Evolution of the brown algae *Fucus radicans* and *F. vesiculosus* in the Baltic Sea

Doctoral Thesis

Daniel Johansson

Department of Biological and Environmental Sciences
University of Gothenburg

Gothenburg, Sweden

2013

UNIVERSITY OF GOTHENBURG

Abstract

The aim of my thesis was to investigate neutral and adaptive evolution of a lineage of macroalga (*Fucus vesiculosus*) that entered the Baltic Sea about 6000 years ago, and later gave rise to a new endemic species, *F. radicans*. To examine whether the two species have adapted to local conditions of the Baltic Sea, or if they were able to establish due to largely plastic phenotypes, we performed a reciprocal transplant experiment with *F. vesiculosus* from the North Sea, *F. radicans*, and *F. vesiculosus* from the Baltic Sea. The results show that both species have adapted to the brackish water conditions by evolving asexual reproduction and faster growth in low compared to high salinity. Noticeably, *F. radicans* was more extremely adapted than the Baltic Sea population of *F. vesiculosus*, which may be due to its endemism and lack of gene flow from populations outside the Baltic Sea. A population genetic survey unveiled that sexual and asexual reproduction are present in Baltic Sea populations of both species, although asexual reproduction is more common in *F. radicans* than in *F. vesiculosus*. We also found a complex population genetic pattern with partly geographic segregation of sexual and asexual reproduction. Furthermore, in *F. radicans*, a few clones were widespread and dominant, while most clones were rare and confined to single sites. Using a modelling approach we tested if such a complex population genetic structure can be the result of stochastic processes, as opposed to natural selection. We found that long-range dispersal in combination with asexual reproduction being successful also in the absence of other individuals, may result in the observed patterns of distribution of clones without invoking fitnesses-differences among clones. To test the alternative hypothesis, if the dominance of one clone may be due to differences in performance among clones, we compared three clones, and found inherited differences in traits that may affect fitness. The most widespread clone grew significantly faster than one of the other two clones, but did not show the highest capacity for asexual reproduction and thus did not show evidence of an overall higher fitness. Notably groups of individuals of the most widespread clone sampled in geographically separated areas, showed large differences in potential for sexual reproduction. We concluded that there were no clear evidence for selection among clones and can therefore not reject a neutral explanation of the observed complex pattern. Finally, in an attempt to assess population aspects of the importance of interclonal variation we compared the performance of three monoclonal groups and a group with unique genotypes.c We found variation in resistance to various stresses to be larger in the group of unique genotypes compared to the monoclonal groups. This suggests that genotypic diversity of a local population may affect its function and resilience. In this way, genotypic diversity may be critical for the adaptation to expected further changes in the Baltic Sea, although stochastic processes seems to have had major impact in structuring the early phase of establishment in the Baltic Sea.

Keywords: local adaptation, Baltic Sea, asexual reproduction, marginal environment

Populärvetenskaplig sammanfattning *Swedish summary*

För att arter ska kunna etablera sig i nya miljöer krävs att de kan anpassa sig till nya förhållanden. Detta kan antingen ske genom en förmåga att justera fysiologiska processer till den nya miljön (fenotypisk plasticitet) eller genom naturligt urval av nya egenskaper. En kombination av båda är också möjlig. För att arter ska kunna utveckla nya anpassningar behövs genetisk variation (dvs att alla individer inte har exakt samma ärftliga egenskaper) och naturligt urval som väljer bland dessa egenskaper. Målet med min avhandling var att undersöka om blåstång, när den etablerade sig i Östersjön efter senaste istiden för ungefär 6000 år sedan, anpassade sig till brackvattenmiljön. Blåstången avknoppade också en ny art, smaltång, vilken är unik till Östersjön. De båda arterna utgör en viktig livsmiljö för andra arter i Östersjön. Framtida förväntade miljöförändringar, som för Östersjön innebär en lägre salthalt, gör det viktigt att öka kunskapen om dessa arters förmåga att anpassa sig till nya förhållanden. I **papper I** undersökte vi huruvida de båda arterna har anpassat sig genom evolution till de extrema miljöförhållandena i Östersjön, eller om de etablerade sig genom en förmåga att justera sin fysiologi. Vi utförde ett experiment där blåstång från svenska västkusten och de båda arterna från Östersjön utsattes för hög och låg salthalt. Resultaten visade att de båda arterna har anpassat sig till förhållandena i Östersjön genom en högre tillväxt i låg salthalt och en förmåga till asexuell reproduktion, vilket saknas hos blåstång från västkusten. Smaltång hade en kraftigare anpassning, vilket kan bero på att den är isolerad till Östersjön och därför inte påverkas av individer utan lokal anpassning. En populationsgenetisk studie i **papper II** visade att båda arterna reproducerar sig sexuellt och asexuellt i Östersjön men att asexuell reproduktion var vanligast hos smaltång. Dessutom fann vi en komplex populationsgenetisk struktur hos smaltång, där några populationer dominerades av sexuell reproduktion, medan andra var nästan helt asexuella. Vidare så hade en klon av smaltång en vidsträckt utbredning och dominerade några populationer, medan de flesta klonerna representerades av få individer och bara återfanns på enskilda lokaler. För att undersöka om ett sådant komplext mönster kan ha uppstått utan skillnader mellan kloner så utvecklade vi en neutral modell i **papper III**. I modellen antog vi att olika kloner hade samma egenskaper vad avser överlevnad och spridning. Genom slump-processer, i samband med långväga spridning och överlevnad, kunde vår modell reproducera ett komplext mönster av kloners utbredning, likt det vi observerat hos smaltång. I **papper IV** undersökte vi den alternativa hypotesen: skillnader mellan kloner påverkar deras överlevnad och förökning, och därmed deras utbredning. Genom att i en kontrollerad miljö jämföra egenskaperna hos den dominanta klonen med två kloner med mer begränsad utbredning fann vi ärftliga skillnader mellan kloner, men att den mest utbredda klonen inte konsekvent hade bäst egenskaper, varför vi inte kunde förkasta att slumpen haft en stor roll i klonernas utbredning. Slutligen, i **papper V** undersökte vi hur bestånd påvkeras av klonsammansättningen. Vi fann att beståndets variation i egenskaper var högre inom en grupp med unika individer än inom grupper bestående av samma klon, vilket kan förväntas ha en betydelse för smaltångens framtida utveckling i Östersjön om miljö förändras ytterligare. Sammantaget så visar våra studier att de båda arterna har anpassat sig till Östersjön, men att speciellt för smaltången, slumpmässiga skeenden kan ha haft stor betydelse för utbredningen av de olika klonerna.

List of papers

This thesis is based on the following papers:

Paper I: Johansson D, Pereyra RT, Johannesson K (manuscript) Local adaptation of fucoid macroalgae to a young marginal environment

Paper II: Johannesson K, Johansson D, Larsson KH, Huenchuñir CJ, Perus J, Forslund H, Kautsky L, Pereyra RT (2011) Frequent clonality in fucoids (*Fucus radicans* and *Fucus vesiculosus*; Fucales, Phaeophyceae) in the Baltic Sea. Journal of Phycology 47:990-998

Paper III: Kleinhans D, Johansson D, Sundqvist L, Pereyra RT, Jonsson PR, Johannesson K (manuscript) A neutral model can explain geographic patterns of sexual and asexual individuals

Paper IV: Johansson D, Pereyra RT, Johannesson K (manuscript) The potential for inter- and intraclonal selection in the macroalga *Fucus radicans*; and on the occurrence of a fit or lucky megaclone

Paper V: Johannesson K, Forslund H, Capetillo NÅ, Kautsky L, Johansson D, Pereyra RT, Råberg S (2012) Phenotypic variation in sexually and asexually recruited individuals of the Baltic Sea endemic macroalga *Fucus radicans*: in the field and after growth in a common-garden. BMC Ecology 12

Table of contents

Introduction

Establishment in marginal environments

Species are usually bound to specific geographical areas or certain types of environments, where they can successfully survive and reproduce. For species to establish in a marginal environment, outside their optimal range of conditions, requires either a capacity of the individual organisms to adjust their phenotype during ontogeny (phenotypic plasticity), or to evolve adaptations to the novel conditions, or a combination of both (Kawecki 2008). Marginal environments can either be permanently dependent on immigration from core areas of species' distribution, or can over time achieve local adaptation, maintaining demographically independent populations (Kawecki 2008). For initial establishment in a young marginal environment, phenotypic plasticity is likely to be beneficial (West Eberhard 2005), however, plasticity is considered to come with potentially high costs (Pigliucci 2001), and may therefore not be a longterm optimal strategy. Studying the colonisation of marginal environments can provide us with important knowledge about what capacity species have to adapt to changing environments – which is urgent knowledge for our understanding of the potential of adaptation to environments under global change. Furthermore, we can study the rate of adaptation as well as the mechanisms involved both at the ecological and genetic levels.

This thesis deals with a lineage of a common macroalga (*Fucus vesiculosus*) that recently (in an evolutionary perspective) established in a marine postglacial environment (the Baltic Sea). This environment is both geographically peripheral and ecologically marginal with, most importantly, a salinity that is constantly much lower than in the surrounding marine environments (Zillén et al. 2008). The establishment of populations of marine species in the Baltic Sea some 6500 years ago (Björck 1995) have been accompanied by marked genetic changes, observed by population genetic analyses (Johannesson & André 2006). However, these differences may either reflect local adaptation or neutral differences caused by demographic isolation and bottlenecks, or possibly both. In this thesis we take a broader approach, combining population genetic analyses with quantitative genetic approaches to elucidate the role of neutral and adaptive drivers shaping the lineages of *F. vesiculosus* that entered the Baltic Sea some 6000 years ago.

The study organisms

Fucus vesiculosus is a brown macroalga found in the Atlantic Ocean and the North Sea. It also established in the Baltic Sea around 6000 years ago, shortly after the formation of this marginal marine environment. Only a few thousand years ago, the endemic *F. radicans* formed from *F. vesiculosus* inside the Baltic Sea (Pereyra et al. 2009). Both are foundation species providing an important habitat for associated organisms (Kautsky et al. 1992, Råberg & Kautsky 2007, Wikström & Kautsky 2007). *Fucus vesiculosus* can be found on hard bottom substratum throughout the

Baltic Sea, except in the northernmost part (the Bay of Bothnia), while *F. radicans* is restricted to the northern Baltic Sea (the Gulf of Bothnia, except the Bay of Bothnia), and is also found in two Estonian sites (Johannesson et al. 2011). A suggested occurrence of *F. radicans* in the Gulf of Finland is not yet confirmed. This means that the two species have a largely overlapping distribution in the northern Baltic Sea (Gulf of Bothnia and Estonia).

In the Baltic Sea, the two species reproduces both sexually and asexually. Male and female reproductive organs are on separate individuals. Sexual reproduction is by means of synchronized release of gamets to the surrounding waters (Fig. 1) from late spring to autumn, and peaks at calm conditions and full moon. Since gametes are negatively buoyant, reproduction is mainly local within a few meters from the mother plant (Serrão et al. 1997), why gene flow is restricted (Tatarenkov et al. 2007). Sexual reproduction is partially inhibited in the low salinity waters of the northern Baltic Sea (Serrão et al. 1996). Asexual reproduction is accomplished by the production of adventitious branches formed from the thallus, which come of and reattach to the bottom through the formation of rhizods (Fig. 1, Tatarenkov et al. 2005). Although adventitious branches are formed also by *F. vesiculosus* outside the Baltic Sea, as a response to tissue damage inflicted by grazers (as has been shown in other fucoid algae; van Alstyne 1989), asexual reproduction has not been observed in these populations (Tatarenkov et al. 2005, Johannesson et al. 2011). Importantly, asexually recruited individuals produce gamets and are sexually functional (Lena Kautsky, pers. comm.).
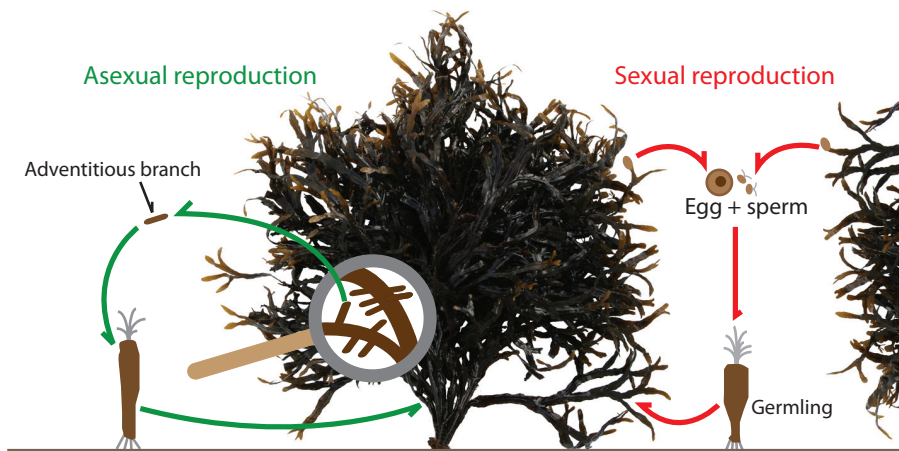
Figure 1. Life-cycle of *F. radicans*. Left hand side: asexual reproduction is by detached adventitious branches that reattach with rhizoids. Right hand side: sexual reproduction by release of gamets. Individuals are either females or males – sperms and eggs are from different individuals.

The Baltic Sea

The Baltic Sea is a postglacial semi-enclosed brackish-water sea that formed only around 8500 years ago (Zillén et al. 2008). The Baltic went through a series of stages with different salinities, starting to acquire its present day salinity only 3000 years ago. Todays' Baltic Sea has a stable salinity gradient ranging from 2 practical salinity units (psu) in the north to 20-25 psu at the transition to the North Sea. Most marine species colonised during the more saline Littorina period, 6000-4500 years ago. Although the Baltic Sea has been colonised by species of both freshwater and marine origin, it remains an ecosystem with low species diversity of most groups of organisms (Ojaveer et al. 2010). For example, of the North Atlantic macroalgae species, only around one fifth are found in the Baltic Sea (Wallentinus 1991, Nielsen et al. 1995). Most species show a steep cline in genetic variation or differentiation at the entrance to the Baltic, indicating either local adaptation or limited gene flow, or both (Johannesson & André 2006).

The Baltic fucoid system offers unique opportunities to study rapid evolution of local adaptation and asexual reproduction, the latter being a trait unique for the Baltic Sea (Tatarenkov et al. 2005). The recent origin of the Baltic Sea makes it a particularly interesting system for studies of evolutionary processes, which gives us good opportunities to study ongoing adaptation. Given the expected decrease in salinity and increase in temperature due to anthropogenically induced environmental changes (Meier 2006), there is an urgent need to understand the potential for rapid adaptation in Baltic Sea organisms in general. For the fucoid species studied in this thesis, it seems as if the distribution of both species is at risk. The situation is especially critical for *F. radicans* because of its endemic status – with an obvious risk of extinction if not able to adapt to the future Baltic Sea environment. Needless to mention, a loss of these habitat-forming species would substantially affect the Baltic Sea ecosystem.

Local adaptation to the Baltic Sea

To investigate the extent of local adaptation in the two species we conducted a reciprocal transplant experiment with *F. vesiculosus* from the North Sea and *F. vesiculosus* and *F. radicans* from a sympatric site (Fig. 2) in the the Baltic Sea (**Paper I**). Adventitious branches from thalli of all three populations were grown in high (24 psu) and low (4 psu) salinity, representing the two sampling sites. After six months we measured fitness-related characters such as growth and capacity for asexual reproduction (formation of adventitious branches and rhizoids for attachment of adventitious branches), as well as capacity for sexual reproduction (formation of receptacles containing sexual organs).

Adventitious branches of all three populations grew faster in their native salinity – *F. vesiculosus* from the west coast grew more in high than in low salinity, while both species sampled in the Baltic Sea grew faster in the low salinity compared to the high salinity (Fig. 3A). Furthermore, in the Baltic Sea, both species

have evolved a capacity for asexual reproduction by formation of adventitious branches (Fig. 3B) and rhizoids, while *F. vesiculosus* from the west coast did not form rhizoids in our experimental conditions (Fig. 3C). Notably, *F. radicans* had the highest capacity to form adventitious branches. Only *F. vesiculosus* from the west coast formed receptacles, and this only in the high salinity. Taken together, our results showed that both species sampled in the Baltic Sea have evolved local adaptations to the novel conditions of the Baltic Sea, and that *F. radicans* seems to be the more extremely adapted species.

Since the Baltic Sea is a relatively young habitat, which formed only some 8500 years ago (Zillén et al 2008), and where *F. vesiculosus* established only 6000 years ago and *F. radicans* formed as late as a few thousand years ago, our conclusion is that the adaptations we observe have arisen relatively rapidly, including the formation of a new species (Pereyra et al. 2009). In the next paragraph we discuss if the difference in capacity for asexual reproduction seen in **paper I** is in concordance with field observations of distribution of asexual reproduction in the two species.
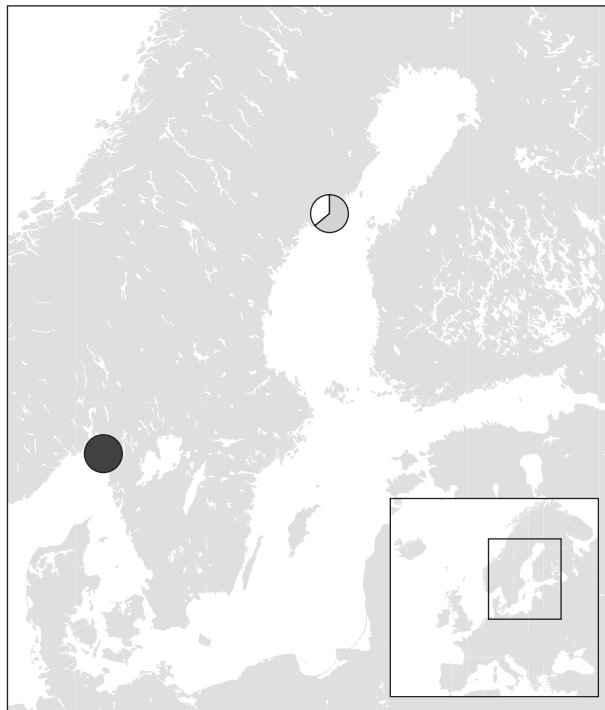
Figure 2. Map showing sampling sites of *F. vesiculosus* in the North Sea (dark grey circle) as well as *F. radicans* and *F. vesiculosus* in the Baltic Sea (circle with light grey and white segments, where the size of the segments indicates the proportion of *F. radicans* and *F. vesiculosus*, respectively, in the sample). Insert map shows Europe.
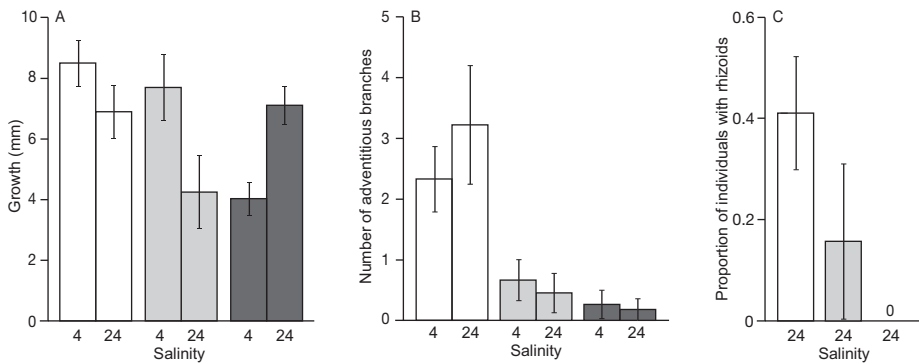
Figure 3. Results from the reciprocal transplant experiment. White bars, *F. radicans*; light-grey bars, *F. vesiculosus* from the Baltic Sea; dark-grey bars, *F. vesiculosus* from the North Sea. (A) Growth of adventitious branches. (B) Number of new adventitious branches formed from adventitious branches. (C) Proportion of individuals that formed rhizoids. Lack of rhizoid formation in *F. vesiculosus* from the North Sea is indicated by a zero. Error bars show exact 95% CI.

Geographic patterns of clonal distribution

It has been known for some time that in addition to sexual reproduction (which is the norm in fucoid species) both *F. radicans* and *F. vesiculosus* in the Baltic Sea may in addition reproduce asexually (Tatarenkov et al. 2005). However, the occurrence of clonality in different parts of the Baltic Sea, and the geographic distribution of single clones, were not earlier investigated. As part of this thesis we performed a study to both describe the occurrence and distribution of clones, and to examine the effects of clonality on genetic variation (**paper II**). Using microsatellites as genetic markers, we genotyped samples of *F. radicans* from ten sites and of *F. vesiculosus* from seven Baltic Sea sites, and from one sample of *F. vesiculosus* from the Swedish west coast. For each sample we estimated genetic variation (e.g. allelic richness) and clonal richness.

Clonality was more common in *F. radicans* compared to *F. vesiculosus*, and tended to increase northwards in both species, although clonality sometimes varied extensively among nearby sites (Fig. 4). In *F. radicans* many of the populations were dominated by asexual reproduction, and while most clones were local (only found in one site) and present in small numbers, one female clone was very widespread and dominated several of the populations along 550 km coastline. However, some populations were instead completely sexual, but these populations were only found in the south east area of the distribution (Estonia). No clones were detected in *F. vesiculosus* from outside the Baltic Sea, and in light of the results of **paper I** this is probably due to that these populations do not have the capacity to form rhizoids from adventitious branches.

It seems to be a general trend that clonality is more common inside the
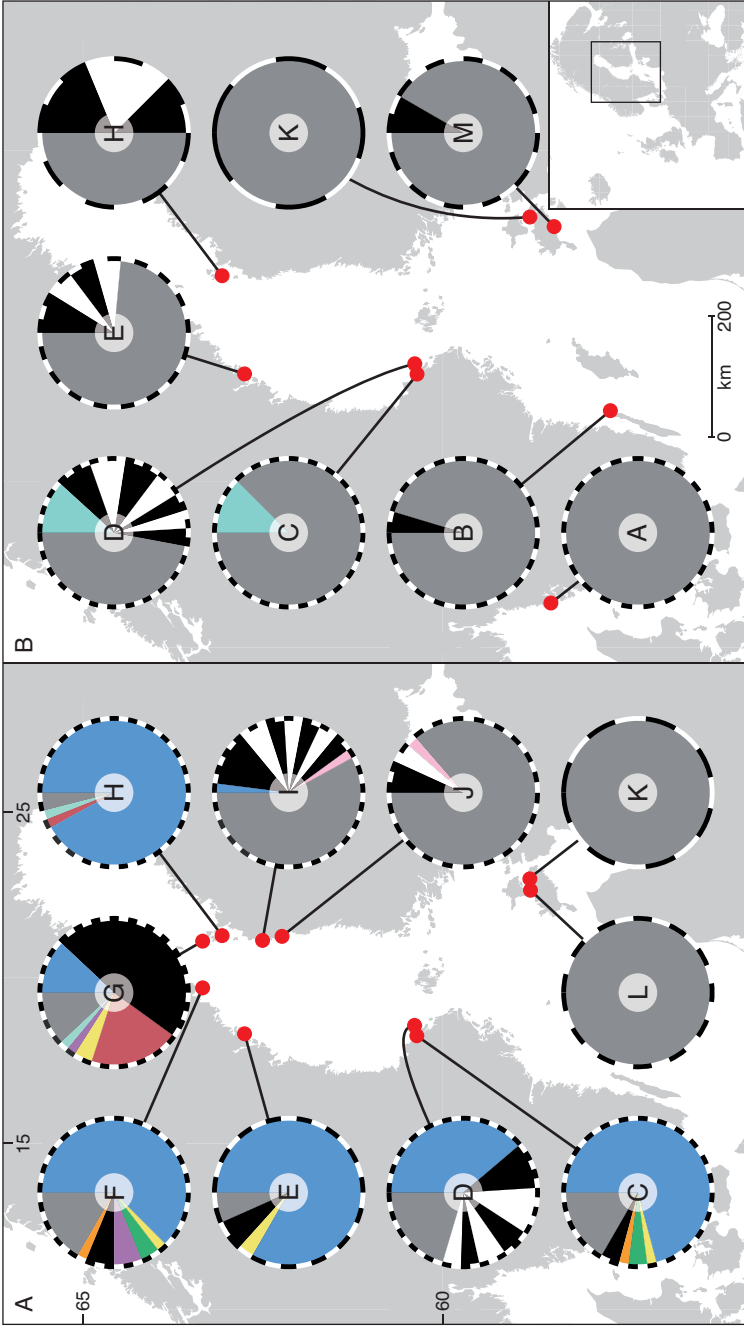
Figure 4. Distribution of unique and multiple (clonal) multilocus genotypes (MLGs) over populations of *F. radicans* (A) and *F. vesiculosus* (B) in the Baltic Sea. Grey segments indicate total proportion of unique MLGs in each site, black and white segments show MLGs found in two or more copies (clones) but unique to the particular site and coloured segments show MLGs distributed over more than one site. Sample sizes (number of individuals analysed) are indicated by black and white frames.

6

Baltic Sea (Hällefors 1981), with two other species being particularly well-documented; *Zostera marina* and *Ceramium tenuicorne* (Reusch et al. 2000, Gabrielsen et al. 2002, Bergström et al. 2003). This fits conclusions from other areas that clonality increases towards marginal areas of species' distributions (Eckert 2001, Kearney et al. 2006, Silvertown 2008), a phenomenon sometimes referred to as geographic parthenogenesis (Hörandl 2006). However, the extensive distribution and numerical dominance of a female clone of *F. radicans* is intriguing, and suggests, in the first place, that this clone is old. Old clones have also been found in *Z. marina* in the Baltic Sea (Reusch et al. 2000). Notably, these observations contrasts with what is known for terrestrial flowering plants where clones are usually not old and widespread (Ellstrand & Roose 1987, Wilk et al. 2009). One hypothesis to explain the observation of few old and widespread clones in terrestrial plants is the accumulation of deleterious mutations (mutational meltdown, Lynch & Gabriel 1990).

Even though it has been suggested that asexual reproduction in itself does not result in a loss of genetic variation, but that this is set by the effective population size – which is not expected to decrease in populations dominated by asexual reproduction (Balloux et al. 2003, Bengtsson 2003, but see Orive 1993) – we found evidence that genetic variation decreases as clonality increases in *F. radicans*. One explanation for this may be that dominance of one clone contributes a disproportionately high amount of gamets, and hence decrease the effective population size. A complex genetic pattern may also arise as a consequence of local adaptation and selection among clones, which we examined in **paper IV**. However, complex patterns as the one observed in *F. radicans* could also arise as a consequence of stochastic processes during establishment, without selection among clones, as discussed in the next paragraph.

A neutral model may explain clonal distribution

To follow up on the study of clone distribution, and the observations made in *F. radicans* in particular (**paper II**), we used a modelling approach to test if these intriguingly complex patterns of clonal distribution, may be the result of stochastic processes during establishment of this young species, without the need to invoke selection among clones.

Our model, where all individuals were allowed to reproduce both sexually and asexually, did not involve adaptation, and genetic differences were assumed to be neutral. Therefore, there was no difference between clones in dispersal and survival capacity, or in capacity to produce sexual or asexual recruits. The model allowed occasional long-range relocation of individuals, and while colonisation of empty sites were only possible by asexual reproduction due to allee effects, recruitment by sexual reproduction could only take place when there were individuals of opposite sex in the neighbouring area. We ran the model both with and without a fixed rate of stochastic extinction.

The modelling results reproduced core features of the patterns we had earlier observed in *F. radicans*, such as a few clones that were widespread while a larger number of clones were local, as well as populations dominated by asexual reproduction while others were completely sexual (Fig. 5). This without invoking fitness differences between clones. Indeed, most earlier attempts to explain similar patterns with geographic differences in the frequency of sexually and asexually reproducing individuals (geographic parthenogenesis) have most often suggested a selective advantage of asexual reproduction in marginal habitats (Kawecki 2008). In contrast, our model suggests that patterns as the one observed in *F. radicans* may instead be a consequence of the interplay between stochastic long-distance transport of individuals and the obvious advantage of asexual reproduction in an earlier empty habitat. Since in *F. radicans*, as in many other organisms, sexual reproduction is a local process, then if a relocated individual, arriving alone at a distant empty site, can reproduce asexually, asexual reproduction can outperform sexual reproduction in the short term and result in genetic patterns depending on random effects of dispersal. However, with a finite extinction rate, sexual reproduction is able to outcompete asexual reproduction in the long run, if it is less costly and therefore more efficient. However, an alternative explanation to the observed patterns in *F. radicans* is that there are differences between clones in fitness-related traits, resulting in selection among clones, which we discuss in the next paragraph.

The alternative explanation: interclonal selection

Although the distribution of clones are usually restricted to one or a few nearby sites while widespread clones are rare (Ellstrand & Roose 1987), a few species contrast in having a few widespread and dominant clones (Kliber & Eckert 2005, Darling et al. 2009, Pfeiffer et al. 2012). Our modelling approach in **paper III** showed that such complex patterns of clonal distribution, where most clones are local and present in small numbers while a few are widespread and dominant, may arise as a consequence of stochastic processes during colonisation, without invoking selection among clones. In **paper IV** we tested the alternative hypothesis: there is competition among clones, where the most widespread clone in *F. radicans*, present over 550 km of coast line, is expected to be the one with the highest overall fitness. Including this particular clone, we investigated the potential for interclonal selection by comparing fitness-related traits among individuals of the widespread clone with that of individuals of two other clones with more restricted distributions, sampled in the northern Baltic Sea (Fig. 6).

Already from comparing phenotypes of the three clones sampled at a sympatric site (and thus grown in a similar natural environment) we found differences in size distributions, while the three clones largely overlapped in shape variation (Fig. 7). However, these results were somewhat inconclusive, as differences may emerge from microhabitat differences rather than inherited differences. Thus, in
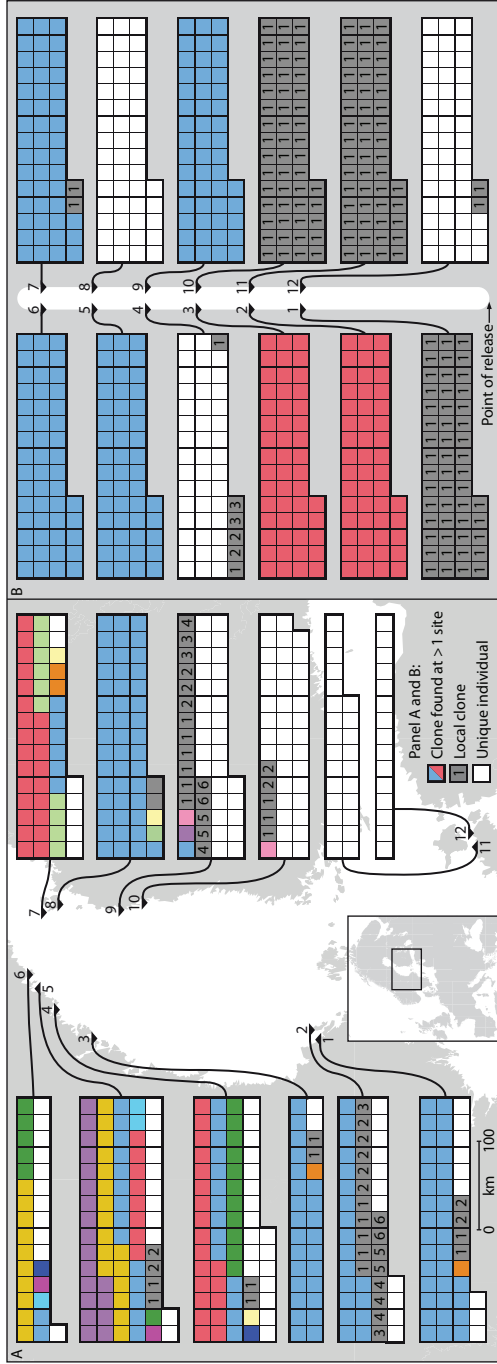
Figure 5. (A) Distribution of clones of *F. radicans* sampled in the Baltic Sea. (B) Representative realisation of our simulations in an artificial circular habitat. Each square box in the respective populations refers to an individual. Boxes remain white if the individuals are unique. Clones encountered at several sampling locations are indicated by a unique colour. Clones unique to respective sampling locations are coloured grey and numbered, with each number referring to a different clone.

9

Figure 6. Map showing sampling sites in the Baltic Sea in northern Europe. The three light grey circles show sampling sites of the widespread clone. The circle with white, light grey and dark grey segments show sampling site of the three clones at a sympatric site, where the size of the segments indicates the proportion of each clone in the sample, and the light grey segment represents the widespread clone.

a next step we incubated adventitious branches from thalli of the three clones in common gardens at two different salinities (4 and 24 psu). After several months of growth under the same conditions we compared growth of new tissue and characters related to asexual reproduction (formation of adventitious branches and rhizoids), to assess the presence of inherited phenotypic differences that may provide a potential for interclonal selection.

We found inherited differences among clones of *F. radicans* in fitness-related traits. For example, the widespread clone grew significantly more than one of the other clones, while not faster than the third clone (Fig. 8A). A faster growth could potentially explain a clone's dominance, since it may confer advantages such as larger surface to form adventitious branches from, ability to outcompete slower-growing clones or other organisms, and to more efficiently compensate for grazing damage. However, the widespread clone did not produce more adventitious branches than the other two clones; on the contrary, it was one of the other clones that were more successful in this respect (Fig. 8B). These results suggests that there is potential for selection among clones that may affect their distribution and frequency. However, we did not find any specific evidence for the widespread clone being at a general advantage to the more local clones with respect to these
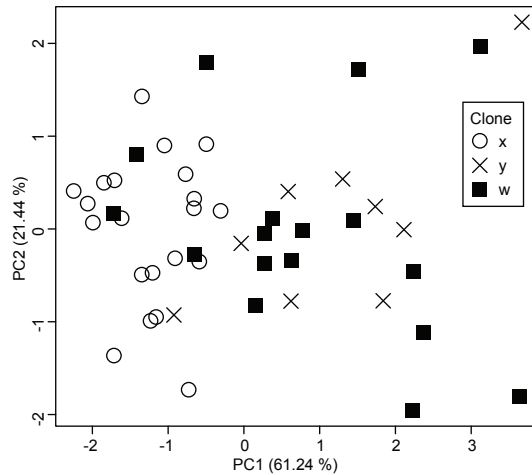
Figure 7. Principal components analysis of morphometric data from the three clones sampled at a sympatric site.

studied traits. Differences between clones were also found in **paper V**, discussed in more detail later, but also these differences indicated inherited differences in traits that may be under natural selection. Previous studies have also found different clones sampled at the same location to show potential for interclonal selection, at least under experimental conditions (Pistevos et al. 2011, Kremp et al. 2012, Lohbeck et al. 2012, McLeod et al. 2012, Schwarzenberger et al. 2012). Selection has also been suggested to be involved in cases when one clone is dominant over a large geographic area, as for example in the aquatic plant *Butomus umbellatus* (Kliber & Eckert 2005). However, despite evidence for inherited differences in many trait that may affect fitness, there is as yet no experimental data showing a positive relationship between fitness and geographic distribution; that is, the most widely distributed clones also being those showing superior fitnesses in key traits. We can therefore not at this stage reject the neutral hypothesis (**paper III**) as an explanation to the distribution of different clones of *F. radicans*.

However, a clone could also become widespread by adapting to local conditions through somatic mutations that may accumulate, specifically in old and large clones (Whitham & Slobodchikoff 1981), providing intraclonal variation. Reusch and Boström (2011), for example, found somatic mutations to be present in half of the investigated clones of *Zostera marina* in the Baltic Sea. However, as variation in neutral genetic markers cannot be directly translated into variation in phenotypic traits, we took another approach and instead assessed degree of inherited variation directly on the level of the phenotype, comparing phenotypic traits among groups of the widespread clone sampled at geographically distant sites (Fig. 6). We thus performed additional common garden tests, this time using four different salinities (2, 4, 5.5 and 24 psu), representing both extreme and
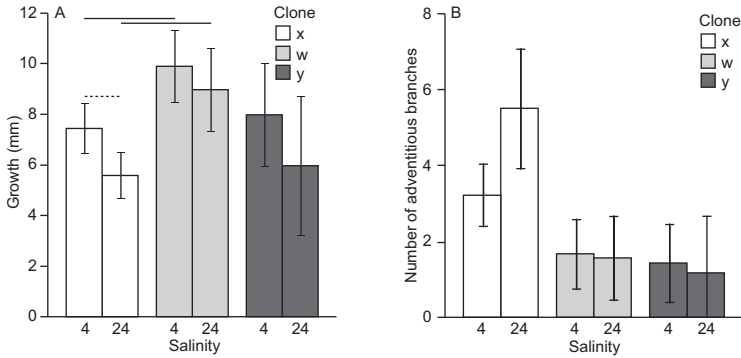
Figure 8. (A) Growth of adventitious branches of three different clones from Skagsudde. Horizontal bars indicate pairwise comparisons, significant at an alpha-level of 0.05 after Holm adjustment. The dashed horizontal bar indicates a marginally significant comparison (p=0.052). Error bars show exact 95% CI. (B) Number of secondary adventitious branches formed by the three clones from Skagsudde. Error bars show exact 95% CI.

normal salinities of *F. radicans*. Among three groups of the widespread clone, one grew less than the other two, while another developed considerably more receptacles than the other two groups (Fig. 9). This suggests inherited differences among groups of the same clone which are most likely due to somatic mutations. With respect to formation of receptacles, the differences were remarkable and may perhaps suggest mutations in control sites rather than in the genes directly responsible for the trait. Although again, our results provide a potential for inter-clonal selection, we have no direct evidence for these traits being in any way correlated with the distribution of the different groups of clones. Indeed, differences in receptacle formation was observed only in a salinity much higher than what is found in the Baltic Sea.
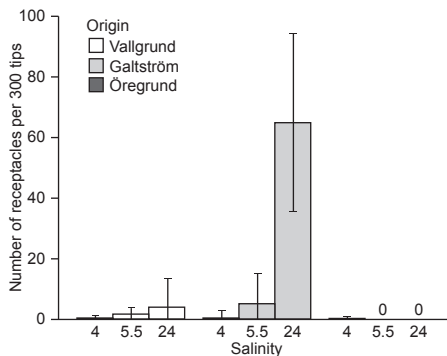
Figure 9. Number of receptacles per 300 tips, formed by the widespread clone sampled at three different sites. Error bars show exact 95% CI.

In conclusion, although we found evidence for inherited differences among different clones in some traits, and among geographically distant groups of the same clone, differences did not correlate with distribution success, and thus we cannot reject the alternative hypothesis of a completely neutral mechanism as an explanation of the complex distribution of clones observed in *F. radicans*.

Ecosystem effects of clonality

In ecosystems dominated by one or a few foundation species, phenotypic diversity within the foundation species may be more important than species diversity, and is likely to affect function and resilience of the ecosystem (Reusch et al. 2005). It has been shown experimentally that increased genotype diversity of plants has positive effects on for example primary production, carbon storage, nutritional load of surrounding soils and diversity of associated species (Bailey 2011). Furthermore, experimentally manipulated seagrass meadows with high genotypic diversity have been shown to better resist grazing, grazing from specific grazers, extreme conditions, and have higher productivity and biomass of associated species than meadows with low genotypic diversity (Hughes & Stachowicz 2004, Reusch et al. 2005, Hughes et al. 2010). The aim of **paper V** was to investigate whether phenotypic diversity within *F. radicans* can be explained by genetic variation. This is important knowledge since populations of *F. radicans* dominated by one or a few clones would than be expected to show lower capacity with respect to ecosystem function and resilience compared to populations of high genotypic diversity (that is, composed of many different clones).

In the lab, we created four groups, where three groups were monoclonal (each group with a different clone), while the fourth group consisted of a mix of unique genotypes. We compared the performance of all the individuals of these groups with respect to nine phenotypic traits, where some trais were assessed directly after forming the groups, while other traits were assessed after some time in lab culture (in a common garden). We measured two morphological traits (thallus width and distance between dichotomies), phlorotannin concentration, and palatability to grazers shortly after sampling. Of these characters we found significant differences among genotypes only in phlorotannin concentration (Fig. 10A), indicating that variation in this trait is at least partly inherited. This has been shown to be the case also for the sister species *F. vesiculosus* (Jormalainen & Honkanen 2004, Jormalainen & Ramsay 2009). While in brown macroalga, including *F. vesiculosus*, phlorotannin concentrations are to some extent also induced by UV light and grazing (Peckol et al. 1996, Pavia et al. 1997, Pavia & Toth 2000).

After treatment in a common garden we measured growth and photochemical yield under normal conditions, photochemical yield and water content after desiccation and photochemical yield after freezing. Of these characters, photochemical yield after freezing and photochemical yield after desiccation showed differences among genotypes that were highly significant and nearly significant,

respectively (Fig. 10B-C).

In the measurements done immediately after collection of the individuals in the field, the differences in traits found among clones may be due to genetic differences but also to clones being non-randomly distributed over different environments that may have affected the phenotypic traits, and thus these results are somewhat inconclusive. In contrast, the measurements done after cultivation in a
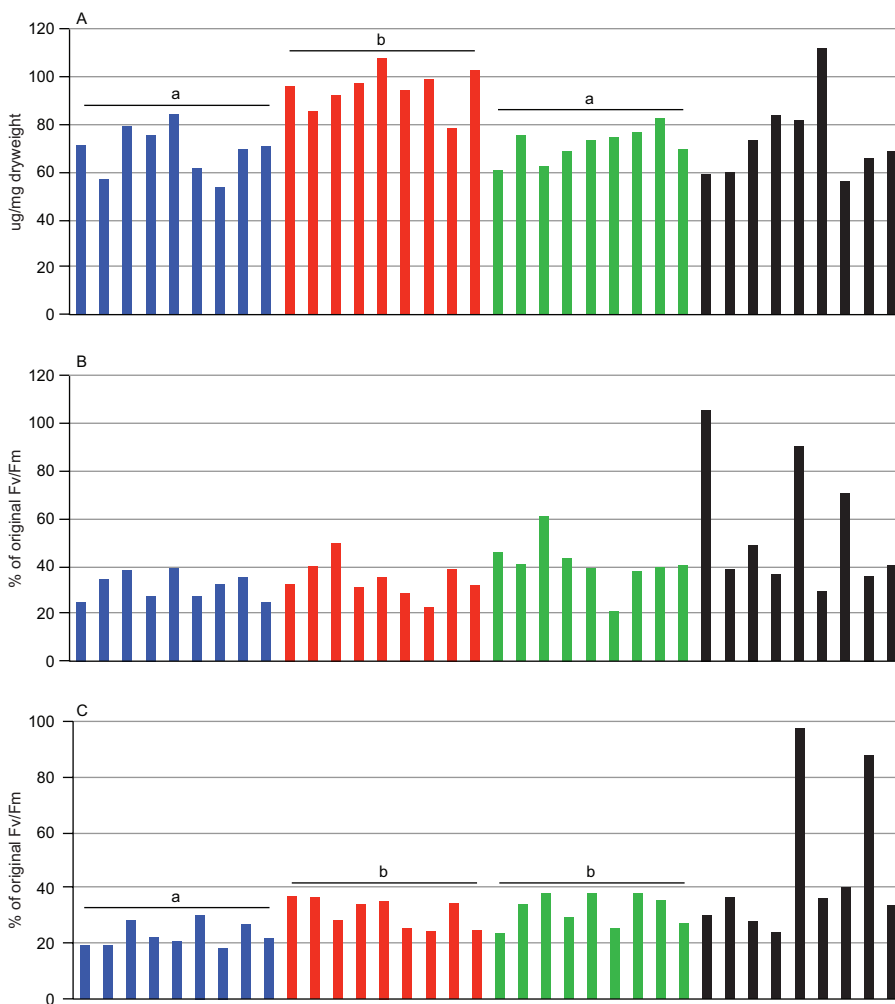
Figure 10. Trait values for traits that showed significant inherited variation. Values for each individual thalli are indicated (separate bars). The coloured bars represent individuals from the three monoclonal groups, while black bars represent unique individuals. Letters above groups indicates significant comparisons between clones. (A) Phlorotannins. (B) Photochemical yield after desiccation and recovery. (C) Photochemical yield after freezing and recovery.

common garden on newly formed tissue, are most likely to reflect differences that were to a large extent genetically determined.

The variation in the same three traits within the groups of unique genotypes was significantly larger compared to within the monoclonal groups, which further supports our conclusion that variation in these traits are largely inherited. Moreover, it suggests that the higher phenotypic variation in groups of unique individuals, compared to the monoclonal groups, there will also be a difference in terms of ecosystem function and resilience between high and low clonality groups. High clonality likely has negative effects on ecosystem function, as has been shown in stands of *Zostera marina* (Reusch et al. 2005, Ehlers et al. 2008, Hughes & Stachowicz 2009). Populations of *F. radicans* dominated by one, or a few clones, may also be more vulnerable to expected environmental changes of the Baltic Sea (Meier 2006).

Conclusions and future prospects

Our results show that, even though the Baltic Sea lineage of *F. vesiculosus* established relatively recently, *F. radicans* and Baltic Sea populations of *F. vesiculosus* have adapted to the local conditions. Furthermore, the results strongly suggest that *F. radicans* is even more adapted to the Baltic Sea than the population of *F. vesiculosus*. A major, and indeed rather unique adaptation among the group of fucoid species in general, is that both species have evolved a capacity to reproduce asexually, and again *F. radicans* being more extreme in this respect than *F. vesiculosus*. Asexual reproduction is likely an adaptation to the low salinity waters of the Baltic Sea, where sexual reproduction is partly inhibited. Another key adaptation is that both species grow better in low salinity compared to high. Thus, there seems to be clear evidence for local adaptation to the Baltic Sea, in general, in these two species. On the other hand, the complex distribution of clones within *F. radicans,* including the occurrence of one clone which is extremely widespread and dominant, seems not easily explained by a selection-based hypothesis. Although we found some evidence of inherited differences among clones, suggesting possibilities for interclonal selection, we did not find the widespread clone to be outstanding in fitness compared to other clones. Instead, our modelling approach showed that the observed pattern may indeed arise as a consequence of stochastic processes during establishment, without invoking selection among clones. Finally, we showed that asexual reproduction resulting in low genotypic diversity decreases phenotypic variation, which might decrease ecosystem services and resilience. Therefore, anticipated environmenal changes (Meier 2006) will likely have more negative consequences in populations dominated by one or a few clones, than in populations of high clonal richness. Even though the Baltic lineages have been able to adapt to the marginal conditions to some extent, future environmental change is expected to occur over shorter time scales. The question is then, will the Baltic populations of fucoids be able to adapt fast enough to withstand even

more extreme conditions? If not, their disappearance will likely have ecosystem consequences, as they constitute a habitat for many species. Therefore, more research is needed to investigate effect of future scenarios on the fucoids, and what can be done to facilitate their continued existence.

This thesis has contributed with pioneering data for an evolutionary lineage and a new endemic species of key importance to the Baltic Sea. Future research initiatives will need to further address both the role of local adaptation of these macroalgae to the extreme conditions of the Baltic Sea, the role of stochastic processes shaping population genetic structures, and how the existing genetic patterns will be able to cope with future environmental changes. For example, there is a need to understand the genetic mechanisms behind the evolved adaptations, and the role of unique genetic elements in the evolution of adaptation to even more extreme conditions than in today's Baltic Sea environment.

References

Bailey JK (2011) From genes to ecosystems: a genetic basis to ecosystem services. Popul Ecol 53:47-52

Balloux F, Lehmann L, de Meeus T (2003) The population genetics of clonal and partially clonal diploids. Genetics 164:1635-1644

Bengtsson BO (2003) Genetic variation in organisms with sexual and asexual reproduction. J Evolution Biol 16:189-199

Bergström L, Bruno E, Eklund B, Kautsky L (2003) Reproductive strategies of *Ceramium tenuicorne* near its inner limit in the brackish Baltic Sea. Bot Mar 46:125-131

Björck S (1995) A review of the history of the Baltic Sea, 13.0-8.0 ka BP. Quatern Int 27:19-40.

Darling JA, Kuenzi A, Reitzel AM (2009) Human-mediated transport determines the non-native distribution of the anemone *Nematostella vectensis*, a dispersal-limited estuarine invertebrate. Mar Ecol Prog Ser 380:137-146

Eckert CG (2001) The loss of sex in clonal plants. Evol Ecol 15:501-520

Ehlers A, Worm B, Reusch TBH (2008) Importance of genetic diversity in eelgrass *Zostera marina* for its resilience to global warming. Mar Ecol Prog Ser 355:1-7

Ellstrand NC, Roose ML (1987) Patterns of genotypic diversity in clonal plant-species. Am J Bot 74:123-131

Gabrielsen TM, Brochmann C, Rueness J (2002) The Baltic Sea as a model system for studying postglacial colonization and ecological differentiation, exemplified by the red alga *Ceramium tenuicorne*. Mol Ecol 11:2083-2095

Hughes AR, Best RJ, Stachowicz JJ (2010) Genotypic diversity and grazer identity interactively influence seagrass and grazer biomass. Mar Ecol Prog Ser 403:43-51

Hughes AR, Stachowicz JJ (2004) Genetic diversity enhances the resistance of a

seagrass ecosystem to disturbance. P Natl Acad Sci USA 101:8998-9002

Hughes AR, Stachowicz JJ (2009) Ecological impacts of genotypic diversity in the clonal seagrass *Zostera marina*. Ecology 90:1412-1419

Hörandl E (2006) The complex causality of geographical parthenogenesis. New Phytol 171:525-538

Johannesson K, Johansson D, Larsson KH, Huenchuñir CJ, Perus J, Forslund H, Kautsky L, Pereyra RT (2011) Frequent clonality in fucoids (*Fucus radicans* and *Fucus vesiculosus*; Fucales, Phaeophyceae) in the Baltic Sea. J Phycol 47:990-998

Jormalainen V, Honkanen T (2004) Variation in natural selection for growth and phlorotannins in the brown alga *Fucus vesiculosus*. J Evolution Biol 17:807-820

Jormalainen V, Ramsay T (2009) Resistance of the brown alga *Fucus vesiculosus* to herbivory. Oikos 118:713-722

Kawecki TJ (2008) Adaptation to marginal habitats. Annual Review of Ecology, Evolution, and Systematics 39:321-342

Kautsky H, Kautsky L, Kautsky N, Kautsky U, Lindblad C (1992) Aspects on *Fucus vesiculosus* in the Baltic Sea. Acta Phytogeo Suec 78:33-48

Kearney M, Blacket MJ, Strasburg JL, Moritz C (2006) Waves of parthenogenesis in the desert: evidence for the parallel loss of sex in a grasshopper and a gecko from Australia. Mol Ecol 15:1743-1748

Kliber A, Eckert CG (2005) Interaction between founder effect and selection during biological invasion in an aquatic plant. Evolution 59:1900-1913

Kremp A, Godhe A, Egardt J, Dupont S, Suikkanen S, Casabianca S, Penna A (2012) Intraspecific variability in the response of bloom-forming marine microalgae to changed climate conditions. Ecol Evol 2:1195-1207

Lohbeck KT, Riebesell U, Reusch TBH (2012) Adaptive evolution of a key phytoplankton species to ocean acidification. Nat Geosci 5:346-351

Lynch M, Gabriel W (1990) Mutation load and the survival of small populations. Evolution 44:1725-1737

McLeod KA, Scascitelli M, Vellend M (2012) Detecting small-scale genotype-environment interactions in apomictic dandelion (*Taraxacum officinale*) populations. J Evolution Biol 25:1667-1675

Meier HEM (2006) Baltic Sea climate in the late twenty-first century: a dynamical downscaling approach using two global models and two emission scenarios. Clim Dynam 27:39-68

Nielsen R, Kristiansen A, Mathiesen L, Mathiesen H (1995) Distributional index of the benthic macroalgae of the Baltic Sea area. Acta Bot Fenn 155:1–51

Orive ME (1993) Effective population-size in organisms with complex life-histories. Theor Popul Biol 44:316-340

Pavia H, Cervin G, Lindgren A, Åberg P (1997) Effects of UV-B radiation and

simulated herbivory on phlorotannins in the brown alga *Ascophyllum nodosum*. Mar Ecol Prog Ser 157:139-146

Peckol P, Krane JM, Yates JL (1996) Interactive effects of inducible defense and resource availability on phlorotannins in the North Atlantic brown alga *Fucus vesiculosus*. Mar Ecol Prog Ser 138:209-217

Pereyra RT, Bergström L, Kautsky L, Johannesson K (2009) Rapid speciation in a newly opened postglacial marine environment, the Baltic Sea. BMC Evol Biol 9

Pfeiffer T, Klahr A, Peterson A, Levichev IG, Schnittler M (2012) No sex at all? Extremely low genetic diversity in *Gagea spathacea* (Liliaceae) across Europe. Flora 207:372-378

Pigliucci M (2001) Phenotypic plasticity: beyond nature and nurture. Johns Hopkins Univ. Press, Baltimore, MD

Pistevos JCA, Calosi P, Widdicombe S, Bishop JDD (2011) Will variation among genetic individuals influence species responses to global climate change? Oikos 120:675-689

Reusch TBH, Boström C (2011) Widespread genetic mosaicism in the marine angiosperm *Zostera marina* is correlated with clonal reproduction. Evol Ecol 25:899-913

Reusch TBH, Ehlers A, Hämmerli A, Worm B (2005) Ecosystem recovery after climatic extremes enhanced by genotypic diversity. P Natl Acad Sci USA 102:2826-2831

Reusch TBH, Stam WT, Olsen JL (2000) A microsatellite-based estimation of clonal diversity and population subdivision in *Zostera marina*, a marine flowering plant. Mol Ecol 9:127-140

Råberg S, Kautsky L (2007) A comparative biodiversity study of the associated fauna of perennial fucoids and filamentous algae. Estuar Coast Shelf S 73:249-258

Schwarzenberger A, Kuster CJ, Elert E (2012) Molecular mechanisms of tolerance to cyanobacterial protease inhibitors revealed by clonal differences in *Daphnia magna*. Mol Ecol 21:4898-4911

Serrão EA, Kautsky L, Lifvergren T, Brawley SH (1997) Gamete dispersal and pre-recruitment mortality in baltic *Fucus vesiculosus*. Phycologia 36(Suppl.):101-102

Serrão EA, Kautsky L, Brawley SH (1996) Distributional success of the marine seaweed *Fucus vesiculosus* L in the brackish Baltic Sea correlates with osmotic capabilities of Baltic gametes. Oecologia 107:1-12

Silvertown J (2008) The evolutionary maintenance of sexual reproduction: Evidence from the ecological distribution of asexual reproduction in clonal plants. Int J Plant Sci 169:157-168

Toth GB, Pavia H (2000) Water-borne cues induce chemical defense in a marine alga (*Ascophyllum nodosum*). P Natl Acad Sci USA 97:14418-14420

Tatarenkov A, Bergström L, Jonsson RB, Serrão EA, Kautsky L, Johannesson K (2005) Intriguing asexual life in marginal populations of the brown seaweed *Fucus vesiculosus*. Mol Ecol 14:647-651

Wallentinus I (1991) The Baltic Sea gradient. In: Mathieson AC, Nienhuis PH (eds) Ecosystems of the World 24 Intertidal and Littoral Ecosystems. Elsevier, Amsterdam

Whitham TG, Slobodchikoff CN (1981) Evolution by individuals, plant-herbivore interactions, and mosaics of genetic-variability - the adaptive significance of somatic mutations in plants. Oecologia 49:287-292

Wikström SA, Kautsky L (2007) Structure and diversity of invertebrate communities in the presence and absence of canopy-forming *Fucus vesiculosus* in the Baltic Sea. Estuar Coast Shelf S 72:168-176

Wilk JA, Kramer AT, Ashley MV (2009) High variation in clonal vs. sexual reproduction in populations of the wild strawberry, *Fragaria virginiana* (Rosaceae). Ann Bot-London 104:1413-1419

Zillén L, Conley DJ, Andren T, Andren E, Björck S (2008) Past occurrences of hypoxia in the Baltic Sea and the role of climate variability, environmental change and human impact. Earth-Sci Rev 91:77-92

Acknowledgements

further. Good luck Lisa!

Thank you **Per** for your commitment as my examiner. Thanks to the **members of the BaltGene** project (Baltic Sea Genetic Biodiversity) for interesting meetings. Thanks also to the **CeMEB community** (The Linnaeus Centre for Marine Evolutionary Biology) for a creative platform. Thanks to **Karin**, director of GENECO (Graduate Research School in Genomic Ecology) and, moreover, my opponent, for a fantastic forum. Also, thanks to **Catarina**, at the MiL Institute, for the mentor program within GENECO, providing an excellent platform for development. Thanks to **Wolfgang** who, as an external mentor through GENECO, widened and challenged my thinking. For advise on statistical analyses I would like to thank **Swantje**, **Gerry**, **Joacim**, **Per J**, **Jon**, **Mats**, **Per B** and **Bo**. Thanks **Marina** for interesting discussions and support when we taught population genetics. Thanks **Eva Marie**, for arranging gatherings and spreading joy. **Inger W**, thanks for suggesting the title used for this thesis! Thanks **Martin** for changing water of the experiments! **Håkan**, at the marine pilot station Skagsudde, greatly facilitated sampling of algae by taking our divers to the site and waiting there with the boat(!) – Tack Håkan! He and his colleague also invited me to stay in the pilot station for the night when I sampled barnacles some years earlier.

Thanks **everyone at the lab** for fika-pauses, knitting-evenings, ragattas, lifewest-storage-room-parties, Inre Vattenholmen-ball, rubrikscube-party, sauna bathing, football-playing and lots of other fun stuff! Thanks also to **Conny** and the **Lovén Centre staff** in the administration, workshop and house keepers for keeping the lab functional and shiny, providing fika and delicious lunches and **Gunilla** for arranging with housing! Special thanks to **Conny** and **Thomas** for helping me repair the rudder!

I will be eternally grateful for the hospitality that **Barbro**, **Sven-Gustav** and my lovely cousins **Sofia**, **Lisa** and **Sara** showed when opening their home for me during my first time of marine biology studies in Gothenburg as well as for the wonderful childhood summers at the west coast, from where I got my fascination for the oceans.

Martin and **Sandra**, thanks for being there although I've spent the last years on a remote island + tack också lilla **Ebba** och **Lisa** för att ni är härliga på ett sätt som bara små barn kan vara! Kram!

Lastly, I thank my **parents** for their support, which has been really important, not only during my PhD, but throughout my upbringing. Spending time in nature as a child led to my fascination for the diversity of life. Tack **Pappa** och **Filomena**! Tack **Mamma** och **Bengt**!

Oh, did I forget someone? ;) All the fascinating friends I've met at Tjärnö and other places means a lot to me! **Elin**! Thanks for being such a good friend and office-mate! Good luck with your thesis! **Swantje**! Thanks for all the adventures, I hope there are many more to come. Very much looking forward to exploring the creations of Slartibartfast with you! I promise not to bring the GPS this time ;) Kram! **Susanna**, thanks for all the kayaking and for all the delicious dinners! **Mårten**, thanks for all the interesting discussions! Thanks **Emma**! **Eva-Lotta**! **Kniven**! **Angelica**!

Thomas! **Laura** – thanks for making the sailing trips so much fun! Hope you'll do your MSc in Gothenburg! Anyhow, I think we might be in the wrong dimension so have some sailing to do this summer! **Anne** – thanks for all the Carcassonne playing, and sorry for always winning ;) Thanks **Artiom** for being such an awesome guy and for talking me into going sailing in a strong gale! Although we broke, and almost lost, the rudder, in addition to capsizing in the big waves, it was totally worth it! That was the most exciting sailing I've experienced, flying on top of the waves! WOW! Lets do it again this summer! Thanks **Karl** for being such a nice guy and working with us in the Fucus-group for a while! **María** och **María**, thanks for all the fun and showing me how to prepare paella and tortilla! Besos! **Abby**, thanks for great company and for introducing me to some great bands! You're awesome! Your bf too! Thanks **Petri** for introducing me to climbing! **Geno**, thanks for taking the initiative for the sailing course at Ursholmen, which made start sailing, finally! Takk **Vigdís** fyrir ævintýri á Íslandi! Takk **Magnea**! Takk **Hildur**! **Per**, thanks for help with R and other stuff. Thanks **Josefin**, for being you! Thanks **Olof**! **Louise**! **Klara**! **Calle**, **Anna-Karin** and **Micke** – it was great fun teaching the marine conservation course with you as well as enjoying your company in the lab! The Ocean! **Zaira**! **Tobias**! **Maren**! **Chris** – thanks for all for the fun times on The island, Eh eh ehhhh. **Micke**! **Danne**! **Marie** och **Anders**! **Per**! **Christian**! **Gunilla**! **Ann**! **Tania** (jag skriver på svenska så att du får öva dig lite), tack för att du bjöd in mig till ditt hem i Las Palmas och för spännande äventyr runt ön, och så klart för en fantastisk tid på Tjärnö! Hoppas vi ses snart igen! Kraaam! **Andreas**! **Anders**! **Eric**, thanks for help in replacing the boom that we snapped in a gale! Haha, vilket äventyr! E**Lisa**beth! **Anna**! **Andrea**! **Elin P**! **Therese**! **Thomas** and **Felix** – your passion for food amazes and inspires me! **Ylva F**! **Ylva** och **Hanna**! Haha, you are so fun! The wind! **Ragnar**! **Finn**, thanks for teaching me bouldering! **Gregg**! **Stina** – good luck! **Joanna**, it was so much fun dancing with you! **Christine**! **Sara**! **Anna-Lisa**, thanks for the encouraging and wise words! **Neva**! **Hanna S**! **Hanna**! **Jimmy**! **Anna**! **Erika N**! **Erika A**! **Elin S**! **Charlotte**! **Mikael T**! Write your name here if forgotten:_____, thanks! Thanks everyone who came sailing, climbing, bouldering, kayaking, skating, skiing, hiking and made my time at the island, and occasionally outside, so much fun!!! Kram :)

PS since the first paper has to begin on recto, I'm gonna fill the following verso with pictures of some beautiful people and places!

Funding

I have too many nice pictures to choose from, so here comes some more.