

Dispersal of Microalgae

The role of Biological and Physical Barriers



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Ph.D. thesis
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Doctoral Thesis

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Abstract

Microalgae are only a few micrometres to a millimetre in size, yet they constitute the base for aquatic food webs and are extremely important drivers in elemental cycling. Long it has been assumed that small organisms (<1 mm) that occur in high abundances, have global dispersal potential facilitated by winds, currents and vectors e.g. birds and insects. A growing body of evidence is however portraying a different story; that several planktonic microalgal species exhibit genetically differentiated populations at regional geographic scales, and that populations can also differ in environmentally important phenotypic traits.

In this thesis I present results from field studies and experiments designed to test various physical and biological dispersal barriers to explain why, despite the high dispersal potential, microalgae exhibit reduced gene flow (exchange of genetic material) between populations. A marine species *Skeletonema marinoi* (Bacillariophyceae) and a freshwater species *Gonyostomum semen* (Raphidophyceae) were used to test the function of geographic distance and hydrographic connectivity (physical barriers) as well as local adaptation and priority effects (biological barriers) on gene flow between populations.

Geographic distance and oceanographic connectivity (currents) have demonstrated suitable predictors for mapping gene flow between populations of various marine microalgae species. Indeed, we also found that over large (>1000 km) regions in the Baltic Sea, population genetic patterns could be correlated with dispersal limitation caused by geographic distance and oceanographic connectivity. Additionally, when genetic data was compared to environmental data, population differentiation was corroborated by differences in salinity and silicate concentrations, indicative of adaptation as a driver of population genetic divergence. Over smaller (<160 km) distances, although reduced gene flow was evident, physical dispersal barriers were poor predictors of local population genetic patterns. Surprisingly, the role of connectivity by currents (marine systems) and rivers (freshwater systems) did not show any signs of facilitating dispersal that resulted in gene flow. In the freshwater study area, we found a weak effect of geographic distance, whereas in the marine study, neither distance nor currents could explain signals of reduced gene flow, suggesting other influences driving population genetic differentiation, e.g. environmental selection against immigrants or persistent historical events reinforced by adaptation (monopolization).

Laboratory experiments using strains of *S. marinoi* confirmed that prior arrival to a vacant resource (founder event) was associated with a competitive advantage compared to later arriving strains (priority effects). In addition, strains were competitively superior in native salinity conditions, compared to non-native strains, supportive of local adaptation. Combined these results provide strong support for monopolization (priority effects reinforced by adaptation) as an important biological barrier to gene flow between nearby populations of planktonic microalgae.

In summary, this thesis provides important insight into the physical and biological barriers that act in concert in driving population genetic differentiation and diversification of these ecologically central actors. In addition, from an evolutionary perspective these results highlight that the history and biology of these organisms are equally important features to consider as well as their physical characteristics that may facilitate dispersal.

Keywords: Phytoplankton | Dispersal | Population genetics | Gene flow | Connectivity
| Diatoms | *Skeletonema marinoi* | *Gonyostomum semen* | bloom-forming |
Monopolization hypothesis | Priority effects | Adaptation

Svensk populärvetenskaplig sammanfattning

Man kan inte se en mikroalg med blotta ögat och ändå utgör de basen för alla näringskedjor i vattenmiljöer och står för 40% av syret vi konsumerar. Länge har det ansetts att på grund av deras ringa storlek och enorma antal så kan de lätt spridas med vindar, strömmar, fåglar och insekter, och därmed har de en global spridning. En växande mängd indikationer pekar dock i en helt annan riktning där populationer (inom samma art) av planktoniska mikroalger är genetiskt åtskilda i närliggande områden, och att dessa populationer även kan skilja sig åt i miljömässigt viktiga egenskaper.

I denna avhandling presenterar jag resultat från fältstudier och laboratorieförsök som syftar till att testa potentiella fysiska och biologiska spridningsbarriärer som kan förklara varför vissa mikroalger uppvisar minskat genflöde (utbyte av genetiskt material) mellan populationer trots hög spridningsförmåga. Fokus har lagts på två arter, som är vanligt förekommande i Skandinavien, den marina kiselalgen *Skeletonema marinoi* och den i sötvatten invasiva nålflagellaten *Gonyostomum semen*. För dessa två arter har vi undersökt hur genflödet mellan populationer påverkas dels av geografiskt avstånd och förbindelse med strömmar och vattendrag (fysiska spridningsbarriärer) samt lokal anpassning och effekten av att komma först till ett område (biologiska barriärer).

Det geografiska avståndet som skiljer mikroalgpopulationer åt och även havsströmmarnas cirkulations mönster har tidigare visat sig stämma väl överens med intensiteten av genflödet. I en storskalig fältstudie (> 1000 km) i Östersjön fann vi också att stora avstånd och regionala strömmar överensstämde väl med populationsgenetiska mönster. Dessutom, när genetiska data jämfördes med miljövariabler som är viktiga för algernas tillväxt kunde vi se en effekt av skillnader i salthalt och kiselkoncentration, vilket tyder på att vissa populationer är anpassade till sin lokala miljö och att detta driver ytterligare på den genetisk särskiljning mellan populationer. Däremot på mindre avstånd (<160 km) kunde vi inte härleda genflöde till varken geografiska avstånd eller vattenförbindelse. Det var överraskande att vattenförbindelse genom strömmar (marin miljö) och vattendrag (sötvattensmiljö) inte visade några tecken på att påverka genflödet. I sötvattenstudien fann vi en svag effekt av geografiskt avstånd där populationer i närliggande sjöar var genetiskt mer lika än de som låg långt ifrån varandra. I den småskaliga marina studien kunde varken avstånd eller lokala strömmar förklara det varierande genflödet mellan olika provtagningspunkter. Detta tyder på att det finns andra spridningsbarriärer som påverkar genflödet, t.ex. så kan olika miljöförhållanden sålla bort sämre anpassade immigranter eller så kan etableringshistoriken i ett område som förstärks genom snabb anpassning orsaka monopolisering av en population i ett specifikt område.

I ett laboratorieförsök kunde vi visa att historisk etableringsordning, alltså ordningen i vilken koloniserare anländer till ett område, kan bestämma vilka som dominerar området. Att anlända först till en livsmiljö gav ett betydande övertag gentemot att anlända senare, detta resulterade i att förstkommande koloniserare dominerade. Vidare kunde vi visa att populationer av *Skeletonema marinoi* som kom från högre respektive lägre salthaltsförhållanden var konkurrensmässigt överlägsna i deras respektive inhemska salthalt. Tillsammans ger dessa resultat starkt stöd för att monopolisering (historiska händelser förstärkta genom lokal anpassning) är en viktig biologisk spridningsbarriär som förhindrar eller minskar genflödet mellan närliggande populationer av planktoniska mikroalger.

Sammantaget ger min avhandling viktiga insikter i samverkan mellan fysiska och biologiska spridningsbarriärer och hur de påverkar genflödet mellan mikroalgpopulationer och därmed påverkar den genetiska variationen i dessa viktiga organismer. Dessutom betonar mina resultat att, ur ett evolutionärt perspektiv, är mikroalgers förmåga till anpassning och historiska händelser lika viktiga funktioner att beakta som habitatets fysiska egenskaper när vi vill förstå hur algbloomningar sprids.

List of papers

This thesis is based on the following papers:

- Paper I:** Sefbom, J., Kremp, A., Jonsson, P., Rengefors, K., Godhe, A. (Submitted) Small-scale population differentiation in a planktonic bloom-forming diatom.
- Paper II:** Godhe, A., Sjöqvist, C., Sildever, S., Sefbom, J. *et al.* (Submitted) Physical barriers and environmental gradients cause spatial and temporal genetic differentiation of an extensive algal bloom.
- Paper III:** Sassenhagen, I., Sefbom, J., Säll, T., Godhe, A., Rengefors, K. (2015) Freshwater protists do not go with the flow: Population structure in *Gonyostomum semen* independent of connectivity among lakes. *Environmental Microbiology* DOI: 10.1111/1462-2920.12987
- Paper IV:** Sefbom, J., Sassenhagen, I., Rengefors, K., Godhe, A. (2015) Priority effects in a planktonic bloom-forming marine diatom. *Biology Letters*, 11 (5); DOI: 10.1098/rsbl.2015.0184
- Paper V:** Sefbom, J., Kremp, A., Johannesson, K., Pettersson, R., Rengefors, K., Godhe, A. (Manuscript) Evidence for population genetic divergence driven by competitive exclusion in a planktonic diatom.

Related publications not included in this thesis:

Sassenhagen, I., Sefbom, J., Godhe, A. & Rengefors, K. (2015) Germination and colonization success of *Gonyostomum semen* (Raphidophyceae) cysts after dispersal to new habitats. *Journal of Plankton Research* DOI: 10.1093/plankt/fbv067

Sildever, S., Sefbom, J., Lips, I., Godhe, A. (Submitted) Local adaptation in a bloom-forming marine diatom.

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Background

Dispersal barriers

The geographic expansion of a species is largely governed by the presence or absence of dispersal barriers. What constitutes a dispersal barrier, however, will vary between species. Sometimes these are easily identified (e.g. oceans, mountains, and deserts), but in other cases the barriers may be less conspicuous and more difficult to recognise. Insight into what physical and biological mechanisms that limit a species expansion is a key component in understanding its ecology and evolutionary biology. The movement of individuals or groups of individuals ultimately allows for the exchange of genetic material between populations, i.e. gene flow (Bohonak, 1999). In evolutionary terms gene flow constitutes a constraining mechanism that prevents populations from evolving into different species. While reduced gene flow, or complete genetic isolation can over time result in the development of population differentiation, local adaptation and eventually speciation (Slatkin, 1987).

Microorganisms— here, there and everywhere?

In 2002, Finlay resurrected the notion that dispersal of microbial unicellular organisms is global, thereby arguing that biogeography in microorganisms is redundant. This idea was originally introduced by Beijerinck (1913) and later formulated into the tenet ‘Everything is everywhere, but the environment selects’ by Baas Becking (1934). The justification being their small size (<1 mm) and vast population sizes, combined with the many natural vectors (e.g. hurricanes, oceanic circulation, damp fur and feather) would facilitate passive transportation. In addition the formation of dormant stages, a common trait amongst microorganisms, is often described as a vehicle for geographic expansion (Anderson, 1989, Matsuoka & Fukuyo, 2000). These dormant stages are hardy and can remain viable for long periods of time under harsh conditions (Huber & Nipkow, 1923, Lundholm, *et al.*, 2011, Ribeiro, *et al.*, 2011). Despite this potential for large scale, or even ubiquitous dispersal, numerous studies using molecular techniques have revealed reduced gene flow between microbial populations at various spatial scales and in different habitats (Foissner, 2006, Martiny, *et al.*, 2006, Medlin, 2007).

Microalgae— the life aquatic

Microalgae are unicellular organisms that can exist as single cells or form chains or colonies. They are only between a few micrometres to a millimetre in size, but they constitute the basis of aquatic food webs, and are responsible for half of the world's primary production (Field, *et al.*, 1998). These ecologically central drivers comprise highly diverse groups of organisms that vary in everything from their phylogeny to their role in elemental cycling (e.g. production of silicate or calcium carbonate), nutrient usage, defence mechanisms (e.g. toxin production) and reproductive strategies (Sournia, 1982). This diversity is not only limited to interspecific variation, but several studies have also been able to show notable intraspecific differences in environmentally important phenotypic traits. A comparison of toxin profiles in the dinoflagellate *Alexandrium ostenfeldii* revealed that strains differed consistently in the proportion of individual toxins depending on their geographic origin (Suikkanen, *et al.*, 2013). Similar intraspecific variability has also been documented for other microalgae species in various phenotypic traits, e.g. different growth optimum for various $p\text{CO}_2$, light and salinity conditions (Ryneckson & Armbrust, 2004, Kremp, *et al.*, 2012, Rengefors, *et al.*, 2015, Sjöqvist, *et al.*, 2015). These phenotypic differences could further be supported by signals of significantly reduced gene flow (Box 1) between the populations. Ultimately, these findings challenge the perception of continuous dispersal in microalgae resulting in gene flow.

Box 1: Dispersal, gene flow and population genetics

Gene flow is the exchange of genetic material often achieved through recombination (sexual reproduction). For this to occur between individuals residing in different locations, dispersal or migration between locations is a prerequisite. Frequent dispersal, resulting in recombination will incorporate similar alleles in similar frequencies and thus the two locations will have a homogeneous gene pool, and little to no differentiation. Alternatively, if dispersal is reduced and recombination between individuals from the respective locations is rare, the two gene pools will most likely differ in allele frequencies and may also have private alleles (alleles only found in one population but not the other). To test for population genetic differentiation, several (often neutral) loci are assayed and then statistically tested against the null hypothesis of identical allele frequencies.

Going the distance — Distance decay

In response to the ‘paradox of high dispersal potential and low gene flow’ among microbial populations, various mechanisms have been proposed. The most well known within the field and most routinely tested is isolation-by-distance (IBD). With IBD there is a distance decay whereby gene flow reduces with increasing geographic distance, and thus population genetic differentiation increases the further apart populations are located (Wright, 1943). The planktonic marine diatom *Pseudonitzschia pungens* clade I is an example whereby population genetic differentiation showed a strong correlation with geographic distance (Casteleyn, *et al.*, 2010). This species is known to have a global distribution and significant population genetic differentiation was detected on a macrogeographic scale. Although, over smaller distances (<650 km) *P. pungens* displays unrestricted gene flow and lacks population structure (Casteleyn, *et al.*, 2009). IBD can, however, also be manifested at smaller spatial scales in other species, e.g. the dinoflagellate *Alexandrium tamarense* (Nagai, *et al.*, 2007). Isolation-by-distance, in the strictest sense, is essentially a neutral process where the underlying mechanism is geographic distance (Figure 1), by which the probability of recombination between individuals from different locations decreases with increasing distance. Thus it should be noted that a

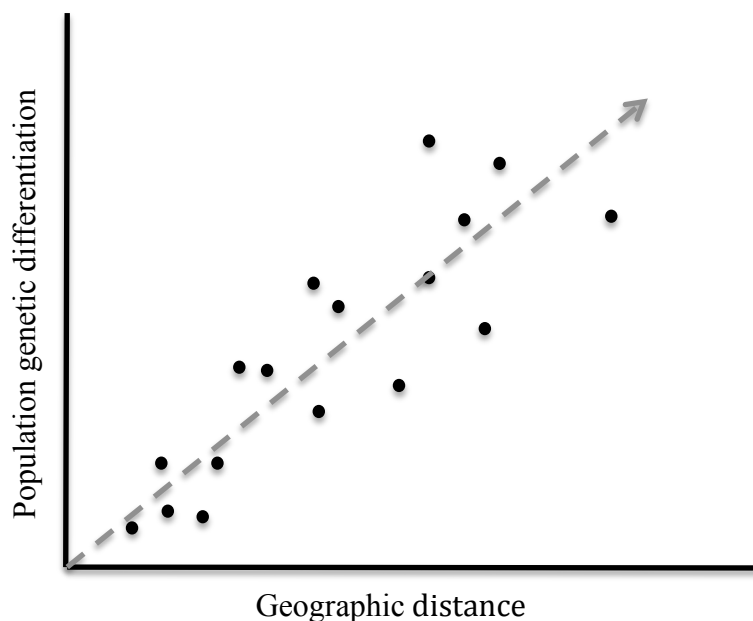


Figure 1. Schematic illustration of a positive isolation-by-distance pattern, whereby population genetic differentiation increases with increasing geographic distance between sampled populations.

pattern of IBD can sometimes be confounded by the presence of an environmental gradient that coincides with the geographic distance (discussed in **Paper II**). Population differentiation along such a transect is then the result of strong selection, and environmental sorting, rather than an effect of distance and dispersal limitation *per se*. Identifying the main responsible driving force(s) can therefore be greatly facilitated by the simultaneous collection of environmental data to compare with population genetic structure (Martiny, *et al.*, 2006).

Going with the flow – Connectivity

For planktonic organisms, which are unable to move against currents, dispersal is largely dependent on hydrographic connectivity as a means of transportation, and therefore also gene flow (Cowen & Sponaugle, 2009). Consequently, Euclidean distance may not always be an adequate predictor of population genetic patterns in planktonic organisms. Two distant sites may be well connected with frequent migration sustained by currents, whereas two adjacent sites may rarely or never exchange individuals due to being separated by an oceanographic front (Gill & Hilbish, 2003). The same is also true in limnic habitats, where adjacent lakes may lack connecting rivers or streams. As a result, several studies have employed modelled movement of passive particles by ocean currents as a measure of estimated connectivity, and compared with gene flow. This approach has proven relevant for several marine species over larger spatial scales, including the diatom *Skeletonema marinoi*, where population genetic differentiation along the Swedish west coast and in the Baltic Sea could be explained by modelled water circulation patterns (Godhe, *et al.*, 2013, Sjöqvist, *et al.*, 2015). In the Mediterranean Sea, the population structure of *Alexandrium minutum* also corresponded with regional circulation patterns (Casabianca, *et al.*, 2012). Similarly, in freshwater systems, it was shown that the most suitable approximation for gene flow in the cladoceran *Daphnia ambigua* was to apply models that incorporated travelling distance, current direction and flow rate of streams and rivers (Michels, *et al.*, 2001). To date, very little literature is available on the role of connectivity in mediating gene flow between microalgal populations at small spatial scales. This is true for both marine and freshwater systems but is addressed in this thesis (discussed in **Paper I and III**).

Monopolization hypothesis

Although dispersal is a prerequisite for gene flow (Bohonak, 1999), micro-evolutionary processes are influenced by an array of environmental and biological features that cause selection and determine the rate at which gene flow can occur. Genetic isolation driven by IBD or lack of hydrographic connectivity are both neutral mechanisms (Orsini, *et al.*, 2013) in the sense that they do not consider the history or biology of a species. Instead, De Meester, *et al.* (2002) proposed the *Monopolization hypothesis* as an explanation to why zooplankton populations in well-connected lakes exhibit significant genetic differentiation. In the monopolization hypothesis it is reasoned that organisms with rapid growth rates will quickly dominate the community after arriving to a vacant resource. In addition, having a resting stage as part of the life cycle can then provide a genetic anchor and reservoir, buffering against future colonizers, through priority effects (discussed in **Paper IV**). Over time, adaptation to local environmental conditions will further reinforce population stability, as they will become better competitors for limited resources (discussed in **Paper V**), and thus monopolize the niche (De Meester *et al.* 2002).

Aims

As a consequence of various anthropogenic activities, such as coastal urbanisation, increased carbon dioxide emissions, aquaculture and shipping, the inhabitants of aquatic ecosystems are now presented with a suite of stressors to take on. Hence, it is of critical importance to understand their biology, ecology and potential for adaptation. Considering the diversity contained within microalgae, there will be those that thrive and those that fall short. At the community level, such stressors may result in a shift in the community composition, where species that are better competitors for limited resources under specific conditions will dominate. At the species level, it may give rise to geographic range expansion as regions that have previously been inhospitable or inaccessible become available. Such range expansion has already been reported for several dinoflagellate species that form intensive and toxic blooms (harmful algal blooms or HABs), which could be traced back to the transportation of ships' ballast water (Hallegraeff, 1998). At the population level, genotypic selection has been shown as a response to environmental stressors and a mechanism for adaptation (Lohbeck, *et al.*, 2012). Predictions for global change are

expected to proceed gradually during decades. For fast growing organisms such as microalgae, this may allow for adaptation whereby species may become genetically and phenotypically different from the present day populations (Hallegraeff, 2010). Ultimately, the aim of this thesis is to contribute with a better understanding and recognition of abiotic and biotic factors that influence dispersal, microevolutionary processes and consequently the potential for adaptation and geographic expansion of microalgae under current and future scenarios.

Objective

In light of these drivers of genetic differentiation in natural populations, the focus of my thesis is to investigate what physical and biological mechanisms may be responsible for reduced gene flow in two different species of microalgae for which genetic differentiation has previously been documented. Two species have been used as model organisms, both being bloom-forming and therefore of particular importance for biogeochemical cycling in their respective habitats (Rost, *et al.*, 2003). For comparative purposes I have used a diatom species found in marine and brackish waters, *Skeletonema marinoi* (Sarno & Zingone) and one that resides in freshwater, the raphidophyte, *Gonyostomum semen* (Ehrenberg). Although *S. marinoi* (**Box 2**) and *G. semen* (**Box 3**) are very different in many respects, they do indeed share some key characteristics central to the mechanisms I wanted to investigate: 1) they exhibit highly structured populations despite being common across large areas, 2) both species are common in Swedish waters 3) they are highly successful in their respective environments and often dominate the phytoplankton community during a bloom, and 4) they form benthic resting stages that sink to the sediment.

Box 2: *Skeletonema marinoi* (Sarno & Zingone)

The centric chain-forming diatom *Skeletonema marinoi* (Bacillariophyceae) is an important member of the planktonic community in many coastal temperate regions (Kooistra *et al.*, 2008). In Swedish waters *S. marinoi* is found all year round, but will dominate during the spring bloom (February-March). When light conditions and nutrients are ample, proliferation mainly occurs asexually by vegetative cell division, at a rate of one division per day (Taylor *et al.*, 2009). During cell division, each daughter cell receives a larger valve (epitheca) from the parent cell, into which a smaller valve (hypotheca) is formed. As a result cells progressively get smaller, until they reach a critical size. Cell size restoration is then generally accomplished by producing gametes for sexual reproduction. *Skeletonema* can, however, also restore cell size asexually by cell enlargement (Gallagher, 1983). When conditions become unfavourable for growth,

S. marinoi can induce a resting stage. These can remain viable in the sediment for decades (Härnström *et al.*, 2011).

Vegetative cells of
Skeletonema marinoi

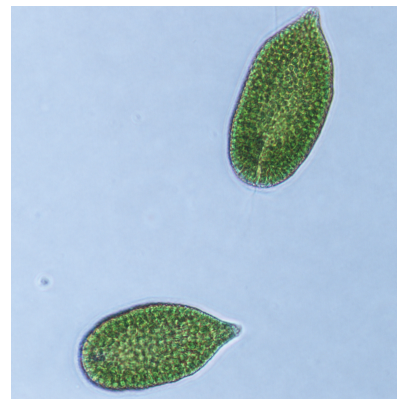


Box 3: *Gonyostomum semen* (Ehrenberg)

G. semen is a freshwater raphidophyte. The vegetative cells are large (32-92 μm), and free swimming cells (Figueroa & Rengefors, 2006). Using the two flagella, located in the apical pit, they can migrate vertically in the water column (Eloranta & Raike, 1995). The cells have mucous threads and trichocysts, which in contact to skin can cause irritation (Sorenson, 1954, Lepisto *et al.*, 1994). Blooms of *G. semen* are therefore considered nuisance blooms and can constitute up to 97% of the phytoplankton community (Lebret *et al.*, 2012).

During the bloom season (summer) they proliferate by cell division, and towards the end of the bloom they undergo sexual reproduction and form resting cysts that sink to the sediment (Figueroa & Rengefors, 2006).

Vegetative cells of *Gonyostomum semen*
Photo taken by Ingrid Sassenhagen



Assessing physical dispersal barriers

Oceanographic connectivity and gene flow at small spatial scales

Unpublished. Not available electronically.

Box 4: Microsatellites

Microsatellite loci are non-coding fragments of repetitive nucleotide sequences in the DNA. Typically, regions of DNA containing repetitions are highly susceptible to replication slippage, which may result in a deletion or insertion of a repeat sequence. Consequently, microsatellites are highly variable in their length and are hence suitable markers for DNA fingerprinting and population genetics.

5' flanking region microsatellite locus 3' flanking region

...CGTAGCCTTGCATCCTT**TATATATATATATA**AATCGGTACTACGTGG...(46 bp)

...CGTAGCCTTGCATCCTT**TATATATATATATATATA**TTCGGTACTACGTGG...(50 bp)

Using primers that attach to the specific flanking regions the microsatellites are amplified with polymerase chain reaction (PCR). The fluorescent-labeled fragments are then run through an electric field and separated according to length by capillary electrophoresis.

Spatial and temporal characterisation of the Baltic Sea spring bloom

Unpublished. Not available electronically.

Hydrological connectivity and gene flow in a limnic microalga

The lakes in northern Europe have within recent years become increasingly populated by the freshwater raphidophyte *Gonyostomum semen*, both in terms of biomass and also geographic expansion (Lepistö, *et al.*, 1994, Rengefors, *et al.*, 2012). In **Paper III**, we investigated the role of connecting rivers in mediating gene flow between populations of *G. semen*. Specifically, we hypothesised that populations of *G. semen* occurring in lakes belonging to different watersheds (i.e. not connected by rivers) would exhibit reduced gene flow compared with populations belonging to the same watershed. Using Amplified Fragment Length Polymorphism (AFLP) markers we genotyped 109 strains originating from six lakes, situated in two bordering watersheds. The distances between lakes varied from only 6 km up to 80 km, which is comparable to distances between sites in **Paper I**. Similar to **Paper I**,

we found evidence for significantly reduced gene flow between lakes, and population differentiation could not be corroborated by connectivity. Instead, there was a weak signal of IBD (Figure 3). This was surprising as an earlier large scale study on *G. semen*, by Le Bret, *et al.* (2013) that incorporated a large area of northern Europe did not find an effect of geographic distance on genetic differentiation among populations. In the case of the cladocera *Daphnia ambigua* populations, models accounting for varying hindrance to dispersal in waterways could effectively better explain patterns of gene flow (Michels, *et al.*, 2001). In **Paper III** we did not account for variation of hindrance in waterways with the motivation that the vegetative cells of *G. semen* are very fragile and are therefore less likely to survive transportation through rapidly moving water, compared to zooplankton. In contrast to the marine environment, lakes are isolated systems and as rivers seem of little importance for gene flow, we suggest that dispersal by vectors is more important at this range. The transportation of freshwater organisms with vectors such as insects and birds has long been recognised (Darwin, 1878). In **Paper III**, we propose that infrequent long distance transportation with e.g. birds has facilitated the geographic expansion of *G. semen*. Intermittent introductions to new lakes have then possibly resulted in several source populations dispersed randomly throughout northern Europe. From these, more frequent transportation between adjacent lakes has resulted in small-scale isolation-by-distance patterns. A drastic decline in population size or colonization by only a few individuals, as would be assumed with dispersal of this type, is referred to as a genetic bottleneck. Range expansion and dispersal, such as we propose, will undoubtedly reveal a signal of bottlenecks in the genetic data. Indeed, our data in **Paper III** supported this hypothesis.

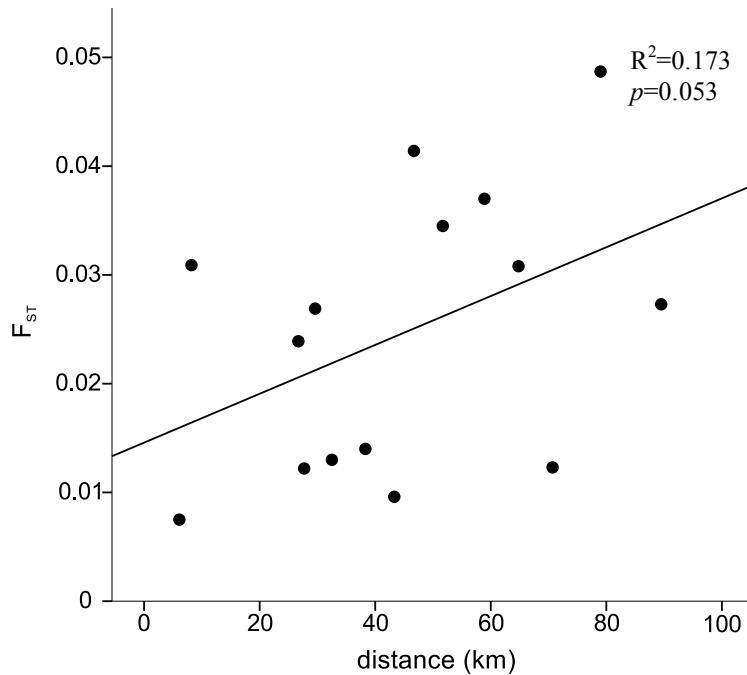


Figure 3. From **Paper III**. Pairwise geographic distances (km) between lakes, versus genetic differentiation (F_{ST}).

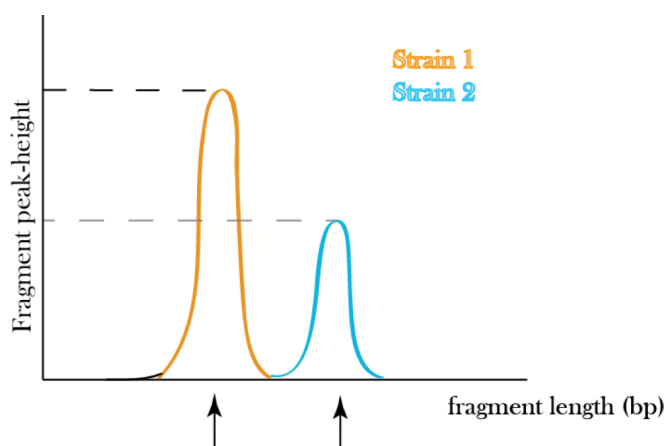
Assessing biological dispersal barriers

Priority effects

Priority effect is the advantage acquired by an organism through its prior arrival to a vacant source of resource (Connell & Slatyer, 1977) Through priority effects, a competitive advantage is gained by allowing the organism with earlier arrival to exploit resources and space before other organisms arrive. This concept has been studied rigorously in the context of interspecific interactions in community assembly (Robinson & Dickerson, 1987, Fukami, *et al.*, 2005, Louette & De Meester, 2007), biological invasions (Case, 1990, Case, 1991, Wainwright, *et al.*, 2012), and restoration ecology (Mergeay, *et al.*, 2011, Martin & Wilsey, 2012, Martin & Wilsey, 2014). Only a limited number of studies have investigated the importance of priority effects at the intraspecific level, e.g. in amphibians, fish and cyanobacteria (Blaustein & Margalit, 1996, Eitam, *et al.*, 2005, Geange & Stier, 2009, van Gremberghe, *et al.*, 2009). In **Paper IV** we designed a microcosm experiment to investigate whether priority effect is an important biological dispersal barrier between planktonic protist populations, as suggested in the monopolization hypothesis (De Meester, *et al.*, 2002). The current imbalance between studies on inter- rather than intraspecific competition is most likely due to the technical issue of differentiating between conspecific individuals, rather than lack of ecological importance. In **Paper IV** we tested, and applied, a molecular method (**Box 5**) for reliably quantifying different strains of

Box 5: Allele-specific quantitative PCR

The respective fragment peak-heights from the two strains were used as a relative quantification measurement. To establish that PCR amplification was not biased toward one strain, we mixed strain pairs in eight known proportions (established by cell counts) and carried out DNA extraction. After PCR amplification, peak-height ratios from the electropherograms (schematic drawing below) were compared to the known admixed proportions. Three different microsatellite markers were tested for unbiased PCR reaction.



S. marinoi when growing together. To test priority effects, we sequentially inoculated two strains into a bottle and let them grow until early stationary phase. Using AsQ-PCR (**Box 2**) we could assess the relative proportion of each strain, i.e. the one that arrived first versus the strain that arrived later.

Different mechanisms have been proposed for earlier documentation of intraspecific priority effects. In the case of the fire salamander (*Salamandra salamandra*), priority effect was explained as a result of more matured early cohort gaining a competitive advantage of size (Eitam, *et al.*, 2005). Geange and Stier (2009) suggested that the priority effects displayed in the coral-reef wrasse (*Thalassom hardwickle*) was due to more experienced prior residents that had better knowledge of the local environment. The competitive advantage demonstrated through sequential arrival of the cyanobacterium *Microcystis* may have been due to an advantage in numbers (van Gremberghe, *et al.*, 2009). Density-dependent priority effects have been argued to play a key role in the structuring of biodiversity (Waters, *et al.*, 2013), and neglecting its importance may cause misinterpretations of species connectivity (Fraser, *et al.*, 2014). In **Paper IV** we omitted the advantage of numbers by adding

the later arriving strain in equal quantity to the existing quantity of the first arriving strain.

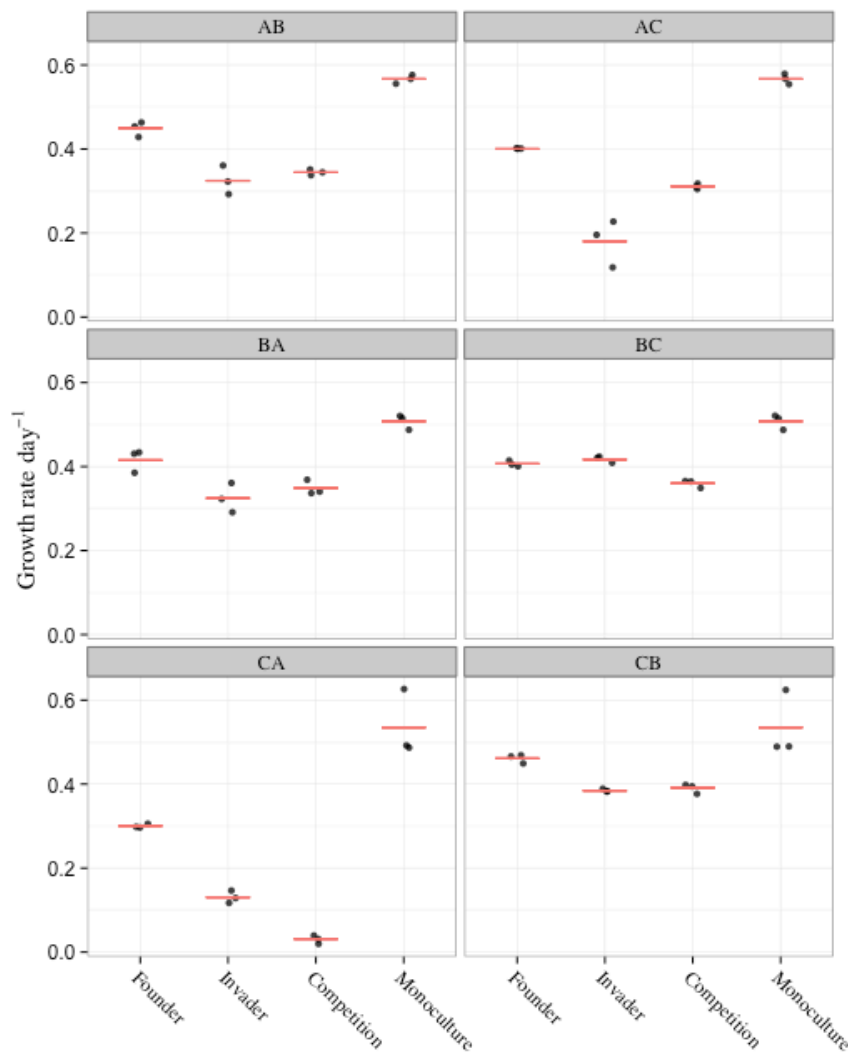


Figure 4. Strain-specific growth rates in monoculture and each experiment in **Paper IV**. The first letter in each header (XX) indicates the measured strain ID and the second letter (XX) indicates the competing strain ID. Raw data are illustrated as dots and the averages as red lines.

Even without an advantage of numbers, we saw an effect of priority whereby 50% of the tested strains increased their biomass significantly when compared with simultaneous arrival. In addition we could show a deceleration in strain specific growth rates when growing together, while arriving first had lessened the negative effect on growth rate (Figure 4). Based on our results we propose in **Paper IV** that prior arrival of a set of genotypes increases their competitive ability and thus restricts establishment of later-arriving genotypes and consequently reduces gene flow.

Local adaptation – a competitive advantage for native populations

Unpublished. Not available electronically .

The dispersal of bloom-forming microalgae

Importance and function of physical and biological dispersal barriers

The assumption that microorganisms have unlimited dispersal has mainly been motivated by their physical characteristics (i.e. small cell size and large populations). And although dispersal and transportation of these organisms is greatly facilitated by connectivity (**Paper II**) (Casabianca, *et al.*, 2012, Godhe, *et al.*, 2013, Sjöqvist, *et al.*, 2015), or birds and insects (**Paper III**) as proposed by Finlay (2002), it does not necessarily translate into sufficient gene flow to allow for homogenization of populations. In this thesis I present results and evidence for reduced gene flow at small geographical scales and at resolutions rarely considered for microevolutionary processes in microalgae. In addition I have found confirmation of additional ‘biological’ mechanisms that may be equally important at driving population genetic differentiation in microalgae. Telford, *et al.* (2006) advocated that despite the enormous dispersal potential of microscopic organisms, it is still too slow at a global scale to override regional metacommunity processes. Based on these findings I propose that dispersal is too slow also at regional scales to override physical and biological barriers.

In the discussion of whether or not biogeography is a relevant concept for microorganisms, i.e. if there are subdivisions and endemic populations, much focus has previously been put on the physical aspects of dispersal and testing IBD and connectivity. These neutral drivers of population divergence can collectively be termed isolation-by-dispersal-limitation (IBDL) (Orsini, *et al.*, 2013). When comparing the studies in **Paper I** and **Paper II** we see that IBDL is a suitable predictor of gene flow across larger distances in marine systems (**Paper II**), but that they become less important or evident over smaller regions (**Paper I**). The signal of reduced gene flow despite an absence of IBDL at smaller spatial scales, suggests influence of one or more biological barrier(s). Such biological barriers may be e.g. inter- and intraspecific competition, phenotypic traits and adaptation through selection or reproductive strategies. Studies on biological dispersal barriers in marine protists are greatly underrepresented in the literature, with the exception of interspecific competition, in comparison to physical dispersal barriers. In this thesis we provide support for two mechanisms whereby intraspecific competition is significantly affected and can potentially restrict gene flow. Ultimately, it is important to remember

that dispersal can be greatly overestimated when the biological characteristics of a species and historical events are neglected (Fukami, *et al.*, 2007).

An organism's ability to disperse may also vary throughout the different life-cycle stages (Okolodkov, 2005). In microalgae, the dormant stages and cysts are generally more resistant to harsh environmental conditions than vegetative cells; therefore their potential to survive long distance dispersal is higher than for vegetative cells (Anderson, 1989, Matsuoka & Fukuyo, 2000). This may be especially true for *G. semen*, since the vegetative cells are extremely fragile. However, instead of being a vehicle for dispersal, the majority of dormant cells will presumably sink to the sediments and later feed the subsequent bloom, thereby anchoring the population within an area. This function of seed banks has been documented for *Skeletonema marinoi*, where dormant stages in the sediment have maintained population stability for thousands of generations (Härnström, *et al.*, 2011). Thus the arrival of a limited number of propagules, as indicated by the signal of genetic bottlenecks in *G. semen* (**Paper III**), to an already occupied lake is likely to be out-numbered and/or out-competed by the resident population anchored in the sediment. Numerous obstacles influence the establishment success of immigrants as they must first overcome the colonization phase, and if ecological factors allow, they can then grow and contribute to the population and community (Smayda, 2002). Populations of *G. semen* show little signs of local adaptation and instead exhibit a high degree of plasticity (Sassenhagen, *et al.*, 2015). Since this species is under current expansion (Lepistö, *et al.*, 1994, Rengefors, *et al.*, 2012), plasticity is probably therefor a favoured trait. Genetic divergence that is established during colonization may initially be sustained over shorter periods of time through priority effects. Competitive exclusion brought on priority effects will, however, over time be subject to decay as new migrants arrive and if dispersal is sufficiently high it will even counterbalance divergence (Hubert, *et al.*, 2015). As indicated in **Paper III**, the rate of dispersal in *G. semen* is however probably too slow to counterbalance divergence. Thus, rather than a locally adapted population facilitating competitive exclusion it may be that their rapid generation time relative to dispersal rate is instead maintaining population differentiation (Meutter, *et al.*, 2007) and reduced gene flow in this species.

In comparison to the relatively isolated systems of lakes, *Skeletonema marinoi* reside in a continuous aqueous environment and hence dispersal between sites is presumably more constant. Nevertheless, as seen in **Paper IV** founder strains of *S.*

marinoi can become dominant with only a few days prior arrival. It is expected that this advantage be further amplified by their rapid growth rate. As founder genotypes exhaust resources, conditions become suboptimal for invading genotypes, and therefore less likely to properly establish (De Meester, *et al.*, 2002, Okamura & Freeland, 2002). In addition, the close coupling of sediment seed banks and the pelagic population documented in *S. marinoi* (Godhe & Härnström, 2010, Härnström, *et al.*, 2011), implies that even short range dispersal is too slow to override recruitment from the seed banks and e.g. priority effects. The importance of sediment seed banks is suggested as an explanatory factor for the population genetic patterns seen in **Paper I**, but was emphasized in **Paper II**, where it was indicated that the spring bloom developed in shallow coastal areas. Over time founder events and priority effects may be enhanced by adaptation to local environmental conditions resulting in monopolization (De Meester, 1996, De Meester, *et al.*, 2002). Contrary to *Gonyostomum semen*, *S. marinoi* demonstrates signs of local adaptation (**Paper II**, Sildever *et al.*, unpublished) and that native populations have an associated competitive advantage in their home conditions (**Paper V**, Sildever *et al.*, unpublished), thus having the potential to monopolize resources (De Meester, *et al.*, 2002, Orsini, *et al.*, 2013).

Conclusion and future directions

The aim of my thesis was to investigate and test physical and biological dispersal barriers that may influence microevolutionary processes, and consequently the potential for adaptation and geographic expansion in planktonic microalgae. The results presented in my thesis provide strong support for that not only physical barriers, such as isolation-by-distance and connectivity, are important drivers of population genetic differentiation but also biological barriers. In *Skeletonema marinoi* we found support for both priority effects as well as competitively superior local populations, which together indicate the potential for monopolization. In *Gonyostomum semen*, genetic data support persistent founder events, which may be sustained by infrequent dispersal by vectors combined with fast propagation rates and local seed banks. These barriers to gene flow are likely non-exclusive but evident at different spatial scales.

Based on our findings we see that biological barriers to gene flow can be equally important in driving microevolutionary processes. One such barrier, which has been mentioned but not addressed, is reproductive strategies. Gene flow is ultimately the result of recombination, and amongst microalgae, reproductive strategies and frequency of sexual events can vary greatly. Yet, little is still known about e.g. frequency of sexual recombination, what induces sex in different species or the degree of selfing. Consequently, even less is known about how reproduction influences gene flow between populations of the same species.

My contribution to the papers

Paper I: Small-scale population differentiation in a planktonic bloom-forming diatom.

KR, AG and AK conceived the study. AG, AK and JS designed the experiment. AK organised the field work. Field work was carried out by JS, AG and AK. JS isolated, cultured and extracted DNA from strains. Modelling was done by PJ. Molecular work and analysis was performed by JS. AG, AK, KR, PJ and JS helped with data interpretation. JS wrote the manuscript with comments from AG, KR, AK and PJ.

Paper II: Physical barriers and environmental selection cause spatial and temporal genetic differentiation of an extensive algal bloom.

Note: There are 19 co-authors on this manuscript and therefor I will only state my contribution to this work.

I helped with data collection in the field, isolation and culturing of algal strains. I was also responsible for logistical organisation between labs, data sorting, data interpretation and commenting on manuscript.

Paper III: Freshwater protists do not go with the flow: Population structure in *Gonyostomum semen* independent of connectivity among lakes.

KR and AG conceived the study. KR, AG and IS designed experiment. IS organised the fieldwork, JS participated in sampling, and JS, AG, IS and KR participated in isolation of algae. IS performed molecular work. IS and TS analysed the data, and JS, AG and KR helped with data interpretation. IS wrote the manuscript with comments from JS, TS, AG and KR.

Paper IV: Priority effects in a planktonic bloom-forming marine diatom.

AG and KR conceived the study. AG, KR and JS designed the experiment. JS carried out experiment and method development as well as analysed the data. IS, KR and AG contributed to data interpretation. JS wrote the manuscript with comments from AG, IS and KR.

Paper V: Competitive exclusion through salinity adaptation in a common marine diatom.

AG and KR conceived the study. AG, AK and KR designed the study with contributions from JS. Competition experiments were carried out by RP, and acclimatisation and growth rates were performed by JS. JS was responsible for data analysis. Data interpretation was done by JS, AG, KR and AK with contribution from KJ. The manuscript was written by JS and commented on by AG, AK, KJ, RP and KR.

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