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Wachstum und Produktion einer Cyanobakterienblüte der Ostsee in Abhängigkeit von der CO_2-Konzentration.

Growth and production of a Baltic Sea cyanobacteria bloom in relation to pCO_2.

Diplomarbeit

vorgelegt von

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Rostock, 10. April 2012

Jenny Fabian

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Abstract

In face of the high impact of cyanobacteria on the Baltic Sea ecosystems it is important to understand how they will react towards predicted changes in ocean carbonate chemistry to quantify the resulting changes within the whole ecosystem. Therefore the aim of this study was to investigate the effect of increasing pCO_2 on a phytoplankton community predominated by cyanobacteria. Across a natural pCO_2 gradient of the Baltic proper (140 to 228 µatm) growth and productivity of cyanobacteria blooms were analyzed. To quantify the impact of predicted increases in pCO_2, surveys made on cruise were supported by a mesocosm study in a pCO_2 gradient from 180 to 950 µatm. As the carbonate system of the Baltic Sea is characterised by a large spatial and seasonal variability phytoplankton communities are familiar with changes in pCO_2 and expected to be well adapted and therefore strong related to changing [CO_2]. Yet, in this study such relationship was not observed for a natural phytoplankton community predominated by cyanobacteria, neither in the field, nor in a mesocosm experiment. It is known than occurrence and magnitude of cyanobacteria blooms are strong related to environmental factors including nutrients, light and temperature. During cruise biomass and fixation rates were strong related to irradiance and temperature but not to pCO_2. This suggests that a positive effect of pCO_2 was cancelled out by negative physical influences like decreasing light intensity (irradiance) or temperature whereas in result a stimulation of growth and production with rising pCO_2, as known from culture studies, could not be observed in the field. Moreover, the studied community was in a late bloom state as indicated by the high POC:POP ratios (120 to 303) that indicate phosphate starvation and the rely on intracellular phosphorous resources.

Cyanobacteria seem to take advantage from the hydrologic and methodological modifications that result from climate change. However if this is supported even further by ocean acidification cannot be approved so far. Yet, as indicated by the results of this study there might be interactive effect between environmental factors and the stimulating effect of rising pCO_2 diminish under nutrient deplete conditions. Hence, it is even more important to reduce the anthropogenic input of inorganic nutrients to act against possible stimulating effects of cyanobacterial blooms.

Zusammenfassung

In Anbetracht des immensen Einflusses von Cyanobakterien auf das Ökosystem der Ostsee ist es wichtig zu verstehen, wie jene auf vorhergesagte Veränderungen des ozeanischen Carbonatsystems reagieren, um mögliche Auswirkungen auf das gesamtem Ökosystems zu quantifizieren. Darin begründet war das Ziel dieser Arbeit mögliche Effekte ansteigender CO_2 Konzentrationen auf eine Phytoplanktongemeinschaft, dominiert bei Cyanobakterien, zu erforschen. Entlang eines natürlichen pCO_2 Gradienten (140 to 228 µatm) durch die zentrale Ostsee wurden Wachstum und Produktion der Phytoplanktongemeinschaft analysiert. Die Feldbeobachtungen wurden mit einem Tankversuch (180 to 950 µatm) untermauert, um auch eine Aussage über zukünftige pCO_2-Konzentrationen treffen zu können. Das Carbonatssystem der Ostsee ist in der Deckschicht durch starke saisonale, sowie lokale Unterschiede charakterisiert. Heimische Spezies sind demnach an unterschiedliche pCO_2 gewöhnt eine starke Stimulierung ansteigender [CO_2] war erwartet. Jedoch wurden derartige Stimulierungen nicht beobachtet, weder im Feld noch im Tankexperiment. Auftreten und Ausmaß N_2-fixierender Cyanobakterien sind bekanntlich von Umweltfaktoren wie Licht, Temperatur und Nährstoffverfügbarkeit abhängig. Im Feld wurde eine starke Abhängigkeit der Fixierungsraten von den Temperatur- und vom Lichtverhältnissen beobachtet. Das lässt

vermuten, dass sich positive Effekte wie das Ansteigenden des CO_2 Partialdruckes und negative Effekte wie die Verringerung der Lichtintensität und Temperatur gegenseitig aufheben wobei netto ein CO_2 Effekt auf Wachstum und Produktion, wie er in anderen Studien beobachtet wurde, verschwindet. Weiterhin befand sich die observierte Gemeinschaft bereits in einem späten Blütestadium, was auch die POC:PON Verhältnissen (120 zu 303) deutlich machen. Sie überragen typische Redfield-Verhältnisse von 106 und lassen vermuten, dass die Gemeinschaft bereits angefangen hat ihren Bedarf an Phosphor aus intrazellulärem Speicher zu decken und daher stark Phosphat limitiert ist.

Cyanobakterien scheinen von den zukünftigen hydrologischen und meteorologischen Veränderungen, welche klimatisch bedingt sind, zu profitieren. Ob dieser positive Effekt durch zukünftig ansteigende CO_2 Partialdrücke unterstützt wird kann mit den Ergebnissen aus dieser Studie nicht beantwortet werden. Jedoch, scheint das Ausmaß der zellulären Stimulierung ansteigender pCO_2 stark von anderen Umweltfaktoren abhängig zu sein und verschwindet sogar unter Nährstofflimitation. Daher ist es um so wichtiger Nährstoffeinträge in die Ostsee zu reduzieren umso einer möglichen starken Stimulierung von Cyanobakterienblüten entgegen zu wirken.

Table of contents

Figures

Tables

Abbreviations

A_T : total Alkalinity
C : Carbon
C_T : Total CO_2, Carbonate alkalinity
Chl a : Chlorophyll a
$CO_{2\ (aq)}$: Dissolved CO_2
DIC : Dissolved inorganic carbon
N : Nitrogen
PN : Particulate nitrogen
PON : Particulate organic nitrogen
POC : Particulate organic carbon
POP : Particulate organic phosphate
T : Temperature
S : Salinity
pCO_2 : CO_2 partial pressure

1. Introduction

During the last 200 years the carbon cycle has been strongly influenced by human activities through massive release of carbon dioxide (CO_2) by fossil fuels burning, cement production and changes in land use (Doney 2010). In result, atmospheric [CO_2] increased from pre-industrial level of approximately 280 µatm to nearly 385 µatm in present days and is suspected to double by the end of this century (Solomon et al. 2007; Doney et al. 2009). Thereby the increase in atmospheric pCO_2 did not only occur much faster than the natural glacial-interglacial cycles (180↔280 µatm) of 100,000 years but also exceeds pCO_2 levels of the past 20 million years by ~100 µatm (Berner 1990) .

About 25 to 30 % of the CO_2 emitted by human activity is absorbed by the ocean because a permanent gas exchange between both compartments exists (Sabine et al. 2004; Canadell et al. 2007). Thus ocean uptake helps to limit the concentration of CO_2 in the atmosphere but it changes the oceans fundamental chemistry (Orr 2011). When CO_2(g) dissolves in the surface water its aqueous form (CO_2(aq)) reacts with water or carbonate ion (CO_3^-) to form true carbonic acid (H_2CO_3) and hydrogen carbonate (HCO_3^-) (*Equation 1.1 and 1.2, buffer reaction*). H_2CO_3 further dissociates to HCO_3^- and CO_3^-, which produces H^+. That in turn increases [H^+] and therefore lowers the pH that is defined as $-\log_{10}[H^+]$ (Doney et al. 2009; Orr 2011).

$$CO_3^{-2} + CO_2 + H_2O \Leftrightarrow 2HCO_3^- \quad (1.1)$$

$$CO_2 + H_2O \Leftrightarrow H_2CO_2 \Leftrightarrow H^+ + HCO_3^- \Leftrightarrow 2H^+ + CO_3^{2-} \quad (1.2)$$

The effect of this rapid increase in dissolved inorganic carbon concentration ([DIC]=[HCO_3^-]+[CO_3^-]+[H_2CO_3]) is already causing measurable changes in oceanic carbonate chemistry in both the mixed layer and deep waters. From preindustrial to present day surface pH dropped by 0.1 units and a further decline by 0.3 to 0.4 units is suggested until year 2100 (Caldeira and Wickett 2003).

pCO_2 and the pH are among the most important factors controlling life in marine systems (Omstedt et al. 2010). Thus, there is growing concern that ocean acidification, together with other human activities, will cause significant changes in the biodiversity and function of marine ecosystems (Barry, Widdicombe, and Hall-spencer 2011). One well-known effect is the shift in the pH dependent equilibrium reaction of the carbonate system (*equation 1.2*) leading to an increase of $CO_{2(aq)}$ in relation to its dissolved products. From preindustrial to present day oceanic [CO_3^{2-}] decreased from 222 to 186 µmol Kg^{-1} and a

further decline by 81 µmol Kg^{-1} until 2100 is expected whereas [CO_2] increased from 9 to 13 µmol Kg^{-1} and is thought reach future concentrations of 25µmol Kg^{-1} (Fabry 2008). The loss of CO_3^- ions causes a decrease in the calcium carbonate (aragonite/calcite) saturation that was shown to have a negative impact on the shell formation of calcifying organisms (*equation 1.3*) like coccolithophorids, foraminifera, molluscs, echinoderm and corals (Bellerby et al. 2008; Bijma 2002; Zondervan 2002; Krug, Schulz, and Riebesell 2010; Hendriks, Duarte, and Álvarez 2010).

$$Ca^{2+} + CO_3^{2-} \Leftrightarrow CaCO_3 \qquad 1.3$$

Instead, photosynthesis of some primary producers appears to benefit from moderate increases in pCO_2 (B Rost, Zondervan, and Wolf-Gladrow 2008). Phytoplankton species are generally carbon limited because the enzyme ribulose-1,5-bisophosphate carboxylase oxygenase (RuBisCO) that catalyses the acquisition of CO_2 is characterised by a very low affinity for its substrate. To ensure an effective carboxylation the intracellular CO_2 is raised by so-called carbonate concentrating mechanisms (Murray R Badger et al. 1998). Since higher [CO_2] allow greater efficiency in carbonate concentrating mechanism it frees up energy that can be shunted towards other metabolic processes like the fixation of CO_2 (S. Kranz, Eichner, and Rost 2010; Raven 1991).

On an ecosystem level increased fixation of CO_2 enhances production, exudation as well as the vertical flux of biogenic material (Arrigo 2007). This process also referred as "biological pump" will potentially increase the CO_2 storage capacity of the ocean but also trigger oxygen minimum zones (Hofmann and Schellnhuber 2009). However, the extent of this CO_2- fertilization depends on the physiology of individual photosynthesizers (Björn Rost et al. 2003). Hence, it is likely that species specific responses will lead to changes in phytoplankton composition favouring algae that gain and affecting calcifying organisms that suffer from acidification processes (Ulf Riebesell 2004). In particular organism with high RuBisCo saturation levels and an inefficient carbon acquisition pathway like for instance cyanobacteria are thought to succeed from increasing pCO_2. (Ulf Riebesell 2004; Gehlen et al. 2011).

Cyanobacteria are gram (+) photo-autotrophic bacteria. Many cyanobacteria are able to reduce dinitrogen (N_2) and are therefore independent of combined nitrogen sources in contrast to most phytoplankton species. As nitrogen is often the nutrient that limits primary productivity in many oceanic environments (M R Mulholland and Capone 2000) cyanobacteria are an important link in the global nitrogen cycle (Breitbarth, Oschlies, and LaRoche 2007). Especially, in the open oligothrophic ocean pelagic N_2 fixation is the

dominant natural source of new nitrogen (M R Mulholland 2007; J LaRoche and Breitbarth 2005).

Cyanbacteria are quite diverse in their physiology and morphology including unicellular and filamentous forms. In some filamentous cyanobacteria the fixation of N_2 is restricted to specialized cells, the so-called heterocyst. Oligotrophic tropical and subtropical waters are dominated by the filamentous, non-heterocystous genius *Trichodesmium sp.* (Douglas G Capone 1997; Douglas G. Capone 2005) and small, unicellular diazothrophs like *Crocosphaera watsonii* accounting for more than 50% of total nitrogen fixation there (Joseph P Montoya et al. 2004; J P Zehr et al. 2001). Instead, heterocystous diazotroph are typically distributed in more specialized environments like the cooler, brackish waters of the Baltic Sea (Sohm, Webb, and Capone 2011).

The Baltic Sea is one of the view brackish water areas where diazotroph cyanobacteria are an important component of phytoplankton (Larsson et al. 2001; Howarth 1988) as they represents about 44% of the community primary production (Lucas J Stal and Walsby 2000; Ploug et al. 2010). Extensive cyanobacteria blooms are recurring phenomena during late summer; in particular in the central Baltic Sea (Baltic Proper), the Bothnian Sea and the Gulf of Finnland. They are favoured by the low ratio of inorganic nitrogen to phosphorous (N:P<8) compared to the Redfield ratio (N:P=16) required for a build-up of biomass that is leading to a general nitrogen- limitation of primary production there (Monika Nausch et al. 2008; M. Raateoja, Kuosa, and Hällfors 2011). A bloom is referred to a biomass development of more than 22 µg C/l (Norbert Wasmund 1997). They are mainly composed of the freshwater species Nodularia spumigena, Aphanizomenon flos-aque and Anabaena sp., whereas dominance of the latter is mostly inconsiderable (Larsson et al. 2001). Small-sized picocyanobacteria are also present in varying proportions (~70 to 80) but have not been observed to fix N_2, so far (L Stal 2003).

The newly fixed nitrogen by cyanobacteria is carried into the food web via cell lyse, grazing or exudation of ammonia (NH_4^+) and dissolved organic nitrogen (DON) (M. Mulholland, Bronk, and Capone 2004) and comprises about 50% of the total nitrogen input in the Baltic Sea (434000 t a^{-1}, Helcom Commission 2009). That in turn underlines the important function of cyanobacteria as it increases the overall productivity of an ecosystem that is already suffering from eutrophication because of high anthropogenic nutrient input (Emil Vahtera et al. 2007; Maren Voss et al. 2011). Moreover, the genera are potentially toxic as they produce hepa- and neurotoxines that tend to accumulate within the food chain thus are a

potential threat for marine life forms and humans (Jonasson et al. 2010; Laamanen, Gugger, and Lehtima 2001; Uronen 2007).

In the last few decades frequency and intensity of blooms have increased, probably as a consequence of anthropogenic eutrophication and climate change (Karjalainen et al. 2007). To date, it is not well understood and barely investigated how ocean acidification will affect diazotroph cyanobacteria. There are several studies, mainly on cultures of the marine genera Trichodesmium, that observed a strong elevation of N_2- and CO_2 fixation rates, as well as growth (D. A. Hutchins, Mulholland, and Fu 2009; D A Hutchins et al. 2007; Barcelos e Ramos et al. 2007). Yet, the magnitude of stimulation varied between species and was shown to depend on physical conditions like temperature and light intensity as well as on nutrient supply (Nathan S Garcia et al. 2011; F. Fu et al. 2008; Sven Kranz et al. 2010; Levitan, Brown, et al. 2010).

Studies on heterocystous cyanobacteria, like those of the Baltic Sea, are still needed. In contrast to ocean waters the carbonate system of the Baltic Sea is characterised by large spatial and seasonal variability in surface pCO_2 (Thomas 1999). The amplitude of the annual cycle varies significantly between regions as a result of their different characteristic in hydrology and biological features (H. Lu, Wallace, and Ko 2004). The largest amplitude (400 µatm) occurs in the north-eastern Baltic Sea ranging from almost 500 µatm in winter to 100 µatm in summer and the smallest amplitude (120 µatm) is observed in the transition area to the North Sea (Figure 1.1, Rutgersson, Norman, Schneider, Pettersson, & Sahlée, 2008; Thomas, 1999). Thus, cyanobacteria of the Baltic Sea are familiar with fluctuations of pCO_2 and should be well adapted and therefore strong related to changing pCO_2 concentrations. However the results of recent studies differ and even conflict each other. A culture study of Nodularia spumigena observed a strong decrease in cell division rate (36 %) and a slight decrease in N_2-fixation when pCO_2 increased from glacial (150 µatm) to predicted levels (750 µatm) (Czerny, Barcelos e Ramos, and Riebesell 2009). In contrast, in two other studies on the same genera a strong stimulation of N_2-fixation (13 to 720 %), CO_2-fixation (36 to 190 %) and growth (44 to 260 %) was observed with increased pCO_2 (Isensee et al., submitted; Wannicke et al., submitted). Furthermore an effect of pCO_2 on Baltic Sea cyanobacteria is only known from cultures and under stable laboratory conditions. Yet, a natural population might react completely different as they experience an everyday changing environment.

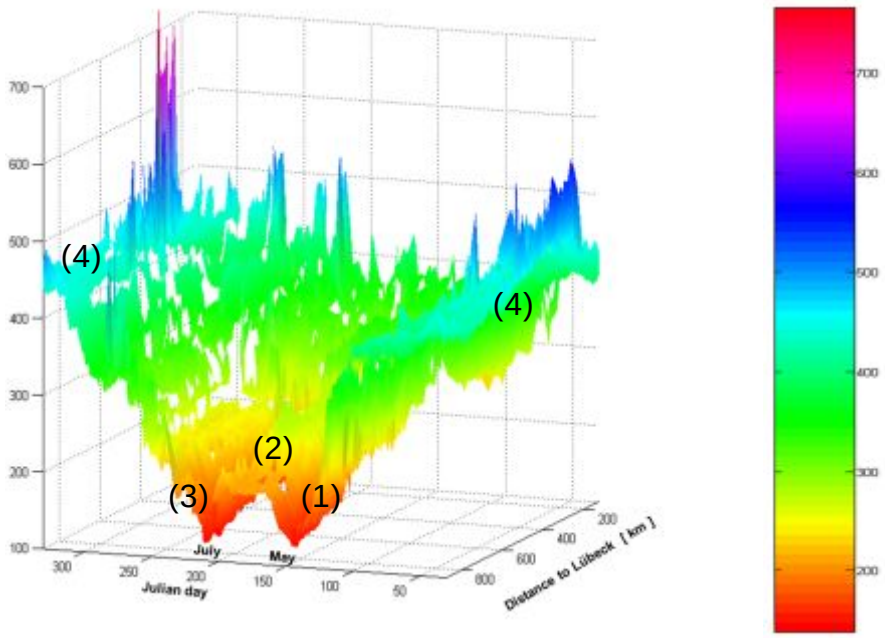

Figure 1.1 Seasonality of the CO_2 partial pressure (pCO_2) between the southern (Lübeck) and the northeastern (Helsinki) Baltic Sea (Bernd Schneider et al. 2009).

In face of the high impact of cyanobacteria on the Baltic Sea ecosystems it is important to understand how they will react towards predicted changes in the oceanic carbonate system to quantify the resulting changes within the whole ecosystem. Therefore the aim of this study was to investigate the effect of pCO_2 on a natural phytoplankton community predominated by cyanobacteria. Across a pCO_2 gradient of the Baltic proper nine stations were sampled and growth and productivity of cyanobacteria blooms analyzed. To quantify the impact of expected increases in pCO_2, surveys made on cruise were supported by a mesocosm study in a pCO_2 gradient from 180 to 950 µatm.

Throughout the course it is hypothesized that cyanobacteria are well adapted to changing pCO_2 concentrations and therefore stimulated by increasing pCO_2.

(1) As the pCO_2 decreases from south to north in the Baltic Proper it is expected to observe higher productivity and growth rates in the southern parts than in the northern once.

(2) As Cyanobacteria of the Baltic Sea naturally experience fluctuations in pCO_2 in a range from 100 to 500 µatm it is expected that the stimulation of N_2/ CO_2-fixation rates and growth in the mesocosm experiment will be strongest within the same range thus between glacial (180µatm) and present day pCO_2 (380µatm) levels.

2. Material and Methods

Study site

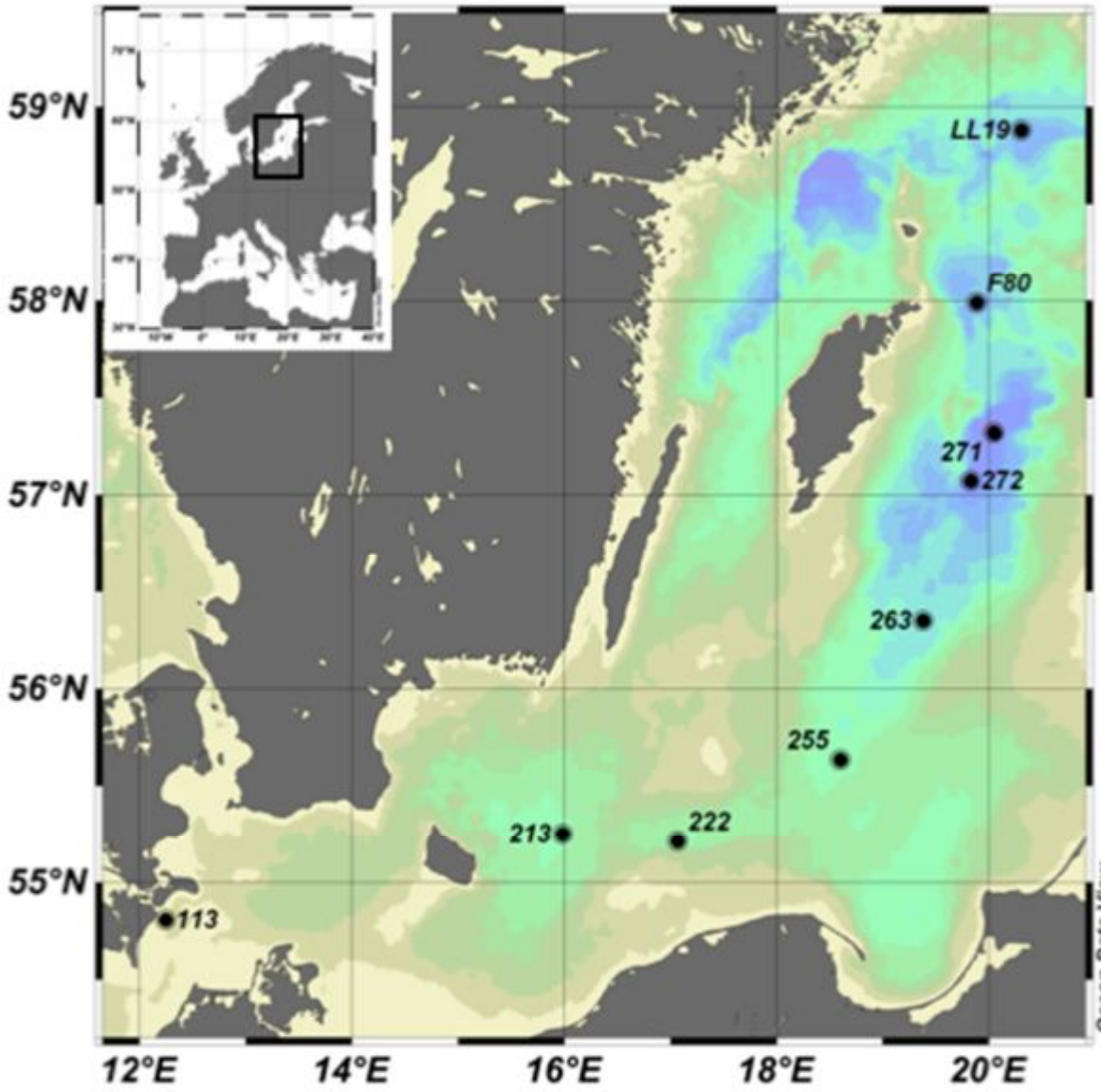

Figure 2.1 Overview of the study area and stations sampled during cruise.

The Baltic Proper represents the central part of the Baltic Sea and ranges from the Åland Island in the north to the Danish sounds in the west. The geographical extension of the studied area ranged from 13.25 to 20.05 °E and from 54.81 to 57.98 °N. Stations were located in the Arkona Basin (station 113), the Bornholm Basin (station 213 and 222) and the Gotland Basin (station 255, 263, 272, 271, LL19 and F80). The Gotland Basin can be subdivided in the northern (station LL19), eastern (station F80, 272 and 271) and southern Gotland Basin (station 263 and 255).

2.1. Sampling at Sea

Sampling was conducted during the Elisabeth Mann Borgese cruise from the 23rd till 31st of July. Weather conditions (PAR, wind speed) were monitored by the ships sensory system. pCO_2 and pH of surface water were monitored every hour throughout the cruise. At each station water from surface to 20m was collected using a Seabird CTD-system containing a rosette water sampler (12x5L). Profiles on pressure (depth), temperature, photosynthetic active radiation and salinity were recorded with CTD sensors. Samples of primary production (N_2-fixation, and CO_2-fixation), POM, nutrients, chlorophyll a and phytoplankton compositions were taken. In addition surface water of each station was sampled to analyse concentration of different carbon species and alkalinity. At station 271 (27.07.11) a profile of pCO_2 and pH was made for the upper 50 m. Station 271 was sampled twice (upon return, 27.07.11) to collect water for a supplementing mesocosm study. An overview of all monitored parameters is given in Table 2.1.

Table 2.1 Overview of parameters monitored during field and mesocosm study.

Parameter
Wind speed
Temperature
PAR (photosynthetic active radiation)
Salinity
Carbonate chemistry (pCO_2, DIC, pH)
Chlorophyll a
Nutrients (NH_4^+, NO_3^-, NO_2^-, PO_4^-)
N_2 and CO_2 fixation
POM (PON, POC, POP) concentration
Phytoplankton composition

2.2. Mesocosm study

2.2.1. Water and phytoplankton enrichment

Mesocosm were rinsed with 1N HCl and MilliQ before usage. Water and phytoplankton was taken from the surface (<5 m) at station 271 (20.05 °E, 57.32 °N). Phytoplankton was collected using a WP2 plankton net (100 µm mesh size) and re-suspended in sterile filtered seawater (0.2 µm Cellulose Acetate Filter, Sartorius) in 2 l polycarbonate bottles. Zooplankton was removed from net samples in a light trap (a black funnel illuminated from the bottom). Zooplankton is positive photoactive therefore moved towards the illuminated bottom whereas the filamentous cyanobacteria are buoyant and floated on top. The zooplankton was removed through the release of the bottom water by opening a plug valve in

the bottom part of the light trap. Phytoplankton enriched in filamentous cyanobacteria was transferred into a water filled mesocosm. At the institute (31.07.2011) aliquots of a given cyanobacteria mass were transferred in each mesocosm to end up with similar chlorophyll a concentration (~3.5 mg m^{-3}) between mesocosm Figure 2.2 and a volume of 1000 l.

2.2.2. Manipulation of mesocosm carbonic chemistry

The pCO_2 of the sample water was 166 µatm and represented the lowest pCO_2 level (180 µatm). Subsequently different pCO_2 levels (380, 750 and 950 µatm) were adjusted by adding calculated amounts of Na_2HCO_3 (Merck), whereas A_T was kept constant for all treatments (A_T=1628 µmol Kg^{-1}) through addition of hydrochloric acid (0.1N HCl, Merck). Both solutions were added using a narrow flexible tube that ended at the ground of the mesocosm to avoid CO_2 gas emission. For optimal homogenisation the mesocosm water was stirred for some minutes using a plastic paddle.

2.2.3. Experimental set up

An overview of the experimental setup and measured parameters is given in Table 2.1 and Figure 2.2. The experiment started about four days after sampling of water and phytoplankton (t=0). Mesocosm are named after their adjusted pCO_2 level: Mesocosm 180=180µatm, Mesocosm 380=380µatm, Mesocosm 750=750µatm, Mesocosm 950=950µatm.

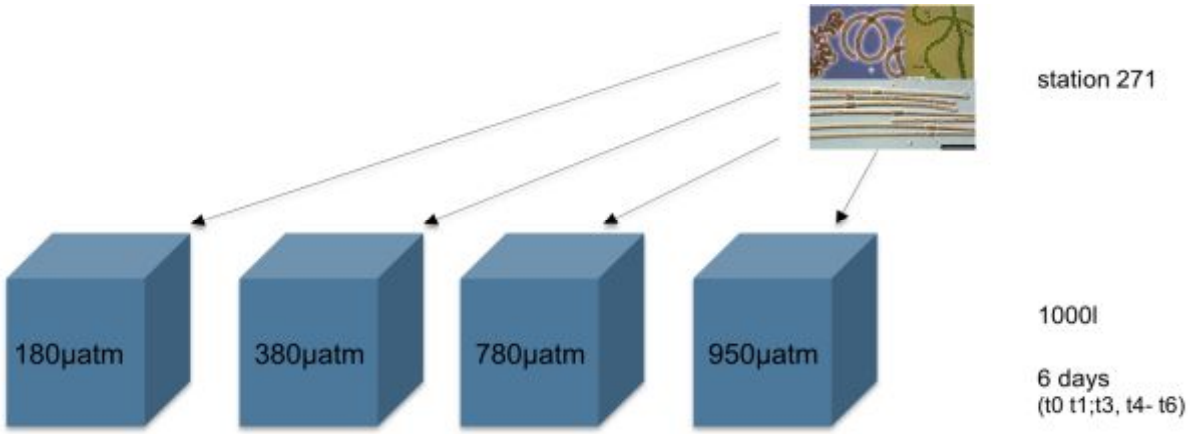

Figure 2.2 Experimental setup of mesocosm study. Water and phytoplankton was sampled during cruise return (station 271). The experiment started about four days after sampling. pCO_2 ranged from 180 to 950µatm. Sampling period was 6 days (t_0 to t_6). Mesocosm are named after their adjusted pCO_2 level: Mesocosm 180=180µatm, Mesocosm 380=380µatm, Mesocosm 750=750µatm, Mesocosm 950=950µatm.

Mesocosm were kept outside the institute but in the shade to avoid direct insolation and therefore unnatural intra-day temperature fluctuations. Besides cooling with water on very hot days no other regulations of physical parameters occurred. The sampling started about 12 hours after the acidification (t=0). Water samples were taken in the morning at about 9'o

clock over a period of six days (t_0 to t_6) using a peristaltic pump) and through a small opening on top of the mesocosm that was closed with a lid. Mesocosm were manually stirred before and during sampling and about two more times a day (morning, mid-day, late afternoon) with the plastic paddle to keep the water column and the phytoplankton composition homogenous. Salinity was measured with start of the experiment. Chlorophyll a, N_2-Fixation, $C0_2$-Fixation, PON and POC concentrations were measured every period (t_x), except for t_1. Carbonic chemistry, nutrients and phytoplankton compositions were measured at t_0,t_2,t_3,t_4,t_6. Irradiance (PAR) was measured at t_3 and t_6. An overview of all monitored parameters is given in Table 2.1.

2.3. Methodical intercomparisson

Nitrogen fixation rates were regular measured using the ^{15}N-Tracer technique after Montoya et al. 1996. The method is based on the direct injection of pure $^{15}N_2$ tracer gas (99% $^{15}N_2$) into a water samples to achieve an appropriate enrichment of about 10atom% ($^{15}N/^{14}N$). A full equilibration between tracer gas bubble and sample water is assumed and the resulting equilibrium concentration of $^{15}N_2$ and $^{14}N_2$ are commonly calculated according to Hamme and Emerson (2004) and R. F. Weiss (1970). This however, was never proven by direct concentration measurements of both isotopes. A recent study observed that the injected $^{15}N_2$ gas bubble does not attain equilibrium with the sample water as quickly as assumed and the enrichment technique was modified Mohr et al. (2010). At station 255, 272, 271 and LL19 N_2 fixation rates were measured with both the original and the modified method to determine if the bubble enrichment technique leads to an underestimation of fixation rates. At each station six bottles were filled with sample water of each sampled depth. Three bottles (triplicates) were enriched after Mohr et al. (2010) and the other three after Montoya et al. (1996). In addition, CO_2 fixation was determined for each sample bottle. The results of both methods were compared. For exact description of both enrichment techniques see 2.3.1. Furthermore, the equilibrium between gas bubble and ambient seawater was observed over a period of 24 hours in a $^{15}N_2$ dissolution experiment (see 2.3.2)

2.3.1. Nitrogen and Carbon fixation- tracer experiments

$^{15}N_2$ enrichment after Montoya et al. 1995

Fixation rates were analysed in triplicates. Sample water was filled to overflowing in 640 ml Nalgene plastic bottles before sealed bubble free with a septum cap (Teflon-lined butyl

rubber). A gas-tight syringe was used to add 0.5 ml $^{15}N_2$ Tracer gas (99% $^{15}N_2$) into the bottle and a second needle to withdraw 0.5 ml of solution to equalize pressure across the septum. All bottles were then gently mixed and incubated in water filled bins covered with neutral-density screening to stimulate in situ light intensities (surface=75%, 10 to 20m=50%). After 24 hours incubation suspended particles of each bottle were fractionated using a 10 µm gaze and collected by gentle vacuum filtration through 0.8µm precombusted (450°C, 4h) Whatman® - GF/F glass microfiber filter which were stored frozen by -20°C until analysis.

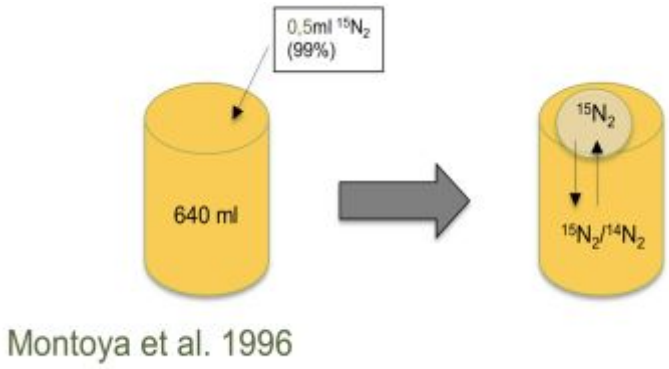

Figure 2.3 Methodical setup of tracer addition after Montoya et al. 1996. A complete dissolution of the $^{15}N_2$ tracer from the gas bubble into the ambient sample water is assumed.

$^{15}N_2$ enrichment after Mohr et al. 2010

Fixation rates were analysed in triplicates. During cruise a tracer mix of degasses water enriched with $^{15}N_2$ was prepared with seawater of each particular station. The seawater (depth <10 m) was degassed by pumping it through a membrane (Minimodule, Membrana GmbH) with a peristaltic pump. The membrane was connected to a vacuum pump pulling about -950 mbar reaching 50-70 mbar absolute end pressure in the inner membrane. The water was degassed for at least 10 minutes at a velocity of 400 ml s^{-1} and filled up bubble free into a gas tight Tedlar bag. A small amount of the degassed water was used to rinse the Tedlar bag before filling. Subsequently the volume of the bag was roughly determined through volume displacement in a water filled bucket. A gas-tight syringe was used to inject a specific volume of $^{15}N_2$- Tracer (10 ml per 1 l degassed filtered sea water) through a septum into the Tedlar bag. To get all added gas completely dissolved, the bag was agitated and hit with a ruler. A subsample of the prepared enriched seawater was filled into an exetainer (Labco) for the exact analysis of the enrichment. 50 ml of the prepared enriched seawater was filled into a 640 ml nalgene bottle together with sample water leaving out a small gas volume to facilitate the mixing of both solutions. Subsequently the bottle was reopened and filled up bubble free. The final sample volume was 590 ml for each bottle. All bottles were incubated together with those prepared after Montoya et al. 1996.

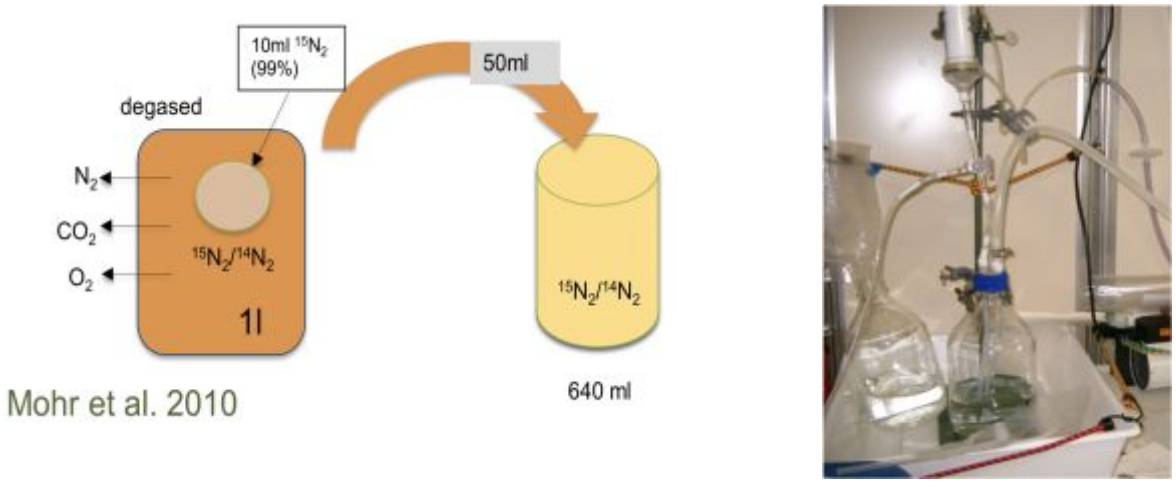

Figure 2.4 Methodical setup of the $^{15}N_2$ tracer addition after Mohr et al. 2010. The tracer mix of degasses seawater enriched with $^{15}N_2$ that prepared for each particular station is marked orange. The finally $^{15}N_2$ labelled seawater sample is marked yellow.

2.3.2. $^{15}N_2$ dissolution experiment

After the mesocosm experiment seawater of mesocosm 180 was filled into 40 nalgene bottles (640 ml) whereas 20 samples were enriched after Montoya et al. 1996 and the other 20 after Mohr et al. 2010 (see 2.3.1). Within a period one to 24 hours bottles in parallels of two were opened and a 12 ml subsample filled into an exetainer (Lanco). The subsample was fixed with zinkchloride (ZnCl, Merck) and stored upside down in the refrigerator (8 °C) until analysis that occurred within two weeks after sampling. An overview of the $^{15}N_2$ dissolution experiment is given in Figure 2.5.

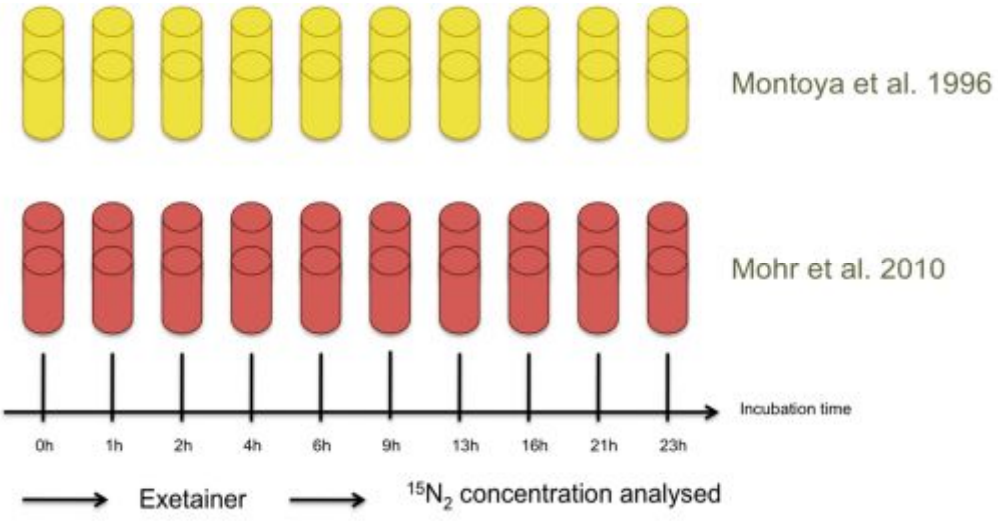

Figure 2.5 Setup of the $^{15}N_2$ dissolution experiment. 40 nalgene bottles (640ml) were filled with seawater (mesocosm 180). 20 were enriched after Montoya et al. 1996 and the other 20 after Mohr et al. 2010. Within a period of one to 24 hours bottles in parallels of two were opened and a 12 ml subsample was filled into an exetainer. The subsample was fixed with zinkchloride (ZnCl, Merck) and stored upside down in the refrigerator (8°C) until analysis.

2.4. Analytical methods

2.4.1. Dissolved inorganic nutrient and Chlorophyll a analysis

The colorimetric determination of nitrate (NO_3^-), nitrite (NO_2^-), ammonia (NH_4^+) and phosphate (PO_4^{3-}) was carried out after Grasshoff, Erhardt, and Kremling (1983) by the chemical department (Birgit Sadkowiak). NO_3^-, NO_2^- and PO_4^{3-} were analysed by an autoanalyser (Evolution III, Allinance Instruments). NH_4^+ was analysed manually using a spectrophotometer U200 (Hitachi- Europe GmbH, Krefeld, Germnay). The sample water was GF/F (0.8 µm precombusted (450 °C, 4 h) Whatman® - GF/F glass microfiber) filtrated before analysis. During cruise nutrients were determined directly. During the mesocosm experiment filtrated samples were stored frozen by -20 °C to be measured within a month after sampling. All nutrients were determined in triplicates.

Nitrate was measured by reducing it to nitrite on the passage through copper- plated cadmium columns in a pH range from 7.5 to 8.4. It reacts with added sulphanilamide and N-(1 napthyl)-ethylamide dihydrochloride solution. After 15 minutes dark incubation at room temperature a red Azo dye develops proportional to the nitrite concentration and can be measured photometrical at 543 nm. Nitrate concentrations are then calculated by subtracting the nitrite concentration. For determination of **nitrite** the same procedure was applied excluding the reduction step. According to Grasshoff et al. (1983) the detection limit of the method is 0.1 µmol l^{-1}.

To measure the concentration of **phosphate** a mixed reagent (molybdate and antimony dissolved in 50 % sulphuric acid) and ascorbic acid solution was added. Under acidic conditions phosphate anions and molybdate form a complex compound (Heteropolyacid) reacting to molybdenium blue. The extinction can be photometrical measured after 20 minutes by 885 nm and is proportional to the phosphate concentration. Detection limit of the method is 0.02 µmol l^{-1}.

Ammonia was measured as Idophenol blue that forms after addition of phenol and a mixed reagent (sodium hydrochlorite and alkaline citrate buffer solution) followed by a dark incubation (6-18 h) at room temperature. The extinction is proportional to the ammonia concentration in the samples and was measured at 630 nm. Precision of the method is 0.05 µmol l^{-1}.

Chlorophyll a was measured flourometrically. Suspended particles of the sample water were fractionated through a 10 µm gaze and collected by gentle vacuum filtration through a Whatman® - GF/F glass microfiber filter (0.8µm). Filters were stored frozen at -20 °C in an

opaque eppendorf tube until analysis. For determination filters were defrosted and extracted in 10 ml 96 % Ethanol for at least three hours at room temperature and in darkness. Pigment extracts were measured using a 10-AU-005 TURNER fluorometer. To distinguish Chlorophyll a from its degradation product Phaeophytin each sample was acidified with 100µl hydrochloric acid and re-measured after 30 seconds (Edler 1979). Chlorohyll a concentrations were calculated according to the method of Jeffrey & Welschmeyer (1997) (Equation 2.1).

$$Chla\left[mg/m^3\right] = F_m * \left(F_m - 1\right)^{-1} * \left(F_o - F_a\right) * K_x * V_E * V_p^{-1} \quad (2.1)$$

F_m is the acidification coefficient, K_x the linear calibration factor, F_0 is the fluorescence before and F_a the fluorescence after acidification. V_E and V_p represent the volume [ml] of extraction and filtration, respectively.

2.4.2. Particulate organic matter (POP, PON, POC)

To determine **particulate organic carbon (POC) and nitrogen (PON)** suspended particles of each bottle were fractionated using a 10 µm gaze and collected by gentle vacuum filtration through 0.8 µm precombusted (450°C, 4h) Whatman® - GF/F glass microfiber filters which were stored frozen by -20°C until analysis. Filters were analysed as described in 2.4.4.
Particulate organic phosphorous (POP) was calculated as the difference between total (TP) and the sum of dissolved inorganic (DIP) and organic phosphorous (DOP). The data was received from the working group "microbial processes and phosphorous cycle" of Monika Nausch.

2.4.3. Carbonate chemistry (pH, Alkalinity, pCO_2, C_T)

Carbonate chemistry (pCO_2, pH, Alkalinity, C_T) was determined by the working group "CO_2 System and Air/Sea Exchange" of Bernd Schneider, Leibniz Institute for Baltic Sea research. During cruise surface pCO_2 was continuously measured by pumping seawater from a depth of about 5 m. A CTD system installed at the water inlet recorded *in situ* temperature and salinity. Seawater and the atmospheric pCO_2 content were determined after Körtzinger et al. (1996) using a bubble-type equilibrator and a non-dispersive IR spectrometer (LI-6262). Calibration of the system was performed using ultra pure air with a certified CO_2 content (453.02 ppm; NOAA Climate Monitoring and Diagnostics Laboratory, Boulder, CO). High-resolution (3-h)

wind speed and atmospheric pressure data were obtained from the ship's meteorological record during the cruises.

Alkalinity (A_T) was analysed according to K. M. Johnson et al. (1993) using the SOMMA system. Total inorganic carbon (C_T) was calculated from the determined A_T and pCO_2 using the programm CO2SYS. The pH was determined with an electrode (Knick Mikroprozessor pH Meter 761, Typ 176 SE 100 glass electrode) that was calibrated before each measurement with NBS buffer giving values of pH in relative to the NBS scale.

2.4.4. Isotopic analysis and concentration of particulate matter (PON, POC) of labelled and unlabeled filters

Prior to analysis filters were dried overnight at 60°, wrapped into tin caps (IVA tin boats 6x6x12 mm) and formed into pellets with a laboratory press. Measurements were done by Iris Liskow with an elemental analyser (Carlo Erba flash EA) coupled to an isotope ratio mass spectrometer (IRMS, Finnigan Delta S). The products of the burning process (CO, CO_2, NO_x, SO_2, H_2O) were carried through a reaction tube (650 °C) with a helium flow as carrier gas were all C- and N containing gaseous compounds were converted into their reduces forms CO_2 and N_2. The gas stream from the elemental analyser was passed through a water trap (phorsphorpentoxide, P_2O_2) before entering the column of a gas chromatograph (85°C, 60cm, Fa.Hekatech) where all gases were separated. A small portion of each gas (1%) was injected into the ion source of a Finnigan Delta-S mass spectrometer where it was ionized, accelerated and focused into one beam. The ions are separated according to their mass-to-charge ratio by passing an electromagnetic field. The hits were recorded by a detector and transferred into electrical signals to calculate concentrations of POC and PON. The δ value is calculated from the different atomic masses 28 and 29(Equation 2.2, example for the calculation of $\delta^{15}N_2$).

$$\delta^{15}N_2[‰] = \left[\frac{\left(\frac{^{15}N}{^{14}N}\right)_{sample}}{\left(\frac{^{15}N}{^{14}N}\right)_{reference}} \right] * 1000 \qquad (2.2)$$

The IRMS determined the difference in ^{15}N and ^{13}C abundance between sample and lab intern reference gas. The reference gasses were calibrated to an international standard of the International Atomic Energy Agency (IAEA, see Table 2.2) that are calibrated to the primary references atmospheric N_2 (Mariotti 1983) and Vienna Pee Dee Belemnite (VPDB) (Coplen

1994) having a δ value of 0‰. To ensure precise analysis a peptone standard is measured after every fourth sample that leads to an overall analytical precision of 0.2 ‰ for this method.

Table 2.2 Overview of used standards

Standard	**$\delta^{15}N$ (‰)**	**$\delta^{13}C$ (‰)**
International laboratory standards		
Peptone	5.8± 0.2	-22.11± 0.17
Acetanilid (C_8H_9NO, 10,36% N, 71,09% C)	-1.7± 0.2	-29.81± 0.19
International standards		
IAEA N1 (Ammonium sulphate)	0.43± 0.07	
IAEA N2 (Ammonium sulphate)	20.32± 0.09	
IAEA N3 (Potassium nitrate)	4.69± 0.09	
IAEA C6 (Saccharose)		-10.43± 0.13
NBS22 (Oil)		-29.74± 0.12

2.4.5. Phytoplankton composition

Cell counts of mesocosm samples were done by Stefanie Linsenbarth. 50 ml Samples were preserved with 200 µl acetic Lugol`s (KI/I2) solution and stored dark at room temperature until analysis. According to Utermöhl (1958) 25 ml of the lugol fixed phytoplankton sample was enriched by sedimentation in a tube chamber over at least 8 hours and subsequently analysed using an inverted microscope (Axiovert S100, Zeiss) at 400x and 100x magnification. 400 individuals of the filamentous main N_2-fixing cyanobacteria species Anabaena, Aphanizomenon and Nodularia taken as a whole were counted giving a precision of ±10% and about 50 individuals of all other species giving a precision of ±28%. Taxonomic groups were determined rather than species. Counted species number was converted in to biovolume assuming species-specific geometrical approximation after Helcom (2006) and to carbon using the following conversion factor after Menden-Deuer and Lessard (2000) (Equation 2.2 and 2.3).

$$pgC\ \ cell^{-1} = 0.288 \times volume(\mu m^3)^{0.811} \text{ (for diatoms)} \quad (2.3)$$

$$pgC\ \ cell^{-1} = 0.760 \times volume(\mu m^3)^{0.819} \text{ (for other phytoplankton)} \quad (2.4)$$

2.5. Calculations

2.5.1. Specific Growth rates

Growth rates were calculated from the increase in particulate organic carbon during the mesocosm experiment (Equation 2.4). For each mesocosm the natural logarithm of particulate organic carbon was plotted against the incubation time (d) whereas the slop of the resulting regression line gives the mean growth rate for the testing period of each mesocosm.

$$\mu_{POC} = \frac{\ln(t_{n+1}) - \ln(t_n)}{\delta t}$$
$$\mu_{POC} = specific \quad growth \quad rate \qquad (2.4)$$
$$t = time \quad in \quad hours$$
$$\delta t = t_{n+1} - t_n$$

2.5.2. N_2 and CO_2 fixation rates

Fixation rates were calculated from enrichment of ^{15}N or ^{13}C in particulate organic matter (Equation 2.5) where x is the atom (Carbon for CO_2 fixation, nitrogen for N_2 fixation) and *A* the atom% of *x* in the particulate organic matter (P_x) at the end (*final*) or beginning (*t=0*) of the incubation or in the dissolved pool of x (*dx*).

$$\frac{\left(A_{Px}^{final} - A_{Px}^{t=0}\right)}{\left(A_{dx} - A_{Px}^{t=0}\right)} \times \frac{[Px]}{\Delta t} \qquad (2.5)$$

2.6. Statistical analysis

Data was analysed using the statistical programm R. Most data sets were not drawn from a normal distribution and not equal in their variances (homogeneity). Therefore non- parametric statistics was applied to compare biological parameters along the pCO_2 gradient. Hence, the field data of stations with similar pCO_2 levels were pooled giving two groups the northern and the southern Baltic Proper (see Table 3.2). The surface pCO_2 of stations in the Bornholm Sea (station 213, 222) and the southern Gotland Sea (station 255, 263) ranged from 208 to 229 µatm and represent the southern Baltic Proper. The surface pCO_2 from stations in the eastern (station 272, 271, F80) and northern Gotland Basin (LL19) ranged from 140 to 166 µatm and represent the northern Baltic Proper. Biological parameters were compared between both areas. During the mesocosm experiment the discussed biological parameters represent the median of the whole testing period from t_0 to t_6. Diazotroph cyanobacteria were assumed to be

present in fraction >10 µm but not in fraction <10 µm. Therefore the N_2- fixation rates measured for fraction <10 µm represent the uptake of $^{15}N_2$ that was released after fixing by diazotroph cyanobacteria of fraction >10µm.

3. Results

To separate diazotroph cyanobacteria from the other phytoplankton community samples were fractionated into subsamples through a 10 µm mesh whereas diazotroph cyanobacteria were assumed to be present only in the fraction >10 µm.

3.1. Comparison of $^{15}N_2$ tracer addition methods

3.1.1. Comparison of N_2 and CO_2-fixation rates measured during cruise

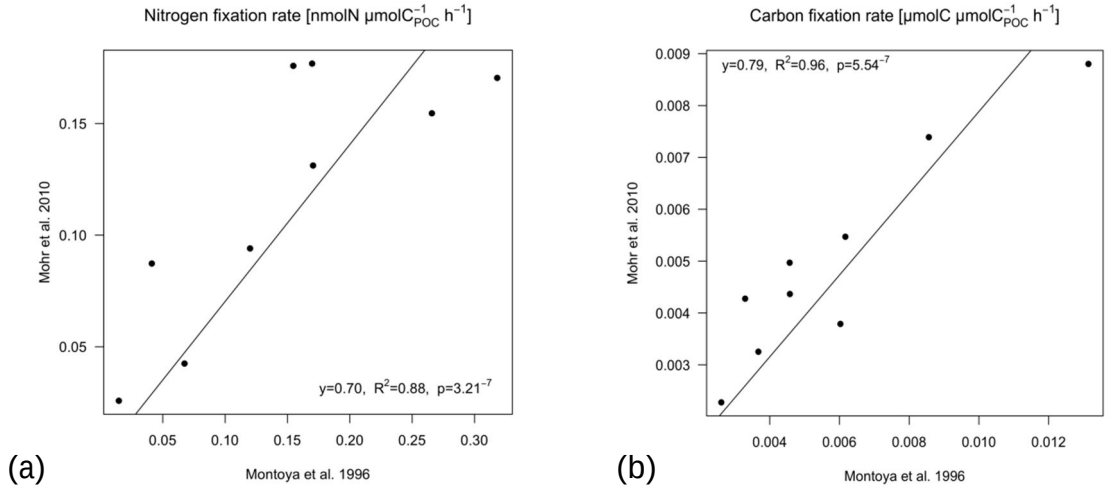

Figure 3.1 At station 255, 272, 271 and LL19 nitrogen (N_2, a) and carbon (CO_2,b) fixation rates were measured using both methods in parallels of samples. Samples were fractionated after the incubation of 24 hours. Fraction >10 µm is represented and analysed. Results of both methods are plotted against each other. Fixation rates are normalized to particulate organic carbon (POC).

Fixation rates at each station were measured in triplicates. Yet, triplicates of fraction >10 µm had to be pooled during filtration because of low biomass concentration. Therefore fixation rates of each station and depth are represented by a single sample. A clear relationship between fixation rates of the two methods was not visible. Fixation rates measured after Mohr et al. 2010 were sometimes higher and sometimes lower than those measured after Montoya et al. 1996. The mean $ratio_{Mohr/Montoya}$ is given by the slope of the linear regression line between the results of both methods. CO_2 and N_2 fixation rates measured after Mohr et al. 2010 were generally lower (67 % and 56 %) than those measured after Montoya et al. 1996. The mean CO_2 fixation $ratio_{Mohr/Montoya}$ was 0.79. In two replicates the CO_2 fixation rates of parallels equalled each other ($ratio_{Mohr/Montoya}$=~1, marked dark green). Mean N_2 fixation $ratio_{Mohr/Montoya}$ was 0.7 whereas parallels equalled each other in one replicate ($ratio_{Mohr/Montoya}$=~1, marked dark green).

Table 3.1 Rates of N_2 and CO_2 fixation of fraction >10µm. Nine Replicates were measured in triplicates after Montoya et al. 1996 and Mohr et al. 2010. Riplicates were pooled during fractionation. The Ratio was calculated by dividing fixation rates determined after both methods (Mohr/Montoya). Ratio >1 are marked light green, ratios~1 are marked dark green.

station	depth [m]	Incubation start [hours]	N_2 fixation [µmolN µmolC-1POC h-1]		CO_2 fixation [µmolC µmolC-1POC h-1]		Ratio (N_2) [ ]	Ratio (CO_2) [ ]
			Mohr et al. 2010	Montoya et al. 1996	Mohr et al. 2010	Montoya et al. 1996	(Mohr/Montoya)	(Mohr/Montoya)
255	1.5	12:53 pm	0.170	0.318	0.007	0.009	0.536	0.863
255	11.2	12:53 pm	0.155	0.266	0.009	0.013	0.581	0.670
272	5.6	17:45 pm	0.131	0.171	0.005	0.006	0.769	0.887
272	11.1	17:45 pm	0.042	0.067	0.004	0.006	0.630	0.629
LL19	1.3	11:16 pm	0.177	0.170	0.004	0.005	1.042	0.954
LL19	11.1	11:16 pm	0.087	0.041	0.004	0.003	2.119	1.298
LL19	20.9	11:16 pm	0.026	0.015	0.002	0.003	1.748	0.872
271	1.6	10:20 pm	0.176	0.155	0.005	0.005	1.137	1.087
271	11.1	10:20 pm	0.094	0.120	0.003	0.004	0.784	0.885

3.1.2. Dissolution of $^{15}N_2$

An enrichment of about 33 µmol l^{-1} was expected for all samples (Figure 3.2). In samples enriched after Montoya et al. 2010 concentration of $^{15}N_2$ increased within the first 12 hours of incubation before it stabilized reaching about 75 % (25 µmol l^{-1}) of the expected enrichment. The $^{15}N_2$ concentration of samples enriched through addition of degassed seawater was ~3 µmol higher than expected (36 µmol l^{-1}) with start of the incubation. [$^{15}N_2$] reached ~33µmol l^{-1} within the first six hours of the incubation representing about 97% of the expected enrichment.

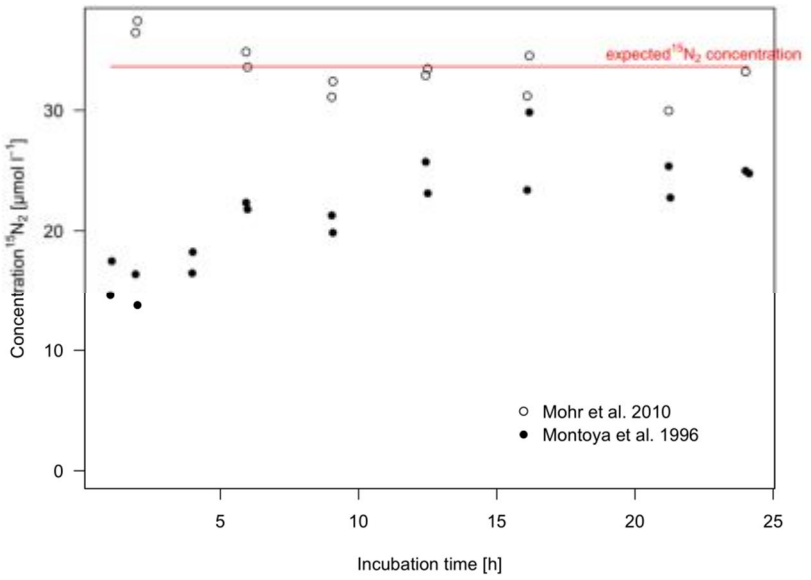

Figure 3.2 $^{15}N_2$ concentration of sample water as function of time. Old sample water of station 271 was enriched with $^{15}N_2$ either through direct in injection of a tracer gas bubble (black symbols) or through the addition of degassed enriched seawater (white symbols). The red line represents the expected enrichment of about 33 µmol$^{15}N_2$ l^{-1}.

3.2. Field surveys

3.2.1. The hydrography, nutrients and chlorophyll

An overview of physical and biological parameters of the surface water is given in Table 3.2 and Table 3.3. All stations, except station 113, were characterized by a thermocline within 10 to 30 m depth. The water column of station 113 was completely mixed. At each station different water layers, more precisely surface (<5 m), above (~10 m) and within/below thermocline (~20 m) were sampled.

Surface temperature increased during cruise ranging from 15°C (Arkona Sea) to 20 °C (northern Gotland Basin). Surface temperature in the southern Baltic Proper was generally lower than in the northern areas (Figure 3.3). Salinity decreased northwards from 7.45 (Bornholm Sea) to 6.62 (northern Gotland Basin). Bottom depth in the Gotland Basin ranged from 162 to 235 m and decreased towards the south (Arkona Basin) to 44 m. Stations in the Gotland basin were characterised by a stable halocline in 60 to 70 m depth. Irradiance determined with the CTD sensory system was mistaken and therefore excluded from further analysis. Mean Irradiance the water surface (75 % light) and subsurface (50% light) was calculated from the irradiance measured by the ship's methodical sensory system. Mean surface irradiance fluctuated between 146 to 753 µE m^2 s^1 during cruise. Mean subsurface irradiance fluctuated between 98 to 502 µE m^2 s^1. Dissolved inorganic nitrogen (NO_3^-, NO_2^- and NH_4^+) were below the detection limit (0.05 to 0,1 µmol l^{-1}) within the upper 20 m and therefore depleted at all stations, except station 272. Chlorophyll a concentration of fraction >10µm was about three times higher that concentration of <10 µm ranging from 3.25 to 1.75 mg m^{-3} and 1.25 to 0.02 mg m^{-3}, respectively.

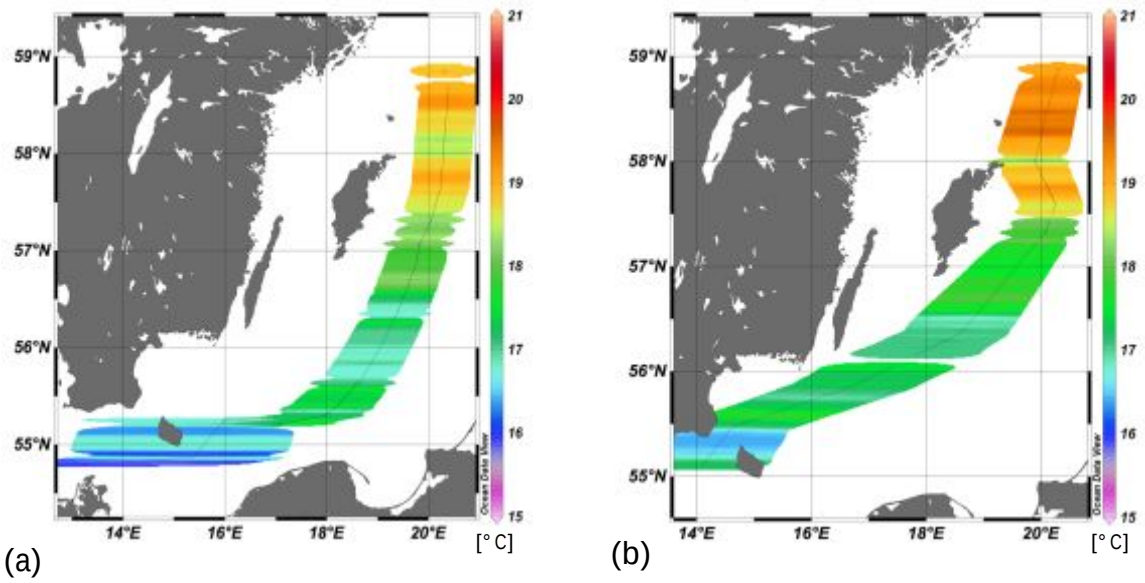

Figure 3.3 Surface water temperature measured from (a) 23th to 28th of July 2011 (Bornholm Basin→ northern Gotland Basin) and from (b) 28th to 30th of July 2011 (northern Gotland Basin → Bornholm Basin).

3.2.2. Carbonate chemistry

Surface pCO_2 was measured along a gradient from the Bornholm Basin

The pCO_2 determined from the 28th to 30th July 2011 (Bornholm Basin→ northern Gotland Basin) and from the 28th to 30th July 2011 (northern Gotland Basin → Bornholm Basin) is represented in Figure 3.4. Surface pCO_2 ranged from 322 µatm (Bornholm Basin) in the south to about 122 µatm (northern Gotland Basin) in the north during sampling from 23rd to 27th of July 2011. During cruise return (28th to 30th July 2011) the surface pCO_2 was measured a second time along a similar gradient. Alkalinity fluctuated between 1428 and 1678 µmol Kg^{-1}.

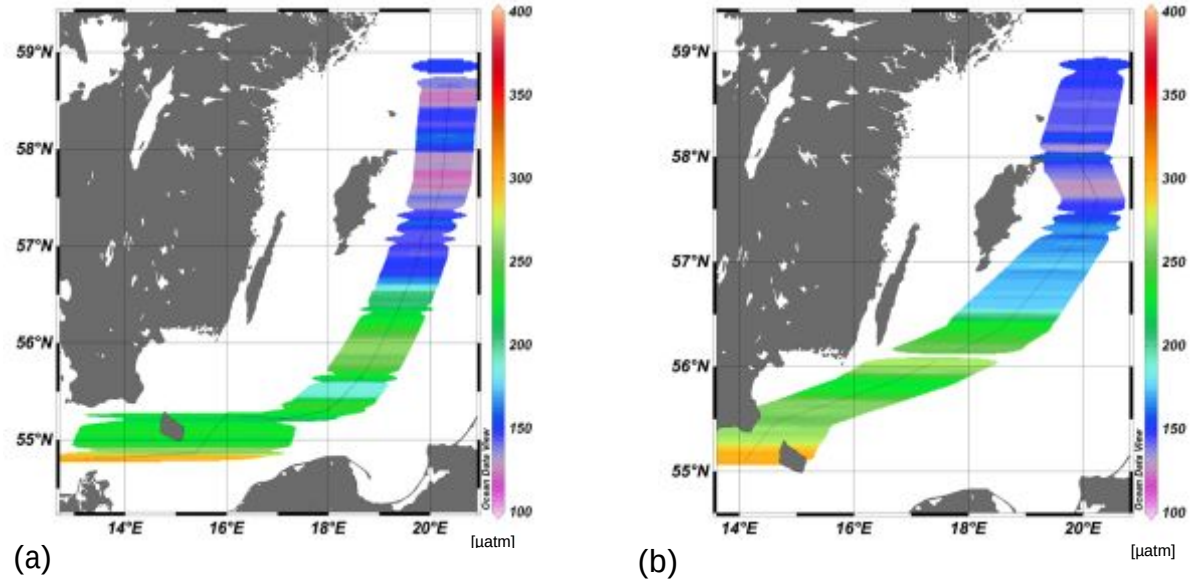

Figure 3.4 Surface water pCO_2 measured during cruise from (a) 23th to 28th of July 2011 (Bornholm Basin→ northern Gotland Basin) and from (b) 28th to 30th of July 2011 (northern Gotland Basin → Bornholm Basin).

3.2.3. The phytoplankton composition

Figure 3.5 gives an overview of phytoplankton composition within the euphotic zones (< 20 m). The phytoplankton community was composed of flagellates and *cyanobacteria* next to *Chlorophyceae* (*Oocystes sp., Planktonema sp.*), *Dinophyceae* and *Lithostomatae* (*Mesodinium rubrum*) and few *Diatomeae* (Figure 3.5). Cyanobacteria of fraction >10 µm were predominated by the flamentous, N_2- fixing genera *Nodularia* and *Aphanizomenon*) and *Anabena* (Table 3.3). Abundance of the latter was negligible. All three are heterocystous cyanobacteria and belong to the group *Nostocales.* Fraction <10 µm was predominated by the small unicellular and colonial cyanobacteria groups *Chroococcales* (60 to 80 %, data not shown). Few other cyanobacteria of the genera Pseudanabaena/ Limnothrix (Oscillatoriales) were also found. Dominance of all phytoplankton species groups varied between stations and different depth as can be seen from Figure 3.5. At surface dominance of *Nostocales* varied between 13 and 80 % of total phytoplankton biomass (Figure 3.5) and decreased with depth. Dominance of *Chroococcales* varied from 3 to 38 %. Observed biomass of *Nostocales* ranged from 1.04 (station 113) to 643.25µg/l (Gotland deep, station 271) within upper water layer (≤10 m). Bloom abundance was only observed at surface (station 213, 222, 255, 272 and 271) and subsurface (<10 m, station 213, 222, 272, and 271). Highest biomass of *Aphanizomenon* sp. and Nodularia sp. were observed at surface in the Bornholm Basin (station 213, 222) and the eastern Gotland Basin (272). At those stations biomass of 301.23, 147.48 and 108.86 µg l^{-1} were observed for Aphanizomenon sp. and 109.12, 140.62 and 227.48 µg l^{-1} for *Nodualaria sp,* respectively. Observed biomasses were higher in the southern Baltic Proper than in the northern Baltic. Yet, a significant increase of biomass with increasing pCO_2 was not observed during the cruise.

3.2.4. Stoichiometrie

POC:PON ratios calculated for the two size fractions did not differ significant with depth (median 8.2±0.5). Overall ratios in the range from 10.4 to 6.6 µmolC µmolN^{-1} thus showed a high variability in the data. The data of phosphate was not fractionated. Therefore total ratios could only be calculated. Total POC:POP and PON:POP ratios ranged from 120 to 272 and from 19 to 37., respectively Table 3.3).

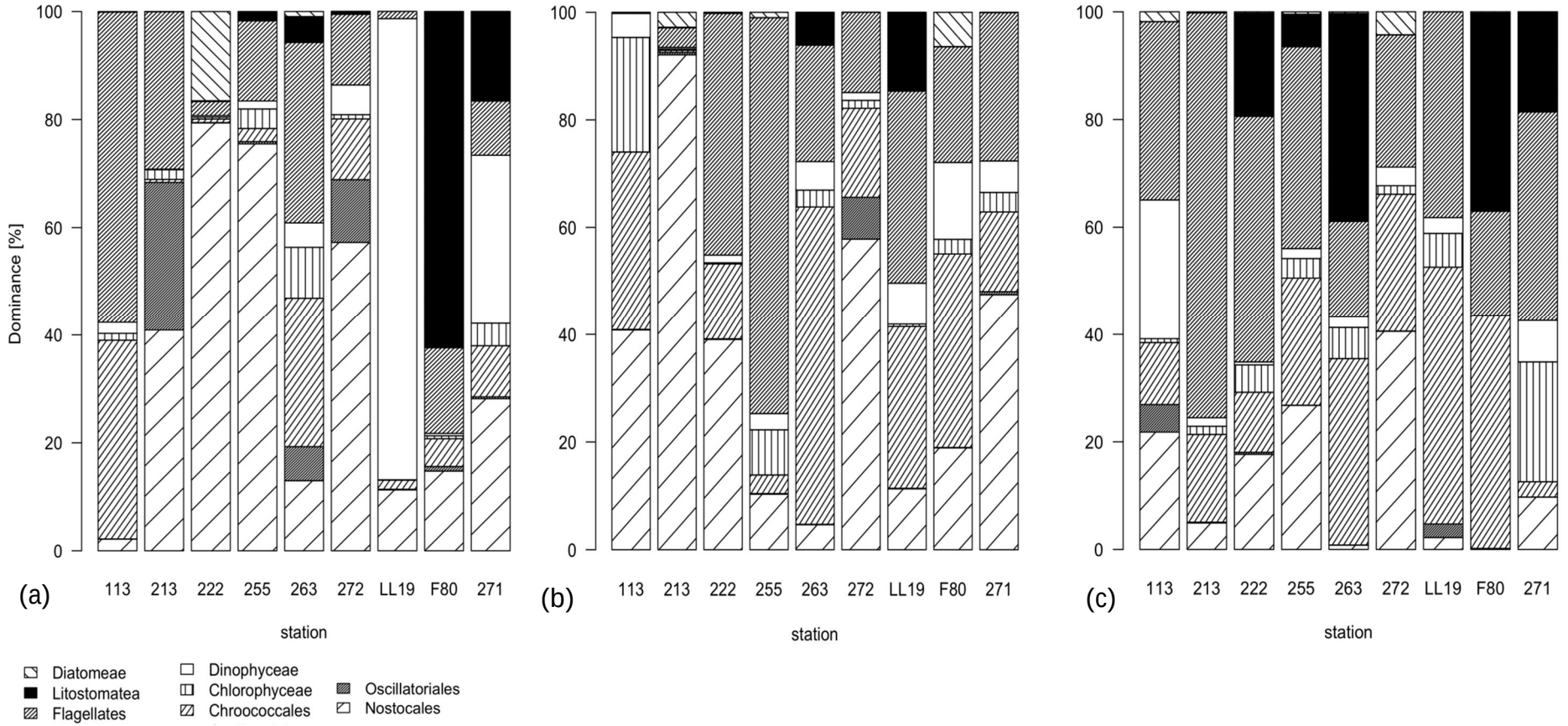

Figure 3.5 Composition of the studied phytoplankton community. Dominance of different phytoplankton species groups is given for each station at surface (a), subsurface (b) and 20m depth. Phytoplankton composition varied between stations. Dominance of N_2-fixing cyanobacteria (*Nostocale*s) decreased with depth.

3.2.5. N_2 and CO_2-fixation

N_2- and CO_2-fixation rates of fraction >10 µm did significantly decrease by 20 and 3-folds with decreasing depth from surface to 20 m, respectively (Kruskal-Wallis rank sum test, $p<0.05$), in contrast fixation rates of fraction > 10 µm that did not decrease. (data not shown). Fractionated fixation rates were not dependent form pCO_2 because of high variability in the data (Wilcox rank sum test, $p<0.05$). However, CO_2 fixation rates of fraction >10 µm were 30 % higher in the southern Baltic Proper (208-228 µatm) than in the northern areas (140-166 µatm). N_2-fixation rates did not differ between stations.

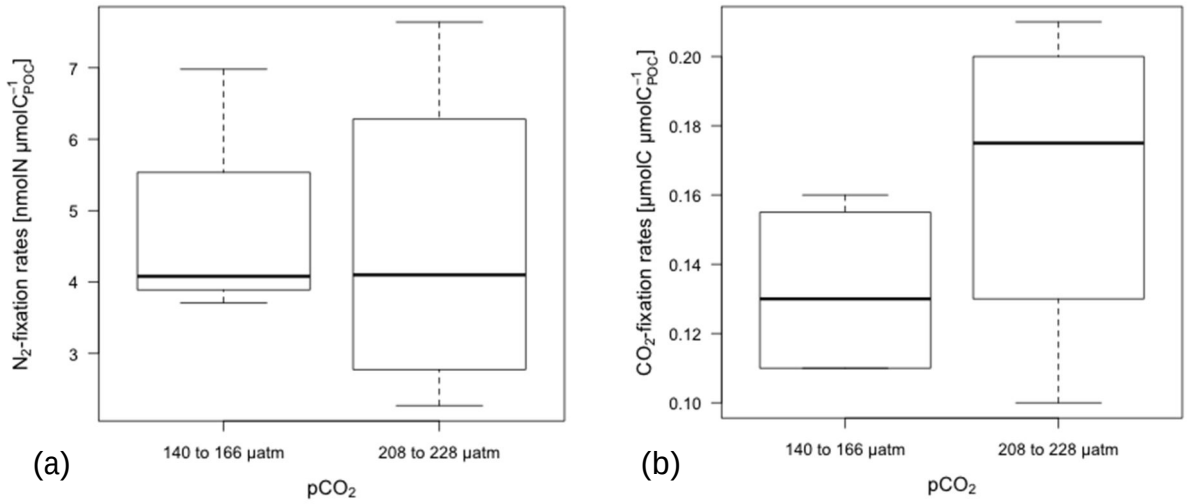

Figure 3.6 N_2- (a) and CO_2-fixation (a) rates of fraction >10 µm .

CO_2-fixation and N_2-uptake rates of fraction <10 µm were 28 % and 33 % higher in the southern Baltic Proper (208-228 µatm) than in the northern areas (140-166 µatm)

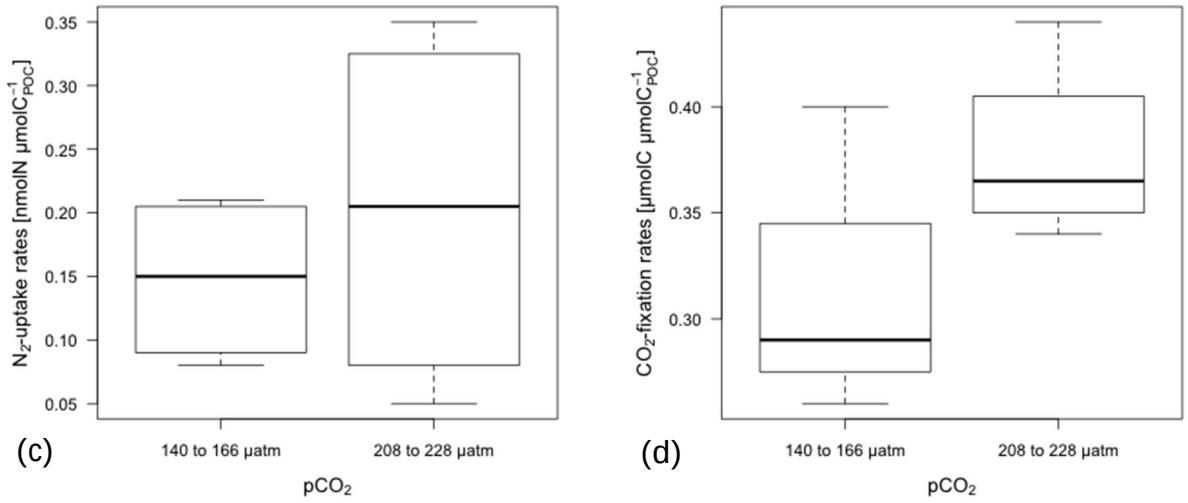

Figure 3.7 N_2- uptake (c) and CO_2-fixation (c) rates of fraction <10 µm

Table 3.2 Overview of physical and biological parameters determined during cuise.

	station	date	Lon	Lat	Temperature	Salinity	Irradiance	Wind	thermocline	pCO2	C_T	A_T	pH	DIN	DIP	O2
			[° E]	[° N]	[°C]	[mg Kg^{-1}]	[µE m^2 s^{-1}]	m s^{-1}	[m]	[µatm]	[µmol Kg^{-1}]	[µmol Kg^{-1}]	[]	[µmol l^{-1}]		[ml l^{-1}]
Southern Baltic Proper	113	24.07.11	13.25	54.81	15.54	7.45	212	7	well mixed	303.0	1642.7	1569.3	8.3	0.05	0.08	6.61
	213	25.07.11	15.98	55.25	16.43	7.21	753	6	16-28	228.4	1626.0	1526.6	8.4	0.06	0.04	6.62
	222	25.07.11	17.07	55.21	16.97	7.19	753	2	16-23	208.6	1621.4	1510.3	8.5	0.09	0.02	6.75
	255	26.07.11	18.60	55.63	16.83	7.08	370	3	15-34	222.2	1629.6	1526.1	8.4	0.09	0.00	6.64
	263	26.07.11	19.38	56.35	15.70	7.04	370	3	12-25	215.2	1628.3	1525.5	8.4	0.08	0.03	6.99
Northern Baltic Proper	272	27.07.11	19.83	57.07	17.80	7.13	319	2	15-38	139.8	1678.0	1510.0	8.6	0.14	0.03	6.63
	LL19	28.07.11	20.31	58.88	18.48	6.62	708	3	10-30	150.4	1570.4	1428.4	8.6	0.07	0.05	6.87
	F80	28.07.11	19.89	57.99	17.48	6.82	708	3	0-25	165.3	1610.5	1476.9	8.5	0.02	0.03	6.87
	271.2	29.07.11	20.05	57.32	17.68	7.07	146	3	15-30	166.2	1638.3	1499.7	8.6	0.03	0.02	n.d.

Table 3.3 Overview of biological parameters determined during cruise; n.d. means not determined; PM means particulate organic matter.

Fraction		Total PM			>10µm						<10µm		
	station	C:P	N:P	C:N	Chl a	N_2-Fixation	CO_2-Fixation	Anabaena	Aphanizomenon	Nodularia	Chl a	N_2-Fixation	CO_2-Fixation
						[nmolN µmolC_{POC} d^{-1}]	[nmolC µmolC_{POC} d^{-1}].		Biomass [µg/l]		mg m^{3-}	[nmolN µmolC_{POC} d^{-1}]	[nmolC µmolC_{POC} d^{-1}].
Southern Baltic Proper	113	120	19	6	0.18	n.d.	n.d.	0.13	0.75	0.15	2.80		
	213	208	32	6	0.46	2.27	0.10	2.23	301.23	109.12	1.88	0.05	0.34
	222	178	28	6	0.67	4.92	0.16	0.04	147.48	140.62	1.72	0.35	0.36
	255	150	25	6	0.65	7.64	0.21	1.47	38.98	66.38	2.18	0.11	0.37
	263	137	37	4	0.29	3.28	0.19	0.25	0.99	1.81	2.40	0.30	0.44
Northern Baltic Proper	272	n.d.	n.d.	2	0.38	4.09	0.15	40.83	108.86	47.26	3.05	0.10	0.40
	LL19	172	22	8	1.13	4.07	0.11	0.16	23.21	69.51	1.82	0.21	0.26
	F80	201	30	7	0.63	6.98	0.16	0.92	3.96	4.55	2.43	0.20	0.29
	271	272	26	10	0.53	3.71	0.11	1.00	4.85	10.92	2.47	0.08	0.29

3.3. Mesocosm study

Table 3.4 and Table 3.5 gives an overview of analysed physical, chemical and biological parameters for each mesocosm during testing.

3.3.1. Physical conditions (Salinity, Irradiance and Temperature), nutrients and chlorophyll a

Mean temperature ranged from 21 to 22 °C. Salinity of the sample water was 7.9 mg Kg^{-1}. Mean surface irradiance (< 50 cm) was calculated from four measurements (t_3 (9 o'clock) and t_6 (9, 12 and 15 o'clock)). Mean irradiance ranged from 223 to 260 µE m^2 s^{-1}. Temperature, Irradiance and Salinity did not significantly differ between mesocosm (Kruskal wallis test, $p<0.05$).

Concentration of PO_4^{3-} differed between mesocosm with start of the experiment. They were ten times higher in mesocosm 750 and 950 (0.261µmol/l, 0.212µmol/l) than mesocosm 180 and 380 (0.036µmol/l, 0.032µmol/l). This difference was balanced by the addition of 25µmol/l PO_4^3 the day before the start of the experiment (t_{-1}). With start of the experiment nutrient concentrations of the sample water were exhausted in all mesocosm (DIP<0.08 µmol l^{-1}, DIN<0.03 µmol l^{-1}, Table 3.5).

Chlorophyll a concentrations were similar in all mesocosm with start of the experiment ranging from 3.31 (mesocosm 750) to 3.65 mg m^{-3} (mesocosm 380). Total Chl a concentrations equally increased in all mesocosm within the first three days of the testing period and declined the following three days Table 3.5.

3.3.2. Carbonate chemistry

An overview of the carbonate chemistry of each mesocosm is given in Table 3.4. The manipulation of the mesocosm carbonate system did not work out completely. With start of the experiment the pCO_2 level of mesocosm 750 and 950 were lower than intended (722 and 788 µatm, respectively). This was adjusted through further addition of HCl and $NaHCO_3$ at day 0. At testing day 2 both mesocosm reached a pCO_2 level of 801 and 938 µatm, respectively. The pCO_2 of mesocosm 380, 750 and 950 decreased throughout the testing period, reaching 340, 637 and 725 µatm, respectively, with the end of the experiment (t_6). The pCO_2 of mesocosm 180 increased during the testing time reaching 199 µatm at day 6. As result of the variability in mesocosm carbonate chemistry median pCO_2 level of mesocosm 750 and 950 did not significantly differ from each other (u-test, $p<0.05$). Yet, both were still

significantly higher than the median pCO_2 level of mesocosm 180 and 380 (Kruskil wallis test, post-hoc u.test, $p<0.05$).

Table 3.4 Overview of determined physical and biological parameters during the mesocosm study.

									Total community		
time	mesocosm	MEAN Irradiance	T	pCO_2	DIC	pH	DIP	DIN	PON/POP	POC/POP	POC/PON
[d]		**[µE m^{-2} s^{-1}]**	**[°C]**	**[µatm]**	**[mmol]**	**[]**	**[µmol l^{-1}]**				
0	180		20.61	199.00	1.506	n.d.	0.3	0.00	22	198	9
2	180		21.25	212.01	1.515	8.31	0.1	0.01	37	330	9
3	180	223	21.55	224.41	1.521	8.32	0.1	0.01	30	242	8
4	180		21.85	228.19	1.522	8.33	0.1	0.01	35	272	8
							0.4	0.03			
6	180		22.05	214.33	1.516	8.37	0.0	0.01	27	211	8
0	380		21.01	347.00	1.550	n.d.	0.1	0.01	21	179	8
2	380		21.55	375.69	1.566	8.10	0.0	0.00	31	245	8
3	380	235	21.45	377.20	1.566	8.12	0.1	0.01	30	223	7
4	380		21.85	353.78	1.561	8.14	0.0	0.01	26	196	8
							0.2	0.01			
6	380		22.15	340.51	1.558	8.17	0.0	0.02	30	236	8
0	750		21.31	722.00	1.608	n.d.	0.1	0.01	22	196	9
2	750		21.55	801.32	1.619	7.76	0.1	0.01	29	238	8
3	750	237	21.85	763.66	1.616	7.79	0.0	0.01	28	224	8
4	750		22.05	697.99	1.610	7.83	0.0	0.01	35	265	8
							0.3	0.01			
6	750		22.05	637.26	1.604	7.90	0.0	0.02	31	234	8
0	950		21.71	788.00	1.614	n.d.	0.1	0.00	25	197	8
2	950		21.85	938.32	1.629	7.69	0.0	0.00	29	234	8
3	950	260	21.25	932.00	1.629	7.72	0.0	0.01	29	223	8
4	950		21.95	777.73	1.617	7.78	0.0	0.01	33	252	8
							0.2	0.01			
6	950		21.95	724.77	1.612	7.84	0.0	0.01	35	235	7

Table 3.5 Overview of determined biological parameters during the mesocosm study. For N_2 and CO_2 fixation rates mean and standard deviations of triplicates are shown.

		Fraction <10 µm						Fraction >10 µm					
time	**mesocosm**	**Chl a**	**PON**	**POC**	POC/PON	N_2 fixation	CO_2 fixation	**Chl a**	**PON**	**POC**	POC/PON	N_2 fixation	CO_2 fixation
[d]		**mg m^{-3}**	**µmol l^{-1}**			(nmolN µmolC_{POC} d^{-1})	(nmolC µmolC_{POC} d^{-1})	**[mg m^{-3}]**	**µmol l^{-1}**			(nmolN µmolC_{POC} d^{-1})	(nmolC µmolC_{POC} d^{-1})
0	180	1.67	4.97	48.58	10	0.32± 0.13	0.17± 0.003	1.86	3.60	27.56	8	7.36	0.14
2	180	1.69	4.62	43.06	9	0.38± 0.24	0.15± 0.007	1.98	2.16	18.03	8	6.86± 1.99	0.13± 0.002
3	180	2.20	5.42	47.27	9	0.35± 0.10	0.19± 0.011	2.21	5.16	38.54	7	5.51± 0.74	0.14± 0.010
4	180	2.41	5.38	46.31	9	0.39± 0.03	0.14± 0.002	2.04	6.02	42.21	7	6.08± 0.91	0.09± 0.001
5						0.30± 0.04	0.20± 0.012					3.54± 0.66	0.12± 0.004
6	180	1.78	5.30	44.55	8	0.14± 0.01	0.13± 0.000	1.32	6.07	43.04	7	2.44± 0.33	0.08± 0.005
0	380	1.65	4.89	43.04	9	0.50± 0.12	0.21± 0.004	1.24	3.82	30.38	8	10.76	0.14
2	380	2.34	4.47	36.54	8	0.45± 0.07	0.17± 0.002	1.61	2.90	21.09	7	8.31± 0.74	0.12± 0.005
3	380	2.22	4.95	39.38	8	0.37± 0.03	0.21± 0.003	2.01	6.03	42.11	7	6.98± 1.93	0.13± 0.008
4	380	2.28	5.69	44.87	8	0.29± 0.08	0.17± 0.017	1.83	5.41	38.52	7	4.09± 1.67	0.10± 0.004
5						0.24± 0.05	0.20± 0.007					4.05± 1.58	0.13± 0.016
6	380	1.86	4.78	37.28	8	0.22	0.14	1.59	6.16	50.07	8	2.62	0.08
0	750	1.62	4.63	43.49	9	0.27± 0.03	0.21± 0.009	2.69	3.23	25.13	8	6.39± 0.58	0.15± 0.012
2	750	1.69	4.47	40.70	9	0.33± 0.09	0.17± 0.009	1.85	2.65	18.74	7	7.47± 1.46	0.12± 0.006
3	750	2.69	5.50	46.93	9	0.59± 0.06	0.25± 0.007	1.76	5.00	37.03	7	6.80± 2.32	0.14± 0.011
4	750	2.59	6.08	51.15	8	0.41± 0.01	0.19± 0.015	1.87	6.35	44.24	7	7.44± 1.73	0.14± 0.008
5						0.15± 0.01	0.18± 0.006					2.71± 0.23	0.14± 0.006
6	750	2.46	6.30	51.26	8	0.15± 0.05	0.14± 0.001	1.46	5.35	37.70	7	3.57± 0.61	0.08± 0.003
0	950	1.44	4.63	38.11	8	0.40± 0.11	0.24± 0.024	3.09	3.84	28.98	8	8.16± 0.69	0.16± 0.004
2	950	1.70	4.86	40.88	8	0.33± 0.05	0.18± 0.012	1.78	2.55	18.79	7	6.25±0.48	0.12± 0.003
3	950	2.13	5.17	42.16	8	0.44± 0.10	0.25± 0.015	1.87	5.42	40.52	7	7.60± 2.11	0.15± 0.017
4	950	1.95	5.49	45.33	8	0.36± 0.02	0.20± 0.009	1.74	6.21	42.94	7	4.87± 1.95	0.12± 0.004
5						0.33± 0.11	0.19± 0.002					5.25± 2.38	0.10± 0.011
6	950	1.74	5.11	36.64	7	0.16± 0.04	0.14± 0.009	1.61	6.34	41.03	6	2.15± 0.46	0.07± 0.004

3.3.3. Growth

Specific growth rates can be calculated from the change in either concentration of particulate organic matter carbon content (Lugol samples) during testing. In this study growth rates were only calculated from the change in particulate organic matter (Table 3.6), because of the high daily variability in the biomass data that was received from counting lugol samples.

Table 3.6 specific growth rates calculated from the change in particulate organic matter during testing.

	Specific growth rate μ_{PON} [d^{-1}]		Specific growth rate μ_{POC} [d^{-1}]	
Fraction	**> 10 µm**	**< 10 µm**	**> 10 µm**	**< 10 µm**
Mesocosm 180	0.19	0.05	0.16	0.00
Mesocosm 380	0.16	0.03	0.16	0.00
Mesocosm 750	0.17	0.08	0.15	0.05
Mesocosm 950	0.17	0.01	0.14	0.02

Specific growth rates of fraction >10 µm ranged from 0.16 to 0.19. They did not significantly differ between mesocosm (Kruskal wallis test, $p<0.05$), thus were not correlated to pCO_2 (spearman rank correlation rho, $p<0.05$). Growth rates of fraction <10 µm calculated from the change in PON during testing were higher in mesocosm 750 than mesocosm 180, 380 and 950. Yet, growth rates calculated from the change in POC during testing were higher in mesocosm of future pCO_2 level (μ_{POC}=0.05/0.02 d^{-1}, mesocosm 750 and 950) compared to glacial and present day pCO_2 level (μ_{POC}=0 d^{-1}, mesocosm 180 and 380). A clear relationship from pCO_2 was not observed for both fractions.

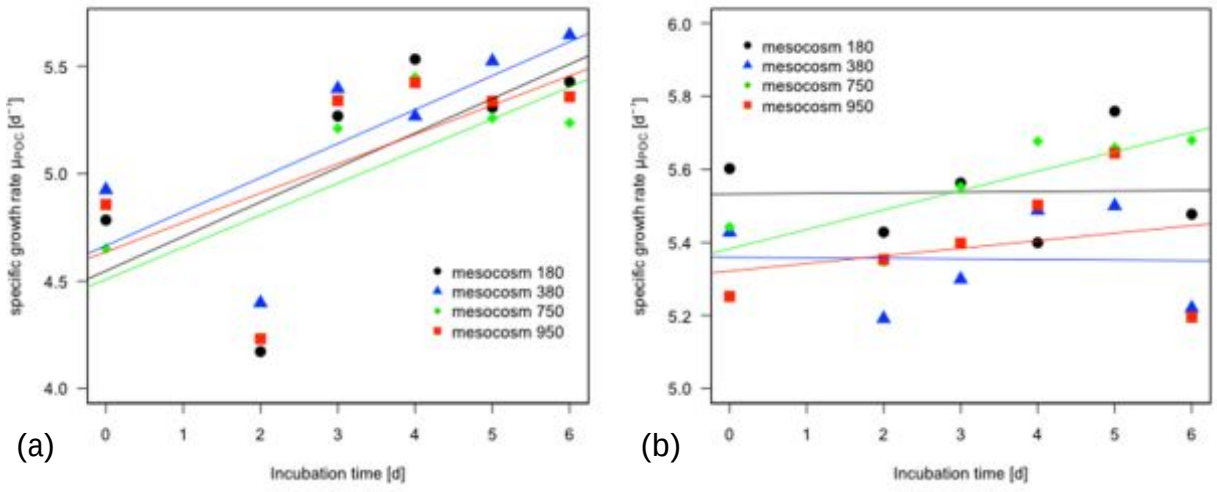

Figure 3.8. Specific growth rate μ_{POC} calculated for fraction > 10 (a) <10µm (b).

3.3.4. N_2 and CO_2-fixation

All rates were normalized to particulate organic carbon. Fractionated N_2- and CO_2 fixation/uptake rates not related to pCO_2 (Kruskal-Wallis test, $p<0.05$). N_2-fixation rates of mesocosm 180, 380 and 950 ranged from 5.54 to 5.8 nmolN $\mu molC_{POC}^{-1}$ (Figure 3.9a). Median N_2-fixation rates of mesocosm 750 (6.60 nmolN $\mu molC_{POC}^{-1}$) were 14 to 19 % higher than median rates of mesocosm 180, 380 and 950. N_2-uptake rates of mesocosm 180, 380 and 950 ranged from 0.35 to 0.33 to nmolN $\mu molC_{POC}^{-1}$ (Figure 3.9b). Median uptake rates of mesocosm 750 were slightly (10 to 15 %) lower than median rates of mesocosm 750. N_2-fixation and uptake rates decreased during testing by 44 to 76 % and 43 to 61 %, respectively.

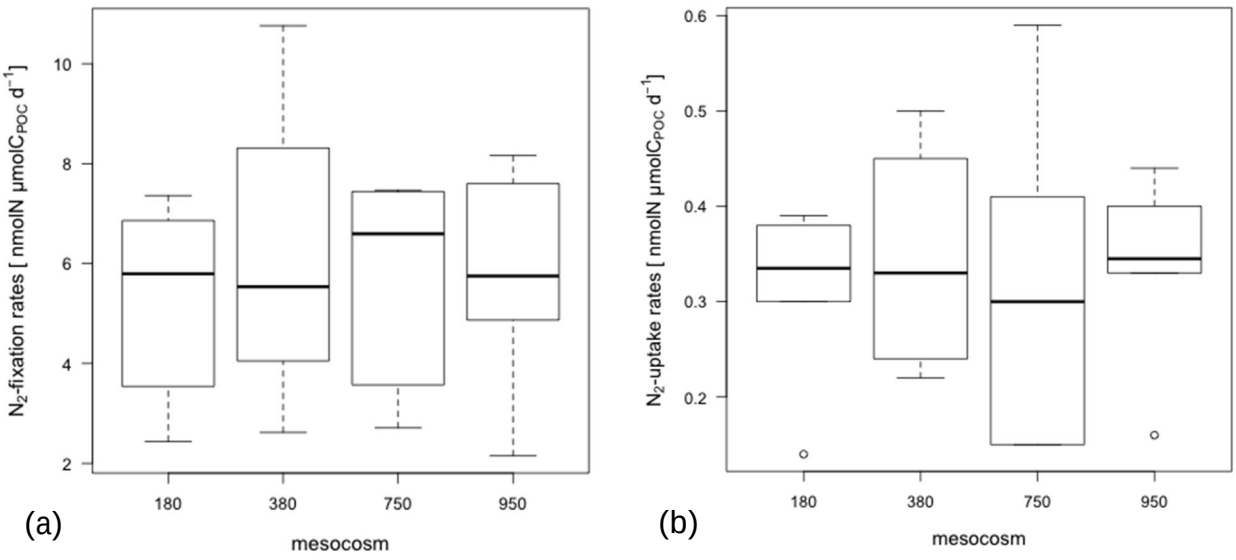

Figure 3.9 N_2-fixation rates of fraction >10 µm (a) and <10 µm (b) normalized to particulate organic carbon. N=6 for all mesocosm and fractions.

CO_2 fixation rates of fraction >10 µm did not differ between mesocosm 180 and 380 (0.13 µmolC $\mu molC_{POC}^{-1}$) and were 8 % lower than mesocosm 750 and 8 % higher than mesocosm 950. CO_2 fixation rates of fraction <10 µm were lowest in mesocosm 180 (0.16 µmolC $\mu molC_{POC}^{-1}$). Median fixation rates of mesocosm 380, 750 and 950 ranged between 0.19 and 0.2 µmolC $\mu molC_{POC}^{-1}$. Thus CO_2 fixation rates increased with increasing pCO_2 and were 25 % higher in mesocosm 950 compared to mesocosm 180. Fractionated CO_2-fixation rates decreased during testing by 43 to 58 % (<10 µm) and 22 to 40 % (>10 µm), respectively. Carbon to nitrogen fixation rates (data not shown) of fraction >10µm did not significantly differ between mesocosm. Fraction >10µm fixed three times more carbon than nitrogen compared to fraction <10µm fixing 70 to 80 times more carbon than nitrogen.

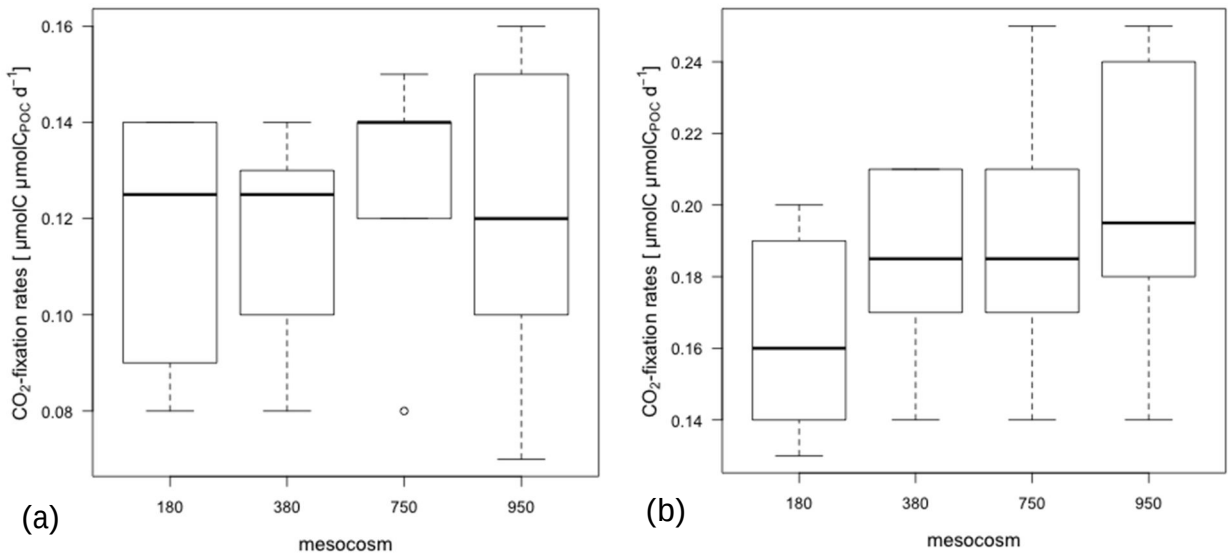

Figure 3.10 CO_2-fixation rates of fraction >10 µm (a) and <10 µm (b) normalized to particulate organic carbon. N=6 for all mesocosm and fractions.

3.3.5. The phytoplankton composition

Phytoplankton community with start of acidification (t_{-1})

Figure 3.11(a) and (b) give an overview of phytoplankton composition and carbon content before the start of the experiment (t_{-1}). The phytoplankton community was predominated by cyanobacteria *(nostophyceae)* with 60 to 80 % of total carbon content. The group itself was predominated by *Nodularia sp.* (80 %) and *chroococcales*. Furthermore, the phytoplankton community was composed of flagelates, *chlorophyceae* (*Oocystes sp.*, *Planktonema sp.*), *dinophyceae*, *lithostomatae* (*Mesodinium rubrum*) and few *diatomeae*. The Species composition was similar in all mesocosm with start of the experiment, except for mesocosm 750. The carbon content of *Nostophyceae*, in particular *Chlrorophyceae* and *Nodularia sp.* were 1.5 to 2-folds higher in mesocosm 750 than in the other mesocosm.

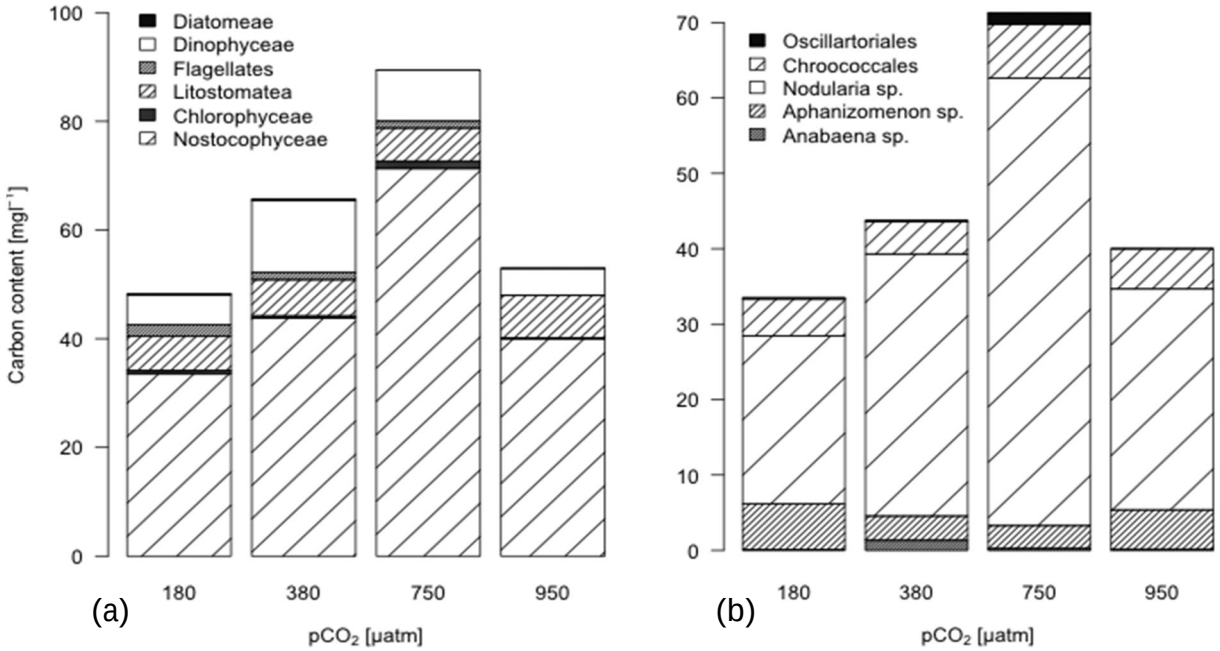

Figure 3.11 Composition of the phytoplankton community (a) and Cyanobacteria (b) prior to the start of the experiment (t_{-1}). Carbon content was calculated from counting of Lugol samples. Each mesocosm is represented by one counted sample. Phytoplankton carbon content was 1.5 to 2-folds higher in mesocosm 750 than in the other mesocosm.

Phytoplankton community during testing (t_0-t_6)

Abundance and biomass of diazotroph cyanobacteria decreased varied during testing but showed an overall decrease from 60 to 80% (t_0) to 30 to 60% (t_6) (Figure 3.12). In contrast, other phytoplankton groups such as flagellates and *dinophyceae* slightly increased. The observed changes could not be tested statistically because each day was only sampled once. Median dominance either calculated from biomass or abundance of cyanobacteria and other phytoplankton genera did not differ between mesocosm thus were not related to pCO_2.

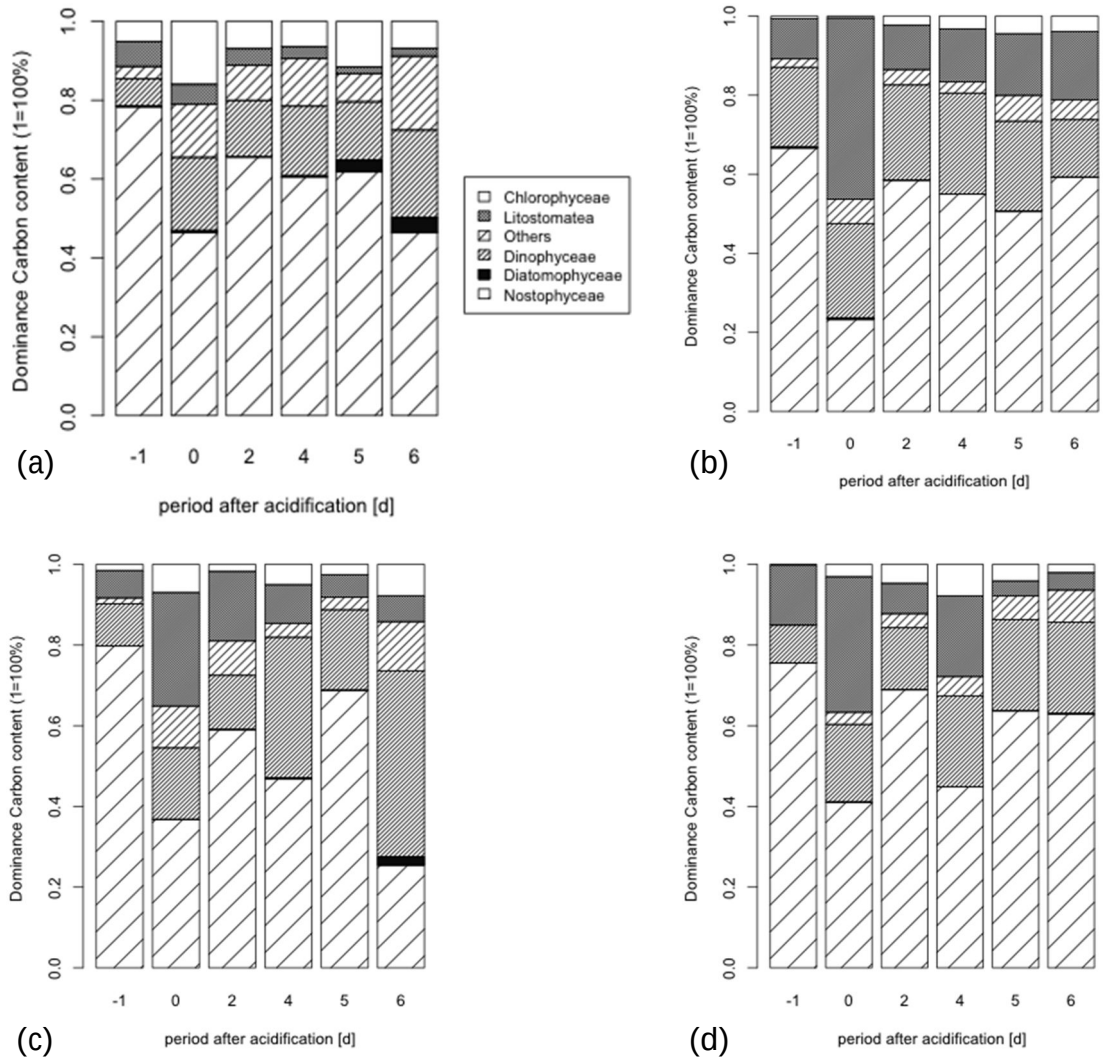

Figure 3.12 Phytoplankton composition of mesocosm 180 (a), 380 (b), 750 (c) and 950 (d). Dominance of each phytoplankton group was calculated from the carbon content of each group. Each day represents one counted Lugol sample. The group other represents flagellates.

3.3.6. Stoichiometry

Median POC:PON ratio of fraction >10µm were about 7 in all mesocom therefore not dependent to pCO_2. In contradiction POC:PON ratios of fraction <10µm were lower in mesocosm 380 and 950 (7.8) than 180 and 750 (8.4).

Concentrations of particulate organic phosphate were not fractionated thus only total POC:POP and PON:POP ratios could be calculated. Median PON:POP was about 29 in all mesocosm therefore above Redfield. Calculated median rations of POC:POP ranged between 179 and 330.

4. Discussion

4.1. Critical discussion of $^{15}N_2$-based methods to determine N_2-fixation rates

The $^{15}N_2$-tracer addition method is based on the direct injection of pure $^{15}N_2$ tracer gas (99 %^{15}N) into a water samples to achieve an appropriate enrichment of about 10atom % ($^{15}N/^{14}N$) (J P Montoya et al. 1996). A full and rapidly equilibration between tracer gas bubble and ambient sample water is assumed and the resulting equilibrium concentrations of $^{15}N_2$ and $^{14}N_2$ are commonly calculated according to (Hamme and Emerson 2004; R. F. Weiss 1970). This however, was never proven by direct concentration measurements of both isotopes. As cyanobacteria fix and incorporate N_2 they get enriched with the stable isotope $^{15}N_2$ during incubation. Therefore fixation rates are calculated by multiplication of the concentration of particulate organic matter with the ratio of the differential atom% in the particulate organic matter between end (A_{PN}^{final}) and start ($A_{PN}^{t=0}$) of the incubation and the differential between the atom% enrichment of the sample (A_{N2}) and the particulate matter with start of the incubation ($A_{PN}^{t=0}$) divided by the incubation time (equation 4.1, (Jonathan P Zehr and Montoya 2007)).

$$N_2\ fixation\ rate = \frac{\left(A_{PN}^{final} - A_{PN}^{t=0}\right)}{\left(A_{N_2} - A_{PN}^{t=0}\right)} \times \frac{[PN]}{\Delta t} \tag{4.1}$$

Yet, a recent study observed that the injected $^{15}N_2$ gas bubble does not attain equilibrium with the sample water as quickly as assumed leading to an overestimated enrichment with $^{15}N_2$ and therefore to underestimated and variable N_2 fixation rates (Mohr et al. 2010). The authors proposed and tested a modified $^{15}N_2$ tracer method based on the addition of $^{15}N_2$-enriched seawater that provides an instantaneous, constant enrichment. They observed up to 40 % higher N_2- fixation rates (pers. comm. Wiebke Mohr) using the modified method compared to parallels measured after Montoya et al. (1996). In this study, the determined dissolution of $^{15}N_2$ during an incubation time of 24 hours confirms findings made by Mohr et al. (2010). The concentration of the stable isotope $^{15}N_2$ strongly increased within 12 hours after start of the incubation reaching about 75 % of the expected atom %. In contrast the concentration of $^{15}N_2$ remained relatively stable and reached about 97% of the calculated enrichment in incubations to which $^{15}N_2$-enriched degassed seawater was added. However, during cruise the assumed overestimation of the $^{15}N_2$ enrichment of the sample water did not lead to a clear underestimation of N_2 fixation rates in comparison to measurements after Mohr et al.

(2010).A stable relationship between both methods was not visible. In 56% of replicates N_2 fixation rates determined after Montoya et al. (1996) were slightly higher than parallels measured after Mohr et al. (2010). For the other 40% the opposite was observed (Table 3.1). Mean N_2 fixation rates were 1.22 times higher when measured after Montoya et al. 1996 than after Mohr et al. 2010 (Equation 4.2 and 4.3).

$$N_2\text{-fixation}_{Mohr} = 0.82*N_2\text{-fixation}_{Montoya} + 0.018 \quad (4.2)$$

$$CO_2\text{-fixation}_{Mohr} = 0.87*CO_2\text{-fixation}_{Montoya} + 0.001 \quad (4.3)$$

One possible explanation is agitation as it was shown to fasten the dissolution between gas bubble and water sample (W E Asher et al. 1996; William E Asher and Pankow 1991). The equilibration between gas and ambient solution takes place between the boundary layer of both. Therefore small bubbles achieved through agitation fasten the equilibration process as the ratio of bubble surface to volume decreases when the bubble size increases. Furthermore, the renewal rate of the water-bubble interface that appeared to have the greatest effect on the isotopic exchange is increased through agitation. For instance, dissolved $^{15}N_2$ concentration achieved about 90% of calculated $^{15}N_2$ within an hour of continuous shaking (Mohr et al. 2010). Thus the discrepancies might decrease when incubations are carried out on board of a research vessel since it provides some agitation of the bubble, especially during stormy days (Mohr et al. 2010). Lowest N_2 fixation $ratios_{Mohr/Montoya}$ were observed within (station 255, 272) during the end (station 271) of the cruise. Wind intensity did not vary much (2 to 3 m s^{-1}) between sampling days of the particular station thus doesn't explain the observed varying pattern in the observed $ratios_{Mohr/Montoya}$ of N_2 fixation rates. Yet, the general shaking of the research vessel might have increased the dissolution of $^{15}N_2$ that in turn would have lead to a more accurate labelling than observed from the $^{15}N_2$ dissolution experiment. Moreover, observations from this study also indicate that the N_2 fixation rates measured after Mohr et al. 2010 were lower than the actual N_2 fixation rates of the studied community. A possible explanation is the dilution of the natural community in the sample through addition of 10 % $^{15}N_2$-enriched degassed seawater. This dilution could have lead to imbalances or changes in the phytoplankton community (including diazotrophs) or other microbes that has not been determined so far. This is also indicated by the observed CO_2 fixation $ratios_{Mohr/Montoya}$. CO_2 fixation rates measured after Mohr et al. were generally lower (67 %) than parallels measured after Montoya et al. 1996.

Since the dissolution of $^{15}N_2$ is time dependent the underestimation of the true N_2 fixation rates will vary with start of the incubation in relation to the dial pattern of N_2 fixation and the incubation time (Mohr et al. 2010). Baltic Sea cyanobacteria fix during the day (L J Stal and Walsby 1998; Marc Staal, Stal, et al. 2003) therefore the degree of error as well as the differential between both methods should increases if the incubation was begun in the morning. However, such relationship was not observed from this study. The $ratio_{Mohr/Montoya}$ varied independent if the incubation started in the evening (17:45 o'clock pm) or in the morning (10:20, o'clock pm).

One more realistic explanation could be the phytoplankton distribution and the overall error of the rate measurements. N_2 fixing cyanobacteria of the Baltic Sea show a patchiness distribution that complicates the sampling. Therefore it is not unusual that parallels deviate from each other by about 30% (comments in N_2 fixation workshop). This represents about the same range by which the N_2 fixation rates of both methods differ from each other. Yet, it was observed that standard deviations between parallels were much smaller in samples measured after Montoya et al. 1996 (e.g. Andy Rees, data shown at N_2 fixation workshop). Since the particulate organic matter on cruise filters (fraction >10 µm) was too low to be measured filters of triplicates had to be pooled ending up with a single sample measurement. Hence, in this study there is no information of a standard deviation between parallels.

To develop a reliable method for future measurements of N_2 fixation rates a workshop was carried out in Kiel in the beginning of the year (6. to 8.07.2011). The studies of workshop participants differed in their results. Some observed clear higher N_2 fixation rates using the modified than the bubble method (e.g. Mathew Church, Andrew White data shown at N_2 fixation workshop) and some observed variations similar to the results from this study (e.g. Andy Rees, Margaret Mulholland, some data shown at N_2 fixation workshop). Clearly the bubble dissolution does not occur completely as was observed from other workshop participants and therefore represents a source of error. However, the preparation of $^{15}N_2$ degassed seawater demands a lot more handling and preparation time and might lead to other errors through higher manipulation of the system (e.g. trace metals/ nutrients). Furthermore, the labelling of the sample is just one small step of many in the measurement of N_2 fixation rates. In summary, further method studies are needed. In the first, to determine if the under enrichment will lead to really major errors in the subsequently determination of N_2–fixation rates and second if there is an alternative method to provide an instantaneous and constant enrichment of the sample water that is simple in handling and without other sources of risk.

4.2. The Carbonate system of the study area

In the end of July (23. - 31.07.2011) nine stations were sampled along a natural pCO_2 gradient across the Baltic Proper. The total CO_2 (C_T) ranged from 1569 µmol Kg^{-1} in the southern to 1428 µmol Kg^{-1} in the northern parts of the sampling area. This gradient is quite small in comparison to the large-scale spatial C_T distribution across the whole Baltic Sea that ranges from 2000 µmol Kg^{-1} in the Kattegat to about 600 µmol Kg^{-1} in the northern part of the Gulf of Bothnia (Beldowski et al. 2010). The wide range of C_T is controlled by the alkalinity (A_T) distribution, which at a given pCO_2 determines C_T. During cruise surface A_T ranged from 1621 to 1678 µmol Kg^{-1}. In the Baltic Sea the salinity and A_T are controlled by the mixing of riverine with North Sea waters in contrast to the ocean where both are mainly controlled by precipitation and respiration. Since precipitation and respiration processes have no instinct effect on A_T and strong relationship between A_T and salinity exists in ocean waters. However, this does not account for the Baltic Sea due to the inflow of high saline and high A_T waters that mix with waters from rivers (Körtzinger et al. 1996). A_T concentrations vary completely between rivers as a result of the different lithogenic characteristic of the Baltic Sea drainage area (Kuliński and Pempkowiak 2011). The southern parts are characterized by lime rich soils which have a low potential for the release of A_T by weathering in opposite to the Scandinavian catchment area that is characterised by igneous rocks (Bernd Schneider 2011). Thus, freshwater inflows in the southern and central regions contain high A_T (~3200 µatm Kg^{-1}) whereas inflows in the northern regions contain low A_T (400 to 800 µmol Kg^{-1}). In addition the southern regions are influenced by the inflow of high saline and high A_T waters from the North Sea (2200µatm Kg^{-1}). An overview of the spatial A_T range as a function of salinity is given in Figure 4.1 (Beldowski et al. 2010). The strait lines describe the different relationships between A_T and salinity. They are intercepting at a salinity of 7.0±0.50 and an A_T of ~1650 µmol Kg^{-1} that represents the observed range during this cruise study (Figure 4.1, solid red cycle). Both the observed salinity and A_T are typical for the central Baltic Sea that can be considered as a mixing chamber for water from the nearby basin (Bernd Schneider 2011).

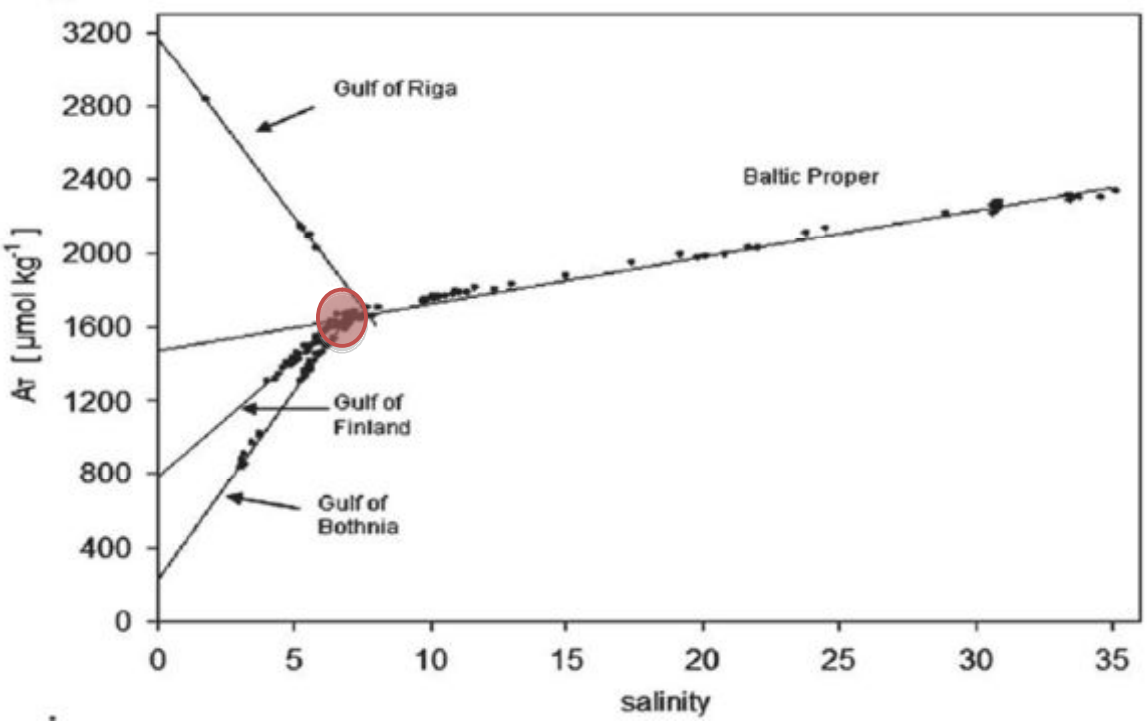

Figure 4.1 gives an overview on the spatial distribution of the total Alkalinity as a function of salinity with regression lines for different subareas of the Baltic Sea. The Alkalinity range observed during cruise is marked red (adopted from Beldowski et al. (2010)).

Surface pCO_2 ranged from 322 to 122 µatm across the sampling area thus the surface water was undersaturated in pCO_2 with respect to the atmosphere (~385µatm). This strong decrease in surface pCO_2 results from high primary production, in particular from cyanobacteria blooms that predominate phytoplankton blooms from mid-June until the beginning of August in that area. The production of particulate organic matter via photosynthesis consumes CO_2 and therefore reduces C_T whereas the respiration (re-mineralisation) of organic matter produces CO_2 and therefore increases the C_T (Körtzinger et al. 1996; B Schneider, Kaitala, and Maunula 2006). In general surface water pCO_2 tends to equilibrate with atmospheric pCO_2. Yet, the disequilibrium caused by biological processes is much faster than the re-equilibration by gas exchange. The reason for that is represented by the buffering reaction of the carbonate system (Equation 1.2). When CO_2 dissolves in the water the CO_2 partial pressure increases that however is buffered by the reaction of $CO_{2(aq)}$ with H_2O reducing the pCO_2. Thus the equilibration of the partial pressure between atmosphere and surface water is much slower for CO_2 in contrast to other gases like N_2 and O_2 and in contrast to changes in pCO_2 by biological processes (personal communication, Bernd Schneider). In addition to the gas exchange with the atmosphere the biological consumption of CO_2 is also buffered by the mixture of re-mineralized CO_2 from deeper water layers into the surface. But, in the Baltic Proper a seasonal thermocline evolves from spring to autumn because of the increase in surface water temperature and fresh water inflow. It acts like a strong barrier that hinders the

mixture of surface and deeper waters leading to an undersaturation of the surface pCO_2 during phytoplankton blooms (Rutgersson et al. 2008). Thus, both slow and hindered physical processes in addition to high biological production during blooms lead to a strong undersaturation of the surface pCO_2 throughout the productive season. The observed minima during cruise represents one in two that characterise the annual cycle of surface pCO_2 in the central Baltic Sea. An overview of this seasonality in surface pCO_2 is given in Figure 1.1 (Bernd Schneider et al. 2009). The first minimum results from the offset of the spring bloom in the beginning of March. With peak production in the beginning of May the pCO_2 decreases to about 130 µatm in the northern and 250 µatm in the southern Baltic Proper (Figure 1.1 (1)). The spring minimum is followed by a slight increase in surface pCO_2 because of the increasing temperature during summer (Figure 1.1 (2)) because the dissolution constant of CO_2 is temperature dependent. When temperature increases the dissolution of CO_2 decreases followed by an increase in pCO_2. The second minimum is observed during July that results from the occurrence of cyanobacteria blooms as was observed from this study (Figure 1.1 (3)). In autumn the production period ends because the mixed water layer increases as a consequence of decreasing temperature and enhanced wind intensities. The resulting input of re- mineralized pCO_2 from deeper water layers leads to an increase of the surface pCO_2 until the end of the year reaching up to 400 µatm in the southern and 500 µatm in the northern Baltic Proper (Figure 1.1 (4)). The amplitude of the biological controlled seasonality in surface pCO_2 decreases from north to south and almost disappears in the south-western regions of the Baltic Sea. This spatial distribution in surface pCO_2 was also observed in this study whereas the pCO_2 were generally lower in the northern Baltic Proper (153µatm) than in the southern parts (219µatm). This large scale spatial variability is partly attributed local difference in production rates as well as the spatial distribution of the A_T (Omstedt et al. 2010). The drop in pCO_2 through biological consumption is a little bit less buffered in the northern parts of the Baltic Sea than in the southern areas that are characterised by higher A_T concentrations. Yet, the major causes are the differences in the hydrographic conditions between the different areas. Whereas the central and northern Baltic Sea is characterised by a stable seasonal thermocline the shallower and hydrographically more dynamic waters in the south-western areas are frequently well mixed (Bernd Schneider 2011). Thus production and respiration of organic matter are not entirely separated by stratification of the water column that in turn result in a less draw down of pCO_2 during the productive period. However hydrographical and biological conditions do not fully explain the observations from this study. Even though all stations, except 113, were characterised by a seasonal thermocline

from 10 to 30 m increases in Chlorophyll a concentrations don't go along with a decrease in surface pCO_2 (Figure 4.2). The wind was very strong the days before the southern parts were sampled exceeding 7 m s^{-1}. That could have led to some mixing processes between deep and surface waters and therefore increases in surface pCO_2. Moreover, the pCO_2 signal represents a long-term signal, which is more consistent than biological activity (personal communication, Bernd Schneider)

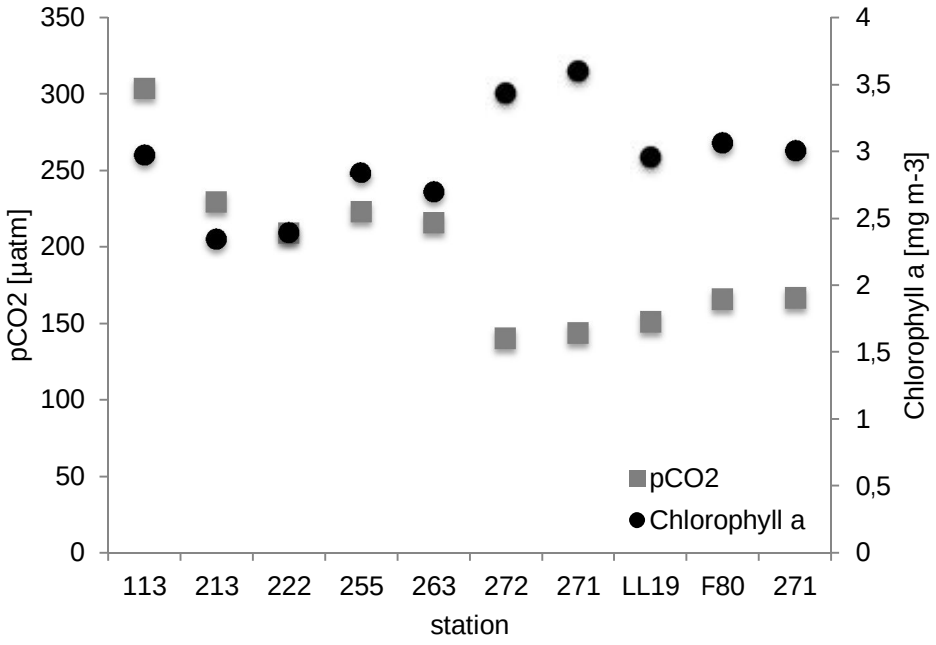

Figure 4.2 Total chlorophyll a concentration and surface pCO_2 determined for each station.

4.3. Bloom stage of the studied community

In the Gotland Basin and in the Bornholm Basin, surface pCO_2 can drop to 100 µatm and 200, µatm , respectively, during the bloom peak. In this study the surface pCO_2 ranged from 208 to 228 µatm and from 140 to 143 µatm, respectively. The surface water was depleted with phosphate and the POC:POP ratios ranged from 137 to 272. The ratios exceed Redfield ratios (106C:16N:1P) that are usually observed under optimal growing conditions. This indicates that the bloom had started to rely on intracellular phosphorous storage to cover their phosphorous demand. It was observed that POC:POP ratios can exceed values of over 400 until the bloom will break down (Kangro et al. 2007).Both pCO_2 level and POC:POP level indicate that the blooms were in a late-bloom stage during cruise sampling and with the start of the mesocosm experiment. This is also underlined by the chlorophyll a concentrations that were mainly below 3 mg m^{-3} that lay below concentrations of 5 mg m^{-3} that are usually observed during high blooms abundance (N. Wasmund and et al. 2005).

4.4. Physiological effects of increasing pCO_2 on a natural phytoplankton community predominated by diazotroph cyanobacteria (fraction >10µm)

Fractionated CO_2- fixation rates of the southern Baltic Proper (208-229 µatm) were 35 % higher in median than rates of the northern Baltic Proper (140-166 µatm). This was not observed for the same pCO_2 gradient during the mesocosm study. Median CO_2 fixation rates did not differ between mesocosm 180 and 380. In addition, median assimilation of CO_2 increased by 8 % when the pCO_2 level increased to 750 µatm but declined by 15% when pCO_2 increased from 750 to 950 µatm. However none of the observations were significant because of high variability in the data. A relationship between pCO_2 and N_2 fixation was also not observed for fraction >10 µm, same as for growth and stoichiometry. Median N_2 fixation rates of stations in the southern and northern Baltic Proper equalled each other. The same was observed between mesocosm 180 and 950. In contrast, median N_2 fixation rates of mesocosm 750 were 14 to 19% higher than mesocosm 180, 380 and 950. Yet, once again none of the observations were significant because of high variability in the data.

Hence, in this study a statistically significant dependence from pCO_2 was not observed for a natural phytoplankton community predominated by N_2 fixing cyanobacteria, neither in the field, nor in a mesocosm experiment. Those results were most unexpected. As described in the previous chapter the Baltic Sea is characterised by seasonal and spatial gradients in surface pCO_2 ranging from 100 to over 500 µatm. Since the phytoplankton community naturally experiences fluctuations in pCO_2 in a magnitude of 400 µatm that are equal to forecast increases until year 2100 a positive stimulation along an increasing pCO_2 gradient was hypothesized. Moreover, it was suggested to observe the strongest stimulation between glacial and present day pCO_2 levels as the amplitude equals the spatial and seasonal amplitude that is naturally observed in the Baltic Sea. However, this was not observed and the hypotheses must be rejected.

The community of fraction >10µm was predominated by filamentous cyanobacteria (nostocales, oscillatoriales) in addition to *chlorophyceae, dinophyceae, litosstomatea, diatomeae* and flagellates (Figure 3.5). The phytoplankton composition differed between stations sampled along the CO_2 gradient during cruise. For instance the abundance of *nostocales, diatomeae* and flagellates ranged from 5 to 80%, 0 to 18% and 8 to 60%, respectively. The determined CO_2-fixation rates represent responses of the whole phytoplankton community. Recent studies indicate that phytoplankton species differ in their CO_2 sensitivity, in particular in their carbon demand as well as their metabolic mechanisms (Ulf Riebesell 2004) that might explain the observed independent variations. Some species

preferable use CO_2 as carbon source whereas others prefer on HCO_3^- (Elzenga, Prins, and Stefels 2000). The equilibrium constant of the buffer reaction (equation 1.2) is pH dependent. An increase in pH shifts the equilibrium of the carbonate system leading to an increase of $CO_{2(aq)}$ in relation to its reactants HCO_3^- and CO_3^-. Thus species that preferably draw their inorganic carbon from HCO_3^- are less stimulated than species that mainly use CO_2. The metabolic process of CO_2 fixation is catalyzed by the enzyme Ribulose-1,5-bisphosphate carboxylase oxygenase (RuBisCO). The RuBisCO of most phytoplankton species is characterised by a half saturation constant (K_M=20 to 185µM CO_2, (Murray R Badger et al. 1998)) above ambient marine [CO_2] of 10 to 20µM. To ensure an effective carboxylation the intracellular [CO_2] is raised through active import of CO_2 and HCO_3^- whereas the latter is converted to CO_2. This so-called carbon-concentrating mechanism (CCM's) is energy demanding and therefore facilitated by the increase of ambient [CO_2] (Price et al. 2008). Yet, species have different RuBisCO saturation constants. Species like diatoms with a saturation constant close to present day [CO_2] (K_M=8-10µM CO_2) are less stimulated by increasing pCO_2 (Burkhardt et al. 2001; Björn Rost et al. 2003) than for instance cyanobacteria whose RuBisCO saturation constant is way below (K_M=105-108µM CO_2, (M. R. Badger 2003; Murray R Badger et al. 1998)). Those species-specific CO_2 sensitivities in addition to varying community compositions between stations might explain the observed variations in CO_2-fixation that result in an insignificant stimulation of the phytoplankton community with increasing pCO_2. Yet, mesocosm were enriched and therefore predominated by filamentous cyanobacteria of the genera Aphanizomenon and Nodularia (60-80 %, Figure 3.12). Culture studies on both genera observed a significant stimulation of CO_2-fixation rates (200 %) when pCO_2 increased from 270 to 780 µatm thus stay in contradiction to the results drawn from this study. Possible explanations for the loss of CO_2 mediated stimulations of CO_2 fixation observed in this mesocosm study will be discussed later together with observed N_2-fixation rates.

In contrast to CO_2 fixation, in the Baltic Sea the acquisition of N_2 is thought to refer to the common filamentous genera Aphanizomenon, Nodularia and Anabaena. Thus changes in N_2 fixation represent physiological responses of diazotroph cyanobacteria. Field and mesocosm samples were co-dominated by the common genera Nodularia and Aphanizomenon. Anabaena species were also present but their dominance negligible (Table 3.3, Figure 3.11). It is suggested that higher CO_2 concentrations allow greater efficiency in carbonate concentrating mechanism and that the freed up energy can be shunted towards other energy consuming processes like the fixation of N_2 by diazotroph cyanobacteria (S. Kranz, Eichner, and Rost

2010; Raven 1991). Yet, once again a relation to pCO_2 was not observed for a natural assemblage of cyanobacteria; neither during cruise nor during the mesocosm experiment. The results agree well with observations of Czerny et al. (2009) but stay in contradiction to the hypothesized adaptation of Baltic Sea inhabitants versus pCO_2. Czerny et al. (2009) observed a very slight, but insignificant decrease of N_2 fixation rates with increasing pCO_2. The author suggests that a possible energy translocation between CCM and N_2 fixation does not account for heterocystous cyanobacteria. Heterocysts are specialized cells in which the enzyme nitrogenase that catalysed the fixation of N_2 is localised and the acquisition of N_2 occurs. Since they lack photosystem II and the RuBisCO (reviewed in Böhme (1998)), they are not directly influenced by changes in pCO_2 (Czerny, Barcelos e Ramos, and Riebesell 2009). Furthermore, Czerny and co-authors assume that the reduction of ambient seawater pH as a result of increased pCO_2 restrain the translocation of amino acids from heterocyst to vegetative cyanobacteria cells. The N_2 fixed by the heterocyst is reduced to ammonia and either released as DIN or assimilated through the production of glutamine and glutamate within the glutamine synthetase/glutamine oxoglutarate aminotransferase (GS/GOGAT) reaction. The subsequent produced amino acids glutamin, glutamat and arginine are transferred to the vegetative cells. As in heterocystous cyanobacteria only the heterocysts are capable of fixing N_2, the vegetative cells of a filament strongly depend on the supply of nitrogen by the N_2 fixing cells. But, as discussed by (Wannicke et al., submitted), the translocation between adjacent cells takes place through intercellular channels, called microplasmodesmata, that link the cytoplasm of cells. As the intracellular pH is kept constant ionic exchange is not directly dependent on extracellular pH (Mullineaux 2008; E Flores and Herrero 2010). Furthermore, the outer membrane of the heterocyst envelope is an efficient permeability barrier, thus it is more likely that amino acids diffuse within a continuous periplasm and are re-imported into the cytoplasm of vegetative cells, instead of passing the inner and outer layers of the heterocyst envelope (Enrique Flores et al. 2006) as suggested by Czerny et al. (2009). Moreover, in contrast to Czerny et al. (2009) and to the results from this study other recent studies observed a stimulating effect of N_2-fixation by increasing pCO_2 in heterocystous cyanobacteria and therefore agree with the expectations of this study. Wannicke et al. (submitted, 2012) observed a positive stimulation by 13 % for a monoculture study on Nodularia spumigena when the pCO_2 increased from 180 to 750 µatm. The same patterns were observed for a co-culture of Nodularia spumigena and Aphanizomenon (Isensee et al., submitted) but the magnitude in reactions differed considerably. N_2-fixation rates were stimulated 420-720%, respectively, when the pCO_2 was increased from 270 to 780µatm.

These large differences in CO_2 sensitivity obtained by the different studies raise questions about their experimental conditions other than the applied pCO_2 levels. An overview of both is given in Table 4.1. It is well known, that occurrence and physiology of diazotroph cyanobacteria are linked to various physical and biological parameters including nutrients, light, temperature, intra- and innerspecies competition, as well as grazing and viral infestation. (Inga Lips and Lips 2008; Giller and Mapfumo 2006; Margaret R Mulholland and Bernhardt 2005; L Stal 2003; Chan et al. 2004). Figure 4.3 gives a small overview of the complex interaction between cyanobacteria with their environment. Thus it is likely that the CO_2 sensitivity of N_2 fixing cyanobacteria is modulated by other environmental factors that in turn would explain the varying observations between all studies. Additionally, the experiments of the present study were carried out in the field under natural conditions (mesocosm were situated outside the institute) that changed within and between sampling days. During cruise biomass and fixation rates were strong related to irradiance and temperature but not to pCO_2 (multiple regression R^2=0.6, $p<0.01$). This suggests that a positive effect of pCO_2 was cancelled by negative physical influences like decreasing light intensity (irradiance) or temperature whereas in result a stimulation of growth and productions by rising pCO_2, as known from culture studies, could not be observed in the field.

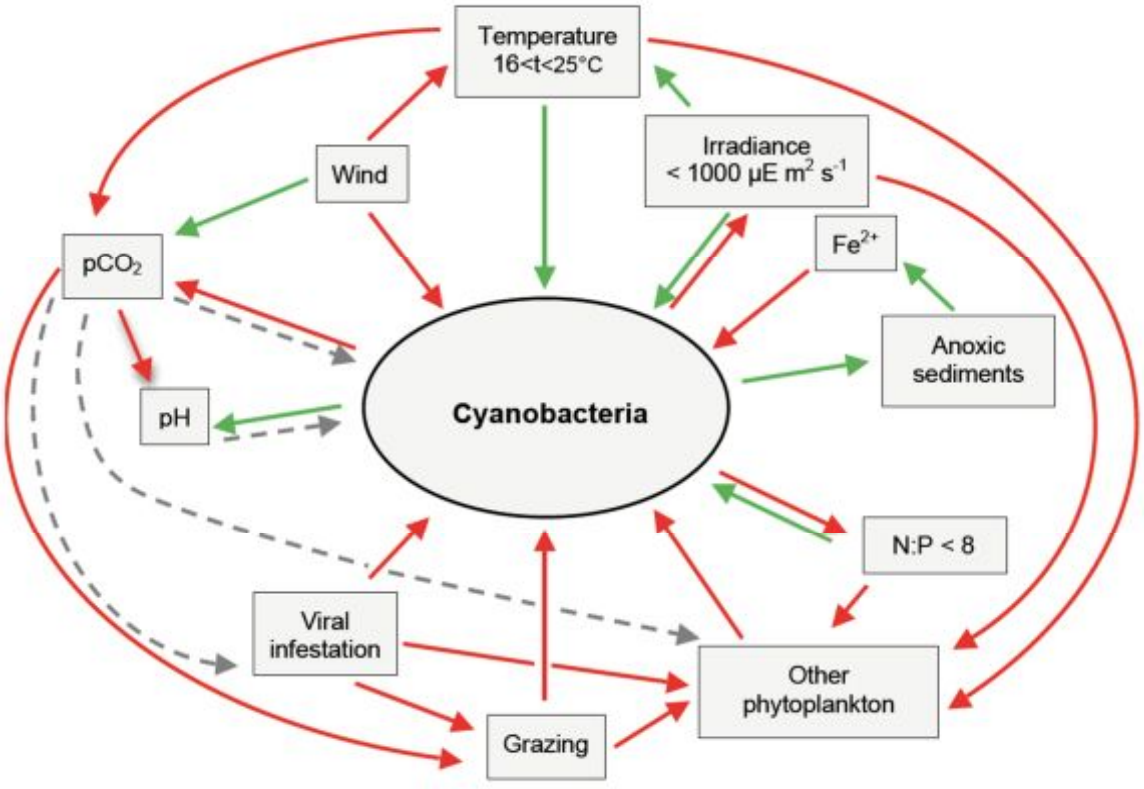

Figure 4.3 Interaction of diazotroph cyanobacteria with their environment. Green arrows represent stimulating effects. Red arrows represent negative effects. Dashed, grey arrows represent unknown effects of increasing pH and pCO2.

Table 4.1 Comparison of studies on N_2 fixing cyanobacteria genera of the Baltic Sea. T is temperature

Conditions (experimental setup)									References
Organism	Media	Homogenization	light/dark cycle	CO_2 manipulation	Acclimation	Irradiance	$Nutrients_{START}$	T	$Chla_{START}$	
			[h]			[µE m^{-2} s^{-1}]	[µM]	[°C]	[µg l^{-1}]	
Nodularia spumigena, Aphanizomenon flos aque Co-culture	Baltic Seawater 10µm filtrated	Manually	16/8	Premixed CO_2 gas	2d	100	0.6 DIP 4.1 DIN	18	5 10	Isensee et al., submitted
Nodularia spumigena [IOW-2000/1] Mono-culture	Artifical seawater 0.2µm filtered	Rotating device bottle rotation (1 rpm)	14/10	HCl NaOH	13-20d	85	5.4 DIP 0 DIN	16	1	Czerny et al. 2009
Nodularia spumigena Mono-culture	Baltic Seawater 0.2µm filtered, UV axenic	Manually	16/8	Premixed CO_2 gas	3d	200	0.35+0.5 DIP 0 DIN	23	0.8	Wannicke et al., submitted
Nostocales Natural assemblage	Baltic Seawater	None	~16/8	HCl NaOH	-	500	<d.l. DIP <d.l. DIN	17±1	-	Current study (cruise)
Nostocales (enriched) Natural assemblage Net sampled (100µm)	Baltic Seawater	Manually 3-5 time d^{-1}	~16/8	HCl NaOH	12h	239±15	0.2 DIP <d.l. DIN	22	3.46±0.15	Current study (mesocosm)

Conditions (experimental setup)			Physiological Responses							References
pCO_2	pH	C_T	POC:PON	POC:POP	PON:POP	Growth	Stoichiometry	N_2-fixation	CO_2-fixation	
[µatm]		[µmol kg^{-1}]				Δ%[d^{-1}]	Δ%	Δ%	Δ%	
270 780	8.23 7.79	1609 1707	6.2 6.4	93.3 101.7	14.9 15.7	0.05 0.17 (+340%)	POC:PON +27 POC:POP ±0 PON:POP -25	722	450	Isensee et al., submitted
150 700	8.57 7.95	1981 1981	5.5 7.0	170 170	32 24	0.52 0.33 (-63%)	POC:PON +27 POC:POP ±0 PON:POP -25	±0 (-)	n.d.	Czerny et al. 2009
180 750	8.15 7.99	1599 1648	7.0 7.0	140 150	20 22	44%	±0	13%	36%	Wannicke et al., submitted
139-166 208-303	8.3-8.4 8.5-8.6	1510-1569 1428-1519	7-8 7-8	56-221 59-321	7-28 8-49	n.d.	±0 (-)	±0 (-)	±0 (+)	Current study (cruise)
180 950	8.31 7.69	1506 1614	7.5 7.5	240 235	29 28	±0	±0	±0 (-)	±0 (+)	Current study (mesocosm)

Along the horizontal pCO_2 gradient of the study area, the southern parts (higher pCO_2, 208-228 µatm) were characterised by lower surface water temperatures ±16 °C than the northern parts (lower pCO_2, 140-166 µatm) with surface water temperatures ±18°C. On a global scale temperature per se does not restrict diazotroph growth as N_2 fixing cyanobacteria can be encountered at temperatures close to freezing (Zielke et al. 2002; Pandey et al. 2004). However, heterocystous cyanobacteria are typically distributed in cooler waters due to the temperature dependence on N_2-fixation, O_2 flux and respiration (Marc Staal, Meysman, and Stal 2003). During cruise cyanobacteria were abundant in all samples with temperatures down to 5 °C whereas diazotroph "blooms" were only observed within a temperature range from 16 to 19°C. The findings accord well with observations made by Wasmund (1997) and the assumed optima for *Aphanizomenon sp.* and *Nodularia sp.* of 16 to 22 °C and 25 to 28 °C, respectively (Lehtimaki et al. 1997). Therefore the decrease from 19 to 16 °C along the increasing pCO_2 gradient could have negatively influenced N_2-fixation rates during cruise and therefore cancelled out a positive stimulation of higher pCO_2. So far, little is known about the combined effects of increasing pCO_2 and temperature on the physiology of cyanobacteria. Yet, studies on *Trichodesmium erythraeum* (ISM101) observed decreased reactions with increased temperatures (Levitan, Sudhaus, LaRoche, et al. 2010). The marine cyanobacterium is abundant in tropical and subtropical waters and thought to be strong related to pCO_2. Its optimal growth temperature ranges from 24 to 30 °C. In a culture experiment gross N_2-fixation rates of *Trichodesmium erythraeum* (ISM101) increased by about 7-fold when the pCO_2 increased from 400 to 900 µatm at a temperature of 25 °C. In addition the fixation period was prolonged from 9 to 13 hours. However, when the temperature was increased up to 31°C, thus above optimal growth conditions, the observed stimulation decreased by about 2-fold and the fixation period was not prolonged. Moreover at 31 °C N_2-fixation rates were not stimulated when the pCO_2 increased from 250 to 400 µatm. Thus, even though the combined effect of pCO_2 and temperature was not significant in the recent study the observations indicate that CO_2 sensitivity is modulated by temperature.

This hypothesis is also supported by the experimental setup of studies on Baltic Sea cyanobacteria. Czerny et al. (2009) observed no stimulation of the physiology of *Nodularia spumigena* by increasing pCO_2 at a temperature of 16 °C, whereas Wannicke et al. (submitted) and Isensee et al. (submitted) observed a strong stimulations of *Nodularia spumigena* and *Aphanizomenon flos-aque* cultures at optimal growth temperatures of 22 to 23 °C. In this field study temperatures (16 to 19°C) were optimal for *Aphanizomenon sp.* but not

for *Nodularia sp*. Thus both species were probably affected differently by temperature along the pCO_2 gradient that in turn could explain the high variations in the data.

Temperature is linked to other environmental factors like irradiance and wind speed and both are linked to cyanobacteria occurrence and physiology (Inga Lips and Lips 2008; Kanoshina 2003; L Stal 2003) (Figure 4.3). When wind intensity increases the depth of the mixed layer increases. This in turn reduces the mean light intensity and temperature of the euphotic water layer as warm surface water is mixed with colder, deeper water layers. As Cyanobacteria are stimulated by increases in irradiance and temperature and tend to accumulate at surface an increase in wind intensity negatively affects them. During cruise wind intensity decreased from about 7 to 2 m s^{-1} with decreasing pCO_2. However the N_2 fixation rates were not linked to wind intensity (multiple regression, R^2=0.25, kruskil wallis rank sum test, $p<0.05$) and cyanobacteria were probably more controlled by temperature and irradiance, because wind conditions were quite calm and below the observed critical value of <6 m s^{-1} (Norbert Wasmund 1997). An increase in temperature favours the stratification of the water layer and leads to a decrease of the mixed layer. This in turn increases the mean light intensity of the mixed layer and changes the light spectra since the intensity of light decreases and the light spectra shifts from red to green light with water depth (Six et al. 2007). Primary production is a very light dependent process since the energy used in metabolic processes is provided by photosynthesis that is fuelled by the capture of light. Especially diazotroph cyanobacteria are easily limited by light as the fixation of N_2 is a very energy demanding process and one of major energy sinks in the cell next to carbon acquisition and CCM (B Bergman et al. 1997; Breitbarth et al. 2008). That explains the observed significant decrease of N_2-fixation and CO_2-fixation by 20 and 3-folds with decreasing depth from surface to 20 m in the field that occur well with the literature (Marc Staal, Stal, et al. 2003). At surface the daily pattern of N_2-fixation is characterised by two maxima one in the morning and one in the afternoon. Since both decrease with depth the daily pattern flattens. That in turn leads to the reduction of N_2 fixation rates with decreasing irradiance, in particular with depth (Evans et al. 2000; L J Stal and Walsby 1998).

A significant difference between southern (mean=492µE m^2 s^{-1}) and northern parts (mean=470 µE m^2 s^{-1}) did not exist for the daily mean of surface irradiance that varied from 146 to 753 µE m^2 s^{-1} between sampling days. Studies on interactive effects of light and pCO_2 on *Trichodesmium* erythraeum (IMS101) cultures determined a declining effect of elevated pCO_2 on N_2-fixation with increasing irradiance (Sven Kranz et al. 2010; Levitan et al. 2010; Nathan S Garcia et al. 2011). Kranz et al. (2010) observed an increase in gross fixation rates

by 200 % under low light (50 µE m^{-2} s) and high pCO_2 (900 µatm) and by 112% under high pCO_2 and high light (200µE m^{-2} s^{-1}). Garcia and co-authors observed the same pattern and suggest that a negative feedback interaction is caused by enhanced retention of cellular nitrogen as the ratio of gross:net fixation was correlated with light. The nitrogenase activity of Baltic Sea cyanobacteria is saturated below a photon flux intensity of 600 µE m^{-2} s^{-1} and consistently inhibited above 1000 µE m^{-2} s^{-1}(Evans et al. 2000). Therefore mean irradiance was quite high and could have cancelled out a positive stimulation of increased pCO_2 as was observed for *Trichodesmium* cultures and natural assemblages. This hypothesis is again underlined by the different experimental setup of studies on Baltic Sea cyanobacteria. The highest stimulating effect was observed for 100 µE m^2 s^{-1} that represent the medium light intensity compared to the irradiance levels used by the other studies. Czerny et al. (2009) used very low light intensities of 80 µE m^2 s^{-1}, that are typically observed at subsurface, when studying CO_2 effects on a *Nodularia spumigena* culture. Yet, the genera is known for its demand of strong light intensities and is usually distributed close to surface. Furthermore, at station 271 (Gotland Deep) pCO_2 was measured along a transect from surface to 50 m depth showing an increase in pCO_2 from 166 to 331 µatm within the upper 20 m (data not shown). Assuming a similar pattern for other sampling areas in the central Baltic proper the observations underline a possible modulation of the CO_2 sensitivity by irradiance as N_2 and CO_2-fixation rates significantly decreased with depth.

It was also discussed that high irradiance increases oxygen production that in turn inhibits N_2-fixation (M Staal, Te Lintel Hekkert, et al. 2003; J. Gallon 1981; Jonathan P Zehr et al. 1993). However elevated oxygen concentration could not be measured (Sven Kranz et al. 2010) and cyanobacteria domestic in the Baltic Sea are all heterocystous. In non-heterocystous cyanobacteria nitrogen fixation is temporal separated by photosynthesis or either down regulated during peaks (I Berman-Frank, Lundgren, et al. 2001). However, in heterocystous cyanobacteria N_2-fixation and photosynthesis are spatially separated, thus both processes can occur within the same period. In addition oxygen concentrations of cruise samples were saturated and did not differ from each other. Therefore light itself might be much more significant than oxygen in regulating nitrogenase activity in heterocystous cyanobacteria of the Baltic sea (Evans et al., 2000).

In conclusion physical parameters changed along the sampled horizontal gradient of decreasing pCO_2 from 228 to 140 µatm. Wind intensity decreased from 7 to 2 m s^{-1}, temperature increased from 16 to 19 °C, respectively, and the daily mean in irradiance varied between 146 and 753 µE m^2 s^{-1} (Table 3.2). Moderate increases in temperature and light

favour cyanobacterial occurrence whereas an increase in wind intensity has a negative effect. This indicates that the positive stimulation by pCO_2 observed in culture studies is strongly modified under varying physical factors and that in this study a possible stimulating effect of increasing pCO_2 on cyanobacterial physiology was cancelled out by the physical conditions.

Yet, in the mesocosm study physical parameters like irradiance and water temperature remained relatively stable throughout the testing period and did not significantly differ between mesocosm. Mean temperature was 21.5±0.5 °C and irradiance ranged from 223 to 260 µE m^2 s^{-1}. As the physical parameters were similar to that of Wannicke et al., (under review) who observed a stimulating effect possible modulations of CO_2 sensitivity by physical parameters don't explain the diminished pCO_2 effect that was observed in the mesocosm experiment.

So far, a stimulation of cyanobacterial growth and production by increasing pCO_2 was only observed under nutrient replete conditions. In contradiction, the sample water of the mesocosm experiment and during field studies was depleted with nitrogen and phosphate (PO_4<0.04, NO_{23}<0.11, NH_4<detection limit). Besides dissolved inorganic compounds cyanobacteria can use intracellular phosphorous storage to cover their phosphate demand (E Vahtera, Laamanen, and Rintala 2007; Monika Nausch et al. 2008). In the four pCO_2 treatments mean biomass ratios of POC:POP and PON:POP ranged from 235 to 240 and 28 to 29, respectively. Similar ratios were observed during cruise. POC:POP and PON:POP ranged from 137 to 272 and from 19 to 37, respectively. In addition biomass, N_2- and CO_2-fixation rates decreased during mesocosm testing by 44 to 76 % and 43 to 56 %, respectively.

Those findings occur well with a study of Isensee et al. (submitted) who also observed a diminished pCO2 effect in a late bloom community that was starving of phosphorous and relied on intracellular phosphorous storage. In addition, phosphate concentration used in the laboratory experiment of Wannicke et al. (submitted) were much lower than in the culture experiment of Isensee et al. (submitted) who observed a much stronger stimulating effect of increased pCO2 Phosphate is essential for growth as it is a component of DNA, RNA and phospholipids that form cell membranes. It is also part of important energy carrying molecules like ATP and NADPH. Thus, the stimulation of metabolic processes and growth might increase the phosphate demand and the energy released from higher CCM efficiency with increasing pCO_2 cannot be used.

Moreover nutrient limitation in general leads to nutrient stress responses that involve morphological and physiological adaptations allowing the cell to survive periods of starvation

(Schwarz and Forchhammer 2005). The cell responds with a progressive deactivation of the photosynthetic apparatus (chlorosis) and the relative abundance of the RuBisCO is down regulated. Even though heterocystous cyanobacteria of the Baltic Sea are though to be mainly limited by phosphate a co-limitation with the trace metal iron (Fe) was observed for marine diazotrophy of the tropical Atlantic waters. (Chappell & Webb, 2010; Mills, Ridame, Davey, La Roche, & Geider, 2004). Since iron is an essential co-factor in the nitrogenase protein complex the requirement of Fe in diazotrophy is ~5-folds higher than the assimilation of NH_4^+. (Ilana Berman-Frank, Cullen, Shaked, Sherrell, & Falkowski, 2001; Kustka, San, Carpenter, & Sunda, 2003). There is no information on trace metal concentrations of the seawater sampled during cruise and used in the mesocosm experiment. Therefore possible limitations stay as a guess. Yet, iron control of primary production has been observed in the Baltic Sea (Paczuska & Kosakowska, 2003; Lj Stal, Staal, & Villbrandt, 1999; Stolte et al., 2006) even though iron concentrations are usually orders of magnitude higher in costal and estuarine waters (Breitbarth et al. 2009). For instance, the addition of iron/EDTA and DOM was shown to increase biomass of *Anabaena cf. inaequalis* and *Nodularia spumigena* cultures by 5 to 10-folds (Stolte et al. 2006). Studies on combined effects of Fe and pCO_2 on Baltic Sea cyanobacteria are still needed. However, in a culture study of the unicellular diazotroph *Crocosphaera watsonii* the positive effect of increased [CO_2] diminished under iron depletion (F.-xue Fu et al., 2008). Diazothroph cyanobacteria of the Baltic Sea tend to agglomerate in surface scums together with heterothrophic organisms. This close association of oxygen producing and consuming processes within surface scums been suggested to improve iron uptake by creating a favourable pH and oxygen depleted microenvironment (Ploug, 2008). In seawater, iron occurs in two oxidation states whereas Fe (II) is more bio available than Fe (III). Fe(II) is highly soluble but rapidly re-oxidizes to Fe(III) in oxygenated seawater (Breitbarth et al. 2009). Therefore a natural assemblage characterised by dispersed filaments like in this study could be iron limited. An increased iron limitation by the dispersal of filamentous cyanobacteria would explain the observed strong decrease in growth with rising pCO_2 by Czerny et al. (2009). The author used a rotating device that permanently rotated the incubation bottles in contrast to the other studies that manually homogenised the samples and therefore not completely avoid the formation of aggregates. High abundance of aggregates was present in the samples of Isensee et al. (2009) and might explain why the stimulating effect by pCO_2 was strongest in this study.

Certainly, in addition to nutrient limitation and other abiotic factors low fixation rates as well as decreasing biomass of the phytoplankton community during the mesocosm experiment could also result from biological factors like intra- and inter species competition, grazing pressure or viral infestation. Yet, as there is not information on those factors and they are only mentioned and won't be discussed any further.

4.5. Physiological effects of increasing pCO_2 on a natural phytoplankton community predominated by picocyanobacteria (fraction <10µm)

A significant stimulating effect of increasing pCO_2 was also not observed for the fraction <10 µm, neither for growth nor production. Yet N_2-uptake increased were 33 % higher in the northern than the southern Baltic Proper. N_2-uptake rates observed during mesocosm study also showed a very slight but insignificant decrease with increasing pCO_2 from 180 to 750 µatm. Median CO_2-fixation rates of the southern Baltic Proper (208-228 µatm, 0.37 nmolN $\mu molC_{POC}^{-1}$ d^{-1}) were 28 % higher than in the northern Baltic Proper (139 to 166 µatm, 0.28 nmolN $\mu molC_{POC}^{-1}$ d^{-1}). During the mesocosm study median CO_2-fixation rates were similar at pCO_2 level of 380, 750 and 950 µm (~0.19 nmolN $\mu molC_{POC}^{-1}$ d^{-1}) and 19 % higher than fixation rates observed at a pCO_2 level of 180 µatm (0.16 µatm). Field CO_2-fixation rates were higher compared to the mesocosm study. Yet, as was observed for fraction > 10 µm a significant physiological response was not observed for small phytoplankton species when faced with increasing pCO_2 because high variability in the data.

In both, field and mesocosm samples, the phytoplankton community of fraction <10 µm was predominated by unicellular and colonial picocyanobacteria (65 to 90 %) next to flagellates, *dinophyceae* and few *oocystes* and *cyclotheca species.* There is no information on species composition of the cyanobacterial community <10 µm. But, it is know that the group mainly composes unicellular species of the group Synechococcus (Lucas J Stal & Walsby, 2000) as well as colonial species like Aphanocapsa delicatissima, Aphanothece minutissima, Chroococcus microscopicus, Coelosphaerium minutissimum, Cyandictyon reticulatum, Cyandictyon planctonicum, Lemmermanniella pallida (Wasmund, Voss, & Lochte, 2001).

So far, there are is no research on physiological responses of non- filamentous Baltic Sea cyanobacteria in relation to pCO_2. Yet, a strong stimulation was observed for some marine, cyanobacterial *synechococcus* strains (F. Fu et al. 2007; Z. Lu, Jiao, and Zhang 2006), as well as for the marine unicellular cyanobacterium *crocosphaera watsonii* (F. Fu et al. 2008). As was suggested for fraction >10 µm the insignificant stimulation by pCO_2 might result from

varying CO_2 sensitivities of the different phytoplankton groups and cyanobacteria species (Björn Rost et al. 2003; Ulf Riebesell 2004). For instance, in a study of Lu et al. (2006) the stimulating effect of pCO_2 varied between different synechococcus strains. Whereas growth of phycocyanin (PC) rich strains increased by 37 % from present day pCO_2 levels to 800 µatm, growth of phycoerythrin (PE) rich synechococcus strains was unaffected. The pigmentation represents the adaption of different light spectra that change with depth. Those that contain high concentrations of the pigment phycoerythrin (PE) absorb green light efficiently and are referred as red strains due to their red appearance in contrast to green picocyanobacteria that contain high concentrations of phycocyanin (PC) and absorb red light efficiently, thus appear green. Green cyanobacteria dominate turbid, red light prevailing waters whereas red ones are more abundant in open ocean waters. In the Baltic Sea both synchenococcus strains coexist. Phycocyanin rich ones slightly predominate the upper 5 m and phycoerythrin rich picocyanobacteria are more abundant in deeper water depth (5-15 m) (T. H. A. Haverkamp et al., 2009; T. Haverkamp et al., 2008). The vertical distribution of the different strains matches the underwater light spectra of the Baltic Sea whereas green light penetrates more deeply than red one (T. H. A. Haverkamp et al., 2009; T. Haverkamp et al., 2008).

Another explanation for the insignificant stimulation by pCO_2 could also a modulation by temperature as an increase in growth by ~2.3-fold was observed under green house conditions (750µatm, 24°C) compared to the control (350µatm, 20°C) in a study on marine synechococcus strains (F.-X. Fu, Warner, Zhang, Feng, & Hutchins, 2007). Yet, those observations were only made on cultures. As the phytoplankton species of <10 µm are not buoyant they are entrained in the mixed layer and exposed to rapid changes in irradiance and temperature. This is also indicated by the N_2- and CO_2-fixation rates and the abundance of small cyanobacteria that did not significantly decrease with depth (Figure 3.5, Figure 3.9 and Figure 3.10) in contrast to the fraction >10 µm. Thus small cyanobacteria might tolerate higher variations in temperatures and light intensities Finkel, Irwin, & Campbell, 2007) and therefore not as strong dependent on physical factors as was observed for the community of fraction > 10 µm and other diazotroph cyanobacteria.

Growth rates of fraction <10 µm were close to zero and the abundance of non-filamentous cyanobacteria decreased during mesocosm testing. This could result from the strong phosphate as was already discussed for fraction > 10 µm. Moreover the community was limited by nitrogen as indicated by the median POC:PON ratios of about 9 as well the CO_2:N_2-fixation (70 to 800) that exceed typical Redfield ratios (C:N=6 to 7). So far, non-

filamentous cyanobacteria of the Baltic Sea have not been observed to fix N_2 and therefore depend on combined nitrogen compounds. Moreover, the community was depleted with flagellates that are known to feed on small cyanobacteria. Increased feeding pressure could also explain the observed decrease in cyanobacterial biomass and CO_2-fixation rates. Thus, a modulation of CO_2 effects by feeding pressure is likely but has not been proven so far.

In result, the high variability in the data, as well as decreasing abundance and low growth rates indicate that the stimulation of rising pCO_2 as was observed for marine non-filamentous cyanobacteria was stimulated by nutrient depletion, grazing pressure and species-specific differences in the CO_2-sensitivity.

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4.6. Conclusion and Implications for further research

In conclusion, a slight stimulating effect of increasing pCO_2 on CO_2- and N_2 –fixation as well as uptake rates was observed from this study. Yet, due to the high variability in the data none of the observations were significant. Physical parameters changed along the sampled horizontal gradient of decreasing pCO_2 from 228 to 140 µatm. Wind intensity decreased from 7 to 2 m s^{-1}, temperature increased from 16 to 19 °C, respectively, and the daily mean in irradiance varied between 146 and 753 µE m^2 s^{-1} (Table 3.2). Moreover the phytoplankton was starving for nutrients, especially phosphate, as indicated by the high Redfield ratios and decreasing abundance during the mesocosm experiment. It is well know that dominance and production of cyanobacteria is related to various environmental factors. That indicates that in this study a possible stimulating effect of increasing pCO_2 on cyanobacterial physiology was cancelled out by the physical conditions.

Stimulations using a Baltic sea ecosystem model show an increase in surface water temperature by 2 to 3.5 °C, a decrease in salinity by 1.5 to 2g Kg^{-1} as well as reduced ice-covering within the next 100 years (Thomas Neumann 2010; Thomas Neumann and Friedland 2011). As warming of surface waters strengthens the vertical stratification mean radiation increases by reducing the mixed layer and the growing season is prolonged (lengthens optimal growth period). Furthermore, global warming will increase intense precipitation that in turn boost surface and groundwater nutrient discharge into the Baltic. Increased nutrient concentration favour plankton growth and potentiate anoxic conditions in the deeper basin when their biomass settle out leading to a further release of phosphate from anoxic sediments and loss of nitrogen by sinking processes such as annamox and dinitrification (Conley et al. 2009; Mort et al. 2010). The resulting low winter N/P ratio favour cyanobacteria blooms as they are independent of particulate and dissolved nitrogen sources. Furthermore cyanobacteria grow better at higher temperatures than other phytoplankton species giving them a competitive advantage (H. W. Paerl and Huisman 2008).

All in all, cyanobacteria seem to take advantage from the hydrologic and methodological modifications that result from climate change. (H. W. Paerl and Huisman 2009; O'Neil et al. 2011). However if this is supported even further by ocean acidification cannot be approved so far. As indicated by the results of this study, there might be interactive effect between different environmental factors like competition between toxic and non-toxic strains (Kardinaal et al. 2007) or virus attack that mediate bloom development and distribution

(Honjo 2006; Proctor and Fuhrman 1990). Moreover, high algal abundance increases the pH and reduces the concentration of CO2, thus cyanobacteria might experience high CO2 conditions only under pre-bloom conditions. The contradicting results, especially on Baltic Sea diazotrophs underline the complexity of the whole ecosystem and the need of further studies in order to ascertain the individual and interactive effect of these environmental factors for both each phytoplankton group itself as well the whole natural assemblage. Moreover factors causing the dominance of one or the other group are often difficult to reveal because several interacting factors are usually involved which are not necessarily the same in different environments (Casamayor et al. 2000). In addition predicted changes will occur over years and not within a time of one day to three month commonly used to acclimate organism to changed water chemistry and other environmental conditions during experiments. As cyanobacteria are known for their variety of adaptations they might react completely different in year 2100 confirming the need of long time experiments. Furthermore a pCO_2 effect on Baltic Sea cyanobacteria, either positive or negative, was only observed in mon- and co-cultures and under stable conditions in the lab, so far. Yet, even though the cultures offspring was ones isolated from a natural assemblage, holding conditions of cultures are usually stable and might change the species physiology as well as its genetic (Casamayor, Núñez-Cardona, Calderón-Paz, Mas, & Pedrós-Alió, 2000; Collins & Bell, 2004). Therefore responses given by a culture might completely differ from those given by a natural population that grows under everyday changing environmental conditions. Moreover, in most studies, including this one, the main focus remains on effects caused by changes in the pCO_2. Yet, as enzymes are characterised by an small optima in pH opposing effects by the decrease in pH with increasing pCO_2 are possible and need to be included in further research.

So far, this is the first study that investigated pCO_2 effects on a Baltic Sea phytoplankton community that was predominated by cyanobacteria. Climate change might favour the abundance of cyanobacteria in the Baltic Sea but as indicated by the results of this study the stimulating effect of forecast increases in light and temperature in addition to rising pCO_2 diminish under nutrient deplete conditions. Hence, it is even more important to reduce the anthropogenic of inorganic nutrients.

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