

**Phenotypic and Genotypic Responses in the  
Planktonic Diatom *Skeletonema marinoi***

Effects of Natural Processes and Anthropogenic Stressors

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Doctoral Thesis



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*Till Johan, Benjamin och Jonathan.*

*“It always seems impossible until it's done.”*

-Nelson Mandela

**Keywords:** Diatom | Genotype | Phenotype | Resting cell | Eutrophication |  
Spring bloom | Diversity | Grazing | Adaptation | Microevolution | Phytoplankton

# ABSTRACT

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Diatoms are one of the most diverse and abundant phytoplankton taxa and are highly important as primary producers, accounting for nearly half of the marine primary production and constituting the base in the marine food web. Despite their high dispersal potential they are genetically diverse and display genetically structured populations.

The high genetic diversity enables quick adaptation to changes in the environment. The ocean is a dynamic habitat, changing rapidly in e.g. temperature, nutrients and salinity and phytoplankton have to adapt in order to persist. Natural processes such as blooming events, predation and resting cell formation are all part of phytoplankton ecology and may all have an effect on the genetic or phenotypic diversity. Eutrophication and other climate changes are stressors in the marine ecosystem; species or populations that fail to adapt will be outcompeted by those who succeed in evolving to the new conditions and thus get better fitness. This process decreases species diversity and can in the same way alter genetic diversity within species and populations, making them sensitive to further changes of the environment. Therefore it is important to understand how natural processes affect the genetic and phenotypic composition of phytoplankton and furthermore, to understand if and how they can adapt to anthropogenic stressors such as climate change, eutrophication etc.

In this thesis I have used the common diatom *Skeletonema marinoi* to study how natural processes and anthropogenic stressors affect the genotypic and phenotypic structures. By geminating natural populations from a sediment core, we have been able to do phenotypic characterization and genetic analysis that demonstrate an adaptation to eutrophic conditions over nearly a century. The phenotyping was done by an optimized method that is more time efficient than other methods and thus can manage large sample sizes.

Moreover, we successfully induced resting cells that remain viable for 12 months. This treatment did not have any negative effects on the growth rate of germinated cells, but induced sexual reproduction, which can facilitate further genetic studies.

The presence of grazers can influence the genetic composition within a phytoplankton population. By creating a population of eight genetically and phenotypically different *S. marinoi* strains and exposing them to three levels of grazing pressure we found that a medium grazing level significantly altered the genetic composition of the population.

Phytoplankton spring bloom dynamics in the Baltic Sea was studied by measuring environmental parameters and isolating *S. marinoi* strains during eight weeks, along a transect across Baltic proper. Genetic analysis showed that the spring bloom was a dynamic event and that it consisted of two genetically differentiated populations. This differentiation was coinciding with a spatial and salinity gradient. Moreover, a shift in the populations was seen as silica concentration decreased, indicating a subpopulation specialized to lower silica concentrations.

The genetic diversity in a population increases the adaptation potential, thus it is of great importance to study and understand how small fluctuations, natural processes and human induced changes in the environment affect the genetic and phenotypic structures in phytoplankton. My thesis contributes to the understanding of how the common diatom *S. marinoi* can adapt to present and future stressors such as eutrophication, and is an important piece of the puzzle to understand on-going changes in the marine environment.

# POPULÄRVETENSKAPLIG SAMMANFATTNING

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Kiselalger är en av de mest artrika växtplanktongrupperna och de beräknas bidra till nästan hälften av primärproduktionen i marina miljöer. Växtplankton är inte bara viktiga för att binda koldioxid från atmosfären och förse oss med syre utan har även en oumbärlig roll som basen i den akvatiska näringskedjan. I haven saknas det synliga barriärer och därför trodde man förr att alla marina mikroorganismer kunde sprida sig obehindrat och att det var en och samma population överallt. Detta har under de senaste 20 åren motbevisats i flera studier och mycket pekar på det motsatta; att det finns väldigt strukturerade samhällen av vitt skilda genetiska populationer, trots till synes obefintliga barriärer.

Havet är ett dynamiskt system där det sker snabba skiftningar i miljön; temperatur, salthalt och närsalter är några av de faktorerna som kan ändras på kort tid. För att kunna överleva i en sådan miljö är det fördelaktigt att vara anpassningsbar och de populationer som anpassat sig bäst blir framgångsrika och kan därmed konkurrera ut andra. Att ha en stor genetisk mångfald inom en population anses vara nyckeln till framgång, på så sätt är det större sannolikhet att någon genotyp har rätt förutsättningar och möjlighet att anpassa sig ifall det sker en förändring i miljön.

I min avhandling har jag använt mig av den i svenska kustvatten vanligt förekommande kiselalgen *Skeletonema marinoi* för att undersöka hur den genetiska sammansättningen (genotyperna) och de uttryckta egenskaperna (fenotyperna) påverkas av olika ekologiska processer. Algbloomning (paper V), predationstryck (paper VI) och långvarig vila i form av viloceller (paper III) är naturliga delar av livscykeln hos flertalet växtplankton och kan ha påverkan på den genetiska sammansättningen i en population. Utöver dessa naturliga processer utsätts även organismer för mänsklig påverkan i form av miljöförändringar t.ex. övergödning. Dessa miljöförändringar sker ofta inom en kort tidsram evolutionärt sett och kräver snabb anpassning för att organismer ska kunna överleva.

För att kunna förstå *om* och *hur* en växtplanktonpopulation kan anpassa sig till övergödning kläckte vi viloceller från sedimentlager från en kraftigt övergödd fjord (paper II). Vi odlade upp populationer som levde innan fjorden blev övergödd (1930-talet), under övergödningen (1950- och 1970-talet) och i modern tid (2010-talet). Genom att utsätta *S. marinoi*-populationerna för miljöförhållanden som liknar de innan övergödningen (låga närsalter och starkt ljus) samt under övergödning (höga närsalter och svagt ljus) mätte vi hur väl de olika individerna växte och kunde på så sätt avgöra hur välanpassade de är till de olika förhållandena. Denna studie gjordes med hjälp av en ny metod (paper I) som är mer tidseffektiv jämfört med andra metoder och på så sätt möjliggör experiment med hundratals individer. Dessutom sekvenserade vi individerna för att kunna se anpassningen på genetisk nivå. Våra resultat överensstämde med vår hypotes: att populationen från modern tid är bättre anpassade till en övergödd miljö. Den moderna populationen växte signifikant snabbare i höga närsalter och kunde nå högre cellantal under svagt ljus jämfört med de äldre populationerna. Detta visar på att de har anpassat sig från ett liv i låga närsalter och starkt ljus till en miljö med höga närsaltshalter och svaga ljusförhållanden. I den genetiska analysen i paper II valde vi att titta på förändringar på nukleotidnivå (baser) i gener som vi tror har betydelse för organismens närings- och ljusupptag och där kunde vi hitta flera

förändringar genom tiden i populationerna. Vi kunde hitta flera sådana nukleotider och se att dessa antingen ökade eller minskade i antal. Genom att jämföra tillväxtkaraktärerna (fenotyperna) med skillnader i nukleotidfrekvenser (ökning eller minskning), kunde vi koppla ihop vissa gener med tillväxtkaraktärerna i de olika behandlingarna. Detta tyder på att förändringarna har skett genom selektion och att fjordpopulationen under de 80 år vi tittat på har anpassat sig till en kraftig övergödd miljö. Den genetiska analysen visade också att det har skett en snabbare förändring på molekylär nivå de senaste 40 åren (mellan 1970 och 2010) än mellan (1930 och 1970).

I papper III ville vi undersöka om vi kunde få *S. marinoi* att bilda viloceller och se hur länge de klarar av att överleva i mörker. Syftet med studien var att undersöka om vi kunde använda denna naturliga egenskap hos algen för att långtidsförvara kulturer på labb. Vi ville också se hur egenskaperna förändrades efter en längre tid i mörker för att säkerställa att det inte har några negativa effekter på algens egenskaper (fenotypen). Vi lyckades att hålla dem vid liv i 12 månader i mörker och när vi mätte tillväxthastighet efter 12 månaders vila kunde vi inte se några negativa effekter.

I papper IV ville vi se om hoppkräftor som livnär sig på växtplankton kunde påverka den genetiska sammansättningen i en algpopulation. För att undersöka detta valde vi åtta genetiskt olika individer (genotyper) med olika egenskaper (fenotyper) i form av tillväxthastighet, PUA (en kemisk substans som har visat negativa effekter på fortplantningen hos hoppkräftor) produktion och kedjelängd och utsatte de för betare i olika koncentrationer. För att efterlikna naturliga populationer blandades de åtta genotyperna i samma proportioner och fick växa i stora behållare (ca 2000 liter) med olika betningstryck; låg (ca 15 betare/liter), medel (ca 80 betare/liter) och hög (ca 170 betare/liter). Efter 12 dagar provtog vi 50 algindivider från varje behållare och tittade på den genetiska sammansättningen. Våra resultat visade att det medelkraftiga betningstrycket förändrade genuppsättningen i populationen. Dels så minskade populationens genetiska diversitet och dels förändrades förhållandet mellan genotyperna (som hade tillförts i samma proportioner). Vi såg inte samma förändring i varken låga, höga eller i kontroll- behandlingen(utan betare).

I papper V följde vi en algblomning under våren längs med en sträcka med start i Finska viken i nordost, till Travemünde i sydväst. Vi mätte olika miljö parametrar och samlade planktonprover på fyra kryssningar under åtta veckor. Från planktonproverna isolerade och odlade vi upp kulturer av *S. marinoi* och genom genetisk analys kunde vi se att vårblomningen bestod av två genetiskt skilda populationer samt att det blev ett skifte av populationer styrt av låga kiselhalter. Våra resultat indikerar att det finns subpopulationer som är anpassade till skillnader i miljö och att dessa kan avlösa mindre välanpassade populationer. På så sätt kan en vårblomning bibehållas en längre tid och bestå av flera subpopulationer som hinner växa till.

Eftersom den genetiska mångfalden i en population är ett mått på hur väl den kan klara sig i en föränderlig miljö är det av stor vikt att veta hur populationer påverkas av naturliga processer och stressande miljöförändringar skapade av människan. Denna avhandling bidrar till en ökad förståelse för hur den globalt vanliga kiselalgen *S. marinoi* klarar framtidens påfrestningar i form av övergödning, vilket är en viktig pusselbit när vi försöker förstå pågående förändringar av den marina miljön.



# LIST OF PAPERS

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This thesis is based on the following papers:

- PAPER I:** Gross S., Kourtchenko O., Rajala T., Andersson B., Fernandez L., Blomberg A., Godhe A. (*in press*). Optimization of a high-throughput phenotyping method for chain-forming phytoplankton species. *Limnol. Oceanogr. Methods*.
- PAPER II:** Töpel M., Gross S., de Wit P., Rajala T., Kourtchenko O., Tesson S., Andersson B., Almstedt A., Axelson-Fisk M., Nilsson S., Fernandez L., Ellegaard M., Joest Andersen T., Nordberg K., Clarke A., Blomberg A., Johannesson K., Godhe A. Evolution of a key phytoplankton species to anthropogenic stress: evidence from revived strains accumulated in fjord sediments over the last century.  
*Manuscript*
- PAPER III:** Gross S., Kourtchenko O., Montresor M., Benvenuto G., Tesson S., Johansson E., Godhe A. Natural preservation – the resting stage of the chain forming marine diatom *Skeletonema marinoi*.  
*Manuscript*
- PAPER IV:** Sjöqvist C., Kremp A., Lindehoff E., Båmstedt U., Egardt J., Gross S., Jönsson M., Larsson H., Pohnert G., Selander E. 2014. Effects of grazer presence on genetic structure of a phenotypically diverse diatom population. *Microb. Ecol.*, 67: 83-95.  
doi:10.1007/s00248-013-0327-8
- PAPER V:** Godhe A., Sjöqvist C., Sildever S., Sefbom J., Harðardóttir S., Bertos-Fortis M., Bunse C., Gross S., Johansson E., Jonsson P R., Khandan S., Legrand C., Lips I., Lundholm N., Rengefors K E., Sassenhagen I., Suikkanen S., Sundqvist L., Kremp A. 2016. Physical barriers and environmental gradients cause spatial and temporal genetic differentiation of an extensive algal bloom. *J. Biogeogr.*, 43: 1130–1142. doi:10.1111/jbi.12722

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**Related publications not included in this thesis:**

Bunse C., Bertos-Fortis M., Sassenhagen I., Sildever S., Sjöqvist C., Godhe A., **Gross S.**, Kremp A., Lips I., Lundholm N., Rengefors K E., Seftom J., Pinhassi J., Legrand, C. 2016. Spatio-Temporal Interdependence of Bacteria and Phytoplankton during a Baltic Sea Spring Bloom. *Front. Microbiol.*7.  
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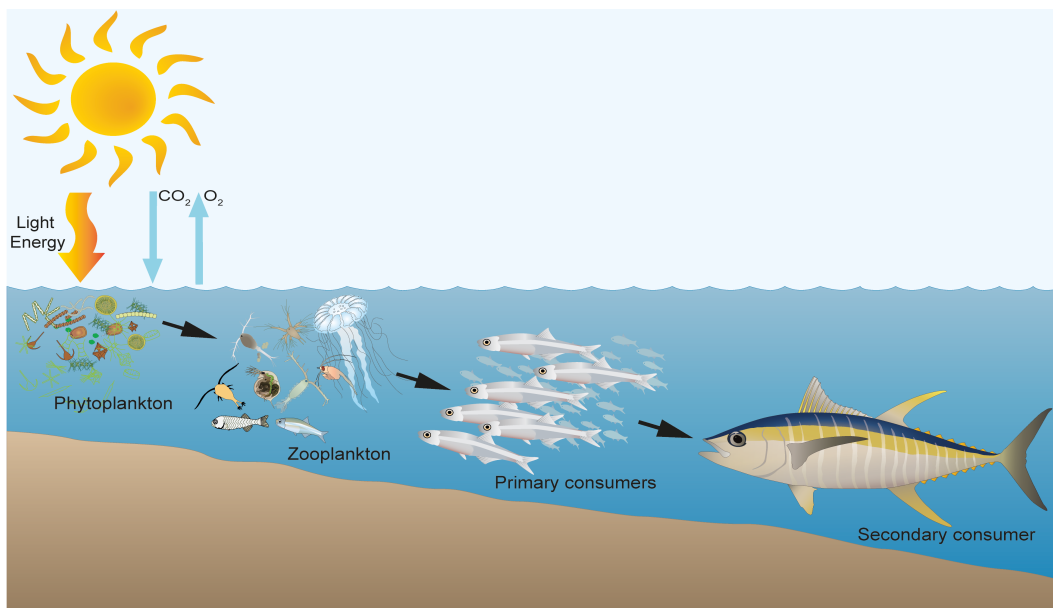
# BACKGROUND

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## Ecological importance of phytoplankton

One of the most dominant and diverse taxa in the phytoplankton community are the diatoms, a unicellular algae with ornamental silica frustules. They have a vast geographical distribution and can be found in aquatic and semi-terrestrial habitats. By dominating nutrient rich areas they are estimated to account for about 45 percent of the marine primary production (Armbrust et al. 2004) and are important for the silica cycle (Nelson et al. 1995). Diatoms, together with other phytoplankton taxa, form the base of the marine food webs as they are consumed by zooplankton, fish larvae, jellyfish and other filter feeders (Fig. 1). Through photosynthesis they convert sunlight and carbon dioxide into nutritious, carbon rich biomass, making the carbon available for higher trophic levels (Field et al. 1998). Phytoplankton are also an important part of the biological carbon pump; when sinking, they carry (vertically transporting) the bound carbon dioxide to the ocean floor, temporarily removing it from the atmosphere (Volk and Hoffert 1985). With this crucial placement in the bottom of the food chain and their important role in biogeochemical cycling, they undoubtedly affect the whole marine ecosystem (Sigman and Hain 2012).



**Fig. 1.** Simplified marine food chain with a mixed phytoplankton community as primary producers, zooplanktons as herbivores, anchovies as primary consumers and tuna as secondary consumer.

## Algal blooms

In temperate areas during early spring as light increases and the water column gets enriched by nutrients from below by vertical mixing (Sommer 1996), it becomes the optimal conditions for rapid phytoplankton growth and they can reach high cell concentrations and biomass (McQuoid et al. 2002; McQuoid 2002). With increasing numbers, algal cells have to compete for nutrients and light, and as the temperature rises, grazers and pathogens becomes more abundant and contribute to the pressure. Finally, when the nutrients are depleted and the light becomes limited the phytoplankton can no longer maintain growth and the bloom will come to an end. Algal blooms are highly dynamic processes (Sommer 1996) and there is a succession within the phytoplankton community; on the Swedish west coast, diatoms are the dominating taxa in early spring and dinoflagellates become more prominent during the later part of spring. In the Baltic Proper, the succession differs slightly; with diatoms and dinoflagellates dominating in early spring and gradually changing into a cyanobacteria bloom during the summer, until diatoms and dinoflagellates reappear again during autumn for a second but smaller bloom (Bianchi et al. 2002). However, there is also a succession within the phytoplankton groups e.g. different species of diatoms with different requirements replacing each other during a bloom (McQuoid and Godhe 2004) and on an even smaller scale, population shifts within diatom species have been seen (Gallagher 1980; Godhe and Rynearson 2017; Rynearson et al. 2006). These population shifts indicate that the high intraspecific genetic variation is expressed as high phenotypic variability that facilitates an adaptation to different environmental conditions, allowing a succession between populations and genotypes during a bloom, which corresponds to our findings in **paper V**.

## Genetic and phenotypic diversity

Due to their small size and high abundance it was long thought that phytoplankton species was unrestricted by biogeographic barriers and thus had an unrestrained pandemic distribution and gene flow (Fenchel and Finlay 2004). Consequently it was believed that there were no or insignificant genetic differences between or within populations of phytoplankton. This theory has since then repeatedly been challenged whereby several phytoplankton species exhibit highly differentiated populations and show strong population structures even on a small spatial scale (Alpermann et al. 2010; Gallagher 1980; Godhe and Härnström 2010; Rynearson and Armbrust 2004; Sjöqvist et al. 2015).

The genotypic variability seen within species and populations may be expressed as physiological traits and responses. Studies have also shown that, even though having no visible boundaries, phytoplankton populations are highly specialised and adapted to their habitat, where pH, salinity, and temperature have been shown to be important drivers (Alpermann et al. 2010; Sildever et al. 2016; Sjöqvist et al. 2015; Sjöqvist and Kremp 2016). With consideration to this phenotypic variability, drawing ecological conclusions based on the phenotypes of only one or a few genotypes may not reveal the true response to stressors in a species or population.

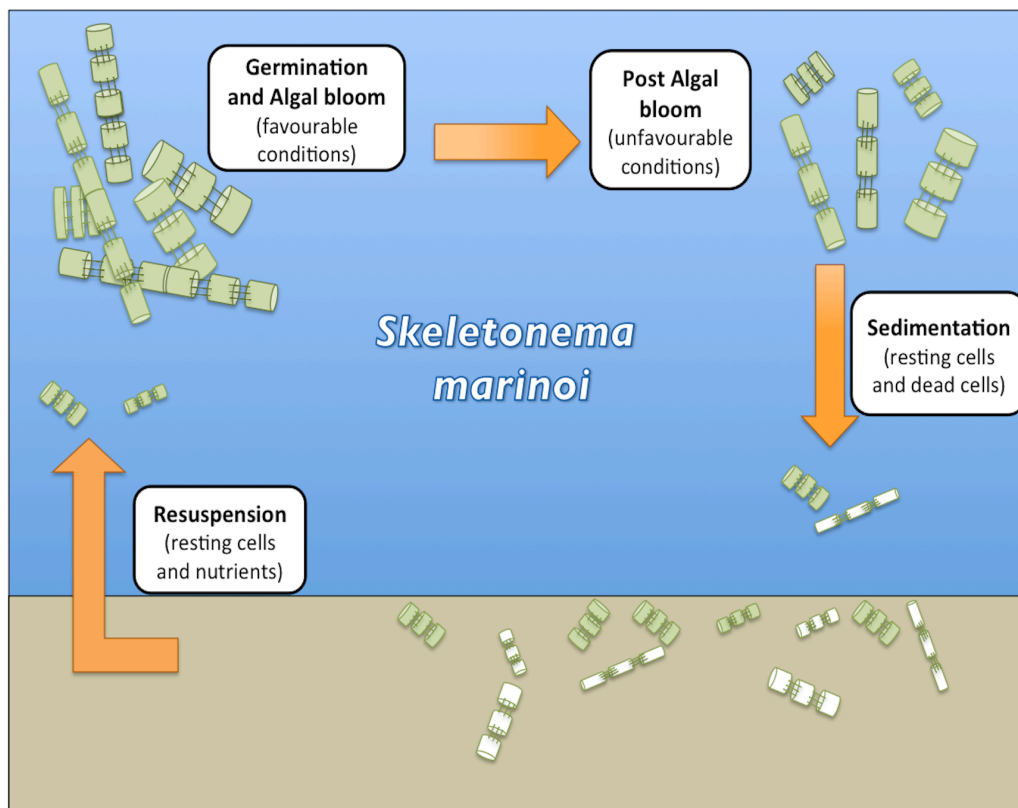
## Characterizing genetic and phenotypic diversity

The majority of the recent phytoplankton population genetic studies have used microsatellites, which are non-coding regions of repetitive nucleotide sequences. These sequences are highly variable among individuals and are thus suitable for genetically distinguishing individual strains (genotypes) and for studying population structures. Since the molecular revolution, the increasing efficiency and lowered cost of molecular methods has also opened up the availability for full genome sequencing which can show individual differences on a nucleotide level (single nucleotide polymorphism; SNP) and thus identifying genes under selection (Luikart et al. 2003). However, generating large amounts of genomic data also increases the demand for high throughput phenotyping, because without the possibility to couple genes to functions and traits, a lot of vital sequencing information is futile. For many organisms current phenotyping methods are time consuming and so the techniques are lagging with a need for optimization and improvements (Granier and Vile 2014). Measuring traits like growth rate can reveal how well a genotype is adapted to a certain environment, and the yield (cell density) indicates efficiency of nutrient utilization (Blomberg 2011). In diatoms, measuring growth patterns are the most common ways to study responses to environmental factors and stress. The systems available for phytoplankton today are usually limited to solitary species (i.e. do not form chains or colonies) or have the drawback of being destructive, i.e. there is a need for sampling which requires larger volumes of cultures. Also, it is still common practice to manually count cells by light microscopy, which also requires sampling and is a highly time consuming and tedious work. In **paper I** we present a high throughput method for studying phenotypes of chain-forming phytoplankton species which makes it possible to measure the growth of hundreds of samples as seen in **paper II**. Consequently this method will assist in allowing for work where phenotypic characteristics can be coupled with genomic data.

## Natural processes

### *Resting cells*

Resting stage formation is a trait seen in many phytoplankton species (McQuoid et al. 2002; McQuoid and Hobson 1996), however, what actually drives this process is not entirely clear but there might be several benefits of forming resting stages (Smetacek 1985). For diatoms, resting stage formation is believed to be an adaptation to avoid unfavourable conditions occurring at the end of an algal bloom, when nutrients are depleted, light is poor and the grazing pressure and pathogens are increasing (Smetacek 1985; Smetacek and Cloern 2008). By sinking and forming dormant resting cells, that can endure long periods of dark and hostile environments, the cells can avoid predation and await more favourable conditions (Fig. 2). Some diatom species have the ability to survive in sediments for ~150 years (Härnström et al. 2011; Lundholm et al. 2011) and by resuspension these seed banks are thought to have the potential to reseed the habitat and in this way anchor the population to a location (McQuoid and Hobson 1996).



**Fig. 2.** In unfavourable conditions, resting cells are formed and sink to the bottom. Resting cells get accumulated in the sediment where they can remain viable for long periods. An upwelling event can resuspend the resting cells and if the environment provides favourable conditions they will germinate and can recolonize the habitat.



Furthermore, by having an accumulation of resting cells these can serve as gene pool storage and provide a present population with a range of diverse alleles. Theoretically this would allow a population to maintain a high genetic diversity and thus making the population more prone to adapt to and persist in a changing environment (Templeton and Levin 1979). In **paper III**, we have studied resting cell formation in the diatom *Skeletonema marinoi* and investigated the germinated cells for any changes in fitness (growth rate) due to dormancy.

### ***Predation-prey interaction***

Centric diatoms are non-motile and cannot actively avoid threats like grazers (Fig. 3) and pathogens. However, some diatoms have developed some other defence mechanisms; in the presence of copepods *S. marinoi* has been observed to reduce their chain-length, which makes them harder to be detected and eaten by the copepods (Bergkvist et al. 2012). Moreover, it has been proposed that some species of diatoms may have chemical defence systems against grazers. Many diatoms can produce polyunsaturated aldehydes (PUA), a compound which has been seen to have a negative effect on the hatching success of copepods and also causing reduced bacterial growth (Pohnert et al. 2002; Ribalet et al. 2008). The direct effects of PUAs is however still questioned as it is not clear if PUAs alone are the accountable compounds responsible for these negative effects (Ianora and Miralto 2010). What is known is that predator-prey interactions are one of the strongest ecological driving forces that consistently alter phenotypic traits (e.g. prey size) and thus may influence the genetic composition of a population (Langerhans 2007; Selander et al. 2011; Selander et al. 2015) as studied in **paper IV**.



**Fig. 3.** Close-up photograph of a copepod grazing on phytoplankton.  
*Photo: Erik Selander*

## Anthropogenic stressors

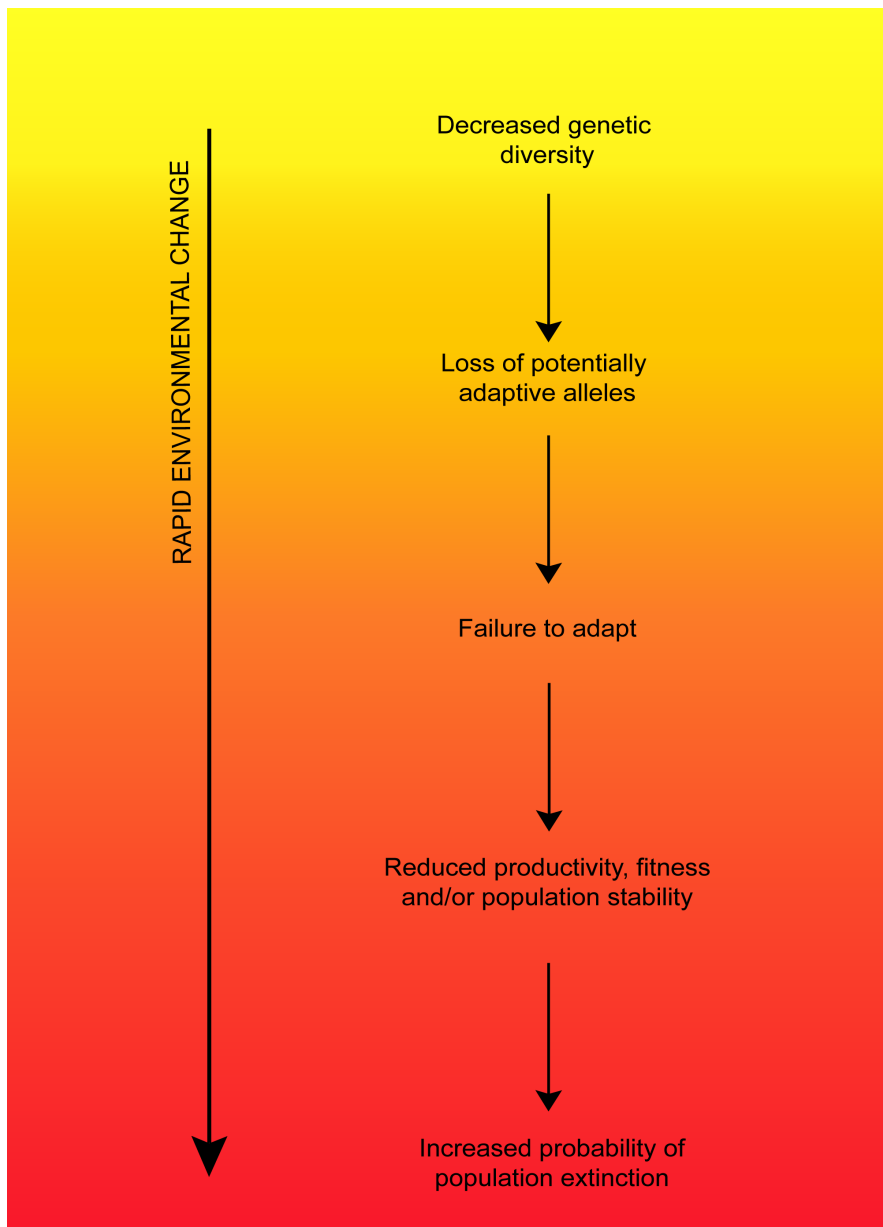
### *Eutrophication- causes and consequences*

Increased input of nutrients from e.g. land use and agriculture during the past century has had large effects on the marine ecosystems. Today, eutrophication is one of many increasing ecological threats to the marine environment. Elevated nutrient levels facilitate phytoplankton blooms and they can grow faster and reach higher cell densities. The increased primary production may however have negative effects, such as higher turbidity and bottom anoxia, which negatively affects the benthic flora and fauna (Bonsdorff et al. 1997; Smith and Schindler 2009; Österblom et al. 2007) causing regime shifts e.g. from slow-growing macrophytes to filamentous macroalgae (Pihl et al. 1995; Smith and Schindler 2009). Furthermore, stressors like eutrophication can lead to lowered biodiversity, as a species that adapts faster to the new environment will outcompete those with lower fitness, and similarly it may also alter the genetic diversity within species or on a population level (Fig. 4). In a population with lower or lost genetic variation, the population is believed to become more sensitive to stressors and environmental changes as there is a reduced “genetic reservoir” to select from, in case of a new disturbance (Marques et al. 1997). Moreover, low genetic variation can also have a negative impact on the population in terms of productivity, as seen in algal cultures where mixed genotypes were more productive compared to monocultures (Bell 1991).

### *Adaptation*

Anthropogenic environmental change is usually a rapid process, which is causing a strong selection pressure on a population. The exposed population will respond to the new regime by adaptation, or fail and get extinct (Chevin et al. 2010; Jump et al. 2009). Adaptation can either be achieved by selection on the genetic variation already present in a population or by novel mutations. Adaptation by newly formed mutations is however likely to be much slower than that of already existing alleles; as the appearance of a beneficial mutation can take time, whereas the alleles fixed within a population are already present and have been selected for in an earlier phase (Barrett and Schluter 2008; Jump et al. 2009). To have a large genetic variation within a population is thus a major advantage and can make the difference between successful adaptation and extinction.

The large genotypic (and phenotypic) diversity within phytoplankton and diatoms especially, is believed to be the key to successful adaptation to changing environments (Godhe and Rynearson 2017) and species with the ability to form resting stages may have an even more diverse allele pool, thus increasing the potential for rapid adaptation (Templeton and Levin 1979).



**Fig. 4.** Schematic figure describing a negative genetic cascade in a population due to rapid environmental change. (Adapted from Jump et al. (2009))

# AIMS AND OBJECTIVES

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The aim of the thesis is to contribute to the knowledge of diatom ecology and evolution in general, but also to increase the understanding of the specific population ecology and evolutionary processes in the non-model organism *Skeletonema marinoi*. I wanted to investigate if and how natural processes and fluctuations such as grazing; dormancy, spring blooms, and anthropogenic stressors such as eutrophication affect the genetic composition and phenotypic expression in *S. marinoi*.

**Paper I:** In this study our aim was to establish an effective high-throughput phenotyping method in order to facilitate growth measurements for hundreds of strains needed for ecological studies such as **paper II**. We evaluated and optimized a method where *S. marinoi* strains were cultured on plates in different treatments and growth parameters were measured in a plate reader by using *in vivo* fluorescence.

**Paper II:** In order to investigate how *S. marinoi* has adapted to human induced eutrophication during the past century we conducted (1) phenotypic and (2) genotypic characterizations of newly hatched populations from four sediment layers originating from 1930, 1950, 1970 and 2010, and finally (3) we coupled the phenotypic traits to the genotypic differences.

**Paper III:** In order to explore the possibility to use the natural feature of resting cell formation as a mean of long term storage we examined resting cell formation by (1) inducing resting cells, (2) examining the viability and (3) looking at what effects the treatment had on the phenotypes.

**Paper IV:** To determine the effect of grazing on population structure we carried out a mesocosm study where grazers were added to a cultured *S. marinoi* population consisting of eight distinct strains. The strains were genetically and phenotypically different in terms of growth rate, PUA production and chain length reduction. After the exponential phase strains were isolated for genetic analyses to see shifts in population structure due to grazing pressure.

**Paper V:** Our aim was to describe the population structure in a phytoplankton spring bloom and to distinguish what environmental conditions are driving the progression of the bloom. During one month, we repeatedly sampled *S. marinoi* strains for genetic analyses and several environmental parameters were monitored along a SW–NE transect across the Baltic Sea.

# METHODS

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## Study species

The marine chain-forming diatom *Skeletonema marinoi* Sarno et Zingone (Fig. 5) was used for all the studies in this thesis. This is a common species in the coastal phytoplankton community in Scandinavian waters (Kooistra et al. 2008; Sarno et al. 2005). As a primary producer it is important for the nutrient cycling and as food for higher trophic levels. During the algal spring bloom, they can reach extremely high concentration (millions of cell per liter), which is also mirrored in the coastal sediments in terms of resting cells. Studies have estimated up to 3.5 million resting cells  $g^{-1}$  sediment with the potential to get resuspended and recolonizing the water column (McQuoid 2002).

*S. marinoi* mainly reproduces asexually through cell divisions, with a gradual reduction of the cell size. As a critical size is reached, where further division is no longer possible, they undergo cell enlargement. The enlargement of cells can be attained by sexual or asexual cell reproduction whereby the cell size is restored (Gallagher 1983).



**Fig. 5.** Vegetative cells of the chain-forming marine diatom *Skeletonema marinoi*.  
*Photo: Josefin Sefbom*

Besides being a key species in the coastal ecosystem, *S. marinoi* has several properties making it a beneficial organism to work with; it is easy to isolate and to maintain in culture. It has high genetic diversity within and between populations (Godhe and Härnström 2010; Sjöqvist and Kremp 2016), which makes it a useful candidate to study adaptation and responses to predation (**paper IV**), and population structures driven by environmental factors (**paper II and V**). Furthermore, the possibility to germinate and study resting cells (**paper III**) makes it an excellent candidate for resurrection biology (Burge et al. 2017; Godhe and Rynearson 2017; Härnström et al. 2011) to shed light on evolutionary processes such as adaptation to environmental stressors (**paper II**).

## Sampling areas

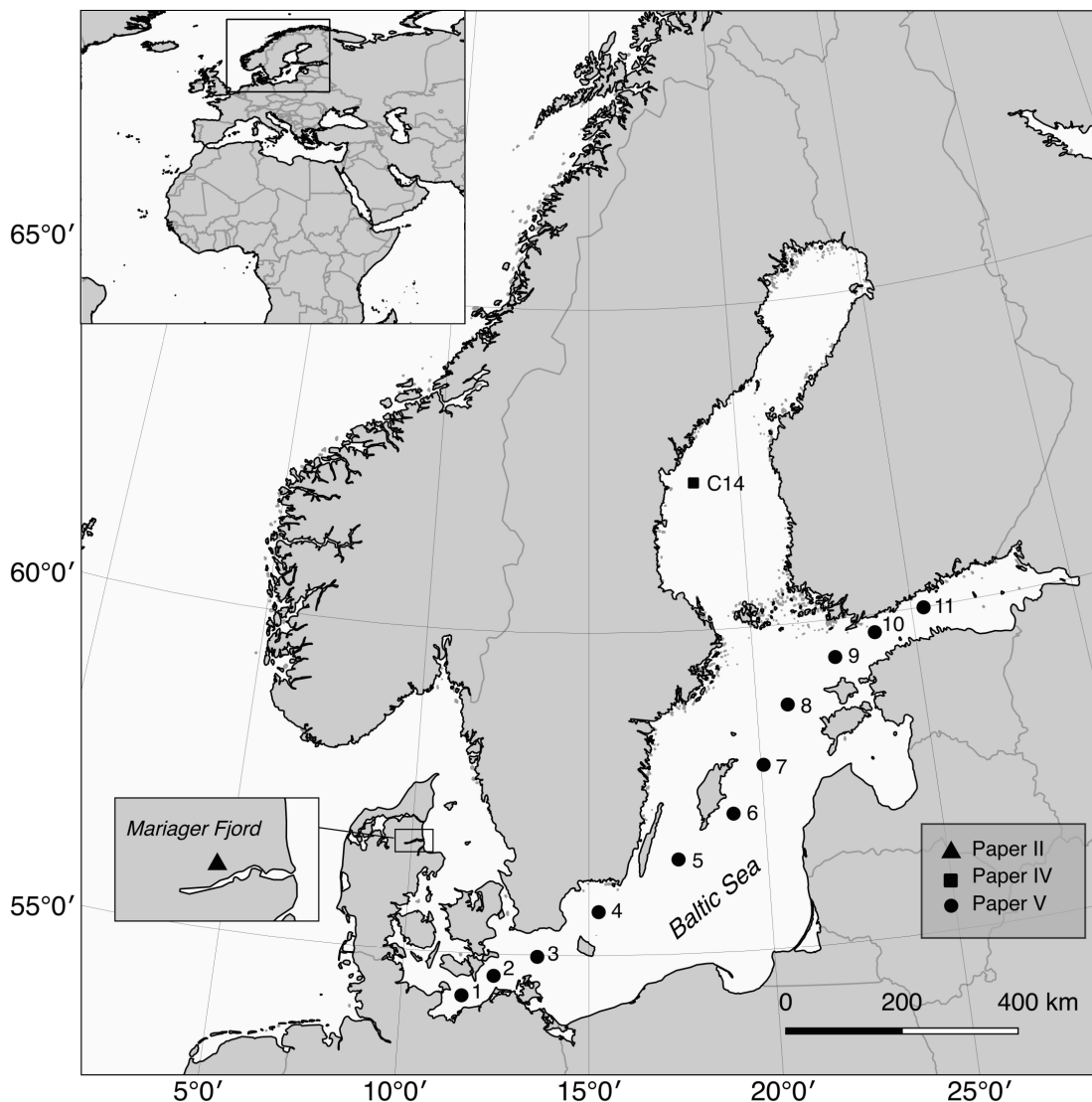
### *Mariager Fjord (paper II)*

Sediment cores were collected in Mariager Fjord (Denmark, 56.678267°N, 10.023517°E) in 2014. The cores were sliced in one-centimetre sections from top to bottom, with the exception of the three upper centimetres, which were sampled as one top layer. To avoid contamination between layers, the outer parts of each slice were removed before sampling. Subsequently, subsamples from the layers were dated by analysis activity of  $^{210}\text{Pb}$ ,  $^{226}\text{Ra}$ , and  $^{137}\text{Cs}$ . This was done at the Gamma Dating Centre, Department of Geography, University of Copenhagen as described by Härnström et al. (2011).

Mariager Fjord (Fig. 6, triangle) is located at the east coast of northern Denmark, adjacent to Kattegat. Due to its properties with a narrow and shallow inlet, water exchange is slow and irregular. The fjord has a long history of high nutrient input that increased with the extensive agriculture during the 1950s (Ellegaard et al. 2006). The deeper basins are permanently anoxic and in the summer of 1997 the anoxia spread throughout the whole water column, killing most of the eukaryotes (Fallesen et al. 2000). In the anoxic basins, the sediment is undisturbed due to the absence of bioturbators and provides laminated sediment suitable for isotope dating and for resurrection biology, thus Mariager Fjord is a valuable system for the study of micro-evolutionary processes. Germinated *S. marinoi* resting cells accumulated in Mariager Fjord sediment has been shown to be genetically isolated from the open sea population for at least the past decade (Härnström et al. 2011), despite the connection to the open sea.

## *Baltic Sea (paper IV and V)*

The strains used in **paper IV** were isolated from the surface (upper 10 cm) sediments of a core collected in 2011 at the monitor station C14 (62.1208°N, 18.5539°E) in the Bothnian Sea. (Fig. 6, square C14). For **paper V** all samples and data were collected from the Baltic Sea in 2013, following a transect from southwest (Travemünde, Germany, 53.9667°N, 10.8667°E) to northeast (Helsinki, Finland 60.1708°N, 24.9375°E). On four cruises during the spring bloom (in March and April) environmental data was recorded by an automatic measuring system installed on the cargo vessel MS Finnmaid and *S. marinoi* strains were isolated from water samples taken from 9-10 stations per cruise (Fig 6, circles 1-11).



**Fig. 6.** Map of sampling areas in **papers II, IV and V**. Mariager fjord (triangle), from which sediment cores were collected for the study in **paper II**. Sampling station C14 in Bothnian Sea (square), where surface sediment was collected for the mesocosm study in **paper IV**. The circles in the Baltic Sea show the fixed sampling stations along a transect following the bloom in **paper V**. Map: Malin Olofsson.

The Baltic Sea is the second largest brackish water body in the world and it consists of several large basins, which all differ in size, depth, and hydrography. Due to a long, shallow and narrow connection with the North Sea across the Danish straits the water exchange is irregular. The limited seawater inflow with a large freshwater input from the catchment area creates a salinity gradient across the Baltic Sea, with the highest surface salinity of 10-7 by the inlet in SW (Danish Straits), 8-6 in the large central basin (Baltic Proper) and to the lowest salinity of 3-2 in N and NE (Bothnian Bay and Gulf of Finland) (Rodhe 1998; Sjöqvist et al. 2015; Stigebrandt 2001). A longitudinal temperature gradient is found and is nearly coinciding with the SW-N/NE salinity gradient. Shifts in nutrient levels can be found on smaller scales, with higher levels usually prevalent at the coastal areas, which are mainly deriving from riverine input (Stålnacke 1996). The Baltic Sea is newly formed (~8000 years) and many of the species are originating from the North Sea and have adapted to a life in brackish water, living close to the margin for their salinity tolerance (Johannesson and Andre 2006). Due to the physical (Danish straits) hydrological barriers (e.g. salinity) there is a lower species diversity observed among a wide range of taxonomic groups and they also display a lower genetic diversity than in the adjacent North Sea (Johannesson and Andre 2006; Sjöqvist et al. 2015). This lower diversity and with species living in this marginal habitat has been predicted to have serious consequences on the ability to recover from increasing environmental stressors such as eutrophication and climate change.



# MAIN FINDING OF THE THESIS

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Method for high-throughput phenotyping

**Unpublished-Not available electronically**

With this method it is possible to phenotype hundreds of strains, as seen in **paper II**, and thus acquire a broader and more accurate estimate of the variation within populations. Only two phenotype features were used in **paper I** but there are also other features that can be investigated by this method, e.g. time to maximum cell density (used in **paper II**), length of lag phase or stationary phase, kill curves etc.

Effects of rapid anthropogenic environmental change

**Unpublished-Not available electronically**

## Response to natural processes

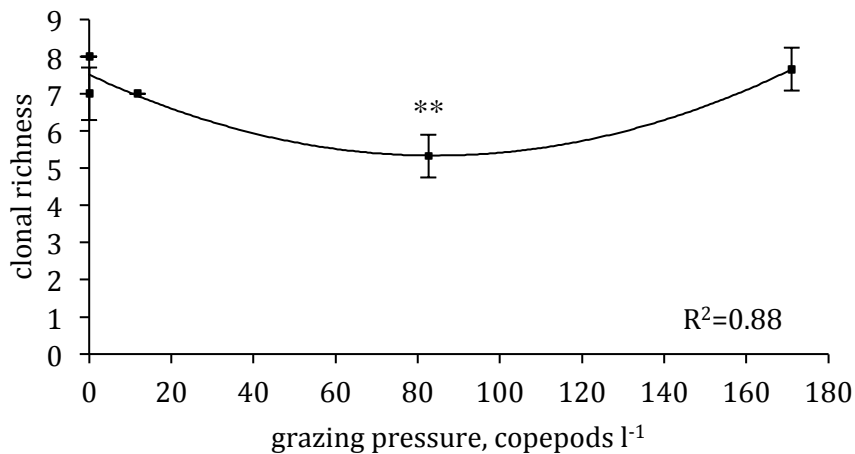
***Resting cells are not negatively affected by short-term dormancy***

**Unpublished- Not available electronically**

## ***Grazers may influence genotypic diversity in phytoplankton populations***

For the study in **paper IV**, eight newly established monoclonal cultures of *S. marinoi* were verified to be genetically distinct genotypes by using eight microsatellite loci (S.mar 1-8; as describe in Godhe and Hårnström 2010). The strains were grown as monocultures and were phenotyped with regards to maximum growth rates, and in the presence of grazers, PUA production and reduction in chain length was measured. The eight strains formed the populations used in the mesocosm study and a mixed population of copepods were added in three different densities; low ( $\sim 15$  copepods  $l^{-1}$ ), medium ( $\sim 80$  copepods  $l^{-1}$ ) and high ( $\sim 170$  copepods  $l^{-1}$ ) to see how the grazing pressure affected the genetic diversity in the *S. marinoi* population.

The strains expressed phenotypic differences in growth rates, PUA production, and chain length reduction. Chain-length was reduced in all strains with grazers present, however the PUA production did not increase in the presence of grazers, and was therefore not considered as a direct defence mechanism against grazing. The mesocosm showed that medium level of grazer pressure significantly lowered the strain diversity in a population (Fig. 9). Low and high grazer levels did not alter the clonal diversity, suggesting that there may be some density dependent defence mechanism initiated by higher grazer densities. By the end of the mesocosm experiment, the two strains that dominated the populations in all the treatments were those with the longest chains in the presence of grazers, which is the opposite of the expected result; as these should be more easily encountered and thus consumed by the grazers. Also, there seemed to be no clear grazing preference towards strains with lower PUA production.

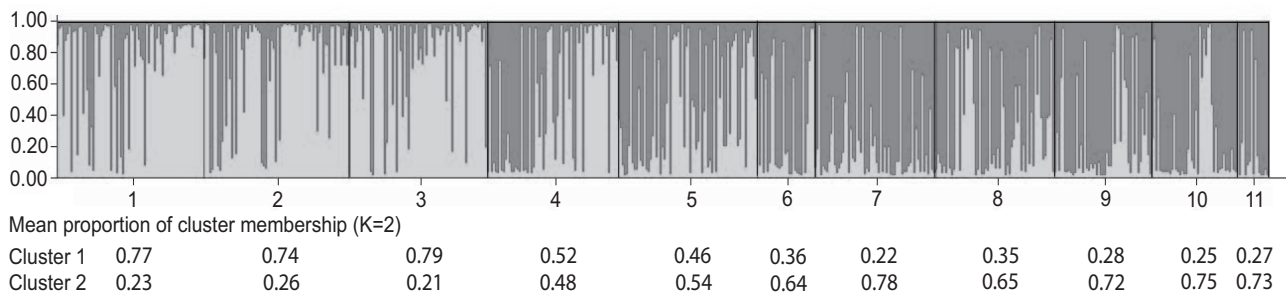


**Fig. 9.** Clonal richness at the end (day 12) of the mesocosm experiment (**paper IV**) based on microsatellite genotyped isolates from the three different treatments with low (~15 copepods l<sup>-1</sup>), medium (~80 copepods l<sup>-1</sup>) and high (~170 copepods l<sup>-1</sup>) grazer pressure. Control (no grazers) and initial values are positioned at zero on the x-axis. \*\*p<0.001

### ***Population structures and shifts during spring bloom***

In **paper V** we saw that the Baltic spring algal bloom in 2013 was initiated simultaneously in each end of the transect; with the German coastal areas in the southwest and the Finnish coast in the northeast. Both coasts had high inorganic nutrient concentrations at the start of the bloom, with highest concentrations seen in the northeast. The temperature was higher in southwest and remained constant during the three first cruises. As the bloom began to decline in the southwest it propagated offshore. By the end of the bloom, nutrients were depleted or low throughout the whole transect. Silica concentrations were significantly lower

during the last cruise. *Skeletonema marinoi* accounted for 33% of the biomass in the bloom and the population genetic study recognized two genetically differentiated populations (Fig. 10). These population genetic patterns were correlated to spatial distance that in this case also is correlated to a salinity gradient. Moreover, in the second half of the bloom, a temporal shift in population structure was observed. This shift was best explained by a reduction in silica concentration. Thus, besides being a spatial structure there is also a temporal succession, where genotypes that may be better adapted to low silica conditions could replace the predecessors.



**Fig. 10.** Genetic clustering of *S. marinoi* based on eight microsatellite loci. Each bar represents one of the 611 individuals that were sampled from the fixed sample locations (1-11) across the Baltic Sea during four cruises conducted during March to April 2013.

# DISCUSSION, CONCLUSIONS AND FUTURE PERSPECTIVES

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This thesis focuses on investigating how different natural processes and anthropogenic induced eutrophication affect the common diatom species *Skeletonema marinoi* in terms of genotypic and phenotypic changes. Algal blooms, resting stages and predation pressure are vital parts of a species' life cycle and are most likely important driving factors, affecting the genotypic and phenotypic diversity. In addition to the natural processes, organisms are also exposed to environmental changes caused by human activities. These changes require organisms to rapidly respond in order to survive in the new environment.

The ocean is a highly dynamic environment with changes in abiotic parameters; variation in light, nutrient and salinity. To sustain a fluctuating environment requires high phenotypic variation and is consequently a strong driving force for genetic differentiation. Indeed, the genotypic succession seen during a bloom (**paper V**) implies that high genetic diversity within a population facilitate adaptations even to small shifts in the habitat. By having specialized subpopulations that are highly adapted to specific conditions (e.g. decreased silica concentrations, as seen in **paper V**), it is possible to maintain the bloom over longer periods even with changes in the surrounding environment. The population structures we observed in the spring bloom coincided with a salinity gradient. However, with the projected climate changes including increased precipitation and temperature in the Baltic Sea, these population structures are likely to change as the environment changes.

Population structures may also be altered by grazers, as revealed in **paper IV**. The results from the mesocosm experiment showed that grazers could affect the genetic diversity within a phytoplankton population. This highlights that grazing can be an important factor influencing population structures of phytoplankton. Chain length was reduced in all grazer densities, whereas an increase was seen in the control without grazers. This indicates that the grazer density in all treatments were high enough to get detected by the algal population. However, only the medium grazing pressure affected the algal genetic diversity, which indicates that at high grazer density the copepods cannot be selective and eat all the individuals they encounter, while in the low density treatment the encounter rate is too low to have a significant effect on the genetic diversity.

Interestingly, the strain that was most abundant in the end of the mesocosm study in all of the treatments had one of the lowest growth rates when growing in optimal conditions as a monoculture, and further more, it had the longest chains in the presence of grazers. This implies that phenotypic traits in monoclonal cultures are not necessarily the same in a multiclonal mix, and there might be mechanisms or factors other than growth rate and chain length that made this particular genotype successful in this predator-prey interaction. It is possible that the strain produces some compounds that repel the grazers or there may be some interactions between the algal strains that favour this particular genotype. This study highlights the complexity of phytoplankton ecology and the importance of studying several strains and phenotypes when performing experiments.

The ability to form resting cells is believed to facilitate the high genetic variation within populations and is probably contributing to the genetic differentiation between populations. Yet, little is known about the resting cell, how they are formed, and how the process is affecting the phenotypic traits. In **paper III** we kept experimentally induced resting cells of *S. marinoi* viable for 12 months, which is longer than previously reported for this genus. The growth rate was not negatively affected by this treatment. In natural sediment cores *S. marinoi* cells can remain viable for ~150 years (Härnström et al. 2011; Lundholm et al. 2011), but in laboratory conditions the viability is limited due to factors still unknown. However we believe that by better mimicking the conditions found in natural sediments, we could further prolong the viability of the induced resting cells. Additionally, we observed that *S. marinoi* was triggered to undergo cell enlargement after dormancy. With further studies (Gross et al. unpublished) we have been able to demonstrate that the post-dormancy cell enlargements were sexually produced, which is an important insight into the ecology of *S. marinoi* and by experimentally controlling sexual reproduction events it allows us to do e.g. pedigree studies to see how alleles are inherited. It would also be informative to do further in depth molecular studies on resting cells in order to see if and what kind of processes are active during the dormancy period.

Based on the findings in my thesis it is clear that *S. marinoi* is genetically and phenotypically diverse. Grazers can alter the diversity by feeding on preferred strains or by avoiding feeding on strains with certain phenotypic properties and this could also be a driving force for genetic differentiation. High genetic diversity allows a population to exist in an environment with fluctuations in e.g. nutrients and temperature and may facilitate rapid adaptation.

We have demonstrated that *S. marinoi* has the potential to adapt to a rapid change in the environment as we could show changes in the phenotypic response through time (**paper II**). By observing these changes on a molecular level and couple it to the phenotypic characters the occurrence of micro-evolutionary events is evident. The study was performed using a fjord population but this setup could also be applied to other well-monitored systems, e.g. power plant basins with cooling water outlets or mining sites and could use other treatments to estimate the evolvability to e.g. changes in temperature, heavy metals etc. Studies like this can increase the understanding and help us estimate how phytoplankton communities are likely to respond to present and future stressors.

To fully understand the ecology and ecosystem functioning of a species is a difficult task. There are many interactions, which controls and influences the population structures and thus change the outcome of a study (as seen in **paper IV**). In order to be able to get a more correct estimation of how a genetically diverse natural population is affected by disturbances it is important to include many individuals in a study. This enables detection of genetic and phenotypic variation found within a species. The high-throughput phenotypic method we developed for *S. marinoi* in **paper I**, makes it possible to include more individuals and get a more reliable estimate of the diverse responses seen in populations and systems, thus we foresee that it will become common practice to use this or similar methods within phytoplankton ecology.

The ecological importance of these primary producers can't be stressed enough and even though every answer gives rise to new questions I am confident that it will gradually bring us to a closer understanding of the ecology of phytoplankton.

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