THESIS ON NATURAL AND EXACT SCIENCES B225

Influence of Physical-Chemical Factors on Community and Populations of the Baltic Sea Spring Bloom Microalgae

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Declaration:

Hereby I declare that this doctoral thesis, my original investigation and achievement, submitted for the doctoral degree at Tallinn University of Technology has not been submitted for doctoral or equivalent academic degree.

Sirje Sildever

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Füüsikalis-keemiliste tegurite mõju kevadõitsengu mikrovetikate kooslustele ja populatsioonidele Läänemeres

SIRJE SILDEVER

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KIRJASTUS

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LIST OF ORIGINAL PUBLICATIONS

This thesis is based on three papers, in the following referred to by their Roman numerals:

I Sildever, S., Kremp, A., Enke, A., Buschmann, F., Maljutenko, I., Lips, I. 2016. Spring bloom dinoflagellate cyst dynamics in three eastern sub-basins of the Baltic Sea, *Continental Shelf Research* [accepted manuscript].
II Sildever, S., Sefbom, J., Lips, I., Godhe, A. 2016. Competitive advantage and higher fitness in native populations of genetically structured planktonic diatoms. *Environmental Microbiology*. DOI: 10.1111/1462-2920.13372
III Godhe, A., Sjöqvist, C., Sildever, S., Sefbom, J., Harðardóttir, S., Bertos-Fortis, M., Bunse, C., Gross, S., Johansson, E., Jonsson, P.R., Khandan, S., Legrand, C., Lips, I., Lundholm, N., Rengefors, K.E., Sassenhagen, I., Suikkanen, S., Sundqvist, L., Kremp, A. 2016. Physical barriers and environmental gradients cause spatial and temporal genetic differentiation of an extensive algal bloom. *Journal of Biogeography*, 43 (6), 1130-1142. DOI: 10.1111/jbi.12722

Author's contribution

I – The author was responsible for sampling, analysis of samples and data, and writing of the manuscript.
II – The author was responsible for conducting the experiments, DNA extraction, data analysis, and writing of the manuscript.
III – The author participated in the sampling, counted and analysed phytoplankton data and contributed to the writing of the manuscript.

ABBREVIATIONS

AsQ-PCR – allele-specific quantitative polymerase chain reaction

Chl *a* – chlorophyll *a*

CI – confidence intervals

Cp - settled cyst percentage

CTAB - cetyltrimethyl ammonium bromide

DNA - deoxyribonucleic acid

fCDOM - fluorescence of coloured dissolved organic matter

F_{ST} - fixation index

GoF - Gulf of Finland

GoR - Gulf of Riga

NEBP - north-eastern Baltic Proper

NO_2 - nitrite

NO_3 - nitrate

OD - optical density

PCR - polymerase chain reaction

PO_4 - phosphate

RDA - redundancy analysis

SiO_2 - silica

1. INTRODUCTION

1.1 Microalgae

Microalgae are unicellular eukaryotic organisms that inhabit different aquatic environments. Their size ranges from a few micrometres to almost a millimetre, and they can live as single cells or form chains or colonies. Despite their minute size, photosynthesizing microalgae constitute the basis of the aquatic food web and generate about 48% of the annual net primary production (Field *et al.*, 1998). Autotrophic microalgae inhabit the photic zone of the water column, where there is enough light and nutrients available for photosynthesis (Figure 1). This study focuses on two groups of microalgae: dinoflagellates and diatoms.

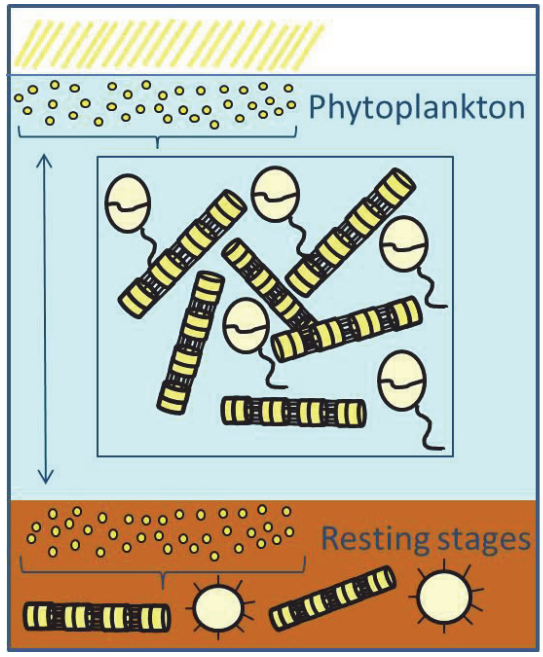

Figure 1. Diatom and dinoflagellate cells in plankton and sediments

There are more than 1000 dinoflagellate species capable of photosynthesis (Gómez, 2012), whereas diatoms are the most species-rich group of microalgae with more than 8000 species (Guiry, 2012). Diatoms contribute around 50% of the total microalgal primary production (Nelson *et al.*, 1995; Rousseaux and Gregg, 2013), and they are characterised by the silica frustule that comprises of two overlapping valves, similarly to a Petri dish. Planktic diatoms are non-motile (Crosta and Koç, 2007), thus depending on buoyancy and water movement to stay suspended in the photic zone. Contrary to diatoms, dinoflagellates have two flagella

that allow them to move in the water column, e.g. to utilise nutrients at different depths (Cullen, 1985). This difference has formed the basis of a classic view that diatoms benefit from turbulence, whereas dinoflagellates are favoured by stratification (Margalef, 1978).

Some species from both groups produce benthic resting stages that provide an escape from unfavourable environmental conditions and also act as a seed population for inoculating future blooms (Dale, 1983; McQuoid and Hobson, 1996; Bravo and Figueroa, 2014). The seed beds can be very abundant, for example up to 50 000 diatom and 200 dinoflagellate resting stages per gram of wet sediment have been reported from Scandinavia (McQuoid *et al.*, 2002). Diatom and dinoflagellate resting stages accumulated in the sediments may remain viable for several decades (McQuoid *et al.*, 2002; Lundholm *et al.*, 2011).

1.2 Spring bloom in the Baltic Sea

In a temperate climate, spring bloom is the time for highest primary production in the aquatic environments. During winter, the water column is deeply mixed through, thus more nutrients become available in the surface layer. The onset of spring bloom is governed by irradiance and mixing depth (Sverdrup, 1953; Mignot *et al.*, 2016). Spring bloom develops when the upper mixed layer becomes shallower than the euphotic zone (Wasmund *et al.*, 1998). In the Baltic Sea, salinity has been reported as the main factor governing the stratification needed for the development of spring bloom. Stratification also influences how the bloom propagates, i.e. from more stratified coastal areas to less stratified central areas (Kahru and Nõmmann, 1990). This spreading pattern corresponds well with the long-term data showing that the spring bloom usually develops first in the southern basins at the end of March or early April, about 12 days later in the Gulf of Finland and the latest in the Baltic Proper (Fleming and Kaitala, 2006; Groetsch *et al.*, 2016). The highest spring bloom intensity has been reported from the Gulf of Finland (Fleming and Kaitala, 2006) and the overall spring bloom intensity of the south-north transect correlates with winter surface nutrient concentrations (Fleming and Kaitala, 2006; Groetsch *et al.*, 2016).

Spring bloom phytoplankton community in the Baltic Sea is governed by diatoms and dinoflagellates (Andersson *et al.*, 1996; Wasmund *et al.*, 1998; Jurgensone *et al.*, 2011; Lips *et al.*, 2014). Chain-forming *Skeletonema marinoi* Sarno & Zingone, is one of the common and abundant spring bloom diatom species in the Baltic Sea (Gasinaite *et al.*, 2005; Spilling, 2007, there as *S. costatum*). From dinoflagellates, *Biecheleria baltica* Moestrup, Lindberg, & Daugbjerg 2009, *Scrippsiella hangoei* (Schiller) Larsen 1995, *Gymnodinium corollarium* Sundström, Kremp & Daugbjerg 2009 and *Peridiniella catenata* (Levander) Balech 1977, are the most dominating spring bloom species in the northern Baltic Sea (Heiskanen and Kononen, 1994; Hällfors, 2013; Klais *et al.*, 2013; Lips *et al.*, 2014). The three first dinoflagellate species cannot be easily separated under light-microscope during

routine phytoplankton analysis. Interestingly, the three-species complex has low abundances in the plankton community in the Gulf of Riga (Estonian Environmental Agency, 2015), although the conditions there are considered suitable for the species (Klais *et al.*, 2013). Dispersal limitation by the currents and encystment as a response to a changing salinity and temperature have been suggested as the main factors inhibiting the establishment of the species in the Gulf of Riga (Klais *et al.*, 2013).

Dinoflagellates have become more dominating over recent decades in some basins of the Baltic Sea (Wasmund and Uhlig, 2003; Klais *et al.*, 2011). This pattern has been associated with the stratification of the water column in winter and early spring that favours dinoflagellates over diatoms (Wasmund and Uhlig, 2003; Klais *et al.*, 2011). The increase in dinoflagellate proportion influences the quality and quantity of the food available for benthos as well as the oxygen consumption at the sediment surface. While diatoms sediment quickly and reach the benthos intact, most dinoflagellate cells disintegrate in the water column or arrive at the sediment surface as cysts (Heiskanen and Kononen, 1994), which are resistant to degradation (Dale, 1983) and grazing in some species (e.g. Kremp and Shull, 2003; Montresor *et al.*, 2003). Thus, the oxygen consumption at the sediment surface reduces with the increase of resting stages proportion compared with the degradation of diatoms and dinoflagellate cells (Spilling and Lindström, 2008).

1.3 Community structures of microalgae resting stages

All of the above-mentioned species produce resting stages (Kremp, 2000b; Mcquoid *et al.*, 2002; Kremp *et al.*, 2005; Moestrup *et al.*, 2009; Sundström *et al.*, 2009). Microalgae resting stages species composition and abundances are influenced by the life cycle events as well as by other biotic and abiotic parameters (Dale, 1996; Kremp, 2000c; Persson and Rosenberg, 2003; Crosta and Koç, 2007; Montresor *et al.*, 2013). Formation of resting stages is usually a response to environmental stress, e.g. depletion of nutrients, unfavourable temperatures or increased grazing (Oku and Kamatani, 1997; Anderson and Rengefors, 2006; Kremp *et al.*, 2009). Thus, resting stages community composition and abundance can be related to different environmental conditions prevailing during their formation (Godhe and Mcquoid, 2003; Weckström and Juggins, 2006). After formation, the resting stages sink to the seabed, however during and after the sedimentation they can be affected by horizontal transport (Wang *et al.*, 2004). For example, the dinoflagellate resting stages have similar hydrodynamic characteristics as fine silt particles (Dale, 1976) and thus can be easily resuspended and transported by the near-bottom currents. Further, the dinoflagellate resting stages are often found in high abundances from areas where fine-grained sediments dominate (Nehring, 1994; Anderson *et al.*, 2005; Narale *et al.*, 2013). The resting stage abundance and species diversity may provide an indication of the magnitude and community composition of future blooms (McQuoid and Godhe, 2004; Anderson *et*

al., 2005). However, it is important to recognise that the proportion of resting stages produced compared to vegetative population varies between species (Kremp, 2000c). In order to inoculate future blooms, the resting stages have to germinate, which can be inhibited in the sediment or at the sediment surface due to low oxygen conditions, presence of hydrogen sulphide or darkness (McQuoid and Hobson, 1996; Kremp and Anderson, 2000). Thus, resuspension of resting stages can be necessary for receiving cues for germination (Eilertsen *et al.*, 1995; Nehring, 1996; Kremp, 2001; McQuoid *et al.*, 2002). In dinoflagellates, increase in empty resting stage abundance between different sampling times has been explained by germination (Giannakourou *et al.*, 2005), whereas an increase in live resting stage abundances after the bloom has been connected with the new production of resting stages (Kremp and Anderson, 2000; Anglès *et al.*, 2010).

1.4 Population structures of microalgae

It has been hypothesized that microbial organisms are homogeneously dispersed due to their large population sizes and continuous dispersal by currents, wind and other organisms (Finlay, 2002). Despite this, intraspecific genetic structuring have been reported for several cosmopolitan microalgae (Rynearson and Armbrust, 2004; Nagai *et al.*, 2009; Casteleyn *et al.*, 2010). In addition to differences in spatial scales, population genetic differentiation in the same locality on a temporal scale has also been reported (Alpermann *et al.*, 2009; Godhe and Härnström, 2010; Härnström *et al.*, 2011; Tesson *et al.*, 2014). Genetic differentiation between sub-populations has been related to isolation by distance (Nagai *et al.*, 2007; Casteleyn *et al.*, 2010), limited connectivity due to circulation (Casabianca *et al.*, 2011; Godhe *et al.*, 2013), as well as to differential environmental selection (Rynearson *et al.*, 2006; Sjöqvist *et al.*, 2015). The latter supports the development of local adaptation in populations (Blanquart *et al.*, 2013; Orsini *et al.*, 2013), which increases the phenotype-environment mismatch for the later immigrants (Marshall *et al.*, 2010). Local adaptation is defined as improved fitness of a population in its native habitat compared to a foreign habitat or as higher fitness in native habitat compared to a foreign population introduced to the same habitat (Kawecki and Ebert, 2004; Blanquart *et al.*, 2013). Local adaptation may develop rapidly after the colonisation of a new habitat, given the quick population growth and the presence of divergent selection between different habitats (Lohbeck *et al.*, 2012). The production of resting stages as a part of life cycle may further enhance reduced connectivity through the priority effects, i.e. higher relative abundances of the first coloniser compared to new migrants (De Meester *et al.*, 2002; Sefbom *et al.*, 2015).

1.5 Motivation and objectives

In the Baltic Sea, the highest amount of new organic matter is produced during the spring bloom. Locations of major cyst beds have been reported for the spring

dinoflagellates in the north-eastern Baltic Sea by Olli and Trunov (2010). However, the contribution of cysts from those areas to the initiation of blooms is not known. They also report differences in the cyst distribution and motile cell abundances, suggesting that cyst production and deposition areas may not be the same (Olli and Trunov, 2010). Better knowledge on the influence of different factors on the cyst distribution and input to the blooms is essential to predict future bloom intensities and dispersal, with emphasis on harmful species (Forrest *et al.*, 2009; Casabianca *et al.*, 2011).

Physical-chemical factors can also shape intraspecific genetic diversity (Johannesson and André, 2006; White *et al.*, 2010; Sjöqvist *et al.*, 2015). High genetic diversity, rapid generation time and large population size provide potential to respond and adapt to the changes in the environment (Bell and Collins, 2008; Lohbeck *et al.*, 2012; Collins *et al.*, 2014). Although the importance of genetic diversity on ecosystem processes has been recognised (Duffy and Stachowicz, 2006; Hughes *et al.*, 2008), more knowledge on the factors generating and forming genetic diversity is needed. This is particularly relevant regarding global change (Pauls *et al.*, 2013). Genetically diverse populations have the potential to cope with disturbances more efficiently than less diverse populations (Hughes and Stachowicz, 2004; Steudel *et al.*, 2013; Sjöqvist and Kremp, 2016) and they also help to maintain higher biodiversity (Menden-Deuer and Rowlett, 2014).

The aim of this PhD study was to investigate how physical and chemical factors influence natural community and populations of the spring bloom microalgae in the Baltic Sea. This was done by testing the following hypotheses:

- The proportion of live and empty dinoflagellate resting stage abundance change in different seasons due to the germination and encystment (**I**);
- The newly formed dinoflagellate resting stages sediment close to the point of formation (**I**);
- Two neighbouring, but genetically differentiated diatom populations are locally adapted and show a competitive advantage in their native environment (**II**);
- The abundant spring bloom diatom species has one panmictic population in the Baltic Sea (**III**)

The main objectives of this thesis were:

- to detect and describe temporal dynamics in the spring dinoflagellate cyst community (**I**);
- to explore the transport of newly formed cysts by currents using modelling approach (**I**);
- to detect if genetically differentiated populations are locally adapted and have a competitive advantage in their native environment (**II**);
- to reveal if phytoplankton bloom in the Baltic Sea consists of one population or several genetically differentiated populations (**III**)

2. MATERIAL AND METHODS

2.1 Sampling

All samples were collected during the year 2013. For the first study (**I**), surface sediment samples from the Gulf of Finland, north-eastern Baltic Proper and Gulf of Riga were collected from 13 stations (Figure 2) during three cruises, using a Niemistö gravity corer. The samples were collected before (January), during (April) and after (May) the spring bloom. For the second study (**II**), surface sediment samples were collected by a box corer from Mariager Fjord and by a modified HAPS corer from Kattegat. For the third study (**III**), water samples were collected from fixed stations at four cruises during the spring bloom (March-April) using the ferrybox system on board MS Finnmaid. Both concentrated (for isolation) and non-concentrated (for phytoplankton analysis) water samples were taken from each station.

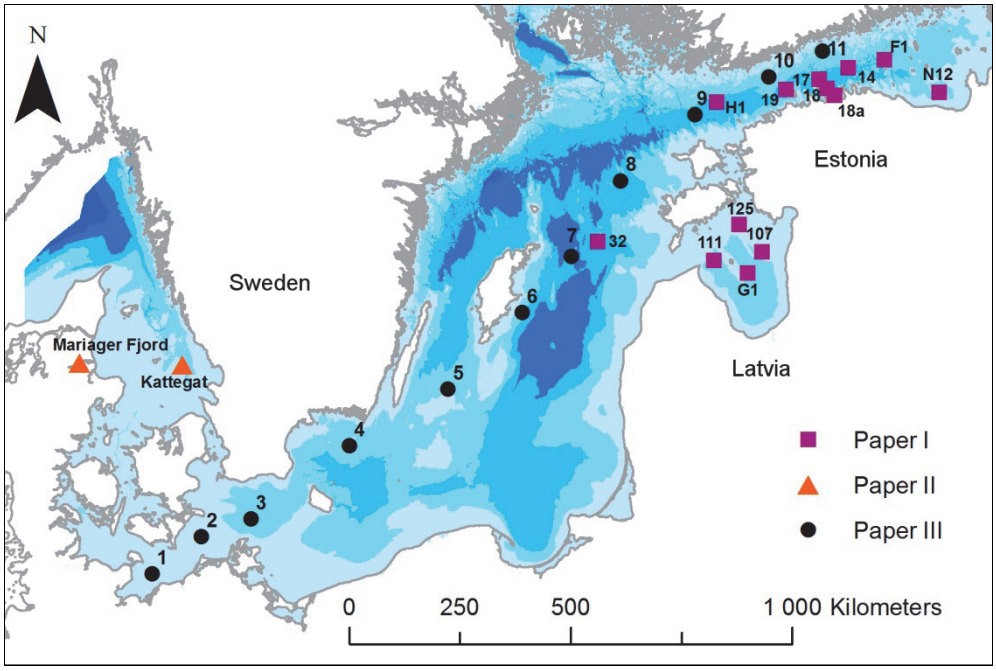

Figure 2. Map of the sampling stations for studies I-III.
Bathymetry layer available from the Baltic Sea Hydrographic Commission (2013)

2.2 Environmental parameters

All sampling stations used in the studies are also monitored under the national marine monitoring programmes of different countries. Thus, data regarding physical, chemical and biological parameters from the sampling campaigns was available through different institutions: the Estonian Environmental Agency, the Swedish Meteorological and Hydrological Institute, the Danish Nature Agency and the Finnish Environment Institute.

2.3 Sample preparation and analysis

For the first study (**I**), sub-samples (1 mL) of homogenised sediment were cleaned by sonication (30 s) and wet-sieving through a 53 µm sieve onto a 15 µm sieve. The final volume of the cleaned samples was adjusted to 10 mL. A minimum of 250 cysts were counted from each sample and from all stations at least one sample was counted in triplicates. Cysts counts were converted into a number of cysts per gram of dried sediment. Sediment dry weight was determined by weighing 1 mL of sediment in triplicates from every station during all sampling months before and after drying for 6 h at 105 °C.

In the third study (**III**), non-concentrated water samples collected from each sampling station were fixed with Lugol's solution for phytoplankton analysis. Samples were settled in a 25 mL Utermöhl chamber (Utermöhl, 1958) and analysed under an inverted microscope at magnifications 200-400x.

2.4 Culturing, DNA extraction and genotyping

For the **II** study, monoclonal *S. marinoi* cultures were established from the surface sediments by mixing 1 g of sediment with nutrient enriched f/2 medium (Guillard, 1975). The media was based on water from the two sampling sites. Aliquots from the sediment slurry were distributed into 24-well plates and incubated at 10 °C on a 12:12-h light:dark cycle at an irradiance of 60 µmol photons m^{-2} s^{-1}. After detecting germination and vegetative growth, one chain from each well was isolated by micropipetting, transferred to a Petri dish and incubated under the same conditions. When the growth continued, contents of each Petri dish were transferred to 40 mL NUNC flasks. For the **III** study, chains of *S. marinoi* were isolated immediately after sampling on board by micropipetting and incubated in separate wells of 24-well plates containing f/2 medium at 5 °C, 50 µmol photons m^{-2} s^{-1}. After vegetative growth was confirmed, contents of each well were transferred to 50 mL NUNC flasks and incubated under the same conditions.

Strains in exponential (**II**, **III**) and in stationary (**II**) phase were filtered onto Versapor-3000 filters with 25-mm diameter and 3-µm pore size (Pall Corporation) and genomic DNA was extracted following a CTAB based protocol (Kooistra *et al.*, 2003). Eight (**II**, **III**) or five (**II**) microsatellite loci (Almany *et al.*, 2009) were

amplified by PCR as described by Godhe and Härnström (2010). The products were analysed in an ABI 3730 (Applied Biosystem) and allele sizes were assigned relative to an internal standard (GS600LIZ). Allele sizes for the individual loci were determined and processed using Genemapper (ABI PrismGeneMapperSoftware v 3.0).

2.5 Experiments and AsQ-PCR

In the **II** study, growth experiments were conducted with *S. marinoi* strains. Ten strains from both locations (Mariager Fjord and Kattegat) were randomly selected and grown in triplicates in their native and non-native water based f/2 media to determine their biomass. The starting concentration was 5000 cells mL^{-1} for each selected strain and the strains were acclimatized for 7 days before the growth experiment in foreign water. Growth was monitored daily by measuring optical density (OD) of 1 mL of culture at 600 nm in a plate reader. To determine the relationship between cell density and OD, standard curves were made for each strain using serial dilutions (1:1 to 1:8). The growth was monitored until the strains reached stationary phase. Maximum OD values were converted to cell numbers per $mL \times 10^3$ and multiplied by carbon volume per cell (ng C), which was calculated based on the relationship proposed by Menden-Deuer and Lessard (2000). The cell volume used in the carbon volume relationship was calculated based on the geometric model (Sun and Liu, 2003). The required width and length was an average of 50 cells for each strain measured from Lugol fixed subsamples from the day of maximum OD.

Six strains from both locations were selected for a reciprocal competition experiment in f/2 media based on each of the two water types. Two strains, one from each site, with equal starting concentrations (5000 cells mL^{-1}) were grown together in triplicates. The mixed strains were selected based on similar growth rates determined in the previous experiment. OD was monitored daily until the strain combinations reached stationary phase. To assess the relative abundance of each strain in the experiment, an allele-specific quantitative PCR (AsQ-PCR) method was used (Meyer *et al.*, 2006). In AsQ-PCR, the respective peak-heights from the two strains in the electropherograms are used as a relative quantification measurement. To confirm that PCR amplification did not favour one strain over another, standard curves were prepared for each pair of strains. The six strain combinations were mixed in five known proportions, ranging from 10:90 to 90:10 (3 replicates each), DNA was extracted, and 5 microsatellite markers were amplified as above to find the least biased PCR reaction. Peak-height relative abundances were plotted against the known relative cell abundances to obtain r^2-values. When the mixed cultures reached stationary phase, the cultures were filtered down, DNA extracted and amplified (5 markers) as above. Allele sizes for the individual loci and the respective peak height ratios were determined using Genemapper.

2.6 Genetic structure

GENEPOP v. 4.0.7 (Raymond and Rousset, 1995) was used to estimate deviations from Hardy-Weinberg equilibrium (10 000 Markov Chain dememorizations, 20 batches and 5000 iterations per batch) of each locus in both populations, inbreeding coefficient (F_{IS}) and genotypic linkage disequilibrium (LD) between pairs of loci in each sample (10 000 dememorizations, 100 batches and 500 iterations per patch) following Bonferroni correction to adjust the level of statistical significance (Rice, 1989) (**II**, **III**). Microsatellite Tools for Excel was used to detect identical eight-loci genotypes and allelic richness (Park, 2001) (**II**, **III**). Microsatellite data set was analysed for null alleles, stuttering and large allele drop out using MicroChecker 2.2.3 (1000 randomization) (**III**). Null allele frequencies were calculated as in Brookfield (1996). Temporal and spatial genetic differentiation between the populations sampled in 2013 and in Härnström *et al.*, (2011) (**II**) as well as between all pairs of cruises and sample locations (**III**) was determined by calculating pairwise F_{ST} using Genetix 4.05 (Belkhir *et al.*, 2004) with 10 000 permutations. Bayesian analysis, as implemented in STRUCTURE (Pritchard *et al.*, 2000; Falush *et al.*, 2003), was used to detect the number of genetically differentiated clusters (K) (**III**). Log likelihoods of the generated data were used to infer the most likely ΔK (Evanno *et al.*, 2005). Isolation by distance (IBD) analysis was performed in GENEPOP v. 4.0.7 (Raymond and Rousset, 1995) (**III**). Directional relative migration rates were calculated from directional genetic differentiation (Sundqvist *et al.*, 2013) using Jost's D (Jost, 2008) as a measure of genetic differentiation (**III**). Calculations were performed using the function divMigrate from the R-package "diversity" (Keenan *et al.*, 2013) (**III**).

2.7 Oceanographic modelling

In the **I** study, a passive tracer extension (Bruggeman and Bolding, 2014) to a General Estuarine Transport Model (Burchard and Bolding, 2002) was used to simulate the transport of newly formed cysts by currents before settling to the seabed. Species-specific settling velocity of 2.5 m d^{-1} (based on Stoke's law) was used (Heiskanen, 1993). To characterize the spread of the highest cyst concentration, 10^{th} and 90^{th} percentiles were calculated from the settled cyst concentration (Cp). Spread distances were calculated as the distance from each station (x0, y0) to the geometrical centroid (cx, cy) of the Cp area. Distance = | (cx, cy) - (x0, y0) |, where geometrical centroid coordinates are defined as $cx = \int Cp\ x\ dA / \int Cp\ dA$ and $cy = \int Cp\ y\ dA / \int Cp\ dA$, where dA is an area element.

In the **III** study, the dispersal of diatoms was simulated using the Lagrangian trajectory code TRACMASS (Döös, 1995). It uses temporal and spatial interpolation of the flow-field data from the BaltiX configuration of the Nucleus for European Modelling of the Ocean circulation model (Hordoir *et al.*, 2013) with a time step of 15 min. Particle transport was simulated for 20 or 30 days in the

surface (0-2 m) or sub-surface (10-12 m) water. Connectivity among the sampling sites was estimated by calculating the proportion of particles released from one site (*i*) that ended up in another site (*j*).

2.8 Statistical analysis

In the **I** study, confidence intervals (CI, 95%) were calculated for the samples counted in triplicates to estimate if the differences in total live cyst abundances per gram of dried sediment between the sampling months are due to natural variability or represent true changes. Chi-square test of independence and *post hoc* tests (pairwise Chi-square test) with Bonferroni correction were used to detect the potential influence of life cycle events to the proportion of empty *versus* live cysts of *B. baltica* during different months (**I**). To measure the strength of association between month and cyst condition (empty *versus* live) Cramer's V was calculated for statistically significant Chi-square test results (**I**). A factorial correspondence analysis (FCA) was done to investigate differences in genotypic data between two years (2008 and 2013) and sampling sites (Mariager Fjord and Kattegat) by using Genetix v. 4.05 (Belkhir *et al.*, 2004) (**II**). One-tailed paired t-test was used for each strain to test if different water had a significant effect on *S. marinoi* biomass (**II**). Two-tailed unequal variance t-test was used to investigate whether the native *S. marinoi* population performed significantly better in its native water compared to the foreign population in the same water (**II**). For the competition experiment, the null hypothesis of equal cell abundance in the two water types in the presence of a competitor was tested by one-tailed paired t-test (**II**). Multiple Mantel tests were conducted to examine the correlations between gene flow and oceanographic connectivity (**III**). Partial Mantel tests were used to investigate correlation between genetic differentiation and environmental parameters (Chl *a*, *S. marinoi* abundance, fCDOM, NO_2-NO_3, PO_4, SiO_2) and redundancy analysis (RDA) with variation partitioning was used to test the effect of different environmental variables on the genetic structure of *S. marinoi* during the spring bloom (**III**). The variation partitioning was calculated to determine the effects of space and environmental factors to the genetic structuring. The significance level was set at $P < 0.05$; 0.005 or 0.001 in the statistical tests (**I**, **II**, **III**).

3. RESULTS AND DISCUSSION

3.1 Community structure of dinoflagellate resting stages

The results of the first study (**I**) provide a detailed overview of dinoflagellate cyst dynamics before, during and after the spring bloom in the north-eastern Baltic Sea. Spring bloom dinoflagellate live cyst abundance differed greatly between the basins and months sampled (Figure 3). Cysts of *B. baltica* dominated the community in all sampling months and basins. Although cysts of *P. catenata* were present at all stations and sampling months, they became more abundant after the spring bloom (May). Cysts of *S. hangoei* were found from all stations in the GoR and only from four stations in the GoF. In both basins, cysts of this species constituted a minor proportion of the overall community.

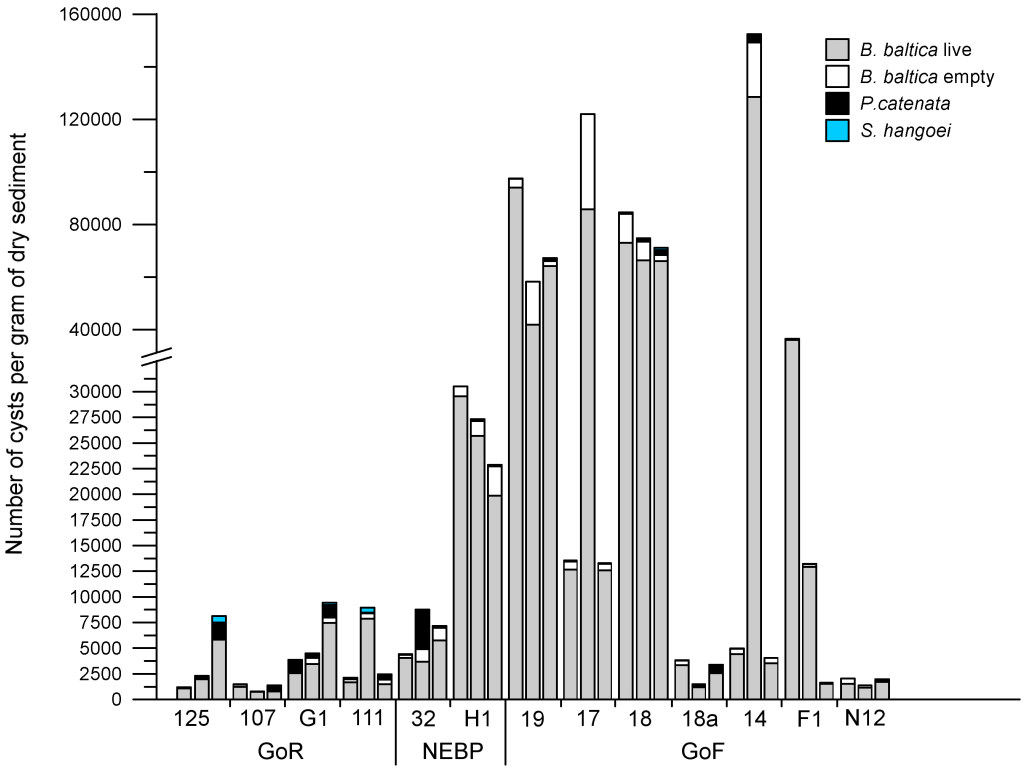

Figure 3. Cyst abundances during different sampling months (first bars = January; second bars = April; third bars = May) at sampling stations

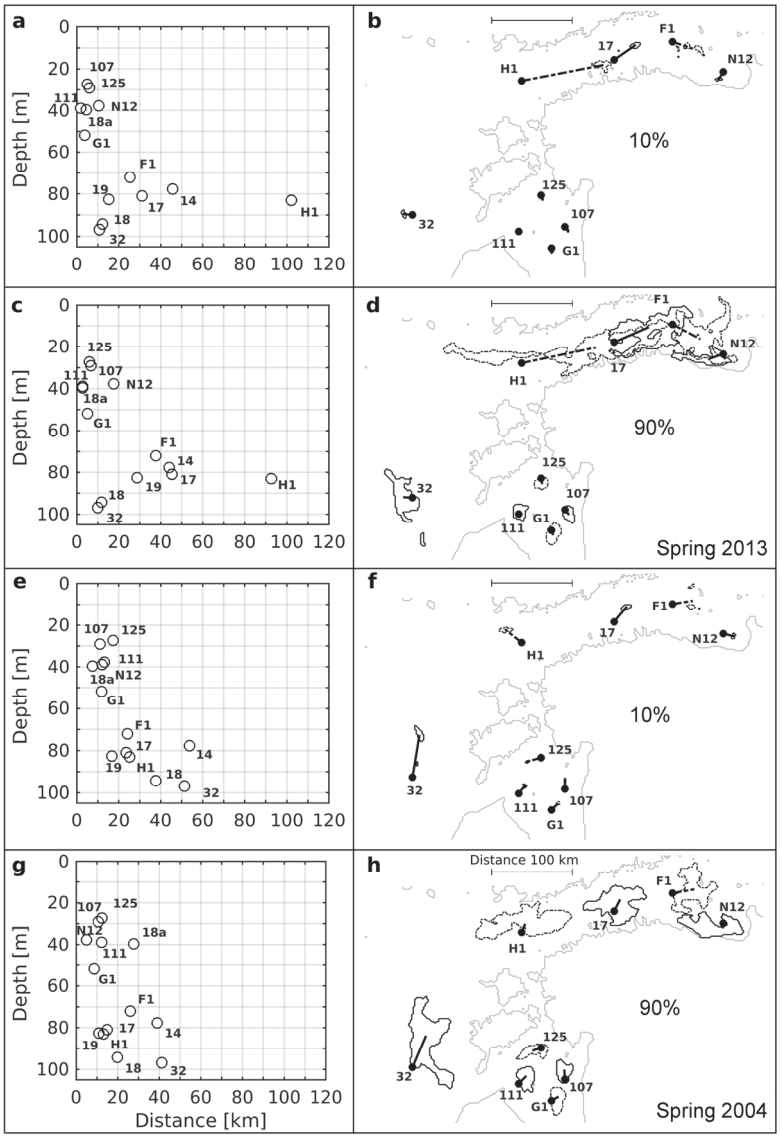

Figure 4. Simulated transport of newly formed cysts during spring 2013 (**a - d**) and 2004 (**e - h**) with transport vectors. First panel (**a, c, e, g**) indicates transportation of cysts relative to the water column depth at the point of origin. **A** and **b** show the distribution of 10 % of the highest abundances of cysts released from the stations during 2013, **c** and **d** denote the distribution of 90 % of the released cysts during 2013. **E** and **f** show the distribution of 10 % of the highest abundances of cysts released from the stations during 2004, **g** and **h** denote the distribution of 90 % of the released cysts during 2004.

The overall cyst abundance was highest in the GoF and lowest in the GoR. A similar pattern in total cyst abundances between the two basins has also been documented by Olli & Trunov (2010). The highest cyst abundances are usually reported from areas with fine-grained sediments, where silt and clay dominate (Wall, 1971; Nehring, 1994; Anderson *et al.*, 2005). Estimated from the sediment map by Carman & Cederwall (2001), the majority of the samples in all basins are taken from such locations. Cyst production in the upper layers of the water column and species-specific encystment patterns are known to influence the cyst abundance and species community in the sediments (Wall, 1971; Dale, 1983; Anderson *et al.*, 2005; Kremp *et al.*, 2009). Dinoflagellate biomass in spring is notably lower in the GoR than in the two other basins, as diatoms dominate the spring bloom community there (Jurgensone *et al.*, 2011; Klais *et al.*, 2011). Furthermore, the planktonic spring dinoflagellate community in the GoR is dominated by the *P. catenata* (Jurgensone *et al.*, 2011), whereas the contribution of *B. baltica* complex is low (Supplementary Figure 1 in Paper **I**). The overall lower cyst abundances found from the GoR most probably reflect this, as *P. catenata* encysts at low frequency (Kremp, 2000c).

Overall spring bloom dinoflagellate cyst abundance decreased during the spring bloom (April) compared to the samples collected before the bloom (January) at the majority of the stations in the GoF (Table 1, Paper **I**). At the same time, a similar pattern was only found from one station in both the NEBP and GoR. The proportion of live cysts of *B. baltica* decreased significantly only at two stations from January to April (Table 2 in Paper **I**). Lack of general pattern of reduction in the total live cyst abundances as well as in the live *B. baltica* cyst proportions do not comply with the expected pattern of recruitment (Kremp, 2000a; Giannakourou *et al.*, 2005). In addition, the majority of the stations in the GoR and NEBP, where reduction of cysts was detected before the bloom, are ≥ 80 m deep. Although, cysts of *B. baltica* and *P. catenata* can germinate in darkness (Kremp, 2001) and the oxygen conditions below the halocline were high in January (Martin *et al.*, 2014), the decrease in majority of the stations was higher than would be expected given the low germination rates in dark (Anderson *et al.*, 1987; Kremp, 2001; Vahtera *et al.*, 2014). This discrepancy indicates that other processes than life cycle events also influence the cyst dynamics in winter and early spring.

Resuspension and horizontal transport by the near-bottom currents could explain the reduction in cyst abundances in the deep stations in the GoF. Relatively small extent of high saline water together with high oxygen levels in the near-bottom layer of the GoF in January compared to April (Figure 2 in Paper **I**) indicate the possibility of a reversed circulation event. Reversed circulation is characterised by the outflow of saline water in the near-bottom layer and by the inflow of less-saline water in the surface layer of the GoF, and its occurrence depends on the prevailing wind direction (Elken *et al.*, 2003; Liblik *et al.*, 2013). The course of along the gulf cumulative wind stress between January and April (Supplementary Figure 2 in Paper **I**) indicates the presence of several shifts favouring the changes between the

usual estuarine and reversed circulation patterns. During reversals, the near-bottom current velocities along the GoF central axis can be particularly high (Liblik *et al.*, 2013). The fluffy sediment surface layer is eroded by the near-bottom currents from 0.62 cm s^{-1} (Ziervogel and Bohling, 2003). Based on the maximum critical shear velocity (1.24 cm s^{-1}) calculated from the velocity measurements at the bottom boundary layer between January and April 2014, the near-bottom currents along the central GoF can be sufficient to initiate resuspension of cysts and keep them suspended.

Influence of transport could also explain the increase in cyst abundances between January and April in the GoR. The increase of live cysts abundances was driven by the cysts of *B. baltica*. However, this species forms a minor part of the spring bloom phytoplankton community in the GoR. Thus, the increase in live cyst abundances probably results from the input of the resuspended *B. baltica* cysts or vegetative cells from the NEBP that would potentially encyst in the GoR due to unknown adverse environmental factors prevailing there. This explanation is further supported by the input of more saline water to the GoR (Figure 2 in Paper **I**), which coincides with the highest cysts abundances found at the station closest to the strait between the NEBP and GoR.

After the spring bloom (May), the live cyst abundances increased at several stations in the GoR and GoF and the live *B. baltica* cyst proportion increased significantly at three stations in the GoF. This coincides with the main cyst formation time in the GoF (Heiskanen and Kononen, 1994; Kremp and Heiskanen, 1999; Spilling *et al.*, 2006). Increase due to the formation and sedimentation of new cysts is further supported by the increase of cysts of all three studied species at the surface sediment and by the decrease in chlorophyll (Chl) *a* concentration (Figure 3 in Paper **I**) in the surface water layer. Interestingly, at some deep stations in the GoF and NEBP, the live cyst abundance declined markedly compared to April. This finding could potentially be explained by the resuspension and transport induced by the shift in the circulation pattern in the second half of May (indicated by the course of favourable along the gulf wind stress) (Supplementary Figure 2 in Paper **I**).

However, at one station (F1) the changes in cyst abundances cannot be explained by the influence of cyst resuspension and transport due to reversals, and thus the influence of other factors to the cyst community needs to be recognised. As a potential explanation for the changes at that station, the fine-scale differences in seabed topography and sediment properties are proposed, as the exact sampling locations at that station were about 100 m apart. Further study is needed to verify this explanation.

The importance of transport was further emphasised by the modelled spread of newly-formed cysts during two different springs. In the GoF, the cysts in spring 2004 were mainly transported northward from the original location, whereas in 2013 the main transport direction was eastward (Figure 4). Notable changes in the simulated transport direction were detected from the NEBP and differences were also present in the GoR. Distance travelled by the cysts before sedimentation in the

GoF and GoR was about the same (10-30 km) during both springs. However, in the NEBP the distance varied remarkably, e.g. from station H1 the cysts were transported within 20-30 km in 2004 and around 100 km in 2013. Those notable differences can be explained by the variability in prevailing wind forcing (Supplementary Figure 3 in Paper **I**). The obtained results indicate that the transport of resting stages in the water column has an influence on spatial cyst distribution and needs to be considered when using dinoflagellate cysts as proxies for the biological productivity of a water body.

3.2 Population structure of *Skeletonema marinoi* in the Mariager Fjord and Kattegat

Populations of one of the dominant spring bloom species, *S. marinoi,* from neighbouring areas (Mariager Fjord and Kattegat) are genetically differentiated despite the absence of physical dispersal barriers (Härnström *et al.*, 2011; **II**). This genetic structuring has been present for more than thousands of generations (Härnström *et al.*, 2011). Thus, reciprocal transplant and common garden experiments were conducted to investigate the presence of local adaptation and competitive advantage to explain the persistent genetic structure.

In the reciprocal transplant experiments both populations produced significantly higher ($P < 0.001$) biomass in their native water than in foreign water. This fulfils the "home *vs*. away" criterion (Kawecki and Ebert, 2004), which is one of the indicators for detecting local adaptation (Blanquart *et al.*, 2013). Another criterion, "local *vs*. foreign" (Kawecki and Ebert, 2004) was only fulfilled by the Mariager Fjord population that showed significantly higher biomass ($P < 0.001$) in its native water than the foreign population in the same water (Figure 5 B in Paper **II**). The presence of local adaptation in both populations was further confirmed by the associated competitive advantage, displayed by the significantly higher relative cell abundances of native strains in their native water in the presence of a competitor (Figure 5).

Differential selection pressure between habitats is required for the development of local adaptation (Blanquart *et al.*, 2013) and this has also been proposed as one of the causes in population differentiation in pelagic organisms (Korpelainen, 1986; Rynearson *et al.*, 2006). There are notable differences in physical, chemical and biological parameters between the Mariager Fjord and Kattegat (Table 1 in Paper **II**). Although local water from both sites was used for the cultivation and experiments, equal amounts of nitrate, phosphate and silica were added to water from both locations, resulting in excess of nitrate and phosphate. Thus, the nitrate and phosphate concentrations did not influence the observed outcome. The concentration of silica was higher in the Mariager Fjord water including the amount added. As the growth of diatoms is dependent on ambient silica concentrations (Egge and Aksnes, 1992) and there is evidence from the Baltic Sea that some *S. marinoi* genotypes are adapted to different silica concentrations (Paper **III**), it is

possible that differences in silica requirements might explain the genetic differentiation and local adaptation found. However, this hypothesis remains to be tested, as currently there is no data regarding the phenotypic traits on silica requirements for the Mariager Fjord and Kattegat genotypes. Differences in average surface salinity were also considered as a potential cause, but the surface salinity in both habitats is well within the range suitable for growth of this species (Balzano *et al.*, 2010; Sjöqvist *et al.*, 2015). Also, the pH between the two studied locations is somewhat different with more variable pH in the Mariager Fjord and more stable in the Kattegat. However, variable conditions should promote phenotypic plasticity and not local adaptation. The water used for cultivation and experiments was not autoclaved and thus intrinsic characteristics, e.g. unidentified organic molecules and/or viruses, that could facilitate the growth of locally adapted populations, were not destroyed.

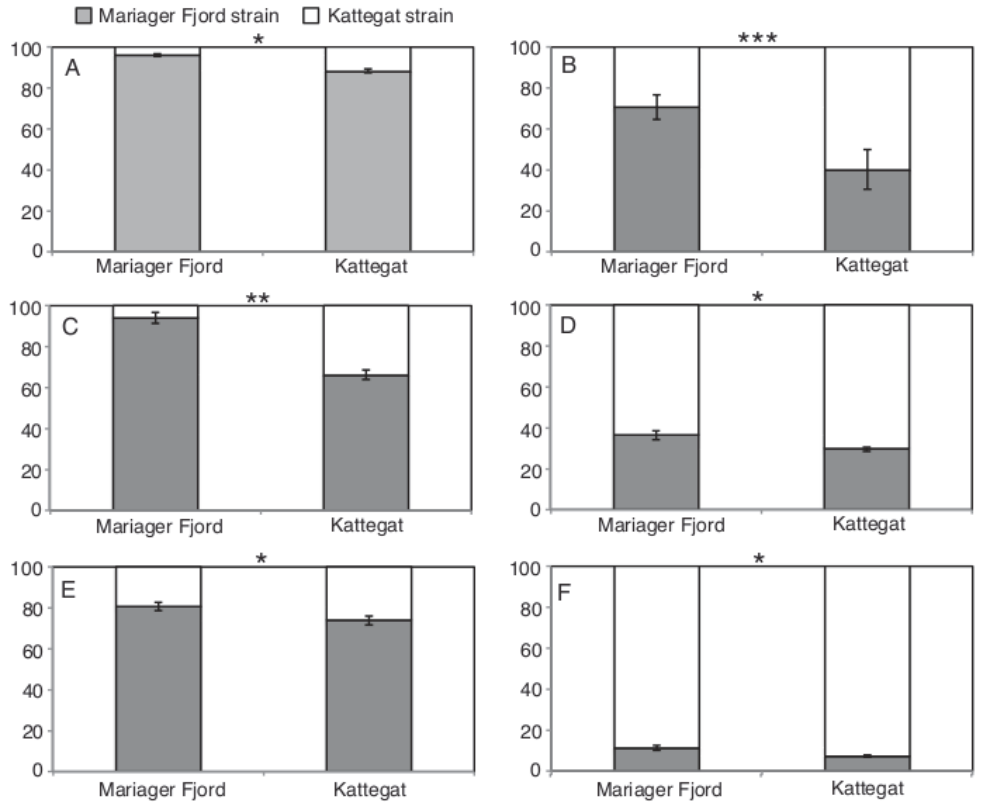

Figure 5. Kattegat and Mariager Fjord strains grown as a mix (six different strains from each site) in a competition experiment in the both types of water. Y-axis indicates relative abundances. Error bars show standard deviation (N=3). Significance is displayed by * $P < 0.05$, ** $P < 0.01$, ***$P < 0.001$.

The presence of reduced gene flow despite high dispersal potential has also been recorded for other aquatic organisms (Pálsson, 2000; Campillo *et al.*, 2009), which can be explained by the quick population growth after a historical founder event, a rapid adaptation to local conditions and an abundant benthic seed bank buffering against immigrants (De Meester *et al.*, 2002). *Skeletonema marinoi* has shown priority effects even in the absence of a numerical advantage (Sefbom *et al.*, 2015). Potentially, a historical founder event might have occurred during complex deglaciation of the Baltic Sea region (Björck, 1995). In the case of differential selection between habitats, selection for individual clonal lineages may be swift (Lohbeck *et al.*, 2012), especially in *S. marinoi* as it reproduces mainly asexually by dividing approximately once per day (Taylor *et al.*, 2009). This may also cause selection against migrants, which reinforces differentiation and local adaptation, thus reducing gene flow between habitats and creating isolation by adaptation (Nosil *et al.*, 2009). Genetic differentiation and local adaptation in *S. marinoi* are further supported by the abundant seed bank (Mcquoid, 2002) and strong coupling between the benthic and pelagic assemblages (Godhe and Härnström, 2010). Thus, varying selection pressure between connected habitats can counteract genetic homogenization, which may be intensified by the development of local adaptation.

3.3 Population structure of *Skeletonema marinoi* during spring bloom in the Baltic Sea

Genetic differentiation between *S. marinoi* populations in the Baltic Sea has been reported based on cultures established from the resting stages (Sefbom, 2015; Sjöqvist *et al.*, 2015). In this study, the presence of genetic structuring within *S. marinoi* population was investigated during a basin-wide spring bloom in the Baltic Sea. Taking into account the proposed movement of water masses from more stratified coastal areas to the less stratified off-shore regions (Kahru and Nõmmann, 1990), the bloom population should be panmictic. However, *S. marinoi* populations from the Baltic Sea have shown differences in salinity optima (Sjöqvist *et al.*, 2015), which might also be visible during the bloom.

The spring bloom in 2013 started to develop during the second half of March in the south-west (off the German coast) and north-east (Gulf of Finland), while in the offshore the bloom started later (Figure 2a in Paper **III**). By mid-April, the bloom had declined in the south-west, whereas in the north-west it was still ongoing. *Skeletonema marinoi* constituted up to 33% of the total biomass during the bloom. The population structure of *S. marinoi* indicated the presence of several genetically differentiated groups (Table S4 in Paper **III**), which show that the *S. marinoi* spring bloom in the Baltic Sea is not panmictic. The observed genetic structure can be explained by the combined effect of isolation by distance, environmental gradients (salinity) and oceanographic connectivity (Table 2 and Figure S2a-b in Paper **III**). Oceanographic features, e.g. currents, have an important role in the genetic structuring of marine planktonic organisms (White *et al.*, 2010; Casabianca *et al.*,

2011) and it has been found to explain a large part of the *S. marinoi* genetic structure (Godhe *et al.*, 2013; Sjöqvist *et al.*, 2015).

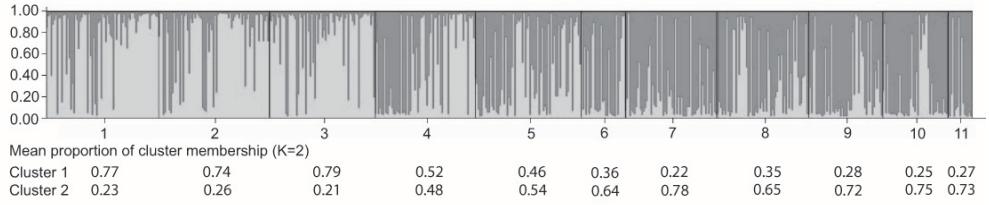

Figure 6. Genetic clustering of *Skeletonema marinoi* individuals (N=611) along the sampling transect in the Baltic Sea into two genetically distinct clusters. Individuals are represented by a vertical bar coloured according to the assigned group.

Based on the gene flow the genotypes from different stations grouped into two genetically distinct clusters along the south-north gradient (Figure 6). The division into two clusters reveals the presence of a spatial barrier to gene flow in the southern part of the Baltic Sea between stations 3 and 5. The presence of a barrier in this area has also been reported by Sjöqvist and others (2015). Over the four cruises, two genetically differentiated populations were detected from this area, which might be explained by the lower retention of cells in this station compared to others and input of cells from the neighbouring areas (Table S7 and Figure 4 in Paper **III**). Thus, the station 4 represents a transition area between the south and north populations, which is seeded by the neighbouring populations.

In addition to spatial differentiation, temporal genetic differentiation was detected between the cruises, except between the first and the second cruise (Table S5 in Paper **III**). Thus, fluctuations between genetically differentiated populations could arise over short temporal scales. Temporal variability was also visible in the correlation between environmental variables and population structure, e.g. salinity was significantly correlated with the genetic structure during the last cruise, whereas silica concentration was significantly associated with the population structure during the last two cruises (Table S8 in Paper **III**). Silica concentrations during the last cruise were notably lower compared with previous cruises (Figure 2h in Paper **III**), and this could influence the growth of diatoms (Egge and Aksnes, 1992). Thus, the *S. marinoi* spring bloom might consist of strains with different growth optima, which shift as the conditions, e.g. silica concentration, change during the bloom. This is supported by the high genotypic diversity detected from each station throughout the cruises (Table 1 in Paper **III**). However, this explanation remains to be tested, as there is no data regarding phenotypic characteristics of the isolated genotypes.

CONCLUSIONS

In this PhD thesis, the influence of physical-chemical factors on community and population structure and dynamics of the Baltic Sea spring bloom microalgae were investigated. This was done by determining the resting stage abundance and community composition, conducting common garden experiments, genetic and statistical analyses, and modelling their dispersal. Better knowledge of the factors influencing microalgae is relevant to understand ecology, evolution and dispersal of those important primary producers.

The main results of this thesis can be summarised as follows:

- Dinoflagellate cyst abundance before, during and after the spring bloom was found to be potentially influenced both by physical processes as well as by the species' life cycle events. In the Gulf of Finland, strong near-bottom currents, induced by the reversals of estuarine circulation, are proposed to resuspend and transport sedimented cysts. In the Gulf of Riga, the input of cysts from the north-eastern Baltic Proper is suggested to influence the cyst abundances.
- High abundances of resting stages are not always indicators of local cyst production intensity as the newly formed cysts may be transported up to 100 km before deposited as well as due to the potential transport between sub-basins.
- The presence of local adaptation and competitive advantage were demonstrated by the two seemingly well-connected diatom populations. Despite the possibility of dispersal, varying selection pressure(s) between habitats can support the development of genetic differentiation. The genetic structuring can be further enhanced by the presence of local seed banks and development of local adaptation. The specific selective factor can be difficult to determine due to the potential interaction of several environmental components.
- Despite the potential for panmixia, spatial and temporal genetic structuring was detected during a basin-wide bloom event. Geographical distance, oceanographic connectivity and environmental parameters were found to explain most of the spatial differentiation, whereas shifts in environmental conditions induced temporal genetic differentiation. Temporal genetic differentiation indicates the presence of various phenotypes and genetic variation. Thus, the bloom might consist of a sequence of short-lived subpopulations, each adapted to particular conditions, e.g. low silica concentration, which may help to maintain the bloom over longer periods.

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PUBLICATIONS

Paper I

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Spring bloom dinoflagellate cyst dynamics in three eastern sub-basins of the Baltic Sea

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Abstract

Dinoflagellate cyst abundance and species composition were investigated before, during and after the spring bloom in the Gulf of Finland, north-eastern Baltic Proper and Gulf of Riga in order to detect spatial and temporal dynamics. Transport of newly formed cysts by currents was modelled to explore the possible distance travelled by cysts before sedimentation. The cyst community of the spring bloom dinoflagellates was dominated by the cysts of *Biecheleria baltica* in all basins, despite its marginal value in the planktonic spring bloom community in the Gulf of Riga. Dinoflagellate cyst abundance in the surface sediments displayed temporal dynamics in all basins, however, this appeared to be also influenced by physical processes. The model simulation showed that newly formed cysts are transported around 10-30 km from the point of origin before deposited. The latter suggests that transport of resting stages in the water column significantly affects spatial cyst distribution in the sediments and thus needs to be considered in the interpretation of temporal biological productivity patterns of a water body from cyst proxies.

Keywords: *Biecheleria baltica*, *Peridiniella catenata*, cyst abundance, resuspension, cyst transport, Baltic Sea

1. Introduction

Dinoflagellates are a diverse group of unicellular organisms that form an important part of planktonic biomass in aquatic habitats (Dale and Dale, 2002). Dinoflagellates can be autotrophic, heterotrophic or mixotrophic (Graham et al., 2008), whereas photosynthetic species are globally important primary producers (Delwiche, 2007). The majority of dinoflagellate species are found in marine habitats, including brackish water and estuaries (Gómez, 2012), and around 10 % of dinoflagellates are known to produce resting cysts to survive unfavourable conditions (Head, 1996). Cyst formation results from asexual and sexual processes (Bravo and Figueroa, 2014) and can be triggered by environmental signals or stress, e.g. changes in temperature or nutrient limitation (Ellegaard et al., 1998; Figueroa et al., 2005; Kremp et al., 2009). Once formed, resting cysts sink through the water column and accumulate into sediments (Dale, 1983), thus building up a seed bank that maintains biodiversity during unfavourable environmental conditions and allows seeding of new pelagic populations (Boero et al., 1996; Kremp, 2001; Nehring, 1996). Depending on the species, cysts may remain viable in the sediments from months up to a century (Lewis et al., 1999; Lundholm et al., 2011; McQuoid et al., 2002).

Extensive cyst formation, dense cyst beds and efficient recruitment of cysts from sediments are among factors supporting the dominance of dinoflagellates over diatoms in some basins of the northern Baltic Sea (Klais et al., 2011). As the input of newly produced organic matter in temperate marine environments is the highest during spring (Blomqvist and Heiskanen, 2001; Heiskanen and Tallberg, 1999), the dominance of dinoflagellates has implications for the availability of food for benthos

(Tamelander and Heiskanen, 2004). Most of the dinoflagellate vegetative cells disintegrate in the water column, thereby providing food to the microbial food web in the upper layer. At the same time, the resting cysts, which are resistant to degradation (e.g. Dale, 1983), and for some species also to grazing (e.g. Kremp and Shull, 2003; Montresor et al., 2003), settle to the seabed (Blomqvist and Heiskanen, 2001; Heiskanen, 1993; Heiskanen and Kononen, 1994).

In the northern Baltic Sea, the dinoflagellates *Biecheleria baltica* Moestrup, Lindberg, & Daugbjerg 2009, S*crippsiella hangoei* (Schiller) Larsen 1995*, Gymnodinium corollarium* Sundström, Kremp & Daugbjerg 2009 and *Peridiniella catenata* (Levander) Balech 1977 form an important part of the spring bloom (Heiskanen and Kononen, 1994; Hällfors et al., 2013; Klais et al., 2013; Lips et al., 2014). These cold-water species also produce resting cysts to survive unfavourable water temperatures and to seed new blooms (Heiskanen, 1993; Kremp, 2000a; Kremp *et al.*, 2005; Moestrup *et al.*, 2009; Sundström *et al.*, 2009). Particularly *B. baltica* is known for massive cyst production, with about 44% of the vegetative population producing cysts (Heiskanen, 1993; Kremp and Heiskanen, 1999). For *P. catenata* the exact magnitude of encystment is not known, as it forms cysts in the deep water layers (Spilling et al., 2006). However, its cumulative sedimentation is reported to be several magnitudes lower compared to *B. baltica* (Tamelander and Heiskanen, 2004). Cysts of *G. corollarium* have approximately equal sedimentation rates than cysts of *B. baltica* (Sundström et al., 2009). There is no information available regarding the proportion of cysts produced by *S. hangoei* relative to its vegetative population. However, the species is assumed to be present in low abundances in the spring bloom (Sundström et al., 2009). Dinoflagellate cyst species composition, relative and total abundances of cysts in the sediment surface layer can be influenced by the seasonal dynamics of different parameters, e.g. temperature, stratification, or availability of nutrients (Harland et al., 2004; Marret and Scourse, 2003; Warns et al., 2013). In the Gulf of Finland cysts of *B. baltica* and *P. catenata* display seasonal dynamics most probably related to germination and input of newly produced cysts (Kremp, 2000a;b) (*B. baltica* cysts then considered to be cysts of *S. hangoei*). Distribution, abundance and species composition of cysts in the sediments provide valuable information regarding previous and future blooms (Anderson et al., 2014; Dale, 2001).

Cyst abundance in the surface sediments and the locations of the main cyst beds consisting of cysts produced by *B. baltica* (there as *Woloszynskia* spp.) and *P. catenata* have been mapped after the spring bloom in the Gulf of Finland, north-eastern Baltic Proper and in the Gulf of Riga previously by Olli and Trunov (2010). Dense cyst beds were detected in the central and eastern Gulf of Finland (Olli and Trunov, 2010), which have been suggested to promote dinoflagellate dominance (Klais et al., 2011). However, to what extent the cysts from different deep and shallow accumulation areas contribute to the seeding of the respective blooms has remained unknown. Furthermore, Olli and Trunov (2010) found inconsistencies between cyst distribution patterns and spatial abundances of motile cells in the overlying water column indicating that the areas of cyst production may not be the same as the areas of cyst deposition.

The Baltic Sea is a shelf sea in the northeast of Europe (Fig. 1) connected to the Atlantic Ocean via the Danish Straits, Kattegat and Skagerrak. It is one of the largest brackish water bodies in the world. The inflow of saline water from the North Sea is limited by the shallow Danish straits (Lass and Matthäus, 2008; Leppäranta and Myrberg, 2009). Northern and eastern basins receive larger freshwater input than basins in the south and west (Bergström and Carlsson, 1994). Thus, the surface water in the northern basins is less saline (e.g. around 3 in the Bay of Bothnia) than in the southern basins (e.g. around 8 in the Arkona basin) (Leppäranta and Myrberg, 2009). The outflow of low salinity water in the surface layer and sporadic major inflows of saline North Sea water through the Danish Straits (Lass and Matthäus, 2008) maintain the salt balance and strong salinity stratification in the Baltic Sea

(e.g. Leppäranta and Myrberg, 2009). The halocline (located at a depth of 60–80 m) separates upper low salinity (6–8) layer and deep more saline (10–14) layer in the central Baltic Sea (Leppäranta and Myrberg, 2009). Changing wind forcing modulate the large scale circulation. In the Gulf of Finland estuarine circulation can be reversed, i.e. outflow in the bottom layer and inflow in the surface layer, when south-westerly winds prevail (Elken et al., 2003; Liblik et al., 2013). Strong and long enough pulses of south-westerly winds in winter may cause estuarine circulation reversals, resulting in the vanishing of stratification and mixing of the entire water column (Elken et al., 2014; Liblik et al., 2013; Lips et al., 2016). Water temperature in the Baltic Sea has a characteristic annual cycle. In winter, the northern Baltic is usually ice-covered, while the average surface temperature is 2–3°C in the south (Leppäranta and Myrberg, 2009). During spring, thermal stratification starts to develop as the surface layer is heated by solar radiation (Leppäranta and Myrberg, 2009). When the upper mixed layer becomes shallower than the euphotic zone the spring bloom starts to develop (Wasmund et al., 1998). Increasing solar radiation strengthens the seasonal thermocline, which allows temperature to rise quickly in the upper mixed layer and prevents input of nutrients from below (Hagaström et al., 2001). The spring bloom ends when mineral nutrients become exhausted above the seasonal thermocline (Hagaström et al., 2001). During summer, the seasonal thermocline is located at a depth of 15–30 m and the average sea surface temperature is 13–18 °C (Leppäranta and Myrberg, 2009).

In this study, the resting cysts of the three most abundant spring dinoflagellate species were investigated in surface sediments of the eastern Baltic Sea to detect spatial and temporal dynamics in cyst abundance and species composition. Ratios of empty *versus* live cysts of *B. baltica* were analysed to detect the potential influence of the spring bloom related life cycle events to the cyst community. To explore the geographical extent of the spread of the newly formed cysts in the water column when settling and to assess the interannual variability of such processes, transport by currents was modelled for two spring situations with different atmospheric forcing using a three-dimensional hydrodynamical model.

2. Method

2.1. Sampling

Surface sediment (0-5 cm) samples from 13 stations in the Gulf of Finland (GoF), north-eastern Baltic Proper (NEBP) and the Gulf of Riga (GoR) were collected during three national open sea monitoring cruises in 2013 (Fig. 1., Table 1) by using a Niemistö gravity corer. The sampled sediments consisted of mud and sand. More details regarding the sediment properties from most of the stations are available in Olli and Trunov (2010). Sediment from 0-5 cm was collected to ensure comparability with a previous study by Olli & Trunov (2010). Although the cysts in this layer represent an integration from several years depending on the sedimentation rates at each specific location, seasonal changes in cyst abundances were expected to be visible in the differences between total cyst abundances in each sampling month. Data from sediment samples taken before (January), during (April) and after the spring bloom (May) was compared to account for temporal cyst dynamics affected by benthic-pelagic coupling during the bloom season. Samples were stored in the dark at 4 °C until processing.

2.2. Physical, chemical and biological background data

Data regarding water temperature, salinity, chlorophyll *a* (Chl *a*), inorganic nutrients and oxygen concentrations during the cruises is available in the data annex of the 2013 national open sea monitoring report (Martin et al., 2014). Sampling and analysis methods of different parameters are described in the national open sea monitoring report (Martin and Lips, 2014). In this study salinity, temperature and oxygen concentrations throughout the water column are shown in Figure 2. The

values for each sub-basin are based on one station best representing the dynamics in the particular basin. For the GoR the data is from station 111, for the NEBP from station H1 and for the GOF from station F1.

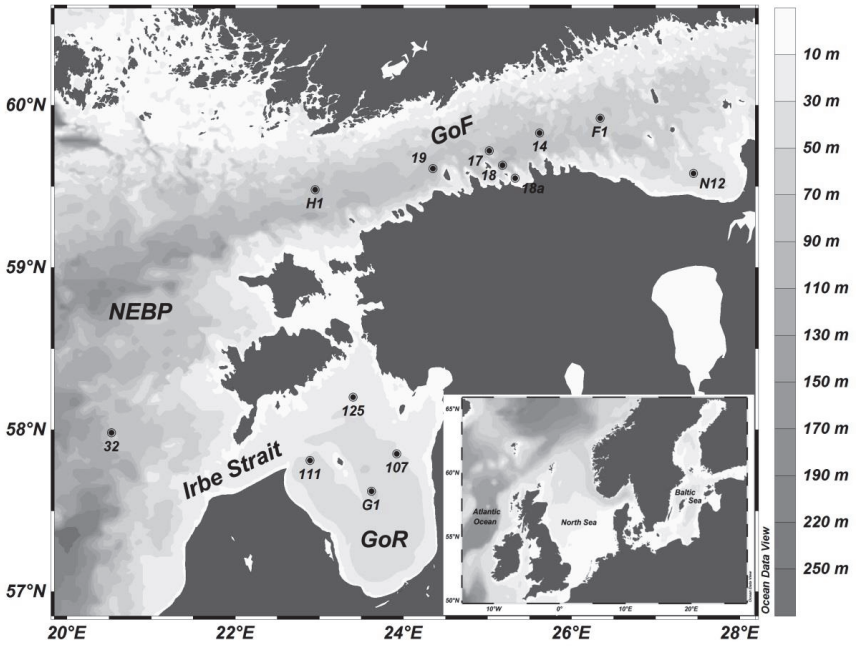

Fig. 1. Sediment sampling stations in the Gulf of Finland (GoF), north-eastern Baltic Proper (NEBP) and the Gulf of Riga (GoR). Scale bar indicates bottom depth. Inset shows the wider spatial context of the area.

2.3. Sediment sample processing and analysis

From each sample 1 mL of sediment was sonicated for 30 s at medium level using a Bandelin Sonoplus sonicator and wet-sieved through a 53 µm sieve onto a 15 µm sieve. Additional sonication (30 s) was applied to samples where aggregates remained. The final volume of the cleaned samples was adjusted to 10 mL. The processed subsamples were examined in a 2 mL Utermöhl chamber under Olympus IX 51 microscope. A minimum of 250 cysts were counted from each sample. From all stations at least one sample was counted in triplicates. Cysts counts were converted into a number of cysts per gram of dried sediment. To determine sediment dry weight, 1 mL of sediment from every station during all sampling months was weighed and dried in triplicates for 6 h at 105 °C. After cooling the samples were weighed again. Abundances of cysts produced by the spring bloom dinoflagellates *B. baltica*, *P. catenata* and *S. hangoei* are presented in this study. Confidence intervals (CI, 95%) were calculated for the samples counted in triplicates to estimate whether the differences in total live cyst abundances per gram of dried sediment are due to natural variability or represent true changes.

To assess the potential influence of life cycle events, i.e. recruitment and cyst formation, on the cyst abundances, the fraction of empty *versus* live cysts was analysed for *B. baltica*, assuming that the empty cyst fraction would increase when germination takes place and relatively decrease when new

cysts settle to the sea floor. In contrast to *P. catenata* and *S. hangoei*, cysts of this species possess distinctive spines on their surfaces which can be recognised on empty walls. Chi-square test of independence (SPSS, IBM) and post hoc tests (pairwise Chi-square test between different months) with Bonferroni correction were used to detect differences between proportions of empty and live cysts before (January), during (April) and after the spring bloom (May). To measure the strength of association between the month and the cyst condition (empty *versus* live) the Cramer's V was calculated for statistically significant Chi-square test results.

2.4. Modelling cyst transport

General Estuarine Transport Model (GETM) (Burchard and Bolding, 2002), a three-dimensional hydrodynamical model, was used to study hydrodynamical conditions in the spring of 2004 and 2013. GETM solves momentum conservation equations together with the continuity equation and equation of state to derive three-dimensional velocity, salinity and temperature fields. Vertical mixing was parameterized via General Ocean Transport Model (Umlauf and Burchard, 2005) using a k-ε model with algebraic closure functions. Horizontal turbulence was described by using constant horizontal viscosity of 10 m^2 s^{-1}. High-order total variation diminishing advection scheme with P2-PDM limiter was chosen for momentum and tracer variables (Pietrzak, 1998). The modelling domain of the Baltic Sea, with a horizontal resolution of a 1 nautical mile, was initiated from the average salinity and temperature field of the climatic simulation for period 1966-2004 (Maljutenko and Raudsepp, 2014). Initial water level and velocities were set to zero. Boundaries at Kattegat were closed for such a short simulation period. The monthly mean river runoff derived from HYPE model (Donnelly et al., 2016) was used. Meteorological fields (heat fluxes, wind, pressure, air temperature) were derived from two datasets: BaltAn65+ reanalysis dataset (Luhamaa et al., 2010) for the year 2004 and from HIRLAM-ETA dataset for the year 2013 (Undén et al., 2002).

For simulation of cyst transport, a passive tracer extension to the GETM model, available via Framework for Aquatic Biogeochemical Models (Bruggeman and Bolding, 2014), was used. At the beginning of simulations, cysts at each station within the 0-10 m surface layer were treated as concentrations of different tracers. During simulations, the cysts settled with a constant velocity of 2.5 m d^{-1} (based on Stoke's law; Heiskanen, 1993). When cysts reached to the seafloor, they were deposited to the sediment surface. After two months of simulation, when more than 99% of initial cysts were settled into the sediment pool, the 10^{th} and the 90^{th} percentiles were calculated from settled cyst concentration (Cp) to characterise the spread of the highest cyst concentration. Spread distances were calculated as the distance from each station (x0, y0) to the geometrical centroid (cx, cy) of the Cp area. Distance = $|(cx, cy) - (x0, y0)|$, where geometrical centroid coordinates are defined as $cx = \int Cp\ x\ dA / \int Cp\ dA$ and $cy = \int Cp\ y\ dA / \int Cp\ dA$, where dA is an area element.

3. Results

3.1. Physical, chemical and biological characteristics

In the GoF, the water column displayed relatively weak salinity stratification in January 2013 (Fig. 2). The entire water column was well oxygenated, even the near-bottom layer. In April, a layered structure developed in the water column and salinity stratification strengthened. In the near-bottom layer, the salinity increased significantly compared to measurements in January. In deeper layers oxygen deficiency started to develop (oxygen concentrations < 2 mg L^{-1}). By the end of May, the whole water column was strongly stratified and the vertical distribution of salinity was similar to April. The water column was well oxygenated down to the halocline, whereas the near-bottom layer was hypoxic.

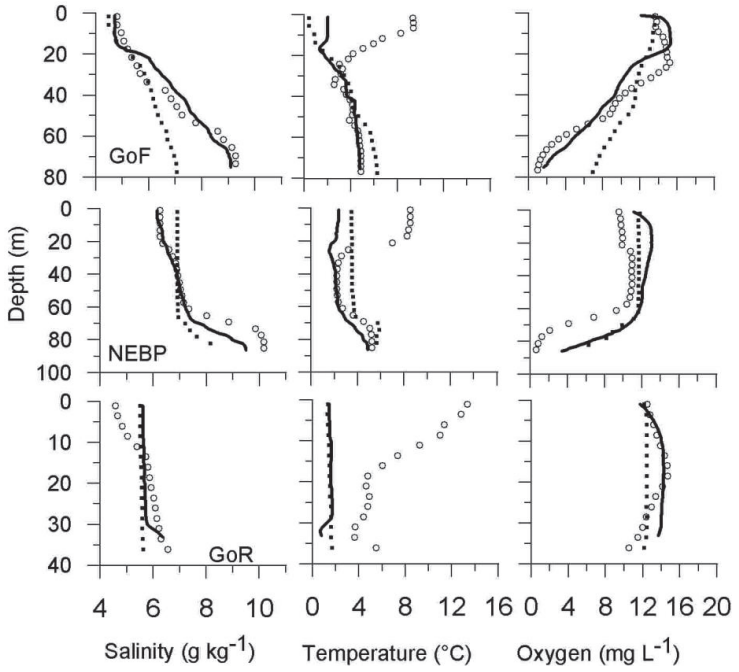

Fig. 2. Vertical distribution of salinity (g kg-1), temperature (°C) and oxygen concentration (mg L-1) in the Gulf of Finland, north-eastern Baltic Proper and the Gulf of Riga in January, April and May 2013. Black squares represent measured values in January, solid line in April and empty circles in May.

In the NEBP, the entire water column was mixed down to the halocline in January (Fig. 2). The oxygen conditions were high throughout the water column. In April, weak salinity stratification was present in the water column above the halocline. In the near-bottom layer the salinity increased compared to January, whereas oxygen concentrations decreased. In May, the seasonal thermocline had developed, strengthening the stratification in the upper water column. Above the halocline the salinity distribution in the water column was similar to April, however, in the near-bottom layer the salinity increased. Oxygen concentrations below the halocline had decreased notably and hypoxia was present.

In the shallower GoR, the entire water column was mixed down to the bottom in January (Fig. 2). In April, the water column was still well mixed to a great extent, however in the near-bottom layer an increase in salinity was observed. In May, both thermal and salinity stratification was present in the water column and oxygen concentrations decreased towards the bottom (Fig. 2).

In January, the Chl *a* values in the surface layer (0-10 m) were low in all basins, the highest values were measured in the Gulf of Riga (Fig. 3). In April, the highest Chl *a* concentration in the surface layer was recorded in the GoR at station G1 (36.77 mg m^{-3}) and high Chl *a* values were present in all basins. The maximum Chl *a* concentrations in May had decreased notably compared to concentrations measured in April in the GoF (19.90 to 3.07 mg m^{-3}), NEBP (5.99 to 2.40 mg m^{-3}) and GoR (36.77 to 8.94 mg m^{-3}).

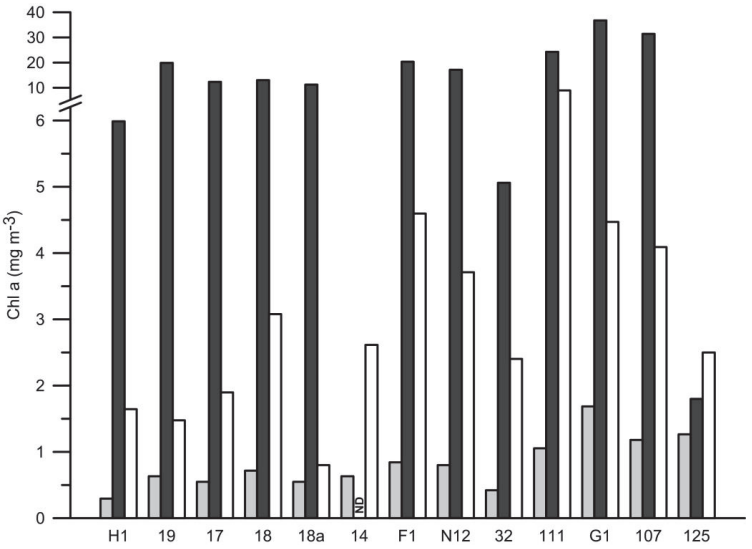

Fig. 3. Chlorophyll *a* concentrations in the surface layer (1-10 m) at different sampling times: January (grey bars), April (black bars) and May (white bars) 2013 in the Gulf of Finland, north-eastern Baltic Proper and the Gulf of Riga. ND = no data. Note that the vertical axis has been cut between 6 and 10 mg m^{-3}.

3.2. Cyst abundances

The total live cyst abundance varied notably in time and space (Table 1). In the GoF, the cyst abundances ranged from 1144 to 128 572 cysts per gram of sediment dry weight (g dw^{-1}) during the study period. In the NEBP, the cyst abundances varied in different seasons from 3678 to 29 538 cysts g dw^{-1} and the lowest abundances of cysts in the surface sediments (748 to 7887 cysts g dw^{-1}) were found in the GoR. At the majority of the stations in the GoFthe live cyst abundance per gram of dry sediment decreased in April (during the spring bloom) compared to January (before the bloom). The total live cyst abundances decreased notably at the GoF open sea stations (by 55 % and 64 % at stations 19 and F1, respectively) as well as at stations closer to the coast (59 % at station 18a and 24% at station N12) from January to April. At station 18 the cyst abundances showed a decrease, but this was probably due to natural variability as the cyst abundances from both months remained within the 95% CI for the April sample. In the NEBP at station H1, the total cyst abundances decreased by 13 % in April, whereas at station 32 the cyst abundances indicated a notable increase. However, it is not known if those patterns represent sampling error or actual changes. In the GoR, the decline in cyst abundance in April was only detected at station 107 (39 %). At stations 125 and 111 the total live cyst abundances increased (49 % and 79 % respectively) in the surface sediments compared to the results obtained in January. At station G1 the increase in the live cyst abundances probably represents natural variability.

In May (after the spring bloom), the total live cyst abundances increased mainly at stations closer to the southern coast of the GoF (stations N12 and 18a the abundance increased by 40 % and 58 % respectively), as well as at one open sea station 19 (increase by 36 %), compared to the abundances in April. In the GoR, the abundance increased at all stations, except at station 111, where the live cyst abundances decreased by 76 % compared to April values. In the NEBP, the live cyst abundances

decreased by 23 % at station H1 and by 21 % at station 32 compared to the counts in April. At stations 19, 14, 17, F1 (GoF) and 111 (GoR) the total cysts abundance displayed notable changes in magnitude during different sampling months.

Table 1. Cyst abundances (g dw^{-1}) at sampling stations in January (1), April (2) and May (3) 2013. Mo=month, D=depth, CI=confidence intervals, *B. bal.* =*Biecheleria baltica*, *P. cat.* = *Peridiniella catenata*, *S. han.* = *Scrippsiella hangoei*

St.	Basin	Lat. (N)	Lon. (E)	Mo.	D. (m)	Live *B. bal.* cysts	*P.cat.* cysts	*S. hang.* cysts	Total nr. of live cysts	SD	95% CI */	Empty *B. bal.* cysts
H1	NEBP	59.48	22.95	1	86	29 538	0	0	29 538			968
				2		25 674	173	0	25 847			1 474
				3		19 841	128	0	19 969	744	1.09	2 883
19	GoF	59.61	24.35	1	82	93 987	139	0	94 126			3 337
				2		41 885	0	0	41 885	2 614	1.69	16 337
				3		64 226	1 176	0	65 402			1 809
17	GoF	59.72	25.02	1	110	12 662	105	0	12 767	636	1.13	782
				2		85 787	0	0	85 787	8 480	1.27	36 247
				3		12 591	68	0	12 659			643
18	GoF	59.63	25.18	1	98	72 990	431	215	73 636			10 981
				2		66 293	1 113	223	67 629	6 843	1.28	7 160
				3		66 099	1 909	881	68 890			2 203
18a	GoF	59.55	25.33	1	45	3 362	27	0	3 389	129	1.10	427
				2		1 177	196	0	1 373			107
				3		2 580	718	10	3 308			91
14	GoF	59.83	25.62	1	75	4 432	18	0	4 450	624	1.40	518
				2		128 572	3 198	0	131 770	14 209	1.29	20 696
				3		3 539	13	0	3 552	199	1.15	501
F1	GoF	59.92	26.34	1	80	36 105	40	0	36 145	2 727	1.21	323
				2		12 923	0	69	12 992	855	1.17	241
				3		1 511	19	0	1 530	187	1.36	98
N12	GoF	59.58	27.45	1	35	1 514	9	0	1 523	77	1.14	523
				2		1 144	0	11	1 155			238
				3		1 711	195	7	1 913			61
32	NEBP	57.98	20.53	1	96	4 044	72	0	4 116			307
				2		3 678	3 832	0	7 510			1 235
				3		5 761	184	0	5 945	133	1.06	1 210
111	GoR	57.81	22.89	1	33	1 660	51	86	1 797			308
				2		7 887	95	473	8 455	84	1.02	500
				3		1 503	525	0	2 028	27	1.03	427
G1	GoR	57.62	23.62	1	55	2 579	1 084	105	3 768	152	1.11	90
				2		3 466	449	0	3 915			594
				3		7 464	1 217	256	8 937			513
107	GoR	57.85	23.92	1	28	1 226	0	24	1 250			244
				2		748	8	13	769	39	1.13	21
				3		783	488	60	1 331	19	1.04	41
125	GoR	58.20	23.40	1	25	1 063	4	33	1 100			94
				2		1 960	102	114	2 176			144
				3		5 817	1 518	654	7 989	98	1.03	164

At all sampling stations and occasions more live than empty cysts of *B. baltica* were present (Table 1). In the Chi-square tests between the sampling month and the cyst condition as well as in the post-hoc pairwise Chi-square tests all expected cell frequencies were greater than five fulfilling an assumption required to provide valid results for the test. The ratio of empty *versus* live cysts changed significantly from January (before the spring bloom) to April (during the spring bloom) only at four stations (Table 2). In May (after the spring bloom), the empty *versus* live cyst ratio was again significantly different at

four stations compared to April (during the spring bloom). The correlation between the sampling month and cyst condition (empty *versus* live) varied between very weak and weak (based on Fowler et al., 1998) depending on the sampling station and the month tested (Table 2).

Table 2. Statistically significant results of the Chi-square test of independence and post-hoc tests between live and empty *B. baltica* cyst proportions and sampling months with Cramer's V indicating the strength of association. Df indicates degrees of freedom and asterisk indicates p-values after applying the Bonferroni correction.

Station	Post-hoc test	χ^2 statistic	df	p-value	Cramér`s V	p-value
19		41.46	2	<0.001	0.372	<0.001
	January *vs* April	23.86	1	<0.001*	0.345	<0.001
	April *vs* May	23.86	1	<0.001*	0.345	<0.001
17		33.96	2	<0.001	0.336	<0.001
	January *vs* April	19.51	1	<0.001*	0.312	<0.001
	April *vs* May	21.64	1	<0.001*	0.329	<0.001
N12		10.89	2	0.004	0.191	0.004
	April *vs* May	10.89	1	0.001*	0.233	0.001
111		10.44	2	0.005	0.187	0.005
	April *vs* May	10.63	1	0.001*	0.231	0.001
G1		10.59	2	0.005	0.188	0.005
	January *vs* April	8.79	1	0.003*	0.210	0.003
107		15.011	2	0.001	0.224	<0.001
	January *vs* April	10.89	1	0.001*	0.233	0.001

3.3. Species composition of the spring bloom dinoflagellate cyst assemblages

The cysts of *B. baltica* dominated the community at the majority of stations and sampling occasions, except in April at station 32 (Table 1). The cysts of *P. catenata* were present at all stations (Table 1). However, their abundances were notably lower compared to the cysts of *B. baltica*. An exception was station 32 in April, where the cysts of *P. catenata* dominated the community. In general, the cysts of *P. catenata* formed a more visible part of the overall cyst assemblage in May (after the spring bloom), except at stations 14 (GoF) and 32 (NEBP), where the cysts of this species had the highest proportions in April (during the spring bloom). At station G1 (GoR) the proportion of cysts of *P. catenata* was the highest in January and remained lower during April and May. Cysts of *S. hangoei* were found at four stations in the GoF and at all stations in the GoR but were not detected from the NEBP. In general, the cysts of this species constituted a minor proportion of the overall cyst community in both basins. The average abundance of live cysts of *B. baltica* was 34 052 cysts g dw^{-1} in the GoF, 3013 cysts g dw^{-1} in the GoR and 14 756 cysts g dw^{-1} in the NEBP. The average abundance of cysts of *P. catenata* was 446 cysts g dw^{-1} in the GoF, 462 cysts g dw^{-1} in the GoR and 732 cysts g dw^{-1} in the NEBP. The average abundance of cysts of *S. hangoei* was 67 cysts g dw^{-1} in the GoF and 151 cysts g dw^{-1} in the GoR.

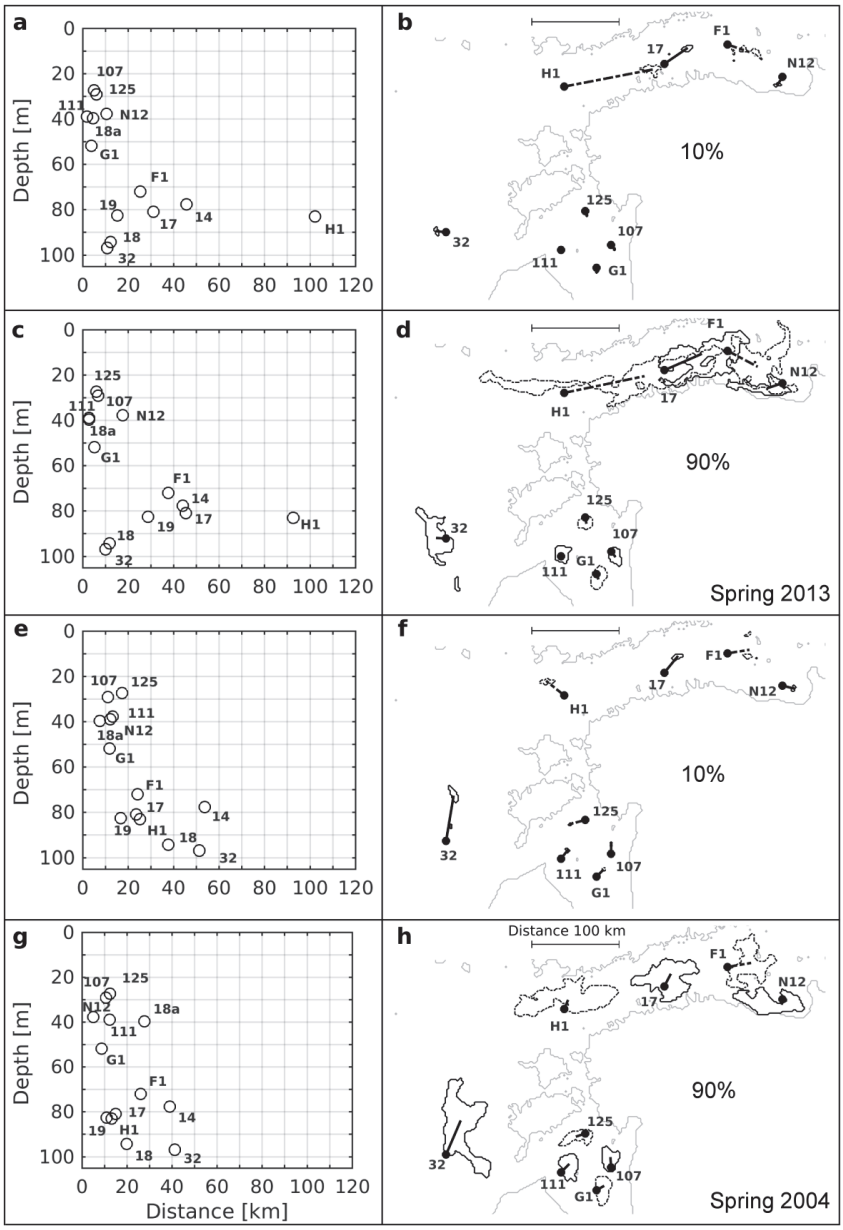

Fig. 4. Simulated transport of newly formed cysts during spring 2013 (**a - d**) and 2004 (**e - h**) with transport vectors. First panel (**a, c, e, g**) indicates transportation of cysts relative to the water column depth at the point of origin. **A** and **b** show the distribution of 10 % of the highest abundances of cysts released from the stations during 2013, **c** and **d** denote the distribution of 90 % of the released cysts during 2013. **E** and **f** show the distribution of 10 % of the highest abundances of cysts released from the stations during 2004, **g** and **h** denote the distribution of 90 % of the released cysts during 2004.

3.4. Modelled cyst transport

Simulated transport of cysts during the springs of 2004 and 2013 displayed different patterns of spread before sedimentation. Simulations indicated mainly northward transport from the original location during the spring of 2004, whereas in 2013 the cysts were mainly transported eastward along the Estonian coast in the GoF (Fig. 4). An exception during both years was the station N12 where the cysts were transported to the south-west in both simulations. In the GoF, the majority of cysts were transported within 30 km from the original location during both years (see insets in Fig. 4). During the same simulation periods the majority of cysts in the GoR displayed spread around 10 km from the original location before sedimentation. A notable difference between years was shown by the modelled transport of cysts from stations H1 and 32 (NEBP) (Fig. 4). For example, in 2004, the highest concentration of cysts was found within 20-30 km from station H1, whereas in 2013 the highest concentration was found about 100 km from the same station. Model results indicated that ten percent of the highest cyst concentrations settled in the area relatively close to the initial location, whereas the rest of the cysts settled within the wider area around the initial location (Fig. 4).

4. Discussion

The present study provides a detailed overview of dinoflagellate cysts abundance, species composition and dynamics before, during and after the spring bloom in the Gulf of Finland, north-eastern Baltic Proper and Gulf of Riga. Total cyst concentrations varied among basins with the highest numbers recorded in the Gulf of Finland and the lowest in the Gulf of Riga. In general, the cysts of *Biecheleria baltica* dominated the cyst community in all basins. Seasonal dynamics in cyst abundances were detected in all basins. However, corresponding changes in the cyst abundances were presumably also influenced by the horizontal transport within and between the sub-basins. Modelled spread of newly formed resting stages indicated high probabilities for cysts to be transported within 30 km from the point of release, i.e. formation, before sedimentation in the Gulf of Finland and north-eastern Baltic Proper, whereas in the Gulf of Riga the cysts settled closer to the origin.

4.1. Spatial differences in cyst abundances between the sub-basins

The average total cyst concentration in the GoF was about two times higher compared to the NEBP and exceeded the numbers in the GoR by an order of magnitude. A notable difference in total cyst abundances between GoF and GoR has previously been reported by Olli and Trunov (2010). Dinoflagellate cysts are in the same size range as silt particles (Dale, 1976). Thus highest cyst abundances are typically found from areas dominated by fine-grained sediment, e.g. silt, clay (Anderson et al., 2005; Sundström et al., 2009; Wall, 1971). Bottom types where silt and clay dominate are well represented in all three basins (Carman and Cederwall, 2001; Winterhalter et al., 1981) and the majority of the samples were taken from the accumulation areas in all basins, based on the map by Carman & Cederwall (2001).

Dinoflagellate cyst distributions in the sediments are also influenced by the cyst production patterns in the overlying water column and specific encystment strategies of the respective dinoflagellate species (Anderson et al., 2005; Dale, 1983; Kremp et al., 2009; Wall, 1971). The decadal average spring bloom biomass values in the GoF and GoR are notably higher (>6 mg L^{-1}) than in the NEBP (around 2 mg L^{-1}) (Klais et al., 2011). However, unlike the NEBP and GoF, where dinoflagellates dominate the spring phytoplankton community (Klais et al., 2011), the GoR spring bloom largely consists of diatoms (Jurgensone et al., 2011; Klais et al., 2011). Therefore, the dinoflagellate biomass is much lower in the GoR than in the two other studied sub-basins (Suppl. Fig. 1). Moreover, the cold-water

dinoflagellate community is dominated by *P. catenata* in the GoR (Jurgensone et al., 2011), while the *Scrippsiella* complex (incl. *B. baltica*) proportion of the dinoflagellate biomass is very low (Suppl. Fig. 1). The vegetative stage of *B. baltica* belongs to the *Scrippsiella* complex in the phytoplankton data as several medium-sized dinoflagellates (*B. baltica*, *Gymnodinium corollarium*, *S. hangoei*) are not identified to species level during routine phytoplankton counting. The exact reason for the low biomass values of the complex is not known, however, the influence of temperature and dispersal limitation due to dominant current patterns are suggested as potential causes (Klais et al., 2013). The low concentrations of spring dinoflagellate cysts in the GoR most likely reflect this, as the two species have different encystment strategies. *Peridiniella catenata* encysts at very low frequency (Kremp, 2000c), and thus even a large standing stock of cells during a bloom will result in a low number of deposited cysts. In comparison, the encysting fraction of a *B. baltica* bloom is large (Kremp and Heiskanen, 1999) and even low abundances of this species in the water column will result in a substantial input of *B. baltica* cysts to the sediments.

The NEBP is represented in this study by two stations located far apart (Fig. 1). Cyst abundances from station 32 could be considered more characteristic of the dynamics prevailing in the NEBP, than abundances at station H1, since the latter is positioned at the entrance of the GoF. The distinct nature of the two stations is also reflected by the notable differences in live cysts abundances during all sampling months (Table 1). The higher average live cysts abundance at station H1 could be explained by the dominance of species belonging to the *Scrippsiella* complex (including *B. baltica*), which is prevailing in the GoF (Klais et al., 2011), as well as by high cyst production of *B. baltica* (Kremp and Heiskanen, 1999). In contrast, the lower abundance of live cysts at station 32 likely reflects the lower biological productivity in the NEBP compared to the GoF and GoR (Klais et al., 2011).

4.2. Temporal cyst dynamics

In the GoF, the total live cyst abundances decreased from January to April at most of the stations, whereas in the GoR an opposite pattern was observed. In the NEBP, the cyst abundances at station H1 decreased, whereas at station 32 the abundances increased. The presence of different patterns between and within basins does not comply with the expected decrease in cyst abundances related to recruitment before the bloom (Kremp, 2000b). Significant increase in empty cyst proportion, an indicator for germination (Giannakourou et al., 2005), was present at four stations. However, this coincided with a decrease in live cyst abundances only at two stations. This could be due to aging of *B. baltica* cysts as they lose the ability to germinate within a few months after completing the dormancy and the general susceptibility to low oxygen conditions and the requirement for light during germination (Kremp, 2001; Kremp and Anderson, 2000). Hence the percentage of cysts that germinate is generally low and reflected by low contribution of empty cysts to the total (Kremp, 2000b). This also explains why more live than empty *B. baltica* cysts were recorded during all sampling months. Differential preservation of cysts with and without cell content cannot be excluded, however, there is currently no information available regarding this.

Some of the stations in the GoF and in the NEBP, where reduction of cysts was detected in early spring, were ≥ 80 m deep. Thus a suppression of germination due to lack of light and oxygen should be expected (Kremp and Anderson, 2000). Although cysts of *B. baltica* and *P. catenata* can germinate in darkness (Kremp, 2001) and the oxygen concentrations below the halocline were high in January 2013 (Martin et al., 2014), the decrease in cyst concentrations at most of the stations in the GoF and NEBP was higher than would be expected given the low germination rates of dinoflagellate species in darkness (Anderson et al., 1987; Kremp, 2001; Vahtera et al., 2014). Thus, recruitment via germination does not seem a sufficient explanation for the observed pattern. The latter strongly

suggests that other factors than recruitment processes govern the cyst dynamics in winter and early spring.

The temporal and spatial dynamics of dinoflagellate cyst abundances in surface sediments are also influenced by the physical processes, e.g. near-bottom currents, leading to resuspension and transport of cysts (Nehring, 1994). The near-bottom current velocities along the GoF central axis are particularly high (up to 40 cm s^{-1}) during shifts between estuarine and reverse circulation (Liblik et al., 2013). A relatively small extent of high saline water and high oxygen levels in the near-bottom layer of the GoF in January compared to April (Fig. 2), a characteristic of the reversed circulation pattern, indicate that such shifts might have taken place. The possible reversed circulation event is supported by the course of along gulf cumulative wind stress between January and April (Suppl. Fig. 2) during which several shifts favouring the changes in circulation patterns were present. During those shifts, the SSW wind component exceeded the average wind speed required to induce the reversal of estuarine circulation in deep layers (data not shown) (Elken et al., 2003).

The maximum velocity of 44 cm s^{-1} (inertial oscillations included) measured at the bottom boundary layer between January and April 2014 resulted in a maximum critical shear velocity of 1.24 cm s^{-1} (see Appendix A for details). The fluffy sediment surface layer is eroded by the near-bottom currents with velocities from 0.62 cm s^{-1} (Ziervogel and Bohling, 2003). Assuming that dinoflagellate cysts act as fine silt particles in the sediments (Dale and Dale, 2002), the near-bottom currents along the central GoF are sufficient to initiate the resuspension and keep the particles suspended. Thus, the reduction in cyst abundances at most of the deep stations and increase at stations 17 and 14 in the GoF could be due to the horizontal transport of cysts by the near-bottom currents.

In the GoR, the total cyst abundances increased in April compared to January at the majority of the stations. Based on the long-term national open sea monitoring data the diatom-dominated spring bloom usually commences in April in the GoR (Jurgensone et al., 2011). The increase in the total live cyst abundances was mainly due to the input of cysts of *B. baltica* (Table 1), which at the same time forms only a minor part of the spring bloom phytoplankton community in the GoR. Thus, the increase found in April is probably due to the input of *B. baltica* cysts or vegetative cells transported from the NEBP that would encyst due to unknown unfavourable environmental factor(s) prevailing in the GoR. The main water exchange between the Baltic Proper and GoR is through the Irbe Strait, with a sill depth of 20 m, which allows only surface water from the Baltic Proper to enter the GoR (Leppäranta and Myrberg, 2009). The input of more saline water through the strait was visible in April (Fig. 2), which also coincides with the highest cyst abundances found from station 111.

In May, the live cysts abundances increased at the majority of the stations in the GoF and at all stations in the GoR, whereas the abundances decreased at both stations in the NEBP. The live *B. baltica* cyst proportion also increased significantly at three stations in the GoF. The latter coincides with the main cyst formation time (at least in the GoF) (Heiskanen, 1993; Heiskanen and Kononen, 1994; Kremp and Heiskanen, 1999; Spilling et al., 2006). The formation and sedimentation of new cysts are further supported by the increased abundances of cysts of all three species at several stations (Table 1) and by the decrease in Chl *a* concentration in the surface layer in May compared to April (Fig. 3), indicating the termination of the spring bloom. However, there were some deep stations in the GoF and NEBP, where the cyst abundance displayed a notable decrease compared to April (F1, 14, 17, H1, 32). These findings could be explained by the resuspension and transport induced by the shift in circulation pattern in the second half of May 2013 (Suppl. Fig. 2). At the same time, the reduction in cyst abundances in May was around eightfold compared to April and about twenty-three-fold compared to January at station F1, suggesting that other factors might also be important. One source of

variability between cyst abundances at the same station could be the exact location where the samples are taken. The exact sampling locations within this station during different months were located about 100 m from each other. Thus, fine-scale differences in seabed topography could also have an impact on the sediment properties, potentially leading to notably different cyst abundances. However, as there is no detailed information regarding seabed topography and sediment properties available from the sampling stations, this explanation needs further verification and could be the scope of a future study.

The importance of transport on cyst communities is further supported by the modelled spread of the newly formed cysts between the two springs. Those simulations displayed notable differences in directions and distance that the cysts were transported during the two springs, which were induced by the differences in prevailing wind forcing (direction and speed) (Suppl. Fig. 3). This has implications for the biological productivity estimates of a water body using dinoflagellate resting stages, as cysts found from specific area seem to be influenced by transport. Furthermore, as cysts in the surface sediments represent an integration of several years, the variability in transport patterns between years has a significant influence on the final cyst community.

Conclusions

Benthic resting stages provide an abundant seed bank for the spring bloom dinoflagellates in the eastern Baltic Sea. The results of this study indicate that changes in the cyst abundances before, during and after the spring bloom are probably influenced by the transport of resuspended cysts in the near-bottom layer and newly formed cysts in the water column. Particularly, the strong near-bottom currents induced by the shifts from estuarine circulation to reversed and *vice versa* are suggested to resuspend and transport resting cysts in the Gulf of Finland. In the Gulf of Riga, an input of resuspended cysts or encystment of vegetative cells transported from the north-eastern Baltic Proper are proposed as the main explanation for the observed changes in cyst abundances. The latter is further supported by the dominance of *Biecheleria baltica* cysts, despite its marginal role in the phytoplankton spring bloom community in the Gulf of Riga. Thus, higher abundances of dinoflagellate resting stages are not always representative of the local cyst production intensity. In May, the input of newly formed cysts was detected in all basins. The simulations displayed transport of newly formed cysts within 10-30 km from the point of formation for the majority of stations. The obtained knowledge provides a new input to the studies where dinoflagellate cyst community species composition and abundance are used to investigate biological productivity of a water body on a temporal scale.

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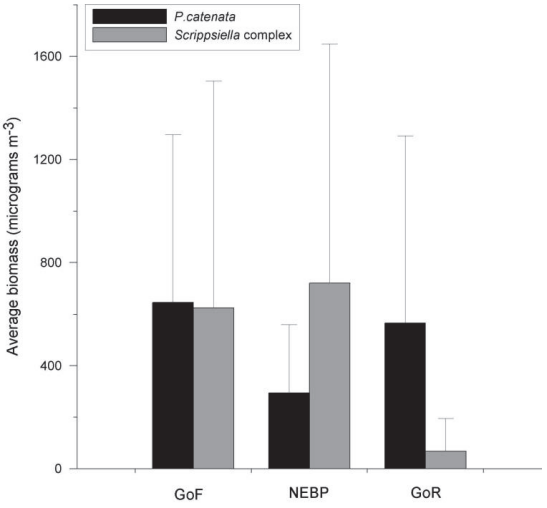
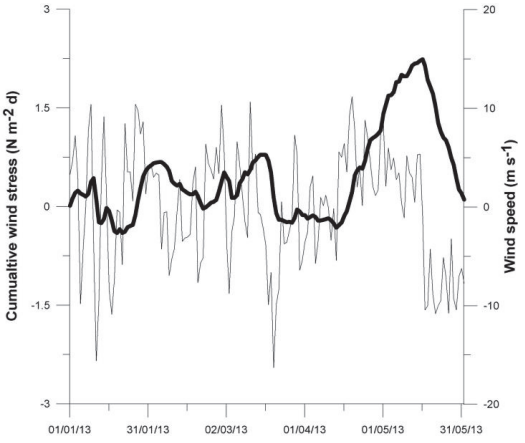
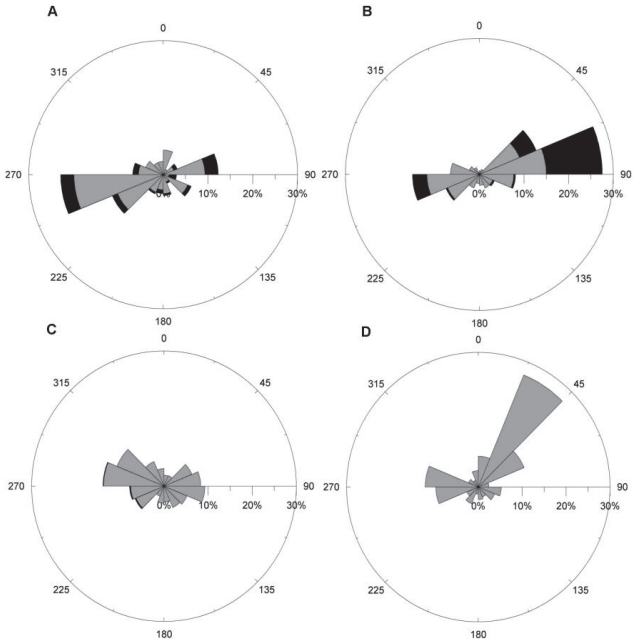
Supplementary figures



Supplementary Fig. 1. Long-term average biomass values of *P. catenata* and *Scrippsiella* complex in the water column in different basins during April and May (2003-2013). Grey colour bars represent the *Scrippsiella* complex biomass and black bars *P. catenata* biomass, error bars represent standard deviation.



Supplementary Fig. 2. Cumulative wind stress (bold line) and wind speed in the Gulf of Finland in January-May 2013. Positive values correspond to the wind from SSW and negative from NNE.



Supplementary Fig. 3. Wind speed and direction distribution in the Gulf of Finland during May 2004 (A) and 2013 (B) and in the Gulf of Riga during the same years (C and D, respectively). Grey colour represents the wind speed up to 10 m s^{-1} and black denotes speeds up to 20 m s^{-1}.

Appendix A

Text A1. Bottom-currents data

Near-bottom current velocities (5 m above the seabed) from the GoF between January-April 2014 measured within the frame of Estonian Science Foundation Research Grant project (ETF 9382) were used to explore the potential for cyst resuspension and transport. Logarithmic velocity profile formula (Soulsby, 1983) was used to estimate shear stress velocity in the bottom boundary layer from the measurements above 5 m of the seabed. Bed roughness length (10^{-3} m) was used from experiments by Arneborg et al. (2007). Critical shear stress velocity (0.62 cm s^{-1}) for fluffy surface material on mud was taken from Ziervogel & Bohling (2003).

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Text A2.Wind data

Data regarding wind speed and directions in the GoF was obtained from the Kalbådagrund weather station (Finnish Meteorological Institute) and in the GoR from Ruhnu weather station (Estonian Weather Service) to gain further understanding of the physical process present in the water column from January to May 2013 as well as to compare wind dynamics between May 2004 and May 2013. The wind speed from Kalbådagrund was multiplied by a height correction coefficient of 0.91 (Launiainen and Saarinen, 1984) to reduce the measured wind speed at 32 m above the sea surface to 10 m reference height. For cumulative wind stress in the GoF, the wind velocity components (January-May 2013) were calculated along the axis from SSW to NNE to follow the central axis of the GoF, with positive values corresponding to SSW.

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Paper II

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Competitive advantage and higher fitness in native populations of genetically structured planktonic diatoms

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Summary

It has been shown that the planktonic diatom *Skeletonema* from neighbouring areas are genetically differentiated despite absence of physical dispersal barriers. We revisited two sites, Mariager Fjord and Kattegat, NE Atlantic, and isolated new strains. Microsatellite genotyping and F-statistics revealed that the populations were genetically differentiated. An experiment was designed to investigate if populations are locally adapted and have a native competitive advantage. Ten strains from each location were grown individually in native and foreign water to investigate differences in produced biomass. Additionally, we mixed six pairs, one strain from each site, and let them grow together in native and foreign water. Strains from Mariager Fjord and Kattegat produced higher biomass in native water. In the competition experiment, strains from both sites displayed higher relative abundance and demonstrated competitive advantage in their native water. The cause of the differentiated growth is unknown, but could possibly be attributed to differences in silica concentration or viruses in the two water types. Our data show that dispersal potential does not influence the genetic structure of the populations. We conclude that genetic adaptation has not been overruled by gene flow, but instead the responses to different selection conditions are enforcing the observed genetic structure.

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Introduction

There is plentiful evidence for intraspecific genetic structuring in marine planktonic microorganisms (Rynearson and Armburst, 2004; Nagai *et al.*, 2007; Casteleyn *et al.*, 2010), which challenges the theory of unlimited dispersal in aquatic microbes (Finlay, 2002). Similar to holoplanktonic microorganisms, genetic structuring and differentiation are also reported for aquatic multicellular animals with a planktonic larval stage (Campillo *et al.*, 2009), despite their high dispersal capacity and rapid colonization of new habitats (Jenkins and Buikema, 1998; Bohonak and Whiteman, 1999). In the marine environment oceanographic features such as currents, fronts and gradients are important in generating heterogeneity among different habitats and consequently structuring genetic populations (White *et al.*, 2010; Sanford and Kelly, 2011; Casabianca *et al.*, 2012). Furthermore, differences in physicochemical and biotic parameters such as salinity, temperature, pH, level of toxic substances, predation and exposure to parasites, are known to induce adaptation and genetic differentiation in aquatic organisms (Hairston *et al.*, 1999; Cousyn *et al.*, 2001; Decaestecker *et al.*, 2007; Weisse *et al.*, 2007; Yampolsky *et al.*, 2013; Defaveri and Merilä, 2014). However, it is often problematic to reliably pinpoint specific hydrographic or biological selective factor(s) that influence relevant ecological traits and generate genetic structure. This is because several intrinsic habitat characteristics may interact and generate local adaptation and native competitive advantage (Korpelainen, 1986; Declerck *et al.*, 2001).

Local adaptation is the improved fitness of a population in its native habitat compared to its fitness in a foreign habitat. It can also be manifested as superior fitness compared to a non-native populations transplanted to the same environment (Kawecki and Ebert, 2004; Blanquart *et al.*, 2013). The fitness of a population can be estimated by the genetic contribution to the next generation, growth rates, or other traits related to performance. To test the presence of local adaptation using a transplant or common garden experiment, populations or genotypes are exposed to native and non-native conditions. Alternatively, natives are placed together with foreign individuals to compete for

limiting resources. If the natives display higher fitness than the foreigners it is considered a demonstration of local adaptation with an associated competitive advantage (Fraser *et al.*, 2011; Sanford and Kelly, 2011).

We used the planktonic chain-forming diatom *Skeletonema marinoi* Sarno *et* Zingone to address the question whether competitive advantage and local adaption to native habitat exists in genetically differentiated phytoplankton populations. *S. marinoi* occurs all year round in the NE Atlantic, but reaches its highest abundance during the spring bloom in February-March (Saravanan and Godhe, 2010). It forms resting stages which sediment to the sea floor, and these propagules may survive for many years in the sediment and thereby re-seed the planktonic population (McQuoid *et al.*, 2002). In this study, populations from Mariager Fjord and from Kattegat were investigated. It was previously found that the two populations are genetically differentiated despite water exchange and fluxes of potential colonizers between the two habitats. Genetic analyses of strains germinated from resting stages, accumulated over more than 100 years and embedded in discrete layers of a sediment core, displayed a uniform structure, which suggests that the populations of the Mariager Fjord and the Kattegat have not mixed for thousands of generations (Härnström *et al.*, 2011). The Mariager Fjord is connected to the Kattegat over a shallow sill (< 6 m, Fig. 1). The water column inside the fjord is stratified and bottom water is separated from the mixed surface layer by the pycnocline. Due to the high level of eutrophication and irregular inflows of saline water from the Kattegat, the bottom water of the fjord is anoxic (Fallesen *et al.*, 2000; Olesen, 2001). During the summer the anoxic zone may extend upwards and for short periods hypoxic conditions may prevail at shallow depths because of stable stratification and increased oxygen consumption (Fallesen *et al.*, 2000).

We revisited these two locations and established fresh isolates by germinating resting stages. In all culturing steps, i.e. isolation, germination and incubation of strains, we used culturing media based on the isolate's native water (Guillard, 1975). The aim was to investigate if the two populations were locally adapted and exhibit a competitive advantage in their native environment, which could serve as an explanation for the strong genetic structure between the two sites. Our first hypothesis was that *S. marinoi* strains grown in their native water would have higher fitness than when they were grown in a foreign environment. Second, we hypothesized that *S. marinoi* strains grown in their native environment would show a competitive advantage in comparison to growth in a foreign environment. To test this, we first examined the accumulated biomass of individual strains grown in batch cultures in native and foreign water respectively. Thereafter, a competition experiment was set up in which we mixed strain pairs from the two sites in equal cell abundances and tested if strains displayed significantly higher final proportion in their native water.

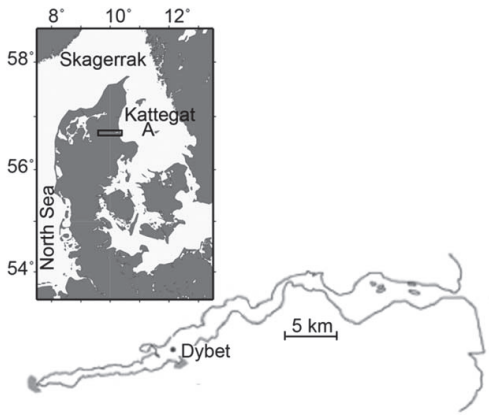

Fig. 1. Denmark with a location map of the Mariager Fjord, and the sampling station (Dybet). Populations from the Kattegat were established from sediment samples collected at Anholt (A).

Results

All isolated strains produced unique multi locus genotypes. We used eight microsatellite loci to genotype the strains and all markers were polymorphic. Locus S.mar5 was the most variable and S.mar3 the least variable. Significant ($P < 0.05$) departure from the Hardy-Weinberg equilibrium was present for all loci except S.mar6 (Supporting Information Table S1). Based on the 69 genotyped strains isolated from Mariager Fjord ($N = 45$) and Kattegat ($N = 24$), we found that the populations were significantly differentiated and displayed a F_{ST} of 0.085 ($P < 0.001$). Additionally, we tested pair-wise F_{ST} between genotypes isolated from the same sites in 2008 (Härnström *et al.*, 2011) and the new isolates from Mariager Fjord and Kattegat respectively. The samples were not genetically differentiated ($P > 0.05$) over time, displaying F_{ST} of −0.00003 (Mariager Fjord) and 0.003 (Kattegat).

A factorial correspondence analysis (FCA) was used to illustrate the differences between the different sampling years (2008 and 2013) and the sampling sites Mariager Fjord and Kattegat. The FCA identified three axes with the eigenvalues of 0.155 (axis 1), 0.107 (axis 2) and 0.08 (axis 3), each explaining 45.92%, 30.05% and 24.03% of the variation respectively. The analysis showed separation, especially along axis 1, in accordance with the significant F_{ST} values between the fjord and the open sea (Fig. 2).

Eighteen of the 20 strains tested, 10 Mariager Fjord strains and 8 Kattegat strains, produced significantly

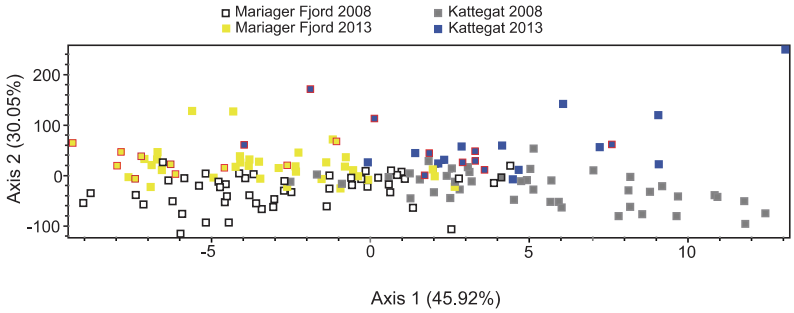

Fig. 2. Two-dimensional FCA of the genetic distances of individual strains belonging to the two habitats. All strains are established from germinated resting stages from undisturbed surface sediment from Mariager Fjord (2008 and 2013) and from Anholt, Kattegat (2008 and 2013). Axis 1 has the largest explanatory power (45.92%) and divides the to habitats: Mariager Fjord to the left, and Kattegat to the right. Mariager Fjord 2008 ($N = 47$, white squares) and Kattegat 2008 ($N = 42$, grey squares) are from Härnström and colleagues (2011). Isolates from Mariager Fjord 2013 ($N = 45$, yellow squares), Kattegat 2013 ($N = 24$, blue squares). Recent Mariager and Kattegat individuals (10 from each population) used in the physiological experiment are highlighted with a red box frame.

higher biomass (ng C ml^{-1}) in their native water compare to foreign water (Fig. 3A and B). The two populations produced significantly higher ($P < 0.001$) biomass in their respective native water. The Mariager Fjord population produced 6.89 ± 1.95 ng C ml^{-1} (mean $\pm$ SD) in native water, and 1.68 ± 1.33 ng C ml^{-1} (mean $\pm$ SD) in foreign water. The Kattegat population produced 1.93 ± 0.95 ng C ml^{-1} (mean $\pm$ SD) in its native water and 0.3 ± 0.44 ng C ml^{-1} (mean$\pm$SD) in foreign water. Comparison of biomass accumulation between populations revealed that the native population produced significantly higher average biomass than the foreign population in Mariager Fjord water. In Kattegat water there was no significant difference in biomass production between the native and the foreign population.

The allele-specific quantitative PCR (AsQ-PCR) standard curves for loci S.mar4, S.mar5 and S.mar6 for the respective strain pairs showed no PCR bias for any of the selected strain combinations. Relative peak-heights plotted against known relative cell abundances yielded r^2-values of 0.82–0.99 (Supporting Information Fig. S1). Thus, we could use this method to assess relative final strain proportions in the two water types of the six pair-wise strain combinations. In all combinations tested (replicated in three), the native strains always had a competitive advantage in their native water and displayed significantly higher relative cell abundances in native compared to foreign water (Fig. 4A–F).

Discussion

Despite water exchange between the two sites, Kattegat and Mariager Fjord, we here report on persistent genetic differentiation of two planktonic diatom populations, in relatively close geographic proximity. Our results from the reciprocal transplant experiments provide strong support for the presence of local adaptation to intrinsic water characteristics in the respective native populations. When tested experimentally, using a common garden set-up, the higher fitness exhibited in native water was associated with a competitive advantage in all of the tested strains.

We tested the 'home vs. away' criterion, which implies that a population has higher fitness in its own habitat (at home) compared to in an alternate habitat (away) (Kawecki and Ebert, 2004; Fig 5A). The two populations, Mariager Fjord and offshore Kattegat, fulfilled this criterion by presenting significantly higher biomass yield in their respective native waters in contrast to the foreign water (Fig. 5B). Another criterion is the 'local vs. foreign' in which the local population in its native habitat is expected to show higher fitness than another population in that same habitat (Fig. 5A). This criterion was fulfilled by the Mariager Fjord population, which displayed a significantly higher average biomass yield in Mariager Fjord water than the Kattegat population (Fig. 5B). Our results contrast previous work as we observed that both populations fulfil the 'home vs. away' criterion, whereas earlier studies on pelagic aquatic microscopic organisms have not demonstrated this pattern (Declerck *et al.*, 2001; Alcántra-Rodríguez *et al.*, 2012; Rengefors *et al.*, 2015). Instead they have reported fulfilment of 'local vs. foreign' criteria in one native population, similarly to the findings presented here. In comparison to earlier work our study has included more strains, which may have facilitated the outcome of the significant 'home vs. away' observation presented here.

Differences in selection pressure between habitats is considered as a perquisite for the development of local adaptation (Blanquart *et al.*, 2013) and it has been proposed to be one of the causes of population differentiation in pelagic organisms (Korpelainen, 1986; Rynearson *et al.*, 2006). The Mariager Fjord site and the off-shore Kattegat

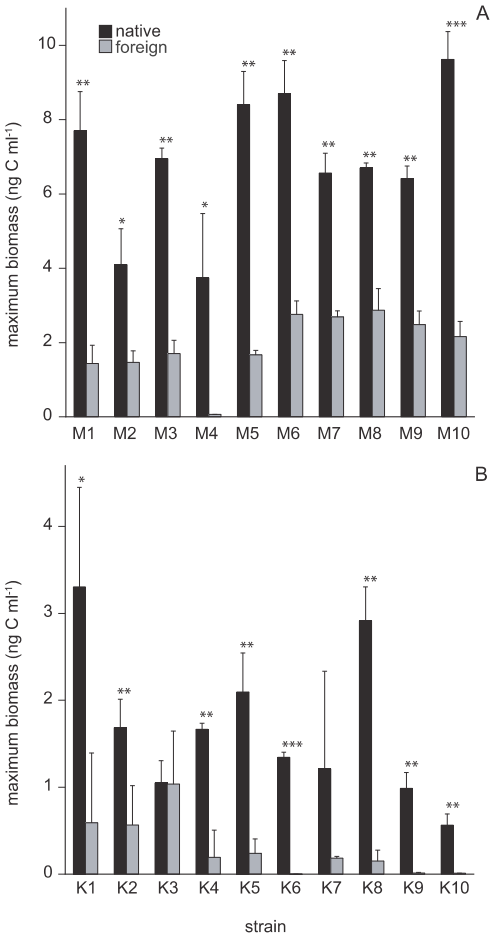

Fig. 3. Monoclonal strains grown in Kattegat and Mariager Fjord water based f/2 medium.
A. Mariager Fjord strains maximum biomass (ng C ml^{-1}) in native and foreign water based medium. All tested strains produced significantly higher biomass in medium based on native water.
B. Kattegat strains maximum biomass (ng C ml^{-1}) in native and foreign water based medium. All strains produced higher biomass in medium based on native water. Eight strains produced significantly higher biomass in the water of their origin. Significance is indicated by $*P < 0.05$, $**P < 0.01$, $***P < 0.001$. Error bars indicate standard deviation ($N = 3$).

differ remarkably in respect of hydrography (Table 1), and therefore the concept of local adaptation in the respective populations is plausible. The average concentrations of inorganic nutrients in the surface water, for example, the winter concentrations of nitrate, phosphate and silicate are, respectively, 12, 4 and 6 times higher in Mariager Fjord (Table 1), which will inevitability induce different growth conditions *in situ*. This is also manifested by the much higher phytoplankton standing stock (seven times higher) and the higher primary production (five times higher) in the Mariager Fjord (Fallesen *et al.*, 2000; Olesen, 2001; ICES, 2015). However, in our experiments the local water was spiked in equal amounts with nutrients, which is equivalent to algal full growth media, that is, 880 μM NO_3, 36.2 μM PO_4 and 106 μM SiO_3 (Guillard, 1975, see Table 1). Therefore, the observations of local adaptation in the two populations are unlikely the results from differences in nitrogen and phosphorous availability, as those added nutrients are in extreme excess. In terms of silica the Mariager Fjord water displayed an overall higher concentration including the extra addition. Growth of diatoms is dependent on ambient silica concentration (Egge and Aksnes, 1992), and in fact we recently documented significant correlations between depletion of silica and a population genetic shift during the spring bloom of *S. marinoi* in the Baltic Sea (Godhe *et al.*, 2016). That correlation indicated that specific genotypes are adapted to different silica concentrations. Nevertheless, as we have no data on phenotypic traits on silica requirements specifically from the Mariager Fjord or the Kattegat genotypes, this hypothesis remains to be tested. The Mariager Fjord and the Kattegat also differ moderately in terms of salinity and pH (Table 1). In the Mariager Fjord, average surface salinity is lower (16–17 PSU) than in the Kattegat (17–22 PSU). This variability is, nevertheless, well within the salinity range (3–35 PSU) for *S. marinoi* growth (Balzano *et al.*, 2010; Sjöqvist *et al.*, 2015). In terms of pH the Kattegat is more stable (8.3-8.6), and the Mariager Fjord is more variable (7.7–9.0), which might provide an advantage to the Mariager Fjord strains. *Skeletonema* grow well over a wide range of pH (6.5–8.5), but show a reduction in growth starting from pH 9 (Taraldsvik and Myklestad, 2000; Hansen, 2002). Drastic pH fluctuations should, however, promote the development of phenotypic plasticity in the Mariager Fjord population, instead of local adaptation. In the present study we took care not to eliminate any intrinsic characteristics of the original water. We therefore refrained from autoclaving the water and only pre-filtered it (0.2 μm) before adding macro and micronutrients in equal amounts in order to enable growth for the transplant and the competition experiment. As such we did not destroy any unidentified organic molecules and/or viruses intrinsic to the two respective water types, which could facilitate growth for the locally adapted populations.

Intriguingly, the Mariager Fjord has maintained an endemic population of a marine planktonic protist for thousands of generations with reduced levels of gene flow between the adjacent populations in the Kattegat. This is despite the continuous water exchange and influx of new potential colonizers (Härnström *et al.*, 2011). The paradox of reduced gene flow despite high dispersal potential has

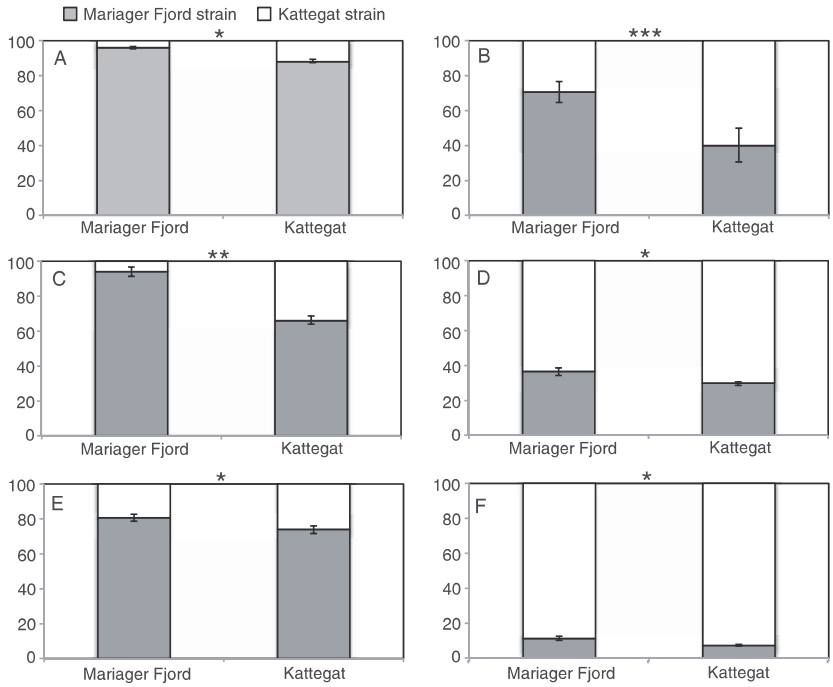

Fig. 4. Six pairs, one from each site in triplicates, of Kattegat and Mariager Fjord strains grown as a mix in a competition experiment in the two water types. Relative cell abundances (%) of clones are indicated on the Y-axis in Mariager Fjord and Kattegat water respectively. In all six pairs the proportion of the native clones is significantly higher in native water compared to foreign water.
A. Strains M10 and K4.
B. Strains M1 and K8.
C. Strains M6 and K4.
D. Strains M6 and K8.
E. Strains M3 and K4.
F. Strains M2 and K4.
Significance is indicated by $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. Error bars indicate standard deviation ($N = 3$).

been recorded for several aquatic organisms (Palsson, 2000; Campillo *et al.*, 2009), and can be explained by quick population growth after a historical founder event, rapid adaptation to the local environment in the resident population, and a large benthic propagule bank which buffers against later immigrants (De Meester *et al.*, 2002). In a previous laboratory experiment, using strains of *S. marinoi*, we documented significant priority effects even in the absence of a numerical advantage (Sefbom *et al.*, 2015). Theoretically, a historical founder event such as a bottle neck may have occurred during the complex deglaciation of the geologically young Baltic Sea region (Björck, 1995). Because *S. marinoi* reproduce mainly asexually, dividing approximately once per day (Taylor *et al.*, 2009), selection for individual clonal lineages can be swift if differential environmental selection in two habitats prevails (Lohbeck *et al.*, 2012). Furthermore, the same mechanism may cause selection against migrants, thus reinforcing differentiation and local adaptation, leading to a reduced realized gene flow and producing a pattern of isolation by adaptation (Nosil *et al.*, 2009). *S. marinoi* produces rich seed banks in Scandinavian sediments, with up to 50 000 propagules per gram of sediment (McQuoid *et al.*, 2002), and the strong link between the benthic and pelagic assemblages (Godhe and Härnström, 2010) may act to maintain the genetic structure. If so, the genetic structure of the benthic seed bank is stable over time and the locally adapted population continuously shifts between the benthic and pelagic phases.

We provide experimental evidence for local adaptation and competitive advantage in marine phytoplankton, and our findings indicate that the varying selection pressure in two connected habitats can counteract genetic homogenization despite high potential for dispersal. Over time,

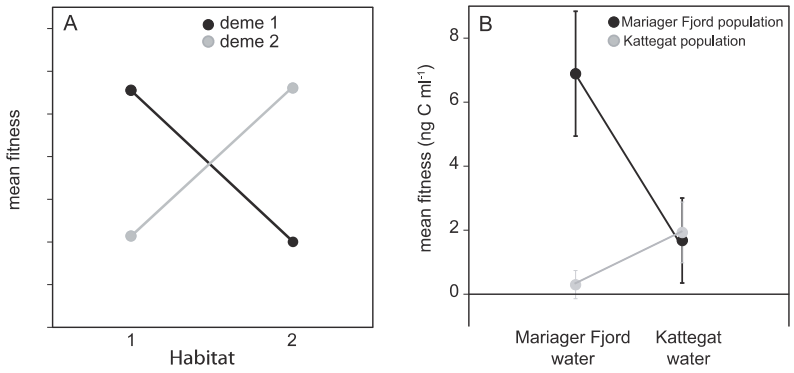

Fig. 5. Pattern of population-habitat interactions.
A. Hypothetical patterns of deme-habitat interaction for fitness (redrawn from Kawecki and Ebert, 2004). Black circle: the mean fitness of deme originating from habitat 1. Grey circles: the mean fitness of deme originating from habitat 2. The patterns satisfy the 'local vs. foreign' and the 'home vs. away' criterion.
B. Fitness comparisons of Mariager Fjord (black circles) and Kattegat (grey circles) populations based on maximum biomass (ng C ml^{-1}) averaged ($N = 10$, error bars indicate standard deviation) over the investigated strains in their native and foreign water based media. The two populations produce significantly higher biomass in their respective native water satisfying the 'home vs. away' criteria. In Mariager Fjord water the local population produced significantly higher biomass than the foreign population satisfying the 'local vs. foreign" criteria. In Kattegat water, the biomass production of the two different populations were not statistically significant.

genetic adaptation in response to local abiotic or biotic conditions can serve as a mechanism that promotes genetic differentiation even further. Our observations on local adaptation and competitive advantage most likely also apply to other planktonic protists with similar strong genetic structure.

Experimental procedures

Surface sediment was collected from Anholt, Kattegat (56°40.00′N, 12°07.00′E) at 53 m depth in May 2013 by a box corer, and from Mariager Fjord in September 2013 (56°40.70′N, 10°01.41′E) at 30 m depth by using a modified HAPS corer (KC Denmark, Fig. 1). The top centimetre of the undisturbed sediment cores was carefully scraped off and transferred to airtight plastic containers. The sediment samples were kept cool and dark until further processing. To establish monoclonal strains, 1 g of sediment was distributed in smaller aliquots into 24-well plates (Nunc), and mixed with nutrient enriched f/2 medium (Guillard, 1975) with a final concentration as stated in Table 1. Media was prepared based from water from the two sampling sites collected at the time of sediment sampling. Nutrient analyses determined the concentrations of NO_3, PO_4 and SiO_3 concentrations in the water from both sites (HELCOM 2015; Grasshoff *et al.*, 1999). For preparing the experimental media, the sea water was double filtered through GF/F glass microfibre filter (pore size 0.7 μm), and through Pall Supor Membrane filter (pore size of 0.2 μm), but not autoclaved. The media based on water from respective sampling locations was used throughout the study for all the different steps, i.e. an individual strain was isolated, incubated and maintained in growth medium based on its native water. The sediment slurries in the well plates were kept at 10°C on a 12:12-h light:dark cycle at an irradiance of 60 μmol photons m^{-2} s^{-1} and checked daily for germination and vegetative

Table 1. Nutrient concentrations (μM) of experimental media, and long-term hydrographic characteristics of the Mariager Fjord and the Kattegat surface water (Fallesen *et al.*, 2000; Hansen 2002; ICES 2015).

Variable	Experimental water Mariager Fjord[a]	Experimental water Kattegat[a]	Mariager Fjord[b]	Kattegat[b]
NO_3 (μM)	882.1	884.2	90	7
PO_4 (μM)	37.2	36.6	3	0.7
SiO_3 (μM)	152.3	114.2	47	8
Chl *a* (mg m^{-3})			15	2
Primary production (g C m^{-2} y^{-1})			800	150
salinity			16–17	17–22
pH			7.7–9	8.3–8.6

a. nutrient concentration after addition of f/2 nutrients (Guillard, 1975).
b. Nutrient concentrations (NO_3, PO_4, SiO_3) are winter estimates (Dec-Feb). Salinity and pH are lowest and highest average values between March and November. Phytoplankton biomass (Chl *a*) and primary production are based on estimates recorded between May and September.

growth of *S. marinoi*. One cell chain from each well was isolated by micropipetting, rinsed three times in growth medium based on respective water, transferred to a Petri dish (50-mm diameter) filled with medium and incubated under the same conditions as above. When growth was confirmed, the cells were transferred to 40-ml Nunc flasks with 10 ml of medium. After further propagation, more medium was added. Each strain was grown in duplicate in order to use one set for genotyping and the other for the experiments.

Twenty-four strains from Kattegat and 45 strains from Mariager Fjord were genotyped. Strains in exponential phase were filtered onto Versapor-3000 filters with 25-mm diameter and 3-μm pore size (Pall Corporation) and genomic DNA was extracted following a CTAB based protocol (Kooistra *et al.*, 2003). Eight microsatellite loci (S.mar 1–8, Almany *et al.*, 2009) were amplified by PCR as described in Godhe and Härnström (2010). The products were analysed in an ABI 3730 (Applied Biosystem) and allele sizes were assigned relative to an internal standard (GS600LIZ). Allele sizes for the individual loci were determined and processed using Genemapper (ABI PrismGeneMapperSoftware v 3.0). Genepop, v. 4.0.7 (Raymond and Rousset, 1995) was used to estimate deviations from Hardy-Weinberg equilibrium (10 000 Markov Chain dememorizations, 20 batches and 5000 iterations per batch) of each locus in both populations. Genetic differentiation between the populations sampled in 2013 and the populations sampled in Härnström and colleagues (2011) was determined by calculating F_{ST} using Genetix v. 4.05 (Belkhir, 2004) with 10 000 permutations. FCA was done using genotypic data (8 microsatellite loci) to display the structural relationships among the Mariager Fjord and the Kattegat individuals using Genetix v. 4.05 (Belkhir, 2004).

From each location, 10 strains were randomly selected in order to determine their biomass in native and non-native water. The abundances of the selected strains were estimated in exponential phase by using a Sedgwick-Rafter chamber and an inverted microscope (Zeiss Axiovert 135). From the stock cultures, a volume equivalent to the starting concentration (5000 cells ml^{-1}) was transferred to 60 ml of fresh growth media prepared from the Mariager Fjord and the Kattegat water respectively. Each of the 10 strains was grown in their native and foreign water in triplicates. Before the growth experiment in foreign water the strains were acclimatized for 7 days until they reached exponential phase, and thereafter re-inoculated into 60 ml fresh foreign water at the starting density of 5000 cells ml^{-1}. We monitored growth with optical density (OD) at 600 nm. In order to efficiently monitor growth during experiments we constructed standard curves for cell density and OD measurements for each of the strains (Supporting Information Fig. 2), using serial dilutions (1:1–1:8), thus allowing us to determine the relationship between the two estimates. Growth (measured as OD) was monitored daily by transferring 1 ml of culture onto 48-well plates and using a plate reader (Varioskan Flash, ThermoFisher Scientific). When strains had entered stationary phase, maximum OD values were converted into cell numbers (cells ml^{-1}) using the respective standard curves. For each strain the length and width of 50 individuals were measured from Lugol fixed subsamples originating from the day of maximum measured OD to obtain average cell volume estimates based on Sun and Liu (2003). Cell volume was then converted to carbon (pg C) per cell (Menden-Deuer and Lessard, 2000) and multiplied by cell abundances in order to establish final biomass (ng C ml^{-1}) per strain and replicate. Maximum yield (ng C ml^{-1}) was then used as a measurement of fitness for the respective strains in native and foreign water. A one-tailed paired *t*-test was used for each strain to test if different water had a significant effect on maximum yield. Two-tailed unequal variance *t*-test was used to investigate whether the native population performed significantly better in its native water compared to foreign population in the same water. Significance level was set at $P < 0.05$.

The reciprocal competition experiments were carried out using six different strain combinations grown in each of the two water types. Strain selection was based on similar growth rates in monoculture. In each of the combinations there were two strains, one from each site, per bottle (3 replicates). To assess the relative abundance of each strain in the mix we used the AsQ-PCR method (Meyer *et al.*, 2006). In AsQ-PCR the respective peak-heights from the two strains in the electropherograms are used as a relative quantification measurement. To confirm that PCR amplification did not favour one strain over the other, we prepared standard curves for each pair of strains (six strain pairs in total). We mixed the six strain combinations, in five known proportions, ranging from 0.1:0.9 to 0.9:0.1 (three replicates each), extracted DNA and amplified five of the microsatellite loci (S.mar1, S.mar2, S.mar4, S.mar5, S.mar6) as described above in order to find the least biased PCR reaction. Peak-height relative abundances were plotted against known relative cell abundances to obtain r^2-values. For the competition experiment, cell densities in exponentially growing cultures were estimated as above. A volume equivalent to the concentration of 5000 cells ml^{-1} for each competing strain was transferred from the stock cultures to 60 ml of fresh f/2 media based on the Mariager Fjord or Kattegat water respectively. Combinations of strains were grown in triplicates in the two types of media (Kattegat or Mariager Fjord water) in conditions as described above. Optical density was measured daily until the strain combinations reached stationary phase. The mixed cultures were subsequently filtered down, genomic DNA was extracted and the five markers used for the AsQ-PCR standard-curves were amplified. Allele sizes for the individual loci and the respective peak-height ratios were determined using Genemapper. The null hypothesis of equal fitness, for example, final cell abundance, in the two water types (native and foreign) was tested by one-tailed *t*-tests.

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Supporting information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Table S1. Summary of number of alleles per loci (N_A), expected (H_e) and observed heterozygotes (H_o) for each group of strains established from germinated resting stages from the two sites, Mariager Fjord and Anholt, Kattegat.
Fig. S1. The relative abundance of one strain, in a two-strain mix, determined by cell count and on the x-axis, and proportional peak heights of respective strains in the electropherogram after fragment amplification on the y-axis. Each proportion was replicated in three.
Fig. S2. The relation between cell abundance (10^3 cells ml^{-1}) and optical density (OD, 600 nm) for the Mariager Fjord strains (M1-M10) and the Kattegat strains (K1-K10) used in the transplant experiment.

Paper III

Godhe, A., Sjöqvist, C., Sildever, S., Sefbom, J., Harðardóttir, S., Bertos-Fortis, M., Bunse, C., Gross, S., Johansson, E., Jonsson, P.R., Khandan, S., Legrand, C., Lips, I., Lundholm, N., Rengefors, K.E., Sassenhagen, I., Suikkanen, S., Sundqvist, L., Kremp, A. 2016. Physical barriers and environmental gradients cause spatial and temporal genetic differentiation of an extensive algal bloom. *Journal of Biogeography*, 43 (6), 1130-1142. DOI: 10.1111/jbi.12722

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ORIGINAL ARTICLE

Physical barriers and environmental gradients cause spatial and temporal genetic differentiation of an extensive algal bloom

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ABSTRACT

Aim To test if a phytoplankton bloom is panmictic, or whether geographical and environmental factors cause spatial and temporal genetic structure.

Location Baltic Sea.

Method During four cruises, we isolated clonal strains of the diatom *Skeletonema marinoi* from 9 to 10 stations along a 1132 km transect and analysed the genetic structure using eight microsatellites. Using *F*-statistics and Bayesian clustering analysis we determined if samples were significantly differentiated. A seascape approach was applied to examine correlations between gene flow and oceanographic connectivity, and combined partial Mantel test and RDA based variation partitioning to investigate associations with environmental gradients.

Results The bloom was initiated during the second half of March in the southern and the northern- parts of the transect, and later propagated offshore. By mid-April the bloom declined in the south, whereas high phytoplankton biomass was recorded northward. We found two significantly differentiated populations along the transect. Genotypes were significantly isolated by distance and by the south–north salinity gradient, which illustrated that the effects of distance and environment were confounded. The gene flow among the sampled stations was significantly correlated with oceanographic connectivity. The depletion of silica during the progression of the bloom was related to a temporal population genetic shift.

Main conclusions A phytoplankton bloom may propagate as a continuous cascade and yet be genetically structured over both spatial and temporal scales. The Baltic Sea spring bloom displayed strong spatial structure driven by oceanographic connectivity and geographical distance, which was enhanced by the pronounced salinity gradient. Temporal transition of conditions important for growth may induce genetic shifts and different phenotypic strategies, which serve to maintain the bloom over longer periods.

Keywords
adaptation, environmental gradient, gene flow, genetic structure, isolation by distance, population, seascape, *Skeletonema*

INTRODUCTION

Algal blooms occur in aquatic systems whenever grazing is low and favourable hydrographic conditions support a quick increase in cell abundance. Often only one or a few phytoplankton species are involved, which rapidly grow to cell densities that may be visible as discoloration of the water (Smayda & Reynolds, 2001). Blooms can be local phenomena in closed embayments or extend over vast ocean basins, and may last from a few days to many weeks (Edwards *et al.*,

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2006; Kremp *et al.*, 2009). Despite the predominant asexual mode of propagation during a phytoplankton bloom, extensive genotypic diversity has been observed throughout bloom periods (Tesson *et al.*, 2014).

Although it is well known that phytoplankton populations may display large genotypic diversity (Rynearson *et al.*, 2006; Erdner *et al.*, 2011), information on spatial and temporal population genetic dynamics in blooms is still sparse. A bloom that is spread out in time and space may consist of one genetic population that propagates over vast areas or, alternatively, of several successive or spatially differentiated populations each adapted to local hydrographic conditions of the variable hydrographic environment. The propagation of a bloom in spatial and temporal dimensions can potentially be explained by a seascape approach, as marine population connectivity depends on oceanographic currents combined with characteristics of dispersive life history stages (Kenchington *et al.*, 2006). Correlations between empirical genetic data and high-resolution oceanographic connectivity models have shown good agreement for marine meroplanktonic (Serra *et al.*, 2010; Sunday *et al.*, 2014) and holoplanktonic organisms, including phytoplankton (Casabianca *et al.*, 2012). Additionally, the environment may affect the genetic structure of a phytoplankton bloom. Phytoplankton populations have been shown to contain high phenotypic diversity, for example, in their response to environmental parameters or gradients (Alpermann *et al.*, 2009; Sjöqvist *et al.*, 2015), indicating that standing genetic variation exists for selection to act upon. Moreover, phenotypic adaptation has been documented from multi-generation lab experiments (Collins & Bell, 2004; Lohbeck *et al.*, 2012). Environmental selective forces can thus act on strains, and generate populations adapted to a specific geographical or temporal niche of the bloom. Recent studies have reported changes in genetic structure of phytoplankton populations and discussed these results in context of changing environmental conditions (Rynearson & Armbrust, 2005; Richlen *et al.*, 2012). However, statistical evidence for a relationship between environmental conditions and population level spatial and temporal dynamics of blooms of planktonic protists is lacking.

In most parts of the Baltic Sea, the spring bloom develops during March and April with vertical stratification, and lasts until late April to May when nutrients are depleted and sedimentation of cells occurs. The bloom commences when the depth of the upper mixed layer becomes shallow enough to coincide with the euphotic zone, and remains stable for a long enough period to allow entrained seed populations to proliferate to bloom concentrations (Wasmund *et al.*, 1998). Vertical stability in spring can result from both thermal and salinity stratification (Smetacek & Passow, 1990). Different hypotheses on the spatial and temporal expansion of the bloom across the Baltic basins have been proposed, including a progression from south to north due to a northward delay in warming of surface waters (Jansson, 1978), as well as a synchronized mosaic-like development due to local salinity stratification including movement of water masses from shallow stratified southern and northern coastal areas to the deep central off-shore regions (Kahru & Nömmann, 1990; Stipa, 2002). Transport of water masses is implied by both scenarios, and suggests a high degree of connectivity in the Baltic spring bloom, which could support a panmictic phytoplankton community.

In this study we investigated whether an extensively distributed basin-wide algal bloom like the Baltic spring bloom is panmictic, as expected by large scale transport processes underlying the suggested bloom expansion scenarios, or whether it is genetically structured in time and space due to temporal and spatial changes in physical and environmental factors. To address these questions we analysed the genetic structure and gene flow of one of the dominant spring diatom species, *Skeletonema marinoi* Sarno et Zingone, during four cruises in March and April 2013 together with a variety of hydrographic and hydrochemical parameters. Additionally, an oceanographic connectivity model was applied to assess the relationship between oceanographic transport and gene flow. The chain-forming marine diatom *S. marinoi* occurs all year round in the Baltic Sea region, but reaches its highest abundance during the spring bloom (Saravanan & Godhe, 2010). It forms resting stages which sediment to the sea floor, and these propagules may survive for years in the sediment and thereby re-seed the planktonic population (McQuoid *et al.*, 2002). Provided a plentiful nutrient supply, the cells exhibit a growth rate of one division per day (Taylor *et al.*, 2009). The predominant means of propagation is through vegetative division, but auxospore formation and sexual reproduction has been documented from Baltic Sea populations (Godhe *et al.*, 2014).

MATERIALS AND METHODS

Sample collection and environmental parameters

All material and data were collected while on board the cargo vessel Finnmaid during four cruises in March and April 2013 (cruise A March 4–7; cruise B March 19–22; cruise C April 4–7; cruise D April 16–19). Water samples were collected during a 48 hour time period for each cruise. The shipping route stretched across a 1132 km transect in the Baltic Sea (Fig. 1, Table S1 in Appendix S1) from Travemünde (Germany, 53.9667° N, 10.8667° E) to Helsinki (Finland, 60.1708° N, 24.9375° E). As a 'ship of opportunity', Finnmaid is equipped with a ferry box, which is an automated measuring system recording chlorophyll fluorescence, salinity, temperature and fluorescence of coloured dissolved organic matter (fCDOM) while moving (Rantajärvi, 2003). Water from 8 m depth is pumped into the system every 100–200 m. Additional water samples are taken by an automatic sampling carousel at 24 fixed stations along the transect for spectrophotometric chlorophyll *a* (Chl *a*) and inorganic nutrients (NO_3-NO_2, PO_4, SiO_2) analyses (Grasshoff *et al.*, 1983).

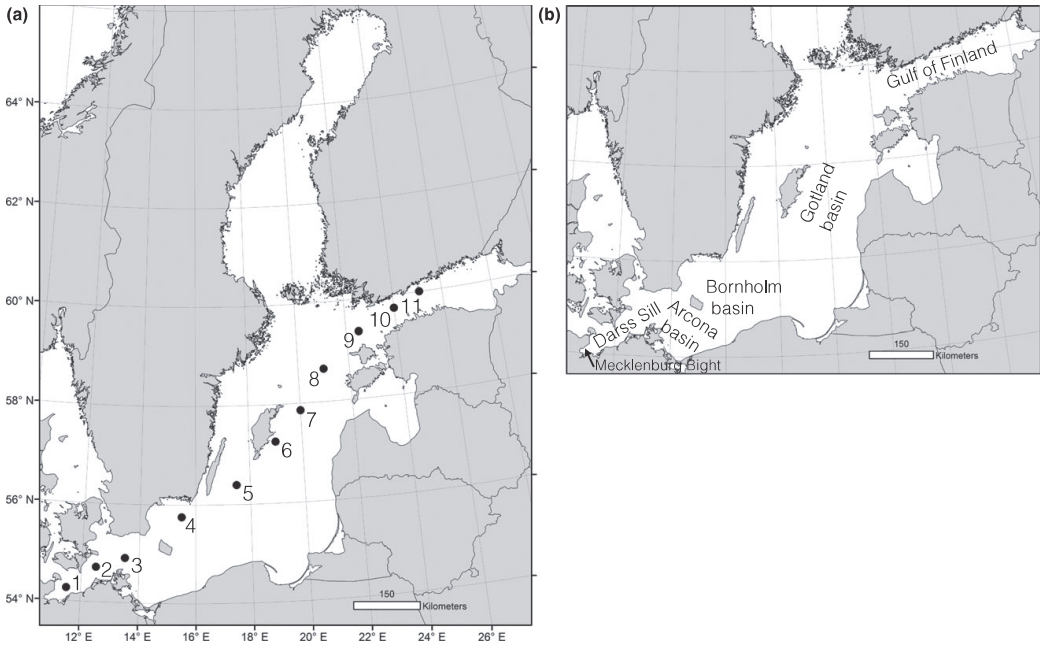

Figure 1 Map of Baltic Sea. (a) Four cruises were conducted on board MS Finnmaid along a south-west–north-east transect during the period March 4 to April 19, 2013. Eleven fixed sampling locations (1–11) were selected for *Skeletonema marinoi* isolation and water sampling. (b) Baltic Sea basins mentioned in the text.

For this study, 9–10 fixed locations per cruise were sampled for *S. marinoi* isolation (Fig. 1a, Table 1). Water was collected either automatically from the sampling carousel or manually from a tap (up to 120 L) depending on the *Skeletonema* abundance. The sample was concentrated by sieving the water through a 10 µm plankton net. An additional non-concentrated water sample was collected and fixed with Lugol's solution for phytoplankton species identification and enumeration. Samples were settled in a 25 mL Utermöhl chamber (Utermöhl, 1958) and analysed under an inverted microscope Leica DM IL Bio at magnifications 200–400X.

Correlation between hydrographic variables, geographical locations and *Skeletonema* abundance was assessed by Spearman correlation using SPSS 21 (IBM Statistics, Armonk, NY, USA).

Culturing, DNA extraction and genotyping

Individual chains of *S. marinoi* were isolated immediately on board by micropipetting. After several washing steps the cells were transferred into separate wells of 24 well NUNC tissue culture plates containing 1 mL of f/2 medium (Guillard, 1975) prepared from local seawater. The plates were incubated at 5 °C, 50 µmol photons m^{-2} s^{-1}. After transfer to the laboratory, plates were regularly monitored for cell growth. When growth was confirmed, the contents were transferred to 50 mL NUNC flasks. Dense cultures were filtered onto 3 µm pore size filters (Ø 25 mm, Versapor®-3000; Pall Corporation, Cortland, NY, USA) for DNA extraction.

Genomic DNA was extracted following a CTAB-based protocol (Kooistra *et al.*, 2003). Eight microsatellite loci (Almany *et al.*, 2009) were amplified by PCR as described in Godhe & Härnström (2010). The products were analysed in an ABI 3730 (Applied Biosystems) and allele sizes were assigned relative to the internal standard (GS600LIZ). Allele sizes for the individual loci were determined and processed using GeneMapper (ABI Prism®GeneMapper™Software 3.0).

Population differentiation and structure

Genepop 4.0.7 (Raymond & Rousset, 1995) was used to estimate deviations from Hardy–Weinberg equilibrium (HWE, 10,000 Markov chain dememorizations, 20 batches and 5000 iterations per batch) of each locus, the inbreeding coefficient F_{IS}, and genotypic linkage disequilibrium between pairs of loci in each sample (10,000 dememorizations, 100 batches and 5000 iterations per batch). Levels of statistical significance were adjusted according to sequential Bonferroni correction (Rice, 1989). Identical eight-loci genotypes were identified in Microsatellite Tools for Excel (Park, 2001). The microsatellite data set was analysed for null alleles, stuttering, and large allele drop out by means of 1000 randomizations using MicroChecker 2.2.3. Null allele frequencies cannot be

Table 1 Estimates of genetic diversity among *Skeletonema marinoi* samples at 8 microsatellite loci collected along a transect in the Baltic Sea during spring bloom of 2013. Cruise A March 4–7; Cruise B March 19–22; Cruise C April 4–7, Cruise D April 16–19. For station locations see Fig 1 and Appendix S1 Table S1. *I*, total number of isolates; *N*, total numbers of genotypes; *G/N*, proportion of unique genotypes; H_E, H_O, expected and observed heterozygosity; *HD,* heterozygote deficiency (*indicates significant HD $P < 0.05$), F_{IS} inbreeding coefficient; LD_{prop} proportion of loci which deviate significantly from linkage equilibrium.

Cruise/Station	1	2	3	4	5	6	7	8	9	10	11
A											
I	26	28	21	26	22	33	32	33	27	38	†
N	20	24	16	21	16	23	28	28	20	18	
G/N	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	
H_E	0.70	0.66	0.65	0.62	0.67	0.69	0.68	0.70	0.64	0.68	
H_O	0.42	0.29	0.41	0.26	0.31	0.25	0.31	0.24	0.33	0.25	
HD	*	*	*	*	*	*	*	*	*	*	
F_{IS}	0.39	0.55	0.39	0.58	0.52	0.65	0.55	0.66	0.48	0.63	
LD_{prop}	0	0.107	0.036	0.036	0.0	0.071	0	0	0.036	0	
B											
I	27	28	20	17	17	‡	‡	22	24	12	19
N	20	16	11	11	8			16	14	4	16
G/N	1.0	1.0	1.0	1.0	1.0			1.0	1.0	1.0	1.0
H_E	0.67	0.74	0.66	0.57	0.52			0.68	0.68	0.67	0.66
H_O	0.40	0.39	0.34	0.25	0.21			0.37	0.33	0.36	0.33
HD	*	*	*	*	*			*	*	*	*
F_{IS}	0.40	0.48	0.46	0.57	0.59			0.46	0.55	0.55	0.49
LD_{prop}	0	0.071	0	0	0			0	0	§	0.036
C											
I	34	36	39	31	29	16	21	28	11	32	
N	16	19	23	13	18	6	7	3	3	8	
G/N	1.0	1.0	1.0	1.0	1.0	0.7	1.0	1.0	1.0	1.0	
H_E	0.68	0.63	0.68	0.60	0.68	0.62	0.61	0.53	0.66	0.68	
H_O	0.34	0.35	0.30	0.35	0.31	0.29	0.20	0.29	0.71	0.25	
HD	*	*	*	*	*	*	*	*	0.65	*	
F_{IS}	0.51	0.46	0.53	0.46	0.52	0.54	0.68	0.52	−0.13	0.65	
LD_{prop}	0	0	0	0.036	0	0	0	§	§	0	
D											
I	19	15	20	26	32	‡	30	14	14	14	
N	18	14	20	21	28		26	13	12	13	
G/N	1.0	1.0	1.0	1.0	1.0		1.0	1.0	1.0	1.0	
H_E	0.63	0.67	0.65	0.64	0.64		0.65	0.68	0.69	0.68	
H_O	0.34	0.34	0.31	0.35	0.30		0.25	0.24	0.24	0.19	
HD	*	*	*	*	*		*	*	*	*	
F_{IS}	0.45	0.44	0.51	0.45	0.53		0.62	0.64	0.62	0.72	
LD_{prop}	0	0	0	0	0.107		0.036	0	0	0	

†*Skeletonema marinoi* strains were only isolated from station 11 during Cruise B.
‡Due to severe wind conditions the ship passed west of the island Gotland on the south (cruise B and D) and north (cruise B) bound journey.
§Cannot be estimated due to low sample size.

accurately estimated in non-HWE loci unless the rate of inbreeding is known (van Oosterhout *et al.*, 2006). Despite susceptibility of heterozygote deficiency in some microsatellite loci (Godhe & Härnström, 2010), and no prior knowledge of the proportion of asexually reproducing individuals, we calculated null allele frequencies according to Brookfield (1996). Hence, we could exclude that heterozygote deficiency in any locus was biased at particular sampling sites.

We investigated temporal and spatial genetic differentiation between all pairs of cruises and sample locations, respectively, by calculating pairwise F_{ST} using GENETIX 4.05 (Belkhir, 2004) with 10,000 permutations. Bonferroni adjustment was used to calculate *P*-values from all multiple tests (Rice, 1989). Bayesian analysis, as implemented in STRUCTURE 2.3.4 (Pritchard *et al.*, 2000; Falush *et al.*, 2003), was used to gain further insight into the gene flow between sampling stations pooled from all cruises, at each individual cruise, and at each separate sampling station over the four cruises. We assessed the number of potential clusters (*K*) among the 11 stations with pooled genotypes from all cruises, from 9 to 10 stations per individual cruise, and from 3 to 4 time points per sampling station. Each analyses consisted of five different runs at each *K* ranging from 1 to 15 for all cruises, 1–10 for individual cruises, and 1–4 for the single stations over time respectively. We calculated the estimated posterior log probability of the data, L(*K*), and the

stability of assignment patterns across runs. For each run, a burn-in period of 50,000 generations and 300,000 Markov chain Monte Carlo replications were applied. We used a model of admixture with the assumption of independent allele frequencies. An ad hoc statistic, ΔK, was calculated on the basis of the rates of change in the log likelihood of data between consecutive K values (Evanno *et al.*, 2005).

Isolation by distance and environment

Isolation by distance (IBD) analysis from matrices of genetic ($F_{ST}/1-F_{ST}$) versus Euclidean distances (Log_e of kilometres) was performed in GENEPOP (Raymond & Rousset, 1995). Euclidean distances were measured as linear distances between pairs of sites. Genetic isolation by salinity differences was estimated by analyses of matrices of genetic ($F_{ST}/1-F_{ST}$) versus pairwise salinity differences (Log_{10} ΔPSU). We tested for significant correlations by using Mantel test in R using the 'vegan' package Oksanen *et al.*, 2011). Statistical significance was assessed by 999 random permutations.

Gene flow and oceanographic connectivity

A new approach was used to calculate directional relative migration. The approach is a directional extension to measures of genetic differentiation and is based on a pool of migrants that is defined for each combination of two samples in pairwise comparison. The pool of migrants is calculated as the geometric means of the frequencies of the respective alleles in the two populations. The pool is then compared to each of the two populations to calculate directional genetic differentiation. Directional differentiation can subsequently be used to calculate directional relative migration. The method is further explained in (Sundqvist *et al.*, 2013). Relative migration rates were calculated using Josts'D (Jost, 2008) as a measure of genetic differentiation. Calculations were performed using the function divMigrate from the R-package 'diversity' (Keenan *et al.*, 2013).

We estimated oceanographic connectivity between the sampling sites with a biophysical model, where velocity fields from an ocean circulation model were combined with a particle-tracking routine to simulate drift trajectories at two different depth intervals representing the dispersal of diatoms. The dispersal of diatoms was simulated using the Lagrangian trajectory model TRACMASS (Döös, 1995). It is a particle-tracking model that calculates transport of particles using temporal and spatial interpolation of flow-field data from the BaltiX circulation model (see Text S1 in Appendix S2) with a time step of 15 min. Each sample site was represented by 15 grid cells closest to the locations given in Table S1 in Appendix S1. Particles were released on the 15th day of March or April over an 8-year period and allowed to drift in surface (0–2 m) or sub-surface (10–12 m) water for 20, or 30 days. Connectivity among the sampling sites was estimated by calculating the proportion of particles released from site i that ended up in site j. In total, the connectivity estimated among the sites was based on 1.98 million released particles.

We tested for significant correlations (Pearson's product-moment correlation coefficient) between estimated migration and oceanographic dispersal probability by using a variant of the Mantel test (Mantel, 1967) but including the full matrix allowing for asymmetric migration and dispersal. Statistical significance ($P < 0.05$) was assessed by 5000 random permutations. The matrix of directional migration consisted of pooled data from all stations during all four cruises (A–D), except stations 6 and 11 which were excluded due to low sample size (< 30 genotyped individuals). The oceanographic connectivity matrices represented (1) cells dispersed in the month of March or April; (2) cells dispersed in surface water (0–2 m) or sub-surface water (10–12 m); (3) cells drifting for 20 or 30 days. Additionally, we tested for significant correlations between the matrix of estimated migration versus an average of all dispersal matrices.

Genetic differentiation and environmental factors

We performed environmental association analyses to test for significant correlations between the genetic variation and the hydrographic variables estimated for the four cruises, that is, Chl *a*, *S. marinoi* abundance, temperature, salinity, fCDOM, NO_2-NO_3, PO_4, SiO_2. In all the analyses, we omitted stations with fewer than 10 genotyped strains. The environmental association analysis was performed using partial Mantel tests and redundancy analysis (RDA). One partial Mantel test per cruise was performed between all pairs of environmental variables and the genetic distance, F_{ST}. All hydrographic variables were $\log_{10}(x + 1)$ transformed before analyses using the software PASSAGE (Rosenberg & Anderson, 2011).

RDA analyses, one for each cruise and one for all cruises together, was used to disentangle the relative contribution of independent variables (biological-hydrographic and spatial components) in driving the genetic structure (dependent variable) of the Baltic Sea spring bloom. For the processing of the genetic data, the spatial data into a spatial matrix (S) and the environmental data into an environmental matrix E, see Text S2 in Appendix S2. We performed RDA analysis according to the double stop criterion (Blanchet *et al.*, 2008), which means that the forward selection is done only when the global model (containing all variables) is significant. For the global RDA, we reported adjusted regression coefficients of multiple determinations (R^2_{adj}). In addition to the global RDA, we calculated variance partitioning (Borcard *et al.*, 1992) to estimate the variance uniquely explained by the environment (E|S) or by space (S|E). Variance decomposition was performed if either the spatial or the environmental model were significant (Borcard *et al.*, 2011). In the partial RDA models, co-variables were included only if significant in either the spatial or the environmental model. All RDA analyses were performed in R (v3.1.2) using the rda and varpart functions of the 'vegan' package (Oksanen *et al.*,

2011). In addition, the forward.sel function of the 'packfor' package was used (Dray *et al.*, 2006).

RESULTS

Hydrography

The Baltic Sea spring bloom of 2013 developed during the second half of March in the south-west end of the transect, off the German coast, and simultaneously in the north-east end of the transect in the Gulf of Finland (Figs 1b & 2a), and later propagated offshore. By mid-April the spring bloom declined in the south-west, whereas high chl *a* fluorescence, was recorded northwards along the transect (Fig. 2a). The spatio-temporal abundance of *S. marinoi* was significantly correlated (Spearman's rho = 0.84, $P < 0.001$) to the chl *a* fluorescence (Fig. 2b, Table S2 in Appendix S1), and constituted up to 33% of the recorded taxa based on microscopic counts converted to biomass (data not shown). The sea surface temperature was homogenous along the transects during the three-first cruises, but in mid-April the temperature had increased in the southern part of the transect (Fig. 2c). Salinity and fCDOM concentration displayed a distinct south–north gradient, and small temporal changes (Fig. 2d,e). Concentrations of inorganic nutrients (NO_3-NO_2, PO_4, SiO_4) were initially high in the coastal areas, and lower in the Baltic proper. By mid-April, the nutrients were depleted, or markedly reduced, at all stations (Fig. 2f–h). Several hydrographic variables co-varied and were significantly correlated with latitude (Table S2 in Appendix S1).

Skeletonema population structure

Out of 611 individuals, two identical eight-loci genotypes were identified at station 6 during the third cruise (April 4–7, Table 1). At all stations the sampled populations were genetically diverse and we observed no increase in clonal structure as the bloom progressed. We observed significant heterozygote deficiency ($P < 0.05$), high $F_{IS,}$ and low proportion of loci deviating from linkage equilibrium in all samples during the four cruises, except from station 9 during cruise C (Table 1). However, from this station we were only able to genotype three individuals. All eight microsatellite loci were polymorphic. Locus S.mar3 was the least variable while S.mar5 and S.mar6 were the most variable (Table S3 in Appendix S1). There was no evidence for large allele drop out or stuttering effects using MicroChecker. Based on the method Brookfield 1, estimates of null alleles frequency were low in S.mar2, S.mar3 and S.mar6; moderate in S.mar4 and S.mar8; and highest in S.mar1, S.mar5 and S.mar7. There was no significant correlation between samples and potential null allele frequencies (2-tailed paired samples *t*-test, $P > 0.05$), and all loci were used in subsequent calculations of genetic differentiation and gene flow.

Spatial genetic differentiation displayed pronounced genetic structure in the south-west to north-east direction based on the Bayesian clustering method and pairwise F_{ST}. The calculated ΔK indicated that two clusters best explained the uppermost hierarchical level of the genetic structuring among the 11 stations (Fig. 3). The pairwise F_{ST} displayed that the southernmost stations (1–3), and the stations 4–5, respectively, were genetically differentiated from all other stations. Among stations 6–11, no or low degree of genetic differentiation was observed (Table S4 in Appendix S1). Besides the spatial structuring, there was temporal genetic differentiation among all paired cruises, except cruise A and B (Table S5 in Appendix S1). The genotypes in stations 1–3 were during all four cruises assigned to one cluster, and strains from stations 5–11 to a separate cluster (Fig. S1 a–d in Appendix S3). When testing for genetic differentiation at each sampling location over time (4 cruises), station 1–3 and 5–10 yielded one or two clusters. In the analyses in which two clusters were identified, the clusters were mixed across all individuals isolated from any cruise (data not shown). Contrary, the strains isolated from sampling station 4 formed two distinct clusters. The first two cruises were assigned to one cluster, and the two last to a second cluster (Fig. S1e in Appendix S3).

Based on the results from the Bayesian clustering analyses and the pairwise F_{ST}, we rejected the model of panmixia for the Baltic Sea spring bloom.

Genetic versus geographical and environmental distance

The Mantel test between matrices of genetic (F_{ST}) and geographical distances (km) was significant ($R = 0.79$, $P < 0.001$) and suggests significant isolation by distance. However, the genetic distance was also significantly correlated with the south–north salinity gradient ($R = 0.57$, $P < 0.001$, Fig. S2 a-b in Appendix S3), which illustrates that the effects of distance and environment were confounded and could not easily be disentangled.

Correlation of oceanographic connectivity and gene flow

Significant correlations between spatio-temporal migration patterns of *S. marinoi* and oceanographic connectivity ranged between 0.24 and 0.50 (Table 2). The analyses between the matrices of relative directional migration pattern from the different sampling stations during the four cruises, and the matrices of oceanographic connectivity (Tables S6 and S7 in Appendix S1) accentuated the importance of oceanographic circulation pattern for the genetic structuring of *S. marinoi*.

Correlation of environmental factors and genetic distance

The partial Mantel test identified a set of environmental variables that significantly co-varied with changes in population structure over the four different cruises (Table S8 in Appendix S1), but significant variables associated varied

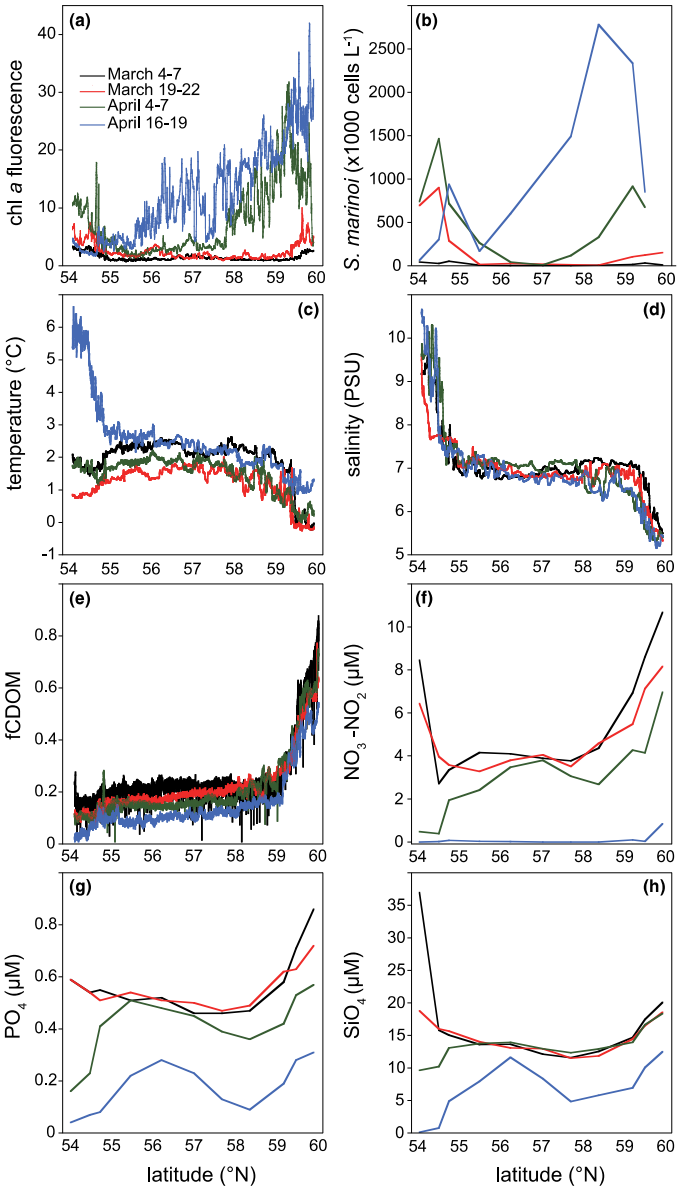

Figure 2 (a–h) Hydrographic variables along the latitudinal transect at each of the four cruises on a south–north transect in the Baltic Sea (March 4–7, March 19–22, April 4–7, April 16–19, 2013) at 8 m depth. (a) Phytoplankton biomass estimated by relative chl *a* fluorescence. (b) *Skeletonema marinoi* abundance ($\times 1000$ cells L^{-1}) (c) Temperature (°C). (d) Salinity (PSU). (e) Dissolved organic matter estimated as relative fCDOM. (f) NO_3-NO_2 concentration (µM). (g) PO_4 concentration (µM). (h) SiO_4 concentration (µM). Data in (a) and (c–e) are registered by automated ferry-box system with spatial resolution of 100–200 m along the transect of each cruise. *S. marinoi* abundance (b) is based on microscopic enumerations from the water samples collected at each of the 9–10 stations per cruise. Data in (f–h) were obtained from water samples collected by an automatic sampler at 24 fixed stations per cruise.

among the cruises. Salinity emerged as an important variable associated with the genetic structure during cruise A and D. SiO_4 concentration was significantly correlated with the F_{ST} during the two last cruises.

The global RDA was significant for the first and the last cruise (Cruise A and D), and for the merged data set including all stations from all cruises (Table 3). Salinity, *Skeletonema* cell density and the spatial matrix (PCNM) ($P < 0.05$) were included in the forward selection for Cruise A. The environmental (salinity and *Skeletonema* cell density) and the spatial matrices explained ~ 30% each of the variability in the genetic data ($P < 0.01$). The E|S and the S|E models for cruise A were not statistically significant, but the population differentiation with a split between southern (higher salinity) and northern (lower salinity) population was evident (Fig. S3a in Appendix S3). For Cruise D, *Skeletonema* cell density and the PCNM were included in the forward selection. Using the variation partitioning and conditioned analyses, the S matrix in

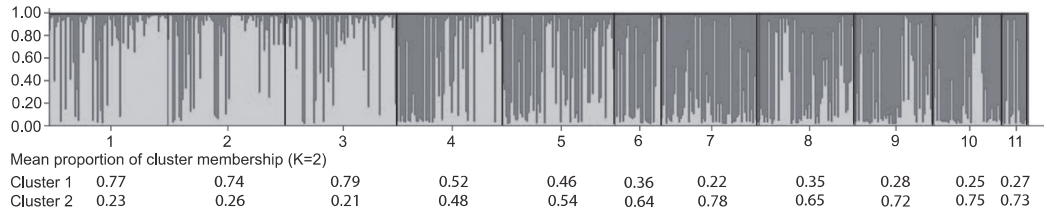

Figure 3 Genetic clustering of *Skeletonema marinoi* based on eight microsatellite loci estimated by Bayesian analyses implemented in STRUCTURE. Samples 1–11 are groups of strains established from fixed locations pooled from four cruises in the Baltic Sea conducted during the period March to April, 2013. Assignment of 611 individuals to $K = 2$ genetically distinguishable clusters. Each individual is represented by a vertical bar coloured according to the assigned group. Mean proportion of cluster membership for each sampling station is given below.

Table 2 Correlations between directional relative migration of *Skeletonema marinoi* and oceanographic connectivity in the Baltic Sea. Directional migration from all stations during all four cruises (Cruise A–D) was tested against connectivity matrices by Mantel test. Connectivity matrices tested were an average matrix based on the 2 months (March and April), the two depths (0–2 m and 10–12 m), and dispersal time of 20 and 30 days; March 0–2 and 10–12 m at dispersal time 20 and 30 days; April 0–2 m and 10–12 m at dispersal time 20 and 30 days. All connectivity matrices were based on particles realized from 15 grid cells per station. Correlation coefficients are given as R.

Migration matrix	Connectivity matrix	Correlation	*P*
Cruise A–D	Average months, depths, dispersal time	0.50	< 0.001
	March, 0–2 m, 20 days	0.15	0.13
	March, 10–12 m, 20 days	0.42	< 0.001
	March, 0–2 m, 30 days	0.24	0.046
	March, 10–12 m, 30 days	0.29	0.02
	April, 0–2 m, 20 days	0.26	0.02
	April, 10–12 m, 20 days	0.31	0.02
	April, 0–2 m, 30 days	0.25	0.047
	April, 10–12 m, 30 days	0.43	0.001

Cruise D explained ~12% of the variability in the genetic data. The E|S model was not statistically significant, but a split between southern (lower cell density) and northern (higher cell density) population was obvious (Table 3, Fig. S3b in Appendix S3). For the merged data set, SiO_4 concentration and PCNM were significant in the forward selection. The E|S model (SiO_4) and the S|E model were statistically significant. The genotypes isolated during the first two cruises grouped together and were separated from the genotypes isolated during the last two cruises. The results indicate that the lower silica concentrations towards the end of the spring bloom selected for different genotypes at all stations (Table 3, Fig. S3c,d in Appendix S3).

DISCUSSION

Until recently it was considered that marine planktonic organisms are made up of vast populations with unlimited dispersal potential due to ocean circulation. Here we show that the *Skeletonema marinoi* spring bloom within the Baltic Sea does not consist of a single panmictic population despite the uninterrupted stretch of several ocean basins. We detected two significantly differentiated populations delimited in space and separated by correlated physical and environmental dispersal barriers. We found a strong signal of genetic isolation by geographical distance. Lack of oceanographic connectivity obstructing gene flow between distant locations, and environmental gradients (salinity) along the Baltic Sea basins, appear to exert a selective pressure that accentuates genetic differentiation. Our data indicate that the spatial population structure remained largely stable throughout the bloom period. However, the depletion of silica during the progression of the bloom was related to a shift in the genetic structure. In response to environmental changes, local genotypes may be selected for and thus cause temporal differentiation.

Provided a significant isolation-by-distance effect we expect that samples collected along a transect consist of a continuum of distributed individuals over space, in which populations in remote locations become differentiated by isolation due to restricted mating probability (Wright, 1943). A significant correlation between geographical and genetic distance was indeed indicated in our data at the larger scale; however, at smaller distances this trend was not as pronounced. In addition, the circulation pattern (not necessarily associated with distance) and the associated gradient of hydrographic variables, essential for microalgal growth, also correlated significantly with the genetic structure. We therefore argue that the basis for the observed genetic structure is more complex than purely a function of distance. The transect was divided into two genetically differentiated populations in which the end locations were genetically distant, but the most important spatial barrier for gene flow was located between sample locations 3 and 5. This dispersal barrier may not be geographically fixed, but rather dynamic and dependent on seasonal circulation, for example, cyclonic gyres in the Arkona and Bornholm Basins during early spring (Leppäranta & Myrberg, 2009). At sampling station 4, two genetically separated populations over the four cruises were

Table 3 Results of the global RDA, RDA with forward selection, and variation partitioning analyses estimating the effect of environmental (E) and spatial (S) components on the genetic distance of *Skeletonema marinoi* populations in the Baltic Sea. E|S, environment controlled for space; S|E, space controlled for environment; sal, salinity; cel, *Skeletonema* cell density; sil, SiO_4 concentration; lat, latitude. Significant *P*-values are in bold. Forward selection and variation partitioning were not performed when the global model (either space or environment) was not significant.

	Cruise A		Cruise B		Cruise C		Cruise D		Cruise A+B+C+D	
	Mar 4–7		Mar 19–22		Apr 4–7		Apr 16–19		Mar 4–Apr 19	
Global RDA	E	S	E	S	E	S	E	S	E	S
R^2	**0.361**	**0.300**	0.138	0.002	0.221	0.061	0.255	**0.409**	**0.142**	**0.337**
P-value	**0.001**	**0.004**	0.275	0.456	0.299	0.160	0.098	**0.001**	**0.021**	**0.001**
Forward selection	E	S	E	S	E	S	E	S	E	S
(variables included)	(sal, cel)	(lat)	–	–	–	–	(cel)	(lat)	(sil)	(lat)
R^2	**0.304**	**0.299**	–	–	–	–	**0.230**	**0.376**	**0.125**	**0.308**
P-value	**0.008**	**0.006**	–	–	–	–	**0.019**	**0.002**	**0.004**	**0.001**
Variation partitioning	E|S	S|E	E|S	S|E	E|S	S|E	E|S	S|E	E|S	S|E
R^2	0.038	0.034	–	–	–	–	−0.023	**0.123**	**0.053**	**0.236**
P-value	0.277	0.309	–	–	–	–	0.816	**0.025**	**0.004**	**0.001**

identified. The dispersal model (averaged through 1995–2002, Fig. 4) supported this, as trajectories seeded from stations 3 and 5 were not continuous. In addition, the oceanographic matrices did not support retention of cells at station 4 as in the other stations, which prevents the build-up of a local buffering seed bank (Tahvanainen *et al.*, 2012). Hence, our data indicate two stable populations; one in the south and another in the north, and in-between a transition zone which is seeded by the two populations.

Oceanographic circulation is undoubtedly important for dispersal and gene flow in marine planktonic organisms (White *et al.*, 2010). We applied a robust biophysical dispersal model based on 8 years of monitoring data, which captures the inter-annual fluctuations in velocities. The high resolution of the BaltiX model permitted us to investigate drift at different depths, duration, and release periods. Thus, we could identify the dispersal conditions, which best correlated with calculated migration data. In the current and previous studies we have found that oceanographic connectivity explains up to 60% of the *S. marinoi* population genetic structure (Godhe *et al.*, 2013; Sjöqvist *et al.*, 2015). This indicates an important impact of oceanographic circulation on gene flow, but also that other factors are important for structuring a phytoplankton bloom. For instance, the Darss Sill (depth 18 m, Fig. 1b) constitutes a topographic and oceanographic borderline between Mecklenburg Bight and the Baltic Proper (HELCOM, 1988). Sampling location 1 and 2 are positioned to the west of the sill, whereas station 3 is positioned east of it. According to the biophysical model the dispersal potential traversing the sill was lower than between the two westernmost stations. Nevertheless, the gene flow appeared not to be affected by the constraints imposed by the sill.

The association between the genetic distances and environmental variables highlights that local hydrographic regimes are also important drivers of population genetic structure, in addition to spatial distribution and oceanographic circulation. Especially salinity was significantly correlated with latitude and disentangling the effects was problematic. The salinity gradient across the Baltic Sea explained the genetic differentiation observed, but only when it was not controlled for the spatial component. Space by itself was not a strong explanatory factor either within this data set. Genetic structure was explained with statistical confidence only when both salinity and space were included. The explicit effect of latitude per se on genetic population structure has no comprehensive biological meaning. It tells us that some factor(s) that vary across the latitudinal gradient is (are) involved in driving the differentiation. One such factor could be local adaptation to a specific salinity regime of the Baltic Sea, which could restrict gene flow and enhance the correlation between genetic and geographical distance.

Another important spatial aspect is the presence of local seed banks of *S. marinoi* and the strong link between the benthic and pelagic assemblages that supports local recruitment of vegetative cells (Godhe & Härnström, 2010). Oceanographic connectivity estimates, in fact indicate local seeding along the investigated transect. Local shallow coastal sites that are well connected to the sampling sites appear as important sources that may seed the spring bloom (Fig. 4). On the contrary, resuspension and seeding from the deeper areas of the Bornholm and Gotland Basins (100–200 m deep) is unlikely as the permanent pycnocline at 60–80 m depth prevents mixing of water and transportation of cells to the euphotic layer. Further, the deep Baltic Sea basins are permanently anoxic (Diaz & Rosenberg, 2008), which prevent resting stage germination (Lundholm *et al.*, 2011).

When all cruises were analysed by RDA and variation partitioning together, a pattern emerged where the populations from the two-first cruises in March grouped together separate from the last two cruises in April. Thus, genetic differences between populations may emerge over short temporal

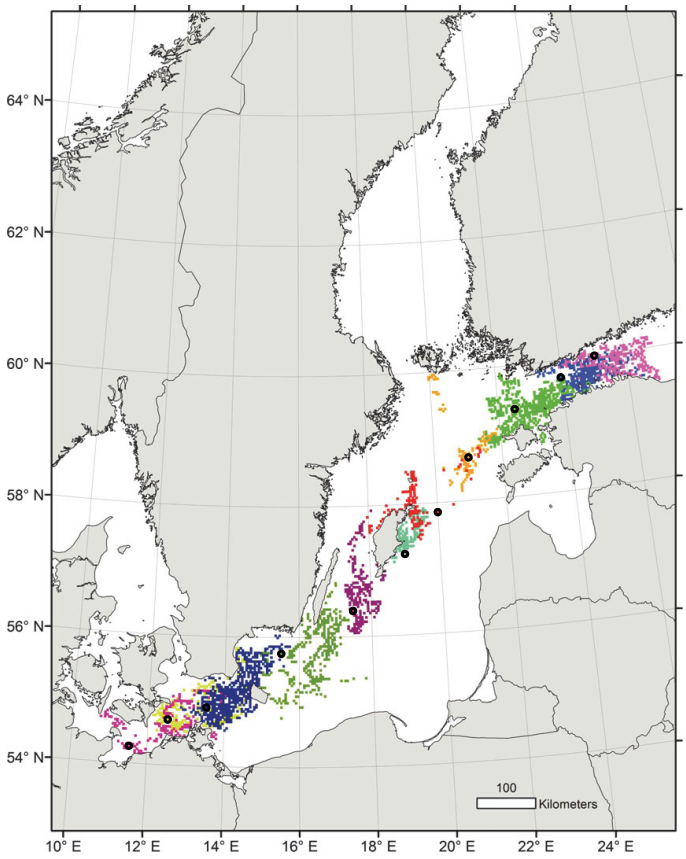

Figure 4 Map showing results from a simulation of diatom dispersal within the studied region in the Baltic Sea based on current velocities from the BaltiX circulation model. The colour-coded grid cells show the sources of simulated algae that end up at the 11 stations sampled during the four cruises (one colour for each station; 1-pink, 2-yellow, 3-blue, 4 green, 5-magenta, 6-turquoise, 7-red, 8- orange, 9-green, 10-blue, 11 pink). A grid cell is colour-coded if at least one trajectory from that grid cell ended up in the 15 grid cells representing each sample station. The simulation showed in the map represents release in April averaged over the years 1995–2002, and a dispersal duration of 20 days with a drift depth of 0–2 m.

scales, that is, a few weeks. This pattern was correlated with the depletion of silica during the course of the bloom. Silica concentrations were on average 50% lower during the last cruise (8.0 μM) compared to the first cruise (16.1 μM). Growth of diatoms, and the extent of blooms can be dependent on ambient silica concentration (Egge & Aksnes, 1992). Our data suggest that the Baltic Sea spring bloom consists of strains with different growth optima, and as the bloom progresses and the hydrographic conditions change there may be a shift towards genotypes with specific physiological adaptations to low silica concentrations. Such shifts in genotype composition imply the presence of diverse phenotypes and large standing genetic variation in the Baltic *S. marinoi* populations. Indeed, high genotypic diversity was recorded at every sampling station during each of the four cruises. Hence, the large genetic diversity observed might be the result of fluctuating selection and subsequent integration of different genotypes as the bloom progressed. Nevertheless, as we have no data on phenotypic traits from the isolated genotypes, such as silica requirements, this hypothesis remains to be tested.

We assumed neutrality of the microsatellites used for identifying the spatio-temporal genetic structure, and therefore we did not expect to see strong effects of the environment. Accordingly, the overall outcome of the RDA analyses was that the environment, that is, spatial and temporal gradients of salinity and silica, had a significant but weaker effect compared to oceanographic and spatial aspects. However, differential selection can provide genetic differentiation also in neutral loci by physical linkage to selected loci (Thibert-Plante & Hendry, 2010). In organisms with low recombination rates, which is applicable for diatoms with primarily vegetative propagation (D'Alelio *et al.*, 2010), a strong selection pressure may yield even greater effects on linked loci (Nosil *et al.*, 2009). Also, selection at certain locally adaptive loci has the effect that immigrants, which lack suitable alleles, are selected against and that this reduces realized gene flow and add to differentiation also in neutral loci (Nosil *et al.*, 2009).

Niche partitioning was recently demonstrated by single-cell genomics of the prokaryote *Prochlorococcus* in which a set of subpopulations had differential fitness and changed their rel-

ative abundance with changing environmental conditions (Kashtan *et al.*, 2014). Based on our discovery of the temporal genetic shift during the Baltic Sea diatom spring bloom, we anticipate that diatom blooms might consist of a sequence of short-lived subpopulations. If so, each subpopulation may be well adapted to particular hydrographic bloom conditions and occupy a distinct temporal-hydrographic niche, which maintain the bloom over longer periods.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1 Supplementary Tables S1–S8.
Appendix S2 Supplementary Text S1–S2.
Appendix S3 Supplementary Figures S1–S3.

DATA ACCESSIBILITY

Microsatellite sequences: GenBank Accession nos EU855763, EU855769-EU855771, EU855775, EU855777, GQ250935, GQ250937.

BIOSKETCH

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Supplementary material to Paper III

Only selected Figures and Tables are shown. All Supplementary material is available from http://onlinelibrary.wiley.com/wol1/doi/10.1111/jbi.12722/suppinfo

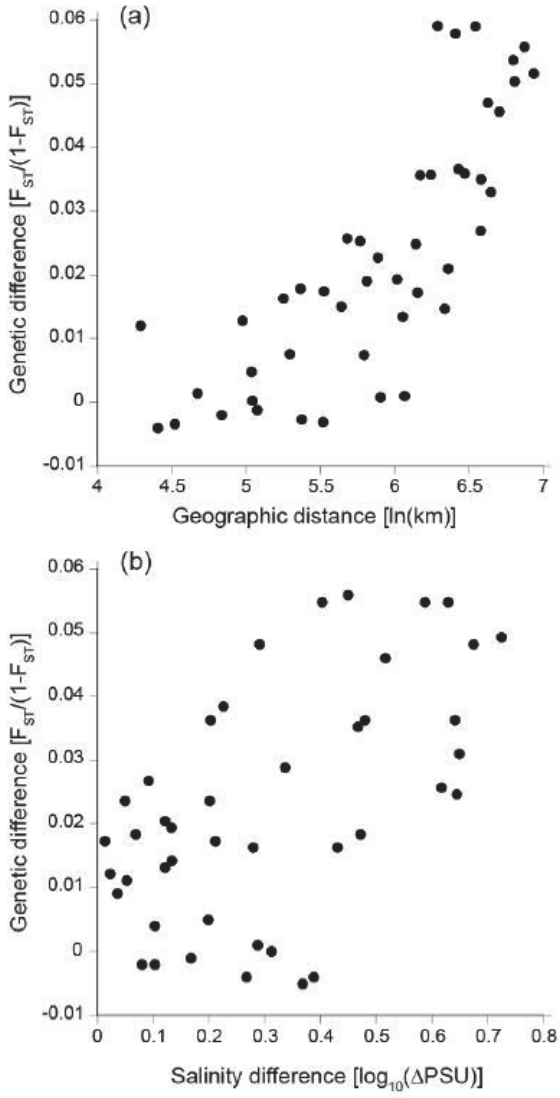

Figure S2. Isolation by distance and by environment among *Skeletonema marinoi* isolated at fixed sample locations during four cruises from March to April, 2013. **(a)** Pair-wise logarithms of geographical distance (km) versus genetic distance (F_{ST}/1- F_{ST}). Linear curve fit of F_{ST} y=-0.105+0.022x, R=0.79, P<0.001.

(b) Pair-wise logarithms of salinity difference (ΔPSU) versus genetic distance (F_{ST}/1- F_{ST}) to assess an example of isolation by environment. Linear curve fit of F_{ST} y=0.0075+0.049x, R=0.57, P<0.001.

Table S4. Genetic differentiation (F_{ST}) between pairs of sample locations pooled from four cruises in the Baltic Sea during March-April 2013. Italic numbers indicate significant (P<0.05) differentiation. Bold numbers denote significant differentiation after Bonferroni correction (P<0.001).

Station	1	2	3	4	5	6	7	8	9	10
2	-0.004	–								
3	0.001	-0.005	–							
4	**0.025**	*0.016*	*0.014*	–						
5	**0.024**	*0.018*	*0.017*	-0.002	–					
6	**0.035**	**0.034**	**0.035**	**0.026**	*0.017*	–				
7	**0.052**	**0.053**	**0.055**	**0.023**	*0.018*	0.011	–			
8	**0.030**	**0.035**	**0.037**	**0.020**	*0.012*	0.009	-0.002	–		
9	**0.046**	**0.044**	**0.046**	**0.023**	*0.013*	*0.019*	-0.001	0.004	–	
10	**0.047**	**0.052**	**0.052**	**0.028**	*0.016*	0.001	0.000	-0.004	0.005	–
11	**0.073**	**0.084**	**0.095**	**0.074**	**0.052**	*0.020*	0.015	0.011	*0.022*	0.004

Table S5. Genetic differentiation (F_{ST}) between pairs of cruises conducted during Baltic Sea spring bloom March-April 2013. Cruise A March 4-7; Cruise B March 19-22; Cruise C April 4-7; Cruise D April 16-19. Italic numbers indicate significant (P<0.05) differentiation. Bold numbers denote significant differentiation after Bonferroni correction (P<0.0083).

	Cruise A	Cruise B	Cruise C
Cruise B	0.0029	–	
Cruise C	**0.0330**	**0.0197**	–
Cruise D	**0.0142**	*0.0056*	**0.0123**

Table S7. Connectivity based on trajectories released from 15 grid cells per sampling station (see text) drifting for 20-30 days in surface water (0-2 m) or at a depth of 10-12 m during the months of March and April. The total number of particle released in the area is 1.98 million, and the trajectories are based on ocean current data gathered during 1995-2002. **A**. Average matrix based on both months, both depths, 20 and 30 days dispersal time. **B**. Dispersal matrix for March at 0-2 m depth during 20 days. **C**. Dispersal matrix for March at 10-12 m depth during 20 days. **D**. Dispersal matrix for April at 0-2 m depth during 20 days. **E**. Dispersal matrix for April at 10-12 m depth during 20 days. **F**. Dispersal matrix for March at 0-2 m depth during 30 days. **G**. Dispersal matrix for March at 10-12 m depth during 30 days. **H**. Dispersal matrix for April at 0-2 m depth during 30 days. **I**. Dispersal matrix for April at 10-12 m depth during 30 days.

A. Average matrix based on both months, both depths and 20 and 30 days dispersal time.

	From										
To	1	2	3	4	5	6	7	8	9	10	11
1	0.03126	0.00860	0.00081	0.00003	0	0	0	0	0	0	0
2	0.00210	0.02265	0.00896	0.00054	0	0	0	0	0	0	0
3	0	0.00045	0.02751	0.00578	0	0	0	0	0	0	0
4	0	0	0	0.00266	0.01181	0	0	0	0	0	0
5	0	0	0	0	0.03770	0.00001	0.00006	0	0	0	0
6	0	0	0	0	0	0.02068	0.01339	0	0	0	0
7	0	0	0	0	0	0.00037	0.03508	0.00159	0	0	0
8	0	0	0	0	0	0	0	0.03530	0.00454	0.00004	0
9	0	0	0	0	0	0	0	0	0.04301	0.00390	0
10	0	0	0	0	0	0	0	0	0	0.02834	0.01389
11	0	0	0	0	0	0	0	0	0	0	0.05696

B. Dispersal matrix for March at 0-2 m depth during 20 days.

	From										
To	1	2	3	4	5	6	7	8	9	10	11
1	0.00867	0.00170	0	0	0	0	0	0	0	0	0
2	0	0.01344	0.00663	0	0	0	0	0	0	0	0
3	0	0	0	0.01429	0	0	0	0	0	0	0
4	0	0	0	0.00578	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0.01446	0	0	0	0
7	0	0	0	0	0	0	0.04626	0	0	0	0
8	0	0	0	0	0	0	0	0.01922	0.00034	0	0
9	0	0	0	0	0	0	0	0	0.00051	0.00629	0
10	0	0	0	0	0	0	0	0	0	0.05425	0.02007
11	0	0	0	0	0	0	0	0	0	0	0.09762

C. Dispersal matrix for March at 10-12 m depth during 20 days.

	From										
To	1	2	3	4	5	6	7	8	9	10	11
1	0.01548	0.00714	0.00306	0	0	0	0	0	0	0	0
2	0.00901	0.01735	0.03963	0	0	0	0	0	0	0	0
3	0	0	0.00714	0.00425	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0.01667	0	0	0	0	0	0
6	0	0	0	0	0	0.04082	0.01395	0	0	0	0
7	0	0	0	0	0	0	0.02381	0	0	0	0
8	0	0	0	0	0	0	0	0.02415	0.00051	0	0
9	0	0	0	0	0	0	0	0	0.02891	0.00289	0
10	0	0	0	0	0	0	0	0	0	0.05612	0.01344
11	0	0	0	0	0	0	0	0	0	0	0.08452

D. Dispersal matrix for April at 0-2 m depth during 20 days.

	From										
To	1	2	3	4	5	6	7	8	9	10	11
1	0.00901	0.01003	0	0	0	0	0	0	0	0	0
2	0	0.02568	0	0	0	0	0	0	0	0	0
3	0	0	0.03265	0.00068	0	0	0	0	0	0	0
4	0	0	0	0	0.00663	0	0	0	0	0	0
5	0	0	0	0	0.00663	0	0	0	0	0	0
6	0	0	0	0	0	0.01139	0.00799	0	0	0	0
7	0	0	0	0	0	0	0.00731	0.00017	0	0	0
8	0	0	0	0	0	0	0	0.00986	0	0	0
9	0	0	0	0	0	0	0	0	0.02653	0.00867	0
10	0	0	0	0	0	0	0	0	0	0.00272	0.00187
11	0	0	0	0	0	0	0	0	0	0	0.03588

E. Dispersal matrix for April at 10-12 m depth during 20 days.

	From										
To	1	2	3	4	5	6	7	8	9	10	11
1	0.05357	0.00510	0	0	0	0	0	0	0	0	0
2	0	0.03605	0.00799	0	0	0	0	0	0	0	0
3	0	0	0.03401	0.00357	0	0	0	0	0	0	0
4	0	0	0	0	0.06054	0	0	0	0	0	0
5	0	0	0	0	0.05017	0	0	0	0	0	0
6	0	0	0	0	0	0.01667	0.02738	0	0	0	0
7	0	0	0	0	0	0	0.02976	0	0	0	0
8	0	0	0	0	0	0	0	0.02568	0.00051	0	0
9	0	0	0	0	0	0	0	0	0.05527	0.00170	0
10	0	0	0	0	0	0	0	0	0	0.02245	0.07211
11	0	0	0	0	0	0	0	0	0	0	0.05493

F. Dispersal matrix for March at 0-2 m depth during 30 days.

	From										
To	1	2	3	4	5	6	7	8	9	10	11
1	0.00068	0.00136	0	0.00034	0	0	0	0	0	0	0
2	0	0.00204	0	0.00442	0	0	0	0	0	0	0
3	0	0	0.00017	0.00119	0	0	0	0	0	0	0
4	0	0	0	0	0.00935	0	0	0	0	0	0
5	0	0	0	0	0	0	0.00068	0	0	0	0
6	0	0	0	0	0	0	0.01905	0	0	0	0
7	0	0	0	0	0	0	0.00170	0	0	0	0
8	0	0	0	0	0	0	0	0	0.00068	0	0
9	0	0	0	0	0	0	0	0	0.00153	0.00374	0
10	0	0	0	0	0	0	0	0	0	0.01071	0.00289
11	0	0	0	0	0	0	0	0	0	0	0.00442

G. Dispersal matrix for March at 10-12 m depth during 30 days.

	From										
To	1	2	3	4	5	6	7	8	9	10	11
1	0.00595	0.00833	0.00391	0	0	0	0	0	0	0	0
2	0.00527	0.00391	0.01633	0.00085	0	0	0	0	0	0	0
3	0	0.00544	0.00697	0.02109	0	0	0	0	0	0	0
4	0	0	0	0	0.00017	0	0	0	0	0	0
5	0	0	0	0	0	0.00017	0	0	0	0	0
6	0	0	0	0	0	0.00102	0.01752	0	0	0	0
7	0	0	0	0	0	0	0.00799	0.01888	0	0	0
8	0	0	0	0	0	0	0	0.01667	0.03384	0	0
9	0	0	0	0	0	0	0	0	0.02347	0.02007	0
10	0	0	0	0	0	0	0	0	0	0.02466	0.00544
11	0	0	0	0	0	0	0	0	0	0	0.04626

H. Dispersal matrix for April at 0-2 m depth during 30 days.

	From										
To	1	2	3	4	5	6	7	8	9	10	11
1	0.00221	0	0	0	0	0	0	0	0	0	0
2	0	0.00034	0.00289	0.00034	0	0	0	0	0	0	0
3	0	0	0.00561	0.01582	0	0	0	0	0	0	0
4	0	0	0	0.00017	0.03197	0	0	0	0	0	0
5	0	0	0	0	0.02959	0	0	0	0	0	0
6	0	0	0	0	0	0.00578	0.01497	0	0	0	0
7	0	0	0	0	0	0.00442	0.01395	0	0	0	0
8	0	0	0	0	0	0	0	0	0.00085	0.00051	0
9	0	0	0	0	0	0	0	0	0.00357	0.00340	0
10	0	0	0	0	0	0	0	0	0	0.00357	0.00629
11	0	0	0	0	0	0	0	0	0	0	0.01310

I. Dispersal matrix for April at 10-12 m depth during 30 days.

	From										
To	1	2	3	4	5	6	7	8	9	10	11
1	0.01241	0.02024	0.00272	0	0	0	0	0	0	0	0
2	0.00136	0.00017	0.00935	0.00085	0	0	0	0	0	0	0
3	0	0	0.02211	0.00850	0	0	0	0	0	0	0
4	0	0	0	0	0.03299	0	0	0	0	0	0
5	0	0	0	0	0.04660	0	0	0	0	0	0
6	0	0	0	0	0	0.02296	0.00850	0	0	0	0
7	0	0	0	0	0	0	0.01344	0	0	0	0
8	0	0	0	0	0	0	0	0.01548	0.01769	0	0
9	0	0	0	0	0	0	0	0	0.01446	0	0
10	0	0	0	0	0	0	0	0	0	0.02398	0.03469
11	0	0	0	0	0	0	0	0	0	0	0.01599

Table S8. Environmental association analysis based on partial Mantel test. Correlations (R) of F_{ST} and environmental gradients at the four different cruises. Significant correlations are indicated in bold numbers within parentheses (P<0.05), marginally significant (P<0.1) correlations are indicated in regular font.

Environmental variables	Cruise A Mar 4-7	Cruise B Mar 19-22	Cruise C Apr 4-7	Cruise D Apr 16-19
Chl *a*	-0.18	0.04	-0.39 (**0.03**)	0.13
Skeletonema abundance	0.42 (0.06)	-0.15	0.27 (0.09)	0.18
Temperature	-0.32 (**0.04**)	0.18	-0.14	-0.17
Salinity	0.37 (0.09)	0.12	0.13	0.39 (**0.04**)
DOM	0.34 (0.09)	-0.13	-0.16	0.004
NO_3	-0.03	-0.04	-0.33 (0.05)	-0.12
PO_4	-0.05	0.05	-0.09	0.42 (**0.04**)
SiO_4	-0.24	0.08	0.29 (**0.04**)	-0.49 (**0.01**)

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ABSTRACT

Influence of physical-chemical factors on community and populations of the Baltic Sea spring bloom microalgae

Photosynthesizing microalgae constitute the basis of food webs in aquatic ecosystems and generate about a half of the annual net primary production on Earth. Some microalgal species produce resting stages as a part of their life cycle, which may contribute to the inoculation of new blooms, thus impacting their magnitude. Therefore, it is relevant to understand how different environmental factors influence the distribution of resting stages and their contribution to the onset of new blooms, particularly regarding harmful species.

The abundance of dinoflagellate resting stages was investigated before, during and after the spring bloom in three sub-basins of the Baltic Sea to identify spatial and temporal dynamics. The abundance of resting stages showed temporal dynamics in relation to the life cycle events but was also potentially influenced by the physical processes such as resuspension and horizontal transport. Transport of newly formed resting stages was simulated to explore the distance travelled by the cysts before sedimentation. Simulation results supported the influence of transport on total cyst abundances, indicating that the majority of cysts were transported further from the point of formation. The latter has also implications for the usage of cysts as proxies for the biological productivity of a water body.

Differences in environmental factors can also drive intraspecific genetic differentiation, which allows species to use various habitats, thus supporting high biodiversity. Therefore, it is important to know which environmental parameters promote genetic structuring to understand the ecology and evolution of microalgae better. Influence of environmental factors on the genetic structure of microalgae was studied by conducting growth and competition experiments between microalgae populations and by investigating the genetic composition of an important spring bloom diatom species, *Skeletonema marinoi*, in time and space during a bloom in the Baltic Sea. Local adaptation and native competitive advantage were detected between populations of the species, despite being seemingly well-connected. Although the factor(s) generating differential selection could not be determined, those findings show that varying selection pressure(s) between habitats can support the development of genetic differentiation. Spatial and temporal genetic structuring was also detected during a basin-wide bloom event. Spatial differentiation was best explained by the geographical distance, oceanographic connectivity and environmental parameters, whereas changes in the environmental conditions could account for temporal genetic differentiation. The presence of various phenotypes and genetic variation may allow the species to bloom over longer periods.

The results of this thesis contribute to a better understanding of the influence of different environmental factors on the community and population dynamics of microalgae.

RESÜMEE

Füüsikalis-keemiliste tegurite mõju kevadõitsengu mikrovetikate kooslustele ja populatsioonidele Läänemeres

Fotosünteesivad mikrovetikad moodustavad veeökosüsteemides toiduahela esimese lüli ning toodavad peaaegu poole kogu planeedi aastasest primaarproduktsioonist. Mõned mikrovetikate liigid moodustavad oma elutsükli käigus puhkestaadiume, mis aitavad neil üle elada kasvuks ebasobivaid keskkonnatingimusi ning loovad võimaluse tingimuste muutudes uute õitsengute moodustumiseks. Eelnevast lähtudes on oluline teada, kuidas erinevad keskkonnategurid mõjutavad puhkestaadiumite levikut ning nende panust uute õitsengute alustamisse. Need teadmised on eriti olulised ohtlike (nt toksiine tootvate) mikrovetika liikide puhul.

Käesolevas töös uuriti dinoflagellaatide puhkestaadiumite arvukust enne kevadõitsengut, õitsengu ajal ja pärast õitsengut kolmes Läänemere alambasseinis. Teostatud uuringu eesmärgiks oli sette pealmises kihis esinevate puhkestaadiumite ajalis-ruumiliste muutuste tuvastamine ja kirjeldamine. Uuritud liikide puhkestaadiumite arvukuses esines elutsükli protsessidega seotud ajaline muutlikkus, aga puhkestaadiumite ajalis-ruumiline varieeruvus oli potentsiaalselt mõjutatud ka füüsikalistest protsessidest nagu näiteks resuspensioon ja horisontaalne transport. Värskelt moodustunud puhkestaadiumite transporti modelleeriti, hindamaks kui pika vahemaa läbivad pinnakihis moodustunud puhkerakud enne merepõhja settimist. Mudeli simulatsioonid kinnitasid horisontaalse transpordi mõju puhkestaadiumite arvukusele, kusjuures selgus, et enamus puhkestaadiume kantakse nende moodustumiskohast eemale. Saadud tulemus mõjutab puhkestaadiumite kasutamise potentsiaali veekogu bioloogilise tootlikkuse indikaatorina.

Erinevused keskkonnatingimustes võivad soodustada liigisisese geneetilise erinevuse teket, mis omakorda võimaldab liigil kasutada erinevaid elupaiku ja toetab seeläbi suuremat bioloogilist mitmekesisust. Mikrovetikate ökoloogia ja evolutsiooni paremaks mõistmiseks on vaja teada, millised keskkonnategurid soodustavad liigisisest geneetilist eristumist. Keskkonnategurite mõju mikrovetikate geneetilisele struktuurile uuriti kasutades kasvu- ja konkurentsieksperimente ühe olulise kevadõitsengus osaleva ränivetika liigi, *Skeletonema marinoi*, erinevate populatsioonide vahel. Lisaks uuriti sama liigi geneetilist struktuuri ajas ja ruumis Läänemere kevadõitsengu vältel. Omavahel näiliselt hästi ühendatud erinevate populatsioonide uurimine näitas, et need on kohastunud oma elupaigaga ja omavad oma elupaigas konkurentsieelist. Kuigi täpsed tegurid, mis antud elupaikades erinevat valikusurvet põhjustavad pole teada, näitavad saadud tulemused, et erinev valikusurve võib toetada populatsioonide geneetilist eristumist. Kevadõitsengu uuringu käigus leiti, et ühe liigi isendite populatsioonid erinevad geneetiliselt nii ajas kui ruumis. Ruumilist geneetilist erinevust selgitasid kõige paremini vahemaa, transport hoovustega ja keskkonnategurite muutlikkus. Tuvastatud ajaline

geneetiline erinevus võib olla seotud muutustega keskkonnatingimustes. Erinevate fenotüüpide esinemine ja suur geneetiline mitmekesisus võivad pikendada liigi ajalist esinemist mikrovetika koosluses.

Käesoleva töö tulemused suurendavad teadmisi erinevate keskkonnategurite mõjust mikrovetikate koosluste ja populatsioonide dünaamikale.

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