GÖTEBORGS UNIVERSITET

The future for microplankton in the Baltic Sea
Effects of SWS and climate change

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Doctoral Thesis

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2017

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Abstract

The Baltic Sea is located between 53°N to 66°N and from 10°E to 30°E and is the second largest brackish water body in the world. It consists of several basins where the Baltic Proper is the major water mass. Around 85 million people live in the catchment area of the Baltic Sea, which subjects it to a range of environmental pressures, such as increased nutrient inputs from human activities (eutrophication), shipping, over-fishing, acid rain and trace metals released from anti-fouling paint. All these stressors, combined with low alkalinity, variable salinity and limited water exchange, makes the Baltic Sea a very sensitive area that may be less resilient to future stressors such as climate change or increased shipping activities. Microplankton communities consist of small heterotrophic bacteria, picoplankton, phytoplankton, cyanobacteria and smaller grazers, such as ciliates and zooplankton. In the Baltic Proper, there is a succession of blooms, within the microplankton community, from diatoms and dinoflagellates in the early spring to cyanobacteria during summer and ending with a second diatom and dinoflagellate bloom in the autumn. The cyanobacteria of the Baltic Proper bloom every summer and are dominated by *Aphanizomenon* sp. and *Nodularia spumigena*. *Dolichospermum* spp. is present but is less abundant. The effects of climate change were tested on a natural microplankton community, as well as on isolated cyanobacteria species from the Baltic Sea. To simulate effects of climate change, the temperature was increased from 12°C to 16°C, salinity decreased from 6-7 to 3-4 and atmospheric pCO$_2$-levels was increased from 380 ppm to 960 ppm. The biovolume of *Aphanizomenon* sp. and *N. spumigena* increased when temperature was increased by 4°C. When salinity was decreased by three units, both the growth and photosynthetic activity of *N. spumigena* were reduced while *Aphanizomenon* sp. was unaffected, and the growth of *Dolichospermum* sp. was increased. Furthermore, present-day salinities were beneficial, in terms of increased biovolumes, of diatoms, dinoflagellates and ciliates, compared to reduced future salinity. Increased atmospheric pCO$_2$ had no effect on any of the species in the microplankton community. These results show that the future microplankton community may be positive, in terms of increased biovolume, for the cyanobacteria species *Aphanizomenon* sp. and *Dolichospermum* spp. An increase of cyanobacteria blooms may open up to the possibility to grow and/or harvest these species as a source of biofuel or fatty acids (FA). *Dolichospermum* sp. yielded higher total FA content per biovolume, compared to the other two cyanobacteria species in phosphorus-depleted medium and *Aphanizomenon* sp. in nitrogen-depleted medium. Natural nutrient levels in the Baltic Proper are low both

in nitrogen and phosphorus, which indicates a possible future market for biofuel and FA technologies.

Additionally, the effects of seawater scrubbing (SWS) were tested on a natural summer-bloom microplankton community. Three different concentrations of scrubber water were added; 1%, 3% and 10%. To elucidate effects of decreased pH alone, water acidified with H_2SO_4 was added in equal concentrations. The six treatments were compared to a control without acidifying substances. SWS or the corresponding pH treatments, did not have a direct effect on microplankton species composition and biovolume. However, the increased amount of Cu and Zn in the scrubber water, combined with significant decrease in pH and alkalinity already at the 1% scrubber water treatment calls for precaution when implementing scrubber units on the shipping fleet of the Baltic Sea. The accumulated effects of long-term repeated addition constantly throughout the year, i.e. in a shipping lane, are yet to be elucidated.

Populärvetenskaplig sammanfattning

1. Bakgrund
1.1 Östersjön

Östersjön är uppdelad i flera olika bassänger, med bl.a. Bottenviken längst i norr, Finska bukten i öster och Egentliga Östersjön, som är den största vattenmassan. I sydväst är Östersjön förenad med Kattegatt genom Danska sundet. Östersjön är världens näst största brackvattenområde, bara Svarta havet är större. Att vattnet är bräckt betyder att det är ett mellanting mellan havets naturliga salthalt på ca 32-33 och sötvatten. Man kan se hela Östersjön som ett jättestort estuarium, där floder och åar kontinuerligt rinner ut med sitt söta vatten. Saltvatten, som är tyngre än sötvatten, rinner längs med botten in i Östersjön från Kattegatt. Detta medför att i hela Östersjön är det en skillnad i salthalten med vattendjupet samt även mellan de olika bassängerna. Längst i norr och öster, dit saltvattnet har svårt att ta sej, är saltvattennivån lägst, bara 2-3. I Egentliga Östersjön är saltnivån 6-7.

Förutom varierande salthalt har Östersjön varierande alkalinitet. Alkalinitet är havets buffringsförmåga. Havsvatten har en alkalinitet på över 2055 μmol kg^{-1}, i Östersjön är alkaliniteten betydligt lägre med 1551 i Egentliga Östersjön och 774 μmol kg^{-1} mot Bottenviken. Vattenmassor med lägre alkalinitet har lägre förmåga att stå emot pH-förändringar. Därför är Östersjön mer känsligt mot försurning än andra vattenmassor, som t.ex. öppet hav.

Ca 85 miljoner människor bor i Östersjöns avrinningsområde. Detta medför att Östersjön utsätts för en mängd stressfaktorer som t.ex. övergödning, överfiske, surt regn och läckande gifter från båtbottenfärger. Alla dessa faktorer, tillsammans med att Östersjön har låg alkalinitet och varierande salthalt, gör att Östersjön har mindre motståndskraft mot eventuella framtida förändringar som klimatförändringar eller ökad fartygstrafik.

Mikroplanktonsamhällen består av växtplankton, cyanobakterier, heterotrofa bakterier och mindre betare som ciliater och djurplankton. Växtplankton och cyanobakterier är s.k. primärproducenter, dvs. att de precis som landväxter använder solljus för att leva och fotosyntetisera. De är därmed basen i näringskedjan och bidrar med energi och föda för betare. Deras tillväxt bestäms av, och begränsas av, tillgången till näring, ljus, temperaturnivå och salthalt.
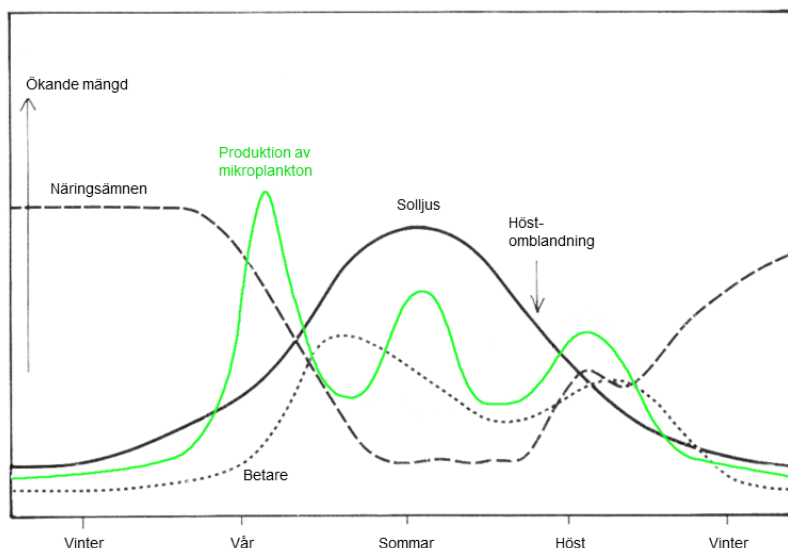
Figur 1. Succession av mängden mikroplankton under ett år i Östersjön jämfört med näring, solljus och betare. Bild bearbetad från Rosenberg 1982, Hällfors & Niemi 1986 och Sundbäck K 2017.

I Östersjön är det en succession i mikroplanktonsamhället under året (Fig. 1). På våren när ljuset är tillräckligt startar vårblomningen med kiselalger och dinoflagellater. De förbrukar nästan all näring i vattnet. På sommaren kan därför de kvävefixerande cyanobakterierna ta över, eftersom de kan leva i låga fosfatnivåer och kan använda (fixera) atmosfärens kväve som löser sig i vattnet. På hösten blir det ofta en omblandning av vattnet till exempel genom stormar och då kan näringsnivån stiga och en ökning av kiselalger och dinoflagellater sker igen.

Cyanobakterierna i Östersjön tillväxer varje sommar, men om förhållandena på sommaren är särskilt gynnsamma, dvs. om temperaturen är hög och det blåser lite, tillväxer de kvävefixerande cyanobakterierna extra mycket och bildar sommarblomningen. Eftersom vissa av cyanobakteriearterna är giftiga, samt att de gärna lägger sig på ytan i stora sjok som sedan flyter in mot land, är dessa sommarblomningar otrevliga för semesterfirare runt hela Östersjön. Giftet är normalt inte farligt för människor, men hundar och boskap som dricker vattnet kan bli sjuka.

Sommarblomningen av cyanobakterier är ett naturligt förekommande fenomen som har funnits ända sedan Östersjön bildades för ca 8000 år sedan, men det finns studier som visar att under de senaste 20 åren har blomningarna blivit värre; dels att de täcker större områden samt att mängden cyanobakterier varje sommar har ökat. Sommarblomningen domineras av arterna *Aphanizomenon* sp. och *Nodularia spumigena*. En tredje art är närvarande, men inte lika dominerande; *Dolichospermum* spp. *Aphanizomenon* sp. föredrar lite kallare vatten så den tillväxer först i blomningen, senare under sommaren tar *N. spumigena* över.

1.2 Klimatförändringar

Klimatförändringar definieras som långtidsförändringar i det globala medelklimatet. Medelklimatet bestäms av den inkommande energin från solen, av jordens egenskaper och atmosfärens reflektion och absorption samt strålningen av energi mellan jordytan och atmosfären. Exempelvis, växthusgaser i atmosfären ökar absorptionen av den utgående strålningen från jorden, vilket resulterar i ett varmare klimat. En växthusgas är koldioxid, CO_2. Mängden CO_2 i atmosfären har ökat de senaste 250 åren, p.g.a. industrialiseringen, från ca 280 ppm till lite över 400 ppm år 2017. FN:s klimatpanel IPCC (International Panel on Climate Change) har med hjälp av olika modeller tagit fram olika scenarier för framtida klimat, beräknat på ekonomisk tillväxt, populationsstorlek och teknologiutveckling. Ett av scenarierna är A1FI, som har den högsta ökningen av CO_2 i atmosfären och av medeltemperaturen. Vid år 2100 skulle då CO_2 halten ökat till 960 ppm och medeltemperaturen med 4,8°C. Regionalt för Östersjöområdet tror man även att nederbörden kommer öka, vilket kommer att öka avrinningen av färskvatten till Östersjön och därmed minska salthalten med ca 3 enheter.

Ökar CO_2 i atmosfären, kan detta leda till havsförsurning. När CO_2 löser sig i vattnet kan det reagera med vatten och bilda kolsyra H_2CO_3. Detta är en svag syra som snabbt löser upp sig i bikarbonatjoner H_2CO_3 och vätejoner H^+. Det sker fler reaktioner, men sammanfattningsvis är det koncentrationen av H^+ i vattnet som definierar pH, där ökande mängd H^+ ger ett lägre pH.

Det finns andra mekanismer som kan sänka pH. En av dem är SWS (seawater scrubbing), eller skrubbning. SWS är en metod för att minska utsläppen av fartygsavgaser till luften, som bl.a. bidrar till surt regn och utsläpp av sotpartiklar. Vid SWS leds avgaserna genom en trumma, där en dimridå av vattendroppar löser svavel- och kväveoxider i avgaserna. Vattendropparna kondenserar inuti trumman och skrubbervattnet får ett pH-värde under 3. Avgaserna leds nu som vanligt ut genom skorstenen, skrubbade från sotpartiklar och svavel- och kväveoxider som orsakar surt regn. Skrubbervattnet släpps ut i vattnet medan fartyget är på öppet vatten. En fördel med att använda SWS är att man då har tillåtelse att använda bränsle med högre svavelhalt. Sedan 1 januari 2015 är Östersjön ett SECA-område (Sulphur Emission Control Area) där högsta svavelhalten i fartygsbränsle sänktes från 1 % till 0,1 %. Har man däremot en skrubber installerad kan man fortsätta använda det billigare högsvavelbränslet.

2. Min avhandling

I denna avhandling har vi studerat hur mikroplanktonsamhället i Egentliga Östersjön påverkas av framtida klimatförändringar, i form av ökad CO_2-halt i atmosfären, ökad temperatur och minskad salthalt. Dessutom har vi studerat effekterna av skrubbervatten på mikroplanktonsamhället, samt ifall cyanobakterier kan nyttjas inom industrin, som biobränsle eller som fettsyraproducenter.

I avhandlingen presenteras experiment både från laboratoriet och ute i fält. Laboratorieexperimenten är utförda på de arter av cyanobakterier som bildar sommarblomningen. Fältexperimenten är utförda på Askölaboratoriet i Trosa skärgård mitt under sommarblomningar. Då har vi använt hela det naturliga mikroplanktonsamhället och sedan utsatt det för olika behandlingar.

2.1 Resultat

Om temperaturen i Egentliga Östersjön ökar med 4°C ökar tillväxten av cyanobakterierna *Aphanizomenon* sp. och *Nodularia spumigena* (**artikel I**). Detta skulle kunna innebära att blomningarna startar tidigare under säsongen, eftersom den optimala vattentemperaturen för cyanobakterierna skulle nås tidigare på försommaren. Om salthalten skulle minska med 3 enheter skulle *Aphanizomenon* sp. inte påverkas alls, medan tillväxten av *N. spumigena* skulle minska (**artikel I**). Varken *Aphanizomenon* sp. eller *N. spumigena* påverkades av ökad CO_2-halt.

När effekterna av minskad salthalt i kombination med ökad CO_2-halt testades på ett naturligt mikroplanktonsamhälle (**artikel II**) fick vi liknande resultat: *N. spumigena* gillar inte minskad salthalt, *Aphanizomenon* sp. påverkades inte alls och *Dolichospermum* spp. gillade kombinationen minskad salthalt och ökad CO_2-halt. Eftersom dessa tre arter alla är trådformiga kvävefixerande arter i Östersjön, samt att många arter generellt gynnas av ökande temperatur, kan man tänka sig att även *Dolichospermum* spp. skulle påverkas i form av ökad tillväxt vid +4°C. I ett framtida mikroplanktonsamhälle i Östersjön, där temperaturen ökat med 4°C, salthalten minskat med 3 enheter och atmosfärens CO_2-halt ökat till 960 ppm, är det möjligt att *Aphanizomenon* sp. börjar blomningen tidigare på säsongen och att den sträcker sig till områden med lägre salthalt. Sedan tar *Dolichospermum* spp. över dominansen, medan *N. spumigena* finns i mindre mängd. Om det skulle bli kraftigare blomningar av cyanobakterier finns det en möjlighet att skörda dem från vattnet och använda som biobränsle eller utvinna deras fettsyror till den industriella marknaden.

Dolichospermum spp. har högst mängd fettsyra per biovolym (**artikel IV**) medan *N. spumigena* är lättast att odla i laboratoriet och ger mest biomassa på kortast tid.

När skrubbervatten tillsattes i tre olika koncentrationer (1 %, 3 % och 10 %) till ett naturligt mikroplanktonsamhälle och jämfördes med behandlingar där försurat vatten tillförts i samma koncentrationer samt en kontrollbehandling där inget skrubber- eller försurat vatten tillsatts. Vi fann ingen effekt i tillväxten av mikroplankton mellan någon av behandlingarna (**artikel III**). Däremot såg vi tydligt att redan vid de lägsta koncentrationerna, 1 %, sänktes pH och alkalinitet, samt att farliga metaller fanns i vattnet. Detta betyder att även om vi inte såg några effekter i vårt 14-dagars experiment, är det sannolikt att effekterna hade kommit senare. Dessutom studerades en engångstillförsel av skrubbervatten. I verkligheten är det flera fartyg med skrubbers installerade som kommer att trafikera Östersjöns fartygsleder dygnet runt, året runt. Denna ständiga tillförsel kan vara negativ för det mikroplanktoniska ekosystemet.

2.2 Framtida studier

I våra experiment har vi studerat hur mikroplanktonsamhället i Egentliga Östersjön påverkas av klimatförändringar och SWS. Fartygstrafiken förväntas fördubblas de närmsta trettio åren, och införandet av skrubbers ombord på fartyg har redan börjat. Däremot saknas det studier på hur ekosystemet påverkas. I denna avhandling presenteras en av de få experimentella studier som har utförts. Flera, och längre, studier på framförallt upprepade utsläpp av skrubbervatten behövs verkligen!

Dessutom har vi inte studerat hur det ser ut på högre nivåer i näringskedjan. Det skulle t.ex. vara intressant att studera hur fettsyranivåerna i mikroplankton förändras med framtida klimat eller av SWS-påverkan. Fettsyranivåerna och deras sammansättning är viktiga för födokvalitén för betare högre upp i näringskedjan. Om betarna påverkas, påverkas även småfisk, som sill, och större fisk som torsk.

Det finns verkligen utrymme för fler studier inom dessa områden!

The truth is out there

X-files

List of papers

This thesis is based on the following papers, referred to in the text by their roman numerals.

PAPER I: <u>Karlberg M</u>, Wulff A (2013) Impact of temperature and species interaction on filamentous cyanobacteria may be more important than salinity and increased pCO$_2$ levels. Marine Biology, 160(8): 2063-2072

PAPER II: Wulff A, <u>Karlberg M</u>, Olofsson M, Torstensson A, Riemann L, Steinhoff FS, Mohlin M, Ekstrand N, Chierici M (submitted to Marine Biology, 2017) Ocean acidification and desalination – Climate-driven change in a Baltic Sea summer microplanktonic community

PAPER III: <u>Karlberg M</u>, Hassellöv I-M, Hedblom M, Nylund A, Tripp L, Turner D, Yong J, Ytreberg E, Wulff A (2017) Effects of seawater scrubbing on a microplanktonic community during a summer-bloom in the Baltic Sea. (Manuscript)

PAPER IV: Steinhoff FS*, <u>Karlberg M</u>*, Graeve M, Wulff A (2014) Cyanobacteria in Scandinavian coastal waters - A potential source for biofuels and fatty acids? Algal Research, 5: 42-51
* Both authors contributed equally.

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1. Introduction

1.1 The Baltic Sea

The Baltic Sea is located between 53°N to 66°N and from 10°E to 30°E and consists of several basins (Fig. 1); e.g. the Bothnian Bay in the north, the Gulf of Finland in the east, and the Baltic Proper that is the major water mass. The Baltic Sea is connected to the Kattegat by the Danish straits in south west. The Baltic Sea is the second largest brackish water body in the world (after the Black Sea), due to riverine inflow of fresh water in the Baltic Sea catchment area and limited water exchange through the narrow and shallow Danish straits. The less saline brackish water from the Baltic Sea flows on the surface through the Danish straits, while saline deep-water from the North Sea, via the Kattegat, periodically flows into the Baltic (Stigebrandt 2001). The Baltic Sea is functionally a large estuary, divided into sub-basins, each with its own characteristic properties.
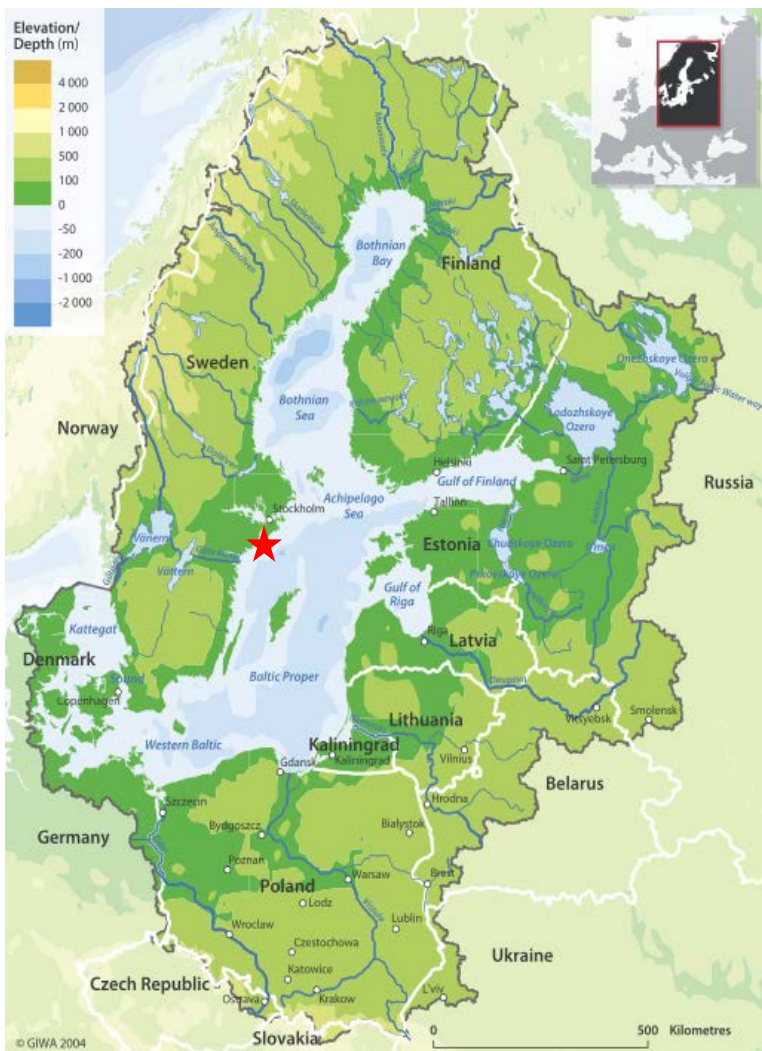
Figure 1. The Baltic Sea, with its basins and catchment area. The red star indicates sampling station B1 just outside Askö Laboratory. Picture © GIWA 2004, in HELCOM 2006.

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This inflow of saline water is sporadic and major inflows, which oxygenate the bottom of the Baltic Sea and mix the water masses, occur only rarely. Therefore, there is a constant halocline in the Baltic Sea. Furthermore, there is a salinity gradient above the halocline in the Baltic Sea. Since saline water flows north-east at the bottom, there is a counter current from river runoffs of fresh water south-west throughout the Baltic Sea. This results in the Bothnian Bay and Gulf of Finland having the lowest salinities of 2-3, around 7 in the Baltic Proper to around 25 in the Kattegat (Rodhe 1998).

The salinity gradient is also reflected in surface alkalinity (A_T), since A_T is closely related to salinity. The Kattegat has an alkalinity of 2055 μmol kg^{-1}, decreasing to 1551 in the Baltic Proper and is 774 μmol kg^{-1} towards the Bothnian Bay (summarized in Hjalmarsson et al. 2008). Seawater's ability to withstand pH change is governed by the A_T of the water. Therefore the brackish Baltic Sea is potentially sensitive to acidification.

Around 85 million people live in the catchment area of the Baltic Sea, which subjects it to a range of environmental pressures, such as increased nutrient inputs from human activities (eutrophication), shipping, over-fishing, acid rain and trace metals released from anti-fouling paint. All these stressors, combined with low alkalinity, variable salinity and limited water exchange, make the Baltic Sea a very sensitive area that may be less resilient to future stressors such as climate change or increased shipping activities.

1.2 Microplankton

Microplankton communities consist of small heterotrophic bacteria, picoplankton, phytoplankton, cyanobacteria and smaller grazers, such as ciliates and zooplankton. Phytoplankton are the primary producers and form the basis of the food chain providing energy for smaller grazers. Phytoplankton, together with picoplankton and cyanobacteria, are limited in their growth by light, temperature, salinity and nutrients.

In the Baltic Proper, there is a succession of blooms, i.e. seasonal high abundance of cells, within the microplankton community, from diatoms and dinoflagellates in the early spring to cyanobacteria during summer and ending with a second diatom and dinoflagellate bloom in the autumn. During winter, low light conditions prevent extensive phytoplankton production resulting in less zooplankton but also nutrient-rich water (Fig. 2). When light conditions are more favourable and nutrients have
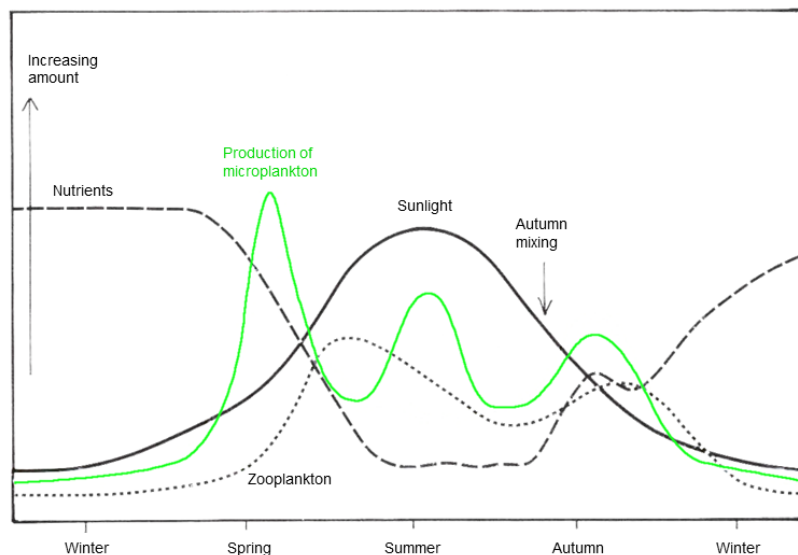
Figure 2. Succession of the amount of phytoplankton in the Baltic Sea compared to nutrients, sunlight and grazing zooplankton. Picture adapted from Rosenberg 1982 and Hällfors & Niemi 1986.

been mixed up from deeper water during winter storms, the phytoplankton spring-bloom starts. During the spring-bloom, diatoms consume nearly all inorganic nitrogen but leave a small amount of phosphate. This situation gives the nitrogen fixing cyanobacteria a competitive advantage over other phytoplankton groups, resulting in massive blooms from June to August. Autumn mixing increases sea surface nutrient concentrations and together with sufficient light, the autumn phytoplankton bloom starts, dominated by diatoms and dinoflagellates with a corresponding later peak in zooplankton. Studies based on time series data from 1979 to 2008 have shown that the total phytoplankton biomass has increased in the Baltic Proper, concurrently temperature has increased and salinity decreased (Suikkanen et al. 2013).

The cyanobacteria of the Baltic Proper bloom every summer and some of the cyanobacteria species causing the blooms are toxic. Therefore, the extensive blooms affect not only recreational activities, but are also lethal for domestic animals that drink the water (Edler et al. 1985, Nehring 1993) as well as animals of higher trophic levels in the Baltic food chain (Kankaanpaa et al. 2002, Sipiä et al. 2002, Karjalainen et al. 2006). The cyanobacterial blooms are a natural phenomenon (Bianchi et al. 2000) but for the last years the blooms have become more frequent and cover larger areas (Kahru et al. 1994, Finni et al. 2001) and starts earlier in the summer, however, with large decadal-scale variations (Kahru & Elmgren 2014).

In the Baltic Proper the summer-blooms are dominated by the cyanobacteria *Aphanizomenon* sp. (Morren ex Bornet & Flahault) and *Nodularia spumigena* (Mertens ex Bornet & Flahault) (Janson & Hayes 2006). *Dolichospermum* spp. (Ralfs

ex Bornet & Flahault, previous *Anabaena* Bory ex Bornet & Flahault (Wacklin et al. 2009)) is also present but is less abundant (Sellner 1997, Stal et al. 2003). *Aphanizomenon* sp. prefers lower temperatures than *N. spumigena* and therefore it is the first cyanobacteria that occurs in early summer in large quantities. Later during the summer *N. spumigena* takes over and can create massive blooms during calm weather (Sellner 1997, Kononen et al. 1998, Jonasson 2006) possibly related to its ability to cope with high intensities of ultraviolet radiation (Mohlin & Wulff 2009). *N. spumigena* produces a toxin, nodularin, which in humans, damages the liver and can be tumour inducing in low doses (Runnegar et al. 1988, Ohta et al. 1994, Humpage & Falconer 1999, Song et al. 1999). Wild and domestic animals have been killed by nodularin (Edler et al. 1985, Nehring 1993) and the toxin might be harmful to fish as well (Kankaanpaa et al. 2002). Nodularin can also be accumulated in the food web (Kankaanpaa et al. 2002, Sipiä et al. 2002, 2008, Karjalainen et al. 2008, Persson et al. 2009). The concentration of nodularin in *N. spumigena* varies under different environmental conditions, such as temperature, salinity, radiation and nutrient concentrations (Lehtimäki et al. 1994, Granéli et al. 1998, Hobson et al. 1999, Repka et al. 2001, Mazur-Marzec et al. 2005, Pattanaik et al. 2010).

1.3 Climate change

1.3.1. Background

Climate change can be defined as the long-term changes in the global mean climate. The mean climate is determined by the incoming energy from the Sun, by the properties of the Earth and the atmosphere´s reflection, absorption and emission of energy within the atmosphere and at the surface. For example, greenhouse gases in the atmosphere increase the absorption of outgoing radiation, resulting in warmer climate on Earth. One such greenhouse gas is CO_2. It occurs naturally in the atmosphere, but due to human activities, i.e. industrialisation, CO_2 has increased in the last 250 years, from about 280 ppm to 380 ppm in 2005 (Solomon et al. 2007) and presently (2017) just over 400 ppm (https://www.co2.earth/). CO_2 emissions from human activities are considered the single largest anthropogenic factor contributing to climate change. 29% of the atmospheric CO_2 is taken up by terrestrial biosphere by photosynthesis, 26% dissolves in the ocean and the rest remains in the atmosphere (Le Quéré et al. 2009). These CO_2 sinks can, however, not compensate for the increase of CO_2 releases from e.g. burning of fossil fuel.
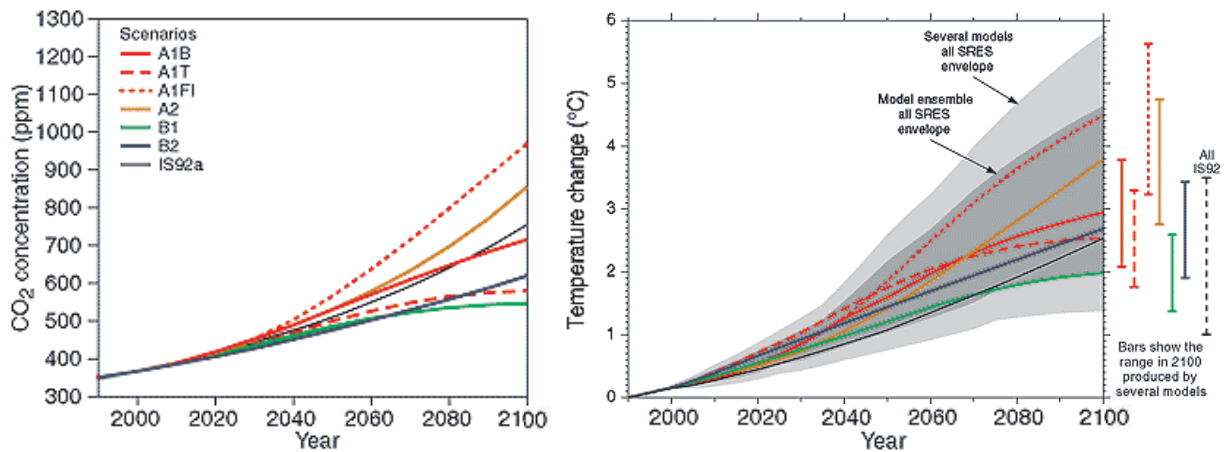
Figure 3. The global climate of the 21st century based on several models and for different scenarios. In **paper I** and **paper II** the A1FI scenario was chosen. Picture adapted from IPCC 2001.

There are several emission scenarios projected for 2100 with differing economic growth, population and technological development. The scenario chosen in **paper I** and **paper II** is the A1FI scenario, with rapid economic growth, a global population that peaks mid-century and with an emphasis on fossil intensive technologies (IPCC 2001). In the aspect of CO_2 emissions and atmospheric CO_2 concentration, the A1FI scenario can be considered the worst case scenario (Fig. 3). By the year 2100 atmospheric CO_2 concentration would then reach 960 ppm and global average temperature would increase by 4.8°C.

1.3.2. Ocean acidification

The definition of ocean acidification (OA) is the reduction of pH over an extended time-period, primarily caused by the uptake of atmospheric CO_2 (Fig. 4), but it can be caused by other chemical additions to the ocean. When atmospheric CO_2 dissolves in the ocean it can remain in the form as dissolved gas and referred to as $CO_2(aq)$, and can be used by plants and phytoplankton in their photosynthesis. Some part of $CO_2(aq)$ reacts with water creating carbonic acid, H_2CO_3. This is a weak acid that quickly dissociates in water forming bicarbonate ions, HCO_3^-, and hydrogen ions, H^+. Some of the H^+ remains in the ocean lowering pH, while others combine with carbonate ions CO_3^{2-}, forming more bicarbonate ions. Seawater pH can be expressed on different scales. Throughout this thesis the total scale, $pH_T = -\log_{10}\{[H^+] + [HSO_4^-]\}$, has been used as recommended by Dickson (2010).

Increased atmospheric CO_2 levels would therefore lead to changes in the carbonate chemistry system of seawater, with increased $CO_2(aq)$, HCO_3^- and H^+ while CO_3^{2-}

would decrease. $CO_2(aq)$, HCO_3^- and CO_3^{2-} are the three inorganic forms that constitutes the dissolved inorganic (DIC) pool in seawater. A typical seawater of pH 8.2 has 89% in the form HCO_3^-, 0.5% CO_2 and 10.5% CO_3^{2-} (Gattuso & Hansson 2011). CO_2 is the ultimate source of DIC for photosynthetic organisms. However, since CO_2 is limited many organisms have carbon concentration mechanisms (CCMs). CCMs use both HCO_3^- and CO_2 to concentrate inorganic carbon intracellularly, which later can be used in photosynthesis. Since both CO_2 and HCO_3^- will increase with increased atmospheric CO_2 levels, ocean acidification may stimulate photosynthesis, but with differing results within and between groups (Riebesell & Tortell 2011, and references within). CO_3^{2-} is used by many calcifying organisms in shells and skeleton as $CaCO_3$, such as molluscs, crustaceans, echinoderms, corals, foraminifera and some phytoplankton. OA will both reduce the amount of CO_3^{2-} in the seawater making it harder to produce $CaCO_3$-structures, while increased H^+ may dissolve $CaCO_3$-structures, resulting in deformed shells and skeletons. Among phytoplankton the Coccolithophores are the main calcifying organisms. These are, however, more or less absent in the Baltic Sea (Hällfors 2004, Thomsen 2016).

1.3.3. Projections for the Baltic Sea

Global climate change is a potential threat to all ecosystems (Fischlin et al. 2007). Changes that occur globally will affect the Baltic Sea as well. But due to its geographical location, large seasonal contrasts and enclosure for example, there are some differences in Baltic Sea projections compared to global ones. For example, global warming was about 0.05°C per decade from 1861 to 2000, while the trend for the Baltic Sea was 0.08°C per decade (HELCOM 2007). Future mean annual temperatures, based on regional modelling studies for the Baltic Sea, projects an increase of 3°C to 5°C by the year 2100.

For the Baltic Proper, the major part of the Baltic Sea, annual precipitation, surface water temperature (HELCOM 2007) and atmospheric partial pressure of CO_2 (pCO_2) levels (Meehl et al. 2007) are projected to increase. There are some uncertainties whether precipitation will increase or decrease in the Baltic Sea catchment area, and precipitation changes will likely affect different parts of the Baltic Sea differently. However, present projections suggest an increase in the entire catchment area during winter, while during summer only the northern part will have increased precipitation (Fig. 5) (HELCOM 2013). Salinity may decrease from current values of 6-7 to 3-4,
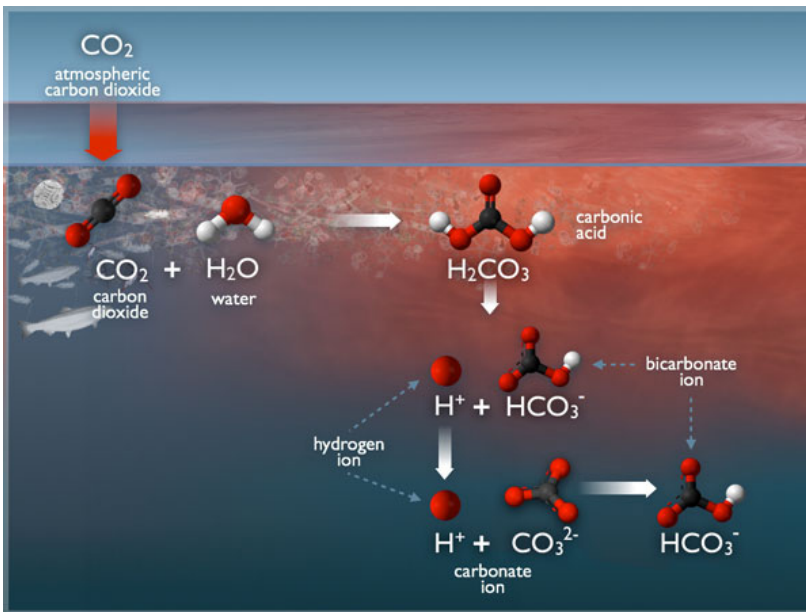
Figure 4. Ocean carbonate chemistry system. Picture from WHOI 2014.
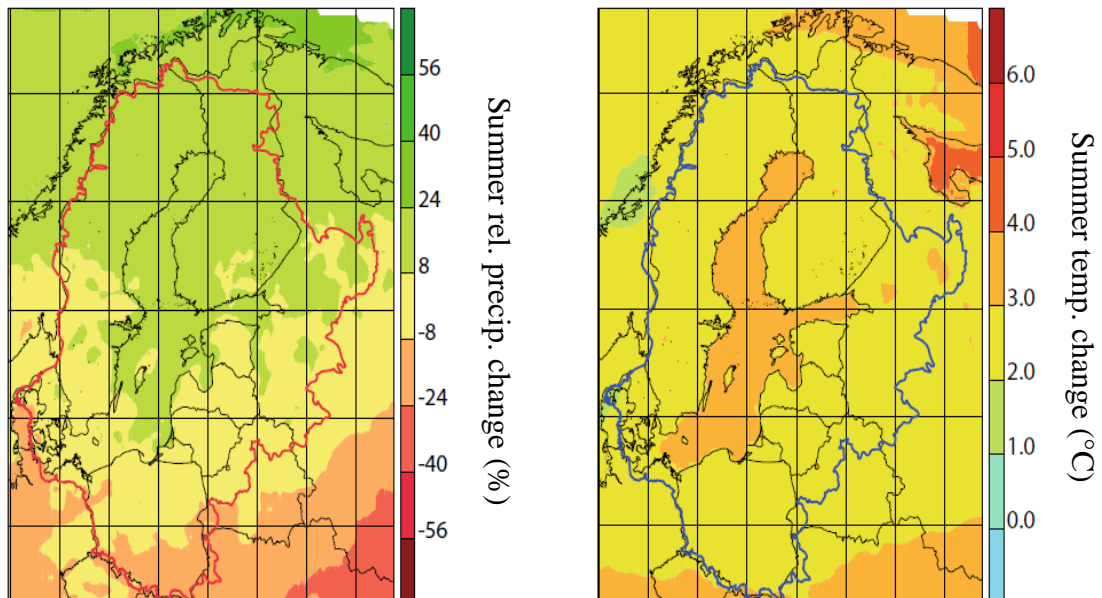
Figure 5. Relative change in percent of average summer precipitation (left), and summer surface air temperature change from 1961-1990 to 2071-2099 (right) as simulated by 13 RCM models. Picture adapted from HELCOM 2013.

and $p\mathrm{CO_2}$ levels may increase from 380 ppm to 960 ppm, by year 2100 (HELCOM 2007).

Increasing surface water temperatures can increase stratification, leading to nutrient depletion and hence a competitive advantage for diazotrophic cyanobacteria in the euphotic zone (e.g. Paerl & Paul 2011, Wells et al. 2015). Moreover, a stagnant water column will expose the species in the euphotic zone to high intensities of ambient

radiation. *N. spumigena* continues to grow under both nutrient deficiency and high ambient radiation intensities including ultraviolet radiation (Mohlin & Wulff 2009, Pattanaik et al. 2010, Mohlin et al. 2012). Furthermore, the stabilization of the water column in association with increasing temperatures may cause the spring-bloom to begin earlier (Hagström & Larsson 1984) and potentially also the cyanobacteria bloom (Kahru & Elmgren 2014). Higher temperatures and stratified water may favour dinoflagellates (Klais et al. 2013) and cyanobacteria over diatoms, thereby affecting the composition of the phytoplankton community (Andersson et al. 1996, Wrona et al. 2006). The increase in water temperature may also increase bacterial activity, change nutrient and carbon recycling in surface waters, and thereby influence phytoplankton species composition and primary production. Changes in the timing and magnitude of phytoplankton blooms and in the species composition will likely affect even higher trophic levels such as fish.

An increase in atmospheric $p\mathrm{CO_2}$ (and a corresponding increase in seawater $p\mathrm{CO_2}$) may increase photosynthesis in phytoplankton (Raven et al. 2005, Hutchins et al. 2007), however, the response differs between groups of microalgae and between cyanobacteria species. For example, $\mathrm{N_2}$ and $\mathrm{CO_2}$ fixation rates of the diazotrophic filamentous cyanobacteria *Trichodesmium* sp. increased with elevated $p\mathrm{CO_2}$ (Hutchins et al. 2007), while the same $p\mathrm{CO_2}$ level resulted in an increase in growth of the picocyanobacteria *Synechococcus* sp. but not *Prochlorococcus* sp. (Fu et al. 2007).

Diazotrophic bloom-forming cyanobacteria in the Baltic Sea are cosmopolitan species existing in fresh and brackish waters. A salinity decrease may therefore not have a negative effect on these cyanobacteria. Laboratory studies on *N. spumigena* and *Aphanizomenon* sp. (Lehtimäki et al. 1997, Pliński & Jóźwiak 1999) have shown highest growth rates of *N. spumigena* in salinities ranging from 5 to 20, but this species can tolerate salinities up to 30. Highest growth rates of *Aphanizomenon* sp. have been observed at salinities between 0 and 10. However, the production rate of the hepatotoxin nodularin was highest in salinities of 5–15. Consequently, a decrease in salinity may increase toxin levels (Blackburn et al. 1996).

1.4 Seawater scrubbing

1.4.1 Background

As previously described, the Baltic Sea is not only exposed to anthropogenic stressors such as increased nutrient inputs from human activities, i.e. eutrophication, over-fishing, acid rain, trace metals released from anti-fouling paint, climate change in the form of increased atmospheric CO_2, reduced salinity and increased temperatures, but also shipping.

The Baltic Sea region is one of the world's most intensively travelled areas, e.g. more than sixty thousand ships pass from Skagerrak to Kattegat annually and the ship traffic is forecasted to be doubled in the coming thirty years (Kadin 2008). Shipping is one of the largest individual polluting industries with respect to emissions of harmful substances; primarily sulphur oxides (SO_X), nitrogen oxides (NO_X) (Corbett & Fischbeck 1997, Hassellöv et al. 2013) but also fine particulate matter (Jalkanen et al. 2014) and PAHs (McElroy et al. 1989). Both SO_X and NO_X have an acidifying effect in seawater, and in addition NO_X results in eutrophication (Doney et al. 2007). Both substances are produced in the fuel combustion process and are released to the atmosphere causing acid rain.

One way to reduce the negative effects of acid rain is to decrease the sulphur content in shipping fuel. Starting from January 1st 2015, the Baltic Sea is part of a SECA-area (Sulphur Emission Control Area) resulting in highest allowed sulphur content in ship fuel was reduced from 1.00% to 0.10% (European Union, 2012). This shift in fuel-type may result in higher costs for shipping companies and therefore an exhaust abatement method called seawater scrubbing (SWS) may be attractive. With SWS ships can continue using cheaper fuel with higher sulphur content, as the scrubber cleans the fumes from SO_X, a small proportion of NO_X and some particles. In the scrubbing process the water inside the scrubber, in which SO_X and NO_X are dissolved, becomes highly acidic. When the scrubber operates in closed-loop mode, this water is collected and processed when the ship reach the harbour, while in open-loop mode the scrubber water is mixed with large volumes of seawater before it is discharged to the sea, causing a decrease in pH. How large decrease in pH this will cause is dependent on the temperature, salinity and alkalinity of the water (Karle & Turner 2007).

1.4.2 Production of scrubber water

In **paper III** the effects of SWS was tested on a natural summer-bloom microplankton community. Ambient seawater, collected at Askö Laboratory was used to produce scrubber water. The scrubbing process was conducted at an engine laboratory at the department of Shipping and Marine Technology, Chalmers University of Technology. The lab was equipped with a four cylinder 100 kW engine from Volvo Penta and a scrubber unit made of stainless steel holding a length of 50 cm and a diameter of 40 cm (Fig. 6). A marine gas oil (MGO) with 1.0 % sulphur was used in the experiment. The exhausts were fed from the engine through an isokinetic heated probe kept at 250°C to the scrubber unit using a vacuum pump. A small portion of the gas flow was sampled before and after the scrubber unit and analysed continuously with respect to SO_2, NO_X, CO, CO_2, and O_2. The engine exhaust flow rate was monitored using a mass flow meter and was kept at 37 l min^{-1} throughout the experiment. Ambient seawater was pumped to the scrubber unit through 7 different nozzles to create a mist. The very fine droplet size creates a substantial surface area extension, allowing for effective gas-water exchange. Similarly, a series of perforated stainless steel plates creates a surface area extension to enable efficient condensation of the wash water. Typical pH for the scrubber discharge water is less than pH 3 and thereby extremely corrosive. Hence the scrubber discharge water is usually diluted with seawater onboard to protect the construction material. The pH of the discharged scrubber water used in **paper III** was 2.8.
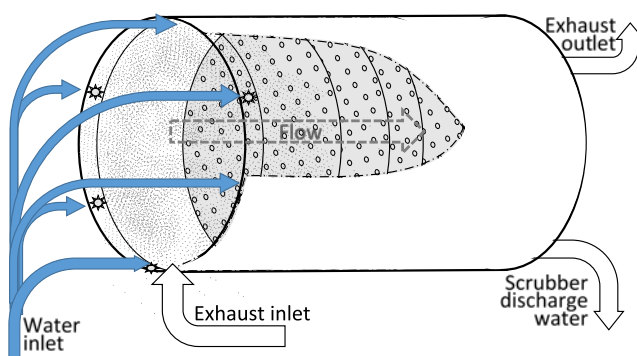
Figure 6. Schematic drawing of Chalmers´ laboratory sized scrubber (left), and the actual scrubber unit (right). Water is pumped into the scrubber through six nozzles creating a mist curtain, which the exhaust gases meet when led into the scrubber. A series of perforated stainless steel plates enables efficient condensation of the wash water. Cleaned exhausts are released and scrubber water is collected or diluted. Illustration by I-M Hassellöv and photo by E Ytreberg.

2. Aims

Phytoplankton, cyanobacteria, flagellates and smaller grazers, are all part of the summer-bloom microplankton ecosystem in the Baltic Sea. They are the basis of the food web and therefore it is important to study possible treatment effects on these organisms. There might be interactive effects between species or taxa of microplankton, and also since those effects will have a cascading effect on all other organisms higher up in the food web. The future microplankton ecosystem of the Baltic Sea will be exposed to a combination of multiple factors of climate change, and also future scrubbing activities. The possible impacts of these stressors are presented in this thesis. The specific aims of each paper were:

Paper I: to determine the projected future impacts of climate change by scenario A1FI on the two cyanobacteria species dominating the summer-bloom in the Baltic Sea, by (1) reduced salinity and increased temperature, and (2) increased temperature and increased atmospheric CO_2 levels. Both species, *Aphanizomenon* sp. and *Nodularia spumigena*, were cultivated as single species and together with the other species to elucidate any inter-specific competition.

Paper II: to determine possible future effects of climate change on a natural summer-bloom microplankton ecosystem, dominated by three species of cyanobacteria, by (1) testing the projected scenario A1FI for the Baltic Proper with reduced salinity and increased atmospheric CO_2 levels, compared to present-day levels.

Paper III: to determine possible future effects of seawater scrubbing on a natural summer-bloom microplankton ecosystem of the Baltic Proper, by (1) adding scrubber water in three concentrations, and (2) exclude effects created by reduced pH alone.

Paper IV: to investigate the application potential of the three bloom-forming cyanobacteria of the Baltic Sea; *Aphanizomenon* sp., *N. spumigena* and *Dolichospermum* sp., for biofuel production and by-products, by (1) screening whether their fatty acid (FA) content is suitable for a potential biofuel production, (2) whether nutrient enrichment can change and enrich total FA content and/or FA composition, and (3) whether these cyanobacteria contain promising marine products, such as lipopeptidic compounds, of importance for future industrial use.

3. Methods

The studies included in this thesis range from laboratory studies with isolated single species of cyanobacteria (**paper IV**), to multifactorial (**paper I**) and interdisciplinary, field experiments on natural microplankton ecosystems (**paper II** and **paper III**). There are a range of published laboratory studies focusing on different treatment effects on isolated single species of phytoplankton including cyanobacteria, trying to find the optimum growth rate regarding salinity, pH, temperature etc. for specific specie. Laboratory experiments with single species are perfect for initial mechanistic studies. For all field experiments presented in this thesis, there has been at least one laboratory experiment prior to the main experiment. The subsequent field experiments, on natural microplankton communities, are the result of collaborations between marine chemists, marine biologists and molecular microbiologists (**paper II**) or marine chemists, marine biologists, oceanographers, modellers, meteorologists and maritime environmental scientists and ecotoxicologists (**paper III**). There are many advantages to interdisciplinary research. For example, already at the planning stage and set-up of experiments everyone brings specific knowledge from their research areas that are often crucial to an improved end result. Interdisciplinary work leads to broadened perspectives and all partners leave the project with new connections and scientific insights into each other´s specific research areas.

3.1 Laboratory vs. field experiments

There are many advantages with laboratory experiments, compared to field experiments. In the laboratory, you have control over the parameters you want to study, and you can keep all other abiotic factors equal. Experiments are set-up in a temperature controlled room where light intensities, and duration, nutrients, salinity are also controlled, all of which are important for microplankton growth. There is even the possibility to study a single strain of a species in different experiments, as with *Nodularia spumigena* (strain KAC12) and *Aphanizomenon* sp. (strain KAC15) in **paper I** and **paper IV**, removing effects of species plasticity while only focusing on the effect of the treatment(s) tested. However, intraspecific variation has been observed between *N. spumigena* strains (Wulff et al. 2007), therefore the best approach would be to also study several strains of a species when working in laboratory environments.

When working with natural microplankton communities in the field, as in **paper II** and **paper III**, the problem with strain specificity is absent, due to the variation both within species, groups and the entire community. There is a possibility to find effects of treatments within groups, such as smaller diatoms being affected differently to larger ones (**paper II**), or on group level: diatoms were found to be more resilient than cyanobacteria (**paper III**).

Natural microplankton communities consist of small heterotrophic bacteria, picoplankton, phytoplankton, cyanobacteria and smaller grazers, such as ciliates and zooplankton. In both field experiments in this thesis, the natural community was filtered prior to the experiments (mesh size 200 or 250 µm) in order to remove large grazers capable of extensively grazing the organisms of interest. Although large phytoplankton, such as *Coscinodiscus* spp., and bundles of *Aphanizomenon* sp. are thus discarded, the feeding rate of copepods was not the focus of this work. However, some juvenile copepods pass through the plankton nets, therefore the water volume required in field experiments are much larger than those in laboratory studies, in order to increase the phytoplankton to copepod ratio.

Another reason for increased volumes in field experiments compared to laboratory experiments, is the generally lower biomass concentration in the field. Therefore, for each parameter measured at each sampling occasion, at least ten times more water volume must be extracted from the experimental container in a field study compared to in the laboratory. For example, when sampling for biovolume for a laboratory experiment, a few millilitres are preserved with Lugol´s solution for subsequent analysis, while in a field experiment duplicate samples of 50 ml each are preserved.

Laboratory studies are conducted in a limited space (a bench in the temperature controlled room, or even in a water bath on the bench there). Therefore, smaller volume cell culture flasks were used (75 ml, **paper I**, and 750 ml, **paper IV**) in laboratory studies and aquaria (~4 l) or plastic bags (~50 l) in field studies (**paper II** and **paper III**, respectively). Since neither the cultures used in laboratory studies are axenic, nor the water in field studies are bacteria free, there is always the risk of bottle-effects (ZoBell & Anderson 1936), even with daily mixing of the experimental water.

While abiotic factors, such as light intensity and temperature can be controlled in laboratory studies, they can instead follow natural cycles in field studies. The microplankton community inside the experimental containers are subjected to natural

diurnal changes in light and temperature. However, in the Baltic Proper some species have the ability to migrate vertically through the water column daily (Hajdu et al. 2007), to avoid damaging radiation intensities as well as to ensure optimum nutrient concentrations. This is not a possibility in confined aquaria or bags, and in both field experiments in this thesis, experimental containers were covered with layers of mesh to mimic light intensities at a water depth of 1-2.5 m. The irradiance must then be monitored, and more mesh added or removed depending on cloud coverage at the site.

In **paper IV**, we present a laboratory study focusing on the bloom-forming cyanobacteria of the Baltic Proper as a potential source for biofuel and fatty acids. The cyanobacteria *Aphanizomenon* sp., *Nodularia spumigena* and *Dolichospermum* sp. were cultivated in full nutrient medium and phosphorus- or nitrogen-depleted medium (Fig. 7), all in order to elucidate the highest biomass or most advantageous fatty acid composition.

3.2 Multifactorial experiment

Natural ecosystems are not subjected to one stressor at a time, but to a combination of multiple stressors. However, not all stressors can be applied at once, so some screening must be done. Therefore, in **paper I**, two laboratory experiments were conducted where combinations of two parameters were studied on single species of cyanobacteria, but also on the two species together. The objective in **paper I** was to study the effects of climate change in the Baltic Proper on the two species dominating the cyanobacteria summer-bloom. For the Baltic Proper, annual precipitation, surface water temperature (HELCOM 2007) and atmospheric pCO$_2$-levels (Meehl et al. 2007) are projected to increase. Therefore, salinity may decrease from current 7 to 4 while mid-summer water temperatures may increase from 12 to 16°C, and pCO$_2$ levels may increase from 380 ppm to 960 ppm, by year 2100. In experiment A (Fig. 7), the effects of temperature and salinity on *N. spumigena* and *Aphanizomenon* sp. were studied. Temperature and salinity were kept at ambient present-day levels, but also combined with future levels, resulting in four treatments. Both species were cultured as single species, and together with the other species. In experiment B (Expt B) the set-up was similar, with the two species alone or together, but this time temperature and pCO$_2$ were tested, both at ambient present-day levels and increased future levels.
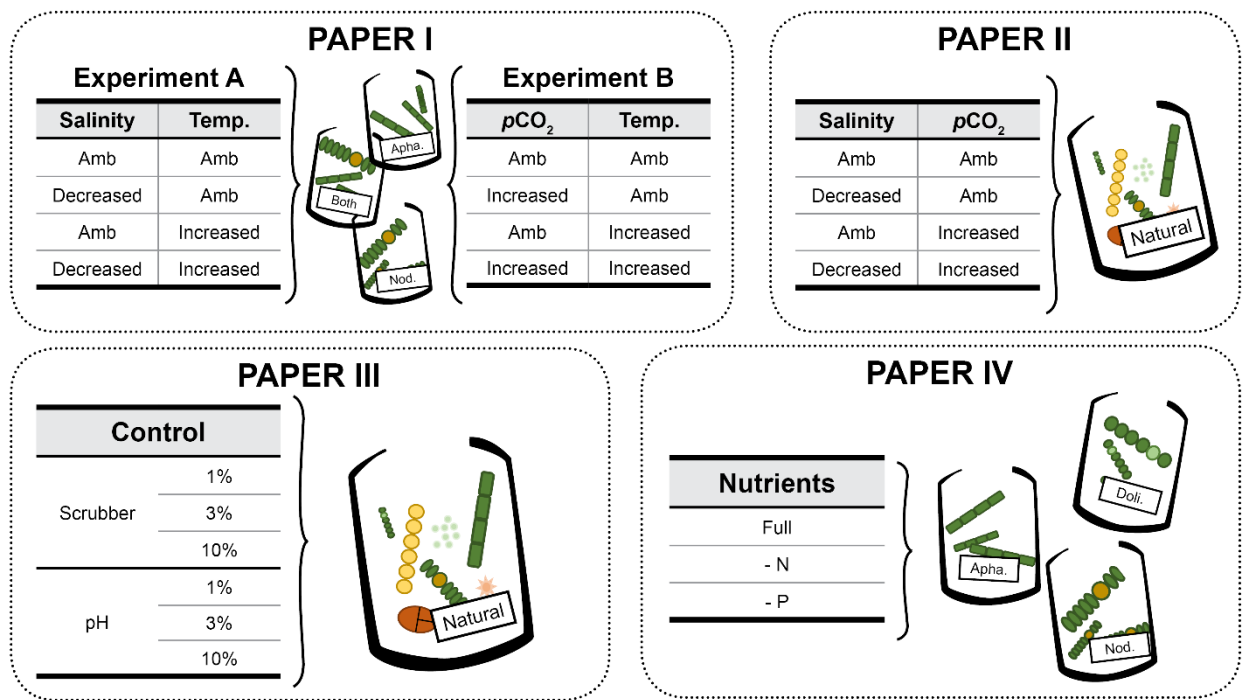
Figure 7. Experimental set-up of Expt A and Expt B in **paper I**, and in **paper II**, **paper III** and **paper IV**. Treatments are ambient present-day levels or future increased temperature and pCO_2 or decreased salinity, three levels of scrubber water and their respective pH-treatments and three levels of nutrient treatments, on three single species of cyanobacteria of the Baltic, *Aphanizomenon* sp., *Nodularia spumigena* or *Dolichospermum* sp., and on the natural microplankton community.

Figure 8. Experimental set-up in **paper II**, with green mesh covering the aquaria and continuous flow through of water (left) and the aquaria being bubbled with CO_2-enriched air (right). Photos by M Karlberg.

3.3 …and interdisciplinary experiments

In **paper II**, the multifactorial approach to study projected climate change effects was continued, examining the combined effect of decreased salinity and increased atmospheric pCO_2, but this time on a natural summer bloom microplankton community (Fig. 7). This time marine chemists, marine biologists and molecular microbiologists collaborated, from both the University of Gothenburg and the University of Copenhagen, in a mix of professors, post-docs, PhD students and master students.

The natural microplankton community was collected using a plankton net (mesh size 25 µm) from sampling station B1 outside of Askö Laboratory (Fig. 1), and inoculated in 0.2 µm filtered water with salinity of either 6 or 3. To mimic atmospheric pCO_2-levels, all aquaria were bubbled with synthetic air containing either 380 µatm CO_2 or 960 µatm (Fig. 8). Since all aquaria were sealed, the headspace became saturated with the two pCO_2-levels, thus mimicking atmospheric pCO_2-levels. In climate change studies, bubbling with CO_2-enriched air is considered as one of the recommended methods to study future increases in atmospheric CO_2-levels (Riebesell et al. 2011).

In **paper III**, a group of scientists from different areas of natural science and from three different universities came together forming the interdisciplinary project SHIpH (http://www.lighthouse.nu/project/shiph). The project group included marine biologists, oceanographers, modellers and marine chemists from the University of Gothenburg (Department of Biological and Environmental Sciences and Department of Marine Sciences), meteorologists from Uppsala University and maritime environmental scientists and ecotoxicologists from Chalmers University of Technology, all focusing on their specific area, but learning from each other at the same time. SHIpH is a project where air, sea, and shipping is connected through modelling and experiments and the results will support future policy development for regulation and monitoring of SO_X and NO_X emissions from shipping. The research focus in **paper III** was to examine the effects of seawater scrubbing (SWS) and the consequences of the acidifying substances SO_X and NO_X from shipping for the Baltic Sea. The project name ShipH is therefore a clever wordplay of "ship" and "pH".

In **paper III** the effect of SWS was studied on a natural microplankton community. SWS reduces the pH, and releases trace metals, soot particles and PAH:s (McElroy et al.1989, Corbett & Fischbeck 1997, Jalkanen et al. 2014), and can therefore add to

the stressors from climate change. Scrubber water was added in 1%, 3% or 10% concentration of the total experimental water volume. To elucidate the effects of reduced pH alone, pH-treatments were established, where H_2SO_4 was added instead. The pH-treatments had equal concentrations of acidified water as the scrubber water treatments; 1%, 3% or 10%. These six treatments were compared to a control, without acidifying substances.

3.4 Methods for microplankton quantification

In all studies included in this thesis, the main result has been which the effect that the different treatments have had on the microplankton species composition, abundance and growth; both in volume and cell numbers. These have been analysed by light microscopy of preserved samples in either a Sedgewick rafter or in a sedimentation chamber according to Utermöhl (1958). In studies where isolated filamentous species were used (**paper I** and **paper IV**), the filaments were measured and all cells, including vegetative and heterocysts, were counted. In **paper II** and **paper III**, where natural microplankton ecosystems were studied, in addition to measuring filaments and counting cells, all other organisms were measured and counted and identified to species, genus or group level. From this the biovolume of each organism could be calculated (Box 1) according to Olenina et al. (2006). The advantage of both measuring biovolume and cell numbers are that in some cases there is no difference in cell numbers between treatments, but there is in biovolume, i.e. the individual cells becomes larger, but not the total cell numbers. In other cases we do not see a treatment effect on the total biomass of diatoms, but there may be one when comparing small and large diatoms (**paper II**). Although light microscopy analysis of microplankton is time-consuming and monotonous work, and novel machines have come out on the market, such as the FlowCam®, there is so much more information to be gained by light microscopy! This gives information not only on biovolumes, species composition etc. but also on unusual species. One treatment may not have an effect on the total biovolume, but on the number of heterocysts, or on the length of each filament. Therefore, measuring and counting gives the option to choose how much data is presented and on which level. There is also the possibility of comparing results with other studies following the same protocols, and with the large amount of stored data from monitoring programs in the Baltic Sea. Not at least, working with light microscopy also confers the privilege of viewing the wonderful and beautiful world of microplankton!

Box 1: Biovolume analysis

Microplankton identification, counting and biovolume calculation are the main variables presented in this thesis. The analysis followed established methods (Utermöhl 1958) and protocols (HELCOM COMBINE 2014). Samples were preserved with Lugol´s solution, stored in the dark and analysed within a few months. A well-mixed sample was poured in a Sedgewick rafter or sedimentation chamber, depending on the microplankton density of the sample. The volume of the sedimentation chamber is also depending on the density of the sample, and in **paper II** and **paper III** chambers of 10 or 25 ml were used. The 50 ml chamber illustrated below was only used to get an overview of the species in **paper II**. The settling time of the sedimentation chambers depends on the volume of the sedimentation chamber, and for 10 and 25 ml chambers minimum settling time is 8 and 18 hours, respectively.

The sample was analysed in an inverted microscope, starting at magnification 100 with large and sparsely distributed species. In this magnification the entire chamber bottom was counted. At higher magnification only a part of the bottom, or one or two transects were counted. The specific species were always counted in the same magnification for all samples.


The diameter and height of all organisms were measured and, where needed, divided into size classes (Olenina et al. 2006). For many organisms, some measurements for biovolume calculations are impossible to make in the microscope. These are the hidden dimensions, such as the thickness (d_2) of *Dinophysis acuminata*. In this case, $d_2 = 0.5 \times d_1$.

Longer chain-forming or filamentous species, such as *Nodularia spumigena*, were measured in 100 µm length units. Cells, heterocysts and akinetes were counted per 100 µm length units, in at least 30 units per sample. Below are examples of species, their geometric shape and formula for biovolume calculation.

Flattened ellipsoid

Volume: $V = \pi/6 \times d_1 \times d_2 \times h$
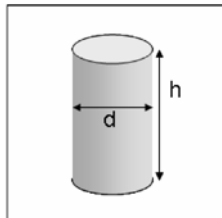
d_1: large diameter
d_2 small diameter
h: height
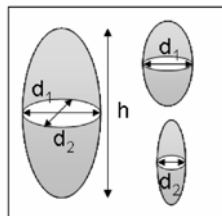
Dinophysis acuminata

Cylinder

Volume: $V = \pi/4 \times d^2 \times h$

d: diameter
h: height

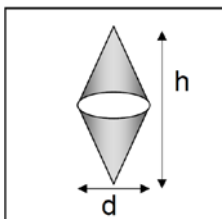*Nodulaia spumigena*

Double cone

Volume: $V = \pi/12 \times d^2 \times h$

d: diameter
h: height

Heterocapsa triquetra

Photos by M Karlberg

Furthermore, microscopy analysis of microplankton samples integrates the results that can be obtained from many other variables, such as photosynthetic pigment composition/concentration, particulate organic carbon (POC), photosynthetic activity and primary productivity. For POC calculations conversion factors between biovolume and carbon content, can be taken into consideration whether the organism is a general phytoplankton, diatom, colony or filament (Menden-Deuer & Lessard 2000, HELCOM COMBINE 2014). Many of the previously mentioned variables offer faster analysis protocols, which gives a snap-shot on how the treatments affect the microplankton, such as the photosynthetic activity measurement with a WATER-PAM. One thing to keep in mind with photosynthetic activity, and primary productivity, is that if you see an effect in these parameters, you will see it subsequently during microscopy analysis by the growth of microplankton.

There are, however, variables that produce information additional to biovolume and species composition. One example is fatty acid (FA) composition in microplankton. FA composition is of importance both when microplankton are used as a food source for higher trophic levels (e.g. Brett & Müller-Navarra 1997, Brown et al. 1997) and for the biofuel industry (e.g. Tamiya 1957, Dismukes et al. 2008). In **paper IV,** FA composition in the three bloom-forming species of the Baltic Proper was analysed in addition to biovolume measurements (Fig. 9). The aims were to find which treatment yielded highest biovolume, total amount of FA, as well as the proportions and amounts of saturated FA (SAFA), monounsaturated FA (MUFA) and polyunsaturated FA (PUFA).

Figure 9. Graphical abstract for **paper IV**, illustrating the possibility to harvest cyanobacteria from the Baltic Sea for biofuel use, and the proportion of fatty acids in the treatment with highest total fatty acid content: *Dolichospermum* sp. in phosphorus depleted medium after 7 days. Illustration by K Karlberg.

4. Main results and discussion

4.1. Effects of climate change on microplankton

4.1.1. Effects of temperature

If temperature alone would increase by 4°C in the Baltic Proper, this would result in an increase in biovolume and photosynthetic activity of both *Aphanizomenon* sp. and *Nodularia spumigena* (**paper I**). The third cyanobacteria species in the Baltic Proper summer-bloom, *Dolichospermum* spp. also had increased biovolume at higher temperatures (Giordanino et al. 2011, Brutemark et al. 2015a). Higher biovolumes at increased temperatures were expected since most organisms have higher metabolic rates at higher temperatures. Furthermore, the elevated temperature of 16°C was in the optimal range for both *Aphanizomenon* sp. and *N. spumigena* (Lehtimäki et al. 1997, Pliński & Jóźwiak 1999). However, *Aphanizomenon* sp. is the first species in the succession of bloom-forming cyanobacteria in the Baltic Proper, and has optimal growth at lower temperatures than at least *N. spumigena* and possibly also *Dolichospermum* spp. Therefore *Aphanizomenon* sp. might not benefit from increased temperatures as much as the other two species. The cyanobacteria bloom in the Baltic Proper starts when the water temperature rises above 16-17°C (Edler 1979, Niemi, 1979). An increase in temperature may result in cyanobacteria blooms starting earlier, perhaps already in the spring (Kahru & Elmgren 2014). Since *Aphanizomenon* sp. both has a higher growth rate than *N. spumigena* at lower temperatures (Lehtimäki et al. 1997), and is a holoplanktonic genus that has vegetative filaments in the water column throughout the year, and do not rely on akinete germination (Suikkanen et al. 2010), it might dominate the future Baltic Proper in late spring when temperatures are not as high as during summer. The end of the bloom, with akinete formation for *Dolichospermum* spp., and some of the *N. spumigena* strains, is initiated by a drop in temperature (Li et al. 1997, Suikkanen et al. 2010). Therefore, an increase in temperature would prolong the cyanobacteria summer-bloom, by delaying the moment of akinete formation. The spring and autumn blooms presently consist of dinoflagellates and diatoms that prefer the colder waters in these seasons. An increase in temperature with mild winters and decreased ice cover in the Baltic Sea, have resulted in an increase of dinoflagellate biomass while diatoms decrease in the Baltic Proper the spring-bloom (Klais et al. 2013). However, further increase of temperature may inhibit these cold water species and favour the warm water cyanobacteria species (Paerl & Paul 2012) and ultimately change the species composition of the Baltic Proper. However, one must keep in

mind that this may be the case when temperature alone is increased, while all other abiotic factors remain at present day levels.

4.1.2. Effects of salinity

If the salinity in the future Baltic Proper were reduced by 3 units compared to present-day values, the photosynthetic activity of *N. spumigena* would be reduced, while *Aphanizomenon* sp. would be unaffected (**paper I**). A reduction in photosynthetic activity may result in a subsequent decrease in growth rate, although this was not seen during the experimental period of seven days in our study. In similar laboratory experiments as in **paper I**, effects of decreased salinities could be viewed first after 7 days, with highest growth of *Aphanizomenon* sp. in salinity 0 or 5, while *N. spumigena* preferred salinity 7-20 (Lehtimäki et al. 1997, Mazur-Marzec et al. 2005). In the experiment in **paper II**, which was conducted for 12 days, there was still no effects of decreased salinity on *Aphanizomenon* sp. while *N. spumigena* had lower biovolumes in decreased salinity, and therefore preferred the present-day salinity in the Baltic Proper. The study in **paper II** was conducted on a natural microplankton community, with all three bloom-forming cyanobacteria species present. Here, *Dolichospermum* spp. had higher biovolumes in lower salinity treatments. Similar results, with *Dolichospermum* sp. cultivated in differing salinity, from 0 to 6, show higher growth rates for strains in lower salinity (Engström-Öst et al. 2011, Brutemark et al. 2015b).

Furthermore, present-day salinities were beneficial, in terms of increased biovolumes, of diatoms, dinoflagellates and ciliates, compared to reduced future salinity (**paper II**). This further indicates that the cyanobacteria species of the Baltic Sea may have a competitive advantage over other groups in the future. However, cyst-forming species, such as the dinoflagellate *Alexandrium ostenfeldii*, can show a high genotypic and phenotypic plasticity resulting in a thriving toxic population when both future salinity and temperature conditions were tested on growth rate and toxin production (Kremp et al. 2016). The toxin of *A. ostenfeldii* has the potential to negatively affect co-occurring dinoflagellates (Hakanen et al. 2014), creating a shift in composition within the dinoflagellate community.

4.1.3. Effects of pCO$_2$

In **paper I** there were no significant effects on either biovolume or photosynthetic activity of *Aphanizomenon* sp. or *N. spumigena* when atmospheric pCO$_2$ levels were increased from present 380 µatm to future 960 µatm. Elevated pCO$_2$ levels in **paper II** stimulated the biovolume of *Dolichospermum* spp., while still having no effect on *Aphanizomenon* sp. or *N. spumigena* (Fig. 10). This is contradictory to other studies

where increased pCO$_2$ levels have had negative effects (Eichner et al. 2014) or positive effects (Wannicke et al. 2012) on the growth of *N. spumigena*. Also for *Dolichospermum* sp. there are studies with differing results than ours, where growth was not increased by elevated pCO$_2$ levels (Brutemark et al. 2015a). It certainly seems that there is a variability between experiments using one strain cultivated alone (Wannicke et al. 2012, Eichner et al. 2014, Brutemark et al. 2015a), or in presence of another species (**paper I**) and in field experiments (**paper II**), making interpretations for future ecosystems more difficult. However, elevated pCO$_2$ levels had no effect on *Aphanizomenon* sp. or *N. spumigena*.

Although there was no statistical interaction effect for salinity and pCO$_2$, the treatment with highest *Dolichospermum* spp. biovolume was the A1FI-treatment; with decreased salinity and increased pCO$_2$ (Fig. 10).

What is notable in **paper II** is the shift in species dominance within the cyanobacteria community. At the start of the experiment, 87% of the cyanobacteria community was *Aphanizomenon* sp., with some *Dolichospermum* spp. and *N. spumigena* (Fig. 11). After 12 days *Dolichospermum* spp. completely dominated the cyanobacterial community. For flagellates, green algae, diatoms or dinoflagellates, there were no difference in biovolume between present-day 380 µatm to future 960 µatm (Fig. 10).
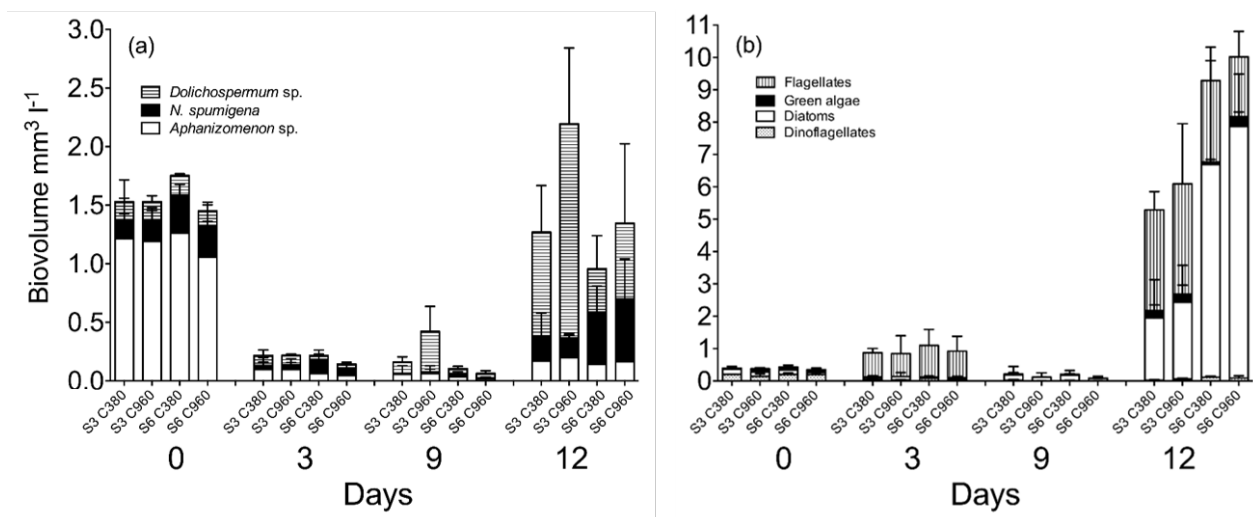

Figure 10. Biovolumes of diazotrophic cyanobacteria (a) and, (b) biovolumes of flagellates, green algae, diatoms and dinoflagellates. The different treatments are four combinations of salinity (S6, S3) and carbon dioxide concentrations (380 µatm, 960 µatm). Vertical lines show standard error (n=4). Redrawn from **paper II**.

2% **11%**

87%

(a)

15%

3%

82%

(b)

☐ *Aphanizomenon* sp.
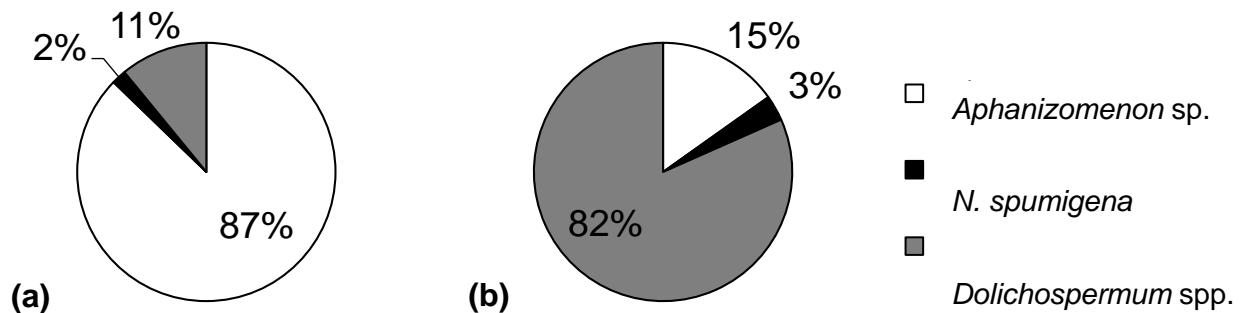
■ *N. spumigena*

■ *Dolichospermum* spp.

Figure 11. Percentage contribution of the three filamentous cyanobacteria species from all treatments in **paper II**; (a) initially, Day 0 and (b) at Day 12.

4.2. Effects of seawater scrubbing on microplankton

In **paper III**, the effects of SWS were tested on a natural summer-bloom microplankton community. Scrubber water was added in 1%, 3% or 10% concentration of the total experimental water volume and compared to treatments with acidified water at the same concentrations as well as a control without acidifying substance.

Initially, the microplankton community was a typical cyanobacterial summer-bloom; dominated by *Aphanizomenon* sp. with some *Nodularia spumigena* and a small proportion of *Dolichospermum* spp., as well as flagellates and ciliates (Fig. 12). After 14 days, *Aphanizomenon* sp. was absent in all treatments, while the biovolume of diatoms had increased in all treatments. There was no difference in biovolume of microplankton between any of the treatments on day 14, but with an indication of more diatoms in the 10% scr treatment. A possible reason for the shift in species dominance between Day 0 and Day 14 was the comparable high amount of NO_2^- $+NO_3^-$ in all treatments. Cyanobacteria have the ability to outcompete diatoms and dinoflagellates when nitrogen is low, such as during the summer-bloom, but not in this experiment, with elevated nutrient-levels.

None of the species or groups seem to be affected by decreased pH, which is consistent with results by Nielsen et al. (2010) and Paul et al. (2015). In our experiment, the pH was initially 8.51 in Ctrl, 6.89 in 10% scr and 7.12 in 10% pH (complete dataset in **paper III**). As seen above, reduced pH by increased
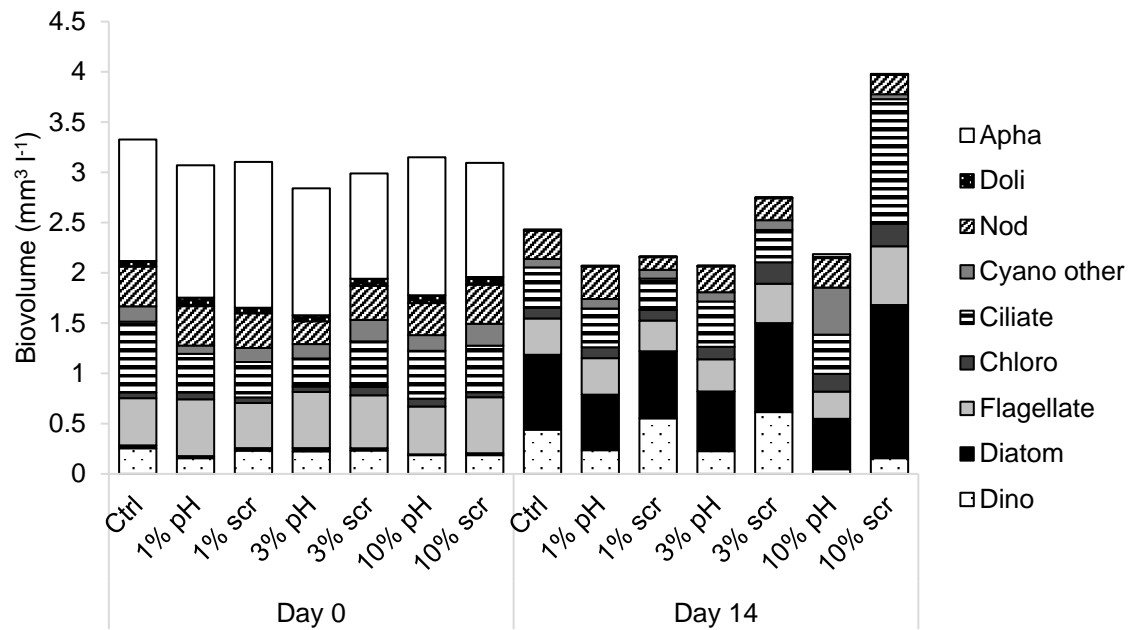
Figure 12. Biovolume ($mm^3 \ l^{-1}$) for all microplankton groups and species; *Aphanizomenon* sp., *Dolichospermum* sp., *Nodularia spumigena*, other cyanobacteria, ciliates, chlorophytes, flagellates, diatoms and dinoflagellates, at Day 0 and Day 14 in the untreated control (Ctrl), 1%, 3% and 10% added scrubber water (1% scr, 3% scr and 10% scr) and their reflective pH treatments (1% pH, 3% pH and 10% pH).

atmospheric CO_2-levels (**paper I** and **paper II**) or by addition of scrubber water or acidified water (**paper III**) may not have an effect on the biovolume of microplankton of the Baltic Proper. These organisms are subjected to diurnal changes in pH, due to photosynthesis and respiration, and was measured both in the field and in experimental bottles during the experiment in **paper II** (Fig. 13). Large variations in pH due to the diurnal cycle of primary productivity have also been observed in coastal surface waters (Fransson et al. 2004, Ploug 2008, Schulz & Riebesell 2013). However, the long-term effects of increased pCO$_2$/decreased pH on microplankton in the Baltic Proper are yet to be studied. For example, when the Antarctic diatom *Nitzschia lecointei* was exposed to increased pCO$_2$ 960 µatm/decreased pH 7.8, a reduction in growth was seen after 147 days (Torstensson et al. 2015). This shows that studies over a few days or weeks may not show the true result of how future microplankton in the Baltic Sea will react to increased pCO$_2$/decreased pH.

Climate change effects, such as increased atmospheric pCO$_2$, are affecting the ecosystem over long decadal time-periods. Adding on the acidifying effects of SWS, may not be the smartest idea, especially with so few studies conducted in this area. As seen in **paper III**, there were significant decrease in pH and alkalinity in the treatment with lowest concentration of scrubber water, compared to the control. This
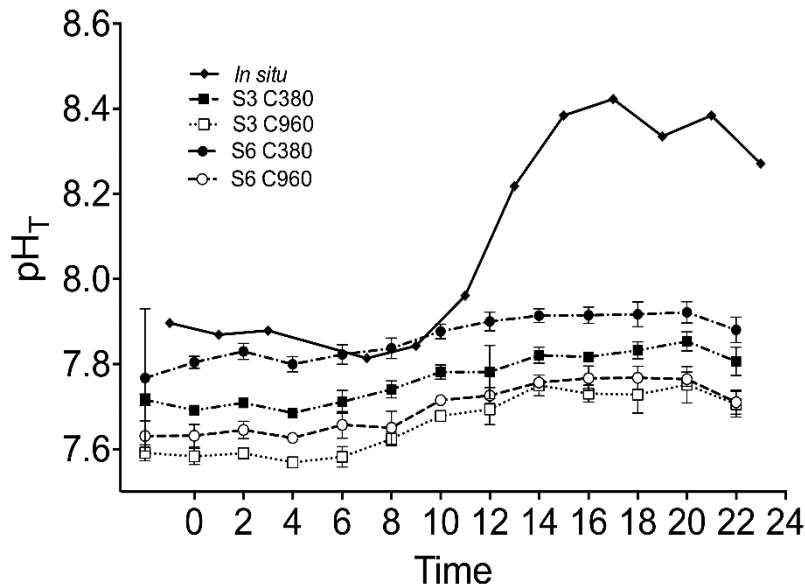
Figure 13. Variation in pH$_T$ over 24 hours. The continuous bubbling of CO$_2$-enriched synthetic air provides a fluctuating pH during the diurnal cycle of primary production. The different treatments are four combinations of salinity (S6, S3) and carbon dioxide concentrations (380 µatm, 960 µatm) plus *in situ* sea surface values. Vertical lines show standard error (n=4).

calls for precautions when implementing scrubber units on the shipping fleet of the Baltic Sea. The Baltic Sea is a sensitive area with low alkalinity, compared to oceanic water. The accumulated effects of long-term repeated addition of SWS, constantly throughout the year, i.e. in a shipping lane, are yet to be elucidated.

Scrubber water is not only acidic, but also contains elevated concentrations of trace metals, particularly, higher concentrations of Al, Cr, Cu, Co, Mn, Mo, Zn and Ni, where several of these have detrimental effects on microorganisms (Sunda 1989). There is a wide range in the sensitivity of different algal species to trace metal toxicity (Fisher & Frood 1980, Brand et al. 1986). In general, cyanobacteria are most sensitive to elevated levels of Cu and Zn, dinoflagellates intermediate and either diatoms (Whitaker et al. 1978, Brand et al. 1986) or chlorophytes (Horne & Goldman 1974, Miao et al. 2005, Levy et al. 2007) least sensitive. The concentration of Cu required to reduce growth or photosynthetic activity varies greatly within groups of microplankton. In Levy et al. (2007) effects could be seen on a few species, of both diatoms and dinoflagellates, at the Cu concentration in the 1% scr treatments in this experiments. In Horne and Goldman (1974), effects of Cu concentrations corresponding to the 3% scr treatment in this experiment (80 nM) could be seen on two species of cyanobacteria; *Aphanizomenon* sp. and *Anabaena* sp. (presently *Dolichospermum* spp.), while having no effects on the diatom *Melosira* sp. and the

chlorophyte *Oocystis* sp. In Brand et al. (1986), the effects were notable on diatoms, dinoflagellates or cyanobacteria first at very high Cu concentrations, approximately 1-5 µM, which is comparable to the pure scrubber water in this experiment; 1.4 µM. For Zn results are similar, there are no negative effects on any groups at Zn concentrations in this experiment, and only if 100% scrubber water would have been used (Jensen et al. 1974, Miao et al. 2005), there might have been reduced growth of photosynthetic activity. For diatoms (Fisher & Frood 1980, Brand et al. 1986, Sunda 1989), chlorophytes (Bilgrami & Kumar 1997), dinoflagellates (Saifullah 1978, Levy 2007) and cyanobacteria (Whitaker et al. 1978) there seems to be an interspecific tolerance to elevated trace metal concentration, where some species are very sensitive and others tolerant. In this 14 days experiment, which can be considered a long term experiment regarding metal exposure since these types of experiments usually run from a few hours to days, we did not see any negative effects on the total biovolume of different groups of microplankton or on their photosynthetic activity. In experiments where $CuSO_4$ have been added to natural microplankton ecosystems in lakes, in order to control the toxic cyanobacterial blooms dominated by *Aphanizomenon flos-aquae*, there was a shift towards a diatom-, chlorophyte- or chrysophyte dominated ecosystem, depending on the amount of $CuSO_4$ added and timing of the addition in the season (Whitaker et al 1978). Increased SWS in the Baltic Sea, in combination with cyanobacteria being most sensitive to increased trace metal concentrations, may result in a shift in species dominance within the Baltic Sea summer microplankton community; from a filamentous cyanobacteria dominated one, towards groups or species with higher tolerance to elevated trace metal concentration.

4.3. Cyanobacteria as biofuel?

In **paper IV**, we focused on the bloom-forming cyanobacteria of the Baltic Proper as a potential source for biofuel and fatty acids (FA). If there is a risk for longer lasting blooms with higher biomass than presently observed, can we benefit from it, turning them into biofuel? Which culture medium yields the most biovolume and FA content and composition? There may even be a potential benefit from increased cyanobacteria blooms and perhaps the Baltic can be relieved from toxic blooms, by harvesting the natural blooms and turning them into biofuels?

In **paper I**, where the future effects of climate change were tested on two species of cyanobacteria, there were no effects on biovolume of *Aphanizomenon* sp. or *N. spumigena* when salinity was decreased or atmospheric pCO$_2$ levels increased. However, for both species the biovolume increased with increased temperature.

Temperature may have similar effect on *Dolichospermum* spp. In **paper II**, where the future effects of climate change were tested on a natural microplankton community, there was a shift in species dominance from *Aphanizomenon* sp. to *Dolichospermum* sp. when salinity was decreased in combination with increased $p\text{CO}_2$ levels. Therefore, it is possible that the future cyanobacterial bloom will be dominated by *Dolichospermum* spp. rather than *Aphanizomenon* sp. and *N. spumigena*. In **paper IV**, these three species were screened for their total FA content as well as proportion and amount of saturated FA (SAFA), monounsaturated FA (MUFA) and polyunsaturated FA (PUFA). *Dolichospermum* sp. had the highest total FA after 7 days in phosphorus-depleted medium, *Aphanizomenon* sp. after 14 days in nitrogen-depleted medium, while none of the media had an impact on the total FA content of *N. spumigena*.

Total FA was measured and calculated as µg mm^{-3}, i.e. depending on the biovolume of cyanobacteria. *Dolichospermum* sp. had the highest amount of FA per biovolume, but *N. spumigena* had in all treatments, throughout the experiment, at least five times the biovolume of the other species. Nevertheless, *N spumigena* would still not have reached the total FA content of *Dolichospermum* sp. Therefore, *N spumigena* would be suitable for biomass production, but not as a FA producer.

Of the SAFA, MUFA and PUFA, there are specific FA within these groups that are of particular interest in commercial production and for use in several anti-cancer and anti-heart disease drugs of the pharmaceutical industry (Plaza et al. 2009). These are the monounsaturated hexadecanoic acid (16:1(n-7)), octadecanoic acid (18:1(n-9)), polyunsaturated octadecatrienoic acid (18:3(n-3)), eicosapentaenoic acid (EPA; 20:5(n-3)) and docosahexaenoic acid (DHA; 22:6(n-3)), which are all present in *Dolichospermum* sp., *Aphanizomenon* sp. and *N. spumigena* (**paper IV**).

5. Conclusion and future prospects

Based on the results from the studies included in this thesis, I conclude that there is clearly a need to further investigate the possible future impacts of climate change and SWS on the natural microplankton community of the Baltic Sea. In the studies presented here, there was a clear result of increased biovolume of *Aphanizomenon* sp. and *Nodularia spumigena* when temperature was increased by 4°C. When salinity was decreased by three units both the growth and photosynthetic activity of *N. spumigena* was reduced while *Aphanizomenon* sp. was unaffected and the growth of *Dolichospermum* spp. was increased. Furthermore, present-day salinities were

beneficial, in terms of increased biovolumes, of diatoms, dinoflagellates and ciliates, compared to reduced future salinity. Increased atmospheric $p\mathrm{CO_2}$ had no effect on any of the species in the microplankton community. These results show that the future microplankton community may be advantageous, in terms of increased biovolume, for the cyanobacteria species *Aphanizomenon* sp. and *Dolichospermum* spp. The long-term effects of future climate change must be further studied.

We have shown that it is possible to extract biomass and fatty acids from the cyanobacteria species in the Baltic Sea, and another study has shown the possibility to harvest the natural summer-bloom in the Baltic Sea (Gröndahl 2009). A possible future increase of the duration, biomass and area of blooms should really push the development of these technologies. Relieving the future ecosystem from toxic blooms, and increasing recreational values throughout the Baltic Sea area is something to look forward to!

Although seawater scrubbing did not have any statistical effects on the biological variables microplankton species composition and biovolume, we believe this may not be the full story. Presented here is a one-time addition of scrubber water to a natural microplanktonic ecosystem. The accumulated effects of long-term repeated addition throughout the year, i.e. in a shipping lane, are yet to be elucidated. Other factors, such as soot particles or PAH:s, and cocktail effects of trace metals, pH reduction, lowered alkalinity and salinity, increased temperature, must also be further studied. Furthermore, here the effects on the microplankton at the base of the food chain were studied, and there is always a potential risk for negative effects at higher trophic levels. For example, the fatty acid composition may be altered (which was not measured in the field experiments), which is essential for growth and recruitment at higher trophic levels (e.g. Brett & Müller-Navarra 1997, Brown et al. 1997).

The Baltic Sea is a sensitive area, which is already exposed to multiple stressors such as eutrophication, over-fishing and climate change. Therefore, the precautionary principle must rule before implementing scrubber units on board the shipping fleet. Preferably, more studies and field experiments should be conducted before any final conclusions can be drawn of the effects of seawater scrubber on the microplanktonic community during summer-blooms in the Baltic Sea.

6. Financial support

7. Acknowledgements

Först och främst vill jag tacka min fantastiska handledare, alfahonan i vår vargflock The Wulff-pack, kvinnan som aldrig backar i en fight och som alltid har min rygg **Angela Wulff**. Innan jag började min master hade jag tänkt att jag i alla fall inte minsann skulle hålla på med nåt litet tjafs man inte kunde se med blotta ögat, men med dej som handledare hoppade jag gladeligen på att studera cyanosar i Östersjön. Tänk om jag hade valt musslor eller sjöstjärnor istället, huvva! Cyanosar, dinosar, kondom-ciliater, fluff, sälar och (jaja…) kiselalger är ju fantastiskt att kika på i mikroskop! Du har varit min klippa i vått och torrt, och även lyckats förmedla din awesomeness till studenter, andra doktorander i nöd och professorer. TACK!

Ett stort tack till:

Min biträdande handledare **Ida-Maja Hassellöv**, som introducerade SHIpH under min doktorandtid, och som dessutom införde traditionen att en väl utförd provtagningsdag avslutas med mousserande. Den traditionen kommer jag föra vidare! Någon gång i framtiden ska vi ta oss till västkusten på hösten för att kika på riktigt kraftig bioluminiscens, istället för Askö på sommaren… **Kristina Sundbäck**, min första examinator, som tyckte att alla kurser jag sökte och konferenser jag åkte

på var vettiga och skulle bredda min kunskap, hur långsökt det än var. När jag blir stor ska jag också flytta till solvärmen! **Anna Godhe**, min nuvarande examinator, som tycker allt går som på räls, även när inte jag kan se det… **Henrik Aronsson** för att du alltid trodde på mej, pushade mej och försökte övertala mej att jag faktiskt är en jättebra lärare. Det är otroligt hur ett möte på 30 minuter två gånger om året kan få mej att må så kanon! Tack till alla i SHIpH-gruppen för ert givande samarbete: **Erik Ytreberg**, **David Turner**, **Anders Omstedt**, **Moa Edman**, **Julian Gallego-Urrea**, **Anna Rutgersson**, **Björn Claremar**. **Erik**, det är dags att sätta igång att hårdträna på att bli av med av Västerås-dialekten. Såhär kan vi inte ha det. Tur att du uppväger det med god whiskysmak… **David**, jag kommer för alltid mäta pH ner till sjuttioelfte decimalen, jag lovar! Alla på Askölaboratoriet måste få ett extra stort tack! **Suss**, **Eva**, **Mattias**, **Carl-Magnus** och **Britt-Marie**: ni är fantastiska och hjälper alltid till med vad man än behöver. Och så kan man åka båt, mäta vatten, pilla på bojen, leka med ROV:n på arbetstid; vilket drömjobb ni måste ha! Den ärade fru **Malin Mohlin**. Angelas stackars doktorand som jag plågade med tusen frågor under mitt masterår, när hon försökte avsluta sin avhandling. Jag ber så hemskt mycket om ursäkt! Jag kan inte föreställa mej vad du måste gått igenom! **Anders Torstensson**, coola killen som introducerade Falafel Marrakesh till 5e våningen. Hur skulle jag överlevt senaste året utan den fantastiska anrättningen? Du är dessutom den enda person jag känner som kan komma hem från en egyptsemester utan att vara solbränd. Det kanske blir så när man spenderar hela semestern i grottor. Under vatten… Alla exjobbare under åren: **Mia**, **Jenny**, **My**, **Lucy**, **Joanne**, **Isabella** och **Gurpreet**. Det har varit ett nöje att vara med er på vägen till examen, lycka till i framtiden till er allihop! And Gurkan, you are the only Indian person I know of that have gotten a sunburn at Askö 2012. In March… **Amanda Nylund**, som med tapperhet och alltid glatt humör provtar dygnet runt, året om. Koller aldrig glömma Askö 2014, vågorna under vattenhämtningen till Kberg 2015, och dina goda veganrecept. Se bara till att hålla dej undan från provtagningar och analyser där man måste stå i labbrock i ett kokhett litet rum där man inte får öppna fönstret! **Mikael Hedblom** för all öl, whisky och intressanta diskussioner under intagandet… Och för att du var så tapper på Askö 2014. Jag kommer för alltid tänka på dej varje gång jag är i ett termokonstantrum, och ska försöka att inte go loco när jag är där... Men vi vet ju vems fel det var och som ville picta utav bara h-vete… **Justin Pearce** för ditt mod att smaka svenska delikatesser som messmör. Och för din brytning; g´day mate! **Kai Lohbeck**, I usually don´t even greet people the first 6 months, then it takes about a year before I truly get to know a person and really like them. It is truly a shame that your post doc is finished so soon, it wold have been great to know you for a year and a half already! Wait a minute, hmmm… **Inger Wallentinus** som vet allt och

dessutom kan rota fram referensen till det på ett par minuter. Vilken skatt att ha dej på Botan! **Monica Appelgren** som man kan prata med allt om. Botan kommer snart aldrig bli sej likt… **Sven Toresson**, Botans egna MacGyver, som kan fixa allt. Outtröttligt. Till mina doktorandkollegor som jag undervisat med under åren: **Lisa A**, **Johanna**, **Alexander**, **Oskar**, **Sten**, **Malin**, **Jonna** samt framför allt **Jenny** och **Emelie**. Vad roligt vi har haft, men J och E, låt oss hoppas att vi aldrig mer behöver rätta labrapporter…

En stor kram till alla doktoranderna på 5e våningen: mina rumskompisar **Lisa Sundqvist**, **Malin Olofsson** och **Louise Eriander**. Vilken tur att vi sällan var där allihopa samtidigt, då hade nog ingen av oss disputerat vid det här laget… Och jag ser verkligen fram emot din trädgårdsblogg/bok/tv-program, Louise! Jag har bestämt att du ska göra nåt sånt och jag är van vid att få min vilja igenom. Så det så! **Daniela de Abreu**, thanks for all the delicious cashews! But especially for letting me and F into your home in Maputo. I will always treasure having seen the real Maputo, where you can buy fresh-squeezed sugar cane juice by the side of the road. Mmm… **Gurpreet Kaur Kahlon** (again) hang in there, you can do it and we're all cheering for you! **Josefin Sefbom**, vad tomt det blev när du försvann! Du har, och kommer alltid att ha en sängplats i vårt hem, var i världen det än är. **Susanna Gross** aka The GIF Master som alltid redan har kollat upp allt som man nånsin skulle velat Googla, som alltid peppar och som för alltid kommer veta den faktiska betydelsen av spank the monkey… **Fabian Roger**, min absoluta favorit tysk-fransos! Det var roligt att träna din svenska och sedan se folks förvirrade miner när de inte förstod vad du sa. Länge var jag den enda som förstod fabianska… Som jag sa till Kai, det tar ett tag att lära känna mej, men nu när du gjort det, och jag faktiskt tycker om dej, kommer du vara fast för livet! Du har gott om tid att försöka övertala mej om att rödvin smakar gott och mer än "ja, det smakar rött". Det har dessutom varit ovärderligt att dela den här sista doktorandtiden tillsammans. Att kunna dela alla panikattacker, diverse gruppdynamiker, fruktstunder och kopp efter kopp med kaffe har varit oersättligt. Och en stor kram som tack för att jag har fått låna din soffa, annars hade den här avhandlingen inte blivit klar…

Ett stort och varmt tack till alla studenter under åren. Jag har haft så roligt och det har varit ett privilegium att inspirera er och visa er tylakoidernas värld, tvärsnitt av växter, dygnsmätningar i PAM, marina mikroalger och mer. Ett av de bästa ljuden jag vet är när man hör ett ja-haaa i kurslabbet, när ni förstår vad jag pratar om eller ser det jag ser i mikroskopet. Tänk på att man måste vilja, kisa och ha fantasi för att se saker i mikroskop… Jag kommer verkligen att sakna undervisningen.

Franciska Steinhoff, jag tyckte det var så fruktansvärt när du flyttade till Norge. Nå er det virkelig grejt, så jeg kan øve skikkelig på min norsk! Det är så roligt att ha en stående inbjudan till sjuttonde maj varje år, även om det inte alltid blir så… Jag saknar dej!

Ett stort tack till **Mats** som har vett att bo på varma platser så vi hade nånstans att fly när det regnade sidledes i Göteborg. Men jag kommer kanske aldrig förlåta dej för att du flyttade från Seychellerna… **Mamma**, **pappa** och syster **Karin** som alltid upprätthållit mitt biologiintresse. Men jag kommer aldrig tycka att man åker och badar i havet när man åker ut i Stockholms skärgård…

Framför allt, ett stort tack till de underbara **Kristian** och **Zelda**. Jag älskar er! Vilket tålamod ni har haft den senaste tiden! Zellis som lärt sej rita gubbar, simma, nästan hela alfabetet, och massa mer, medan mamma bara har varit på jobbet. Tur att Skype finns och att du har världens bästa pappa som tagit hand om allt! K min älskling, mitt livs kärlek, min bästa vän, tack för ditt otroligt stora tålamod. Nu ska vi spela brädspel som fa-an! Tack för att du har torkat tårar i jobbiga stunder, både irl och över Skype. Tack för att du kommer med råd när det behövs och har vett att vara tyst när jag bara behöver raljera och prata av mej… Ingen känner mej så väl som du. Snart kanske vi hamnar i Australien, eller San Diego, eller Japan, eller eh Mölnlycke… Oavsett kommer vi vara tillsammans igen. Du är bäst!

8. References

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

Bilgrami KS, Kumar S (1997) Effects of copper, lead and zinc on phytoplankton growth. Biol Plant 39(2):315-317

Blackburn SI, McCausland MA, Bolch CJS, Newman SJ, Jones GJ (1996) Effect of salinity and growth and toxin production in cultures of the bloom-forming cyanobacterium *Nodularia spumigena* from Australian waters. Phycologia 35(6):511-522

Brand LE, Sunda WG, Guillard RRL (1986) reduction of marine phytoplankton reproduction rates by copper and cadmium. J Exp Mar Biol Ecol 96:225-250

Brett MT, Müller-Navarra DC (1997) The role of highly unsaturated fatty acids in aquatic foodweb processes. Freshw Biol 38:483-499

Brown MR, Jeffrey SW, Volkman JK, Dunstan GA (1997) Nutritional properties of microalgae for mariculture. Aquaculture 151:315-331

Brutemark A, Engström-Öst J, Vehmaa A, Gorokhova E (2015a) Growth, toxicity and oxidative stress of a cultured cyanobacterium (*Dolichospermum* sp.) under different CO_2/pH and temperature conditions. Phycol Res 63: 56-63

Brutemark A, Vandelannoote A, Engström-Öst J, Suikkanen S (2015b) A less saline Baltic Sea promotes cyanobacterial growth, hampers intracellular microcystin production, and leads to strain-specific differences in allelopathy. PLOS ONE 10(6): e0128904

Corbett JJ, Fischbeck P (1997) Emissions from ships. Science 278(5339):823-824

Dickson A (2010) The carbon dioxide system is seawater: equilibrium chemistry and measurements. In: Riebesell U, Fabry V, Hansson L, Gattuso J-P (eds) Guide for best practises in ocean acidification research and data reporting. Office for Official Publications of the European Communities, Luxembourg, pp 17-40

Dismukes GC, Carrieri D, Bennette N, Ananyev GM, Posewitz MC (2008) Aquatic phototrophs: efficient alternatives to land-based crops for biofuels. Curr Opin Biotechnol 19(3):235-240

Doney SC, Mahowald N, Lima I, Feely RA, Mackenzie FT, Lamarque J-F, Rasch PJ (2007) Impact of anthropogenic atmospheric nitrogen and sulfur deposition on ocean acidification and the inorganic carbon system. Proc Natl Acad Sci USA 104(37):14580-14585

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

Edler L (1979) Phytoplankton succession in the Baltic Sea. Acta Bot Fennica 110:75-78

Eichner M, Rost B, Kranz SA (2014) Diversity of ocean acidification effects on marine N_2 fixers. J Exp Mar Biol Ecol 457:199-207

Engström-Öst J, Repka S, Mikkonen M (2011) Interactions between plankton and cyanobacterium *Anabaena* with focus on salinity, growth and toxin production. Harmful Algae 10: 530-535

Fischlin A, Midgley GF, Price JT et al (2007) Ecosystems, their properties, goods, and services. In: Parry ML, Canziani OF, Palutikof JP et al (eds) Climate change 2007: impacts, adaptation and vulnerability. Contribution of working group II to the fourth assessment report of the intergovernmental panel on climate change, Cambridge University Press, Cambridge and New York

Fisher NS, Frood D (1980) Heavy metals and marine diatoms: Influence of dissolved organic compounds on toxicity and selection for metal tolerance among four species. Mar Biol 59:85-93

Fransson A, Chierici M, Anderson LG (2004) Diurnal variability in the oceanic carbon dioxide system and oxygen in the Southern Ocean surface water. Deep Sea Res II 51:2827-2839

Fu F-X, Warner ME, Zhang Y, Feng Y, Hutchins DA (2007) Effects of increased temperature and CO_2 on photosynthesis, growth, and elemental ratios in marine *Synechococcus* and *Prochlorococcus* (cyanobacteria). J Phycol 43:485-496

Gattuso J-P, Hansson L (2011) Ocean acidification: background and history. In: Gattuso J-P, Hansson L (eds) Ocean acidification. Oxford University Press, Oxford, pp 1-20

Giordanino MV, Strauch SM, Villafane VE, Helbling EW (2011) Influence of temperature and UVR on photosynthesis and morphology of four species of cyanobacteria. J Photochem Photobiol B 103:68-77

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

Gröndahl F (2009) Removal of surface blooms of the cyanobacteria Nodularia spumigena: a pilot project conducted in the Baltic Sea. AMBIO 38(2):79-84

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

Hakanen P, Suikkanen S, Kremp A (2014) Allelopathic activity of the toxic dinoflagellate *Alexandrium ostenfeldii*: Intra-population variability and response of co-occurring dinoflagellates. Harmful Algae 39:287-294

Hassellöv I-M, Turner DR, Lauer A, Corbett JJ (2013) Shipping contributes to ocean acidification. Geophys Res Lett 40:2731-2736

HELCOM (2007) Climate change in the Baltic Sea area - HELCOM thematic assessment 2007. Balt. Sea Environ. Proc. No. 111

HELCOM (2013) Climate change in the Baltic Sea Area: HELCOM thematic assessment in 2013. Balt. Sea Environ. Proc. No. 137

HELCOM COMBINE (2014) Manual for marine monitoring in the COMBINE program of HELCOM. 416 pp

Hjalmarsson S, Wesslander K, Anderson LG, Omstedt A, Perttilä M, Mintrop L (2008) Distribution, long-term development and mass balance calculation of total alkalinity in the Baltic Sea. Continental Shelf Research 28:593-601

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

Horne AJ, Goldman CR (1974) Suppression of Nitrogen Fixation by Blue-Green Algae in a Eutrophic Lake with Trace Additions of Copper. Science 183(4123):409-411

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

Hutchins DA, Fu FX, Zhang Y, Warner ME, Feng Y, Portune K, Bernhardt PW, Mulholland MR (2007) CO_2 control of *Trichodesmium* N_2 fixation, photosynthesis, growth rates, and elemental ratios: Implications for past, present, and future ocean biogeochemistry. Limnol Oceanogr 53:1293-1304

Hällfors G (2004) Checklist of Baltic Sea phytoplankton species (including some heterotrophic protistan groups). HELCOM Baltic marine environment protection commission. Balt. Sea Environ. Proc. No. 95

Jalkanen J-P, Johansson L, Kukkonen J (2014) A comprehensive inventory of the ship traffic exhaust emissions in the Baltic Sea from 2006 to 2009. AMBIO 43:311-324

Janson S, Hayes PK (2006) Molecular taxonomy of harmful algae. In: Granéli E, Turner JT (eds) Ecology of harmful algae. Ecological studies 189. Springer, Berlin, pp 9-21

Jensen A, Rystad B, Melsom S (1974) Heavy metal tolerance of marine phytoplankton. I. The tolerance of three algal species to zinc in coastal sea water. J Exp Mar Biol Ecol 15:145-157

Kahru M, Elmgren R (2014) Multidecadal time series of satellite-detected accumulations of cyanobacteria in the Baltic Sea. Biogeosciences 11:3619-3633

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

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

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

Karle I-M, Turner DR (2007) Seawater scrubbing - reduction of SOx emissions from ship exhausts. AGS Office at Chalmers, Gothenburg

Klais R, Tamminen T, Kremp A, Spilling K, An BW, Hajdu S, Olli K (2013) Spring phytoplankton communities shaped by interannual weather variability and dispersal limitation: Mechanisms of climate change effects on key coastal primary producers. Limnol Oceanogr 58(2): 753-762

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

Kremp A, Oja J, LeTortorec AH, Hakanen P, Tahvanainen P, Tuimala J, Suikkanen A (2016) Diverse seed banks favour adaptation of microalgal populations to future climate conditions. Environ Microbiol 18(2):679-691

Lehtimäki J, Moisander P, Sivonen K, Kononen K (1997) Growth, nitrogen fixation and nodularin production by two Baltic Sea cyanobacteria. Appl Environ Microbiol 63(5):1647-1656

Levy JL, Stauber JL, Jolley DF (2007) Sensitivity of marine microalgae to copper: The effect of biotic factors on copper adsorption and toxicity. Sci Total Environ 387:141-154

Li R, Watanabe M, Watanabe MM (1997) Akinete formation in planktonic *Anabaena* spp. (Cyanobacteria) by treatment with low temperature. J Phycol 33:576-584

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

McElroy AE, Farrington JW, Teal JM (1989) Bioavailability of polycyclic aromatic hydrocarbons in the aquatic environment. In: Vanarasi U (ed) Metabolism of polycyclic aromatic hydrocarbons in the aquatic environment. CRC press, Boca Raton, Chapter 1, pp 1-40

Meehl GA, Stocker TF, Collins WD et al (2007) Global climate projections. In: Solomon S, Qin D, Manning M et al (eds) Climate change 2007: the physical science basis. Contribution of working group I to the fourth assessment report of the intergovernmental panel on climate change, Cambridge University Press, Cambridge and New York

Menden-Deuer S, Lessard EJ (2000) Carbon to volume relationships for dinoflagellates, diatoms and other protist plankton. Limnol. Oceanogr. 45: 569-579

Miao A-J, Wang W-X, Juneau P (2005) Comparison of Cd, Cu, and Zn toxic effects on four marine phytoplankton by pulse-amplitude-modulated fluorometry. Environ Toxicol Chem 24(10) 2603-2611

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

Mohlin M, Roleda MY, Pattanaik B, Tenne S-J, Wulff A (2012) Interspecific resource competition – combined effects of radiation and nutrient limitation on filamentous cyanobacteria. Microb Ecol 63: 736-750

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

Niemi Å (1979) Blue-green algal blooms and N:P ratio in the Baltic Sea. Acta Bot Fennica 110:57-61

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

Olenina I, Hajdu S, Edler L, Andersson A, Wasmund N, Busch S, Göbel J, Gromisz S, Huseby S, Huttunen M, Jaanus A, Kokkonen P, Ledaine I, Niemkiewicz E (2006) Biovolumes and size-classes of phytoplankton in the Baltic Sea. HELCOM Balt. Sea Environ. Proc. No. 106, 144 pp

Paerl HW, Paul VJ (2012) Climate change: Links to global expansion of harmful cyanobacteria. Water Research 46:1349-1363

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

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

Plaza M, Herrero M, Cifuentes A, Ibáñez E (2009) Innovative natural functional ingredients from microalgae. J. Agric. Food Chem 57(16):7159-7170

Pliński M, Jóźwiak T (1999) Temperature and N:P ratio as factors causing blooms of blue-green algae in the Gulf of Gdańsk. Oceanologia 41:73-80

Raven J, Caldeira K, Elderfield H, Hoegh-Guldberg O, Liess P, Riebesell U, Shephard J, Turley C, Watson A (2005) Ocean acidification due to increasing atmospheric carbon dioxide. Policy document 12/05 by The Royal Society, London, UK, 60 pp

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

Riebesell U, Fabry VJ, Hansson L, Gattuso J-P (eds) (2010) Guide to best practices for ocean acidification research and data reporting. Luxembourg: Publications Office of the European Union, 260 pp

Riebesell U, Tortell PD (2011) Effects of ocean acidification on pelagic organisms and ecosystems. In: Gattuso J-P, Hansson L (eds) Ocean acidification. Oxford University Press, Oxford, pp 99-121

Rodhe J (1998) The Baltic and the North Seas: a process-oriented review of the Physical Oceanography. In: Robinson AR, Brink K (eds) The Sea, vol. 11. Wiley New York, pp 699-732

Runnegar MT, Jackson AR, Falconer IR (1988) Toxicity of the cyanobacterium *Nodularia spumigena* Mertens. Toxicon 26:145-151

Saifullah SM (1978) Inhibitory effects of copper on marine dinoflagellates. Mar Biol 44:299-308

Schulz KG, Riebesell U (2013) Diurnal changes in seawater carbonate chemistry speciation at increasing atmospheric carbon dioxide. Mar Biol 160:1889-1899

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

Sipiä VO, Kankaanpaa HT, Pflugmacher S, Flinkman J, Furey A, James KJ (2002) Bioaccumulation and detoxication of nodularin in tissues of flounder (*Platichthys flesus*), mussels (*Mytilus edulis*, *Dreissena polymorpha*), and

clams (*Macoma balthica*) from the northern Baltic Sea. Ecotoxicol Environ Saf 53:305-311

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

Solomon S, Qin D, Manning M, Alley RB, Berntsen T, Bindoff NL, Chen Z, Chidthaisong A, Gregory JM, Hegerl GC, Heimann M, Hewitson B, Hoskins BJ, Joos F, Jouzel J, Kattsov V, Lohmann U, Matsuno T, Molina M, Nicholls N, Overpeck J, Raga G, Ramaswamy V, Ren J, Rusticucci M, Somerville R, Stocker TF, Whetton P, Wood RA, Wratt D (2007) Technical summary. In: Solomon S, Qin D, Manning M, Chen Z, Marquis M, Averyt KB, Tignor M, Miller HL (eds) Climate change 2007: The physical science basis. Contribution of working group I to the fourth assessment report of the intergovernmental panel on climate change. Cambridge University Press, Cambridge and New York

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

Stal LJ, Albertano P, Bergman B et al. (2003) BASIC: Baltic Sea cyanobacteria. An investigation of the structure and dynamics of water blooms of cyanobacteria in the Baltic Sea – responses to a changing environment. Cont Shelf Res 23:1695-1714

Stigebrandt A (2001) Physical Oceanography of the Baltic Sea. In: Wulff FV, Rahm L, Larsson P (eds) A Systems Analysis of the Baltic Sea. Springer-Verlag Berlin Heidelberg, pp 19-74

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

Sunda WG (1989) Trace metal interactions with marine phytoplankton. Biol Oceanogr 6(5-6):411-442

Tamiya H (1957) Mass culture of algae. Annu Rev Plant Physiol 8:309-334

Thomsen HA (2016) Baltic Sea coccolithophores - an overview of insights into their taxonomy and ecology from the last 40 years. J Nannoplankton Res 36(2):97-119

Torstensson A, Hedblom M, Mattsdotter Björk M, Chierici M, Wulff A (2015) Long-term acclimation to elevated pCO2 alters carbon metabolism and reduces growth in the Antarctic diatom *Nitzschia lecointei*. Proc R Soc B 282:20151513

Utermöhl H (1958) Zur Vervollkommnung der quantitativen Phytoplankton-Methodik. Mitt int. Verein. theor. angew. Limnol. 9:1-38

Wacklin P, Hoffmann L, Komárek J (2009) Nomenclatural validation of the genetically revised cyanobacterial genus *Dolichospermum* (Ralfs ex Bornet et Flahault) comb. nova. Fottea 9:59-64

Wannicke N, Endres S, Engel A, Grossart H-P, Nausch M, Unger J, Voss M (2012) Response of Nodularia spumigena to pCO$_2$ – Part 1: Growth, production and nitrogen cycling. Biogeosciences 9:2973-2988

Wells ML, Trainer VL, Smayda TJ, Karlson BSO, Trick CG, Kudela RM, Ishikawa A, Bernard S, Wulff A, Anderson DM, Cochlan WP (2015) Harmful algal blooms (HAB) and climate change; What do we know and where do we go from here? Harmful Algae 49:68-93

Whitaker J, Barica J, Kling H, Buckley M (1978) Efficacy of copper sulphate in the suppression of Aphanizomenon flos-aquae blooms in prairie lakes. Environ Pollut 15(3):185-194

Wrona FJ, Prowse TD, Reist JD, Hobbie JE, Lévesque LMJ, Vincent WF (2006) Climate change effects on aquatic biota, ecosystem structure and function. AMBIO 35(7):359-369

Wulff A, Mohlin M, Sundbäck K (2007) Intraspecific variation in the response of the cyanobacterium Nodularia spumigena to moderate UV-B radiation. Harmful Algae 6(3):388-399

ZoBell CE, Anderson Q (1936) Observations on the multiplication of bacteria in different volumes of stored sea water and the influence of oxygen tension and solid surfaces. Biol Bull 71:324-342