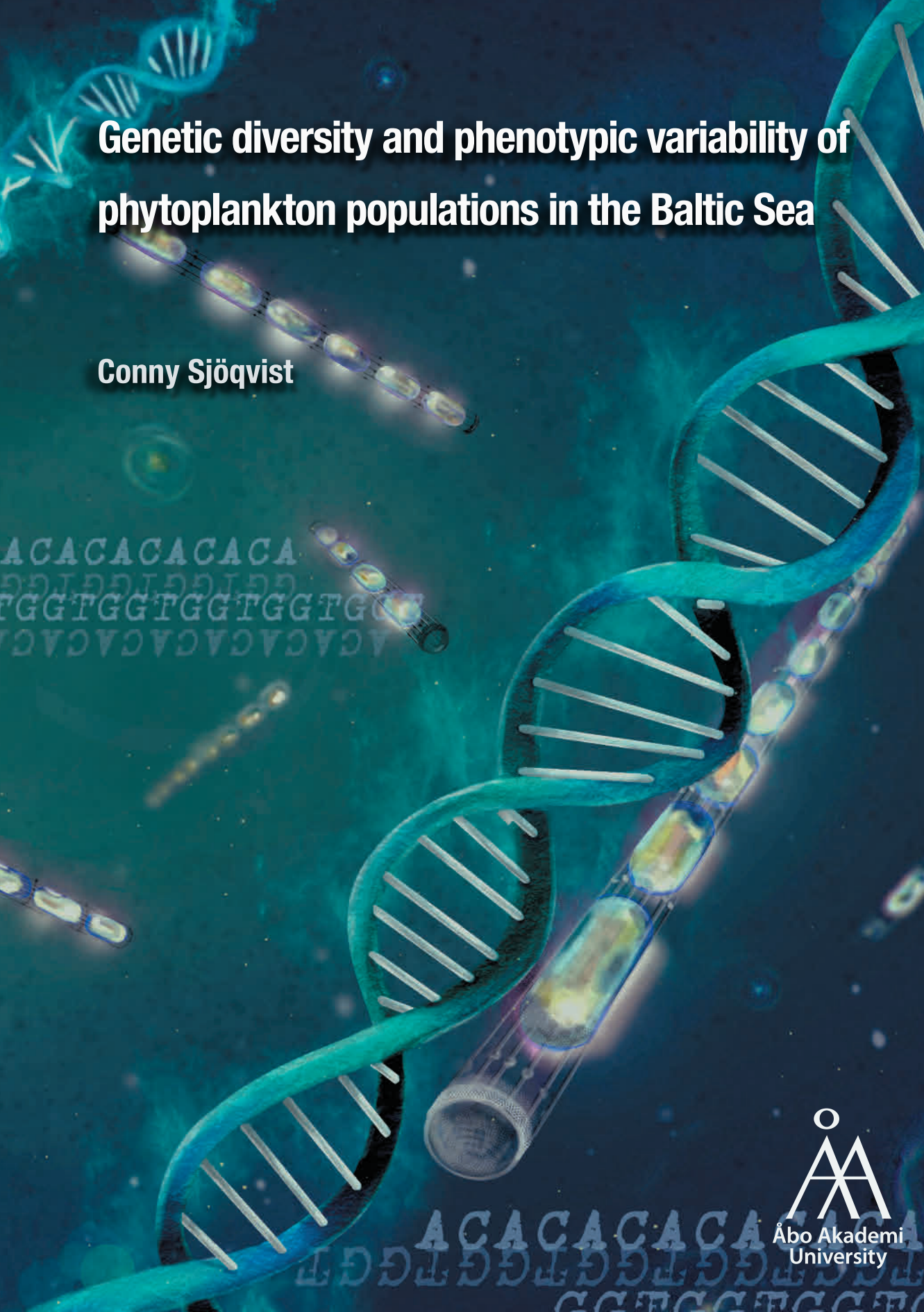
Genetic diversity and phenotypic variability of phytoplankton populations in the Baltic Sea

Conny Sjöqvist



Åbo Akademi University

GENETIC DIVERSITY AND PHENOTYPIC VARIABILITY OF PHYTOPLANKTON POPULATIONS IN THE BALTIC SEA

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”I tried my best to embrace the darkness in which I swim, to realize, all I was searching for was me”

Ben Howard, Keep your head up

Abstract

A fundamental aspect of nature is the immense quantity of variation that exists between organisms and the ecosystems they inhabit. This source of variation leads us to classify organisms into separate species based on morphologic differences or more recently by observing differences at the individual, genetic, level. The development of molecular techniques has substantially increased our understanding of sub-species level variation.
One of the most common phytoplankton species in the Baltic Sea during the productively important spring bloom is the marine diatom *Skeletonema marinoi*. I used this species as a model for phytoplankton populations in the area, with the aim of unraveling genetic and phenotypic diversity along environmental gradients over spatial and temporal scales. An important aspect of this thesis was the investigation of factors that may configure the level of genetic diversity and differentiation. I also studied the link between the level of genetic diversity and important ecophysiological parameters such as, primary production and nutrient content.

By using microsatellite markers I found that the level of genetic diversity in *S. marinoi* is reduced in the Baltic Sea compared to neighboring populations in the eastern part of North Sea. The species is genetically structured displaying one distinct population in the Baltic Sea and another population north of the Danish Straits. Adaptation to local salinity regimes of genetically differentiated populations was observed experimentally. The gene flow between populations was strongly correlated with an oceanographic connectivity model. The genetic structure of an extensive bloom across the Baltic proper was spatially stable during March and April. The sub-populations within this bloom also correlated with environmental variables such as, salinity and dissolved silica. Genetic diversity of Baltic Skeletonema was coupled with phenotypic diversity – my studies revealed extensive variation of ecologically important traits among clones. I experimentally showed that grazers are able to modify the clonal richness of phenotypically diverse *S. marinoi* populations. Increasing levels of clonal richness led to higher performance in the question of primary production and enhanced stability of ecophysiological functions.

As shown in this thesis, a species most often consists of numerous genetic variants associated with phenotypic differences. Knowledge about such intra-specific variation is a prerequisite if we want to understand why and where species occur and forms the basis for predicting adaptation of species to future environmental conditions.

Keywords: marine phytoplankton, genetic diversity, population structure, phenotypic variability, ecophysiology

Sammanfattning

En av naturens mest grundläggande aspekter är den enorma mängd av variation som existerar emellan arter samt de ekosystem arter lever i. Denna variation har lett oss till att klassificera olika organismer på basis av morfologiska skillnader och på senare tid till att jämföra genetiska skillnader på individens nivå. Utvecklingen av molekylära metoder har haft en avsevärd betydelse för anammandet av den variation som existerar på en intra-specifik nivå.

Den marina kiselalgen *Skeletonema marinoi* är en av de vanligaste växtplanktonarter i Östersjön under vårblomningen som anses viktig för den årliga produktionen. Jag utnyttjade denna art som en modell för växtplankton i detta område. En av de främsta målsättningarna var att beskriva den genetiska och fenotypiska diversiteten längs med miljögradienter över rum och tid. En viktig aspekt med denna avhandling var även att klargöra de faktorer som eventuellt är involverade i konfigurationen av genetisk diversitet och differentiering. Jag studerade även kopplingen mellan genetisk diversitet och viktiga ekofysiologiska funktioner så som primär produktion och näringsinnehåll.

Med hjälp av mikrosatellit-markörer visade jag att den genetiska diversiteten hos *S. marinoi* populationer i Östersjön är lägre jämfört med populationer i östra delen av Nordsjön. Arten är genetiskt uppdelad så att en utpräglad population förekommer i Östersjön och en annan, genetiskt åtskild population förekommer norr om de Danska sunden. Experimentella resultat visade att de genetiskt åtskilda populationerna var anpassade till lokala salinitetsförhållanden. Genflödet mellan populationerna korrelerade kraftigt med en oceanografisk konnektivitets-modell. Den genetiska strukturen av en omfattande blomning tvärsöver Östersjön var stabil under mars-april. Delpopulationer inom denna vårblomning korrelerade med olika miljövariabler så som salinitet och upplöst kisel. Den genetiska diversiteten hos Skeletonema i Östersjön var kopplad till fenotypisk diversitet – mina studier avslöjade omfattande variation av ekologiskt vikitga särdrag hos olika kloner. Mina experimentella resultat visade att betare kunde modifiera den klonala mångfaldigheten av fenotypiskt variabla *S. marinoi* populationer. En ökad klonal mångfaldighet ledde till högre prestationsförmåga i fråga om primär produktion och stabiliserade ekofysiologiska funktioner.

Som visats i denna avhandling består en art allt som oftast av åtskilliga genetiska varianter med fenotypiska skillnader. Kunskap om sådana intra-specifika skillnader är en förutsättning för att vi skall kunna förstå var och varför arter förekommer. Denna kunskap utgör även en grund för de prognoser som siktar på att förutspå hur arter kan anpassa sig till framtida miljöförhållanden.

Nyckelord: växtplankton, genetisk diversitet, populationsstruktur, fenotypisk variation, ekofysiologi

Yhteenveto

Suunnaton määrä variaatioita eliölajien ja niiden asuttamien ekosysteemien välillä on perustavanlaatuinen ominaisuus luonnossa. Perinteisesti tätä monimuotoisuutta on käytetty organismien luokittelemiseen eri lajeihin niiden morfologisten eroavaisuuksien perusteella. Hiljattain myös geneettisten erojen huomioimista yksilötasolla on hyödynnetty lajien luokittelemisessa; molekyylisten menetelmien kehityksellä onkin ollut oleellinen vaikutus lajien sisäisten muunnelmien ymmärtämiselle.
Merialueilla esiintyvä piilevä, *Skeletonema marinoi* on yksi Itämeren tavallisimmista kasviplanktonlajeista kevätkukinnan aikana. Käytin tätä lajia mallina muille Itämeren alueen kasviplanktonlajejille. Tavoitteenani oli selventää geneettistä ja fenotyyppistä monimuotoisuutta pitkin Itämeren ympäristögradientteja. Geneettisen monimuotoisuuteen ja erkaantumiseen vaikuttavien tekijöiden selvittäminen oli tärkeä aspekti väitöstutkimuksessani. Tutkin myös geneettisen monimuotoisuuden tason ja tärkeiden ekofysiologisten toimintojen kuten perustuotannon ja ravinnesisällön välistä yhteyttä.

Mikrosatelliitti-markkereita käyttämällä pystyin toteamaan, että *S. marinoi* levän geneettinen monimuotoisuus on Itämeressä merkittävästi alhaisempi kuin läheisessä Pohjanmeren itäosassa. Tutkittu laji jakautuu geneettisesti yhteen erilliseen populaatioon Itämeressä ja toiseen selvästi erottuvaan populaatioon Tanskan salmien pohjoispuolella. Kokeellisten tulosten perusteella nämä geneettisesti erilaistuneet populaatiot ovat kumpikin sopeutuneet paikalliseen veden suolapitoisuuteen. Populaatioiden välisen geenivirran ja merivirtojen luoman yhteyden välillä havaittiin vahva korrelaatio. Itämeren pääaltaan halki muodostunut laajamittaisen kukinnan geneettinen struktuuri oli vakaa maaliskuun ja huhtikuun aikana. Kukinnan ala-populaatiot korreloivat myöskin muutamien ympäristötekijöiden, kuten suolaisuuden ja silikaatin määrän kanssa. Tutkimukseni paljastivat myös laajaa vaihtelua Skeletonema-kloonien ekologisesti tärkeissä ominaisuuksissa. Kokeellisten tutkimusteni perusteella laiduntajat pystyivät muuttamaan geneettisten kloonien lukumäärää fenotyyppisesti monimuotoisissa *S. marinoi* populaatioissa. Lisääntynyt kloonien lukumäärä paransi perustuotantokykyä ja vakautti ekofysiologisia toimintoja.

Kuten tässä väitöstutkimuksessa osoitetaan, lajit koostuvat useimmiten lukuisista geneettisistä muunnelmista, jotka eroavat usein fenotyypeiltään. Ymmärtääksemme missä tietyt lajit esiintyvät ja miksi; tarvitsemme tietoa lajien sisäisistä vaihteluista. Tämä tieto on tarpeellista, jotta voimme ennustaa lajien sopeutumista tuleviin ympäristönmuutoksiin.

Avainsanat: kasviplankton, geneettinen diversiteetti, populaatiostruktuuri, fenotyyppinen variaatio, ekofysiologia

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LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications and manuscripts, which are referred to in the text by their Roman numerals (I-IV). In addition, unpublished results are included.

I. **Sjöqvist, C.,** Godhe, A., Jonsson, P.R., Sundqvist, L. & Kremp, A. Local adaptation and oceanographic connectivity patterns explain genetic differentiation of a marine diatom across the North Sea-Baltic Sea salinity gradient. Molecular Ecology, 24:2871-2885.

II. Godhe, A., **Sjöqvist, C.,** Sildever, S., Sefbom, J., Harðardóttir, S., Bertos, M., Bunse, C., Gross, S., Johansson, E., Jonsson, P.R., Khandan, S., Legrand, C., Lips, I., Lundholm, N., Rengefors, K., Sassenhagen, I., Suikkanen, S., Sundqvist, L. & Kremp, A. Physical barriers and environmental selection cause spatial and temporal genetic differentiation of an extensive algal bloom. *Manuscript.*

III. **Sjöqvist, C.,** Kremp, A., Lindehoff, E., Båmstedt, U., Egardt, J., Gross, S., Jönsson, M., Larsson, H., Pohnert, G., Richter, H., Selander, E. & Godhe, A., (2014). Effects of grazer presence on the genetic structure of a phenotypically diverse diatom population. Microbial Ecology, 67: 83-95.

IV. **Sjöqvist, C.** & Kremp, A,. Genetic diversity affects ecological performance and stress response of marine diatom populations. *Manuscript.*

1. INTRODUCTION

1.1 Brief history

How a species responds to the constant changes in the environment is partly determined by its level of genetic diversity (Hedrick 1986, Hughes et al. 2004). Numerous ecological and evolutionary questions related to speciation, gene flow, metapopulation dynamics etc. may be clarified by characterizing the genetic structure within a species and its populations. Knowledge about genetically diverse phytoplankton species has been available for at least half a century (Guillard and Ryther 1962). Nevertheless, classical studies within the field of phytoplankton ecology mostly assumed that a clonal strain is representative of the whole species in question of physiology (Brand 1989). If this were true, data from experimental studies of a single clone could be used to explain the distribution of respective species across the marine environment. That conception was proven erroneous by studies demonstrating noticeable genetic differences within phytoplankton populations that were coupled with physiological variability in different environmental conditions (Brand 1989). The perception of a genetic basis for phenotypic variability in marine phytoplankton populations had implications for the study of genetically differentiated blooms over time and space (Gallagher 1980) and was considered an important aspect in understanding how blooms are initiated, maintained and terminated. However, it was not until the work published by Rynearson and Armbrust (2000), using microsatellite markers to describe the level of genetic diversity within a bloom of diatoms, that the interest in phytoplankton from a population genetic perspective increased substantially. Since then, numerous studies

using genetic markers of phytoplankton species have emerged confirming the presence of high intra-specific diversity and genetic differentiation (Rynearson et al. 2006, Rynearson and Armbrust 2004, Adams et al. 2009, Masseret et al. 2009, Bell 1991, Casabianca et al. 2012, Casteleyn et al. 2010, Evans et al. 2005, Godhe and Härnström 2010, Godhe et al. 2013, Härnström et al. 2011, Nagai et al. 2007, Saravanan and Godhe 2010, Tahvanainen et al. 2012).

The principle of ”everything is everywhere, but the environment selects”, initially suggested by Baas Becking and Beijerinck in 1934 for microorganisms was later resurrected by Finlay (2002) and Fenchel (2005). Therefore, the question of whether species of microscopic unicellular phytoplankton, are ubiquitously distributed and panmictic or if there is intra-specific genetic differentiation of ecological relevance has become a major objective of study in phytoplankton ecology. The view of panmictic micro-organism (body size <1.0 μm) populations (Fenchel and Finlay 2004) has been challenged by numerous studies (Alpermann et al. 2009, Casabianca et al. 2012, Casteleyn et al. 2010, Godhe and Härnström 2010, Godhe et al. 2013, Härnström et al. 2011, Nagai et al. 2007, Rynearson and Armbrust 2000, Rynearson and Armbrust 2004, Tahvanainen et al. 2012). Currently, there is strong evidence supporting the existence of population genetic structure despite large population sizes and high potential for dispersal in these organisms. The genetic patterns of smaller organisms may potentially be governed by other factors than those of macro-organisms. For instance, micro-organisms may experience a more dynamic surrounding with rapidly shifting micro-habitats which potentially could increase the occurrence of

subtly different niches and offer habitats for a greater number of genetic variants (Fukami et al. 2007). It has also been shown that frequency dependent selection (Minter et al. 2015) and priority effects (Sefbom et al. 2015) can promote genetic diversity in protists. A better comprehension of what governs genetic diversity in micro-organisms could potentially add another layer to the discussion of local adaptation and increase the understanding of the ecosystem in an evolutionary perspective.

1.2 Genetic diversity and differentiation of Baltic Sea organisms

The Baltic Sea is a geographically and hydrographically enclosed brackish water estuary located in northern Europe. It is a marginal habitat with naturally low levels of biodiversity (Johannesson and André 2006). It contrasts more or less, environmentally homogenous oceanic areas, as it contains strong environmental gradients (both horizontal and vertical) of for instance salinity, temperature and nutrient levels at different spatial scales (Leppäkoski et al. 2002). There is a pronounced salinity and temperature gradient traversing the entire sea from NE to SW (1000-1500 km), but gradients of nutrient levels may be found on significantly smaller spatial scales (1-10 km) where near shore water masses protrude into more open and exposed areas. The environmental gradients are strong drivers of species diversity (Bonsdorff and Pearson 1999, Herlemann et al. 2011) which, in combination with oceanographic connectivity patterns and anthropogenic perturbations, such as nutrient pollution, climate change and ship traffic creates highly dynamic habitats in the Baltic Sea with rapid changes of hydrographic variables (Jansson and Dahlberg 1999, Ducrotoy and Elliot

2008). An important aspect to acknowledge is also the relatively short geological history of the Baltic Sea (~8000 years) which may have consequences for the interpretation of genetic patterns in species inhabiting this area. Whitlock and McCauley (1999) stated that non-equilibrium populations that recently experienced range expansions may show biased F_{ST} estimates. It is especially difficult to estimate gene flow based on F_{ST} since the values do not necessarily imply the current situation, but the situation as it was earlier. Low migration rates and large population sizes will increase the time required for a metapopulation to reach equilibrium (Crow and Aoki 1984, Whitlock 1992).

The population genetic diversity and structure of marine species in the Baltic Sea share certain general patterns, including reduced intra-specific variability in geographically isolated and low salinity parts of the sea and genetic differentiation across the transition area (Danish Straits) to the adjacent North Atlantic (Johannesson and André 2006). A low level of genetic diversity in combination with geographic isolation is generally assumed to hamper adaptation to changing environmental conditions (Heschel and Paige 1995, Lande 1988) and increase the risk of ecosystem instability (Hughes and Stachowicz 2004, Neuhauser et al. 2003). In cases where Baltic Sea populations consist of small populations with high levels of clonality, such effects may be even more pronounced (Johannesson et al. 2011). However, data on high levels of clonality is largely based on information obtained from macro-organisms such as macro-algae (Tatarenkov et al. 2005) and invertebrates (Riginos and Cunningham 2005). Only a few studies on micro-organisms in the Baltic Sea cover information

about genetic variability and differentiation. The cyanobacterium *Nodularia spumigena* (Hayes and Barker 1997) show genetic variability at the phycocyanin operon intergenic spacer (PC-IGS) inside the Baltic Sea but another cyanobacterium *Aphanizomenon flos-aquae* seem genetically homogenous in the same region (Barker et al. 2000). Tahvanainen et al. (2012) studied the dinoflagellate *Alexandrium ostenfeldii* and showed that it is genetically differentiated (F_{ST}=0.05-0.28) between five localities inside the Baltic Sea and exhibits low expected heterozygosity (H_E, 0.1) in AFLP loci. However, a comprehensive understanding about the level of genetic diversity in this species is hard to derive as a comparison to marine populations is lacking.

1.3 Factors configuring genetic patterns

The genetic diversity of natural populations is mainly driven by gene flow, random genetic drift, potential inbreeding, mutation rates and natural selection (Wright 1949). Genetic structure across metapopulations may, however, be altered by several factors. Isolation by distance (IBD), initially introduced by Wright (1943) represents a scenario where the genetic differentiation increases with geographic distance. Most individuals cannot disperse throughout their species ranges. This reduced dispersal leads to greater genetic drift in subpopulations with increasing geographic distance (Slatkin 1993). Isolation by distance has been shown for marine species (Teacher et al. 2013, Planes and Fauvelot 2007), including phytoplankton that has been analyzed for population genetic structure over regional scales (Nagai et al. 2007, Casteleyn et al. 2010). Gradients of environmental variables may configure the level of genetic diversity by causing local adaptation to distinct

conditions (Grahame et al. 2006). In other words, environmental gradients have the potential to cause genetic population structure in a species, a phenomenon called isolation by environment (IBE) (Orsini et al. 2013, Wang and Bradburd 2014). In principle this has been shown for *S. marinoi* on the Swedish west coast where a fjord population was genetically differentiated from the nearby open sea population (Godhe and Härnström 2010). Likewise, dinoflagellate populations of *Alexandrium fundyense* in the open Gulf of Maine were genetically different from nearby coastal pond populations (Richlen et al. 2013). Biotic factors are also potential drivers of genetic structure. For instance, selective grazing may shift the genetic structure of the prey population by exerting a directional selection pressure, as shown for predator-prey interactions in terrestrial systems (Punzalan et al. 2005). A similar mechanism is known from marine bacterioplankton that are subjected to size-selective grazing by flagellates and ciliates resulting in smaller mean sizes of bacteria relative to cultured cells (Gonzalez et al. 1990). Gonzalez et al. (1990) did not assess the genetic structure of the grazed populations, however, it is likely that the phenotypic response was linked to a genetic shift. Other mechanisms, such as, intra-specific competition has been shown to affect the population structure by promoting specialization of clones to different niches in the environment leading to genetically differentiated populations between seasons (King 1972, Dia et al. 2014, Tesson et al. 2014). This may also be viewed as a positive sampling effect resulting in numerical dominance of the most competitive clone in a genetically diverse population (Loreau and Hector 2001). This has been experimentally confirmed with the marine coccolithophore *Emiliana huxleyi*

displaying strong competition between clones in stressful environmental conditions leading to a predominance of a single clone in genotypic mixtures (Lohbeck et al. 2012). The relative importance of intra-specific competition in natural populations is, however, not known. Other mechanisms such as frequency-dependent selection and priority effects have also been shown to promote genetic diversity in marine phytoplankton populations (Minter et al. 2015, Sefbom et al. 2015).

In pelagic organisms living in marine systems, oceanographic connectivity may potentially affect the population genetic structure to a greater extent than acknowledged so far. For instance, White et al. (2010) showed that larval dispersal and resulting genetically differentiated sub-populations may be decoupled from the Euclidean distance and show more robust correlation with oceanographic connectivity models. Water currents have been shown to affect the population structure of marine phytoplankton species in the Mediterranean and on the Swedish west coast (Casabianca et al. 2012, Godhe et al. 2013). Connectivity in the sense of metapopulations may be further divided into genetic connectivity and demographic connectivity (Lowe and Allendorf 2010). However, regarding genetic connectivity *per se*, it is an estimation of the gene flow within or between sub-populations and may be expressed as a value of F_{ST} (Wright 1949, Weir and Cockerham 1984). The F_{ST} value ranging from 0-1 may elucidate the nature of genetic connectivity as values close to zero imply high gene flow that maintains similar allele frequencies in subpopulations whereas high F_{ST} is a sign of reduced gene flow between sub-populations. However, values near 1 are unrealistic for highly polymorphic loci such as microsatellites, because the range of values that F_{ST}

can take is restricted by the allele-frequency distribution (Jakobsson et al. 2012). Estimates of the genetic connectivity may provide information about the adaptability of marginal populations. Most often reduced genetic connectivity and subsequent isolation of a subpopulation is a prerequisite for local adaptation (Kawecki 2008). The combination of modelling oceanographic connectivity and multi-locus data of genetic sequences is a powerful approach within seascape genetics to detect barriers to gene flow and increase the understanding of current mechanisms underlying population structure in marine pelagic species (Selkoe et al. 2010). However, in order to grasp population structure patterns of species in an evolutionary perspective we need to include the effect of environmental variables on adaptation. Isolation by adaptation (IBA) (Nosil et al. 2009) means that genetically differentiated populations across an environmentally variable space are confined to local conditions and homogenization of sub-populations is counteracted by poor recruitment and establishment success of allocthonous migrants. The strong environmental gradients of the Baltic Sea are potentially creating such 'selection against immigrants' (Hendry 2004). Even though for instance the northern and southern parts of the Baltic Sea display oceanographic connectivity and exhibit reciprocal gene flow, genetic variants may not establish in a neighbouring area because of being out-competed by a stronger locally adapted population. In this sense, the environmental gradients of the Baltic Sea may have a profound effect on population structure in an evolutionary perspective.

1.4 Genetic diversity and ecosystem function

Studies of marine biodiversity have focused to a high degree on species diversity, the state of it in various habitats, and its importance for essential ecosystem services (Loreau et al. 2001, Stachowicz et al. 1999, Solan et al. 2004, Worm et al. 2006). Numerous scientific efforts imply that the on-going decline of species diversity in marine habitats may render negative effects on for instance primary production. A deterioration of species diversity may also involve a decrease in ecosystem resilience and slower recovery from various perturbations (Worm et al. 2006). However, studies where biodiversity on the sub-species level are viewed in relation to ecosystem services are less common, despite clear linkage of genetic diversity levels to for instance primary production and fluxes of energy and nutrients (Hughes et al. 2008).

Genetic variability of marine phytoplankton species may be reflected as physiological differences between genotypes. For instance the biovolume and growth rate have been found to differ between strains of the same species (Godhe and Härnström 2010). Likewise, some harmful algal species display intra-specific variation in the cellular content of various toxins (Alpermann et al. 2009). Kremp et al. (2012) showed that the differential response between isolates in question of growth rate and toxin production of *A. ostenfeldii*, to increased temperature and CO_2 had a genetic basis. Thus, it is of crucial importance to recognize the level of genetic and phenotypic variability that a species contains in the framework of diversity-function studies.

The level of genetic diversity within a species influences how an assemblage of individuals responds to changes in the surrounding

environment (Reed and Frankham 2003). Consequently, intra-specific diversity (genotypic and phenotypic) may be a significant aspect of the ecological performance of a species or a community within an ecosystem (Hughes et al. 2008). The study of genetic diversity has traditionally been an interest of evolutionary biologists, however, it is becoming increasingly evident that genetic variability has profound ecological consequences (Hughes et al. 2008). An inclusion of this diversity level in Biodiversity and Ecosystem Functioning (BEF) studies is important if we want to understand potential negative effects of impoverished communities and habitats on ecosystem services. When the level of genetic diversity in a species is known, we may predict its adaptive potential and produce estimates of future scenarios (Kawecki 2008). Such exercises are impossible with species data alone. We may certainly test species and their response to perturbations even without genetic information, but the specific mechanism of for instance lowered fitness remains unclear until we understand the respective genetic basis and link it to environmental change. Ecosystems comprising natural environmental gradients may prove valuable if we aspire to establish links between genetic, species and habitat diversity and their joint effects on ecosystem services in natural populations. In relation to this it is worth to remember that the current gap of knowledge about the relative importance of genetic diversity in comparison to other factors that influence ecological processes, for example primary productivity, complicates the interpretation of such studies. However, some studies have shown that the role of genetic diversity may be comparable to that of species diversity (Schweitzer et al. 2005, Crutsinger et al. 2006).

1.5 *Skeletonema marinoi*

The diverse diatom genus *Skeletonema* is found from temperate to tropical ecosystems, but in Scandinavian waters *S. marinoi* is the exclusively dominant species (Kooistra et al. 2008). *Skeletonema marinoi* (Fig. 1) occurs throughout the Baltic Sea and the North Sea coasts of Scandinavia. The species is an important part of the spring bloom community and may contribute up to 60% of the total primary production in some areas (Jochem 1989). When the spring bloom is terminated, planktonic cells of *S. marinoi* produce resting stages that sink to the sea floor where they may remain viable in the sediments for decades (Härnström *et al.* 2011). These seed banks represent ”genetic libraries”, including the genetic variability of numerous generations at a respective site. *Skeletonema marinoi* has been shown to display a wide salinity tolerance (Saravanan and Godhe 2010), which in part may explain its broad distribution and dominance across the Baltic Sea salinity gradient. Diatoms display asexual reproduction with a reduction in cell size after each cell division. At a critical size, diploid vegetative cells undergo sexual reproduction and the original cell size is restored (Davis et al. 1973). Environmental parameters may also trigger the initiation of sexual reproduction. This has been shown to occur in Baltic Sea isolates of *S. marinoi* (Godhe *et al.* 2014). At the same time, size restoration may be achieved asexually (Godhe et al. 2014). Despite irregular sexual events, this mode of reproduction is expected to support genetic diversity within Baltic Sea populations. In fact Balloux et al. (2003) stipulated that populations with predominantly asexual reproduction maintain higher levels of heterozygosity at separate loci compared to sexual populations because of accumulated

mutations. However, the same authors stated that increasing rates of asexual reproduction decreases genotypic diversity and leads to weakened population differentiation (lower F_{ST} values). Studies on genetic structure in coastal waters of Kattegat and Skagerrak adjacent to the Baltic Sea indicate that regional *S. marinoi* populations are genetically differentiated (F_{ST} derived from microsatellite loci) and temporally stable. These populations exhibit high genotypic diversity and significant heterozygote deficiency (Godhe and Härnström 2010, Härnström et al. 2011), indicative of sexual reproduction (Balloux et al. 2003).

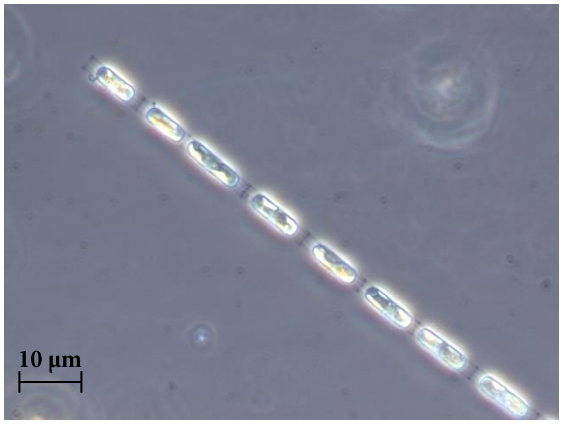

Figure 1. Light microscopy photograph of *Skeletonema marinoi* from the Baltic Sea.

1.6 Studying population structure by microsatellite markers

Genetic diversity may be defined as 'any measure that quantifies the magnitude of genetic variability within a population' (Hughes et al. 2008). The most commonly used measures are observed heterozygosity (H_O), expected heterozygosity (H_E), clonal and allelic richness. Genetic differentiation refers to the arrangement of genetic variability among multiple samples and is used to express the differences in allele frequencies

between two or more populations (Wright 1949). Measures of genetic differentiation are for example the fixation index (F_{ST}) (Wright 1949), which is based on the variance of allele frequencies between populations and G_{ST} (Nei 1973) which is defined as the proportion of genetic diversity that resides among populations. G_{ST} is similar to Weir and Cockerham's (1984) θ (=F_{ST}) which accounts for effects of uneven sample sizes and number of sampled populations. Weir and Cockerham's (1984) F_{ST} and the recently emerged Jost's D (Jost 2008) were used in the papers of this thesis. Jost (2008) criticized the standard measures (F_{ST}, G_{ST}) of being severely biased, specifically if the gene diversity is high.

Different approaches have been developed to characterize individuals genetically and study population structure. The use of genetic markers such as Amplified Fragment Length Polymorphisms (AFLPs) (Vos et al. 1995), microsatellites (reviewed in Sunnucks 2000, Schlötterer 2004) and later on single nucleotide polymorphisms (SNPs) (Baird et al. 2008) have substantially contributed to the understanding of intra-specific diversity patterns in phytoplankton species. The extent of genetic variability in a population must be known before one can study differentiation between populations. Useful neutral types of markers are the microsatellites, initially discovered by (Tautz and Renz 1984) as being single sequence repeats (SSRs). These repetitive regions (2-6 bp repeat motifs) that are prone to high mutation rates and are dispersed throughout the genome have proven specifically useful for studying minute differences in closely related individuals. One benefit of codominant microsatellites compared to for example the dominant AFLP markers are that both alleles at one locus may

be observed, enabling identification of hetero- and homozygotes. Microsatellites are often neutral, i.e. non-affected by natural selection and highly polymorphic. However, neutrality cannot always be assumed as microsatellites may for instance be linked to expressed sequence tags (EST) (Vasemägi et al. 2005). The theoretical process of mutation in microsatellites follow the stepwise mutation model (SMM) whereby each mutational event results in the gain or loss of a single repeat unit (Slatkin 1995). The length of microsatellite fragments in multiple loci provides information about the relatedness of individuals and may be used to establish a set of multilocus genotypes in a population. Important to remember is that mutations that occur under the SMM are not necessarily new to the population, which means that using microsatellites in population genetic studies may underestimate the level of genetic diversity and potentially reduce the genetic differentiation between two populations. This may also be expressed as homoplasy, referring to a phenomenon whereby identical alleles arise independent of descent (Kimura and Crow 1964). It is of critical importance to assess the power of the microsatellite loci to be used when identifying individuals of the same species. A genotype accumulation curve given a random sample of *n* loci can show the number of required loci in order to discriminate between individuals in a data set (Kamvar et al. 2013).

2. AIMS OF THE THESIS

The overall objective of this thesis was to investigate spatial and temporal patterns of genetic diversity and differentiation of the important spring bloom diatom species, *Skeletonema marinoi*, in the Baltic Sea area and examine the factors behind the patterns. Specifically, I wanted to find out

whether patterns of genetic differentiation and reduced diversity documented for marine macro-organism populations across the Baltic Sea-North Sea transition can also be observed in pelagic micro-organisms. Furthermore, I studied whether genetic diversity was coupled with variation of phenotypic traits and whether phenotypic diversity was relevant for ecophysiological parameters.

In the four papers included in this thesis I studied:

Salinity as a potential driver of population structuring in *S. marinoi* (Paper I)

In the first part I investigated the spatial arrangement of neutral genetic variability in *S. marinoi* along the Baltic Sea salinity gradient ranging from 5 psu to 30 psu. By isolating individuals from the sediment I accounted for potential temporal variability as the resting stages accumulated at the ocean floor represents a "genetic library" of the specific area. Is the Baltic Sea/North Sea population of *S. marinoi* panmictic or are there any potential barriers to gene flow? What is the role of oceanographic connectivity in driving potential genetic differentiation? Does the genetic structure coincide with the salinity gradient across a 1500 km transect? If genetic differentiation occurs, can adaptation to local salinity conditions between genetically distinct populations be observed?

The Baltic Sea spring bloom – from south to north (Paper II)

I studied the spatial and temporal genetic structure of an extended spring bloom of *S. marinoi*, hypothesizing that it is panmictic and originating from the same genetic population that is transported from the south to north

during March-April. Alternatively, genetically differentiated populations of *S. marinoi* could be present simultaneously across the entire area, which would indicate local seeding from coastal areas along the S-N transect. If genetic structure was observed, we wanted to understand the drivers of differentiation and disentangle potential spatial and environmental factors from each other which often is a challenge when the two aspects are covarying.

Effects of grazers on the genetic diversity in *S. marinoi* (Paper III)

I studied the impact of zooplankton on clonal richness and clonal evenness of *S. marinoi* during a short time window of exponential growth. Are there any implications of selective grazing on a genetically and phenotypically diverse diatom population?

Ecophysiological parameters and genetic diversity (Paper IV)

In this part I investigated the effects of clonal richness on ecophysiological parameters under different salinity conditions. What is the level of primary production and nutrient content in a monoclonal population compared to a multiclonal population at suboptimal (3 psu) and native (5 psu) salinity conditions? What is the role of clonal richness in stabilizing ecological performance?

3. MATERIAL AND METHODS

3.1 Sample collection and establishment of clonal cultures

The studies were conducted in the Baltic Sea and the adjacent NE part of the North Sea in **Paper I** (Fig. 2). The border between the Baltic Sea and the North Sea follows the ICES definition where Kattegat and Skagerrak are parts

of the North Sea (ICES 2004). Clonal strains used in experiments in **Paper III** and **IV** originated from the Bothnian Sea station (BS).

Surface sediment samples (1-10 cm top layer) were collected with a gravity corer from ten different locations along the salinity gradient for **Paper I**. One gram of wet sediment containing resting stages of *S. marinoi* were incubated in F/2+Si media (Guillard 1975) prepared from filtered seawater (0.2 µm) of respective salinity. Growth conditions were set to +4°C with a 12:12h light:dark cycle (40 µmol m^{-2} s^{-1}). After seven days individual chains of *S. marinoi* were isolated by micropipetting and washed in three drops of filtered seawater, to ensure single-cell isolation, before they were transferred to 24-well tissue culture plates (one chain per well) containing 1.5 ml of F/2+Si media with respective salinity. Once growth was confirmed in well plates, cultures were transferred to and grown in 50 ml tissue culture flasks. The sampling for **Paper II** was conducted on a ship of opportunity where water samples were taken using an integrated sampling device mounted on the ship. The sampling depth was 8 m. The establishment of clonal cultures for **Paper II** was identical to the procedure in **Paper I** except that vegetative cells were isolated from a concentrated net sample taken from the surface water bloom. Growth conditions were as described above.

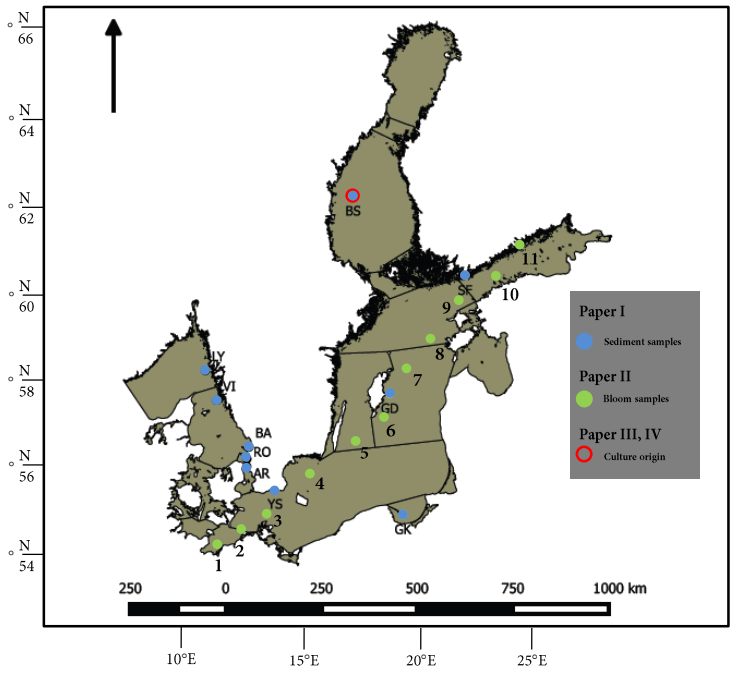

Figure 2. Stations for all field sampling included in this thesis. **Paper I** included five stations from the Baltic Sea (BS=Bothnian Sea, SF=Gulf of Finland, GD=Gotland, GK=Bay of Gdansk, YS=Ystad) and five stations from the NE North Sea (RO=Öresund, AR=Arild, BA=Båstad, VI=Vinga, LY=Lysekil). **Paper II** included eleven stations (green circles) between Travemünde and Helsinki that were sampled four times each during the spring bloom. The isolates used in experimental work for **Papers III** and **IV** originated from the Bothnian Sea (BS).

3.2 DNA extraction and genotyping

All clonal cultures were filtered on Versapor 3000 membrane filters (pore size 3.0 µm, 25 mm Ø) and stored in -80°C. DNA was extracted according to a previoulsy described CTAB-protocol (Kooistra et al. 2003). Eight previoulsy developed polymorphic microsatellite markers were used (Almany et al. 2009), fragments were PCR-amplified according to the protocol described in (Godhe and Härnström 2010). The PCR products were analyzed in an ABI 3730 (Applied Biosystems) using an internal standard (GS600LIZ). GeneMapper (ABI Prism GeneMapper Software v.3.0) was used to determine the allele sizes for the individual loci. Previously genotyped strains were used

in **Papers III** and **IV**. About 30-50 individuals per replicate were isolated by micropipetting followed by reculturing and the steps of DNA extraction and re-genotyped as described above with the exception of only using a limited set of loci sufficient in identifying the genotypes present at the end of the experiments.

3.3 Population genetic analyses

Microsatellite Tools for Excel was used to detect identical eight-loci genotypes and allelic richness (Park 2001) (**Papers I-IV**). Estimated deviations from Hardy-Weinberg equilibrium (HWE) and genotypic linkage disequilibrium (LD) were calculated using the software GENEPOP v. 4.2 (Raymond and Rousset 1995) (10 000 Markov Chain dememorizations, 20 batches and 5000 iterations per batch) including each locus in each local population following Bonferroni correction to adjust the level of statistical significance (Rice 1989). Technical artifacts, such as, potential large allele drop out, stuttering or presence of null alleles were computed in MICROCHECKER v. 2.2 (Van Oosterhout et al. 2004) (95% confidence interval and 1000 randomizations). The R package POPPR (v. 3.0.2), developed for analyzing population genetic data on partially asexual species, was used to test the power of microsatellite loci (not included in the articles) (Kamvar et al. 2013) (Fig. 3). Null allele frequencies of non-HWE loci were calculated as in Brookfield (1996) (**Papers I**, **II**). FSTAT v. 2.9.3 (Goudet 1995) or GENEPOP were used to calculate the observed heterozygosity (H_O), expected heterozygosity/gene diversity (H_E/H_S) per locus and for each local

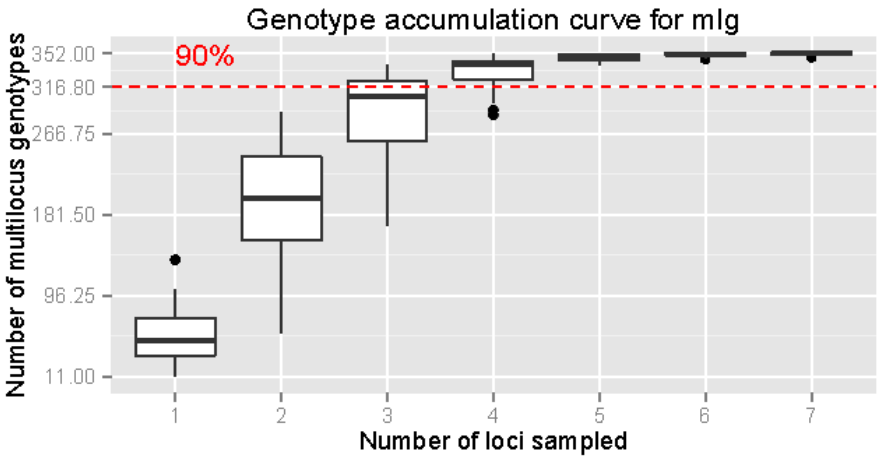

Figure 3. Genotype accumulation curve for the microsatellite data set in Paper I. This analysis tested the power of discrimination between unique individuals given the number of multilocus genotypes (MLG) that was included. The result showed that 5 loci were enough to discriminate between the individuals in the current data set.

population separately (**Papers I-IV**). FSTAT was also used for calculating the inbreeding coeffcient (F_{IS}) (**Papers I, II**). Confidence limits (95%) for H_O, H_E/H_S and F_{IS} were obtained by 2000 bootstrapping replicates. The assessment of some genetic diversity indices in populations may be biased by unequal sample size. This was avoided by performing rarefraction on the allellic diversity using HP-Rare, version 1.1 (Kalinowski 2005) (**Paper I**). GENEPOP was used to calculate pairwise F_{ST} following Fisher's method where probabilities of exact tests are combined (**Papers I, II**). SMOGD, version 1.2.5 (Crawford 2010) was used to calculate pairwise Jost D values (Dest) (CI 95% was obtained by 1000 bootstrapping replicates) (Jost 2008). The Bayesian probabilistic population assignment approach implemented in STRUCTURE (Falush et al. 2003, Pritchard et al. 2000) using the admixture model and correlated allele frequencies was used to infer the value of K, indicating the number of genetically differentiated populations (**Papers I, II**).

The log likelihoods of the generated data were used to infer the most likely Δ*K* (Evanno et al. 2005). Nei's genetic distance (Nei 1972) was calculated in GENETIX 4.0.5.2 (Belkhir et al. 2004) (**Paper II**). Isolation by distance analyses were performed by Mantel tests in the ISOLDE (Rousset 2000) subprogram embedded within GENEPOP.

3.4 Experiments

Experiments were conducted in order to test three main hypotheses 1) Genetically differentiated populations across the Baltic Sea-North Sea transition are adapted to local salinity conditions (**Paper I**). 2) The genotypic diversity of a phenotypically variable *S. marinoi* population is affected by grazer presence (**Paper III**). 3) There is a linkage between clonal richness and ecophysiological parameters in populations of *S. marinoi* (**Paper IV**).

For **Paper I** ten strains per station (BS, GD, BA) were grown in 0-35 psu until the end of the exponential phase was reached. Strains were acclimatized to a "new" salinity condition for one week prior to the growth experiment. The first growth experiment was run in native salinity conditions. From here, the respective strains were successively inoculated to the next higher or lower salinity levels and experiments were run after an adaptation phase. Growth experiments were always inoculated with cells in the exponential growth phase. Growth was measured daily using a Varian 2000 estimating *in vivo* fluorescence of subsamples in 300 µl wells. Calculation of the intrinsic growth rate based on the longest period of exponential growth was conducted as in (Wood et al. 2005).

For **Paper III** a mesocosm experiment was conducted in order to investigate the effect of grazer presence on the genetic patterns of a

phenotypically diverse *S. marinoi* population. The experimental setup included a control without any added copepods and three grazer treatments with increasing levels of copepods (low 1 µg C l^{-1}, medium 10 µg C l^{-1}, high 20 µg C l^{-1}). We added a natural copepod community dominated by *Acartia bifilosa* and *Eurytemora affinis*. All mesocosms (volume per mesocosm ~2000 l) contained initially 100 µg C l^{-1} of eight different *S. marinoi* genotypes in identical proportions. The experiment was run for two weeks until the stationary phase of the diatom population was reached. At the onset of stationary phase, samples were collected and 50 *S. marinoi* individuals per mesocosm were collected with single-cell isolation by micropipetting as described above and identified using three microsatellite loci. Genotype data were used to infer clonal composition, changes in clonal richness and evenness. Chain length (number of cells per chain) of *S. marinoi* was measured in Lugol preserved samples taken on days 0, 5, 8 and 12. A side experiment was conducted with the aim of establishing genotype specific polyunsaturated aldehyde (PUA) profiles considered a potential trait for grazing deterrence. Individual strains were incubated in 250 ml glass bottles (3 replicates) for three days with and without grazers on a rotating plankton wheel. Light conditions were set to 40 µmol photons m^{-2} s^{-1} and temperature was constant at 10°C. On day three, cells were filtered onto GF/F filters and further processed for PUA analysis according to the procedure described in (Wichard et al. 2005).

The importance of clonal richness for ecophysiological parameters was assessed in an experiment comparing growth, primary production and nutrient content in *S. marinoi* cultures at three diversity and

two salinity levels. Richness levels of one, five or twenty strains were incubated in replicates of three at 3 and 5 psu to test for the impact of salinity stress. From each experimental unit growth (cell number and chl a), primary production (C^{14}-method), particulate carbon, nitrogen and phosphorus (POC, PON, POP) and biogenic silica (Bsi) was determined. The experiment lasted until the end of exponential phase (~day 7) when 30 individuals per replicate was isolated by micropipetting to determine clonal composition as described above. These isolates were identified using microsatellite markers and genotype data was analyzed together with the measured parameters for effects of clonal richness on growth, nutrient assimilation and response to salinity stress.

3.5 Statistical analyses

Analyses of variance (One way ANOVA/Two way ANOVA) with sequential Bonferroni correction were used in **Papers I** and **IV** to test the effect of environmental variables on phenotypic traits and genetic patterns. Two-way ANOVA was used to test for significant phenotypic differences between individuals and grazer treatments in **Paper III**. The analyses were conducted in GraphPad Prism, version 6. Differences in clonal proportions, clonal richness and evenness in **Paper III** were anaylzed by permutational multivariate analysis of variance (PERMANOVA) in the software Primer 6 with the PERMANOVA + add-on package (9999 permutations).

Multiple Mantel tests conducted in R (R Core Team) were used in **Papers I** and **II** to examine potential correlation between gene flow and connectivity matrices. Partial Mantel tests were used in **Paper II** to study the correlation between genetic differentiation and environmental correlates.

Mantel tests were conducted in R using the 'vegan package'. Redundancy analysis (RDA) with variation partitioning was used in **Paper II** to test the effect of various environmental variables on the genetic patterning of *S. marinoi* during a spring bloom. The variation partitioning was calculated in order to disentangle the effects imposed by space vs. environmental variables. General liner models (GLM) and linear mixed effect models (LME) were used in **Paper IV** to detect effects of diversity, salinity or strains on the response variables. Analyses of transgressive overyielding (Paper IV) were conducted according to Schmid et al. (2008) in R.

Table 1. Summary of methodological aspects in **Papers I-IV**.

	Paper I	**Paper II**	**Paper III**	**Paper IV**
Study environment	Field/Exp.	Field	Exp.	Exp.
Strains isolated from sediment/pelagic?	sediment	pelagic	sediment	sediment
culturing success	80%	50%	N/A	N/A
re-isolation success	N/A	N/A	90%	99%
in laboratory conditions	30-60 days	30-60 days	12 months	36 months
DNA extraction	CTAB	CTAB	CTAB	CTAB
# genotyped individuals	354	611	431	545
# genotyped loci	8	8	8	8
# re-genotyped loci	N/A	N/A	3	6
genotyping success	75%	76%	92%	98%
diversity indices	H_O, H_E, allelic richness	H_O, H_E, allelic richness	clonal richness, clonal evenness	clonal richness, clonal evenness
measure of differentiation	F_{ST}, Jost D	F_{ST}, Jost D	N/A	N/A
population genetic software	GENEMAPPER, GENEPOP, FSTAT, ISOLDE, M.CHECKER, R, SMOGD, STRUCTURE	GENEMAPPER, GENEPOP, GENETIX, FSTAT, M.CHECKER, R, STRUCTURE	M.SAT TOOLS FOR EXCEL	M.SAT TOOLS FOR EXCEL, R
phenotyping	salinity tolerance	N/A	growth, PUA production, chain length	growth, C uptake, nutrient content

4. MAIN FINDINGS OF THE THESIS

In this thesis, genetic diversity and differentiation of an important spring bloom diatom species, *S. marinoi*, was assessed across the entire Baltic Sea using neutral genetic markers. I investigated the population genetic structure of this species over spatial (**I, II**) and temporal scales (**II**) in its natural environment. Further, I estimated the potential effect of biotic (**III**) and abiotic factors (**IV**) on the population genetic patterns and assessed the role of clonal richness in primary production and nutrient assimilation (**IV**).

4.1 Genetic diversity in *S. marinoi* populations is reduced in the low salinity Baltic Sea

The examination of eight polymorphic microsatellite loci originating from 354 individuals and ten sediment samples along a 1500 km salinity gradient in the Baltic Sea/North Sea area revealed significantly reduced levels of heterozygosity and allelic richness towards the NE part of the gradient (Fig. 4) (**Paper I**). Genetic diversity patterns based on another set of 611 individuals sampled during the *S. marinoi* bloom (**Paper II**) were comparable to the values established from seed beds inside the Baltic Sea. The genetic diversity (H_E) was not significantly different across the sampling gradient, neither did it change significantly over time, despite a minor decrease in H_E towards the end of the bloom.

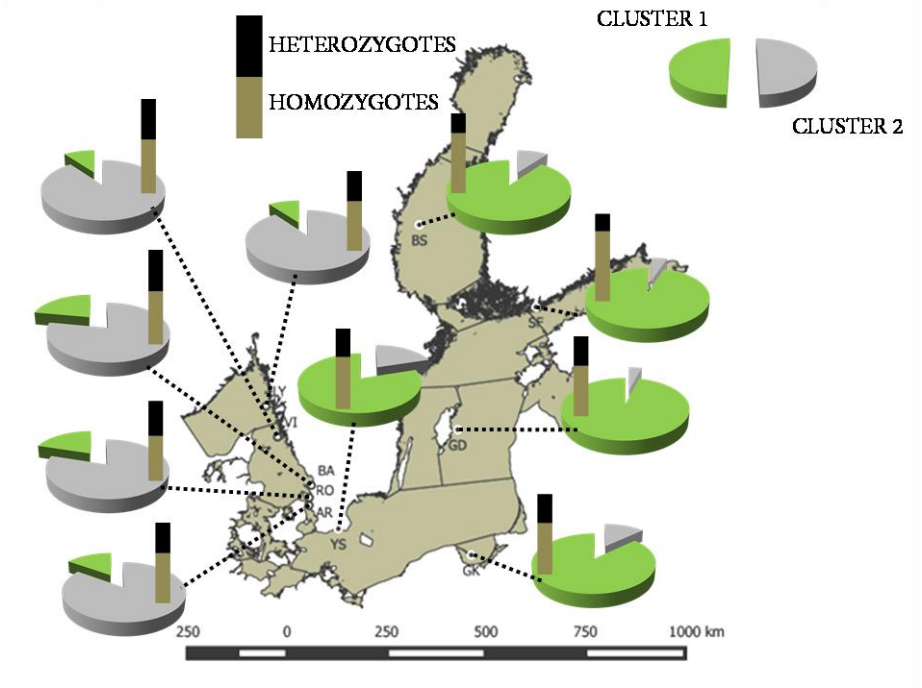

Figure 4. Summary of genetic diversity and differentiation patterns in *Skeletonema marinoi* across the Baltic Sea/North Sea based on strains isolated from sediment samples (**Paper I**). The average observed frequency of heterozygotes was significantly lower in Baltic Sea populations. The average allelic richness was significantly lower in Baltic Sea populations (Student's t-test, $p<0.05$, corrected for sample size) (see **Paper I**). Clusters are derived from Bayesian probability assignment indicating strong differentiation across the transition area.

4.2 Baltic Sea *S. marinoi* is genetically distinct from North Sea populations

Structure analyses revealed that Baltic Sea *S. marinoi* formed a distinct genetic population, separated by the transition zone (Fig. 4) from North Sea *S. marinoi*. A directional migration network revealed that gene flow was highly impeded across the Danish straits. The asymmetric pattern in the gene flow data correlated with oceanographic connectivity in the area (**Paper I**). Oceanographic connectivity also affected the observed population genetic substructure inside the Baltic Sea. When observing the patterns revealed from both seed bed and bloom data (**Papers I, II**) reduced gene flow was detected

south of Gotland, dividing the species into a northern and a southern cluster. This pattern remained stable during the spring bloom over time and correlated with modeled oceanographic trajectories (**Paper II**). The RDA with variation partitioning revealed that the northern cluster was significantly associated with low levels of salinity.

4.3 Baltic *S. marinoi* is adapted to local salinities

I found *S. marinoi* originating from a low salinity habitat (stations BS and GD in **Paper I**) to express maximum growth rates in salinities between 5 psu and 7 psu (Fig. 5, only part of the data shown). These were significantly higher compared to the growth rate of strains isolated from a high salinity area (station BA) which displayed highest growth rate in 30-35 psu. I infer the significantly different growth rates of strains with different origin as an indication of local adaptation to different salinity regimes.

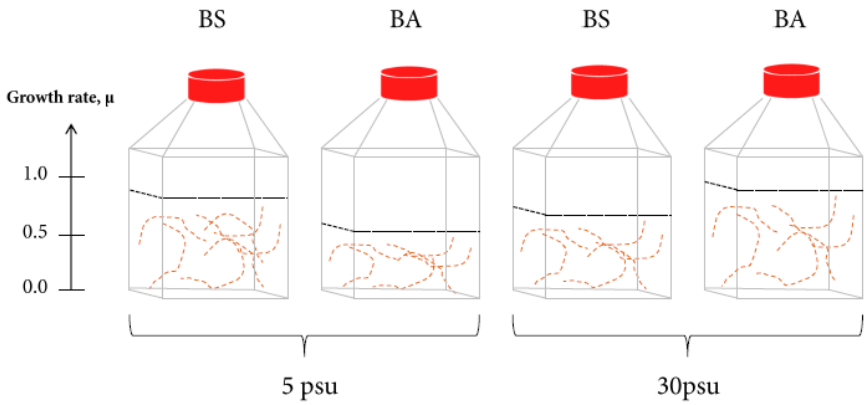

Figure 5. Results of local adaptation experiments in **Paper I**. Ten strains from the BS station in northern Baltic Sea showed significantly higher growth in low salinities compared to strains from the BA station on the Swedish west coast. Isolates from the Swedish west coast displayed higher growth in high salinity conditions. Growth rate (μ) calculated as the intrinsic rate of increase. Only a subset of the data shown. See **Paper I** for detailed results.

4.4 Genetic diversity indices are configured by grazer presence

Experimental data showed that a medium grazer levels (~80 copepods l^{-1}) significantly reduced the level of clonal richness and clonal evenness of genetically and phenotypically diverse *S. marinoi* populations (**Paper III**). This level of grazer density is within the natural range in the N Baltic Sea during spring (Diekmann and Möllmann 2010). Diversity indices were unaffected by low (~15 copepods l^{-1}) and high grazer levels (~170 copepods l^{-1}). I also observed a significant clone specific reduction in chain length as a consequence of grazer presence indicating that copepods were selectively grazing on the phenotypically diverse *S. marinoi* population (**Paper III**). The concentration of PUA was not correlated to direct defense mechanisms in *S. marinoi*.

4.5 Genetic diversity affects ecological performance

Experimental data showed that primary production and acquisition of specific nutrients were significantly reduced in monoclonal populations compared to multiclonal populations (**Paper IV**). Growth of *S. marinoi* was not coupled to different genotypic diversity levels. However, I observed transgressive overyielding with regard to primary production in a low diverse population (5 genotypes) compared to a monoclonal population (Fig. 6). Transgressive overyielding could not be calculated for the high diversity level as the strain-specific primary production values for all 20 strains were unknown. Particulate nutrient content (POC/PON/POP) of *S. marinoi* was significantly higher in multiclonal populations compared to monoclonal populations.

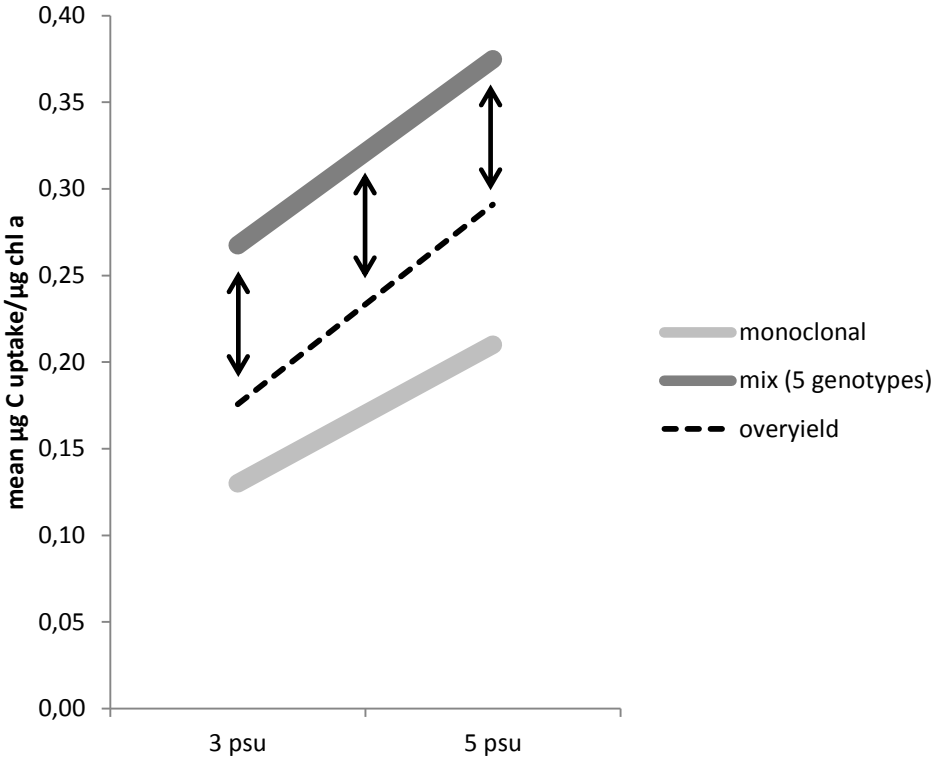

Figure 6. Results of mean µg C uptake/µg chl a in *S. marinoi* strains at different diversity levels and salinity conditions. The rate of C uptake was significantly lower in monoclonal cultures compared to mixtures of the same 5 genotypes. Overyielding was observed in the mix with 5 strains (uptake above dashed line represented by arrows).

4.6 Significant clonality detected in the Bothnian Sea

The primary mode of reproduction was assessed by studying the index of association (I_A) and the r_d. The latter is corrected for number of loci. Significant departure from the null hypothesis of sexual recombination was only significant for the BS population (p=0.007) (Fig. 7). The null hypothesis could not be rejected for any of the other populations (SF, GD, GK, YS, RO, AR, BA, VI and LY).

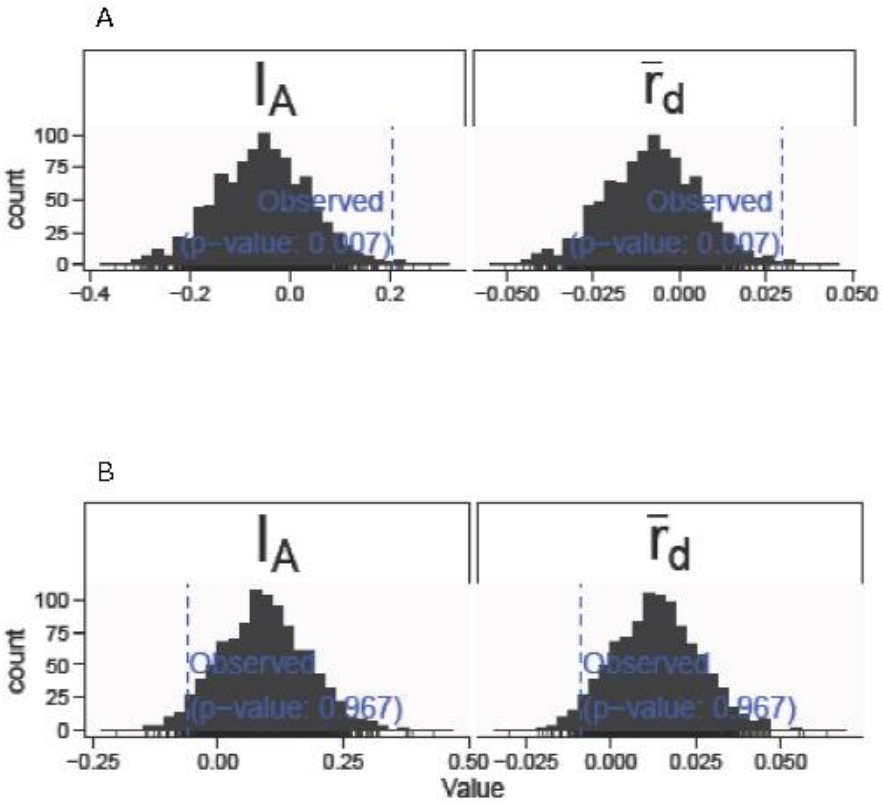

Figure 7. The index of association (*IA*) and r_d for the BS population (A) and LY (B). The tests for both *IA* and r_d with a null hypothesis of recombination were rejected for the BS population (5000 permutations, p=0.007) but not for the LY population (5000 permutations, p=0.967) (LY used as an example of all remaining populations) suggesting that the frequency of asexual reproduction is higher in the Bothnian Sea compared to the North Sea.

5 DISCUSSION

5.1 Genetic diversity and differentiation of Baltic *S. marinoi*

This thesis provides the first analysis of genetic diversity in a micro-organism species across the Baltic Sea-North Sea transition zone. The results show that the level of genetic diversity of a marine diatom species across the Baltic Sea-North Sea transition conform to the general pattern of reduced genetic

diversity in the Baltic Sea (Johansson and André 2006). There are likely several reasons behind the diversity cline in *S. marinoi* across the Danish straits. The geographic isolation of the Baltic Sea since the last glaciation period (~8000 years ago) (Björck 1995) may have lead to a population bottleneck, a phenomenon that results in reduced genetic diversity (Chakraborty and Nei 1977). An example of this from the Baltic Sea is the Atlantic salmon (*Salmo salar*) which has experienced genetic bottlenecks after its postglacial range expansion (Langefors 2005). The marginal Baltic Sea habitat with brackish water is also a ground for extreme selection pressures which according to theoretical expectations would lead to reduced genetic diversity (Kaplan et al. 1989). Additionally, marginal habitats that are geographically isolated tends to host peripheral populations that have been shown to display reduced levels of genetic diversity compared to central populations of the same species (Lesica and Allendorf 1995, Schwartz et al. 2003). However, with regard to the directional relative migration pattern in *S. marinoi*, Baltic populations are not peripheral sink populations (Figure 5 in **Paper I**).

The variation of genetic diversity across spatial and temporal scales has potentially important implications for the adaptation of species to changes in the environment. Low genetic diversity is usually coupled with low adaptability (Templeton 1981) and a homogenization of physiological properties within populations may also disrupt the functionality of ecosystem processes such as primary production and nutrient acquisition (Hughes et al. 2008, Olden et al. 2004). It is well established that high species diversity of phytoplankton communities stabilize ecosystem processes and promote

efficient resource use (Ptacnik et al. 2008). In the Baltic Sea, which is considered an ecosystem with low species diversity (Segerstråle 1957), the role of intra-specific diversity in stabilizing various ecosystem processes may be even more important (Crutsinger et al. 2008). Experimental data within this thesis supported a link between genetic diversity and ecophysiological parameters as increasing levels of clonal richness resulted in higher primary productivity (**Paper IV**). Clonal richness also increased the stability of ecological performance as the effect size was significantly smaller in mixed populations compared to monoclonal cultures. Also, the coefficient of variation (CV) was lower in multiclonal cultures, suggesting increased stability of ecophysiological parameters with increasing genotypic diversity.

The general conception of reduced genetic diversity in Baltic Sea populations has also been challenged by Wennerström et al. 2013 indicating species-specific patterns of genetic differentiation and heterogeneity. Events of secondary contact between geographically isolated populations in the Baltic Sea and central populations (North Atlantic) that postglacially recolonized brackish areas may have led to recovered levels of genetic diversity in some species. Similar postglacial events including secondary contact leading to increased levels of genetic diversity have been documented in terrestrial systems (Pauls et al. 2013).

The results also show a clear genetic differentiation across the transition area, with one genetic population inhabiting the Baltic Sea and another genetic population inhabiting parts north of the Danish Straits further confirming the general pattern that has been emphasized for Baltic macro-organisms earlier (Johannesson and André 2006). The drivers that for

instance create genetic differentiation of fish species across the transition zone, i.e. the salinity gradient and reduced oceanographic connectivity (Nielsen et al. 2004) are also relevant for *S. marinoi*. The benthic seed banks which may contain up 50 000 *S. marinoi* resting stages per gram in Scandinavian waters (McQuoid 2002) have been proposed to anchor *S. marinoi* populations to local areas (Godhe and Härnström 2010) leading to population genetic structure. This was supported by the results of this thesis since all ten populations originating from resting stages isolated from the sediment were significantly differentiated by F_{ST} (**Paper I**). F_{ST} values of the bloom were lower (**Paper II**) compared to populations isolated from sediment samples, indicating more admixture. However, *S. marinoi* was genetically differentiated in a S-N pattern suggesting that populations were not transported from south to north, but local blooms developed independently of each other (**Paper II**). The genetic differentiation within the Baltic Sea indicate a certain stability over time as a reduced gene flow was observed at the Bornholm basin both in populations sampled from a bloom (**Paper II**) and from resting stages deposited in the bottom sediment (**Paper I**). The factors driving the observed differentiation patterns were coupled to geographic distance (IBD), variable environmental conditions and oceanographic connectivity. Genetic differentiation within the Baltic Sea of for example fish species (Teacher et al. 2013) have also been linked to oceanographic connectivity patterns. The most extreme example of genetic differentiation within the Baltic Sea is the case of the brown seaweeds *Fucus vesiculosus* and *Fucus radicans* which led to sympatric speciation. *F. radicans* is reproductively isolated from *F.vesiculosus* and is endemic for the Baltic Sea.

The speciation event has occurred in the last 400 years (Pereyra et al. 2009). The occurrence of these two Fucus species also follow a S-N pattern in which *F. radicans* is more common in low salinity northern parts of the Baltic Sea and shows high prevalence of clonality (Johannesson et al. 2011). The index of association (I_A and r_d) analysis provided in this thesis conforms to the pattern observed in *F. radicans*/*F. vesiculosus* as the highest frequency of clonality in *S. marinoi* also was found in the Bothnian Bay population (BS). The strong genetic differentiation between the BS population and other Baltic populations and significant clonality of the BS population as by r_d could be interpreted as ongoing speciation in Baltic *S. marinoi*. However, this needs to be studied in more detail before any conclusions can be made.

To understand adaptation of marine pelagic species in the Baltic Sea from an evolutionary perspective we need to know something about the nature of gene flow between central (source) populations and marginal (sink) populations (Kawecki 2008). The presupposed scenario is that a central population living in the species core habitat is well adapted and display a large population size. In the case of Baltic *S. marinoi*, the central population would be located in the NE parts of the North Sea given the historical development of the area since the last glaciation period (Björck 1995). Marginal populations are numerically reduced and live on the edge of the species range suffering from maladaption. By definition, marginal populations are demographic sinks receiving large numbers of poorly adapted migrants from the central source populations. Such a theoretical scenario in a marine habitat is, however, only possible if it is supported by a strong oceanographic connectivity pattern favouring migration towards the

marginal population (Kawecki 2008). The results from this thesis indicate that the migration pattern was reversed, i.e. we observed higher relative gene flow from the presupposed sink populations towards the central populations (from Baltic towards North Sea) (**Paper I**). This would favour adaptation to local conditions in the marginal populations because it is less affected by maladapted alleles originating from the central population. In the light of the genetic and oceanographic connectivity data it seems possible that a self-sustaining and well adapted Baltic population has emerged over time. We have used a seascape genetic approach (Galindo et al. 2006) that integrates high resolution spatial data with genetic indices of the focal species. Recent studies within seascape genetics (for example White et al. 2010, Casabianca et al. 2012, Godhe et al. 2013) support our results showing strong correlation between modeled oceanographic connectivity and gene flow. However, the relative importance of oceanographic connectivity compared to environmental variables in driving patterns of gene flow is not well understood. As mentioned earlier, adaptation to new environmental conditions is also dependent of the population size of an adapting population. Bell and Gonzalez (2009) observed a threshold in population size for evolutionary rescue (ER) to take place. Populations with a size above the threshold are more likely to contain resistant genotypes that can survive through times of rapid environmental change.

5.2 The role of environmental conditions as drivers of genetic structure

The role of the abiotic and biotic factors dictating patterns of genetic diversity and structure was studied from several perspectives in this thesis, including

environmental gradients, impact of grazers and stress related conditions. Several of the studied factors proved to have an impact on the genetic patterns of *S. marinoi*.

The role of salinity was investigated in **Paper I** showing that different salinity regimes resulted in local adaptation of populations. Baltic Sea isolates displayed higher growth rates in low salinity conditions compared to North Sea derived isolates and vice versa. The difference in fitness (growth rates) across salinity conditions may have developed since the end of the last glaciation period after which the salinity has gradually declined (Björck 1995) and the species range has presumably extended further into the low salinity habitats of the Baltic Sea. Despite the observed gene flow between North Sea and Baltic Sea populations it seems likely that Baltic *S. marinoi* have adapted to the low salinity conditions and the high salinity populations seem unable to establish because of low salinity adapted populations prevail in the Baltic Sea. This may be a result of selection in combination with reduced connectivity to the North Sea population. The differentiation may be strengthened by selection against immigrants where gene flow from allocthonous individuals is hampered. Such processes may lead to rapid evolution of ecologically dependent reproductive isolation (Hendry 2004). The analyses within this thesis does not concern or quantify reproductive isolation directly, but the genetic differentiation pattern across the transition area and also evidence for population structure inside the Baltic Sea (**Paper II**) are solid indicators of reduced gene flow between the sampled populations. The shift in salinity conditions across the Danish Straits, accompanied by a split in genetic resemblance across the same area supports

the notion that salinity is a strong structuring factor of biodiversity in this area. However, it is hard to disentangle the relative importance of salinity and oceanographic connectivity which both were significantly reduced across the Danish Straits (**Paper I**).

Oceanographic connectivity correlated well with gene flow across the Baltic Sea-North Sea transition (**Paper I**) and also inside the Baltic Sea (**Paper II**). On the contrary, IBD was statistically insignificant inside the Baltic Sea (**Paper I**) suggesting that the effects of geographic distance on genetic differentiation may be overruled by connectivity patterns. Also, the large gene pool in the sediment may to some extent diminish the pattern of IBD. Isolation by distance based on data from a significantly smaller gene pool, the Baltic *S. marinoi* bloom (**Paper II**), was significant, as well as the relationship between oceanographic connectivity and genetic differentiation. Thus, patterns of genetic differentiation may be governed by different factors depending on what time scales are taken into consideration. Also, the relative contribution of various factors, for example horizontal salinity shifts or grazing, may be different at different time points. The lower F_{ST} values within the bloom population compared to seed bank populations may be a result of lower environmental heterogeneity in a "snapshot-study" (**Paper II**). When the species is subjected to long-term spatial differences in environmental parameters the genetic differentiation is reinforced which is manifested in considerably stronger differentiation within seed bank populations (**Paper I**).

The genetic differentiation of a *S. marinoi* bloom was not only governed by salinity where a southern and a northern Baltic population displayed reduced reciprocal gene flow, but also at the later phase of the

bloom by different levels of dissolved silica (**Paper II**). The genetic shift over time (early vs. late bloom) correlated with declining silica levels towards the end of the bloom suggesting niche partitioning where specialized genotypes dominate the bloom in a successive mode. Such different phenotypic strategies within a *S. marinoi* bloom population may enable the dominant role of this species in the community during most of the Baltic spring bloom. Similar genetic shifts over temporal scales of marine diatom species have been documented in the Mediterranean (Tesson, et al. 2014, Ruggiero et al. 2015) and may be a mechanism supporting the high genotypic diversity found in blooms of phytoplankton species (Rynearson and Armbrust 2000).

5.3 Grazers and intra-specific prey diversity

Experimental data in this thesis show that the presence of grazers affects the genetic diversity of *S.marinoi* populations. Both clonal richness and clonal evenness was reduced in treatments with a medium level of grazers (10 µg C l^{-1}). These results suggest that genotypes of *S. marinoi* were grazed upon selectively, likely because of different phenotypic traits. No visible effects on the genetic patterns in the treatment with a high level of grazers may be coupled to anti-predatory strategies in *S. marinoi* that are switched on only at a certain threshold (level of grazers). Such strategies, induced at a certain predator density have been discussed as a possibility in Rotifers (Gilbert 2013). Anti-predatory responses in marine phytoplankton that alternate the phenotype have been documented in for example *Phaeocystis* sp. that adjusts the colony size to minimize losses to grazers (Long et al. 2007). One may speculate that the relative importance of grazer induced changes in genetic patterns of *S. marinoi*, compared to for instance strong environmental

gradients or oceanographic connectivity patterns may be of only local importance or very short-termed phenomena, which are unable to affect the total genetic diversity of a species. Selective grazing on phenotypically different *S. marinoi* genotypes in a Baltic bloom population could potentially have a homogenizing effect on the diverse prey population and may explain the lower F_{ST} values in the bloom data (**Paper II**) compared to seed bank populations (**Paper I**). It is, however, difficult to estimate the extent of grazing impact and how that would translate into genetically different populations of *S. marinoi* over time. It is known from grassland species that grazing promotes successive ecotypes and thus, maintains the genetic diversity of a species (Reisch and Poschlod 2009). Knowledge about how grazing impacts the genetic signature of phytoplankton species in marine systems is very limited.

5.4 Comparison to macro-organisms

No clear differences of my results emerge when compared against the pattern of genetic diversity of macro-organisms (Johannesson and André 2006). A slightly less dramatic reduction of genetic diversity is present across the transition zone but still it is within the range of what has been observed in macro-organisms. However, there are several aspects potentially affecting levels of genetic diversity differently in micro-organisms compared to macro-organisms. These are for instance related to the characteristics of habitats, mobility potential and different modes of reproduction. For example, marine diatoms are faced with a wealth of rapidly changing micro-habitats possibly enabling a higher level of genetic diversity to co-occur (Gsell et al. 2013). Diversifying selection of micro-organisms may operate on a much smaller

scale compared to macro-organisms. For example, the presence of microsites lead to differences in the genetic composition of the wild flower-living yeast *Metschnikowia gruessii* sampled from various parts of the same plant (Herrera et al. 2011). This type of natural selection, acting on very small spatial scales (and temporal) could be a reason for relatively high intra-specific diversity in micro-organisms (Gerstein and Moore 2011). Also, the large population sizes of marine phytoplankton may be an aspect that leads to high genetic diversity (Reusch and Boyd 2013). However, in relation to this even bird populations have been observed to display repeated adaptive divergence within a single population on relatively small spatial scales (Langin et al. 2015) demonstrating that adaptive divergence on a microgeographic scale may be more common than previously thought, also within macro-organisms. The high dispersal potential of micro-organisms should redistribute genetic diversity as opposed to macro-organisms that often exhibit genetic patterns affected by historical events. However, it seems likely that the dispersal potential in micro-organisms is not always realized, leading to historic genetic signatures of for instance range expansions comparable to signatures in macro-organisms (Lowe et al. 2010).

The argument of Finlay and Fenchel (2004) that micro-organisms are more or less panmictic is in clear contrast with the results of this thesis. On the contrary, my results suggest that differentiation patterns in micro-organisms in general are principally driven by the same factors as in macro-organisms. Additionally, as a consequence of fine-scale heterogeneity in microhabitats it seems that genetic differences in micro-organisms arise more or less spontaneously and are maintained by specialized genotypes

resulting in adaptive radiation over very short time scales (days or weeks) (Rainey and Travisano 1998, Fukami et al. 2007). Such mechanisms may also explain the high genotypic diversity of the *S. marinoi* bloom population (**Paper II**) in this study.

5.5 Mode of reproduction and consequences for genetic diversity

Skeletonema marinoi and other phytoplankton species in the Baltic Sea have been proposed to reproduce mainly asexually (Tahvanainen et al. 2012, Godhe et al. 2014) which in theory has been shown to reduce genotypic diversity (Balloux et al. 2003). However, such a conclusion is not straightforward and may be biased by the molecular marker itself (Arnaud-Haond et al. 2005). The aspect of high prevalence of asexuality in Baltic Sea populations is often used to explain the pattern of low genetic diversity in combination with the fact that the area is geologically young (Johannesson and André 2006). This implies that species are still adapting to the Baltic Sea and are in many cases represented by populations at the leading edge characterized by reduced genetic diversity. Data collected so far and subsequent implications almost entirely exclude a major component of the ecosystem, namely the micro-organisms.

A significant proportion of the productively important spring bloom consists of diatoms that mainly reproduce asexually. However, the diatom life cycle involves an obligate sexual phase. This has been shown in *S. marinoi* using strains from station BS (Godhe et al. 2014). The linkage disequilibrium (LD) of all populations in this thesis also suggests that sexual reproduction occurs, however, the frequency of sexuality in *S. marinoi* in natural

environments is unknown. It is known that when a critical cell size is reached the individual *S. marinoi* cell undergoes sexual reproduction. Sexual reproduction in *S. marinoi* may also be induced by salinity shifts (Godhe et al. 2014). Bengtsson (2003) showed that only sporadic events of sexual reproduction in mainly asexual species would result in a genomic pattern indistinguishable from strictly sexual species. Diatoms are mainly asexual but have to undergo sexual events in order to stay viable, alternatively undertake asexual cell enlargement (Gallagher 1983). I performed an additional analysis on the genotype material collected for this thesis to further investigate sexuality in Baltic populations by looking at the degree of association between alleles (Fig. 6), which provide insights into the frequency of sexual events. The index of association (I_A) and r_d is expected to be zero if populations are recombining freely. The value of I_A and r_d will be greater than zero if there is association between alleles which is a potential sign of asexual reproduction. The I_A tends to increase when the number of included loci is higher, thus it is usually not comparable between different studies. The r_d is corrected for the number of loci and is considered a more robust measure of association. In my genotype material the distribution of these indices and the permutation test (5000 permutations) show that sexual reproduction occurs in both Baltic Sea and North Sea populations. However, the null hypothesis was refuted in the case of the BS population suggesting that the rate of random recombination is significantly lower in the Bothnian Sea. The value of r_d may be confounded by population genetic structure, however, this was avoided by treating the populations separately in the analysis. I could not exclude the possibility that the r_d value was affected by other factors, such as genetic drift or mutation.

However, significant deviations from r_d may suggest asexual reproduction (Taylor et al. 1999). This aspect would benefit from further statistical analyses (as in de Meeûs and Balloux 2004). Nevertheless, an asexually reproducing population is usually considered an evolutionary dead-end (Kawecki 2008), thus the higher prevalence of clonality in the BS population may involve reduced adaptive potential in comparison to the sexually reproducing populations. On the other hand, there are several existing examples of asexual organisms with long and successful evolutionary histories (reviewed in Lynch and Gabriel 1983).

It is generally thought that organisms living in marginal habitats employ mainly asexual reproduction because of cost-benefit issues (Kearney 2005, Meirmans et al. 2012). However, a fascinating aspect of the reproductive modes in many organisms living in the Baltic Sea is that asexuality also may be an evolutionary strategy to maintain genetic heterozygosity. Such a statement may seem counterintuitive but in combination with a small population size, meiosis leads by definition to balanced allele frequencies and thus a genetic homogenization of a population. By asexual reproduction populations may maintain the heterozygosity originated in genetically diverse and large central populations. Also, locally adapted alleles that are beneficial for a genetically differentiated population may be preserved more reliably at low recombination rates (i.e. reduced genetic drift).

Asexuality is often also coupled to a larger amount of initial progeny compared to the number of sexually derived offspring. Thus, when a population reproduces asexually in an environment it is optimally adapted to,

as the BS population, it is evolutionarily favoured over sexually reproducing individuals. A sexually derived individual/population may involve an improved adaptive potential, but as long as the environmental gradients of the Baltic Sea are stable, such a strategy produces lots of redundancy in question of genetic material. Thus, environmentally stable conditions are likely to favour asexual reproduction. Additionally, it is worth to recognize that oceanographic connectivity may certainly introduce new alleles even to locally adapted asexually reproducing populations that are of no use and thus not incorporated as long as the environment is stable. During rapid environmental change, connectivity may improve the evolutionary path of asexually reproducing populations.

5.6 Genotypic diversity and ecological performance

The results of this thesis indicate that genotypic diversity in *S. marinoi* is linked to diversity of ecologically important traits such as primary production and nutrient assimilation. In **Paper IV** we found that clonal richness affected the physiological performance of populations. The level of primary production and nutrient content was significantly lower in monoclonal incubations compared to multiclonal incubations with 5 and 20 initially added genotypes in a respective experiment. Thus, the importance of genotypic and associated phenotypic variability for ecosystem functions was shown experimentally. Important ecophysiological parameters such as, primary production and nutrient content, were of higher quality in genetically diverse populations compared to monocultures. A low level of genetic diversity (5 genotypes) resulted in transgressive overyielding in the question of primary productivity. Transgressive overyielding means that a

mixture of genotypes performs better than the best monoculture and indicates the presence of positive inter-clonal interactions (Schmid et al. 2008).

It is known from studies of micro-organisms that natural populations may be represented by thousands of different genotypes co-occurring during a season (Kashtan et al. 2014). A set of genotypes usually dominate the population given a specific time point, but the population may undergo succession on the genetic level when surrounding conditions change (Lebret et al. 2012). The 'portfolio effect' (Tilman 1999, Tilman et al. 2006), enhancing the physiological responsiveness of a population to environmental changes, may be an explanation to why populations with high genotypic richness may have a stabilizing effect on ecosystem processes. The inclusion of sub-species level diversity increases the predictability of related ecosystem services and should be taken into consideration when models of future climate scenarios are used to describe the level of species diversity and its impact on ecosystem services.

In the currently eutrophicated Baltic Sea, a better understanding of especially nutrient uptake dynamics in primary producers is a central issue. The results of this thesis suggest that predicted reductions in salinity over the coming century (Meier et al. 2012) in the Baltic Sea will not decrease the level of clonal richness in *S. marinoi*, neither will the ecological performance with respect to primary production be at risk (**Paper IV**). However, it is worth remembering that our hypothesis of a link between genotypic diversity and ecophysiological parameters was only tested in a short-term experiment with only one included abiotic stressor. Long-term

experiments may highlight different results, for instance reduction in clonal diversity in alternated conditions (Lohbeck et al. 2012). Multiple stressors, for instance combined temperature and salinity stress, may lead to total eradication of certain *S. marinoi* genotypes (Roger et al. 2012). However, the most important result with respect to diversity and functioning provided by this thesis is that clonal richness increases the ecological performance and stability of ecological functions irrespective of salinity conditions.

6. CONCLUSIONS AND FUTURE RESEARCH

6.1 Main conclusions of this thesis

I have shown that the level of neutral genetic diversity in an important spring bloom diatom is reduced in the Baltic Sea compared to the sister populations in the North Sea. Genetic differentiation occurs both over spatial and temporal scales following a S-N transect across the study area. The genetically distinct population of *S. marinoi* in the Baltic Sea is adapted to local low salinity conditions. Gene flow is reduced by the Danish Straits and largely correlated with modelled oceanographic connectivity trajectories. Apart from salinity, concentrations of silica in the water were associated with specific genetic assemblages. Grazer presence in certain densities had significant effects on clonal richness and evenness. Selective grazing was likely since I observed different proportional reduction in specific clones during a two week mesocosm experiment using a genetically and phenotypically diverse *S. marinoi* population. Important ecophysiological parameters, such as primary production and nutrient content in *S. marinoi* were higher in multiclonal populations compared to monoclonal populations irrespective of salinity condition emphasizing the importance of genotypic diversity for the

ecological performance of the populations. An interesting result that was not included in any of the associated **Papers I-IV** showed that the frequency of asexual reproduction may be higher in the Bothnian Sea population compared to all other populations in the Baltic Sea and the North Sea.

6.2 Future perspectives

Studying biodiversity along spatial gradients in the Baltic Sea not only increases our understanding of responses to current environmental conditions, but it is a useful test bed for responses over temporal scales. The level of genetic diversity a species displays in the northern Baltic Sea today may be the reality in more southern areas a century from now because of predicted lower salinity conditions (Meier et al. 2012). Such information, when linked to the level of ecophysiological parameters (for instance primary production) may shed light on the adaptive potential of species and their ecological performance in years to come. The level of genetic diversity is indeed reduced in Baltic *S. marinoi* populations compared to the North Sea; still I observed ~1000 different genotypes in the Baltic Sea, suggesting that ecological risks coupled to climate change may be relatively limited compared to the vulnerability of low diverse macro-organism species (for example *F. radicans*).

The field of ecological genetics that has used DNA fingerprinting techniques (RFLP, AFLP) and neutral genetic markers such as, microsatellites during the last 10-15 years is currently experiencing a revolution as nucleotide sequencing is becoming faster and cheaper with orders of magnitude. This has lead to a significant increase in the use of single nucleotide polymorphisms (SNPs) which not only results in more cost-

effective solutions, but also opens up true potential for linking genes to ecosystem functions that are important for the services humanity depends on. A study including for instance 50 000 SNPs compared to a study evaluating only 8 microsatellite loci has a far greater potential of finding the genomic regions (or genes) important for the functionality of a species across environmental gradients. This is especially true for non-model organisms that most of the environmental genetic studies focus on.

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This PhD-thesis describes patterns of genetic diversity and structure of a marine diatom species, *Skeletonema marinoi* in the Baltic Sea and across the transition zone to the North Sea. The thesis assesses potential drivers of population genetic structure exhibiting one genetic population in the Baltic Sea and another genetically distinct population in the North Sea. The results suggest that presence of grazers affect the genotypic composition of phenotypically diverse *S. marinoi* populations. The results also demonstrate that genetically diverse populations of *S. marinoi* sustain higher primary production than compared to monocultures.

THE AUTHOR

Conny Sjöqvist received his MSc in Environmental Biology from Åbo Akademi University in 2009. Since 2010 he has worked as a PhD student in Marine Biology at the university and at the Finnish Environment Institute/Marine Research Centre in Helsinki. In his research he has collected material through field sampling, established clonal cultures of *S. marinoi* and conducted experimental studies at Tvärminne Zoological Station and at the Umeå Marine Sciences Centre. He has also been a member of the Graduate Research School in Genomic Ecology (GENECO) at Lund University, where he received training in contemporary molecular techniques.