteria are able to maintain growth with DOP as a P source, shown as increasing activity of the nutrient sequestration enzyme alkaline phosphatise (enzyme for the use of DOP) at depleted DIP concentrations (Button 1985). In particular, several studies revealed enhanced alkaline phosphatase activity of the specie *N. spumigena* at diminished DIP concentrations (Degerholm et al. 2006, Kangro et al. 2007).

The strong ability to deal with depleted DIP concentrations is also mirrored in the remarkable plasticity in the ratio of particulate organic C (POC) to particulate organic P (POP) with regards to intracellular P stores (Larsson et al. 2001) (b). Characteristic POC:POP ratios for a cyanobacteria bloom vary between 231 in the beginning of the bloom and can be doubled at a senescent state (Nausch et al. 2004). Due to that, the composition of the particulate organic matter (POM) is an applicable tool to follow up the altering nutrient supply and utilization during the progression of the bloom.

The fast utilization of additional DIP is proofed for *Aphanizomenon* sp. (c). It is known to utilize short time DIP inputs (for example by upwelling) directly, whereas an immediate incorporation/reaction by *Nodularia* sp. has not been reported so far (Degerholm et al. 2006). In consequence a preferred growth of *Aphanizomenon* sp. compared to *Nodularia* sp. after upwelling events is observed.

1.3.4. N_2 fixation – adapatation to DIN deficiency

Biological N_2 fixation, reduction of atmospheric N_2 to ammonium (NH_4^+), is certainly the most important "invention" made by cyanobacteria. With this ability cyanobacteria are independent of inorganic nutrient supply of N. Under optimal conditions the stoichometry of N_2 reduction can be described as followed:

$$N_2 + 8\,e^- + 8\,H^+ + 16\,MgATP \rightarrow 2\,NH_3 + H_2 + 16\,MgADP + 16\,P_i$$

N_2 fixation is promoted by the enzyme complex nitrogenase. Nitrogenase is composed of complex metalloenzymes with conserved structural and mechanistic features (Rees & Howard 2000, Lawson & Smith 2002). It contains two main components. One is the Fe protein, operating as an ATP-dependent electron donor and the other, called MoFe protein, includes the enzyme catalytic site. Both components are characterized by extraordinary oxygen (O_2) sensitivity. Processing of the enzyme includes reduction of the Fe protein by electron donors such as ferredoxin and flavodoxin, transport of electrons from the Fe protein to the MoFe protein and in the end internal electron-transfer in the MoFe protein. The electron-transfer is preceded by the Fe and MoFe proteins forming a complex and subsequently dissociating. Nevertheless the catalytic process is quite slow, with an approximate turnover time of 5 s^{-1}. Due to the relatively slow regeneration of nitrogenase, diazotrophic cyanobacteria have to synthesize large quantities of the different enzyme compounds, resulting in high nutrient demands. Nitrogenase can make up to 20 % of the total protein concentration in the cell. Because it is obligate to protect the enzyme from oxygen damage, different physiological adaptations developed among diazotrophs. Simple strategies like avoidance of O_2 through anaerobic growth as well as consumption of excess oxygen by respiration, oxygen diffusion barriers or compartmentalization of the enzyme temporally or spatially occur.

The filamentous cyanobacteria common in the Baltic Sea developed special cells to maintain spatial separation of the enzyme. These cells are named heterocysts. In *N. spumigena*, heterocysts constitute approximately 5 % of all cells in the filament, while for *Aphanizomenon* sp. lower numbers (1 %) are estimated. They are the only cells where N_2 is fixed. Overall, under conditions of N limitation vegetative cells differentiate to heterocystic, non-growing cells in which N_2 is transformed into organic N like cyanophycin (Wolk 1996). The heterocysts exhibit characteristical features, e.g. thick walls and a poor pigmentation. The physical isolation from the vegetative cells is formed in order to protect the O_2 sensitive enzyme nitrogenase. However the "envelope" of the heterocysts is not complete, microplasmodesmata permit a rapid exchange of organic material between heterocysts and vegetative cells (Flores et al. 2005, Popa et al. 2007) (Fig. 5). Heterocysts receive carbohydrates and provide N compounds for the ancient cells (Jüttner 1983). At the molecular level, the different physiology of the heterocysts and vegetative cells is encoded by different genes, for example genes which are involved in the formation of the heterocyst envelope (Buikema & Haselkorn 1993). Changes in the thylakoid structure of heterocysts are associated with synthesis of a glycolipid layer which is also important in protection of nitrogenase from O_2 (Soriente et al. 1993). Both O_2 and N_2 diffuse into the heterocysts, but increased respiratory activity in membranes near to the polar ends depletes the O_2 concentration. The reductants of N_2 fixation are almost exclusively channelled into the reduction of N_2 to ammonia (NH_3), which in turn reacts with glutamate derived from the imported carbohydrates to form glutamine. Especially during the first hours of light in the course of the day the N demands of vegetative cells is met by a rapid transport of glutamine from heterocysts to vegetative cells (Fig. 5). The lack of N and carbon (C) gradients in the vegetative cells suggests an immediate utilization of the transferred N and C compounds.

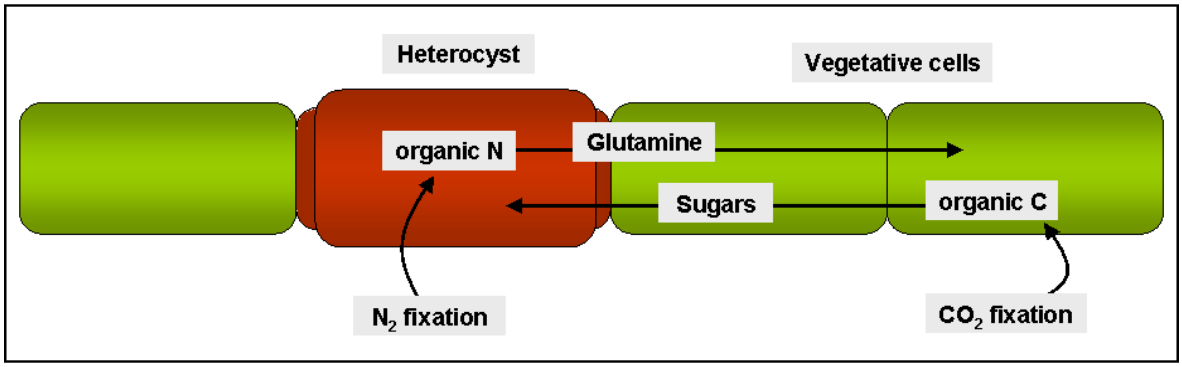


Fig. 5. Scheme of filamentous cyanobacteria showing different types of cells, heterocysts and vegetative cells, including transport directions of organic nutrients and places for N_2 and CO_2 fixation (figure redrawn from Popa et al. 2007).

In this way *Nodularia* sp. and *Aphanizomenon* sp. provide a source of freshly fixed N not only for their own growth but also for non N_2 fixing organisms like picoplankton by facilitating a quick transfer (Ohlendieck et al. 2000, Wannicke et al. 2009). Another study showed that 50 % of reactive available N in the water column is imported by N_2 fixation (Gruber & Galloway 2008).

1.3.5. Adaptation of C acquisition

An efficient and regulated acquisition of dissolved inorganic C (DIC) is a prerequisite for high growth rates and for the ability to form wide spread blooms. But this is not as trivial as proposed. The C fixing enzyme ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) requires CO_2 as substrate. Due to its low affinity to this substrate (K_M > 100 µmol l^{-1}, (Badger et al. 1998)) filamentous cyanoabacteria might suffer from CO_2 limitation at modern CO_2 concentrations of 5 to 25 µmol l^{-1} in surface waters (Wolf-Gladrow et al. 1999). For that reason enhanced CO_2 concentration is assumed to stimulate C fixation by reducing the undersaturation of RuBisCO (Wolf-Gladrow et al. 1999). Based on different structures of RuBisCO two groups of cyanobacteria can be classified, α and β cyanobacteria (Badger et al. 2002) (Fig. 6). These groups developed different kinds of C concentration mechanisms (CCM), which enrich CO_2 at the catalytic site of RuBisCO to circumvent a sub-optimal supply of inorganic C (Badger et al. 1998). CCMs consist of CO_2 uptake systems, DIC transporter, and carboxysomes (Fig. 6). β cyanobacteria posess one CO_2

uptake system more than α cyanobacteria. The different transporters can be either located at the inner cell membrane or thylacoid membrane. So far three bicarbonate (HCO_3^-) transporters (BCT1, SbtA, BicA) and two CO_2 uptake systems (NDH-1_3, NDH-1_4) were identified (Fig. 6). The brackish species *Nodularia* sp. and *Aphanizomenon* sp. belong to the group β cyanobacteria.

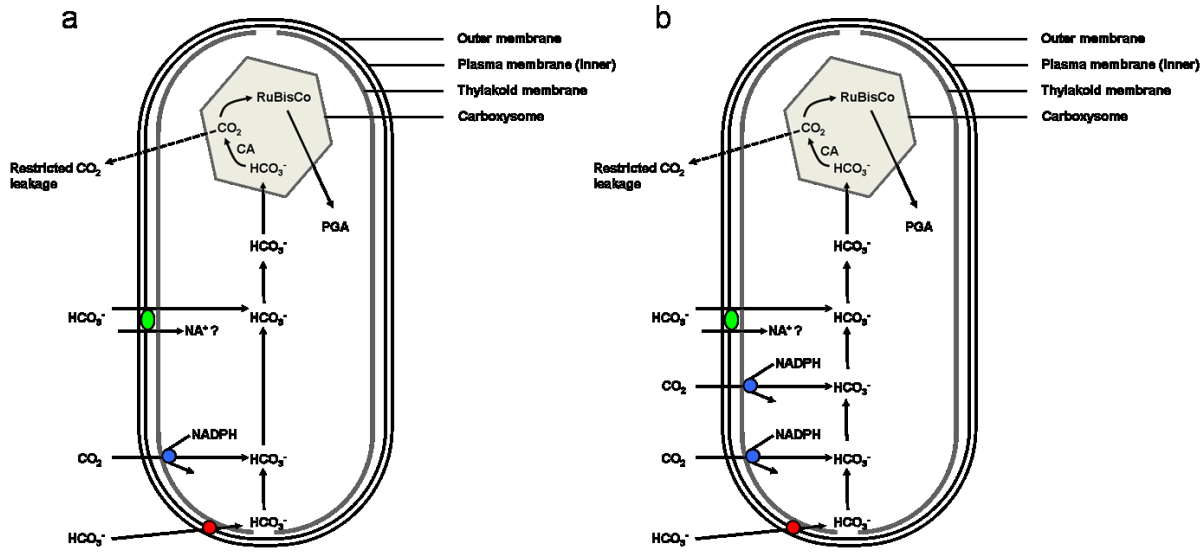


Fig. 6. Carbon concentration mechanisms of a) α cyanobacteria and b) β cyanobacteria. Green – active bicarbonate transporter, blue – CO_2 transporter, red – diffusive bicarbonate transporter, CA – carbonic anhydrase (changed after Price et al. 2008).

The different uptake systems and transporter control different functions within the CCMS. HCO_3^- and CO_2 pass through the outer cell membrane into the peptidoglucan layer. CO_2 diffuses into the cytosol, where NDH-1_3 and NDH-1_4 convert it into HCO_3^- via utilization of NADPH or ferrodoxin. This is necessary to keep the CO_2 concentration within the cells below that one of the surrounding media, to avoid CO_2 diffusion/leakage. The loaded HCO_3^- ions cross the inner membrane by reducing ATP (BCT1) or using the Na^+ gradient (SbtA, BicA). Comparing HCO_3^- concentrations inside and outside the cell, enrichment factors of up to 1000 were measured (Price et al. 2008). The stored HCO_3^- diffuses into the carboxysomes, where the enzyme carbonic anhydrase (CA) accelerates the otherwise slow

interconversion between HCO_3^- and CO_2, which can be used by RuBisCo (Price et al. 2008). It is assumed that parts of different CCMs can be deactivated at high CO_2 concentrations, at which excess energy can be reallocated to N_2 fixation (Barcelos e Ramos et al. 2007, Levitan et al. 2007, Rost et al. 2008). Recent results certified these assumptions and revealed changes in CCM efficiency at increasing CO_2 concentrations and pointed to improved resource allocation between photosynthesis, C and N_2 fixation (Kranz et al. 2009). In conclusion, elevated CO_2 concentrations might reduce the energy needed for protein synthesis to generate DIC transporters and for keeping those transporters working.

1.4. INTERACTION BETWEEN FILAMENTOUS DIAZOTROPHIC CYANOBACTERIA, PICOPLANKTON AND VIRUSES

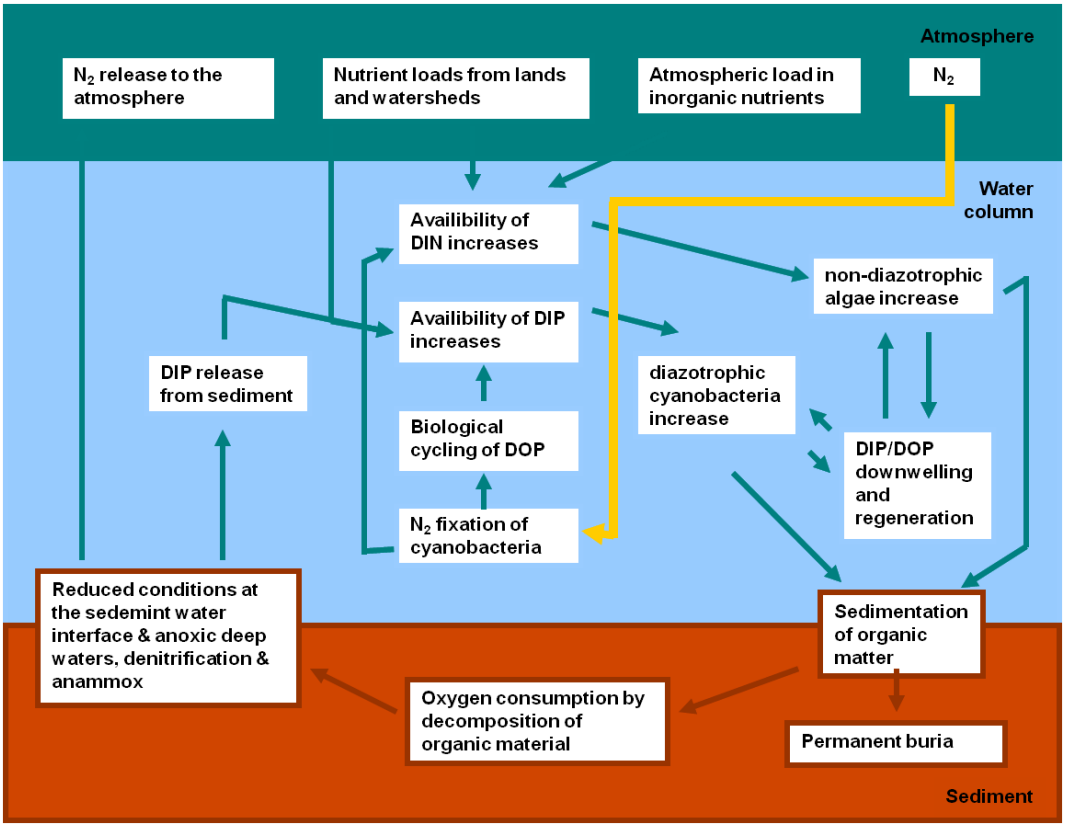


Fig. 7. Interactions and pathways of inorganic and organic nutrients related to filamentous cyanobacteria, influences towards N limited algae inside the water column, the sediment and the atmosphere (figure adapted and modulated from Vahtera et al. 2007a adding sediment information).

Filamentous diazotrophic cyanobacteria interact with other marine and brackish taxa at different trophic levels. The generation of reactive N and the enormous biomass build-up during the bloom emphasize the importance of diazotrophic cyanobacteria for the broader food web (Fig. 7).

The effect on higher trophic levels and nanoplankton species is mainly mediated by secreted bioactive compounds (e.g. Kankaanpää et al. 2002, Suikkanen et al. 2005, Møgelhøj et al. 2006). These impacts are so called direct effects. Despite the toxin production the poor nutritional value of filamentous cyanobacteria is responsible for low grazing activities on these kinds of species by zooplankton (Reinikainen et al. 1994, Koski et al. 1999). But aging and degradation convert the filamentous cyanobacteria into more suitable food (Meyer-Harms et al. 1999). In addition, aggregations with high nutritional food like diatoms, bacteria and flagellates increase the benefit for predators (Engström-Öst et al. 2002). Indirect effects of filamentous cyanobacteria occur as mechanical and visual interference. One example of this is that filamentous cyanobacteria provide ideal conditions as a refuge for small prey (Utne-Palm 2002). In summary, the relations of filamentous cyanobacteria to higher trophic levels are more or less restricted.

First of all, blooms of *N. spumigena* and *Aphanizomenon sp.* offer a rich source of decomposition for picoplankton species (Engström-Öst et al. 2002). As previously mentioned the scums of diazotrophic cyanobacteria are associated with different plankton groups (e.g. flagellates, picocyanobacteria, heterotrophic bacteria and phages or viruses). Colonization of cyanobacterial aggregates by heterotrophic bacteria offers mutual benefits. Largely, the phytoplankton-bacteria interaction is mediated by dissolved organic matter (DOM). Dissolved organic nutrients are resources for heterotrophic growth, introduced into the water column by degradation of diazotrophic cyanobacteria and by the direct release by N_2 fixing cyanobacteria (Hoppe 1981, Capone et al. 1990, Gilbert & Bronk 1994, Nausch et al. 2004). Due to short and fast interrelations, late summer blooms of *N. spumigena* in particular represent a hot spot of microbial activity (Hoppe 1981, Heinänen et al. 1995). Even though a high proportion of the inorganic nutrients, which are released by degradation, are directly taken up by filamentous cyanobacteria, picocyanobacteria

(*Synechoccocus* spp.), heterotrophic bacteria act as effective competitors for nutrients under nutrient depleted conditions (Raven 1987). N and P have been also recognized as elements whose scarcity might restrict bacterial production in some aquatic ecosystems, too (Caron 1994, Kirchman 1994, Elser et al. 1995, Rivkin & Anderson 1997). Therefore algal-bacterial feedbacks vary with changing concentrations of inorganic nutrients. Consequently it is important to combine investigations of autotrophic activities with analyses of heterotrophic plankton to take into account the competition for inorganic nutrients and the release of labile DOM by protists (Johannes 1965, Taylor et al. 1985).

Another component of the plankton community is the group of phages/viruses. It is the most abundant biological entity in marine waters, as their sheer number accounts for about 10^5 to 10^8 particles ml^{-1} (Suttle 2005). Even though viruses were hardly analyzed during the studies presented here, it is important to examine the role of viruses briefly. Viruses act as important agents of mortality for phytoplankton, and they are responsible for the release of bound nutrients and relieve competitive pressure, which can be caused by species domination (Larsen et al. 2001, Martinez et al. 2007). Previous investigations examined a diverse community of *Nodularia*-specific cyanophages in surface waters during summer and autumn months. To date, 17 different hostspecific viruses for *Nodularia* sp. have been identified (Jenkins & Hayes 2006). Nevertheless, artificial conditions, provided during almost every experiment, have been suggested to increase the impact of viruses on autotrophic phytoplankton (Larsen et al. 2008). Thus, an overestimation of the role of viruses for cell lysis and degradation in enclosures compared to the natural environment is likely.

1.5. ANTHROPOGENIC INDUCED ENVIRONMENTAL CHANGES

1.5.1. Acidification of surface waters - Carbonate system

Acidification of surface waters is a result of the CO_2 enrichment in the atmosphere, which contributes to the so called greenhouse effect. In fact natural fluctuations of atmospheric CO_2 concentrations between 180 and 280 μatm coupled with oscillating temperatures were reported for the transition from glacial to

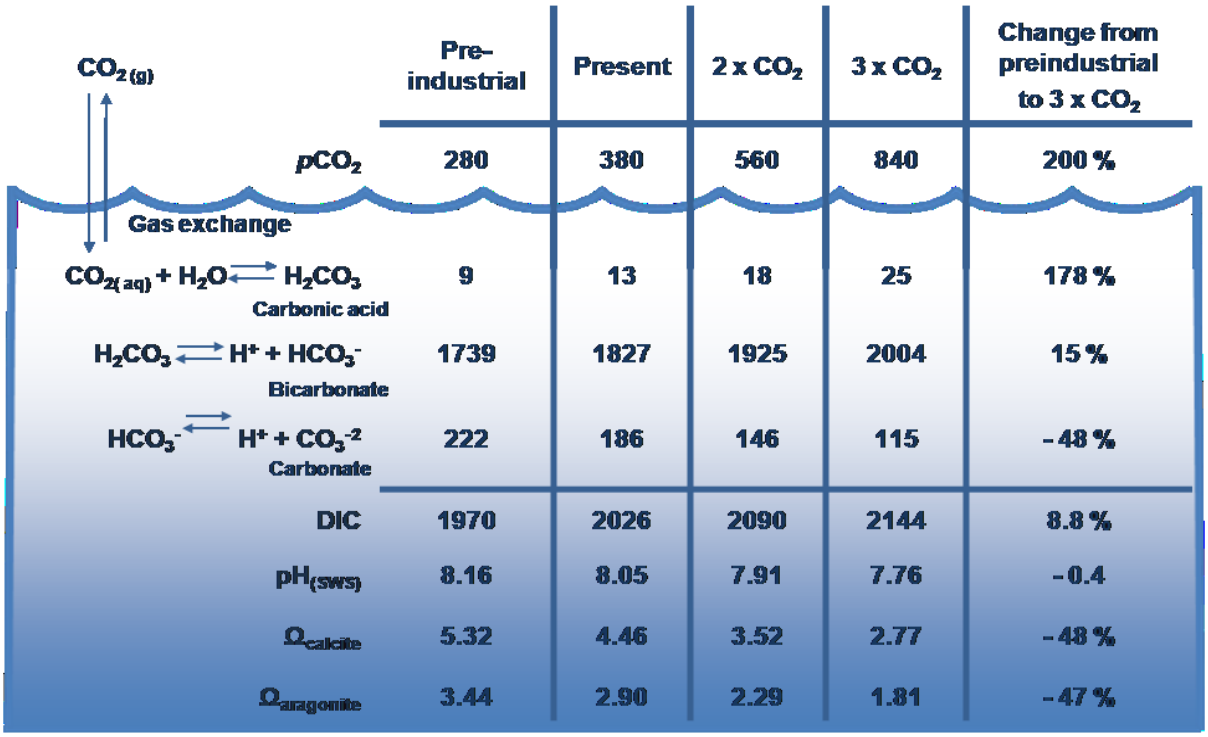
interglacial periods on earth (Siegenthaler et al. 2005). But present concentrations of CO_2 (380 µatm) are significantly higher than preindustrial values and cannot be explained by natural variations. Intensive fossil fuel burning and changing land use induced this remarkable rise (Keeling & Whorf 2005, Forster et al. 2007). Since preindustrial times the atmospheric CO_2 concentration increased about 0.4 % per year. By the year 2100 the atmospheric partial pressure of CO_2 (pCO_2) is predicted to be 700 µatm or even higher under the assumption of "business as usual" (Boer et al. 2001, Alley et al. 2007). The oceans are known to absorb approximately 50 % of this anthropogenically produced CO_2 (Sabine et al. 2004). An alteration of the inorganic carbon buffer system and lowering of ocean pH be the consequences.

But what happens really in the water column. The polar CO_2 molecule is characterized by a high solubility in water. It reacts with water molecules according to the equations described in figure 8. The evolving protons react instantaneously with anions present in seawater. Carbonate (CO_3^{2-}) and HCO_3^-, account for approximately 95 % of the total anions in seawater. The remaining anions like the boric acid anion and hydroxide are found in minor proportions. The value of the total alkalinity (TA) accounts for the sum of protons, which can be neutralized by these anions. The total amount of all carbon species in this equilibrium is defined as DIC.

Since the elevation of pCO_2 is linked with a decreasing pH value rising CO_2 concentrations in the oceans are commonly referred to as ocean acidification. The surface ocean has a great buffering capacity for pH changes, which causes a less drastic decrease of the pH than calculated for unbuffered media. Nevertheless, ocean pH has already decreased about 0.1 units due to the uptake of anthropogenic CO_2 since preindustrial times. The decline is expected to continue by 0.3 to 0.4 units until the end of this century (Meehl et al. 2007). In detail, the increase of pCO_2 will cause elevated carbonic acid (H_2CO_3) and HCO_3^- concentrations and a lower CO_3^{2-} concentration, resulting in reduced calcite and aragonite saturations (Fabry et al. 2008). Enhanced CO_2 concentrations will provoke several feedback effects in the future ocean, for example the buffer capacity will be reduced and this will result in lower CO_2 uptake rates, the decline of the pH value will be enhanced. Additionally, the decrease of the buffering ability of surface waters will be reinforced by global

warming (Friedlingstein et al. 2006). The Revelle factor, which is defined as the ratio of change in pCO_2 per change in DIC versus actual pCO_2 per DIC concentrations (Takahashi et al. 1993), is an ideal indicator defining the buffer capacity of CO_2 uptake.

Even at present conditions the Revelle factor displays enormous differences between the open ocean and the Baltic Sea. In the Baltic Sea natural low values of TA, DIC and salinity cause low values of the Revelle factor, indicating a lower buffering capacity in the Baltic Sea. Nowadays, this reduced buffering ability can be observed by a strong seasonality of the CO_2 concentration. Enhanced CO_2 consumption during the productive summer season is associated with higher pH values > 8 in the Baltic Sea (Thomas & Schneider 1999, Hansen 2002), which antagonizes ocean acidification.



	Pre-industrial	Present	2 x CO_2	3 x CO_2	Change from preindustrial to 3 x CO_2
pCO_2	280	380	560	840	200 %
$CO_{2(aq)} + H_2O \rightleftharpoons H_2CO_3$ Carbonic acid	9	13	18	25	178 %
$H_2CO_3 \rightleftharpoons H^+ + HCO_3^-$ Bicarbonate	1739	1827	1925	2004	15 %
$HCO_3^- \rightleftharpoons H^+ + CO_3^{-2}$ Carbonate	222	186	146	115	-48 %
DIC	1970	2026	2090	2144	8.8 %
$pH_{(sws)}$	8.16	8.05	7.91	7.76	-0.4
$\Omega_{calcite}$	5.32	4.46	3.52	2.77	-48 %
$\Omega_{aragonite}$	3.44	2.90	2.29	1.81	-47 %

Fig. 8. Effects of different atmospheric CO_2 concentrations (preindustrial, present, enhanced: twice of preindustrial conditions, three times of preindustrial conditions), with respect to shifts in ionic composition and altered pH, as well as changes of calcite and aragonite saturations (Ω) in surface waters of the open ocean. Used units: pCO_2 - µatm, CO_2, HCO_3^-, CO_3^{2-} in µmol kg^{-1}. Figure adapted from Fabry et al. 2008.

1.5.2. Acidification of surface waters - Organismic response

With regard to these considerable impacts of elevated CO_2 concentrations on marine ecosystems the objective of several studies was to reveal detailed species specific responses in the changing environment (Tortell et al. 2002, Barcelos e Ramos et al. 2007, Levitan et al. 2007, Riebesell et al. 2007, Czerny et al. 2009). It is presumed that changes in CO_2 and pH may exert strong influences on algal physiology, marine nutrient dynamics and ecological interactions (Fu et al. 2008). Previous phytoplankton investigations mainly concentrated on coccolithophorids and diatoms affected by ocean acidification (Tortell et al. 2002, Sciandra et al. 2003, Riebesell 2004, Rost & Riebesell 2004, Delille et al. 2005, Iglesias-Rodriguez et al. 2008). Due to the expected declines of calcite and aragonite saturations especially coccolithophorids were in the focus of several reports (Fabry et al. 2008).

In addition, elevated activities, concerning the direct utilization of CO_2 by non calcifying autotrophic bacteria and eukaryotic algae during photosynthesis, are estimated. They indicate elevated growth in acidified water bodies (e.g. Barcelos e Ramos et al. 2007, Levitan et al. 2007, Riebesell et al. 2007, Iglesias-Rodriguez et al. 2008). Nevertheless, species specific reduced or elevated autotrophic activities, indicate that increasing CO_2 concentrations will alter interspecies activities and species composition (Barcelos e Ramos et al. 2007, Hutchins et al. 2007). Even though the investigation of different phytoplankton and their response to elevated CO_2 concentrations is intensified, culture experiments dealing with filamentous cyanobacteria are scarce. Czerny et al. (2009) showed decreasing N_2 fixation and cell division rates, which is not in line with investigations of the subtropical species *Trichodesmium* sp. (Barcelos e Ramos et al. 2007, Levitan et al. 2007, Riebesell et al. 2007). Former studies investigating contemporary filamentous cyanobacteria in the Baltic Sea showed that adaptations towards rising CO_2 concentrations already exist (Ibelings & Maberly 1998, Lass et al. 2010). Upwelling processes go along with enhanced CO_2 concentrations and enhanced nutrient conditions. Therefore, areas with upwelling might be used for investigations examining impacts of these changing environmental parameters.

1.5.3. Effects of elevated water temperatures and nutrient availability

Elevated temperatures, also predicted for the future ocean, will change modern ocean currents and reduce the circulation within the water column. Increasing temperatures in the atmosphere and therefore also in the surface water of the oceans are described as a direct consequence of elevated atmospheric pCO_2. In the Baltic Sea the annual warming is predicted to be 0.08 °C. This exceeds estimates for the entire globe, caused by the enormous direct anthrophogenic impacts and due to the low buffering capacity of the water column in the Baltic Sea. The increasing temperature in the water column might result in a prolonging of the growing season, which is defined with a threshold temperature of 5 °C (Leppäkoski et al. 2002). Obviously this also affects the nutrient availability. The responses of filamentous cyanobacteria growth to climate change will probably depend on the available phosphorus pool in the future. Nowadays the main sources for P in the Baltic Sea are discharge by rivers and release by anoxic sediments (Savchuk 2005). Concerning predictions within the scope of CO_2 enhancement, the physical exchange of surface water with dissolved inorganic phosphorus (DIP) rich deep waters and changing sequestration by the sediments has to be taken into account (Conley et al. 2002). In addition the DIP concentration is affected by nutrient uptake rates, transfer and settling velocities, as well as species composition of primary producers. All these factors might change in the future. Contradicting predictions reported for future nutrient concentrations in the Baltic Sea make it difficult to obtain prospects concerning future conditions (Bergström et al. 2001, Conley et al. 2002, Graham 2004). By now observations of upwelling events show that CO_2 together with DIP might prolong the growth period and increase aggregate development of filamentous cyanobacteria in the Baltic Sea (Ibelings & Maberly 1998, Nausch et al. 2009, Lass et al. 2010).

Climate change is definitely taking place. Besides the mentioned primary changes, secondary effects will occur, causing e.g. melting of the polar ice caps and as a secondary effect the sea level will rise (Christoffersen & Hambrey 2006, Otto-Bliesner et al. 2006). Forster & Taylor (2006) calculated that these effects can be accredited to human activity with a certainty of more than 90 %. On the other hand climate change is predicted to increase the albedo effect, which will result in reduced

autotrophic production (e.g. Rosenzweig et al. 2008). Therefore, further wide spread impacts on autotrophic growth and shifts in species composition are anticipated.

1.6. THESIS OUTLINE

Former experiments mainly concentrated on calcifying phytoplankton species and diatoms. It was demonstrated that acidified conditions influence photosynthesis, calcification, as well as elemental composition of marine phytoplankton, and might also affect phytoplankton species composition and succession (Tortell et al. 2002, Riebesell 2004, Hare et al. 2007). Detailed information for diazotrophic cyanobacteria common in the Baltic Sea was missing so far. Thus, the goal of this dissertation was the investigation of filamentous diazotrophic cyanobacteria and their response to enhanced CO_2 concentrations in the surface waters of the Baltic Sea. Observations of diurnal differences of metabolic activities inside a cyanobacteria bloom were accomplished to provide information about the natural variability during the day. Hence, it was possible to compare the extent and the directions of natural versus human induced changes within the diazotrophic bloom.

All studies were done under conditions simulating those occurring in the ecosystem where interactions between autotrophic and heterotrophic organisms take place. According to the diurnal observations, laboratory and field experiments evaluated the development of the diazotrophic cyanobacteria *Aphanizomenon* sp. and *N. spumigena* with respect to nutrient limitations and enhanced CO_2 concentrations as well as of activities of the associated heterotrophic picoplankton. All experimental setups described here allowed conclusions to be drawn regarding the reactions of heterotrophic picoplankton as well.

The experiments included nutrient uptake rates by different size classes. It was shown that growth and development of diazotrophic cyanobacteria might be primarily driven by DIP availability. DIP concentrations affected the impact of changing CO_2 levels and diurnal variations within a cyanobacterial bloom. Therefore, analyses of the examined data concentrated on the impacts of natural and

anthropogenic induced changes on the interaction between different nutrient incorporations.

The obtained results demonstrated distinct differences between autotrophic and heterotrophic responses. Furthermore, investigating different stages of a summer phytoplankton bloom indicated that the strong DIP dependency predominates against the advantages of CO_2 enrichment for filamentous cyanobacteria. Nevertheless, it can be assumed that increasing autotrophic growth at high CO_2 concentrations probably also promoted heterotrophic production indirectly. Further implications on nutrient cycling and power of the biological pump were suggested. The findings presented here might contribute to predictions concerning impacts of increasing CO_2 concentrations and changing DIP availability on the ecosystem of the Baltic Sea.

2. MATERIAL AND METHODS

The investigations conducted during this dissertation included three experimental campaigns. All three studies concentrated on autotrophic processes of filamentous diazotrophic cyanobacteria. We present observations of diurnal cycles, describing diurnal variations of different nutrient incorporation rates, related to changes in abiotic and biotic parameters. Furtherance during a laboratory experiment the influence of enhanced CO_2 concentrations on two filamentous cyanobacteria, *Aphanizomenon* sp. and *N. spumigena* was studied. Similar impacts were investigated during the third study, but this time a natural cyanobacterial bloom was affected. Partly the observations included the reactions of the associated nano- and picoplankton. Slightly different procedures during the measurements are specified in the following paragraphs.

2.1. SITE DESCRIPTION AND SAMPLING FOR DIURNAL OBSERVATIONS (CHAPTER 3.1.)

In 2008 diurnal observations were conducted in the Southern Gotland Sea in the Baltic Proper (Table 1; Fig. 9). The stations, located close to the polish coast, were about 25 to 30 m deep. A shift in temperature of approximately 4 °C between 20 and 22 m depth indicated a thermocline. A significant difference in temperature and salinity between the different samplings and stations could not be detected (Table 1).

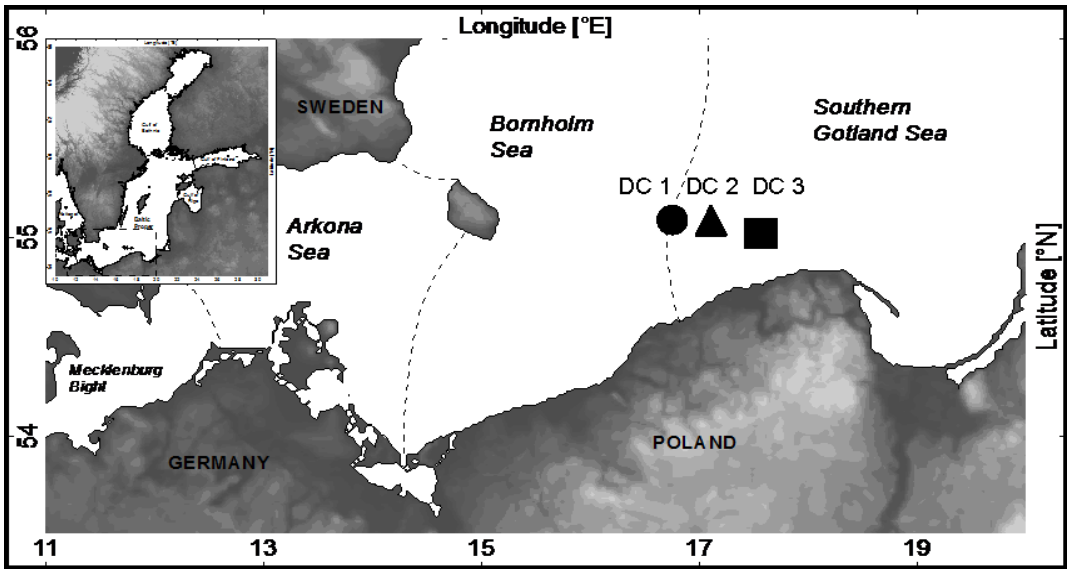


Fig. 9. Baltic Sea Proper, showing the sub-basins and stations of the daily cycles DC 1, DC 2 and DC 3 in the **Southern Gotland Sea. Location numbers correspond to those in table 1.**

Table 1. Hydrographical conditions during water sampling for the three daily cycles (DC 1, DC 2 and DC 3) in July 2008. Samples were taken every 4 h. (lat. – latitude, long. – longitude, T – temperature, S – salinity, pCO2 – partial pressure CO2, v- speed, G – global radiation).

	date	lat.; long.	T [°C]	S [PSU]	pCO_2 [µatm]	mean wind v [m s^{-1}]	mean G [W m^{-2}]
DC 1	12 – 13	55°07; 16°8	17.81 ± 0.12	7.44 ± 0.04	159 ± 11	5.5	198.1
DC 2	15 – 16	55°06; 17°09	17.62 ± 0.11	7.39 ± 0.01	174 ± 9	9.4	167.9
DC 3	18 – 19	55°04; 17°48	17.80 ± 0.12	7.37 ± 0.02	155 ± 6	5.04	192.6

A comprehensive measurement program was carried out during a cruise with the R/V Heincke in July 2008. Samples were taken from 3 m depth in 4 h intervals over three daily cycles (DC 1 – July 12th-13th; DC 2 – July 15th-16th; DC 3 July 18th-19th, starting at 6 a.m or 10 a.m. in the morning until the following morning. Temperature and salinity were recorded using a CTD probe (Seabird 911) combined with a HydroBios rosette sampler containing twelve 5 l bottles. The gained samples

were used for chemical and biological analysis. Global radiation and wind speed were obtained from ship board sensors summarized by the DAVIS-SHIP system.

2.2. SETUP OF LABORATORY EXPERIMENTS (CHAPTER 3.2.)

Axenic strains of the diazotrophic cyanobacteria *Aphanizomenon sp.*, isolated by S. Busch (IOW) and *N. spumigena*, isolated by L. Stal and coworkers from blooms in the Baltic Sea, were grown in liquid BA$^-$ growth medium (1/3 ASN-III and 2/3 BG-11 media) without additional DIN. The experiment was done twice, experiment A and experiment B. Abiotic conditions were set at a water temperature of 18 °C, a salinity of 10 PSU and a 16 h : 8 h light:dark cycle with an irradiance of 100 µmol photons m^{-2} s^{-1} during the light phase.

For the incubations, winter seawater (SW) was obtained at a monitoring station located along the Baltic Sea coast (54°8.55'N, 11°50.6'E Heiligendamm, Germany). Aliquots of the cyanobacterial strains containing ~5, respectively ~10 µg chlorophyll *a* (Chl*a*) l^{-1} were transferred to acid-washed 2300 ml Schott Duran bottles, which were filled with GF/C-filtered (pore size 1.2 µm) seawater, containing picoplankton. Starting concentrations of 1.1 to 1.6 µmol l^{-1} DIP avoided growth limitations by P. The natural not altered nitrate (NO_3^-) concentration was approximately 4.3 µmol l^{-1}. Two days before the experiment was started, the two axenic strains were transferred from nutrient repleted media into this seawater to allow an adaptation to the environment.

The experiments consisted of seven different CO_2 treatments. One treatment was left without any CO_2 addition. It had the CO_2 concentration as found at the onset of a cyanobacterial bloom in the Baltic Sea (Thomas & Schneider 1999). The remaining six bioassay samples were exposed to pCO_2 at six increasing concentrations (Table 1). These were established by calculating amounts of pure CO_2 gas (Air Liquide Deutschland GmbH), using the computer program CO2Sys (Lewis & Wallace 1998), which were then injected through a gas-tight septum to yield final concentrations between 280 and 780 µatm pCO_2. In parallel with the experiment, a control bottle, without any CO_2 amendment, was used to follow the growth of the

filamentous cyanobacteria in order to define the growth phase in which the parameter were measured. Chl*a* analyses and cell counts were conducted to determine autotrophic growth. At day 4 the community reached the end of the exponential growth phase (Fig. 10).

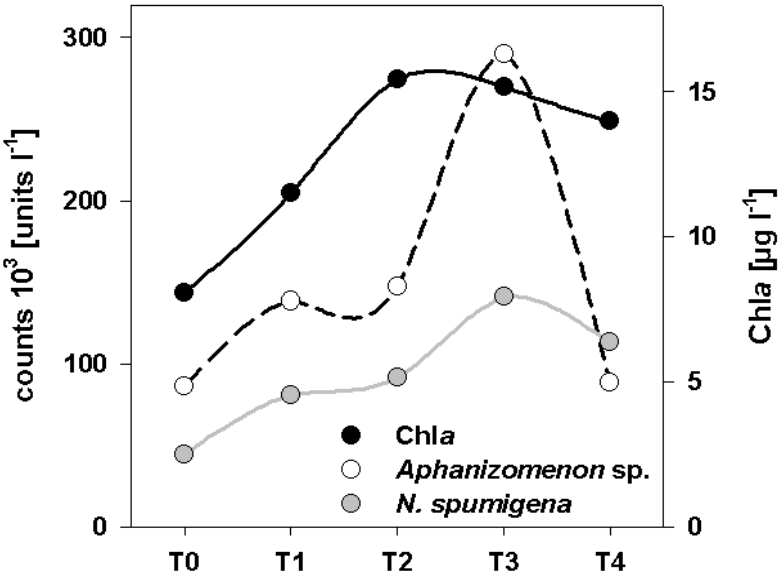


Fig. 10. Growth of filamentous cyanobacteria during 4 days of incubation (T0 to T4). Bulk Chl*a* concentration (black circles, solid line); *Aphanizomenon* sp. cell counts (open circles, dashed line), *N. spumigena* cell counts (grey circles, grey line).

Additionally two short term experiments, experiment C and experiment D, were conducted. Here, 300 ml portions of the CO_2 acclimated autotrophic community, obtained from the experiment B, were exposed to a gradient of slightly different CO_2 concentrations (250 – 950 µatm). The incubation period for experiment C and experiment D were four hours.

2.3. SETUP OF EXPERIMENTS WITH A NATURAL COMMUNITY (CHAPTER 3.3)

These investigations were conducted on board of the R/V Alkor during a cruise at the central Baltic Sea in July 2009. Water was taken at 58°35.0503' N, 18°13.9769.6'

E (Fig. 10), using a HydroBios rosette sampler with twelve 5 l bottles. Light, temperature and salinity were recorded by a CTD probe (Seabird 911). 50 l seawater were filtered through GF/C filters (pore size approximately 1.2 µm). The plankton community in the filtrate comprised autotrophic picoplankton, free heterotrophic bacteria and viruses or phages, checked by flow cytometry afterwards.

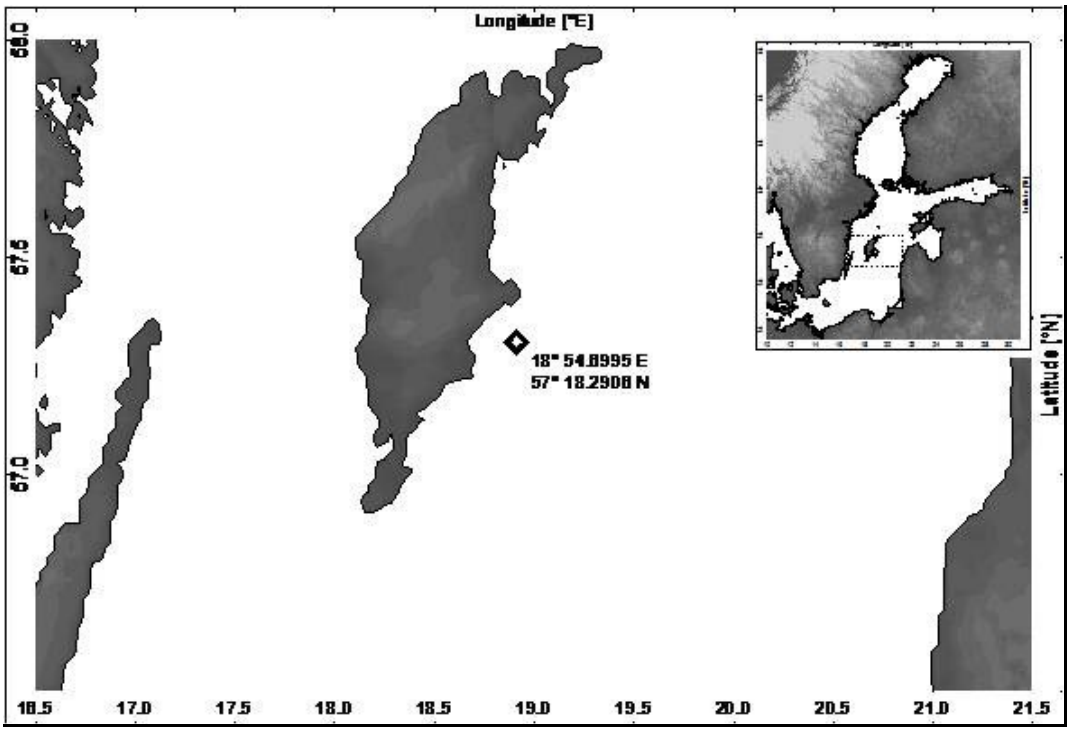


Fig. 11. Map of the Baltic Sea, square refers to the sampling station in 2009.

Filamentous cyanobacteria were collected from the surface (0 – 1 m) with a WP2 plankton net (100 µm mesh size). To eliminate zooplankton, the samples were resuspended in approx. 1.9 l GF/C-filtered seawater in 2 l polycarbonate bottles. The upper part, about two third, of each bottle was covered with black wrapping for 2 to 3 hours. Zooplankton is known to be positive phototactic (e.g. Forward 1988). Therefore a phytoplankton community without grazers could be sampled at the water surface inside the bottle, while the zooplankton moved towards the illuminated bottom. Subsequently this was checked by microscopy.

Aliquots of the isolated cyanobacteria (~20 µg Chl*a* l^{-1}) were transferred into 21 precleaned (1 mol l^{-1} HCl) 2.3 l glass bottles (Schott). Each bottle was filled up with 2 l GF/C-filtered seawater.

The experiment comprised six different CO_2 treatments in triplicates. One treatment served as a control and was kept at *in situ* CO_2 concentrations (210 µatm) without any CO_2 addition, while the remaining five treatments were exposed to an increasing pCO_2 (Table 3). For establishing the five increasing CO_2 concentrations calculated amounts (using the computer program "CO2sys" (Lewis & Wallace 1998) of pure CO_2 gas (Air Liquide Deutschland GmbH) were injected through a gas-tight septum to reach final CO_2 concentrations of 300, 450, 600, 800 and 1000 µatm. The bottles were incubated in a deck incubator at sea surface temperature. Neutral density screens provided 50 % of surface radiation. Light intensity was measured in the 400 - 700 nm range (photosynthetically available radiation, PAR) using a spherical quantum sensor (QSL-101, Biospherical Instruments, San Diego, CA).

The initial conditions (T0) were determined from three untreated bottles harvested immediately after filling (Table 2). The sampling of the 6 different treatments (18 bottles) took place after one day (T1), four (T4) and six (T6) days of incubation.

2.4. DISSOLVED INORGANIC NUTRIENTS

Filtered water samples (pre-combusted Whatman GF/F) were used for DIP, silicate (SiO_4^{2-}), nitrite (NO_2^-) and NO_3^- measurements carried out in an autoanalyser system ("Evolution III") using standard colorimetric methods (Grasshoff et al. 1983). The detection limit was 0.02 µmol l^{-1} for DIP, 0.1 µmol l^{-1} for SiO_4^{4-} and 0.02 µmol l^{-1} for NO_2^- and NO_3^-.

Investigations presented in chapter 3.2 incluced analyses of NH_4^+ concentrations. These were measured by an indophenol method, with a precision of 0.1 µmol l^{-1}, according to Grasshoff et al. (1983).

2.5. DISSOLVED AND PARTICULATE ORGANIC PHOSPHORUS

In general the analyses of DOP followed one protocol during all experiments and investigations. Briefly for the determination of DOP, 40 ml of water was filtered through a 0.2 μm cellulose acetate filter and stored at –20 °C until further processing. The thawed samples were oxidized with potassium peroxydisulfate in an alkaline medium, using a laboratory microwave (Grasshoff et al. 1983). Measurements were made in duplicate from each sampling. DOP was calculated as the difference between the total P concentration in the 0.2 μm filtered water and the corresponding DIP concentration.

For POP measurements differed slightly regarding the investigations of different phytoplankton groups during the specific experiment. Results presented in chapter 3.1 were obtained with samples of 40 ml of unfiltered sample water and < 10 μm filtered samples, stored at -20 °C. The samples were analyzed using the method described above. POP concentration for the total community was calculated as the difference between the P concentrations in the non-filtered water and the 0.2 μm filtered water, and POP concentration for the > 10 μm fraction as the difference between the P concentrations in the non-filtered water and the 10 μm prefiltered water.

POP measurements presentend in chapter 3.2 were not fractionated. The POP values are the difference between the unfiltered sample and the 0.2 μm filtered sample.

For fractionated POP determinations presented in chapter 3.3 two 40 ml aliquots of unfiltered sample water and two 40 ml aliquots of water filtered through 2.7 μm pore size polycarbonate filters were stored at -20 °C. The phosphorus content of filamentous cyanobacteria is the difference between the phosphorus concentrations of the unfiltered seawater and the 2.7 μm filtered seawater. The phosphorus content of picoplankton was calculated by subtracting the phosphorus concentration in 0.2 μm filtered water from that in 2.7 μm filtered one. The analytical precision for DOP and POP measurements was 0.2 μmol l^{-1}.

2.6. DETERMINATION OF THE DISSOLVED INORGANIC CARBON SYSTEM

During the diurnal observations, presented in chapter 3.1, the partial pressure of carbon dioxide (pCO_2) was determined. pCO_2 was measured by non-dispersive infrared spectrometry of the altered seawater before the experiment was started. pCO_2 measurements were corrected to the *in-situ* temperature and to 100 % humidity (Körtzinger et al. 1996). The error in the estimated method accounted for approximately ± 1 µatm.

During the acidification experiments (chapter 3.2 and 3.3) the carbonate system was examined by the pH and pCO_2 values using the program CO2Sys (Lewis & Wallace 1998). The equilibrium constants of Mehrbach et al. (1973), refitted by Dickson & Millero (1987), were chosen. The pCO_2 was determined by non-dispersive infrared spectrometry of the altered seawater before the experiment was started. pCO_2 measurements were corrected to the *in-situ* temperature and to 100 % humidity (Körtzinger et al. 1996). The error in the estimated method accounted for approximately ± 1 µatm. The pH measurements were obtained with a pH meter („Knick Mikroprozessor" 761), using a combined glass/ reference electrode type SE 100. Before and after the incubations, the carbonate system was assessed by measuring pH and pCO_2. Partly also DIC was determined by the coulometric method according to Johnson et al. (1993).

2.7. ENZYME EFFICIENCY

Enzyme efficiency of the alkaline phosphatase was measured by using the substrate MUF-Phosphate. We measured time and enzyme kinetics (duration: 30 min; frequency 3 min; concentrations: 0.8, 1.5, 3.0, 7.5, 15.0, 30.0, 112.5, 225.0 µM final; excitation wavelength: 335 nm; emission wavelength: 460 nm). The fluorescence of the sample was corrected for a blank. The detected intensity increase over time was used for the calculation of the enzyme efficiency ($V_{max}\ K_m^{-1}$) by using a hyperbola fit (Michaelis Menten, Sigma Plot 10.0). Flurorescence units were calibrated using MUF standard solutions in Baltic seawater.

2.8. RATE MEASUREMENTS

The ^{15}N and ^{13}C tracer techniques were applied to determine N_2 fixation rates and primary production (Montoya et al. 1996). For $^{15}N_2$ fixation and ^{13}C incorporation, two replicate subsamplings were filled bubble-free into 660 ml incubation bottles and spiked with 0.57 ml $^{15}N_2$ (99 % $^{15}N_2$, Campro Scientific) via a gas-tight septum and 0.106 ml of a 0.1 mol l^{-1} ^{13}C labelled bicarbonate solution (99 % $H^{13}CO_3^{2-}$). The bottles were incubated under *in-situ* light and temperature conditions. During the experiments described in chapter 3.1 and 3.3 bottles were incubated in a deck incubator at sea surface temperature. Neutral density screens provided *in situ* light levels (50 %). After four hours of incubation, samples were gently vacuum filtered through pre-combusted Whatman GF/F (pore size 0.8 µm), 10 µm gauze (chapter 3.1) and GF/D filters (pore size 2.7 µm) (chapter 3.3) respectively, to divide between size class specific reactions. The filters were stored frozen until analysis. Stable nitrogen and C isotope ratios ($\delta^{15}N$-PON, δ^{13}POC) as well as PON and POC concentrations were measured by means of flash combustion in a Carlo Erba EA 1108 at 1020 °C in a Thermo Finnigan Delta S mass-spectrometer. Filters were trimmed, sectioned and loaded into tin capsules and pelletized for isotopic analysis. After measuring stable N and C isotope ratios for each sample, the values were corrected against standards with defined N and C element and isotopic compositions (International Atomic Energy Agency [IAEA]: IAEA-N1, IAEA-N2, NBS22 and IAEA-CH-6). Values are reported relative to atmospheric dinitrogen ($\delta^{15}N$) and Vienna PeeDee Belemnite (VDPDB, $\delta^{13}C$). The analytical precision for both stable isotope ratios was ± 0.2 ‰. Previous comparisons of photosynthetic rates determined by the ^{13}C or ^{14}C method showed good agreements between the two methods (e.g. Legendre & Gosselin 1997).

For N_2 and carbon fixation measurements in experiment D (chapter 3.2), 1.950 ml $^{15}N_2$ (99 % $^{15}N_2$, Campro Scientific) and 0.357 ml of a 0.1 mol l^{-1} ^{13}C-labelled bicarbonate solution (99 % $H^{13}CO_3^{2-}$) were directly injected into the acidified bioassays. Additionally NO_3^- uptake rates were examined for experiment C, described in chapter 3.2. In this case, each bioassay (seven different CO_2 settings) was directly spiked with 69.7 µl of ^{15}N-$NaNO_3$ (0.01 mol l^{-1}, 99 %) and 0.357 ml of a 0.1 mol l^{-1}

^{13}C-labelled bicarbonate solution (99 % $H^{13}CO_3^{2-}$) for detecting primary production measurements.

For DIP uptake, 60 ml samples in polycarbonate bottles were spiked with [^{33}P]PO_4 (Hartmann Analytics) at a specific activity of 111 TBq mmol^{-1} to yield a 50 pmol l^{-1} ^{33}P final concentration. To suppress biological uptake, formaldehyde was added to the blank samples before spiking with the radiotracer. During the incubation time of 2 h, subsamples of 5 ml were taken at time intervals: 5 min, 15 min, 30 min, 60 min and 120 min. [^{33}P]PO_4 incorporation was directly stopped by filtration onto 0.22 μm, 10 μm (chapter 3.1) or 2.7 μm (chapter 3.3) polycarbonate filters (Millipore) pre-soaked with carrier free KH_2PO_4 (Thingstad et al. 1993). Counting proceeded in a liquid scintillation counter (TriCarb 1600 TR) using Lumasafe Plus (Packard) as scintillation cocktail. All analyses were at least performed in duplicate. The [^{33}P]PO_4 uptake rate was determined from the straight slope of radioactivity incorporated into particulate matter versus incubation time. The phosphate uptake rate (P uptake) was derived from the [^{33}P]PO_4 uptake rate and the ambient DIP concentrations. Specific DIP affinities (α) were calculated using the following equations (1) and (2) (T_t = turnover time, t = incubation time, r = consumed fraction of added [^{33}P]PO_4, and B = POP) (Thingstad et al. 1993, Tanaka et al. 2006):

$$T_t = -\frac{t}{\ln(1 - r(t))} \qquad (1)$$

$$\alpha = \frac{1}{T_t B} \qquad (2)$$

During the diurnal observations in 2008 (chapter 3.1.) heterotrophic bacterial biomass production and cell division were analyzed by the incorporation of ^{3}[H]-leucine and ^{3}[H]-thymidine respectively, using the microcentrifugation method. Briefly, triplicate 1.5 ml samples and a prefixed blank were incubated at the *in-situ* temperature in the dark or 1 h. One set of samples was amended with ^{3}H-Leu (5.29 x 10^{12} Bq mmol^{-1}; Moravek Biochemicals, Inc., California, USA, final

concentration 25 nmol l^{-1}) and a second set with ^{3}H-TdR (2.52 x 10^{12} Bq $mmol^{-1}$; Moravek Biochemicals, Inc., California, USA, final concentration 25 nmol l^{-1}). Incubations were stopped by the addition of formaldehyde (8 % final concentration) buffered with 4 % (w/v) boric acid. After 15 min of fixation, the samples were centrifuged with 6240 × *g* at 4°C for 10 min and the supernatants gently removed by suction. Pellets were resuspended in ice-cold 5 % trichloroacetic acid (TCA), centrifuged at 4°C for 10 min, and aspirated again. Leucine samples were additionally washed with 80 % ethanol, centrifuged at 4°C for 10 min, and aspirated again. Finally, the samples were dissolved in 1.5 ml of scintillation cocktail (Ultima Gold, PerkinElmer) and kept refrigerated until radio-assay analysis (within 4 weeks) using a TriCarb (1600 TR) liquid scintillation counter.

During 2009 (chapter 3.3) heterotrophic bacterial production was analyzed by incorporation of ^{3}H-TdR, too. After spiking with the radioactive isotope, triplicate samples (10 ml) were incubated for at least one hour at *in situ* temperature in the dark. A fourth sample, serving as a blank, was fixed for at least 10 min prior to the addition of the radioactively labeled substrates. Incorporation was stopped with adding formaldehyde (10 % v/w). Afterwards the samples were stored in the dark overnight at 5 °C. The next day, samples were filtered on polycarbonate filters (pore size 0.22 µm) (Millipore). The scintillation cocktail Lumasafe Plus (Packard) was added to the filters. Counts per minute (CPM) of the radiotracer were counted in a liquid scintillation counter (TriCarb 1600 TR). In consideration of the quench corrections of the CPMs, disintegrations per minute (DPM) were automatically calculated. DPM values were used to analyse heterotrophic activity.

2.9. PLANKTON COMPOSITION

For the estimation of the phytoplankton composition, 100 ml subsamples were preserved with acidic Lugol's iodine solution (final concentration 0.5 %). The samples were counted using an inverted microscope according to Utermöhl (1958) and determination of the phytoplankton biomass was performed according HELCOM COMBINE recommendations (HELCOM 2001). Each colony and unicellular

organism was defined as one unit. In the filamentous form the length of 100 μm was considered as one unit.

Picoplankton samples were preserved with glutardialdehyde (GDA) (4 % v/v final concentration), shock frozen in liquid nitrogen, and stored at -80 °C until enumeration by flow cytometry (Gasol et al. 2004). The samples were counted in a "FacsCalibur" (Becton Dickinson) flow cytometer equipped with a laser emitting at 488 nm after adding. Fluoresbrite microspheres (latex beads) (Polysciences) served as the internal volume calibration standard. Heterotrophic bacteria and phages (analyzed only during summer 2009) were stained with SYBR-GreenI (Molecular Probes) (2.5 μmol l^{-1}, 0.5 μmol l^{-1} final concentration). The samples of heterotrophic bacteria and phages were analyzed for 180 s at a high flow rate (105 μl min^{-1}). Bacteria were detected by their signature in a plot of side scatter (SSC) versus green fluorescence (FL1). During the same measurement, using the same protocol, also phages were detected and counted. Analyses of autotrophic picoplankton were made without staining and at medium speed (35 μl min^{-1}). Detection was performed by the signature in a plot of orange (FL2) versus red fluorescence (FL3). Calculations for the enumeration of picocyanobacteria, heterotrophic bacteria and phages relied on the flow rate.

Chl*a* concentrations were measured for the whole phytoplankton community as well as for various fractions: > 10 μm (carefully rinsed back from a 10 μm gauze on a GF/F filter) (chapter 3.1), > 2.7μm (GF/D filters, pore size 2.7 μm) (chapter 3.3) and, the total community (GF/F filter pore size 0.8 μm). Samples were stored at -80 °C until further procession. Chl*a* was extracted in 10 ml 96 % ethanol (three hours), analysed (excitation 450 nm, emission 670 nm) (Wasmund et al. 2006) using a Turner-AV 100 fluorometer, and calculated after correction for phaeopigments (Holm-Hansen et al. 1965, UNESCO 1994).

2.10. DISSOLVED ORGANIC NITROGEN AND DISSOLVED ORGANIC CARBON

Two different methods were used to determine dissolved organic nitrogen (DON) in laboratory and field experiments. Whereas dissolved organic carbon

(DOC) was determined only in the laboratory experiment. Following methods were applied for the laboratory incubations. Total dissolved nitrogen (TDN) and DOC concentrations were determined simultaneously in GF/F-filtered water samples by high-temperature catalytic oxidation with a Shimadzu TOC-VCPN analyzer. A sample volume of 6 ml (in pre-combusted vials) was acidified with 0.12 ml HCl (2 mol l^{-1}) and sparked with O_2 to remove inorganic C. A 50 µl sample was then directly injected onto the catalyst (heated to 680 °C) and the CO_2 thus generated detected with an infrared detector. Final DOC concentrations were averaged from triplicate measurements. Total N was quantified using a chemiluminescence detector (gas flow O_2: 0.6 l min^{-1}). Every six samples, one blank and one standard were measured to guarantee the precision (2 µmol l^{-1}) and quality of the measurements. The DON concentration was calculated by subtracting the DIN concentration from the total dissolved nitrogen (TDN) content.

Concentrations of the TDN during the incubation experiments in summer 2009 were determined using the persulfate oxidation method (Grasshoff et al. 1983, Knapp et al. 2005). For the oxidation of TDN to NO_3^-, persulfate oxidising reagent (POR) was added to the sample and filled into a Teflon bottle. All samples were treated for 60 min in a MarsXPress (CEM) digestion microwave oven following an application for acid digestion. To determine the reagent blank, each batch of samples included a Teflon bottle with deionised water and POR. Concentrations of nitrate and nitrite after oxidation were determined using standard colorimetric techniques (Grasshoff et al. 1983). In this study the DON concentrations were calculated by subtracting the concentrations of NO_3^- and NO_2^-, determined before digestion, from the total nitrogen concentration of the persulfate digested samples. Due to missing measurements of ammonia, values of DON concentrations include ammonia. The reagent blank was always < 2 µmol l^{-1}. Reagent blanks were subtracted.

2.11. STATISTICAL ANALYSES

The statistical significance was tested by the Student's *t*-test (Sokal & Rohlf 1995) using the computer program 'Sigma Plot 10'. The statistical significance levels

were $p > 0.1$– not significant; $p \leq 0.1$ – significant; $p \leq 0.05$ – high significant; $p \leq 0.01$ highly significant.

3. RESULTS AND DISCUSSION

3.1. DIURNAL PATTERNS INSIDE A SUMMER PHYTOPLANKTON BLOOM IN THE BALTIC SEA – NATURAL VARIABILITY

3.1.1. Results

Abiotic environmental conditions and community composition

In summer 2008, investigations comprising three daily cycles (DC) were conducted in the Baltic Sea at sites closely located to the shore in the south (Fig. 8). Comparing the daily cycles no remarkable changes of hydrographical environmental factors like temperature and salinity were detected (Table 1). Slightly higher CO_2 concentrations of 174 µatm were measured in the water column during DC 2 compared to 155 µatm at DC 1 and 159 µatm at DC 3. Higher wind speeds at DC 2 than at DC 1 may have caused an elevated air-seawater gas exchange and therefore rising CO_2 concentrations. The reduced global radiation also indicated different weather conditions at DC 2. During DC 3 wind speeds and global radiations were similar values to those during DC 1.

The autotrophic phytoplankton consisted of cyanobacteria (three filamentous taxa and *Synechococcus* type cells), cryptophyceae (two taxa), dinoflagellates (four taxa), diatoms (four taxa), prasinophyceae (one taxon), and chlorophyceae (two taxa). While filamentous cyanobacteria accounted only for 4 % of the cell counts, this group represented up to 20 % of the total biomass (Fig. 12a).

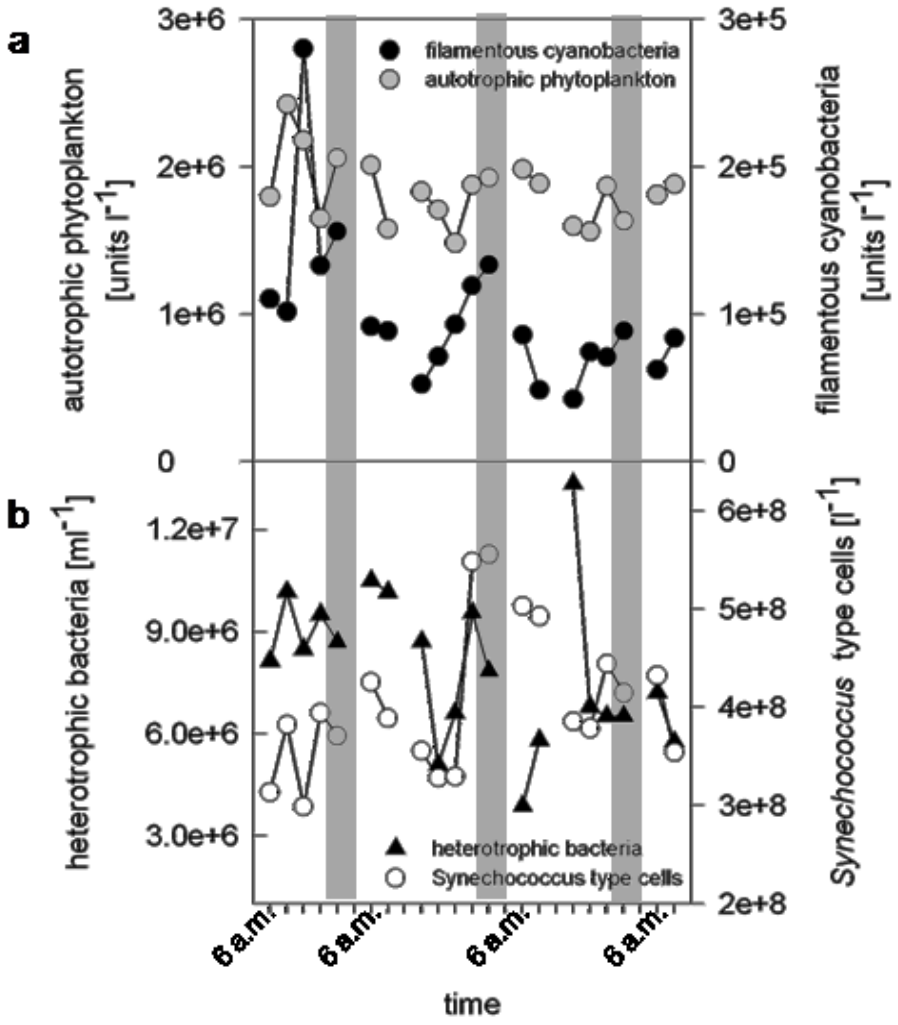


Fig. 12. Phytoplankton and bacterioplankton community composition (cell counts) during the three daily cycles a – filamentous cyanobacteria and other autotrophic phytoplankton groups; b – heterotrophic bacteria and *Synechococcus* type bacteria. Shaded areas represent night time during the observation period.

Filamentous cyanobacteria composition did not vary significantly during the three daily cycles. Cell counts were dominated by *Aphanizomenon* "baltica" (max. 93 %). *Anabaena* sp. (17 ± 7.6 %) was the second most frequent filamentous cyanobacteria. *N. spumigena* showed only low cell numbers, but represented up to 63 % of the filamentous cyanobacteria biomass. The proportions of the individual filamentous cyanobacterial specie did not change throughout the day.

The eukaryotic nanoplankton represented the majority of the total biomass and therefore the reactions measured for this group are mainly reflected by analyses of the size fraction < 10 µm. At DC 1 and DC 3 *Synecchococcus*-type cell concentrations (picoplankton) did not show huge variations, whereas during DC 2 significant higher abundances were measured (Fig. 12b). Heterotrophic bacterial enumerations revealed a parallel pattern to the concentration of *Synecchococcus* type cells at DC 1 and DC 3, except the measurement at 6 a.m.. At DC 2 the maximum concentration of heterotrophic bacteria at 6 p.m. was followed by minimum values 4 hours later and an increase again thereafter (Fig. 12b).

Inorganic nutrient concentrations (DIP, NO_2^-, NO_3^-, SiO_4^{4-}) declined slightly during the period of all three cycles (Fig. 13). The DOP concentration decreased highly significantly from DC 1 to DC 2 ($p < 0.01$), while from DC 2 to DC 3 the content rose again significantly ($p < 0.05$) (Fig. 13a). Along with low DOP concentrations an enhancement of the phosphatase enzyme efficiency was observed. While NO_2^- concentrations were below the detection limit during DC 1 and DC 2, the content slightly increased to approximately 0.02 µmol l^{-1} at DC 3 (Fig. 13b). Strong elevated phosphatase enzyme efficiency was also measured together with maximum concentration of filamentous cyanobacteria and NO_3^- concentrations at DC 1 (Fig. 12a, 13a, 13b).

Autotrophic activity and biomass were described by nutrient uptake rates, and Chl*a*, POM concentrations, respectively. During the observations a discrimination between two size fractions was applied, one bigger than > 10 µm, corresponding to the fraction of filamentous cyanobacteria, and the other fraction < 10 µm represented pico- and nanoplankton. The POM concentrations of filamentous cyanobacteria and the fraction < 10 µm developed differently (Fig. 13). During the survey no impacts of daytime or nutrient concentrations on POC, PON and Chl*a* within the fraction < 10 µm were detected. In contrast, the size fraction > 10 µm showed increasing tendencies of PON and POC concentrations along the observation period, especially comparing DC 1 and DC 2 (highly significant difference PON $p < 0.01$, POC $p < 0.01$). In addition POC and PON concentrations were enhanced during all three nights (Fig. 14b, 14c). The increase of POC at night was also reflected by the POP

and Chl*a* concentrations, especially at DC 2 and DC 3 (Fig. 14d). Furthermore, POP concentrations of the fraction < 10 µm showed higher values at DC 2 than at DC 1 or DC 3 (highly significant $p < 0.01$), which was not indicated to this extend by POC and PON concentrations.

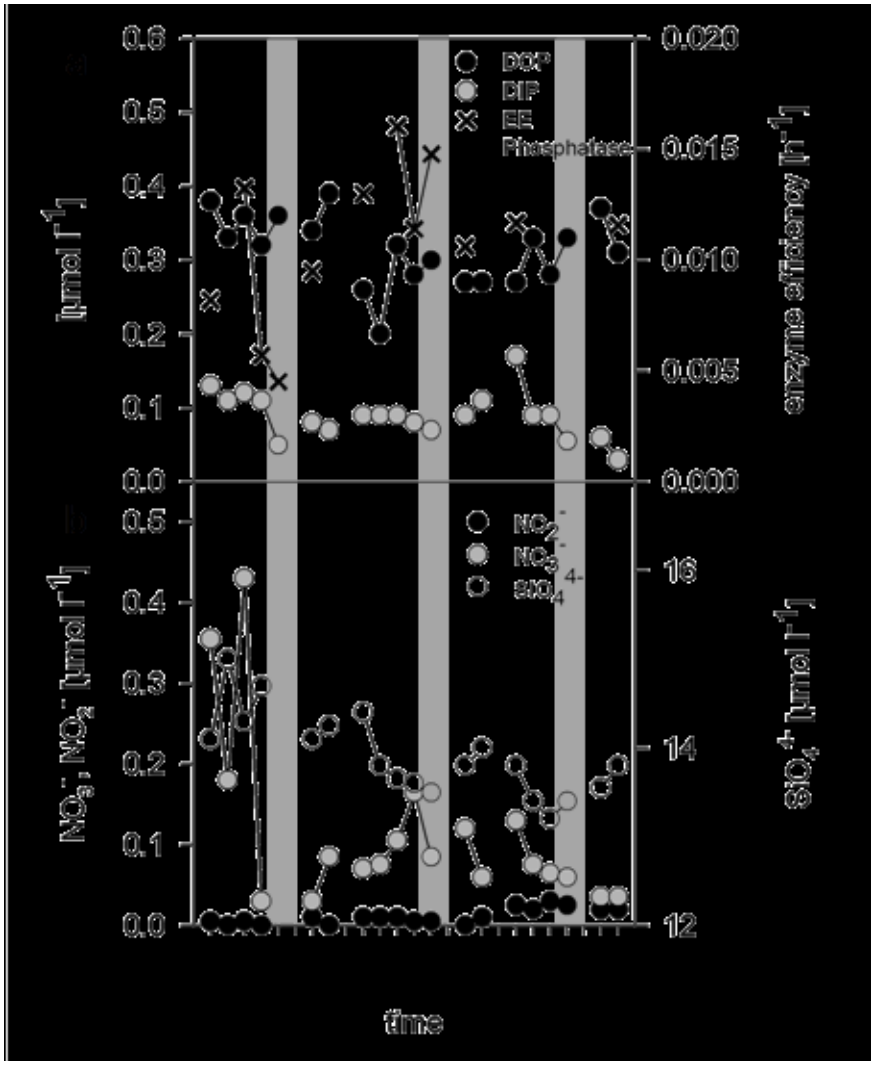


Fig. 13. Nutrient concentrations and enzyme efficiency during the three daily cycles; a – DOP, DIP concentrations [µmol l^{-1}] and phosphatase efficiency [Vmax Km-1]; b – NO_2-, NO_3- and SIO_4^{4-} concentrations [µmol l^{-1}]. Shaded areas represent night time during the observation period

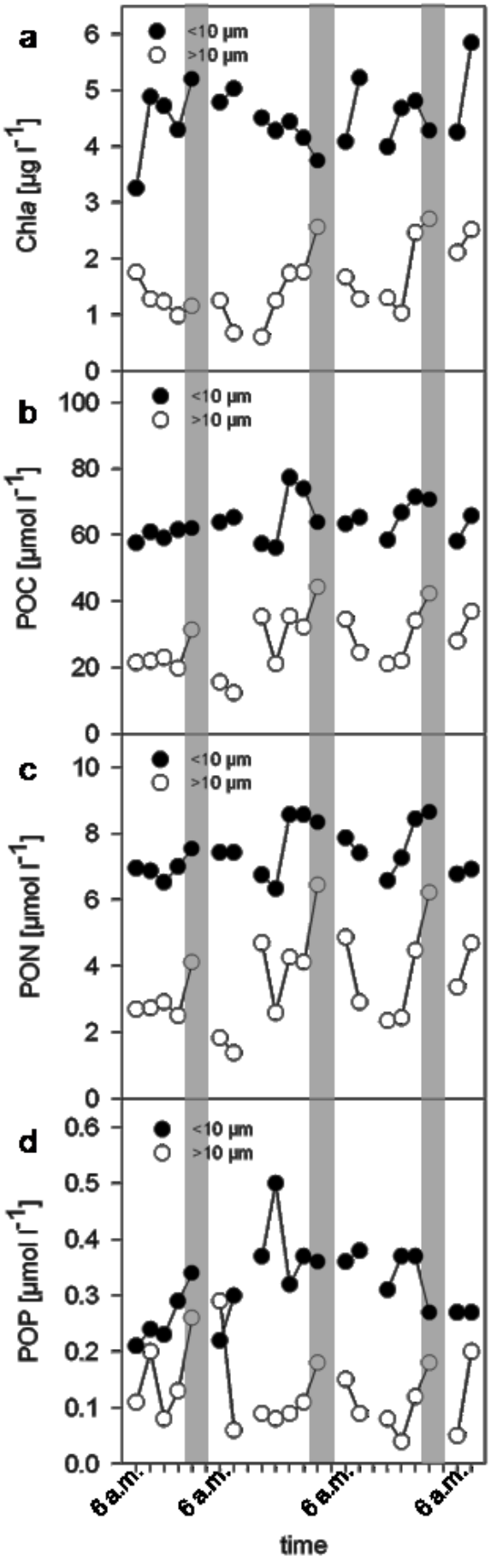


Fig. 14. Particulate organic matter of two size fractions (< and > 10 µm) during three daily cycles; a – Chl*a* content [µg l^{-1}]; b – POC concentration [µmol l^{-1}]; c – PON concentration [µmol l-1]; c – POP concentration [µmol l^{-1}]. Shaded areas represent night time during the observation period.

During the observation period linear correlations between the POC and PON concentrations for both size fractions were detected (Fig. 15a). The calculated POC:PON ratio for the fraction < 10 µm was 6.3 during all three daily cycles (Fig. 14a). The values of the size fraction > 10 µm (range: 6.8 – 9.1), exceeded the Redfield Ratio (6.6) in many cases. Furthermore, variations between day and night time were observed. The lowest ratios were detected during night for each daily cycle. In general, the POC:POP as well as the PON:POP ratio of the size fraction > 10 µm increased along the period from DC 1 to DC 3, with high significant differences between DC 1 and DC 2 ($p < 0.001$), and a less significant difference between DC 2 and DC 3 (POC:POP $p < 0.05$; PON:POP $p < 0.05$). The average value of the POC:POP ratio started with 161 at DC 1, 299 at DC 2 and ended with 348 at DC 3. In both fractions no linear relationship between PON and POP concentrations was detected (Fig. 15b). Measurements within the fraction < 10 µm showed elevated POP at mostly constant PON concentrations from DC 1 to DC 3 (Fig. 14b).

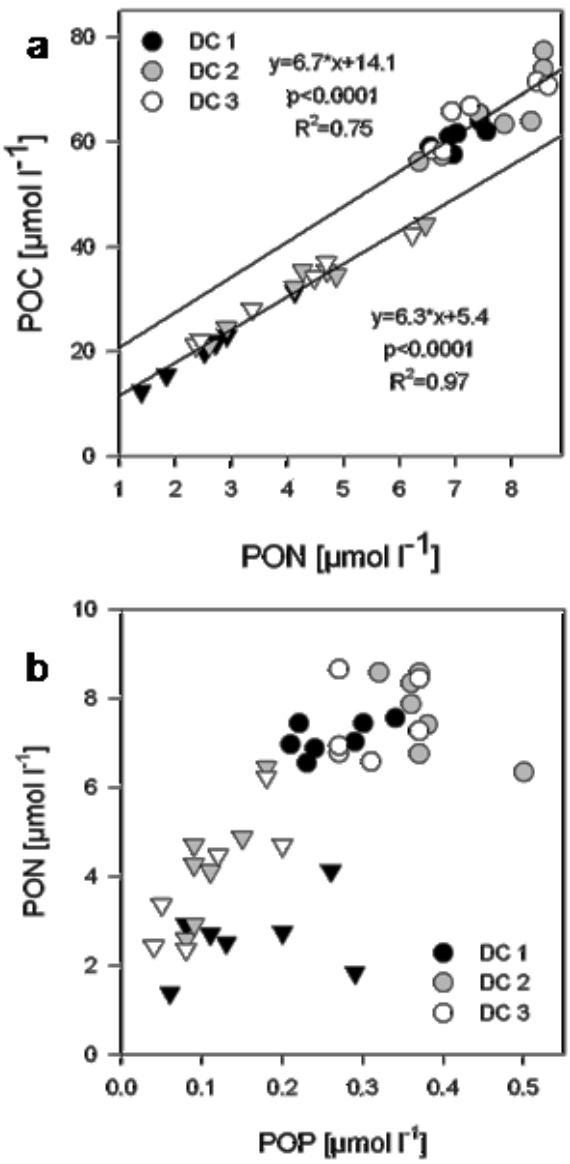


Fig. 15 Correlations between two biomass parameters for two size fraction, < and > 10 µm, during the three daily cycles; a – correlation between PON and POC concentrations [µmol l^{-1}]; b – correlation between POP and PON concentrations [µmol l^{-1}]; circle – fraction < 10 µm, triangle – fraction > 10 µm.

Diurnal patterns of autotrophic and heterotrophic bacterial processes

Diurnal patterns of C, N_2 fixation, and DIP uptake rates were observed. Overall, absolute values of C fixation, were dominated by the fraction < 10 µm similar to POC, Chl*a* concentrations and cell counts. Normalized C fixation rates showed clear diurnal patterns for both size fractions (Fig. 16a). At night (10 p.m.) C

fixation was barely measurable, whereas it immediately increased with sunrise (Fig. 16a).

N_2 fixation in the fraction > 10 μm, decreased towards night, nevertheless it was still detectable (Fig. 16b). Maximum values, measured during the day, coincided with higher diazotrophic cyanobacteria counts at DC 1 (Fig. 12a, 16b). Anyhow a general time dependence, similar hour, for observed N_2 fixation maxima could not be identified for the three cycles. N_2 fixation measured in the size fraction < 10 μm was negligible.

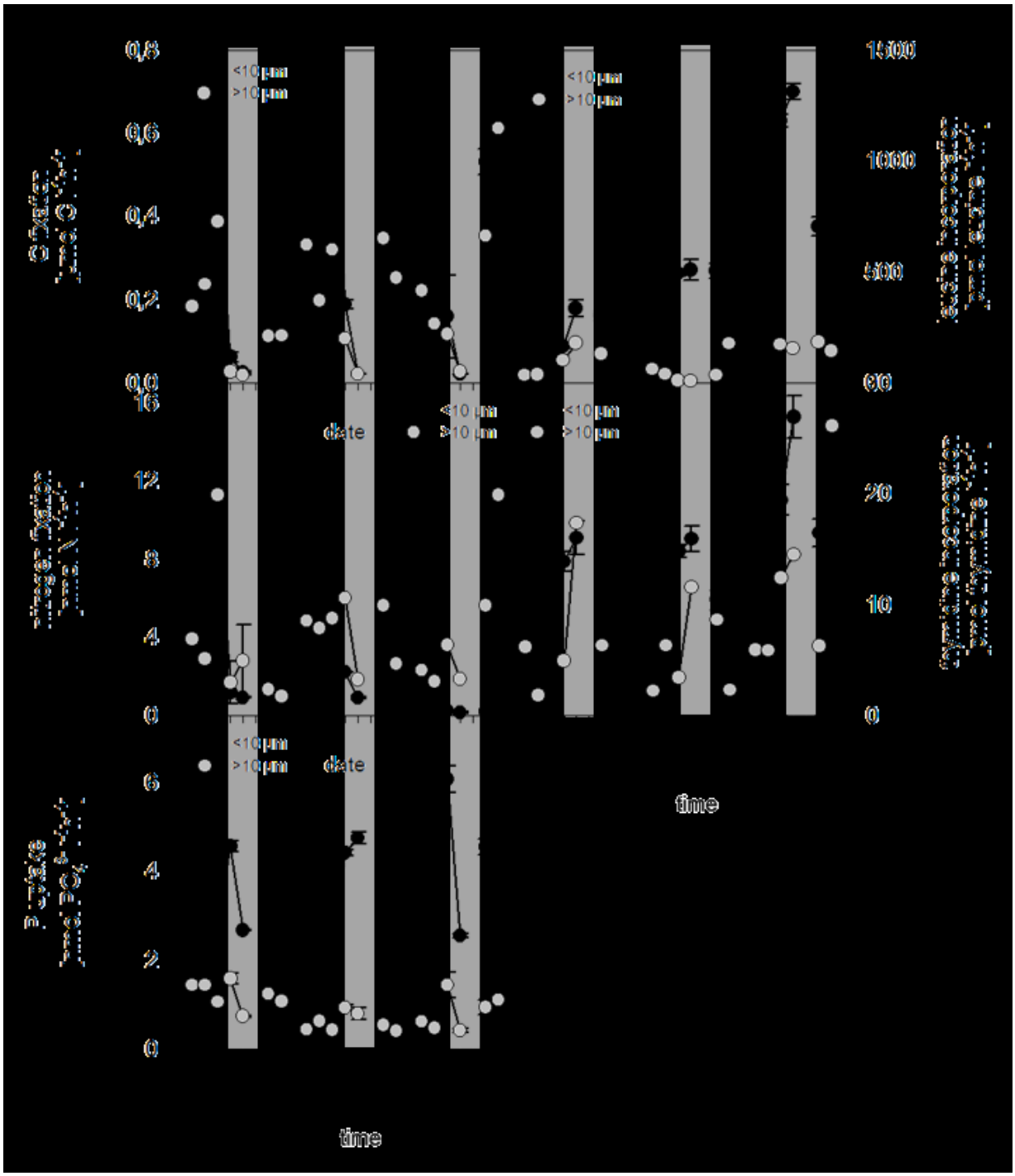


Fig. 16. Autotrophic and heterotrophic fixation rates detected during the three DCs for the fractions > and < 10 µm; a – C fixation measured by the incorporation of ^{13}C [µmol C l^{-1} h^{-1}]; b – nitrogen fixation measured by the incorporation of ^{15}N [nmol N l^{-1} h^{-1}]; c – phosphorus uptake measured by the incorporation of [^{33}P]PO_4^{3-} [nmol PO_4^{3-} l^{-1} h^{-1}], d – leucine incorporation measured by the incorporation of [^3H]leucine [pmol leucine l^{-1} h^{-1}]; e – thymidine incorporation measured by the incorporation of [^3H]thymidine [pmol thymidine l^{-1} h^{-1}]. Shaded areas represent night time during the observation period.

Diurnal variations occurred also for the P uptake rates (Fig. 16c). Again the fraction < 10 µm dominated the absolute DIP incorporation rates. During DC 1 and DC 3 lowest values were detected at 2 p.m. and 10 p.m. in both fractions. The course differed for DC 2. The coincidence of DIP incorporation and the concentrations of *Synecchococcus*-type cells indicated a high influence of this group on DIP incorporation during DC 2 (Fig. 12b).

The comparison of the relationship between the specific DIP affinity and the POC:POP ratio of both fractions showed, that the large fraction regulate its specific DIP affinity to less as the small fraction (Fig. 17). Day light provoked an increase of DIP affinity in both fractions, enhanced values were obtained during day. Anyhow the size fraction > 10 µm was more affected, indicated by a 3 to 4 fold lower affinity of the large fraction than the affinity of the small fraction during night.

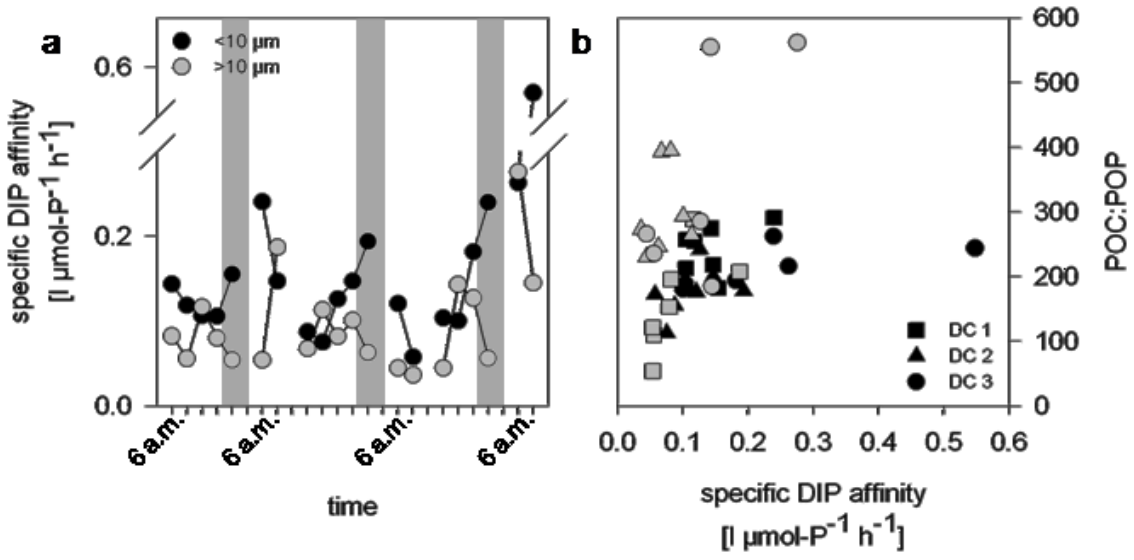


Fig. 17. Changes of the specific DIP affinity [l µmol P^{-1} h^{-1}] during the observation period; a - temporal changes of specific DIP affinity. Shaded areas represent night time during the observation period; b – Changes of the specific DIP affinity towards the corresponding POC:POP ratios; black symbols - < 10 µm, grey symbols - > 10 µm.

The general importance of P for the phytoplankton community was also highlighted by its fast turnover rates (Table 2). The proportion of active P uptake

compared to the particulate matter was about one order higher than N_2 fixation compared to PON in the size fraction > 10 µm. Nevertheless, a higher DIP affinity of cells < 10 µm compared to filamentous cyanobacteria provokes higher proportions of P uptake on the turnover of POP for the fraction < 10 µm (Table 2). Average values of P uptake rates compared to the POP pool showed a turnover of 14 % for the size fraction > 10µm, whereas for the size fraction < 10 µm about 21 % of the corresponding POP pool are replaced within one day.

Leucine and thymidine incorporation were used to study heterotrophic growth. Over the course of observation the leucine incorporation (fraction < 10 µm) increased (difference DC 1 and DC 2 very highly significant $p < 0.001$; difference DC 1 and DC 3 significant $p < 0.05$; difference DC 2 and DC 3 highly significant $p < 0.01$). The leucine incorporation of the fraction > 10 µm showed no significant rise and no differences between night and day could be identified (Fig. 16d). Thymidine incorporation showed highest values at night in both size fractions. The level of thymidine incorporation inside the fraction > 10 µm was similar in all three DCs. Similar to leucine incorporation, thymidine incorporation of the small size fraction increased along the investigation period from DC 1 to DC 3 (difference DC 1 and DC 3 significant $p < 0.05$, difference DC 2 and DC 3 high significant $p < 0.01$) (Fig. 15e).

Table 2. Daily turnover of PON or POP per day deduced by N_2 fixation and P uptake. Calculations of N_2 fixation were based on 16 h active incorporation per day with respect to the maximum or minimum rates during all three daily cycles, while P uptake can be measured during at day and night. P uptake turnover were calculated for the size fractions < and > 10 µm.

	uptake rate	min/ max	potential N_2 fixation or P uptake [nmol l^{-1} 16 h^{-1}]	PON ($\bar{x}$) [µmol l^{-1}]	POP ($\bar{x}$) [µmol l^{-1}]	turnover of PON or POP [%]	
DC 1	N_2 fixation	max	180.2	27.0		6.7	
	(> 10 µm)	min	15.7	27.0		0.6	
DC 2	N_2 fixation	max	95.9	4.3		2.2	
	(> 10 µm)	min	42.4	4.3		1.0	
DC 3	N_2 fixation	max	180.1	3.9		4.6	
	(> 10 µm)	min	27.8	3.9		0.7	Ø **2.6**
DC 1	P uptake	max	25.3		0.13	19.4	
	(> 10 µm)	min	17.0		0.13	13.0	
DC 2	P uptake	max	14.7		0.09	16.4	
	(> 10 µm)	min	6.4		0.09	7.1	
DC 3	P uptake	max	22.9		0.1	22.8	
	(> 10 µm)	min	7.5		0.1	7.5	**Ø 13.6**
DC 1	P uptake	max	73.0		0.24	30.4	
	(< 10 µm)	min	43.4		0.24	18.1	
DC 2	P uptake	max	70.6		0.37	19.1	
	(< 10 µm)	min	36.8		0.37	9.9	
DC 3	P uptake	max	97.1		0.29	33.5	
	(< 10 µm)	min	48.2		0.29	16.6	**Ø 21.3**

3.1.2. Discussion

Temperatures > 17 °C and a stratified water column provide an ideal hydrographical environment for the growth of diazotrophic cyanobacteria (Kononen 2001, Stal et al. 2003, Wasmund et al. 2005). These conditions were met during the investigation period. During the three daily cycles pCO_2 values of approximately 160 µatm were measured, which is below the present atmosphere concentrations. Due to the intensive phytoplankton blooms in spring and summer the CO_2 uptake is enhanced and the water column gets highly under saturated with CO_2 compared to the atmosphere (Thomas & Schneider 1999). Increasing wind velocities at DC 2 resulted in a higher gas exchange and therefore slightly higher CO_2 concentrations in contrast to DC 1 or DC 3.

As mentioned before most of the measurements discriminated between two size fractions. The intention was to separate filamentous cyanobacteria and the remaining phytoplankton community. The size fraction > 10 µm corresponded to filamentous cyanobacteria, which were the only taxa of this size class in the phytoplankton community (Walsby et al. 1995, Congestri et al. 2000). The reactions of the remaining community (< 10 µm) were captured by bulk signals. Besides, specific activity measurements for heterotrophic bacteria and cell counts were approved to give further information.

Filamentous cyanobacteria

Total phytoplankton biomass was not dominated by filamentous cyanobacteria. Like former investigations showed, too, filamentous cyanobacterial blooms are accompanied by high abundances of eukaryotic autotrophs and picocyanobacteria (Andersson et al. 1996, Stal et al. 2003). The sampling depth of 3 m was in between previous reported biomass peaks for *Aphanizomenon* or *Nodularia* (Walsby et al. 1995, Stal & Walsby 2000, Walve & Larsson 2007). Therefore a mixed community was investigated concerning interspecific relations, focussing on nutrient demands and utilization inside the euphotic zone.

The ability to fix N_2 prevents diazotrophic cyanobacteria of DIN limitation, visible in the linear relationship of POC and PON during the investigation period (Fig. 15a). Maximum POC and PON concentrations during night were probably caused by vertical migration from the water surface of filamentous cyanobacteria; especially *Aphanizomenon*, to 3 m (Hajdu et al. 2007). The continuous increase of POC and PON concentrations during the observation indicated a higher production than degradation rate. In contrast a similar accumulation of POP was not measured, giving evidence for a P limited system.

During the day higher DIP affinities and P uptake rates were obtained than during night. We suppose that DIP uptake by diazotrophic filamentous cyanobacteria is also light driven like C and N_2 fixation (Wannicke et al. 2009). Until now DIP uptake was not known to be enhanced by light (Nausch et al. 2004, Sohm et al. 2006). Why we detected this pattern, cannot be definitely anwered. The diurnal pattern of C fixation with midday maxima is in accordance with previous observations (e.g. Gallon et al. 2002). Even though filamentous cyanobacteria accounted only for 20 % of the biomass, they contributed on average with 34 % to the total C fixation pointing to their importance in fueling the system with newly fixed C. N_2 fixation by filamentous cyanobacteria was reduced but still detectable during night, confirming results of depressed N_2 fixation activities in darkness (Stal et al. 1999, Evans et al. 2000, Gallon et al. 2002, Moisander et al. 2007). The nighttime N_2 fixation activities can be explained by the utilization of stored carbohydrates to generate ATP and reductants by a light-independent metabolism (Evans et al. 2000). The POC:PON ratio did not change significantly during the incubation, indicating a constant N supply. In contrast the POC:POP or PON:POP ratios for the size fraction > 10 μm increased during the period of investigation, which referred to DIP limited growth of filamentous cyanobacteria (Larsson et al. 2001).

Eukaryotic autotrophs and picocyanobacteria

Eukaryotic algae represented most of the phytoplankton biomass during the here presented investigation. A characteristic diurnal pattern of promoted C fixation rates during daylight was also observed in this fraction. So far one study reported N_2

fixation in phytoplankton taxa besides filamentous cyanobacteria in the Baltic Sea (Wasmund et al. 2001), anyhow this was not confirmed in subsequent studies (Stal et al. 2003, Ohlendieck et al. 2007). It can be assumed that the constant POC:PON ratios of eukaryotic autotrophs and picocyanobacteria (Fig. 15a) were a result of released NH_4^+ and DON by diazotrophic cyanobacteria, prohibiting a N limitation (Ohlendieck et al. 2000, Bronk et al. 2007, Ohlendieck et al. 2007). Furthermore the DON uptake was demonstrated indirectly by measurements of $^{15}N_2$ in the fraction < 10 µm in our study (Fig. 15a). Diurnal variations were missing and therefore indicated an uptake of released labeled DON instead of N_2 fixation.

The higher surface to volume ratio resulted in higher P uptake rates and DIP affinities for the small size fraction compared to filamentous cyanobacteria (Fig. 15c, 16a, 16 b) (Stal & Walsby 2000, Tanaka et al. 2004).

Synecchococcus-type cells dominated the autotrophic picoplankton. The abundance changed between the three daily cycles. At DC 2 increasing wind velocities and lower light intensities (Table 1) probably favoured growth of picocyanobacteria compared to eukaryotic autotrophs and filamentous cyanobacteria (Stal & Walsby 2000). During DC 2 picocyanobacteria counts correlated with the P uptake and the diurnal variability of this process disappeared. Former investigations already showed, that they are successful competitors at depleted DIP concentrations (Andersson et al. 2009). The increasing influence of picocyanobacteria at instable water conditions was underlined by the obviously elevated enzyme efficiency of phosphatase and the decreasing DOP concentrations during DC 2, which is the main source satisfying the P demand of *Synechococcus*-type cells under P limiting conditions (Fu et al. 2006).

Heterotrophic bacteria

The growth of heterotrophic bacteria is mainly limited by low DON and DOC concentration (Søndergaard et al. 2000, Børsheim et al. 2005). On the other hand diazotrophic cyanobacteria release high amounts of DON and DOC during their growing season (Ohlendieck et al. 2007). Thus, neither a N nor a C limitation of heterotrophic bacteria occured, indicated by stable POC:PON ratios. But due to the sufficient supply of C and N, also the DIP demand might have increased. Therefore it

can be assumed, that the low DIP concentrations (0.1 M) also controlled the growth of heterotrophic bacteria. Due to this fact and high DIP affinities the three groups of the size fractions < 10 µm, eukaryotic autotrophs, picocyanobacteria and heterotrophic bacteria, exhibited a fast daily turnover of the POP, faster than the ones of filamentous cyanobacteria. High DIP affinities and the small size enable heterotrophic bacteria to compete successfully for DIP against filamentous cyanobacteria under P limiting conditions. The advantage in competition for inorganic nutrients between heterotrophic and autotrophic phytoplankton might explain higher rates of heterotrophic growth (thymidine incorporation) during night (Fig. 16e). The observation of elevated heterotrophic activity during night was also supported by the increasing specific DIP affinity of the small size fraction (Fig. 17a). Primarly the thymidine incorporation rates were used for analyzing hetetropthic activity. Thymidine is is exclusively taken up by heterotrophic cyanobacteria (Fuhrman & Azam 1982, Robarts & Wicks 1989, Lehtimäki et al. 1997). While it seems to be, that leucine analyses were not suitable to divide exactly between activities of diazotrophic and heterotrophic bacteria, because minor proportions of leucine can also be incorporated by *Nodularia* strains (Hietanen et al. 2002). However, a significant increase for both, leucine and thymidine incorporation occurred in the course of the observation, indicating enhanced heterotrophic activity. The increase of heterotrophic growth indicated enhanced N and C supplies by filamentous cyanobacteria, probably due to the alteration of the bloom.

3.1.3. Conclusions

Consistent diurnal patterns were detected for the incorporation of the three major nutrients, C, N, and P, within a summer phytoplankton bloom in the Baltic Sea. Light energy is the motor of autotrophic growth. Maximum nutrient uptake rates (noon) can be double of mean values during the day. Therefore the time point of measuring autotrophic rates can be important for calculating bulk production. Interestingly, also heterotrophic bacterial production followed a daily (24 hours) cycle with higher activities at night. The missing light during night seems to be without negative influence for heterotrophic activity. Contrariwise the missing concurrence of autotrophic phytoplankton for inorganic nutrients appears to facilitate the nutrient uptake by heterotrophic bacteria.

We propose that during the course of the bloom the phytoplankton community changed from a primarily N limited system, originally triggering the rise of diazotrophic cyanobacteria, to a P limited system. It was indicated by constant POC:PON ratios for the whole phytoplankton and increasing POC:POP ratios for the size fraction > 10 μm during the period of observation. We showed that the shift is caused by an additional input of "new" N by N_2 fixation and its transfer to other non diazotrophic groups within the community. The transfer was tracked, by enhanced DON concentrations in the water column and increasing ^{15}N levels also in the not N_2 fixing phytoplankton. Thus, eukaryotic autotrophs, picocyanobacteria and heterotrophic bacteria were not N limited anymore. Most of these taxa are also characterized by a higher affinity to DIP, which we assume gives these groups a major growth advantage. This was indicated by higher P uptake rates and increasing heterotrophic activity. Due to that the filamentous cyanobacteria themselves, as bad competitors for inorganic nutrients, are deprived of DIP. Probably their input of "new" N highly contributes to their own collapse, due to DIP limitation. This shift from primarily DIN to DIP limitation results for filamentous cyanobacteria in decreasing autotrophic C fixation and N_2 fixation rates. Depleted nutrient conditions provide ideal conditions for autotrophic picoplankton and heterotrophic bacteria ruling out large filamentous cyanobacteria.

3.2. EFFECTS OF ELEVATED CO_2 CONCENTRATIONS ON THE PRODUCTIVITY OF HETEROCYSTIC CYANOBACTERIA

3.2.1. Results

Variations of the CO_2 system

A gradient of seven different CO_2 concentrations was used to test the effects of elevated pCO_2 on a mixed plankton community, composed of diazotrophic cyanobacteria and picoplankton, common in the Baltic Sea. The addition of CO_2 gas led to higher CO_2 concentrations, increased DIC contents and decreasing pH values (Table 3). TA was constant during the experiments due to a lack of calcifying organisms (~ 1730 µmol kg SW^{-1}).

Table 3. Values of the carbonate system during the two four days lasting experiments A and B. TA (total alkalinity) was 1730 µmol kg SW^{-1}.

	Start			End		
	pCO_2 [µatm]	pH	TCO_2 [µmol kg SW^{-1}]	pCO_2 [µatm]	pH	TCO_2 [µmol kg SW^{-1}]
A	268	8.22	1612	241	8.26	1601
	280	8.20	1617	268	8.22	1612
	380	8.08	1647	365	8.10	1644
	480	7.99	1668	494	7.98	1671
	580	7.92	1684	668	7.86	1695
	680	7.85	1697	680	7.85	1697
	780	7.79	1707	787	7.79	1708
B	261	8.23	1609	175	8.38	1562
	280	8.20	1617	166	8.40	1556
	380	8.08	1647	190	8.35	1573
	480	7.99	1668	235	8.27	1598
	580	7.92	1684	282	8.20	1618
	680	7.85	1697	313	8.16	1628
	780	7.79	1707	356	8.11	1641

Table 4. Starting conditions of Chla, POC, PON, POP and inorganic nutrients (DIN, DIP) of the two experiment A and B.

	Exp A	Exp B
Chla [µg l^{-1}]	5.2 ± 0.2	10.1 ± 1.1
POC [µmol l^{-1}]	198.9 ± 6.4	110.9 ± 4.6
PON [µmol l^{-1}]	35.4 ± 1.0	15.4 ± 0.5
POP [µmol l^{-1}]	1.1 ± 0.3	0.9 ± 0.1
DIN [µmol l^{-1}]	5.9 ± 0.2	4.4 ± 0.3
DIP [µmol l^{-1}]	1.08 ± 0.11	1.57 ± 0.01

Inorganic nutrient concentrations at the beginning of the experiments and the C-,N- and P-characteristics of the added filamentous cyanobacteria differed for the two experiments A and B (Table 4).

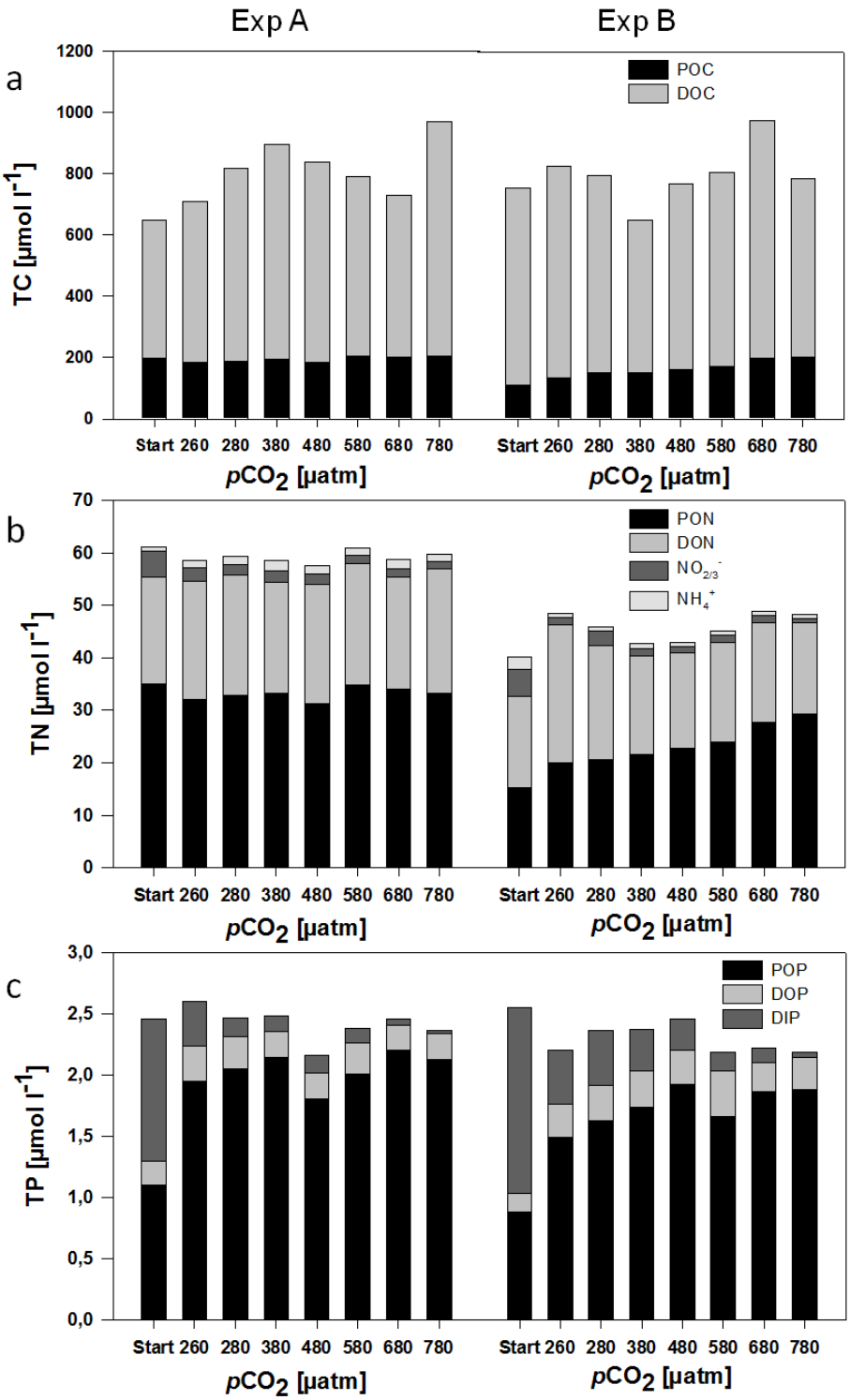


Fig. 18. Concentrations of dissolved and particulate carbon, nitrogen and phosphorus after 4 days at different pCO_2. Left side – experiment A, right side – experiment B. a – Total carbon concentrations (TC), combined particulate organic carbon (POC), and dissolved organic carbon (DOC); b – Total nitrogen concentrations (TN), combined particulate organic nitrogen (PON), nitrite and nitrate ($NO_{2/3}^-$), ammonia (NH_4^+) and dissolved organic nitrogen (DON); c – Total phosphorus concentration (TP), particulate organic phosphorus (POP), dissolved inorganic phosphorus (DIP) and dissolved organic phosphorus (DOP).

Figure 18 illustrates the significant elevations in nutrient uptake and biomass production induced by increasing pCO_2, as revealed by dissolved and particulate phases of C, N and P. The tendencies were more apparent for experiment B than experiment A.

Species composition

The increase of POM and the utilization of inorganic nutrients mainly arose from growth of *N. spumigena*, as indicated by its biomass. In contrast after 4 days of incubation the biomass of *Aphanizomenon* sp. decreased by approximately 50 % during the experiments A and B (Table 5). Consequently, the ratio of the two diazotrophic cyanobacteria species (*Aphanizomenon* sp. : *N. spumigena*) decreased.

Table 5. Phytoplanktion composition and corresponding Chla and POC conetrations for the experiments A and B.

EXP		Chl*a* [µg l^{-1}]	Cell counts [units l^{-1}]		Ratio	Biomass C [µg l^{-1}]		Ratio
			Apha	***Nodu***	cell counts	***Apha***	***Nodu***	Biomass
A	Start	5.2 ± 0.24	65 ± 21 E+03	26 ± 7 E+04	0.25	7.7 ± 2.5	258.1 ± 73.1	0.12
	End	10.4 ± 1.1	33 ± 13 E+03	28 ± 4 E+04	0.12	4.0 ± 1.1	278.1 ± 39.0	0.01
B	Start	10.6 ± 1.1	14 ± 3 E+04	8 ± 1 E+04	1.85	17.1 ± 3.0	76.1 ± 12.5	0.22
	End	11.8 ± 2.4	62 ± 6 E+03	13 ± 3 E+04	0.49	7.6 ± 0.8	126.7 ± 28.2	0.06

No significant changes in the cell counts or growth of heterotrophic bacteria and *Synechococcus* sp. (picocyanobacteria) were observed along the pCO_2 gradient between the beginning and end of the incubations (data not shown), ruling out potential influences of these taxa.

Particulate organic matter

The results of experiment B show, that the particulate organic matter, consisting of POC, PON and POP, increased significantly after 4 days of incubation along the gradient of increasing CO_2 concentrations ($R^2 = 0.93$ $p < 0.001$, $R^2 = 0.95$ $p < 0.001$, and $R^2 = 0.57$ $p < 0.05$ respectively) (Fig. 18, 19). Equal elevations in the concentrations of POC and PON resulted in constant C:N ratios at different CO_2 concentrations (Fig. 18). The highly significant alteration of POC:POP ($R^2 = 0.82$ $p < 0.01$) and PON:POP ($R^2 = 0.83$ $p < 0.01$) along the pCO_2 gradient was caused by a slower build up of POP than of POC or PON. The ratio varied between 91 and 107 for POC:POP along the gradient (Fig. 19). No significant changes or correlations could be applied for the POM development during experiment A.

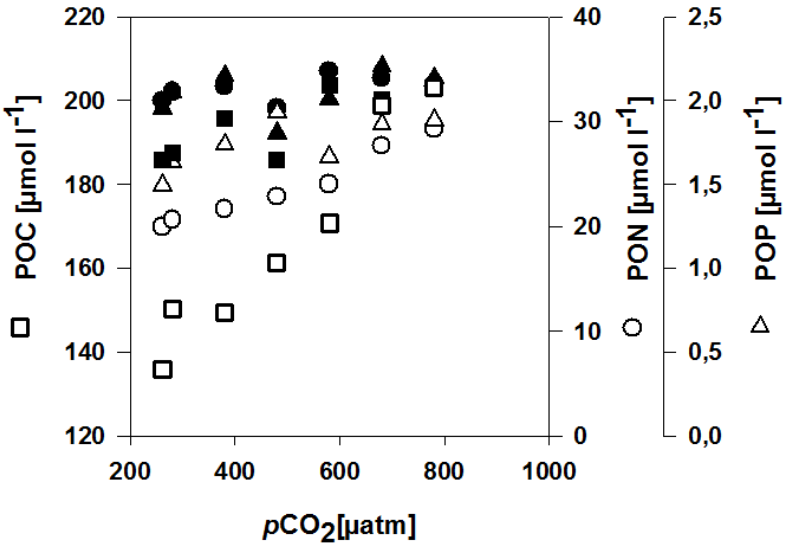


Fig. 19. Net increase of particulate organic matter (POC, PON, POP) after incubation for 4 days at different pCO_2. Experiment A black symbols, experiment B white symbols.

Dissolved inorganic nutrients and CO_2 concentrations

The concentrations of dissolved inorganic nutrients developed similar for both experiments. DIP and $NO_{2/3}^-$ concentrations decreased significantly. The reduction was enhanced with increasing CO_2 concentrations (Fig. 18b, 18c, 20b) and could be described by a linear regression (Fig. 20b). NH_4^+ concentrations did not vary

significantly along the pCO_2 gradient (Fig. 18b, 20b). The observed decrease of pCO_2 values during the incubation was attributed to direct incorporation of $CO_{2(aq)}$. During the experiment B the $CO_{2(aq)}$ incorporation increased with elevated pCO_2, as indicated by the net change in CO_2 concentrations (Fig. 20a), the bulk parameter DIC did not change significantly over the different pCO_2 settings (Fig. 20a). Comparable results were not detected during experiment A.

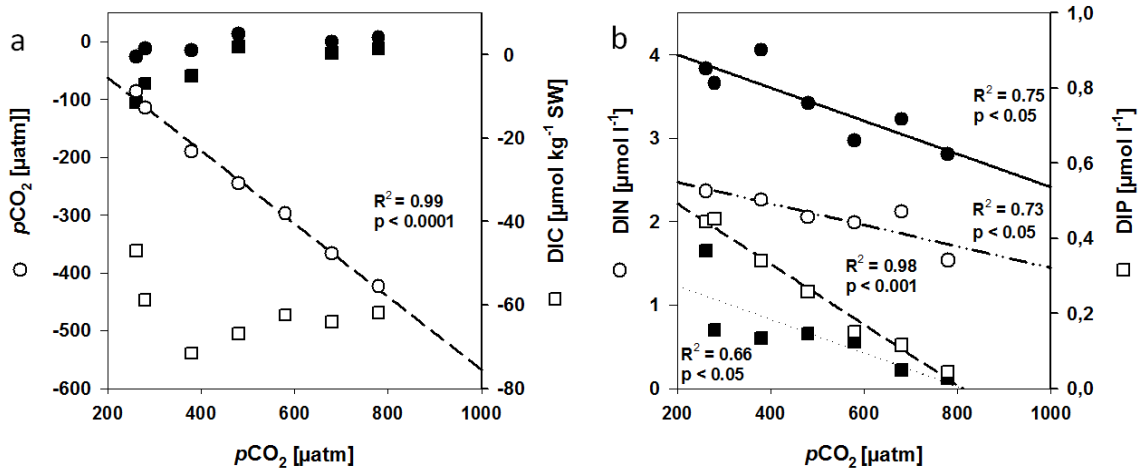


Fig. 20. a – Net change of CO_2 (dashed line) and dissolved inorganic carbon concentrations (DIC) (grey circles) after 4 days of incubation at different pCO_2, b – Concentrations of dissolved inorganic nitrogen (DIN) (solid line) and dissolved inorganic phosphorus (DIP) (dashed line) after incubation for 4 days at different pCO_2. Experiment A black symbols, experiment B white symbols.

Dissolved organic matter

The concentrations of dissolved organic nutrients (DOC, DON, DOP) showed no relation to changing pCO_2 values (Fig. 17). DOC exceeded the POC concentrations, while DON and DOP concentrations were below that of PON and POP respectively (Fig. 17). For both experiments significantly higher DOP concentrations than the initial ones were measured after 4 days of incubation along the entire CO_2 gradient (Table 5). DOC and DON concentrations were only enhanced in experiment A (Table 6).

Table 6. Dissolved organic matter concentrations (DOC=carbon, DON=nitrogen, DOP=phosphorus) at the start and after 4 days of incubation along the pCO_2 gradient for experiment A and B.

pCO_2 [µatm]	DOC [µmol l^{-1}]		DON [µmol l^{-1}]		DOP [µmol l^{-1}]	
	A	B	A	B	A	B
Start	450 ± 94	618 ± 62	20.4 ± 0.6	18.6 ± 0.9	0.20 ± 0.03	0.13 ± 0.02
261	522	690	20.85	25.2	0.29	0.27
280	631	643	21.45	21.7	0.26	0.28
380	700	595	21.83	18.8	0.21	0.30
480	651	608	20.10	18.1	0.21	0.27
580	586	633	21.04	19.0	0.25	0.37
680	530	583	23.78	22. 1	0.21	0.25
780	766	580	24.56	17.3	0.20	0.26

Rate measurements

At high CO_2 concentrations increasing dissolved and particulate organic concentrations of the elements C, N and P were probably caused by an enhanced nutrient utilization by diazotrophic cyanobacteria. Increasing fixation of C and N_2 as well as DIP turnover rates were found, indicating stimulated autotrophic growth (Fig. 21). The enhanced specific DIP affinity accelerated the incorporation of DIP, which resulted in a fast DIP turnover time (Fig. 21c, 21d).

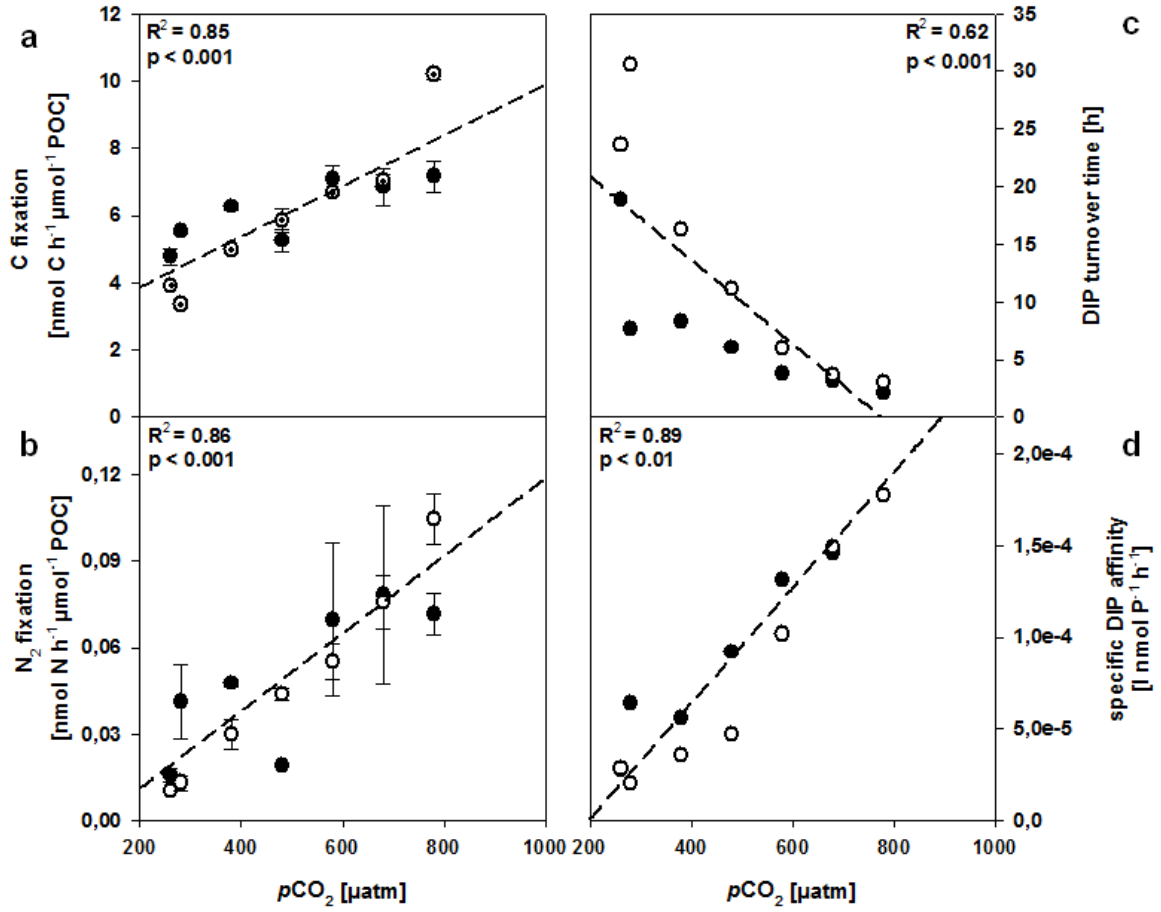


Fig. 21. Responses after 4 days of incubation at different pCO_2. a – C fixation rate normalised to POC; b – N_2 fixation rate normalised to POC; c – DIP turnover time; d – specific DIP affinity normalised to POP (P uptake rates not shown, due to reduced DIP concentrations constant along the gradient). Dashed lines indicate linear regressions. Experiment A black symbols, experiment B white symbols.

The slope of C fixation was lower than the slope of N_2 fixation rates along the CO_2 gradient, leading to a decreasing ratio between these uptake rates (Fig. 22). That implies a stronger stimulation of N_2 than C fixation and thus results in less C per N incorporation. The relationship between pCO_2 levels and the ratio of C fixation to N_2 fixation rates was not linear.

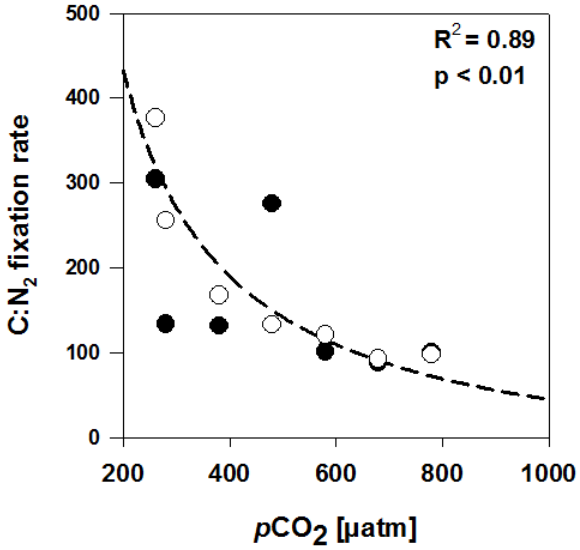


Fig. 22. pCO_2 dependency of the ratio C and N_2 fixation rates after 4 days of incubation. Dashed line indicates a regression of first inverse order. Experiment A black symbols, experiment B white symbols.

Short time incubations

Even after only 4 h of incubation at the ends of experiment C and D similar influences of CO_2 concentration on C and N_2 fixation rates were observed (data not shown). Briefly C and N_2 fixation rates were accelerated with respect to increasing CO_2 concentrations. Additionally, rate measurements of NO_3^- incorporation rates showed a direct positive correlation between pCO_2 and uptake of NO_3^-. Much higher absolute values were obtained for the NO_3^- incorporation rate than for the N_2 fixation rate (factor: 8 – 45). Both processes were stimulated at high CO_2 concentrations, indeed to a different degree. Anyhow, an extraordinary high stimulation of the overall lower N_2 fixation rate was observed, illustrated by an increasing ratio of N_2 fixation to NO_3^- incorporation along a pCO_2 gradient ranging from 250 to 950 µatm (Fig. 23).

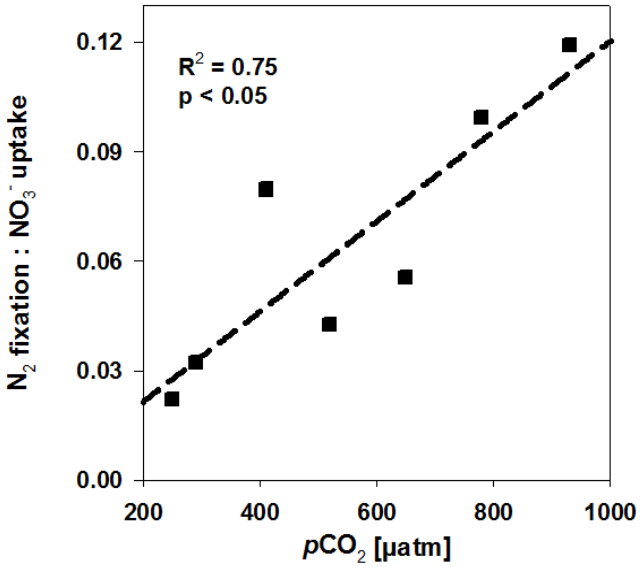


Fig. 23. Responses of N uptake rates after 4 h of incubation; ratio of N_2 fixation to NO_3^- uptake, examined during experiment C and D respectively. Dashed lines indicate a linear regression.

3.2.2. Discussion

Effects of elevated CO_2 concentrations on autotrophic activity

The diazotrophic filamentous cyanobacteria *Trichodesmium* sp. is known to respond to elevated CO_2 concentrations with enhanced primary production and N_2 fixation (Riebesell 2004, Barcelos e Ramos et al. 2007, Hutchins et al. 2007, Levitan et al. 2007, Kranz et al. 2009). The here presented investigation showed similar effects for batch cultures of *N. spumigena* and *Aphanizomenon* sp., which grew in altered seawater containing picoplankton (Fig. 21). Constant temperature values of 18 °C, a 16:8 h light dark cycle and reduced movement of the seawater fulfilled theoretically the hydrographical requirements for optimal growth rates for these diazotrophic cyanobacteria. In contrast, Czerny et al. (2009) investigated the effects of elevated pCO_2 on *N. spumigena* under completely different conditions. They studied the impact of enhanced CO_2 concentration, having the incubation bottles continuously moving. This might have destroyed the filamentous colonies and thus presumably changed CO_2 uptake rates. Accordingly, the here presented laboratory study is hardly comparable with that of Czerny et al. (2009). They showed no positive correlation between pCO_2 and autotrophic growth of N_2 fixation rates.

Nevertheless, the comparison of our results with data of a survey of the literature suggested that heterocystic cyanobacteria derive greater benefit from elevated CO_2 concentrations than do non-heterocystic cyanobacteria (e.g. *Trichodesmium* sp.) (Table 7).

Table 7 Comparison of diazotrophic cyanobacteria species, using the increase of N_2 and C fixation rates [nmol l^{-1} µg Chl*a*$^{-1}$ h^{-1}]under the influence of elevated pCO_2.

Gradient of pCO_2 increase [µatm]	Increase of N_2 fixation	Increase of C fixation	Species	Source
380 - 850	3	-	*Trichodesmium* sp.	(Barcelos e Ramos et al. 2007)
400 - 900	3	-	*Trichodesmium* sp.	(Levitan et al. 2007)
380 - 750	1.35 - 2	1.3 - 1.4	*Trichodesmium* sp.	(Hutchins et al. 2007)
150 - 1000	1.4	1.4	*Trichodesmium* sp.	(Kranz et al. 2009)
270 - 780	7.0	1.8	*N. spumigena* and *Aphanizomenon* sp.	this study

By investigating heterotcystic cyanobacteria we measured a seven-fold higher increase of N_2 fixation at a pCO_2 of 780 µatm than at 270 µatm, which indicated that the spatial separation of C fixation and N_2 fixation probably causes a greater increase of N_2 fixation than temporal separation typical for non-heterocystic diazotroph. It is known that *N. spumigena* and *Aphanizomenon* sp. perform DIC fixation inside vegetative cells and N_2 fixation inside heterocyst, in order to protect the oxygen-sensitive enzyme nitrogenase from the O_2 generated during photosynthesis. Therefore, heterocysts do not contain RuBisCo and the photosystem (PS) II; consequently, they must derive additional energy in the form of reduced carbon compounds from adjacent vegetative cells (Böhme 1998). N_2 fixation inside heterocystic cyanobacteria is likely stimulated firstly by the immediate utilisation of energy produced primarily by PS I (inside heterocysts and vegetative cells) and secondarily by PS II (inside vegetative cells). Additional energy supply may lead to a higher and instantaneous stimulation of N_2 vs. C fixation. In contrast, non-heterocystic cyanobacteria must cope with a down-regulation of photosynthesis at maximum N_2 fixation (e.g. Berman-Frank et al. 2001), as well as diel patterns of nitrogenase activity (Levitan et al. 2010). These differences might lead to a higher energy demand for N_2 fixation in non-heterocystic cyanobacteria.

But how does an enhanced CO_2 concentration influence cellular activities? Both processes, photosynthetic C fixation and N_2 fixation, are energy demanding such that changes in uptake efficiency can cause differences in the amount of fixed material. Due to the characteristics of cyanobacterial RuBisCo, with its low affinity for CO_2 (Km > 100 μmol kg^{-1}) and low half-saturation constant (20 – 150 μmol kg^{-1}) (Badger et al. 1998), cyanobacteria have been shown to develop different CCMS to increase intracellular DIC concentrations. Elevated CO_2 concentrations reduce the demand for energy to carry out protein synthesis directed at the generation and maintenance of the DIC transporter (Barcelos e Ramos et al. 2007). Consequently, a greater allocation of energy and reducing power for CCM activity as well as increasing substrates for N_2 fixation and for growth may be expected (Levitan et al. 2007). The increase in C and N_2 fixation rates measured in this study supports this hypothesis of energy reallocation inside the cells.

The enhanced utilisation of DIP in response to high pCO_2 (Fig. 21c, 21d) suggested that filamentous cyanobacteria increase DIP incorporation as long the substrate is available. Previous studies showed that diazotrophic cyanobacteria accumulate a large supply of phosphate prior to the development of a bloom. In early summer, POC: POP ratios of filamentous cyanobacteria start below the Redfield ratio (106) but by the end of the bloom exceeds values over 400 (Larsson et al. 2001). According to our results, there will be a higher DIP demand of filamentous cyanobacteria at pCO_2 levels predicted by climate-change models for the future. Whereas, Riebesell et al. (2007) proposed that only C fixation will increase under future CO_2 conditions, whereas nutrient uptake will remain at the same level.

Because the incorporation of DIP seems to be not such an energy-demanding process compared to C and N_2 fixation (Thingstad et al. 1993), the reallocation of energy inside the cells probably does not directly influence the DIP uptake rate. Rather, higher DIP incorporation rates may arise from a higher P demand in response to increased POC and PON concentrations. A high specific DIP affinity (Fig. 21d) was previously shown to determine competitive ability among osmotrophs (Vadstein 2000). Our study is the first, providing evidence of facilitated C and N_2 fixation at

elevated pCO_2 levels and of superior competition by the filamentous cyanobacteria *N. spumigena* and *Aphanizomenon* sp. at low DIP and high CO_2 concentrations.

Species competition

In this study, impacts of increasing pCO_2 values on nutrient requisition of two filamentous cyanobacteria, *N. spumigena* and *Aphanizomenon* sp, were observed. They did not respond equally. The biomass of *N. spumigena* increased whereas that of *Aphanizomenon* sp. decreased simultaneously (Table 5). One possible explanation would be that *Aphanizomenon* sp. might not successfully compete for DIP against *N. spumigena*. The low DIP concentration at the end of the experiment matched conditions typical for surface waters during summer in the Baltic Sea (Kahru et al. 1994). A different strategy of *N. spumigena* to satisfy the P demand might be the major reason for the dominance of this species over *Aphanizomenon* sp. While *N. spumigena* is known to grow under P-depleted conditions and to use DOP as a nutrient source, *Aphanizomenon* sp. requires DIP as the main P source for growth (Vahtera et al. 2007b). Therefore, lower DIP availability might have limited the growth of *Aphanizomenon* sp. but not that of *N. spumgiena*. This nutrient-dependent species selection was visible across the range of pCO_2 settings, while impacts of enhanced CO_2 concentrations on the species composition and abundance were not indicated. *In situ* observations have further indicated that *N. spumigena* dominates the species biomass under conditions of relatively constant physical settings, similar to the ones of the here presented investigation, e.g. reduced mixing activities and depleted nutrient concentrations (Kononen & Leppänen 1996, Vahtera et al. 2005). However, a direct, negative species-specific response of *Aphanizomenon* sp. to elevated pCO_2 cannot be ruled out or confirmed and remains to be addressed in further.

The impact of elevated CO_2 concentrations was monitored not only in autotrophic cyanobacteria but also in heterotrophic bacteria. The latter are known to react indirectly to elevated CO_2 concentrations by, for example, enhanced bacterial production at the end of a bloom (Allgaier et al. 2008).

Changes in stoichiometry due to elevated CO_2 concentrations

The rise of POP concentrations was not as pronounced as that of POC and PON (Fig. 19), perhaps due to DIP limitation. The accumulation of POC and PON partly relied on the not limiting nutrients DIC and N_2. Although, C fixation is not restricted by the absolute substrate (DIC) concentrations, it is presumably stimulated by higher CO_2 concentrations, caused by the low CO_2 affinity of the photosynthetic enzyme RuBisCo (Raven 1991, Riebesell 2004). Undoubtedly, the growth of *N. spumigena* was positively influenced by CO_2 during experiment B. Since DIC concentrations remained unchanged, CO_2 rather than DIC was the preferred substrate for C fixation (Fig. 20a).

Probably due to differences in nutrient availability, POC:POP and PON:POP ratios increased at higher CO_2 concentrations, while the POC:PON ratio did not change along the CO_2 gradient. A constant POC:PON ratio was not expected, based on the decreasing C:N_2 fixation ratio observed during the experiment. The surplus of N by N_2 fixation, which was not incorporated into PON, was not be found in rising DON or DIN concentrations. Short linkages between microbial degradation and accumulation processes might have prohibited tracking these newly fixed N in enhanced DON and DIN concentrations during this experiment. In contrast, previous investigations found increasing POC:PON values and therefore assumed enhanced formation of TEP (transparent exopolymer particles). Accordingly increased export of organic C from the upper layers of the water column to the deep ocean at elevated CO_2 concentrations is estimated (Engel et al. 2004, Riebesell et al. 2007).

As noted, seawater used during this investigation still contained NO_3^-, such that *N. spumigena* and *Aphanizomenon* sp. partly satisfied their N demand by NO_3^- incorporation. Nevertheless, NO_3^- concentrations during the experiment were low enough to allow neglecting a negative influence on N_2 fixation rates (Mulholland et al. 2001). The insignificant impact of the NO_3 content was further indicated by a high stimulation of N_2 fixation along the CO_2 gradient. This pattern was observed during our study, after 4 days and after 4 h of incubation respectively (Fig. 22, Fig. 23).

Our results show that high pCO_2 levels cause elevated incorporation rates of dissolved inorganic nutrients as well as N_2 and CO_2 into particulate matter. Active exudation of DOM by growing diazotrophic cyanobacteria (Ohlendieck et al. 2000, Wannicke et al. 2009) was not obviously effected by changing CO_2 concentrations, suggesting that the quality and quantity of nutrient supply for heterotrophic bacteria did not vary since there was no significant change in heterotrophic bacteria growth along the CO_2 gradient. However, Riebesell et al. (2007) were unable to follow the DIC drawdown at increasing POC contents and therefore suggested enhanced DOC export under elevated CO_2 concentrations.

Potential impacts of elevated CO_2 concentrations on the central Baltic Sea

To anticipate the impacts of elevated CO_2 concentrations on the Baltic Sea ecosystem, direct effects of CO_2 enhancement have to be combined with other future abiotic alterations such as temperature increase. Nonetheless, the here presented investigation concentrated on possible responses inside the Baltic Sea on high pCO_2 levels. The measurements show, that elevated pCO_2 values might enhance N_2 and C fixation, thus increasing organic N and C input into the euphotic zone can be suggested. An enhanced POC and PON accumulation could result in a higher DIP demand, causing an earlier onset of the P limitation typical at the end of the bloom season (Moisander et al. 2007). Under the conditions modelled in this study, the development of a diazotrophic bloom might be accelerated due to faster P depletion, higher sedimentation rates may increase the oxygen demand, and higher N_2 fixation rates may cause higher DIN and DON concentrations in the water column.

However, even today, in areas were upwelling takes place, the combined impact of elevated CO_2 and DIP concentrations stimulating cyanobacterial blooms, can be studied (Ibelings & Maberly 1998, Nausch et al. 2009). Anyhow it has to be taken into account that the upwelling water also contains a different phytoplankton community. However, these observations together with the here presented experiments let us assume, that an extension of the growth period and the aggregate development of filamentous cyanobacteria in the Baltic Sea can be expected in the future.

As mentioned before the scenarios discussed herein are based on studying the effects of pCO_2 changes. Further on, the shift in temperature with an increase of 2 – 6 °C, expected within the next 100 years (Boer et al. 2001), probably also causes large-scale changes. Probably the mean sea level will rise and species composition will change. Therefore further laboratory and *in situ* experiments, in which the influences of nutrient availability, temperature, and CO_2 concentration are jointly considered, will be needed to provide a better understanding of ecosystem responses to climate change.

3.3. CO_2 MEDIATED VARIATIONS WITHIN A NATURAL SUMMER PHYTOPLANKTON BLOOM IN THE BALTIC SEA

3.3.1. Results

CO_2 independent development of the plankton community - Size fraction > 2.7µm

We observed specific, CO_2 independent developments of the plankton community in the treated as well as in the untreated bottles.

Table 8. Starting conditions of biotic and abiotic parameters (average values ± standard deviations). Particulate organic matter (POC, PON, POP) and Chl*a* concentrations measurements were divided for the two size fractions > 2.7 µm and < 2.7 µm.

	> 2.7 µm	< 2.7 µm
POC [µmol l^{-1}]	208.2 ± 14.9	23.72 ± 0.74
PON [µmol l^{-1}]	30.2 ± 0.6	4.6 ± 0.3
POP [µmol l^{-1}]	0.90 ± 0.01	0.26 ± 0.01
Chl*a* [µg l^{-1}]	20.03 ± 1.69	0.56 ± 0.14
Heterotrophic bacteria concentration [ml^{-1}]	$4.57 \times 10^6 \pm 9.6 \times 10^4$	
Picocyanobacteria [l^{-1}]	$1.23 \times 10^8 \pm 4.68 \times 10^6$	
DIN [µmol l^{-1}]	0.12 ± 0.02	
DIP [µmol l^{-1}]	< 0.02	
T [°C]	21.2 ± 0.3	
S [PSU]	7	
pCO_2 [µatm]	~ 210	

Organisms of the size fraction > 2.7 µm (mainly filamentous cyanobacteria) accounted for 90 % of the total POC content, at the beginning of the experiment (T0). Low concentrations of DIN and DIP (n = 3 average: 0.12 ± 0.02 µmol l^{-1},

0.01 µmol l^{-1} respectively), corresponded to *in situ* concentrations. A summary of the initial conditions, concerning plankton and environmental characterization, is presented in table 8.

The phytoplankton community mainly consisted of *Aphanizomenon* sp., *N. spumigena* and *Anabaena* sp. (Fig. 24). The quantitative species composition of the cyanobacteria community clearly changed during the experiment. The initial dominance of *Aphanizomenon* sp. decreased during the six days of incubation. Compared to T0 significantly biomasses declined at T4 and T6 ($p < 0.05$) (Fig. 24).

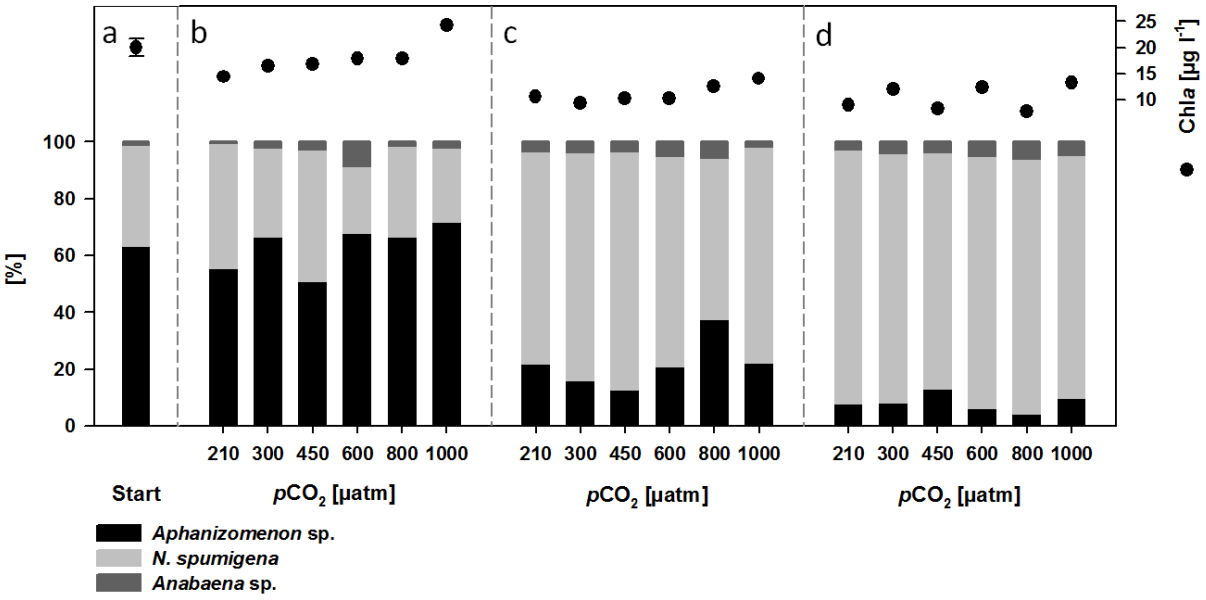


Fig. 24. Relative carbon concentration of *Aphanizomenon* sp., *N. spumigena* and *Anabaena* sp. compared to the total carbon concentration of the filamentous cyanobacteria and Chl*a* concentrations of the size fraction > 2.7 µm (filled circles). a –starting conditions (T0); b – at different CO_2 concentrations after one day of incubation (T1); c – at different CO_2 concentrations after four days of incubation (T4); c –at different CO_2 concentrations after six days of incubation (T6).

The abundance of *N. spumigena* remained rather constant throughout the incubation. The abundance of *Anabaena* sp. was always lower and showed no significant changes. Due to the different developments of the filamentous cyanobacteria, the biomass proportions of the three species shifted towards a

dominance of *N. spumigena* (Fig. 24). Other autotrophic phytoplankton species (> 2.7 µm) were abundant only in single counts until T1. During T4 and T6 increasing concentrations of a filamentous chlorophyta > 2.7 µm (*Planktonema lauterbornii*) were detected (T4: $4.75 \times 10^4 \pm 2.83 \times 10^4$ cells l^{-1}; T6: $1.10 \times 10^5 \pm 6.77 \times 10^4$ cells l^{1}). Anyhow this species only accounted for 10 % of the total phytoplankton counts during T4 and for 36 % during T6. In addition, dinoflagellates occurred in concentrations of $2.44 \times 10^7 \pm 1.28 \times 10^7$ counts l^{-1} at T4 and of $5.66 \times 10^6 \pm 1.53 \times 10^6$ counts l^{-1} at T6.

The declining concentrations of *Aphanizomenon* sp. from T0 to T6 resulted in a decrease of Chl*a* concentrations of this size fraction (Table 10, Fig. 24). Along with the Chl*a* concentrations, POC and PON concentrations declined also in the course of the experiment, while POP concentrations remained at the same level (Fig. 25). The POC:PON ratio was close to the Redfield ratio during the whole investigation, nevertheless a slight but significant decline was detected during the incubation (T0: 6.9 ± 0.5; T1: 6.5 ± 0.2; T4: 6.3 ± 0.1; T6: 6.1 ± 0.0; $R^2 = 0.89$ $p < 0.1$). In contrast, POC:POP ratio showed values much higher than the Redfield ratio right from the start. Significant changes during the incubation were not detected (T0: 232 ± 19; T1: 243 ± 18; T4: 244 ± 2; T6: 197 ± 22; $p > 0.1$).

C and N_2 fixation rates were dominated by the fraction > 2.7 µm with 98 % and 94 % respectively. But as the POC and PON concentrations decreased during the experiment also these rates declined (Fig. 26). N_2 fixation was tenfold higher at T1 than at the end of the experiment. The reduction of C fixation was less dramatic, 40 % of the initial fixation rates were measured at T6. An increase of the ratio of C to N_2 fixation was shown from T1 to T6. This was the result of an accelerated diminishment of N_2 fixation compared to that of C fixation. In contrast to C and N_2 fixation, DIP uptake rates of the size fraction > 2.7 µm accounted for only 22 % of the total DIP uptake. A slight decrease was detected during the incubation (Fig. 27).

CO_2 independent development of the plankton community – Size fraction < 2.7 µm

In the size fraction < 2.7 µm the sum of autotrophic picoplankton groups exceeded those of heterotrophic bacteria. Nanoeucaryotes and *Synechococcus* sp. occurred at relative constant concentrations during the whole incubation period (n = 21; average values: $2.32 \times 10^6 \pm 6.05 \times 10^5$ cells l^{-1}, $1.15 \times 10^8 \pm 2.42 \times 10^7$ cells l^{-1} respectively), whereas picoeucaryotes increased (T0: $2.29 \times 10^6 \pm 1.04 \times 10^5$ cells l^{-1}, T1: $2.95 \times 10^6 \pm 1.47 \times 10^5$ cells l^{-1}, T4: $3.40 \times 10^6 \pm 2.92 \times 10^5$ cells l^{-1}, T6: $4.95 \times 10^6 \pm 1.35 \times 10^6$ cells l^{-1}). Heterotrophic bacteria counts decreased slightly from T1 to T6 (T1: $6.89 \times 10^9 \pm 8.3 \times 10^8$ cells l^{-1}, T6: $4.07 \times 10^9 \pm 2.9 \times 10^8$ cells l^{-1}).

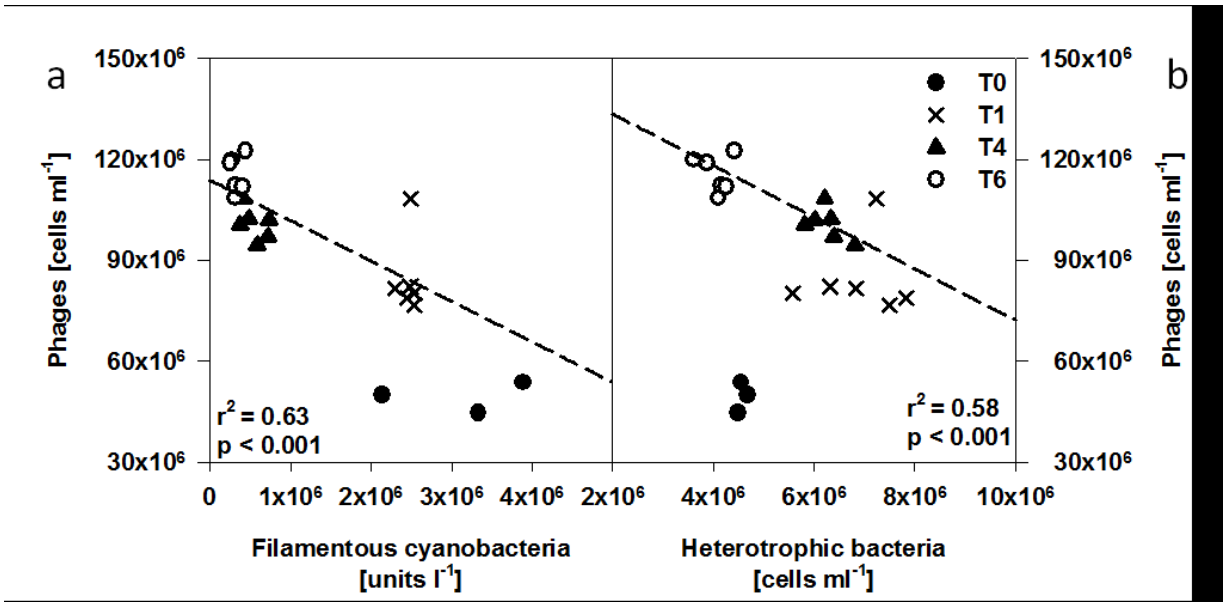


Fig. 25. Relationship between phages concentration and different plankton compounds during the experiment (T0 – filled circles, T1 – cross, T4 – triangle, T6 – open circle), regressions included data from T1, T4 and T6. a – Phages concentrations related to concentration of filamentous cyanobacteria; b – Phages concentrations related to heterotrophic bacteria concentrations.

In addition, increasing concentrations of phages or viruses were detected during the incubation (Fig. 25). A negative correlation was calculated between phages abundance and filamentous cyanobacteria as well as between phages and

heterotrophic bacteria for the experimental time ($R^2 = 0.63$ $p < 0.01$; $R^2 = 0.58$ $p < 0.01$, respectively).

The reduction of heterotrophic bacteria abundances was accompanied by declining POC, PON and POP concentrations in the size fraction < 2.7 µm during the incubation of six days (Table 10). POC:POP ratios were below the Redfield Ratio (Fig. 28). The picoplankton fraction dominated the DIP uptake rate at all sampling times (average 78 %). These rates significantly decreased from the start to the end of the incubation (Fig. 28). In contrast to the size fraction > 2.7 µm, the picoplankton size fraction only constituted 2 % of the C fixation and 6 % of the N_2 fixation.

Responses of the summer plankton community on elevated CO_2 concentrations

The addition of CO_2 gas resulted in higher $CO_{2(aq)}$ concentrations and decreasing pH values (Table 9). Due to biological consumption of CO_2 and its respiration, the CO_2 concentration in the bottles changed over time, shown as net change of pCO_2 in table 8 (T1: $R^2 = 0.96$ $p < 0.001$; T4: $R^2 = 0.89$ $p < 0.01$; T6: $R^2 = 0.89$ $p < 0.01$). Correlations between the pCO_2 net changes and initially installed pCO_2 (T1: $R^2 = 0.96$ $p < 0.001$; T4: $R^2 = 0.89$ $p < 0.01$; T6: $R^2 = 0.89$ $p < 0.01$) showed decreasing slopes from T1 to T6. Similar patterns could also be observed for dissolved CO_2 concentrations, while HCO_3^- concentrations were not related to increasing pCO_2 values (Table 9).

Table 9. Values of the carbonate system, pH, pCO_2 installed at the beginning (T0), pCO_2 measured at the particular sampling, net change of pCO_2 between the sampling and T0 - Δ pCO_2, net change of HCO_3^- between the sampling and T0, net change of CO_2 between the sampling and T0 at the different samplings (T0 - start, T1 – one day incubation, T4 – four days incubation, T6 – six days incubation).

	pH	pCO_2 at T0 [µatm]	pCO_2 [µatm]	ΔpCO_2 [µatm]	ΔHCO_3^- [µmol kg SW^{-1}]	ΔCO_2 [µmol kg SW^{-1}]
T0	8.32	210	210	-	-	-
T1	8.30	210	217	7	-50	0.2
	8.30	300	214	-86	-147	-3.5
	8.12	450	351	-99	-61	-3.8
	8.19	600	286	-314	-187	-12.5
	8.05	800	417	-383	-151	-15.3
	8.02	1000	461	-539	-121	-21.6
T4	8.43	210	194	-16	106	-1.1
	8.24	300	280	-20	-70	-1.2
	8.21	450	300	-150	-107	-6.5
	8.01	600	508	-92	-7	-4.3
	8.05	800	466	-334	-64	-14.2
	8.05	1000	480	-520	-56	-21.7
T6	8.21	210	278	68	27	2.7
	8.24	300	248	-52	-155	-2.1
	8.10	450	361	-89	-132	-3.5
	7.93	600	541	-59	-107	-2.2
	7.90	800	611	-189	-86	-7.6
	7.83	1000	699	-301	-131	-12.0

Inorganic nutrient concentrations (DIN, DIP) were low or below detection limit during the whole incubation experiment and showed no consistent pattern during the experiment (Table 10). In contrast, DON & NH_4^+ and DOP concentrations increased from T0 to T6 (Table 10). The concentrations of inorganic nutrients and

dissolved organic nutrients were not linked to changes in the pCO_2 level ($p > 0.1$) (Table 10).

Table 10 Concentrations of dissolved inorganic nutrients (DIN, DIP), dissolved organic nutrients (DON and NH_4^+), particulate organic matter of the size fractions > and < 2.7 µm (POC, PON, POP) as well as Chl*a* of the size fraction > and < 2.7 µm at the different sampling dates (T0, T1, T4, T6). Standard deviations calculated based on measurements from the different CO_2 settings.

	T0	T1	T4	T6
DIP [µmol l^{-1}]	0.01	0.01	0.01	0.01
DIN [µmol l^{-1}]	0.12 ± 0.02	0.02 ± 0.03	0.14 ± 0.02	0.11 ± 0.04
DOP [µmol l^{-1}]	0.23 ± 0.01	0.25 ± 0.01	0.26 ± 0.01	0.28 ± 0.01
DON & NH_4^+ [µmol l^{-1}]	15.53 ± 0.19	19.28 ± 0.81	24.08 ± 2.29	28.04 ± 2.50
POC > 2.7 µm [µmol l^{-1}]	208.2 ± 14.9	213.6 ± 12.3	193.3 ± 18.4	164.5 ± 9.9
POC < 2.7 µm [µmol l^{-1}]	22.6 ± 2.1	17.9 ± 1.7	15.5 ± 0.7	8.4 ± 4.2
PON > 2.7 µm [µmol l^{-1}]	30.2 ± 0.6	32.8 ± 1.1	30.8 ± 2.8	26.8 ± 1.6
PON < 2.7 µm [µmol l^{-1}]	4.4 ± 0.5	3.2 ± 0.3	2.7 ± 0.2	1.7 ± 0.1
POP > 2.7 µm [µmol l^{-1}]	0.90 ± 0.01	0.88 ± 0.06	0.79 ± 0.06	0.84 ± 0.08
POP < 2.7 µm [µmol l^{-1}]	0.26 ± 0.01	0.25 ± 0.01	0.25 ± 0.02	0.19 ± 0.02
Chl*a* > 2.7 µm [µg l^{-1}]	20.0 ± 1.7	18.0 ± 3.3	11.2 ± 1.8	10.5 ± 2.4
Chl*a* < 2.7 µm [µg l^{-1}]	0.6 ± 0.1	0.3 ± 0.0	0.4 ± 0.1	0.6 ± 0.2

Responses of the summer plankton community on elevated CO_2 concentrations - Size fraction > 2.7 µm

The species composition of the size fraction > 2.7 µm changed scarcely towards CO_2 concentrations ($p > 0.1$), exclusively *Aphanizomenon* sp. showed slightly higher abundances with elevated CO_2 concentrations at T4 ($R^2 = 0.62$ $p < 0.1$).

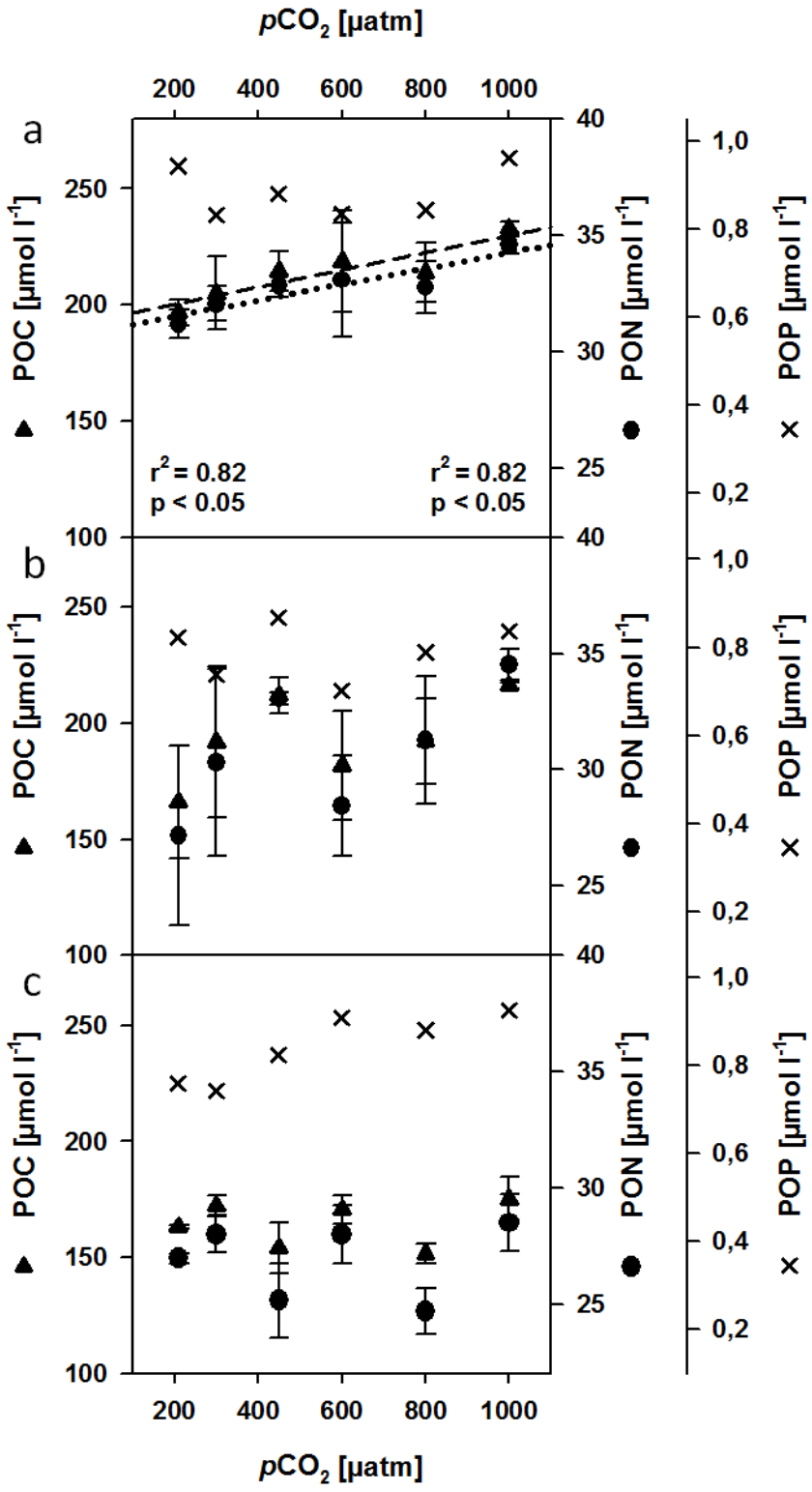


Fig. 26 Concentrations of particulate organic matter in the fraction > 2.7 µm, (POC – triangle, PON – circle, POP – cross) at different CO_2 concentrations. a – after one day incubation, linear regression for POC (dashed line) and PON (dotted line); b –after 4 days of incubation, c – after 6 days of incubation.

In contrast several biomass characteristics and activities changed with respect to CO_2 concentrations. At T1 and T4 increasing C fixation rates and Chl*a* concentrations along the gradient of CO_2 concentrations indicated enhanced CO_2 utilization (Chl*a* and CO_2: T1: $R^2 = 0.79$ $p < 0.05$; T4: $R^2 = 0.76$ $p < 0.05$) (Fig. 24b, 24c). While at T6, the positive impact of enhanced CO_2 concentrations on Chl*a* values was not detected anymore (Fig. 24d). Until T1, POC and PON, increased along the gradient of elevated CO_2 concentrations (Fig. 26a). Significant correlations of $R^2 = 0.82$ $p < 0.01$ for POC and $R^2 = 0.82$ $p < 0.01$ for PON were found.

The increasing POC concentrations at elevated CO_2 concentrations can be partly explained by enhanced C fixation rates at T1 ($R^2 = 0.88$ $p < 0.01$) (Fig. 27a). By contrast the increase of PON was not accompanied by elevated N_2 fixation rates (Fig. 27a). Therefore at T1 an increase in the ratio of C:N_2 fixation rates was detected along the gradient of pCO_2 levels (Fig. 29a). The relationship between CO_2 and POC, PON, and C fixation rates disappeared at T4 and T6 (Fig. 26b, 26c, 27b, 27c). No changes in the POC:PON, ratio were observed along the CO_2 gradient at T4 and T6.

Patterns of CO_2 dependent POP changes differed from those of POC (Fig. 26). Only at T6 a positive correlation between the CO_2 concentrations and the POP content was observed ($R^2 = 0.81$ $p < 0.05$). However, changes of the POP concentration were only small (less than 10 %).

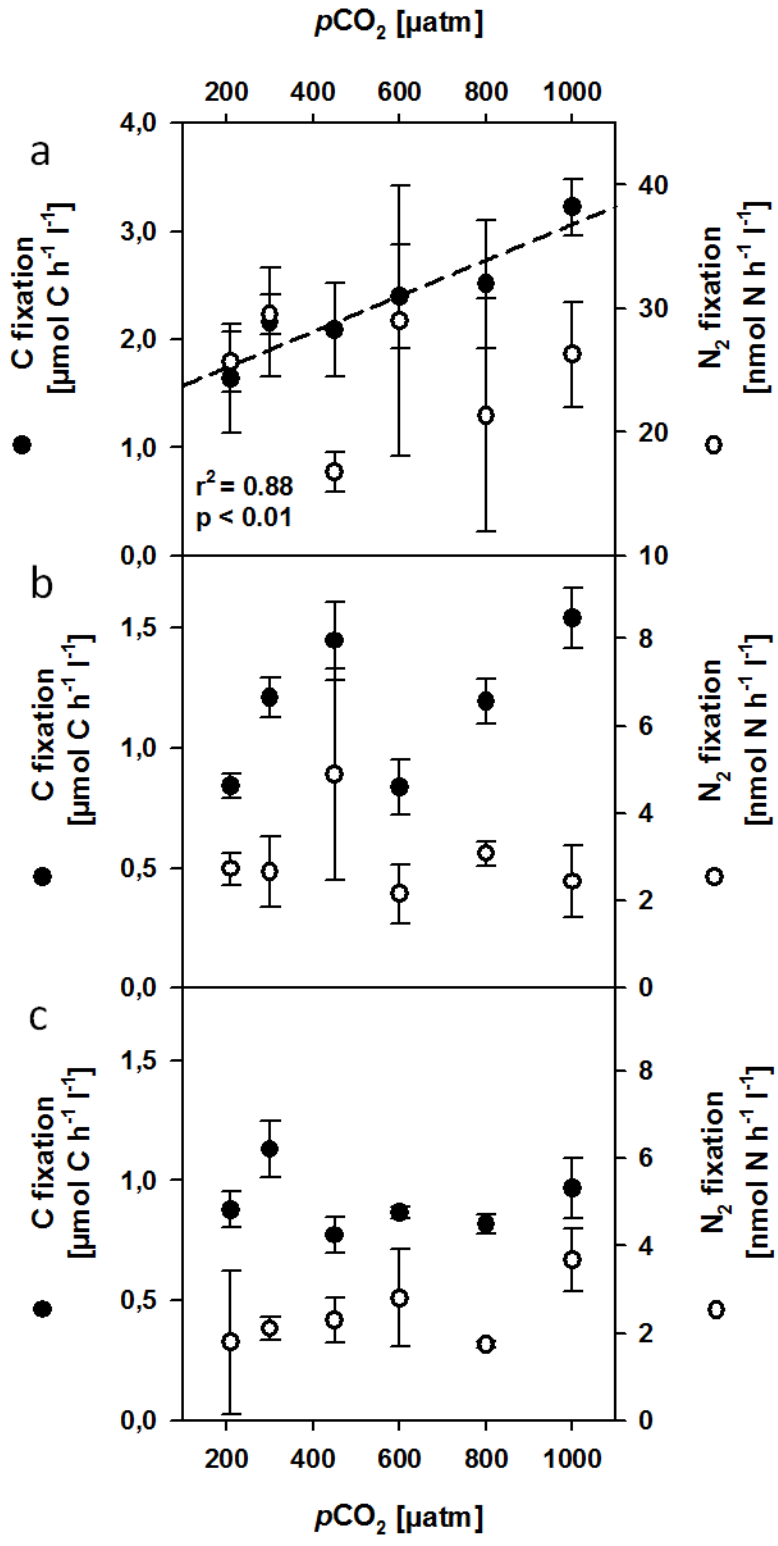


Fig. 27 Responses of C fixation and N_2 fixation rates of the size fraction > 2.7 µm on different pCO_2 levels; C fixation – filled circle, N_2 fixation – open circle. a – after one day of incubation (T1); b – after four days of incubation (T4); C – after six days of incubation (T6).

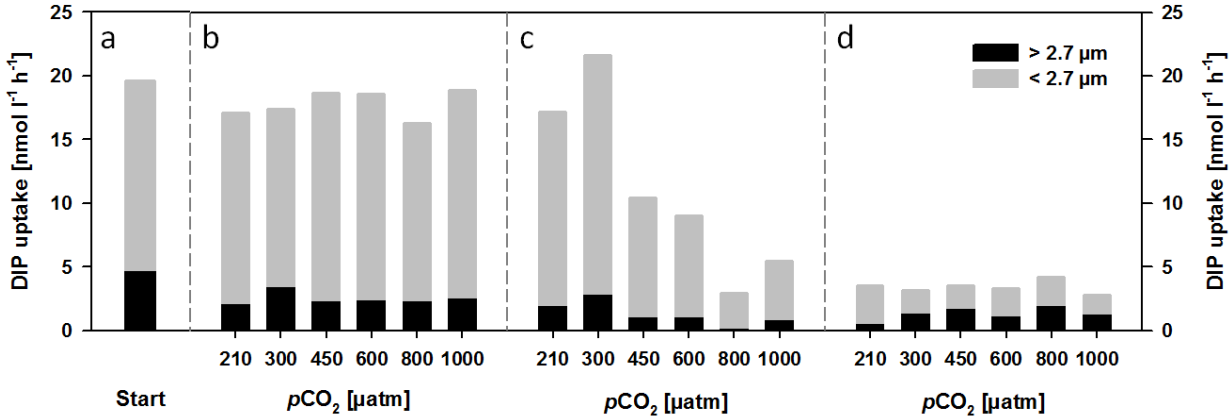


Fig. 28 DIP uptake rate divided in size classes > 2.7 µm (black stack) and < 2.7 µm (grey stack) related to different pCO_2 levels in the course of the experiment. A – starting conditions (T0); black ; B – after one day of incubation; C – after four days of incubation, d – after six days of incubation.

Similar to the N_2 fixation rates, DIP uptake rate did not change significantly along increasing CO_2 concentrations (Fig. 28, $p > 0.1$). The values of DIP uptake rates at T1 were similar to the initial value (Fig. 28a, 28b). At T4 and T6 the DIP uptake rates were reduced (Fig. 28c, 28d).

Responses of the summer plankton community on elevated CO_2 concentrations - Size fraction < 2.7 µm

No species or group related response towards elevated CO_2 concentrations was observed for the picoplankton as well as phages/virues. No CO_2 related changes in POC, PON and POP concentrations were detected. In contrast cell specific bacterial production (thymidine incorporation) increased significantly with CO_2 at T1 and at T4 (the value at pCO_2 800 µatm was not included, due to high activities in the blank). At T6 this influence of elevated CO_2 concentrations towards cell specific bacterial production was not found anymore (Fig. 29b).

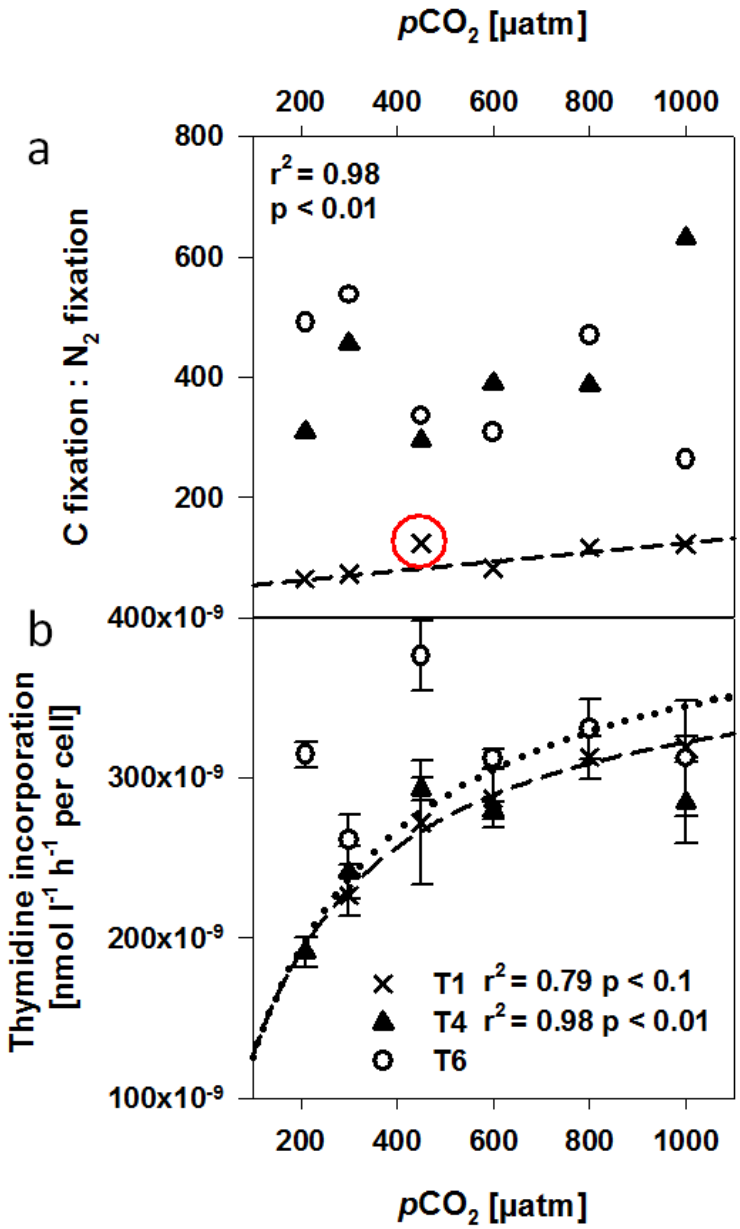


Fig. 29 a – Ratio of C fixation and N_2 fixation rates for the size fraction > 2.7 µm at different pCO_2 levels and at T1 (cross), T4 (triangle), T6 (circle); ratio at T1 – dashed line (data point inside the red circle was excluded). b – thymidine incorporation rate at different pCO_2 levels at T1 (cross), T4 (triangle), T6 (circle); regressions for thymidine incorporation at T1 (dashed line) and T4 (dotted line).

Bacterial production rates at T6 were in the range of maximum values from T1 and T4. At T1 and T4 the correlation between cell specific bacterial production and CO_2 concentrations was not linear and showed a Monod kinetic rising to an optimum (T1: $R^2 = 0.98$ $p < 0.01$; T4: $R^2 = 0.79$ $p < 0.05$) (Fig. 29b). Compared to the bacterial

production the DIP uptake rates showed a contrary behaviour. At T1 CO_2 alteration did not affect DIP uptake rates ($p > 0.1$). However at T4 DIP uptake rates of the size fraction < 2.7 µm decreased (Fig. 27c, $R^2 = 0.78$ $p < 0.5$). This effect disappeared again at T6 ($p > 0.1$) (Fig. 28d). The increasing effect of bacterial production during the incubation was also shown by decreasing values within the ratio C fixation : thymidine incroporation (T0: 4.15 ± 0.69, T1:n 1.2 ± 0.37, T4: 0.76 ± 0.16, T6: 0.71 ± 0.17).

3.3.2. Discussion

In the Baltic Sea, the occurrence of diazotrophic cyanobacteria is a natural phenomenon. Due to the undersaturation of their intracellular carbon requirements, it can be assumed that they react sensitively to pCO_2 elevation (Barcelos e Ramos et al. 2007, Levitan et al. 2007, Czerny et al. 2009, Levitan et al. 2010). Previous experiments indicated species specific responses for autotrophic activity to changing CO_2 concentrations, i.e. increasing N_2 fixation rates for *Trichodesmium* sp. and decreasing rates for *N. spumigena*, (Barcelos e Ramos et al. 2007, Czerny et al. 2009). We suppose that a complementation of these results with those of a field community provide more specific information about the response of cyanobacteria and changes of their metabolic activities to future conditions. Effects of increasing pCO_2 were investigated at specific growth conditions – the progression of a diazotrophic bloom including the switch from the active to the senescent/degradation state.

Bloom characteristics throughout the experiments

The described changes within metabolic activities of autotrophic and heterotrophic organisms along the incubation period can be classified into three stages:

-Stage 1, observed at the start and the first day of incubation, characterized by similar levels of C fixation and N_2 fixation rates. Nevertheless the ratio between C:N fixation (> 100), already indicates reduced N_2 fixation compared to ratios reported for growing filamentous cyanobacteria (< 10) (Gallon et al. 2002).

-Stage 2 occurred after four days of incubation (T4). Compared to stage 1 it was characterized by reduced POM concentrations, elevated DOM concentrations, lower C fixation and totally reduced N_2 fixation rates. These were indications for decreasing autotrophic processes, for example lower values for the ratio C fixation to thymidine incorporation.

-Stage 3, at day six (T6), was dominated by high heterotrophic activities. It was specified by: highest phages concentrations and maximum thymidine incorporation

rates. Comparing with previous investigations these were still in the range of natural occurring rates in surface waters of the Baltic Sea (e.g. Kuparinen & Heinänen 1993). Furthermore highest DOM and low POM concentrations of the size fraction > 2.7 µm indicated lower autotrophic activity. The diazotrophic cyanobacteria exhibited decreased C and N_2 fixation rates compared to stage 1 and to former *in situ* measurements (Evans et al. 2000, Gallon et al. 2002).

This short classification of the different stages and the reasons for the ongoing senescence of diazotrophic cyanobacteria will be explained in more detail in the following paragraphs.

Abiotic conditions, applied during the here presented study, were similar to *in situ* conditions. In surface waters equal nutrient concentrations were observed during the growth season (Wasmund et al. 2005, Nausch et al. 2008, Nausch et al. 2009). No nutrients were added in the experiments, therefore DIN and DIP concentrations were depleted right from the start. However, the ability of filamentous cyanobacteria to fix N_2, avoided an insufficient nitrogen supply for the community, observed in constant or even decreasing POC:PON values in the size fraction > 2.7 µm (Table 10). The detected phosphorus limitation is a characteristic feature for the late summer phytoplankton bloom in the Baltic Sea (Larsson et al. 2001). It was evident in the experiments by low cellular phosphorus contents, which in turn caused POC:POP and PON:POP ratios above the Redfield ratio as observed for the fraction of filamentous cyanobacteria. Thus, inorganic phosphorus deficiency may have caused the dieback of diazotrophs during this study comparable to that observed for surface waters in previous studies (Grönlund et al. 1996). The continuing decline of Chl*a* concentrations from T0 to T6 supported this assumption and indicated that the filamentous cyanobacteria population was already in a stagnant stage at the first day of incubation. This was also mirrored by decreasing C and N_2 fixation rates of the filamentous cyanobacteria during the subsequent days. The decline of the C fixation rate occurred three days later (T4) than the reduction of N_2 fixation, which already started after one day of incubation. The straight reaction of N_2 fixation might be explained by the high sensitivity of N_2 fixation to DIP availability. Previous studies showed that low phosphorus availability and low light are supposed to suppress

heterocyst formation with a subsequent decrease in N_2 fixation activity (Healey 1973, Thompson et al. 1994).

Among the three filamentous cyanobacteria species, which are characteristic for this season and place of sampling (Kahru et al. 1994, Wasmund et al. 2005), the biomass of *Aphanizomenon* sp. decreased already after 24 h of incubation, while that of *N. spumigena* remained at a constant level in all treatments independently from the CO_2 level (Fig. 23). Obviously the prefiltration influenced the species composition and might have also excluded small sized *Aphanizomenon* sp. and *Anabaena* sp. colonies as well. Further one we suppose that different P acquisition strategies of *N. spumigena* and *Aphanizomenon* sp explain the decline of *Aphanizomenon* sp. counts during the experiment. While *N. spumigena* has been revealed to grow under phosphorus deplete conditions by using DOP as a phosphorus source, *Aphanizomenon* sp. relies on DIP solely (Vahtera et al. 2007b). In consequence, the DIP depleted conditions during this state of bloom development were likely less unfavourable for *Aphanizomenon* sp.. In addition also the applied light and temperature conditions might have promoted the growth of *N. spumigena*. Still increasing concentrations of DOP are probably due to higher degradation rates of POP than DOP utilization.

In addition to nutrient availability, viral infection contributes to the overall extent of the cyanobacterial population and influences the composition of the phytoplankton community (e.g Bratbak et al. 1993). Because the abundances of viruses/ phages increased during the experiment, they likely participated to the decline of filamentous cyanobacteria and heterotrophic bacteria in our study, too (Fig. 24). For *Nodularia* sp. 17 different host specific viruses are identified (Jenkins & Hayes 2006). However, it has been suggested that the impact of phages is higher in enclosures than in the natural environment (Larsen et al. 2001), and may thus lead to an overestimation of the role of phages for cell lysis and degradation in ecosystems, applying the here presented results to a natural environment.

CO_2 effects on autotrophic processes

At optimal growth conditions (optimal temperatures, repleted macro- and micronutrient concentrations) autotrophic diazotrophic phytoplankton species are

supposed to respond with enhanced C and N_2 fixation rates on elevated CO_2 concentrations (Riebesell 2004, Barcelos e Ramos et al. 2007, Levitan et al. 2007, Kranz et al. 2009). Until now most studies, investigating the response of diazotrophic phytoplankton to CO_2, focused on *Trichodesmium* sp..

Here, CO_2 effects on a Baltic Sea cyanobacterial community, dominated by *Aphanizomenon sp. and N. spumigena,* were examined under natural conditions. As already mentioned incubations were done at *in situ* light and temperature conditions as well as at *in situ* nutrient concentrations. Despite growth of most of the phytoplankton is not limited by DIC availability at present time; though a higher CO_2 concentration was shown to increase their C fixation rates (Raven 1991, Riebesell 2004). Especially filamentous cyanobacteria are assumed to benefit from elevated CO_2 concentrations in the water column, due to the low affinity of the cyanobacterial photosynthetic enzyme RuBisCo to CO_2 ($Km > 100\ \mu mol\ kg^{-1}$) and its low half saturation constant ($20–150\ \mu mol\ kg^{-1}$) (Badger et al. 1998). At present CO_2 concentrations CCMs facilitate the CO_2 supply at the catalytic side of RuBisCO. Increased CO_2 concentrations might ensue to a decreasing demand of energy and nutrients for protein synthesis to generate DIC transporter and for maintaining the transporters working (Barcelos e Ramos et al. 2007). We assume this reduced nutrient need, because even under low nutrient conditions, as encountered during this study, we observed stimulated C fixation by enhanced pCO_2 (Fig. 25).

Several studies reported that an increase of the CO_2 concentration would increase N_2 fixation rates, too (Barcelos e Ramos et al. 2007, Hutchins et al. 2007, Levitan et al. 2007). Lower CCM activity is assumed to lead to escalating energy sources available as substrates for the high energy demanding N_2 fixation and subsequent growth. These observations were derived from studies with sufficient DIP supply, whereas at depleted DIP conditions we could not find any correlation between N_2 fixation and increasing CO_2 concentrations. Therefore, we deduce that DIP depletion can disrupt this mechanism and the availability of DIP probably highly influences the impact of enhanced CO_2 concentrations. Exhaustion of DIP provokes a reduction of cellular phosphorus, essential for energy carrying phosphate compounds (ATP, NADPH), nucleic acids, essential coenzymes and phospholipids

(Marschner 1995). It can be assumed that the DIP need, necessary to sustain the forgoing production of nitrogenise, might not be satisfied, resulting in an inhibition/stagnation of N_2 fixation during this incubation. Czerny et al. (2009) even reported reduced cell division and lower N_2 fixation rates at high CO_2 for *N. spumigena*, although sufficiently high nutrient concentrations were applied. But in contrast to our study, Czerny et al. (2009) included constant mixing of cells, indicating that high turbulence negatively co-affects N_2 fixation. This assumption is supported by studies, which explored, that aggregation of diazotrophic cyanobacteria, in the absence of turbulence, leads to higher C and N_2 fixation as well as enhanced nitrogen storage e.g. (Ohmori et al. 1992). During our investigation, the positive impact of increasing CO_2 levels on autotrophic growth, especially C fixation rates, was also indicated by elevated Chl*a* and POC concentrations along the CO_2 gradient at stage 1. Thus, the impact of CO_2 elevation on filamentous cyanobacteria is measureable as long as the abiotic conditions provide a sufficient growth and reproduction environment. As soon as growth is restricted, possibly due to the described DIP limitation, the positive influence of enhanced CO_2 concentrations disappears.

CO_2 effects on heterotrophic processes

Late summer phytoplankton blooms in the Baltic Sea have been identified as hot spots of microbial activity (Hoppe 1981, Heinänen et al. 1995). During the here presented experimental study, we observed that cell specific heterotrophic bacterial production rates increased with CO_2 concentrations at stage 1 and 2. It proceeded in parallel to higher C fixation rates and POC concentrations of the autotrophic cyanobacterial community at high pCO_2 levels. Therefore, we assume that the reaction of heterotrophic bacterial activities was an indirect response on rising substrate concentrations in particulate or dissolved form (Grossart et al. 2006, Allgaier et al. 2008).

Further we assume, that enhanced heterotrophic bacterial production might be combined with higher phosphorus utilization by heterotrophic bacteria. However, this was not indicated during stage 1. The picoplankton in stage 2 and 3 showed a reduced phosphorus uptake rate, measured along the gradient of CO_2 concentrations

(Fig. 25). Probably changes within the microenvironment, means total depletion of DIP, are the reasons for that. Measured changes in bulk DIP and DOP concentrations presumably did not affected DIP uptake rates. Nevertheless, phosphorus limitation might be also prevent the heterotrophic thymidine incorporation to be linear increasing. We assume, that due to insufficient phosphorus supply the thymidine incorporation followed a Mono-Kinetic pattern, reaching an optimum at high CO_2 levels during stage 1 and 2 (Fig. 28b). Conversely this implies that repleted DIP concentrations would lead to a linear rise of the thymidine incorporation along increasing CO_2 concentrations.

Development of phages and viruses appeared to be independent of elevated pCO_2 levels during the investigation. Larsen et al. (2008) showed reduced viral abundance of different virus types at high CO_2 concentrations. Here, this effect was not observed, as we did not discriminate between certain groups of viruses.

To sum up, during stage 1 and 2 a positive correlation between the CO_2 concentration and heterotrophic bacterial activity was detected. Nevertheless, the response diminished with decreasing autotrophic activity. We assume an accelerated DIP limitation, low DON and DOC supplies at high CO_2 concentrations, probably limited heterotrophic activity. No response of viral abundance related to the CO_2 concentrations was found.

Implications for the Baltic Sea

Our results showed that elevated CO_2 concentrations may enhance C fixation in the surface water of the Baltic Sea during summer when plankton organisms are growing under present nutrient conditions to a certain extend. Thus, a stimulated CO_2 flux from the atmosphere into the water column can be expected in this season. Laboratory studies showed that under nutrient repleted conditions this will result in enhanced primary production and also higher N_2 fixation rates (Barcelos e Ramos et al. 2007, Levitan et al. 2007, Rost et al. 2008). Assuming, that enhanced C and N_2 fixation are associated with a higher DIP demand, DIP will be consumed faster and DIP limitation might be predated followed by an earlier collapsing of the bloom. Furthermore, an additional bloom event can occur based on regenerated nutrients.

Nevertheless, the here presented study also showed, that the effect of enhanced CO_2 might be anticipated due to insufficient autotrophic growth conditions. We presume that depleted DIP concentrations circumvent a stimulating impact on filamtentous cyanobacteria. Therefore, it can be suggested, that the earlier DIP depletion, due to enhanced primary production, may hamper N_2 fixation at the end of summer in the future. Similar impacts on C fixation probably also occur later in the progression, means the stimulation of autotrophic C fixation will disappear, too. Thus, occasional measurements during the bloom may lead to over- or underestimations concerning the influence of enhanced CO_2 concentrations within the filamentous cyanobacterial bloom.

Phosphorus is assumed to and was also shown during the here presented investigations to hold a key position whether enhanced pCO_2 enhances autotrophic growth and N_2 fixation. Uncertainties exist when and how sources of phosphorus in the Baltic Sea will change in the future. Predictions and calculations of biological responses get more complicated, due to contrary calculations of rivers discharge and phosphorus released by anoxic sediments under future climate conditions (Graham 2004, Conley et al. 2009). Definitely the deep anoxic basins inside the Baltic Sea form an enormous DIP reservoir. Enhanced vertical mixing (up and down welling processes) would provide huge amounts of inorganic nutrients, which likely change the progression of the diazotrophic bloom and postpone DIP limitation (Nausch et al. 2009). On the other hand, the predicted increase of temperature will lead to a stronger vertical stratification and hamper mixing events. Nevertheless, it is hard to speculate about future convection patterns and therefore also about the impact of up and down welling processes on the nutrient concentrations in the future Baltic Sea.

At high CO_2 levels, elevated POC build-up, due to enhanced primary production of filamentous cyanobacteria, likely stimulates heterotrophic growth. In case of strong autotrophic phosphorus deficiency this POC build up might not take place and the autotrophic nutrient limitation may also circumvent enhanced heterotrophic activity. We assume the impact of enhanced CO_2 concentrations on heterotrophic bacteria is mainly mediated by autotrophs. During this study acidification of the water column showed no direct effects on heterotrophic bacteria.

In general, the group of viruses and phages can be assumed to be unaffected by elevated CO_2 concentrations. Nevertheless, certain groups might reduce or enhance activities, probably also due to indirect responses to enhanced autotrophic growth.

4. CONCLUSIONS

4.1. NATURAL VERSUS ANTHROPOGENIC REGULATION OF FILAMENTOUS CYANOBACTERIA IN THE BALTIC SEA

The summer phytoplankton bloom in the Baltic Sea mainly consists of filamentous cyanobacteria. Bloom duration and occurrence are well described and follow similar patterns every year (e.g. Kononen & Leppänen 1996). The investigations presented herein focussed on the adaptations and responses of filamentous diazotrophic cyanobacteria typical in the Baltic Sea (Howarth et al. 1988). Mainly the two dominant diazotrophic cyanobacteria species were studied, *N. spumigena* and *Aphanizomenon* sp. (Wasmund et al. 2005). The concentrations of these species start to increase at the end of April, beginning of May. During this time the NO_2^-/NO_3^- concentrations are below the detection limit and the phytoplankton community is assumed to be nitrogen limited, while sufficient DIP concentrations of 0.1 μmol l^{-1} are measured. This is considered to be essential for widespread bloom development to occur. Within one to two months the DIP is depleted due to consumption by phyto- and bacterioplankton, especially cyanobacteria, which are known to store enormous amounts of DIP inside the cell (Larsson et al. 2001). The following bloom development with widespread surface scums relies on this intracellular storage – so called polyphosphates. At the end of the bloom the intracellular pool is exhausted, and the cyanobacterial bloom is assumed to collapse due to DIP limitation. This development was also shown during the diurnal field observations presented in chapter 3.1. But how this progression might be influenced by future increasing pCO_2 levels, predicted for the end of this century, has not been investigated until now.

We revealed that increasing CO_2 concentrations enhance primary production and N_2 fixation as long as DIP is detectable in the water column or media. The laboratory experiment showed a clear relationship between higher pCO_2 levels and

elevated C fixation and N_2 fixation rates. Due to the enhanced incorporation of carbon and nitrogen the demand of phosphorus also raised which resulted in increasing DIP affinities of filamentous cyanobacteria. Similar effects, less pronounced, were observed during the field study. While C fixation rates were still positively correlated to increasing CO_2 concentration, N_2 fixation rates were not affected. Concurrently, no influence on DIP incorporation by filamentous cyanobacteria was detected. Therefore, we hypothesize that the N_2 fixation requires more phosphorus than C fixation and/or insufficient DIP supply represses the stimulation of N_2 fixation. It seems to be that C fixation relied for a certain time on the intracellularly stored phosphorus, while N_2 fixation probably utilized disposable DIP. We also showed that as soon as DIP concentrations drastically diminished the positive relationship of high CO_2 levels to autotrophic mechanisms disappeared. During the senescence of the bloom the limiting effect of missing nutrients exceeded the stimulating impulse of increasing CO_2 concentrations. This highlights the central role of phosphorus in maintaining autotrophic growth and especially the essential character of DIP in a diazotrophic bloom, when nitrogen is not limiting.

If due to upwelling events additional DIP is introduced, the examined positive effect of higher CO_2 concentrations will be enhanced or if not visible before, observed. Due to spatial and temporal variations of upwelling events, we propose that also the effects of enhanced CO_2 concentrations might be restricted to certain regions and seasons.

In general, the growth stimulation of C and N_2 fixation by elevated CO_2 concentrations along the gradient of 280 to 780 μatm was measured with a factor of 2 to 4 which approximates the same range as daily variations as shown during the study presented in chapter 3.1. Therefore, investigations designed to study impacts of ocean acidification have to sample at fixed times. Different time points would lead to misinterpretation and the discrimination between present and future variations in autotrophic growth would be impossible.

4.2. NATURAL VERSUS ANTHROPOGENIC REGULATION OF NANO- AND PICOPLANKTON IN THE BALTIC SEA

Alongside the filamentous cyanobacteria, unicellular cyanobacteria also exist in the Baltic Sea. Active N_2 fixation has not been verified for these organisms. Current investigations found high concentrations of nifH gene expressions dominated by unicellular cyanobacteria, nevertheless rate measurements to verify diazotrophy by these cells are missing (Farnelid et al. 2011). High densities of the cyanobacteria *Synechoccocus* spp occur with increasing wind velocities and decreasing irradiance. This specie dominates the autotrophic picoplankton in summer. *Synechoccocus* spp. dominated C fixation rates during a bloom of filamentous cyanobacteria, if the environmental conditions, like wind etc., did not fit optimal the prerequisites of *N. spumigena.*, respectively *Aphanizomenon* sp.. No direct effects of enhanced CO_2 concentrations on *Synechoccocus* spp. were found.

Additionally, filamentous diazotrophic cyanobacteria also provide optimal growth conditions for heterotrophic bacteria. Currently wide-spread late summer surface blooms are known to be a hot spot of bacterial activity (Hoppe 1981, Heinänänen et al. 1995). The diurnal study presented herein confirmed this observation. Surprisingly measurements of heterotrophic metabolic activities also showed daily variations. In contrast to autotrophic processes, we examined slightly higher heterotrophic activity during the night. Anyhow variations during the day and within a few days, showed again how fluctuating metabolic rates can be and how important similar sampling times are. During the laboratory experiment and the field study heterotrophic activities are mainly indirectly altered by enhanced CO_2 concentrations. Specifically, higher POM concentrations, due to stimulated primary production based on higher CO_2 levels, probably also indirectly facilitate heterotrophic activity and therefore accelerate degradation processes. Stimulated thymidine incorporation rates with enhanced CO_2 concentrations were measured when autotrophic C fixation was affected by CO_2 alteration and DIP was available. CO_2 related changes in concentration or size distribution within the heterotrophic bacteria were not detectable. Like for autotrophic cyanobacteria in the Baltic Sea, we

assume that the observed alterations of heterotrophic activity are driven by a combined effect of pCO_2 and DIP.

Rough estimates of phage development during the field study showed no indirect or direct influence of pCO_2 enhancement for this group. Nevertheless, more detailed distinction might provide different information. The bulk phage community was not affected by changes in the carbon system.

4.3. POSSIBLE SCENARIOS FOR FUTURE CYANOBACTERIA BLOOMS IN THE BALTIC SEA

As mentioned before, the surface scums of filamentous cyanobacteria provide several habitats for different trophic levels. Therefore, changes in metabolic rates of filamentous cyanobacteria affect links and connections within the food web. Diurnal observations showed strong dependencies between heterotrophic and autotrophic bacterial activity. For example, it can be presumed that lower nutrient uptake rates by filamentous cyanobacteria during the night allow the heterotrophs to increase their metabolism.

These strong connections might change under future environmental conditions. Obviously, cyanobacterial response to enhanced CO_2 concentrations is mediated by nutrient supply. During the studies presented herein strong dependencies on DIP concentrations were observed. Optimal growth conditions, sufficient DIP concentrations, together with rising CO_2 concentrations stimulate C and N_2 fixation rates of filamentous cyanobacteria. Therefore also the substrates for heterotrophic bacterial growth, POM and DOM, increase. It can be assumed that the observed positive correlation of heterotrophic activity (enhanced thymidine incorporation) is not a direct effect of rising pCO_2 levels, rather it is indirectly affected by enhanced substrate concentrations. On the other hand, insufficient DIP supply suppresses the expected stimulation of C fixation and N_2 fixation rates due to rising CO_2 concentrations inside a filamentous cyanobacterial bloom. Insufficient DIP supply restricts the build-up of more POM, which would act as a substrate for heterotrophic

bacteria. In summary, three scenarios can be proposed for the future with respect to changes in DIP and CO_2 concentrations (Fig. 30).

A – Neglecting future changes in CO_2 concentrations but assuming enhanced nutrient input, due to changes in water mass circulation, would stimulate diazotrophic growth, means higher DIP incorporation, C and N_2 fixation rates. Phosphorus is known to be the main limiting nutrient for diazotrophic growth. Further on increasing DIP as well as higher POM concentrations would result in higher heterotrophic bacterial activity, too.

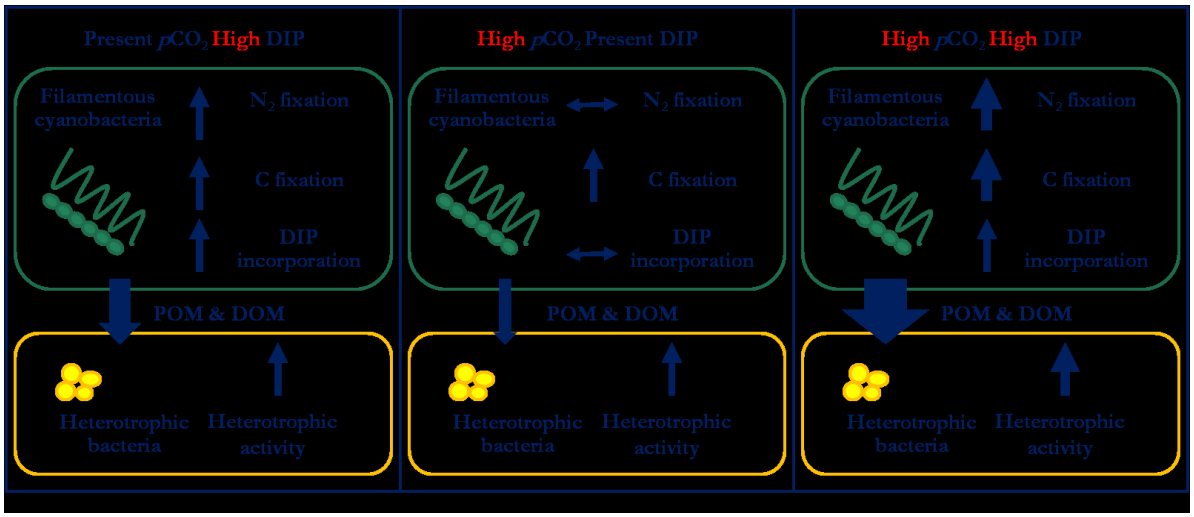


Fig. 30. Impacts and influences of changing pCO2 and DIP concentrations on filamentous diazotrophic cyanobacteria and heterotrophic bacteria. The arrows indicate stable (↔)or enhanced activity(↑). The size of the arrows indicates the magnitude of alteration.

B – higher pCO_2 levels and no changes in DIP concentrations would initially also generate higher C fixation rates of filamentous cyanobacteria but restriction by DIP availability would hamper a prolonging growth period. Analyzes presented here, showed that increasing CO_2 concentrations hardly stimulate N_2 fixation if DIP concentrations are low. Like filamentous cyanobacteria, heterotrophic bacteria would also be stimulated only in the short term. While for autotrophic C fixation several sources exist, which will never be depleted and only differ in "utilization costs", the P sources are restricted.

C – in the case of higher CO_2 and DIP concentrations, a prolonging growth period, higher actual C and N_2 fixation rates, for diazotrophs can be assumed. Optimal growth conditions circumvent inhibition of POM build up. High $CO_{2(aq)}$ concentrations result in reduced CCM activity, diffusive CO_2 support for the photosynthetic enzyme RuBisCo, more energy can be attributed for N_2 fixation and increasing DIP concentrations positively affect the POP build up. But the enhanced autotrophic growth not only results in higher POM concentrations, more DOM is excreted. In the end the enhanced POM and DOM concentrations at high CO_2 and DIP concentrations provide optimal growth conditions for the heterotrophic bacterioplankton. Therefore, high pCO_2 levels would indirectly lead to higher heterotrophic growth.

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6. APPENDIX I – CHAPTER 3.1

Date	Time	depth [m]	T	S	pCO_2 [µatm]	Chl*a* [mg/m³] total	Phaeo [mg/m³] total	Chl*a* [mg/m³] >10 µm	Phaeo [mg/m³] >10 µm	$NO_{2/3}$ [µmol/l]	NO_2 [µmol/l]
12. Jul. 08	06:00	3	17.7	7.5	172	5.01	1.19	1.75	0.34	0.36	0.005
12. Jul. 08	10:00	3	17.7	7.5		6.17	0.60	1.29	0.43	0.18	0
12. Jul. 08	14:00	3	17.9	7.5	165	5.96	0.64	1.23	0.38	0.435	0.005
12. Jul. 08	18:00	3	18.0	7.5		5.28	0.86	0.98	0.32	0.03	0
12. Jul. 08	22:00	3	18.0	7.4	148	6.36	0.95	1.16	0.34		
13. Jul. 08	06:00	3	17.8	7.4	154	6.04	1.02	1.25	0.54	0.04	0.01
13. Jul. 08	10:00	3	17.7	7.4		5.71	0.85	0.68	0.30	0.085	0
15. Jul. 08	06:00	3	17.5	7.4	179	5.11	0.65	0.61	0.28	0.08	0.01
15. Jul. 08	10:00	3	17.5	7.4	182	5.54	0.64	1.26	0.42	0.085	0.01
15. Jul. 08	14:00	3	17.6	7.4	170	6.18	0.32	1.74	0.54	0.115	0.01
15. Jul. 08	18:00	3	17.8	7.4		5.92	0.72	1.77	0.45	0.17	0.005
15. Jul. 08	22:00	3	17.7	7.4	159	6.31	0.78	2.56	0.65	0.09	0.005
16. Jul. 08	06:00	3	17.6	7.4	179	5.75	0.95	1.67	0.51	0.12	0
16. Jul. 08	10:00	3	17.7	7.4		6.51	1.02	1.28	0.48	0.07	0.01
18. Jul. 08	10:00	3	17.6	7.4		5.29	0.75	1.30	0.46	0.155	0.025
18. Jul. 08	14:00	3	18.0	7.4		5.71	0.67	1.03	0.36	0.095	0.02
18. Jul. 08	18:00	3	17.9	7.4	154	7.27	0.89	2.46	0.57	0.095	0.03
18. Jul. 08	22:00	3	17.9	7.4	152	6.99	1.15	2.71	0.72	0.085	0.025
19. Jul. 08	06:00	3	17.7	7.4	164	6.37	1.02	2.11	0.50	0.055	0.02
19. Jul. 08	10:00	3	17.8	7.3	153	8.36	0.70	2.51	0.59	0.055	0.02

Date	Time	NO_3 [µmol/l]	PO_4 [µmol/l]	SiO_4 [µmol/l]	P-uptake [nmol/l h]	P-uptake >10 µm [nmol/l h]	DOP [µmol/l]	POP [µmol/l]	POP >10 µm [µmol/l]	N fix <10 [nmol l^{-1} h^{-1}]
12. Jul. 08	06:00	0.355	0.13	14.1	4.93	1.43	0.38	0.32	0.11	1.57
12. Jul. 08	10:00	0.18	0.11	15	5.05	1.43	0.33	0.44	0.2	1.69
12. Jul. 08	14:00	0.43	0.12	14.3	3.77	1.06	0.36	0.31	0.08	2.04
12. Jul. 08	18:00	0.03	0.11	14.7	6.14	1.58	0.32	0.42	0.13	1.17
12. Jul. 08	22:00		0.05		3.4	0.73	0.36	0.6	0.26	0.91
13. Jul. 08	06:00	0.03	0.08	14.1	5.28	1.23	0.34	0.51	0.29	1.49
13. Jul. 08	10:00	0.085	0.07	14.25	5.18	1.07	0.39	0.36	0.06	1.38
15. Jul. 08	06:00	0.07	0.09	14.4	2.73	0.43	0.26	0.46	0.09	1.53
15. Jul. 08	10:00	0.075	0.09	13.8	3.15	0.62	0.2	0.58	0.08	1.62
15. Jul. 08	14:00	0.105	0.09	13.65	2.72	0.42	0.32	0.41	0.09	1.95
15. Jul. 08	18:00	0.165	0.08	13.6	5.33	0.92	0.28	0.48	0.11	2.21
15. Jul. 08	22:00	0.085	0.07	13.5	5.54	0.79	0.3	0.54	0.18	0.90
16. Jul. 08	06:00	0.12	0.09	13.8	3.93	0.53	0.27	0.51	0.15	1.59
16. Jul. 08	10:00	0.06	0.11	14	3	0.4	0.27	0.47	0.09	1.30
18. Jul. 08	10:00	0.13	0.17	13.8	6.13	0.61	0.27	0.39	0.08	0.20
18. Jul. 08	14:00	0.075	0.09	13.4	3.48	0.47	0.33	0.41	0.04	0.21
18. Jul. 08	18:00	0.065	0.09	13.2	7.5	1.43	0.28	0.49	0.12	0.24
18. Jul. 08	22:00	0.06	0.055	13.4	2.96	0.41	0.33	0.45	0.18	0.16
19. Jul. 08	06:00	0.035	0.06	13.55	5.48	0.93	0.37	0.32	0.05	0.21
19. Jul. 08	10:00	0.035	0.03	13.8	6.08	1.1	0.31	0.47	0.2	0.31

Date	Time	C fix <10 [µmol l^{-1} h^{-1}]	N fix >10 [nmol l^{-1} h^{-1}]	C fix >10 [µmol l^{-1} h^{-1}]	C fix total [µmol l^{-1} h^{-1}]	PON <10µm [µmol l^{-1}]	POC <10µm [µmol l^{-1}]	PON >10µm [µmol l^{-1}]	POC >10µm [µmol l^{-1}]	PON [µmol l^{-1}]	POC [µmol l^{-1}]
12. Jul. 08	06:00	0.333	3.91	1.8E-01	0.52	6.956	57.616	2.709	21.518	9.665	79.134
12. Jul. 08	10:00	0.551	2.90	2.4E-01	0.79	6.880	60.980	2.738	21.962	9.618	82.942
12. Jul. 08	14:00	0.506	11.26	3.9E-01	0.89	6.540	59.080	2.915	23.046	9.455	82.126
12. Jul. 08	18:00	0.062	1.69	2.7E-02	0.09	7.015	61.635	2.510	19.880	9.525	81.516
12. Jul. 08	22:00	0.025	2.80	1.8E-02	0.04	7.552	61.996	4.123	31.446	11.675	93.442
13. Jul. 08	06:00	0.412	1.32	1.1E-01	0.52	7.433	63.946	1.834	15.594	9.267	79.540
13. Jul. 08	10:00	0.574	0.98	1.1E-01	0.69	7.436	65.350	1.386	12.427	8.821	77.777
15. Jul. 08	06:00	0.451	**4.83**	3.3E-01	0.78	6.754	57.420	4.703	35.371	11.457	92.791
15. Jul. 08	10:00	0.588	4.46	2.0E-01	0.79	6.344	56.187	2.598	21.134	8.942	77.320
15. Jul. 08	14:00	0.675	4.96	3.2E-01	0.99	8.571	77.342	4.265	35.505	12.836	112.847
15. Jul. 08	18:00	0.188	5.99	1.1E-01	0.29	8.566	74.009	4.119	32.216	12.685	106.225
15. Jul. 08	22:00	0.021	1.84	2.1E-02	0.04	8.339	63.931	6.453	44.267	14.792	108.198
16. Jul. 08	06:00	0.484	5.62	3.5E-01	0.83	7.864	63.437	4.868	34.584	12.733	98.021
16. Jul. 08	10:00	0.621	2.65	2.5E-01	0.87	7.410	65.408	2.912	24.560	10.323	89.968
18. Jul. 08	10:00	0.572	2.33	2.2E-01	0.79	6.579	58.526	2.362	21.237	8.941	79.762
18. Jul. 08	14:00	0.371	1.74	1.4E-01	0.51	7.264	66.769	2.440	22.185	9.704	88.954
18. Jul. 08	18:00	0.159	3.60	1.2E-01	0.28	8.444	71.542	4.479	34.214	12.923	105.756
18. Jul. 08	22:00	0.021	1.86	2.6E-02	0.05	8.653	70.760	6.227	42.360	14.881	113.120
19. Jul. 08	06:00	0.531	5.63	3.5E-01	0.88	6.773	58.196	3.367	28.099	10.140	86.296
19. Jul. 08	10:00	0.682	11.26	6.1E-01	1.29	6.927	65.840	4.692	37.008	11.618	102.848

Date	Time	Colony form. cyanos [units l^{-1}]	*Anabaena* sp. [units l^{-1}]	*Aphanizomenon* "baltica" [units l^{-1}]	*Nodularia spumigena* [units l^{-1}]	Cryptomonadales 5-9 µm [units l^{-1}]	Cryptomonadales 7-14 µm [units l^{-1}]	Dinoflag. 15x30 [units l^{-1}]	Dinoflag. 20x18 [units l^{-1}]	Dinophysis norvegica [units l^{-1}]
12. Jul. 08	06:00	1.67E+07	1.25E+04	9.17E+04	5.94E+03	8.67E+05	7.91E+04	2.77E+05	3.16E+04	
12. Jul. 08	10:00	1.89E+07	1.16E+04	8.02E+04	9.90E+03	1.07E+06	9.49E+04	3.82E+05	6.32E+04	
12. Jul. 08	14:00	1.98E+07	1.29E+04	2.62E+05	5.28E+03	8.59E+05	1.29E+05	3.00E+05	7.38E+04	
12. Jul. 08	18:00	2.34E+07	1.35E+04	1.15E+05	4.95E+03	6.90E+05	6.32E+04	2.85E+05	3.43E+04	
12. Jul. 08	22:00	1.89E+07	2.31E+04	1.25E+05	8.25E+03	7.38E+05	7.38E+04	3.08E+05	2.90E+04	
13. Jul. 08	06:00	2.01E+07	1.75E+04	5.28E+04	2.15E+04	7.51E+05	7.91E+04	4.16E+05	5.53E+04	6.60E+02
13. Jul. 08	10:00	1.47E+07	1.12E+04	7.39E+04	3.63E+03	6.77E+05	6.85E+04	2.69E+05	2.90E+04	
15. Jul. 08	06:00	3.12E+07	1.82E+04	3.14E+04	2.97E+03	7.38E+05	1.37E+05	2.21E+05	2.64E+04	
15. Jul. 08	10:00	1.39E+07	2.28E+04	4.69E+04	1.65E+03	7.91E+05	1.58E+04	1.61E+05	3.95E+04	6.60E+02
15. Jul. 08	14:00	2.53E+07	2.57E+04	6.44E+04	2.97E+03	6.22E+05	9.75E+04	1.50E+05	4.22E+04	6.60E+02
15. Jul. 08	18:00	1.59E+07	2.24E+04	9.21E+04	4.95E+03	6.59E+05	1.50E+05	2.37E+05	3.95E+04	3.30E+02
15. Jul. 08	22:00	1.03E+07	2.01E+04	9.97E+04	1.35E+04	7.01E+05	1.61E+05	2.79E+05	2.11E+04	
16. Jul. 08	06:00	3.00E+07	1.25E+04	6.40E+04	9.57E+03	7.46E+05	1.87E+05	1.50E+05	2.64E+04	
16. Jul. 08	10:00	1.26E+07	8.58E+03	3.63E+04	3.63E+03	7.04E+05	1.24E+05	2.00E+05	2.64E+04	
18. Jul. 08	10:00	1.17E+07	9.57E+03	2.77E+04	4.95E+03	6.75E+05	6.59E+04	2.42E+05	1.58E+04	
18. Jul. 08	14:00	1.02E+07	9.24E+03	6.11E+04	3.96E+03	7.06E+05	7.38E+04	2.48E+05	1.58E+04	
18. Jul. 08	18:00	2.84E+07	6.27E+03	5.58E+04	8.58E+03	6.56E+05	7.64E+04	2.35E+05	1.05E+04	
18. Jul. 08	22:00	2.20E+07	1.22E+04	6.53E+04	1.12E+04	5.22E+05	7.11E+04	2.37E+05	2.90E+04	3.30E+02
19. Jul. 08	06:00	2.12E+07	1.39E+04	3.70E+04	1.16E+04	6.43E+05	1.00E+05	2.35E+05	3.69E+04	
19. Jul. 08	10:00	2.42E+07	1.35E+04	5.94E+04	1.06E+04	6.98E+05	6.06E+04	1.95E+05	3.69E+04	3.30E+02

Date	Time	*Heterocapsa rotundata* [units l^{-1}]	*Actinocyclus octonarius* 30x15 [units l^{-1}]	*Actinocyclus octonarius* 40x20 [units l^{-1}]	*Chaetoceros impressus* 30x15 µm [units l^{-1}]	*Coscinodiscus radiates* [units l^{-1}]	Centric diatomae [units l^{-1}]	*Cylindrotheca closterium* 25x2 [units l^{-1}]	*Pyramimonas* sp. 5x6 µm [units l^{-1}]	*Crucigenia tetrapedia* [units l^{-1}]
12. Jul. 08	06:00	2.64E+03			1980	330	2.64E+04	1.42E+05	3.16E+04	
12. Jul. 08	10:00	5.27E+03			1320		4.74E+04	2.66E+05	2.90E+04	
12. Jul. 08	14:00	5.27E+03			3630	330	2.90E+04	1.53E+05	4.74E+04	
12. Jul. 08	18:00	2.11E+04			990		1.84E+04	1.26E+05	5.53E+04	
12. Jul. 08	22:00	2.64E+03				330	7.38E+04	3.11E+05	1.29E+05	
13. Jul. 08	06:00	2.64E+03			330		8.96E+04	2.35E+05	1.11E+05	
13. Jul. 08	10:00						7.38E+04	1.03E+05	1.00E+05	
15. Jul. 08	06:00	1.05E+04	660	330	3630			5.80E+04	2.21E+05	
15. Jul. 08	10:00	7.91E+03		660			3.16E+04	8.96E+04	1.77E+05	
15. Jul. 08	14:00	2.64E+03					4.22E+04	6.85E+04	9.49E+04	
15. Jul. 08	18:00	1.05E+04					3.16E+04	3.69E+04	1.34E+05	
15. Jul. 08	22:00	1.32E+04					5.27E+04	1.11E+05	1.98E+05	
16. Jul. 08	06:00	7.91E+03		330			8.70E+04	1.61E+05	1.53E+05	
16. Jul. 08	10:00	1.05E+04			4290	1320	4.48E+04	2.69E+05	1.16E+05	1.05E+04
18. Jul. 08	10:00			330	660		9.75E+04	1.42E+05	7.11E+04	
18. Jul. 08	14:00	1.58E+04					1.03E+05	7.11E+04	1.05E+05	
18. Jul. 08	18:00	1.05E+04					5.80E+04	2.85E+05	1.24E+05	
18. Jul. 08	22:00	3.16E+04				660	5.80E+04	1.45E+05	8.70E+04	
19. Jul. 08	06:00	1.05E+04		990			7.38E+04	3.21E+05	1.45E+05	
19. Jul. 08	10:00	7.91E+03				330	9.75E+04	1.58E+05	2.56E+05	

Date	Time	*Oocystis* sp. 8x4 µm [units l^{-1}]	*Planctonema lauterbornii* [units l^{-1}]	CYST 12 µm [units l^{-1}]	UNIDENTIFIED < 2 µm, 10 cell [units l^{-1}]	UNIDENTIFIED 2-5 µm, 10 cell [units l^{-1}]	heterotrophic bacteria [ml^{-1}]	*Synechococcus* [l^{-1}]	Picoeukaryota [l^{-1}]	Nanoeukaryota 1 [l^{-1}]	Nanoeukaryota 2 [l^{-1}]
12. Jul. 08	06:00	1.21E+05	660		2.90E+05	1.05E+05	2.27E+06	5.75E+06	3.81E+07	6.90E+06	8.01E+05
12. Jul. 08	10:00	1.77E+05	1650	7.91E+03	4.74E+05	1.84E+05	2.44E+06	6.44E+06	3.66E+07	5.15E+06	4.92E+05
12. Jul. 08	14:00	8.70E+04	330	7.91E+03	3.43E+05	2.63E+04	2.25E+06	6.01E+06	3.67E+07	4.95E+06	5.88E+05
12. Jul. 08	18:00	8.70E+04	2310	2.64E+03	3.69E+05	1.32E+05	2.34E+06	4.40E+06	3.70E+07	5.03E+06	3.10E+05
12. Jul. 08	22:00	7.64E+04	3960	2.64E+03	4.48E+05	1.58E+05	1.98E+06	4.92E+06	4.68E+07	6.43E+06	4.06E+05
13. Jul. 08	06:00	1.19E+05	4950	2.64E+03	2.37E+05	5.27E+04	2.11E+06	4.90E+06	4.92E+07	7.83E+06	6.73E+05
13. Jul. 08	10:00	8.70E+04	2970		3.43E+05	7.90E+04	2.00E+06	3.40E+06	4.65E+07	6.43E+06	5.88E+05
15. Jul. 08	06:00	6.85E+04	2640		5.53E+05	2.90E+05	2.61E+06	2.67E+06	3.36E+07	3.79E+06	3.63E+05
15. Jul. 08	10:00	5.53E+04	3960		3.69E+05	2.63E+05	2.72E+06	2.77E+06	3.36E+07	3.91E+06	3.95E+05
15. Jul. 08	14:00	8.17E+04	3300		3.43E+05	1.84E+05	2.69E+06	3.32E+06	3.34E+07	4.84E+06	4.81E+05
15. Jul. 08	18:00	5.80E+04	6600	7.91E+03	6.85E+05	3.95E+05	2.94E+06	2.24E+06	3.29E+07	4.38E+06	4.49E+05
15. Jul. 08	22:00	1.08E+05	2970	1.32E+04	3.56E+05	1.45E+05	2.99E+06	2.35E+06	3.47E+07	4.15E+06	3.74E+05
16. Jul. 08	06:00	1.11E+05	4620	2.37E+04	5.01E+05	2.63E+05	2.99E+06	3.39E+06	4.38E+07	4.00E+06	4.92E+05
16. Jul. 08	10:00	1.29E+05	14190		4.48E+05	1.84E+05	2.69E+06	1.07E+07	5.16E+07	5.07E+06	4.92E+05
18. Jul. 08	10:00	1.32E+05	7590		4.48E+05	1.05E+05	2.88E+06	1.23E+07	4.35E+07	4.57E+06	3.53E+05
18. Jul. 08	14:00	9.22E+04	4290		2.90E+05	5.27E+04	2.86E+06	6.88E+06	3.89E+07	6.33E+06	2.46E+05
18. Jul. 08	18:00	1.32E+05	1650	7.91E+03	4.48E+05	2.11E+05	2.93E+06	4.04E+06	3.18E+07	4.61E+06	2.24E+05
18. Jul. 08	22:00	9.49E+04	4620	2.64E+03	1.00E+06	2.63E+05	2.93E+06	4.43E+06	2.92E+07	4.76E+06	2.99E+05
19. Jul. 08	06:00	9.75E+04	5940	2.64E+03	5.80E+05	7.90E+04	2.77E+06	2.46E+06	2.93E+07	4.94E+06	4.49E+05
19. Jul. 08	10:00	1.00E+05	1650		5.27E+05	1.84E+05	3.25E+06	1.45E+06	2.37E+07	5.75E+06	3.95E+05

Date	Time	EE Phos total	EE Peptidase total	Leu uptake total [pmol l^{-1} h^{-1}]	sd Leu uptake total [pmol l^{-1} h^{-1}]	Thy uptake total [pmol l^{-1} h^{-1}]	sd Thy uptake total [pmol l^{-1} h^{-1}]	Bac GDA total	EE Phos <10	EE Peptidase <10	Leu uptake <10 [pmol l^{-1} h^{-1}]
12. Jul. 08	06:00	0.010	0.055	94.264	25.333	12.905	2.935	8.14E+06	0.008	0.047	59.291
12. Jul. 08	10:00			141.696	10.423	2.962	0.418	1.02E+07			106.019
12. Jul. 08	14:00	0.008	0.046					8.48E+06	0.013	0.048	
12. Jul. 08	18:00	0.010	0.031	263.182	14.247	18.824	3.786	9.51E+06	0.006	0.028	162.292
12. Jul. 08	22:00	0.006	0.058	513.113	43.955	33.369	3.134	8.70E+06	0.005	0.027	335.333
13. Jul. 08	06:00	0.005	0.032	433.270	24.312	14.614	1.454	1.05E+07	0.009	0.035	303.496
13. Jul. 08	10:00			329.738	13.564			1.02E+07			345.434
15. Jul. 08	06:00	0.014	0.065	170.424	9.989			8.71E+06	0.013	0.046	194.213
15. Jul. 08	10:00		0.044	348.909	4.810	15.027	2.655	5.09E+06		0.048	288.746
15. Jul. 08	14:00	0.011	0.066	484.478	32.939	15.510	4.255	6.61E+06	0.016	0.050	444.677
15. Jul. 08	18:00	0.013	0.058	503.250	5.318	18.230	2.309	9.56E+06	0.011	0.053	492.039
15. Jul. 08	22:00	0.010	0.049	514.279	3.504	27.471	2.448	7.85E+06	0.015	0.041	507.489
16. Jul. 08	06:00	0.011	0.073	538.822	4.069	19.095	3.608	3.87E+06	0.011	0.045	503.707
16. Jul. 08	10:00			706.491	5.431	15.267	1.648	5.80E+06			529.171
18. Jul. 08	10:00	0.013	0.072	850.087	58.481	25.991	1.888	1.34E+07	0.012	0.072	1290.389
18. Jul. 08	14:00			914.612	12.810	25.885	0.639	6.79E+06			953.318
18. Jul. 08	18:00			1350.216	133.749	31.833	2.669	6.51E+06			1178.648
18. Jul. 08	22:00			1464.402	140.940	41.435	4.653	6.52E+06			1310.263
19. Jul. 08	06:00			886.319	9.174	22.727	0.098	7.20E+06			703.640
19. Jul. 08	10:00	0.012	0.031	821.547	68.122	40.936	3.461	5.76E+06	0.012	0.044	679.059

Date	Time	sd Leu uptake <10 [pmol l^{-1} h^{-1}]	Thy uptake <10 [pmol l^{-1} h^{-1}]	sd Thy uptake <10 [pmol l^{-1} h^{-1}]	Bac GDA <10
12. Jul. 08	06:00	3.526	6.695	0.534	5.99E+06
12. Jul. 08	10:00	8.775	1.153	0.258	9.72E+06
12. Jul. 08	14:00				9.11E+06
12. Jul. 08	18:00	7.695	13.874	0.883	7.83E+06
12. Jul. 08	22:00	37.242	16.026	1.483	7.00E+06
13. Jul. 08	06:00	22.482	8.297	1.516	7.61E+06
13. Jul. 08	10:00	41.286	10.240	0.933	1.70E+07
15. Jul. 08	06:00	6.925	9.537	0.584	3.54E+06
15. Jul. 08	10:00	12.762	12.793	2.298	3.25E+06
15. Jul. 08	14:00	41.038	9.193	1.381	7.91E+06
15. Jul. 08	18:00	11.698	14.812	0.607	7.67E+06
15. Jul. 08	22:00	47.124	15.907	1.161	5.68E+06
16. Jul. 08	06:00	32.123	10.451	0.895	7.84E+06
16. Jul. 08	10:00	31.228	12.969	0.324	7.64E+06
18. Jul. 08	10:00	14.812	20.062	1.354	7.59E+06
18. Jul. 08	14:00	33.395	20.010	2.105	8.67E+06
18. Jul. 08	18:00	27.749	19.411	1.393	9.11E+06
18. Jul. 08	22:00	34.703	26.924	1.897	8.13E+06
19. Jul. 08	06:00	43.150	16.463	1.251	9.70E+06
19. Jul. 08	10:00	46.905	14.846	3.820	9.61E+06

7. APPENDIX II – CHAPTER 3.2

Bottle	Chl*a* [µg/l]	Phaeo [µg/l]	$NO_{2/3}$ [µmol/l]	NO_2 [µmol/l]	NO_3 [µmol/l]	PO_4 [µmol/l]	Si [µmol/l]	NH_4 [µmol/l]	TCO_2 in [□ mol/kgSW]	pCO_2 [□ atm]
1a start	5.23	-0.09	5.07	0.77	4.30	1.46	11.50	0.95	1612.2	267
2a start	4.87	0.12	4.80	0.74	4.06	1.08	11.00	0.99	1619.2	280
3a start	5.36	0.23	5.24	0.77	4.47	1.16	12.10	0.60	1648.6	380
4a start	5.21	0.06	4.94	0.77	4.17	1.11	11.40	0.60	1668.7	480
5a start	5.10	0.23	5.10	0.77	4.33	1.11	11.95	0.92	1684.1	580
6a start	4.94	-0.10	4.95	0.77	4.18	1.07	11.60	0.99	1696.3	680
7a start	5.57	0.04	5.20	0.77	4.43	1.10	11.75	0.88	1706.6	780
1a	8.79	-0.03	2.60	0.52	2.09	0.37	10.00	1.23	1600.7	241
2a	10.02	-0.01	2.04	0.52	1.52	0.15	9.75	1.62	1612.2	268
3a	10.99	0.07	2.13	0.51	1.62	0.11	9.90	1.93	1643.5	365
4a	9.26	0.40	1.87	0.48	1.39	0.15	10.35	1.55	1670.6	494
5a	9.26	5.57	1.67	0.54	1.13	0.14	12.20	1.30	1694.8	664
6a	10.33	0.45						1.69	1696.7	680
7a	10.65	0.65						1.37	1708.0	787
1b start	10.07	0.11	5.72	0.78	4.95	1.54	10.40	2.84	1609.4	261
2b start	10.10	-0.08	4.94	0.74	4.20	1.49	9.05	2.24	1614.3	280
3b start	10.33	0.36	4.97	0.82	4.15	1.57	10.90	2.28	1644.2	380
4b start	9.63	0.11	5.35	0.77	4.58	1.51	9.25	2.35	1664.7	480
5b start	10.80	0.44	5.47	0.82	4.65	1.61	11.50	2.17	1680.2	580
6b start	12.82	-2.41	5.00	0.80	4.20	1.36	10.90	2.24	1692.5	680
7b start	10.12	0.05	5.05	0.84	4.21	1.59	11.20	2.56	1702.9	780
1b	9.08	0.17	1.55	0.31	1.25	0.48	10.20	0.81	1562.4	175
2b	9.08	0.21	2.77	0.28	2.49	0.50	11.30	0.85	1555.5	166
3b	11.54	0.01	1.34	0.24	1.10	0.42	10.75	0.92	1572.6	190
4b	13.45	0.20	1.24	0.22	1.02	0.33	9.80	0.81	1597.8	235
5b	11.69	0.51	1.28	0.24	1.04	0.17	11.60	0.71	1617.8	282
6b	11.93	0.30	1.31	0.23	1.08	0.13	9.60	0.81	1628.4	313
7b	16.01	0.71	0.90	0.18	0.72	0.08	9.45	0.64	1641.0	356

Bottle	pH	PON [µmol L^{-1}]	POC [µmol L^{-1}]	POC:PON	Nodularia [10^3 counts L^{-1}]	Aphanizomenon [10^3 counts L^{-1}]	Nodularia cell counts [10x3 L^{-1}]	failure Nodularia (12,6%)	Aphanizomenon cell counts [10x3 L^{-1}]	failure Apha [13,6%]
1a start	8.220	35.95	200.99	5.59	5.18E+03	9.45E+02	3.47E+04	4.37E+03	8.51E+03	1.16E+03
2a start	8.195	34.71	199.95	5.76	6.54E+03	1.89E+03	4.38E+04	5.52E+03	1.70E+04	2.32E+03
3a start	8.079	36.32	209.03	5.75	5.73E+03	1.11E+03	3.84E+04	4.84E+03	9.97E+03	1.36E+03
4a start	7.989	34.68	194.92	5.62	3.49E+03	7.76E+02	2.34E+04	2.95E+03	6.98E+03	9.49E+02
5a start	7.914	33.64	188.50	5.60	6.57E+03	1.77E+03	4.40E+04	5.55E+03	1.60E+04	2.17E+03
6a start	7.852	35.93	202.13	5.62	5.05E+03	1.23E+03	3.39E+04	4.27E+03	1.11E+04	1.50E+03
7a start	7.798	34.61	196.59	5.68	2.82E+03	1.16E+03	1.89E+04	2.38E+03	1.05E+04	1.43E+03
1a	8.26	31.99	185.94	5.81	4.69E+03	7.27E+02	3.14E+04	3.96E+03	6.54E+03	8.89E+02
2a	8.22	32.84	187.57	5.71	6.07E+03	4.88E+02	4.07E+04	5.12E+03	4.39E+03	5.97E+02
3a	8.1	33.36	195.67	5.87	6.58E+03	1.17E+03	4.41E+04	5.56E+03	1.06E+04	1.44E+03
4a	7.98	31.26	185.89	5.95	4.76E+03	4.24E+02	3.19E+04	4.01E+03	3.82E+03	5.19E+02
5a	7.86	34.77	203.55	5.85	4.73E+03	5.25E+02	3.17E+04	3.99E+03	4.72E+03	6.42E+02
6a	7.85	34.14	200.11	5.86	5.96E+03	5.97E+02	3.99E+04	5.03E+03	5.37E+03	7.30E+02
7a	7.79	33.34	203.37	6.10	5.42E+03	6.38E+02	3.63E+04	4.57E+03	5.74E+03	7.81E+02
1b start	8.230	15.56	115.41	7.42	1.73E+03	1.91E+03	1.16E+04	1.46E+03	1.72E+04	2.34E+03
2b start	8.213	15.59	113.73	7.30	1.26E+03	3.47E+03	8.46E+03	1.07E+03	3.12E+04	4.24E+03
3b start	8.097	14.67	105.62	7.20	1.29E+03	2.66E+03	8.66E+03	1.09E+03	2.40E+04	3.26E+03
4b start	8.007	15.65	111.76	7.14	1.31E+03	2.61E+03	8.80E+03	1.11E+03	2.35E+04	3.20E+03
5b start	7.934	14.96	106.07	7.09	1.56E+03	2.93E+03	1.04E+04	1.31E+03	2.63E+04	3.58E+03
6b start	7.872	14.96	107.01	7.15	2.13E+03	3.13E+03	1.43E+04	1.80E+03	2.82E+04	3.83E+03
7b start	7.817	16.17	116.46	7.20	1.09E+02	1.59E+02	7.27E+02	9.16E+01	1.43E+03	1.95E+02
1b	8.38	19.98	135.89	6.80	2.21E+03	1.22E+03	1.48E+04	1.86E+03	1.10E+04	1.49E+03
2b	8.4	20.65	150.30	7.28	2.56E+03	1.37E+03	1.72E+04	2.16E+03	1.24E+04	1.68E+03
3b	8.35	21.66	149.47	6.90	3.02E+03	1.19E+03	2.03E+04	2.55E+03	1.07E+04	1.46E+03
4b	8.27	22.81	161.30	7.07	1.75E+03	1.01E+03	1.18E+04	1.48E+03	9.08E+03	1.24E+03
5b	8.2	24.04	170.75	7.10	2.54E+03	1.16E+03	1.70E+04	2.15E+03	1.04E+04	1.42E+03
6b	8.16	27.68	198.76	7.18	3.34E+03	1.32E+03	2.23E+04	2.82E+03	1.19E+04	1.61E+03
7b	8.11	29.34	203.03	6.92	2.03E+03	1.30E+03	1.36E+04	1.71E+03	1.17E+04	1.60E+03

Bottle	N fixation [nmol N L^{-1} h^{-1}] (total)	SD N fixation [nmol N L^{-1} h^{-1}]	C tixation [µmol C L^{-1} h^{-1}] (total)	SD C fixation [µmol C L^{-1} h^{-1}]	TP [µM]	POP [µM]	POC:POP	DP [µM]	DOP [µM]
1a start					2.56	1.10	183.31	1.46	0.14
2a start					1.80	0.55	364.96	1.26	0.22
3a start					2.86	1.56	134.37	1.30	0.20
4a start					2.30	1.03	189.42	1.27	0.24
5a start					2.16	0.89	211.99	1.27	0.23
6a start					2.60	1.41	142.94	1.19	0.18
7a start					2.40	1.19	165.41	1.21	0.17
1a	2.270	0.222	0.69	0.02	2.60	1.95	95.48	0.66	0.29
2a	5.907	1.307	0.79	0.00	2.46	2.05	91.42	0.41	0.26
3a	6.740	0.107	0.89	0.01	2.48	2.14	91.27	0.34	0.21
4a	2.613	0.033	0.72	0.03	2.16	1.80	103.09	0.36	0.21
5a	11.120	2.659	1.13	0.04	2.38	2.01	101.50	0.37	0.25
6a	11.977	3.092	1.05	0.05	2.46	2.20	90.98	0.26	0.21
7a	10.521	0.728	1.05	0.05	2.36	2.13	95.50	0.23	0.20
1b start					2.61	0.93	124.08	1.68	0.14
2b start					2.67	0.83	137.73	1.84	0.28
3b start					2.54	0.82	129.13	1.72	0.15
4b start					2.72	1.02	110.01	1.70	0.12
5b start					2.52	0.84	126.58	1.68	0.10
6b start					2.61	0.90	119.04	1.71	0.13
7b start					2.54	0.86	134.74	1.67	0.10
1b	1.498	0.095	0.561	0.009	2.20	1.49	91.05	0.71	0.27
2b	1.802	0.357	0.461	0.018	2.36	1.63	92.16	0.73	0.28
3b	4.330	0.751	0.724	0.017	2.38	1.74	86.02	0.64	0.30
4b	6.725	0.305	0.895	0.055	2.45	1.93	83.73	0.52	0.27
5b	8.924	0.985	1.079	0.014	2.19	1.66	102.77	0.52	0.37
6b	12.080	1.463	1.120	0.031	2.23	1.86	106.81	0.37	0.25
7b	18.444	1.514	1.797	0.027	2.18	1.88	107.91	0.30	0.26

Bottle	DOC [µM]	DOC [µM] stdev	TDN [µM]	TDN [µM] stdev	DON [µM]	P uptake total [nmol l^{-1} h^{-1}]	P uptake total [nmol l^{-1} h^{-1}] stdev	P uptake > 3µm [nmol l^{-1} h^{-1}]	P uptake > 3µm [nmol l^{-1} h^{-1}] stdev	Bac [ml^{-1}]
1a start	414.65	86.34	25.89	0.82	19.87					2.88E+06
2a start	476.85	161.57	26.07	0.32	20.28					2.69E+06
3a start	469.80	81.88	26.24	0.38	20.40					3.01E+06
4a start	640.65	63.14	27.00	2.00	21.45					2.48E+06
5a start	361.85	4.45	25.90	0.11	19.89					2.66E+06
6a start	394.95	43.77	25.91	0.46	19.97					2.52E+06
7a start	392.85	31.32	26.97	1.20	20.89					2.67E+06
1a	522.75	179.53	24.68	0.76	20.85	27.26	0.69	11.03	0.59	1.65E+06
2a	631.45	338.21	25.11	0.26	21.45	23.03	4.19	15.02	0.14	1.60E+06
3a	700.80		25.89		21.83	25.76		15.96		1.88E+06
4a	651.50	29.27	23.52	0.81	20.10	20.23	2.65	14.07	5.36	1.69E+06
5a	586.95	78.84	24.01	0.10	21.04	31.26	1.87	20.77	1.61	1.82E+06
6a	530.85	61.16	25.47	0.40	23.78	15.20	1.11	4.83	0.43	1.93E+06
7a	766.05	159.31	25.93	1.32	24.56	13.63	2.15	7.15	1.18	1.73E+06
1b start	795.05	210.79	30.67	5.94	22.11					4.75E+05
2b start	612.45	1.63	26.60	0.70	19.41					4.50E+05
3b start	545.00	83.72	25.13	0.00	17.88					5.44E+05
4b start	600.35	59.89	26.26	0.47	18.56					4.50E+05
5b start	569.10	80.47	27.33	1.78	19.69					5.41E+05
6b start	710.20	7.21	24.55	0.42	17.31					5.20E+05
7b start	670.60	8.77	26.44	0.56	18.83					5.20E+05
1b	690.20	30.12	27.59	1.42	25.22	24.08	0.78	14.80	2.18	2.52E+06
2b	642.95	207.82	25.34	0.04	21.72	21.94	4.33	11.78	1.34	3.12E+06
3b	500.40	133.64	21.08	0.28	18.82	22.75	0.20	13.72	2.09	2.59E+06
4b	608.30	0.85	20.12	0.66	18.07	20.87	0.66	14.25	1.75	3.07E+06
5b	632.60	9.62	20.98	0.01	18.99	34.90	7.90	21.44	3.81	2.99E+06
6b	775.60	272.24	24.21	4.33	22.09	32.13	0.81	15.71	4.17	2.91E+06
7b	580.10	27.29	18.87	1.10	17.33	25.75	3.56	14.61	1.02	3.08E+06

Bottle	DIP turnover [h] total	DIP turnover [h] >3µm	DIP turnover [h] <3µm	affinity total normalised on nmol P	affinity total normalised on µmol C
1a start					
2a start					
3a start					
4a start					
5a start					
6a start					
7a start					
1a	18.89	47.45	32.07	2.72E-05	3.66E-07
2a	7.60	11.83	23.16	6.41E-05	9.19E-07
3a	8.32	11.96	15.20	5.60E-05	8.49E-07
4a	6.04	11.58	14.38	9.18E-05	1.21E-06
5a	3.81	6.01	12.36	1.31E-04	1.65E-06
6a	3.12	10.97	4.83	1.46E-04	2.09E-06
7a	2.10	4.51	5.02	2.24E-04	3.25E-06
1b start					
2b start					
3b start					
4b start					
5b start					
6b start					
7b start					
1b	23.62	39.75	59.72	2.84E-05	2.95E-07
2b	30.58	55.29	69.69	2.01E-05	2.38E-07
3b	16.27	27.66	41.00	3.54E-05	4.23E-07
4b	11.08	16.32	36.77	4.68E-05	5.90E-07
5b	5.93	9.89	16.41	1.02E-04	1.04E-06
6b	3.61	8.05	7.48	1.49E-04	1.74E-06
7b	3.00	5.65	7.73	1.77E-04	1.89E-06

Bottle	Chl*a* [µg/l]	Phaeo [µg/l]	$NO_{2/3}$ [µmol/l]	NO_2 [µmol/l]	NO_3 [µmol/l]	PO_4 [µmol/l]	SiO_4 [µmol/l]	NH_4^+ [µmol/l]	TCO_2 in (µmol/kgSW)	pCO_2 in (µatm)	pH
1c start	0.701	0.496	5.86	0.77	5.09	1.93	8.7	3.85	1623.2	298	8.180
2c start	0.840	0.087	5.215	0.72	4.495	1.88	7.5	3.26	1626.0	310	8.169
3c start	0.958	0.107	2.16	0.3	1.86	1.085	2.7	5.45	1656.8	420	8.043
4c start	0.992	0.094	5.12	0.72	4.4	1.87	6.9	2.66	1681.2	560	7.929
5c start	0.853	0.074	5.155	0.78	4.375	1.85	6.7	2.77	1700.4	710	7.831
6c start	1.269	0.070	5.05	0.7	4.35	1.835	7.15	2.35	1711.4	820	7.772
7c start	1.682	0.081	5.05	0.73	4.32	1.785	8.8	2.87	1722.8	950	7.710
1c	0.806	0.084	5.545	0.735	4.81	1.88	9.7	2.91	1612.2	268	8.22
2c	0.709	0.088	5.665	0.705	4.96	1.91	9.3	2.98	1617.8	282	8.2
3c	0.950	-0.037	5.32	0.73	4.59	1.89	11.25	2.77	1650.6	394	8.07
4c	0.903	0.138	2.61	0.36	2.25	1.455	5.5	2.59	1664.1	458	8.01
5c	0.866	0.063	3.46	0.49	2.97	1.41	6.1	2.70	1694.8	664	7.86
6c	1.363	0.102	4.855	0.665	4.19	1.74	11.15	2.73	1700.5	714	7.83
7c	1.499	-0.016	3.365	0.46	2.905	1.47	7.4	2.66	1709.9	806	7.78
1d start	0.578	0.146	3.03	0.5	2.53	1.49	6.4	2.38	1606.6	250	8.240
2d start	0.455	0.018	4.82	0.72	4.1	1.6	10.4	2.56	1622.2	290	8.184
3d start	0.610	0.005	2.51	0.46	2.05	1.27	5.4	2.49	1654.8	410	8.052
4d start	0.837	-0.080	4.37	0.68	3.69	1.72	9.4	2.59	1674.3	520	7.962
5d start	0.746	0.009	5.01	0.76	4.25	1.58	10.2	2.66	1693.6	650	7.866
6d start	1.345	-0.166	4.73	0.73	4	1.56	10.2	2.70	1707.6	780	7.792
7d start	1.690	-0.190	4.37	0.68	3.69	1.69	9.4	2.42	1721.1	930	7.720
1d	0.515	0.160	4.88	0.74	4.14	1.94	10.6	2.31	1603.7	248	8.25
2d	0.351	0.064	4.88	0.73	4.15	2.02	10.7	2.42	1612.2	268	8.22
3d	0.539	0.032	4.93	0.74	4.19	1.44	10.5	2.07	1652.9	404	8.06
4d	0.827	0.016	4.8	0.74	4.76	2.05	10.5	2.42	1664.1	458	8.01
5d	0.866	0.019	4.86	0.74	4.12	1.85	10.1	2.14	1680.9	559	7.93
6d	1.253	-0.086	4.72	0.73	3.99	1.57	10.8	2.14	1698.6	697	7.84
7d	1.604	-0.097	4.72	0.74	3.98	1.7	10.2	2.03	1706.2	768	7.8

Bottle	PON [µmol L^{-1}]	POC [µmol L^{-1}]	C:N	PON >3µm [µmol L^{-1}]	PON < 3µm [µmol L^{-1}]	POC > 3µm [µmol L^{-1}]	POC < 3µm [µmol L^{-1}]	C:N >3	C:N <3	Nodularia [10^3 counts L^{-1}]	Aphanizomenon [10^3 counts L^{-1}]
1c start										2.40E+02	5.31E+01
2c start										1.11E+02	3.15E+01
3c start										2.18E+02	2.62E+01
4c start											
5c start										1.13E+02	2.89E+01
6c start										1.59E+02	2.45E+01
7c start										3.97E+02	3.79E+01
1c				1.57	1.37	13.37	10.75	8.53	7.86	1.16E+02	4.50E+01
2c				1.27	1.54	9.98	13.27	7.87	8.61	1.69E+02	1.65E+01
3c				1.54	1.31	10.70	10.68	6.94	8.14	1.70E+02	3.44E+01
4c				1.66	1.24	12.42	9.42	7.46	7.58	1.63E+02	2.71E+01
5c				1.52	1.18	10.97	9.69	7.19	8.24	1.83E+02	2.80E+01
6c				2.13	1.36	14.95	11.11	7.02	8.17	2.09E+02	2.26E+01
7c				2.25		16.60		7.39		2.76E+02	4.40E+01
1d start										1.49E+02	1.33E+01
2d start										1.26E+02	5.86E+00
3d start										1.77E+02	6.46E+00
4d start										2.34E+02	9.36E+00
5d start										1.28E+02	6.70E+00
6d start										2.41E+02	7.54E+00
7d start										2.05E+02	1.40E+01
1d	4.13	26.75	6.47	1.81	1.21	14.14	9.51	7.79	7.88	2.02E+02	1.09E+01
2d	4.38	30.85	7.04	1.58	1.05	14.13	6.50	8.96	6.18	2.35E+02	7.48E+00
3d	3.57	26.27	7.36	1.59	0.99	11.07	6.59	6.96	6.66	2.53E+02	8.38E+00
4d	4.09	26.19	6.41	1.74	1.05	12.87	7.23	7.39	6.89	2.22E+02	1.47E+01
5d	3.72	24.27	6.52	1.61	1.88	11.28	11.55	6.99	6.14	1.97E+02	8.65E+00
6d	4.19	26.54	6.33	2.27	1.22	15.31	8.92	6.74	7.33	2.32E+02	6.70E+00
7d	4.79	28.57	5.96	2.33	1.45	15.12	9.71	6.48	6.69	2.33E+02	1.03E+01

Bottle	Nodularia cell counts (10x3 L^{-1})	failure Nodularia (12,6%)	Aphanizomenon cell counts (10x3 L^{-1})	failure Apha (13,6%)	N fixation [nmol N L^{-1} h^{-1}] (total)	N fixation [nmol N L^{-1} h^{-1}] (>3µm)	N uptake < 3 µm [nmol N L^{-1} h^{-1}]	C fixation [µmol C L^{-1} h^{-1}] (total)	C fixation [µmol C L^{-1} h^{-1}] (>3µm)	C uptake < 3 µm [µmol C L^{-1} h^{-1}]
1c start	1.61E+03	2.02E+02	4.78E+02	6.50E+01						
2c start	7.42E+02	9.35E+01	2.84E+02	3.86E+01						
3c start	1.46E+03	1.84E+02	2.36E+02	3.20E+01						
4c start	0.00E+00	0.00E+00	0.00E+00	0.00E+00						
5c start	7.54E+02	9.50E+01	2.60E+02	3.54E+01						
6c start	1.06E+03	1.34E+02	2.21E+02	3.00E+01						
7c start	2.66E+03	3.35E+02	3.41E+02	4.64E+01						
1c	7.76E+02	9.78E+01	4.05E+02	5.50E+01				0.014	0.009	0.005
2c	1.13E+03	1.43E+02	1.48E+02	2.01E+01				0.016	0.007	0.008
3c	1.14E+03	1.43E+02	3.09E+02	4.21E+01				0.023	0.014	0.009
4c	1.09E+03	1.37E+02	2.44E+02	3.32E+01				0.027	0.018	0.009
5c	1.23E+03	1.55E+02	2.52E+02	3.43E+01				0.030	0.021	0.009
6c	1.40E+03	1.76E+02	2.04E+02	2.77E+01				0.045	0.032	0.012
7c	1.85E+03	2.33E+02	3.96E+02	5.38E+01				0.042	0.042	
1d start	1.00E+03	1.26E+02	1.20E+02	1.63E+01						
2d start	8.45E+02	1.06E+02	5.28E+01	7.18E+00						
3d start	1.19E+03	1.50E+02	5.82E+01	7.91E+00						
4d start	1.57E+03	1.97E+02	8.43E+01	1.15E+01						
5d start	8.56E+02	1.08E+02	6.03E+01	8.20E+00						
6d start	1.61E+03	2.03E+02	6.78E+01	9.23E+00						
7d start	1.37E+03	1.73E+02	1.26E+02	1.71E+01						
1d	1.36E+03	1.71E+02	9.80E+01	1.33E+01	0.054	0.033	0.021	0.007	0.006	0.001
2d	1.57E+03	1.98E+02	6.73E+01	9.15E+00	0.042	0.029	0.013	0.007	0.005	0.002
3d	1.69E+03	2.13E+02	7.54E+01	1.03E+01	0.104	0.092	0.012	0.014	0.012	0.002
4d	1.49E+03	1.87E+02	1.32E+02	1.80E+01	0.201	0.186	0.015	0.019	0.016	0.003
5d	1.32E+03	1.66E+02	7.79E+01	1.06E+01	0.313	0.271	0.042	0.023	0.019	0.004
6d	1.55E+03	1.96E+02	6.03E+01	8.20E+00	0.707	0.695	0.012	0.039	0.035	0.004
7d	1.56E+03	1.97E+02	9.23E+01	1.26E+01	0.944	0.907	0.037	0.050	0.046	0.005

Bottle	NO_3 uptake total [nmol L^{-1} h^{-1}]	NO_3 uptake total >3µm [nmol L^{-1} h^{-1}]	NO_3 uptake <3µm [nmol L^{-1} h^{-1}]	TP [µM]	POP [µM]	DP [µM]	DOP [µM]	DOC [µM]	DOC [µM] stdev	TDN [µM]
1c start				2.39	0.32	2.06	0.09	408.95	25.67	26.88
2c start				2.32	0.24	2.08	0.09	547.88	32.03	25.06
3c start				2.39	0.43	1.97	0.02	511.63	79.20	25.15
4c start				2.41	0.29	2.11	0.14	526.08	270.47	26.53
5c start				2.25	0.22	2.02	0.11	666.18	156.62	27.24
6c start				2.30	0.31	1.99	0.09	407.58	120.99	23.78
7c start				2.26	0.33	1.92	0.05	513.76	44.18	24.27
1c	4.180	1.50	2.68	2.32	0.25	2.07	0.17	635.70	172.25	27.40
2c	4.550	0.90	3.65	2.22	0.18	2.04	0.11	537.55	34.58	24.15
3c	2.604	1.16	1.45	2.32	0.16	2.17	0.24	511.40	39.32	24.59
4c	6.720	4.35	2.37	2.27	0.29	1.98	0.05	558.60	0.42	25.08
5c	7.590	4.87	2.72	2.21	0.18	2.03	0.13	474.90	3.68	25.20
6c	10.310	6.99	3.32	2.27	0.22	2.06	0.21	599.90	37.19	24.85
7c	7.610	7.61		2.20	0.04	2.16	0.32	691.90	211.14	28.10
1d start				2.34	0.25	2.10	0.11			
2d start				2.32	0.16	2.16	0.15			
3d start				2.28	0.24	2.04	0.05			
4d start				2.45	0.33	2.12	0.13			
5d start				2.41	0.35	2.06	0.14			
6d start				2.33	0.29	2.04	0.13			
7d start				2.33	0.34	1.99	0.14			
1d				2.37	0.28	2.08	0.13			
2d				2.32	0.21	2.12	0.12			
3d				2.33	0.29	2.04	0.04			
4d				2.40	0.29	2.11	0.12			
5d				2.50	0.26	2.24	0.16			
6d				2.32	0.34	1.99	0.09			
7d				2.31	0.39	1.92	0.11			

Bottle	TDN [µM] stdev	DON [µM]	P uptake total [nmol l^{-1} h^{-1}]	P uptake total [nmol l^{-1} h^{-1}] Stabw	P uptake > 3µm [nmol l^{-1} h^{-1}]	P uptake > 3µm [nmol l^{-1} h^{-1}] stabw	Leu uptake [nM h^{-1} l^{-1}]	sd Leu uptake	Thy uptake [nM h^{-1} l^{-1}]	leu:thy	sd Thy uptake
1c start	0.75	17.17					7.01E-02	2.26E-03	1.67E-02	4.2	4.16E-03
2c start	2.55	16.58					8.54E-02	7.37E-03	1.27E-02	6.7	9.47E-04
3c start	0.16	17.53					7.89E-02	3.95E-03	1.20E-02	6.6	3.56E-04
4c start	1.22	18.75					8.46E-02	1.33E-02	1.39E-02	6.1	8.19E-04
5c start	4.66	19.31					8.57E-02	4.28E-03	1.39E-02	6.2	2.44E-03
6c start	1.24	16.38					7.48E-02	2.99E-03	1.19E-02	6.3	7.65E-04
7c start	1.45	16.34					7.69E-02	1.07E-02	1.04E-02	7.4	1.51E-03
1c	0.88	18.94	8.84	1.05	0.74	0.01	8.79E-02	2.27E-02	2.68E-02	3.3	3.90E-03
2c	0.71	15.51	6.02	0.33	0.24	0.04	1.00E-01	3.33E-03	1.61E-02	6.2	2.73E-03
3c	0.29	16.50	8.97	1.06	1.05	1.26	6.67E-02	2.83E-02	1.47E-02	4.5	1.33E-03
4c	1.30	19.88	6.54	1.23	0.36	0.13	7.25E-02	1.77E-02	2.02E-02	3.6	3.21E-03
5c	0.59	19.04	6.21	0.08	0.17	0.71	9.05E-02	9.40E-03	1.81E-02	5.0	3.03E-03
6c		17.26	9.44	0.31	1.29	0.61	9.73E-02	9.48E-03	1.90E-02	5.1	2.43E-03
7c	3.80	22.07	5.96	0.47	1.22	0.80	7.28E-02	1.55E-03	1.40E-02	5.2	2.43E-03
1d start							3.56E-02	6.69E-03	8.89E-03	4.0	1.08E-03
2d start							8.63E-02	1.10E-02	9.44E-03	9.1	3.56E-03
3d start							9.60E-02	1.52E-03	8.19E-03	11.7	5.82E-04
4d start							8.95E-02	1.08E-02	9.38E-03	9.5	2.82E-03
5d start							8.74E-02	1.12E-02	8.85E-03	9.9	1.31E-03
6d start							8.10E-02	1.03E-02	7.77E-03	10.4	1.07E-03
7d start							5.90E-02	6.30E-03	7.87E-03	7.5	3.37E-03
1d			8.24	0.50	0.78	0.05	9.76E-02	1.18E-02	4.30E-02	2.3	5.88E-03
2d			5.28	0.34	0.39		1.38E-01	4.44E-03	2.60E-02	5.3	5.60E-04
3d			5.10	0.42	0.33	0.13	1.33E-01	1.87E-02	1.86E-02	7.2	1.78E-03
4d			7.52	0.00	0.27	0.04	7.37E-02	1.63E-02	1.90E-02	3.9	1.78E-03
5d			3.55	0.16	0.97	0.21	1.17E-01	8.76E-03	1.84E-02	6.3	8.14E-04
6d			4.56	0.16	1.43		1.02E-01	1.56E-02	2.46E-02	4.1	9.37E-03
7d			5.00	0.46	0.59	0.09	1.33E-01	1.92E-02	2.89E-02	4.6	2.91E-03

Bottle	Glucosidase Vmax/Km	Peptidase Vmax/Km	Phosphatase Vmax/Km	Bac [ml^{-1}]	Hred [ml^{-1}]	Lred [ml^{-1}]	Horange [ml^{-1}]	Picoeukaryota [l^{-1}]	Synechoccocus spp. [l^{-1}]	big cyanos [l^{-1}]
1c start	1.04E-03	4.71E-02	1.27E-02	7.25E+05	1.96E+03	3.47E+02	2.07E+03	2.82E+06	1.38E+06	9.40E+06
2c start	1.12E-03	4.22E-02	1.24E-02	7.21E+05	2.94E+03	4.95E+02	2.43E+03	2.70E+06	1.29E+06	8.99E+06
3c start	7.94E-04	4.13E-02	1.18E-02	7.67E+05	2.59E+03	1.00E+03	3.13E+03	3.36E+06	4.73E+06	5.83E+06
4c start	1.09E-03	4.12E-02	1.21E-02	6.97E+05	2.44E+03	7.15E+02	2.88E+03	2.57E+06	1.84E+06	7.02E+06
5c start	1.14E-03	4.06E-02	1.14E-02	6.85E+05	2.28E+03	4.87E+02	3.24E+03	2.60E+06	1.61E+06	5.00E+06
6c start	1.09E-03	4.29E-02	1.32E-02	7.26E+05	2.55E+03	6.01E+02	3.18E+03	3.71E+06	2.21E+06	4.51E+06
7c start	1.37E-03	3.70E-02	1.13E-02	7.75E+05	3.11E+03	6.92E+02	3.24E+03	2.76E+06	2.45E+06	8.82E+06
1c	9.04E-04	3.90E-02	1.43E-02	9.01E+05	2.34E+03	1.87E+02	1.11E+03	2.31E+06	1.99E+06	4.61E+07
2c	1.08E-03	4.04E-02	1.41E-02	8.48E+05	2.59E+03	6.50E+02	2.47E+03	3.58E+06	1.63E+06	6.86E+06
3c	1.11E-03	4.24E-02	1.24E-02	8.65E+05	2.38E+03	7.13E+02	2.77E+03	3.25E+06	1.70E+06	4.10E+06
4c	1.09E-03	3.94E-02	1.36E-02	8.40E+05	2.16E+03	8.85E+02	3.03E+03	2.65E+06	2.27E+06	1.30E+07
5c	1.15E-03	3.80E-02	1.44E-02	8.59E+05	1.93E+03	7.20E+02	2.75E+03	2.57E+06	1.80E+06	4.84E+06
6c	9.74E-04	3.47E-02	1.49E-02	9.18E+05	2.76E+03	8.19E+02	2.90E+03	3.62E+06	2.23E+06	5.54E+06
7c	1.15E-03	3.68E-02	1.42E-02	8.14E+05	2.97E+03	7.03E+02	2.33E+03	3.80E+06	2.78E+06	9.10E+06
1d start	1.17E-03	3.87E-02	1.51E-02	9.35E+05	1.13E+03	6.63E+02	1.76E+03	1.08E+06	1.43E+06	9.27E+06
2d start	1.11E-03	4.04E-02	1.32E-02	9.83E+05	8.28E+02	7.97E+02	2.01E+03	7.76E+05	1.52E+06	3.74E+06
3d start	1.09E-03	4.25E-02	1.31E-02	1.02E+06	8.24E+02	6.20E+02	9.88E+02	9.33E+05	1.81E+06	1.14E+07
4d start	1.09E-03	4.00E-02	1.22E-02	1.04E+06	1.50E+03	1.14E+03	2.50E+03	1.51E+06	2.05E+06	4.44E+06
5d start	9.43E-04	4.21E-02	1.23E-02	1.12E+06	1.58E+03	8.58E+02	2.60E+03	1.52E+06	2.11E+06	5.62E+06
6d start	1.05E-03	3.88E-02	1.35E-02	9.12E+05	1.49E+03	1.11E+03	2.57E+03	1.59E+06	2.90E+06	9.56E+06
7d start	1.12E-03	4.02E-02	1.45E-02	9.51E+05	1.41E+03	8.71E+02	2.52E+03	1.35E+06	3.09E+06	1.95E+07
1d	7.59E-04	3.63E-02	1.08E-02	1.09E+06	1.10E+03	7.38E+02	1.55E+03	9.27E+05	1.48E+06	2.23E+07
2d	9.37E-04	3.71E-02	1.17E-02	9.25E+05	7.65E+02	8.32E+02	1.30E+03	8.88E+05	2.41E+06	1.83E+07
3d	7.85E-04	3.71E-02	1.13E-02	1.16E+06	9.99E+02	9.59E+02	1.84E+03	1.32E+06	2.03E+06	5.24E+06
4d	7.81E-04	3.66E-02	1.06E-02	1.04E+06	1.37E+03	1.13E+03	2.43E+03	1.45E+06	2.24E+06	3.46E+07
5d	8.28E-04	3.42E-02	1.19E-02	9.98E+05	1.39E+03	9.61E+02	1.86E+03	1.59E+06	1.81E+06	7.26E+06
6d	7.91E-04	4.08E-02	1.01E-02	1.00E+06	1.54E+03	1.39E+03	2.03E+03	1.40E+06	2.87E+06	7.33E+06
7d	9.22E-04	3.58E-02	1.32E-02	1.09E+06	1.24E+03	1.02E+03	1.91E+03	1.16E+06	2.01E+06	2.57E+07

Bottle	DIP turnover [h] total	DIP turnover [h] >3µm	DIP turnover [h] <3µm	affinity total normalised on nmol P	affinity total normalised on C
1c start					
2c start					
3c start					
4c start					
5c start					
6c start					
7c start					
1c	247.525424	2974.75732	270.127554	1.6097E-05	1.6745E-07
2c	405.259502	10239.5648	422.021671	1.3702E-05	1.0612E-07
3c	239.085416	1145.76833	269.84235	2.6815E-05	1.9566E-07
4c	270.735048	4778.09375	287.087129	1.2605E-05	1.6912E-07
5c	265.361755	2272.78359	274.728611	2.0882E-05	1.8241E-07
6c	167.184017	1235.40976	193.585177	2.728E-05	2.2948E-07
7c	281.7409	1409.36155	352.510864	9.5984E-05	2.1375E-07
1d start					
2d start					
3d start					
4d start					
5d start					
6d start					
7d start					
1d	248.040708	2683.65915	273.454122	1.416E-05	1.7044E-07
2d	399.373549	5375.41067	416.490511	1.2211E-05	1.2141E-07
3d	328.616203	5055.42705	351.566701	1.0517E-05	1.7232E-07
4d	272.065964	7702.1226	282.083265	1.2644E-05	1.8283E-07
5d	629.920183	2309.60271	866.71817	6.0449E-06	6.9521E-08
6d	499.308959	3166.09969	593.076919	5.9518E-06	8.2674E-08
7d	317.668315	2711.55777	360.022313	7.9845E-06	1.2679E-07

8. APPENDIX III – CHAPTER 3.3

Bottle	PON >3 av [µM]	PON>3 sd	PON <3 [µM]	POC >3 av [µM]	POC>3 sd	POC <3 [µM]	C:N total	C:N >3	C:N <3	Nfix >3 [nmol/l h]	Nfix >3 sd	Nfix <3 [nmol/l h]
1	30.27	1.93	4.42	225.29	42.23	23.20	7.16	7.44	5.24	26.16	4.66	0.29
2	30.73	3.13	4.82	201.15	39.60	24.24	6.34	6.55	5.03	33.18	15.95	0.87
3	29.48	1.76	3.91	198.03	10.93	20.21	6.54	6.72	5.17	25.48	0.11	0.59
1a	31.18	0.60	3.59	196.64	5.44	20.30	6.24	6.31	5.65	25.70	2.41	0.16
2a	32.05	0.75	3.48	204.95	15.75	19.09	6.31	6.40	5.48	29.53	1.59	0.38
3a	32.82	0.47	3.35	214.50	8.29	18.82	6.45	6.54	5.62	16.78	1.60	0.27
4a	33.08	2.46	2.83	218.82	21.81	15.74	6.53	6.62	5.56	28.99	10.90	0.32
5a	32.76	1.11	3.00	213.97	12.86	16.79	6.45	6.53	5.60	21.34	9.46	0.21
6a	34.61	0.39	3.00	232.74	3.19	16.83	6.64	6.73	5.61	26.26	4.28	0.27
1b	27.15	3.86	2.82	165.89	24.32	16.47	6.08	6.11	5.84	2.73	0.37	0.06
2b	30.30	4.03	2.70	191.98	32.55	14.98	6.27	6.34	5.56	2.65	0.81	0.09
3b	33.05	0.28	2.41	211.82	7.50	15.08	6.40	6.41	6.26	4.90	2.42	0.10
4b	28.42	6.17	2.83	181.70	43.28	15.38	6.30	6.39	5.43	2.15	0.68	-0.09
5b	31.24	2.74	2.82	192.15	18.38	16.35	6.12	6.15	5.80	3.08	0.28	0.07
6b	34.53	0.66	2.55	216.11	1.22	14.97	6.23	6.26	5.88	2.44	0.81	0.13
1c	26.97	0.21	1.62	163.26	0.84	9.88	6.06	6.05	6.09	1.79	1.64	0.18
2c	27.98	0.79	1.67	172.15	4.29	10.42	6.16	6.15	6.25	2.10	0.27	0.08
3c	25.14	1.61	1.61	154.02	10.91	0.00	5.76	6.13	0.00	2.30	0.52	0.13
4c	27.98	1.26	1.80	170.61	6.34	11.03	6.10	6.10	6.12	2.80	1.11	0.05
5c	24.68	0.96	1.58	151.67	4.37	9.44	6.14	6.14	5.99	1.74	0.08	0.02
6c	28.48	1.24	1.67	175.15	9.26	9.62	6.13	6.15	5.77	3.67	0.71	0.05

Bottle	Cfix >3 [µmol/l h]	Cfix >3 sd	Cfix <3 [µmol/l h]	P uptake total [nmol/l h]	P uptake total sd	P uptake >3 [nmol/l h]	P uptake <3	turnover total [h]	turnover total sd
1	2.05	0.16	0.07	20.65		9.04	11.61	0.30	0.07
2	2.62	2.12	0.07	16.56	2.85	4.70	11.86	0.23	0.01
3	2.54	0.19	0.06	16.44	6.09	4.68	11.76	0.23	0.00
1a	1.64	0.96	0.08	17.11	0.86	2.16	14.95	0.23	0.00
2a	2.16	0.50	0.08	17.43	3.44	3.44	13.99	0.22	0.00
3a	2.09	0.43	0.09	18.66	2.16	2.39	16.27	0.25	0.00
4a	2.39	0.88	0.08	18.58	2.69	2.41	16.17	0.25	0.00
5a	2.51	0.59	0.09	16.32	1.29	2.40	13.92	0.28	0.01
6a	3.22	0.26	0.10	18.88	1.36	2.56	16.32	0.30	0.03
1b	0.84	0.05	0.07	17.18	1.49	1.96	15.22	0.23	0.00
2b	1.21	0.08	0.07	21.60	3.66	2.89	18.71	0.22	0.00
3b	1.45	0.16	0.08	10.47	5.51	1.11	9.36	0.25	0.00
4b	0.84	0.12	0.08	9.06	0.60	1.09	7.97	0.25	0.00
5b	1.19	0.09	0.10	2.97	1.94	0.25	2.72	0.28	0.00
6b	1.54	0.12	0.09	5.51	2.40	0.86	4.65	0.28	0.00
1c	0.88	0.07	0.06	3.55	0.27	0.58	2.97	0.28	0.00
2c	1.13	0.12	0.16	3.20	0.22	1.37	1.83	0.25	0.01
3c	0.77	0.07	0.00	3.54	0.03	1.78	1.76	0.31	0.01
4c	0.87	0.02	0.08	3.29	0.14	1.15	2.14	0.26	0.00
5c	0.82	0.04	0.09	4.20	0.06	1.97	2.23	0.33	0.01
6c	0.97	0.12	0.12	2.77	0.18	1.31	1.46	0.31	0.01

Bottle	turnover >3 [h]	turnover >3 sd	turnover <3 [h]	turnover <3 sd	DIP affinity >3	DIP affinity <3	DIP affnity total	POP>3 µM [µM]
1	0.35	0.04	0.34	0.13	0.003	0.011	0.003	0.89
2	0.34	0.02	0.25	0.02	0.003	0.016	0.004	0.90
3	0.33	0.02	0.25	0.01	0.003	0.016	0.004	0.91
1a	0.45	0.04	0.23	0.00	0.002	0.016	0.004	0.94
2a	0.43	0.01	0.23	0.00	0.003	0.018	0.004	0.83
3a	0.47	0.01	0.23	0.00	0.002	0.018	0.004	0.88
4a	0.48	0.01	0.23	0.00	0.002	0.018	0.004	0.84
5a	0.51	0.01	0.24	0.01	0.002	0.017	0.003	0.84
6a	0.49	0.00	0.26	0.03	0.002	0.015	0.003	0.96
1b	0.46	0.03	0.23	0.00	0.003	0.020	0.004	0.82
2b	0.41	0.02	0.23	0.00	0.003	0.017	0.005	0.74
3b	0.55	0.06	0.23	0.00	0.002	0.017	0.004	0.87
4b	0.49	0.02	0.23	0.00	0.003	0.017	0.004	0.70
5b	0.70	0.05	0.23	0.00	0.002	0.016	0.003	0.79
6b	0.42	0.14	0.24	0.00	0.003	0.019	0.003	0.84
1c	0.37	0.02	0.34	0.02	0.004	0.019	0.004	0.76
2c	0.33	0.01	0.30	0.01	0.004	0.018	0.004	0.74
3c	0.39	0.01	0.36	0.05	0.003	0.013	0.003	0.82
4c	0.35	0.02	0.27	0.00	0.003	0.018	0.003	0.91
5c	0.41	0.01	0.32	0.03	0.003	0.017	0.003	0.88
6c	0.38	0.00	0.29	0.02	0.003	0.016	0.003	0.92

Bottle	POP<3 [µM]	POP total [µM]	DIP [µM]	DOP [µM]	C:P total	C:P >3	C:P <3	N:P total	N:P >3	N:P <3
1	0.27	1.16	0.02	0.23	214.25	254.45	84.53	29.92	34.19	16.12
2	0.25	1.15	0.01	0.24	196.45	224.74	96.09	30.98	34.33	19.10
3	0.25	1.16	0.01	0.22	187.59	216.72	80.95	28.70	32.27	15.66
1a	0.27	1.22	0.01	0.26	178.39	208.39	74.50	28.59	33.05	13.18
2a	0.23	1.07	0.01	0.25	210.22	246.27	81.75	33.34	38.50	14.92
3a	0.25	1.13	0.01	0.24	207.31	243.64	76.79	32.13	37.28	13.66
4a	0.24	1.08	0.01	0.27	217.80	261.94	65.16	33.34	39.59	11.73
5a	0.25	1.10	0.01	0.25	210.30	253.85	65.99	32.59	38.86	11.79
6a	0.26	1.22	0.01	0.23	204.41	241.96	64.98	30.80	35.98	11.58
1b	0.22	1.04	0.01	0.26	175.29	201.42	75.99	28.81	32.97	13.00
2b	0.26	0.99	0.01	0.26	208.27	260.18	58.57	33.21	41.07	10.54
3b	0.25	1.12	0.01	0.23	202.84	244.12	60.08	31.70	38.09	9.60
4b	0.26	0.96	0.01	0.28	204.36	258.49	58.83	32.41	40.44	10.84
5b	0.27	1.05	0.01	0.26	198.03	244.00	61.61	32.35	39.67	10.62
6b	0.23	1.06	0.01	0.25	217.76	258.58	66.41	34.94	41.32	11.29
1c	0.16	0.91	0.01	0.30	189.42	215.10	63.72	31.28	35.53	10.46
2c	0.18	0.92	0.01	0.28	197.91	232.14	57.62	32.14	37.74	9.21
3c	0.21	1.04	0.01	0.28	148.72	186.96	0.00	25.82	30.51	7.58
4c	0.20	1.11	0.00	0.27	163.27	187.83	54.01	26.78	30.81	8.83
5c	0.19	1.07	0.01	0.29	151.24	172.47	50.80	24.65	28.07	8.48
6c	0.22	1.14	0.00	0.26	161.43	189.37	43.79	26.34	30.80	7.59

Bottle	Phaeo [µg/L] >3	Chl*a* [µg/L] >3	Phaeo [µg/L] <3	Chl*a* [µg/L] <3	Phaeo [µg/L]	Chl*a* [µg/L]	HDNA [ml^{-1}]	LDNA [ml^{-1}]	Pico [l^{-1}]
1	3.87	18.94	0.06	0.71	3.93	19.65	2.77E+06	1.78E+06	2.37E+06
2	3.63	19.18	0.09	0.47	3.72	19.65	2.80E+06	1.88E+06	2.22E+06
3	4.24	21.97	-0.03	0.49	4.21	22.46	2.70E+06	1.79E+06	1.46E+06
1a	3.75	14.45	0.04	0.28	3.79	14.72	5.05E+06	2.19E+06	2.89E+06
2a	3.87	16.51	0.03	0.29	3.91	16.80	5.49E+06	2.35E+06	2.87E+06
3a	3.51	16.87	0.03	0.20	3.54	17.08	4.70E+06	2.14E+06	2.62E+06
4a	2.53	17.92	0.03	0.23	2.56	18.15	5.26E+06	2.25E+06	2.94E+06
5a	2.62	17.83	0.04	0.27	2.65	18.10	4.36E+06	1.96E+06	4.00E+06
6a	-7.09	24.28	0.04	0.20	-7.05	24.48	3.92E+06	1.67E+06	2.36E+06
1b	2.94	10.67	0.03	0.25	2.97	10.91	4.08E+06	2.25E+06	2.51E+06
2b	2.86	9.36	0.03	0.33	2.89	9.69	3.80E+06	2.02E+06	3.08E+06
3b	2.59	10.32	0.02	0.29	2.61	10.61	4.40E+06	2.40E+06	2.90E+06
4b	2.68	10.22	0.05	0.40	2.73	10.62	3.95E+06	2.26E+06	1.86E+06
5b	3.11	12.57	0.09	0.42	3.21	12.99	3.72E+06	2.67E+06	5.05E+06
6b	3.72	14.09	0.10	0.40	3.82	14.50	3.57E+06	2.45E+06	4.99E+06
1c	2.60	9.02	0.42	0.39	3.02	9.41	2.49E+06	1.92E+06	3.82E+06
2c	3.20	12.11	0.14	0.59	3.34	12.70	2.00E+06	2.15E+06	4.45E+06
3c	2.94	8.32	-0.13	0.84	2.81	9.17	1.56E+06	2.06E+06	6.40E+06
4c	2.94	12.39	0.16	0.44	3.10	12.84	2.04E+06	2.06E+06	5.78E+06
5c	2.62	7.79	-0.03	0.66	2.59	8.45	1.90E+06	1.96E+06	5.07E+06
6c	3.98	13.34	0.12	0.50	4.10	13.84	2.04E+06	2.22E+06	4.17E+06

Bottle	Nano big [l^{-1}]	Syns total [l^{-1}]	small Nano [l^{-1}]	Syns early stage [l^{-1}]	Syns late stage [l^{-1}]	big cyano-bacteria [l^{-1}]	Phages [ml^{-1}]	$NO_{2/3}$ [µM]	NO_2 [µM]	NO_3 [µM]
1	2.35E+06	1.26E+08	3.91E+05	1.03E+08	2.27E+07	2.75E+07	5.37E+07	0.11	0.02	0.09
2	1.99E+06	1.19E+08	2.31E+05	8.85E+07	3.08E+07	2.83E+07	5.00E+07	0.10	0.01	0.09
3	2.84E+06	3.70E+07	2.63E+05	2.59E+07	1.12E+07	2.34E+07	4.46E+07	0.15	0.03	0.12
1a	1.24E+06	1.41E+08	5.83E+05	1.27E+08	1.48E+07	1.94E+07	1.08E+08	0.00	0.01	0.01
2a	2.32E+06	1.43E+08	1.10E+06	1.28E+08	1.55E+07	2.16E+07	7.88E+07	0.07	0.01	0.06
3a	2.42E+06	9.53E+07	8.08E+05	7.90E+07	1.62E+07	2.24E+07	8.17E+07	0.08	0.01	0.07
4a	1.87E+06	1.23E+08	5.83E+05	1.10E+08	1.23E+07	1.88E+07	7.66E+07	0.01	0.01	0.01
5a	2.75E+06	9.89E+07	6.86E+05	7.64E+07	2.25E+07	2.37E+07	8.22E+07	0.01	0.01	0.01
6a	1.91E+06	8.95E+07	7.82E+05	7.25E+07	1.70E+07	2.19E+07	8.04E+07	0.02	0.02	0.02
1b	3.98E+05	1.28E+08	1.28E+06	1.03E+08	2.46E+07		1.02E+08	0.16	0.03	0.13
2b	2.95E+05	2.63E+08	1.03E+06	2.18E+08	4.50E+07		1.01E+08	0.13	0.02	0.11
3b	6.73E+05	1.10E+08	1.19E+06	8.18E+07	2.80E+07		9.44E+07	0.15	0.03	0.12
4b	4.23E+05	2.93E+08	1.05E+06	2.43E+08	4.92E+07		1.08E+08	0.12	0.02	0.10
5b	8.46E+05	1.04E+08	9.23E+05	7.24E+07	3.20E+07		9.71E+07	0.16	0.03	0.13
6b	7.69E+05	9.53E+07	1.44E+06	5.29E+07	4.24E+07		1.02E+08	0.14	0.02	0.12
1c	3.21E+05	9.59E+07	2.21E+06	4.92E+07	4.67E+07		1.23E+08	0.09	0.02	0.07
2c	3.72E+05	8.27E+07	1.70E+06	3.43E+07	4.83E+07		1.12E+08	0.08	0.02	0.07
3c	2.50E+05	8.54E+07	1.80E+06	3.63E+07	4.91E+07		1.20E+08	0.34	0.02	0.32
4c	2.31E+05	1.30E+08	2.01E+06	6.64E+07	6.35E+07		1.09E+08	0.19	0.01	0.18
5c	2.44E+05	1.74E+08	2.08E+06	9.77E+07	7.58E+07		1.19E+08	0.11	0.03	0.09
6c	4.30E+05	1.34E+08	1.71E+06	6.67E+07	6.74E+07		1.12E+08	0.09	0.03	0.07

Bottle	PO_4 [µM]	SiO_4 [µM]	Thymidin uptake [nmol $l^{-1}h^{-1}$]	Thymidin uptake stdv [nmol $l^{-1}h^{-1}$]	T [°C]	S [PSU]	pCO_2 [µatm]	pCO_2 start [µatm]	TA [µmol/kg]	TCO_2 [µmol/kg]
1	0.02	10.30	0.55	0.01	21.50	7.00	210.00		1766.52	1520.06
2	0.01	10.55	0.53	0.03	21.00	7.00	203.00		1695.76	1465.33
3	0.01	7.90	0.67	0.06	21.00	7.00	226.00		1657.86	1423.99
1a	0.01	7.60	2.54	0.03	18.40	7.00	217.00	210.00	1714.07	1461.29
2a	0.01	8.70	1.78	0.10	18.10	7.00	214.00	300.00	1714.07	1467.55
3a	0.01	11.10	1.86	0.26	17.70	7.00	351.00	450.00	1695.76	1588.00
4a	0.01	11.30	2.15	0.14	18.00	7.00	286.00	600.00	1618.17	1496.14
5a	0.01	9.90	1.97	0.09	18.00	7.00	417.00	800.00	1618.17	1541.74
6a	0.01	8.70	1.78	0.04	18.20	7.00	461.00	1000.00	1657.86	1587.21
1b	0.01	10.35	1.21	0.06	20.30	7.00	194.00	210.00	1943.60	1665.38
2b	0.01	12.15	1.40	0.10	19.40	7.00	280.00	300.00	1677.03	1536.78
3b	0.01	12.10	1.99	0.05	19.60	7.00	300.00	450.00	1677.03	1552.79
4b	0.01	8.15	1.73	0.03	19.00	7.00	508.00	600.00	1731.96	1664.49
5b	0.01	10.95	1.34	0.20	19.70	7.00	466.00	800.00	1714.07	1635.30
6b	0.01	8.70	1.71	0.15	19.80	7.00	480.00	1000.00	1731.96	1658.46
1c	0.01	8.50	1.39	0.04	18.10	7.00	278.00	210.00	1749.44	1527.25
2c	0.01	15.00	1.09	0.06	18.00	7.00	248.00	300.00	1618.17	1446.10
3c	0.01	9.30	1.36	0.08	17.90	7.00	361.00	450.00	1618.17	1509.14
4c	0.00	10.90	1.28	0.02	17.70	7.00	541.00	600.00	1618.17	1553.84
5c	0.01	14.85	1.28	0.07	18.10	7.00	611.00	800.00	1657.86	1600.29
6c	0.00	9.10	1.33	0.15	18.00	7.00	699.00	1000.00	1618.17	1567.00

Bottle	*Aphanizomenon* [units/l]	*Aphanizomenon* heterocysts [units/l]	*Nodularia* [units/l]	*Nodularia* heterocysts [units/l]	*Anabaena* [units/l]	*Anabaena* hetrocysts [units/l]	*Anabaena* akinets [units/l]
1	3.50E+06	6.93E+05	3.15E+05	6.68E+05	7.35E+04	7.35E+04	0.00E+00
2	1.97E+06	3.07E+05	1.34E+05	2.88E+05	3.94E+04	7.88E+03	0.00E+00
3	3.09E+06	4.73E+05	2.08E+05	4.66E+05	3.15E+04	3.15E+04	0.00E+00
1a	2.26E+06	3.78E+05	2.16E+05	3.65E+05	2.10E+04	1.05E+04	4.20E+04
2a	2.27E+06	3.42E+05	1.31E+05	3.20E+05	5.25E+04	0.00E+00	6.30E+04
3a	2.00E+06	3.39E+05	2.21E+05	0.00E+00	8.67E+04	0.00E+00	0.00E+00
4a	2.24E+06	4.41E+05	9.45E+04	1.98E+05	2.10E+05	1.37E+05	0.00E+00
5a	2.32E+06	3.26E+05	1.34E+05	0.00E+00	4.20E+04	0.00E+00	0.00E+00
6a	2.39E+06	3.73E+05	1.05E+05	2.26E+05	5.25E+04	5.25E+04	0.00E+00
1b	3.16E+05	3.69E+04	1.30E+05	2.23E+05	3.78E+04	3.15E+03	8.51E+04
2b	2.08E+05	1.74E+04	1.27E+05	1.65E+05	3.78E+04	1.26E+04	7.56E+04
3b	2.93E+05	3.78E+04	2.33E+05	3.53E+05	5.99E+04	3.15E+03	1.45E+05
4b	2.59E+05	2.56E+04	1.12E+05	1.96E+05	4.80E+04	2.40E+04	0.00E+00
5b	5.55E+05	7.01E+04	1.02E+05	1.83E+05	6.30E+04	5.25E+03	1.47E+05
6b	4.98E+05	5.99E+04	2.06E+05	3.45E+05	3.15E+04	5.25E+03	3.68E+04
1c	1.66E+06	6.87E+05	2.33E+05	3.24E+05	4.41E+04	3.15E+03	1.54E+05
2c	1.15E+05	1.72E+04	1.52E+05	2.35E+05	4.41E+04	0.00E+00	1.05E+05
3c	1.35E+05	1.68E+04	1.06E+05	1.67E+05	2.94E+04	0.00E+00	1.18E+05
4c	9.32E+04	1.20E+04	1.64E+05	2.33E+05	5.67E+04	2.10E+03	1.51E+05
5c	5.49E+04	5.85E+03	1.39E+05	2.17E+05	5.67E+04	2.10E+03	4.32E+04
6c	1.66E+05	2.05E+04	1.76E+05	2.81E+05	5.88E+04	2.10E+03	2.21E+05

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STATEMENT ON MY CONTRIBUTION TO THE INVESTIGATIONS

CHAPTER 3.1

The presented experiments were planned, conducted and analyzed by me, Maren Voss and Marco Lunau. Concluding examination and summarising of the results were done by me. It was written by me with scientific advise of Maren Voss, Monika Nausch and Mirko Lunau

CHAPTER 3.2

The presented experiments were planned, conducted and analyzed by me, Maren Voss, Marco Lunau and Andreas Weiss. TDC and TN analyzes were perfomed by Boris Koch and his working group. It was written by me with scientific advise of Maren Voss, Monika Nausch and Mirko Lunau.

CHAPTER 3.3

The presented studies were planned by me. The experiments were conducted together with Nicola Wannicke. It was written by me with the scientific advise of Maren Voss, Monika Nausch and Anja Engel.

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Presentations, Talks, Publications

Isensee, K.; Nausch, M..; Weiss, A.; Wannicke, N.; Lunau, M. & Voss, M. Elevated CO_2 concentrations increase the productivity of heterocystic cyanobacteria in the Baltic Sea – evidence from field investigations and laboratory experiments, Marine Biology (in prep.)

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ERKLÄRUNG

Ich versichere hiermit an Eides statt, dass ich die vorliegende Arbeit selbstständig angefärtigt und ohne fremde Hilfe verfasst habe, keine außer den von mir angegebenen Hilfsmitteln und Quellen dazu verwendet habe und die den benutzten Werken inhaltlich und wörtlich entnommenen Stellen als solche kenntlich gemacht habe.

Oviedo, den