



On the endemic *Fucus radicans*  
in the Baltic Sea

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*Till Lena  
Handledare, mentor,  
Förebild & vän*



# Abstract

The brown macroalgae *Fucus radicans* is endemic to the Baltic Sea, but little is known about this newly described species. This thesis investigates the ecology and role of *F. radicans* within the species poor Baltic Sea ecosystem. The thallus of *F. radicans* had a more complex structure but was smaller than *F. vesiculosus*, the other important foundation species with which it grows in sympatry at several sites. The variability of the associated flora and fauna communities of these two *Fucus* species, however, was explained by the thallus size, not the complexity. Comparisons between the populations of *F. radicans* in the Bothnian Sea with those in Väinameri Sea on the Estonian coast, showed that the Estonian thalli were smaller, less complex and lacking the numerous adventitious branches which occur extensively in the Bothnian Sea populations.

The distribution of *F. radicans* in Sweden is limited to the Bothnian Sea coast. The low salinity at the northern limit prevented successful fertilization, while increased salinity did not restrict *F. radicans* but improved its reproductive success. The southern distribution limit was instead shown to be negatively impacted by a combination of grazing and competition. The asexual reproduction through settling of detached fragments was favoured by high light levels and high temperature in laboratory conditions. Re-attachment occurred by basally formed rhizoids but settling also occurred through a calcium-rich substance, seemingly secreted by the fragment. Genetic spatial distribution of *F. radicans* showed a dominance of a few widespread clones both within and between sites with an intermingled rather than clustered pattern. The extensive female clone, common in most sites, is most likely old and several clonal lineages have derived from her. Although more clearly expressed in the clonal populations, the macroscopic sexual dimorphism discovered appears to be a species specific trait in *F. radicans*.

This thesis presents further insight in *F. radicans* role within the Baltic Sea ecosystem and its value as a study species for adaptation, clonality and speciation.

# Populärvetenskaplig sammanfattning

Blåstången i Östersjön kan uppträda i många olika former, allt ifrån stora meterhöga buskar med stora flytblåsor till små, nästan sytrådstunna tussar utan blåsor som flyter fritt. En dvärgform av blåstång med smal, rufsig bål, troligtvis orsakad av den låga salthalten, var typisk för Bottenhavet. Genetiska undersökningar visade dock att det inte var blåstång utan en egen art, som fick namnet smaltång. Då smaltång är en nyss upptäckt art, saknas kunskap om artens roll i Östersjöns ekosystem och vilka faktorer som påverkar artens utbredning. Detta är information som behövs för att kunna bedöma artens tillstånd och ta fram relevanta skötselplaner för det som kan vara den första unika arten för Östersjön. Smaltången har bildats från blåstången och detta tros ha skett relativt nyligen, kanske bara för några tusen år sedan. Det finns därför många likheter mellan dessa två arter, tidigare behandlade som en, vilket innebär att många jämförelser för att visa på likheter eller skillnader mellan smaltång och blåstång tas upp i mitt arbete.

Smaltång återfinns ofta tillsammans med blåstång på 2 – 8 meters djup. Både smaltång och blåstång är viktiga grundstenar i Östersjöns artfattiga ekosystem. De har ofta kallats havens skog eftersom deras fleråriga bål skapar varaktiga habitat som ger skydd, boplats och mat åt många arter av flora och fauna. Kända faktorer som påverkar artsammansättning och individantal av det associerade samhället är bland annat algbålens storlek och komplexitet. För att ta reda på om detta associerade flora- och faunasamhälle skiljer sig åt mellan smaltång och blåstång genomfördes en insamling av dessa två arter från flera lokaler längs den svenska kusten och i Estland (artikel 1).

Smaltången återfinns i Bottenhavet, längs hela dess svenska kust och längs de nordligare delarna av den finska kusten. Den har inte påträffats i Ålands hav, men återfinns runt ön Ösel (Saaremaa) i Estland, som är den enda idag kända lokalen för smaltång utanför Bottenhavet. Om smaltången har bildats inne i Östersjön, kan den mycket väl vara anpassad för de låga salthalterna och dess utbredning söderut kan då hindras av stigande salthalt. Ett experiment utfördes därför för att undersöka hur smaltångens sexuella reproduktion påverkas av olika salthalter (artikel 2). Det kan dock finnas sekundära effekter av stigande salthalt, som ökat betningstryck från bland annat tånggråsugga och ökad skuggning från blåstång, som blir större med högre salthalt. För att få ytterligare klarhet kring varför smaltången inte återfinns längre söderut längs den svenska kusten genomfördes således även ett experiment i fält som

jämförde effekterna av betning och skuggning på smaltångens tillväxt (artikel 2).

Det kanske mest fascinerande med smaltång är dess kapacitet till vegetativ förökning via fragment, något som tidigare inte dokumenterats hos släktet *Fucus*, dit både blåstång och smaltång hör. Denna förmåga gav smaltång sitt latinska namn, *Fucus radicans*, som betyder ”rotbildande”. För att kunna bedöma sårbarheten hos en art, behöver man veta när den fortplantar sig, eftersom detta oftast är den mest känsliga delen i livscykeln. Den sexuella fortplantningen hos smaltång längs svenska kusten sker under mitten av sommaren, under juli-augusti, lite olika beroende på vattnets temperatur. För att ta reda på om den asexuella fortplantningen, framförallt då själva vidhäftningen av fragment, också är kopplad till årstid genomfördes ett experiment där temperatur och ljus manipulerades för att återskapa förhållanden motsvarande de fyra årstiderna i Bottenhavet (artikel 3).

Klonalitet, dvs att föröka sig utan sexuell fortplantning, har visat sig förekomma i mycket liten skala även hos blåstången på dess allra nordligaste lokaler, men smaltången uppvisar klonalitet både på liten skala inom lokaler och storskaligt inom hela Bottenhavet. En hon-klon har återfunnits på i stort sett samtliga provtagna platser längs 550 km av den svenska Bottenhavskusten. Underligt nog har det inte dokumenterats någon klonalitet i populationerna i Estland För att se om de olika klonerna av smaltång skiljde sig åt med avseende på djuputbredning samt om de satt närmare medlemmar av den egna klonen (klumpade) eller om de var helt slumpmässigt spridda, utfördes därför en fältstudie med syftet att undersöka den rumsliga fördelningen av olika kloner på tre lokaler, Drivan I, Drivan II och Järnäs (artikel 4). Lokalerna ligger i norra Bottenhavet vid nordgränsen för smaltångens utbredning. Den låga salthalten medför att den sexuella fortplantningen inte alltid lyckas här (se artikel 2) och andelen klonalt förökade tångruskor har visat sig vara mycket hög på dessa lokaler.

Under flertalet expeditioner till Bottenhavet för att undersöka smaltång tyckte vi oss märka en skillnad i storlek på fortplantningstopparna mellan hanar och honor, urskiljbar med blotta ögat. Sexuell dimorfism förekommer på mikroskopisk skala, där könscellerna hos hanar och honor skiljer sig både i färg och storlek, men har inte dokumenterats på makroskopisk skala för släktet *Fucus*. Således genomfördes en undersökning kring morfologiska skillnader mellan könen hos smaltång (artikel 5). Hanar och honor av smaltång samlades in längs flera lokaler längs den svenska kusten och i Estland för att kunna avgöra om det var en effekt av den höga halten klonalitet i Bottenhavet, eller om det även förekom hos populationer som endast förökar sig sexuellt. För att ytterligare bedöma om denna storleksskillnad är en artspecifik egenskap för smaltång, jämfördes storlek och vikt på fortplantningsorganen hos blåstång och sågtång, båda två skildkönade och inom släktet *Fucus*.

Resultaten i artikel I visar att smaltången har en mer komplex bålstruktur än blåstången men trots detta återfinns fler arter i blåstången, beroende på dess

större storlek. Studien visar även att både blåstång och smaltång i Bottenhavet är större och mer komplexa än i Estland, där de båda arterna är mer lika varandra. Storlek visade sig vara en bra proxy för det associerade samhället och eftersom några arter endast återfanns på den ena av de båda arterna demonstrerar även studien vikten av att skilja mellan smaltång och blåstång vid inventeringar.

Av resultaten i artikel II kan vi dra slutsatsen att den låga salthalten begränsar smaltångens sexuella förökning i norr, men att högre salthalt inte orsakar utbredningsgränsen i söder, snarare tvärtom. Smaltången bildar fler ägg per förökningstopp ju högre salthalten är och andelen befruktade ägg stiger även den med salthalten. Det är mer troligt att den södra utbredningen bromsas av en kombination av att blåstången växer sig större och skuggar smaltången, vilket minskar dess tillväxt. Skuggning kan även medföra att tånggråsuggan, en betare av tång i Östersjön, finner smaltången smakligare än blåstången, då tidigare studier visat att skuggad blåstång innehåller lägre halter av avskräckande kemikalier. Dessutom ökar antalet tånggråsuggor med stigande salthalt och därmed betetrycket på smaltången.

Experimentet i artikel III bekräftade att ljus och temperatur påverkar graden av vidhäftning hos smaltången. Behandlingen som imiterade sommarförhållanden hade högst andel fastsittande fragment efter försökets slut. Vid undersökning av fragmenten under lupp visade det sig att många hade fäst sig mycket hårt mot underlaget genom att utsöndra en vitaktig substans. En närmare undersökning under svepelektronmikroskop (ESEM) visade att den verkade bildas på tångens bål. En grundämnesanalys (EDS) visade att substansen till största delen bestod av kalcium. Något sådant har aldrig tidigare rapporterats för *Fucus*-släktet, och även om flera algararter lagrar kalk i vävnaden så har ingen förklaring hittats till vad detta kan vara.

Den genetiska kartläggningen av kloner i artikel 4 visade att det inte är mer sannolikt att två tångruskor bredvid varandra tillhör samma klon än två långt ifrån varandra. Fördelningen av kloner inom en lokal är således helt slumpmässig både med avseende på kön och djup. Detta är viktigt att veta för framtida studier, eftersom det vid arbete med klonala organismer inte alltid är möjligt att bestämma materialet genetiskt. Vid en provtagning vill man ofta försöka få med så många olika kloner som möjligt för att få största möjliga genetiska variation i materialet, även om man inte kan använda individbegreppet. Jämförelsen med flera lokaler över hela smaltångens utbredningsområde bekräftade den dominanta hon-klonens utbredning. Ett framtaget familjetråd av kloner visade att hon-klonen troligtvis existerat över lång tid, då hon var trolig ”förälder” till många olika kloner som bildats genom somatiska mutationer. En han-klon var också väl representerad i ett område på mer än 100 km och ”förälder” till flera kloner.

Smaltång kan således föröka sig både klonalt och sexuellt. Det visade sig att fortplantningsorganen hos smaltångshonor är större och tyngre än hos smaltångshonar, både i Bottenhavet och i Estland. Resultaten i artikel 5



bekräftar att det inte var en synvilla utan en faktisk skillnad mellan könen hos smaltång. I Bottenhavet var skillnaden något tydligare, där även bålen hos hanar var smalare än hos honor. Ingen skillnad mellan könen återfanns hos vare sig blåstång eller sågtång, utan verkar vara en unik artkaraktär för smaltång.

Sammanfattningsvis har den här avhandlingen visat att smaltång är en viktig art i Östersjöns ekosystem. Smaltången skulle mycket väl kunna sprida sig längre söderut, till Egentliga Östersjön eftersom den sexuella fortplantningen gynnas av högre salthalt. Dock verkar konkurrens och betning begränsa smaltången till Bottenhavet, med undantaget runt Ösel på den estniska kusten. Den asexuella delen av livscykeln verkar gynnas av hög temperatur och ljusmängd och ”nya” kloner bildas ur gamla genom ansamling av somatiska mutationer, något som bidrar till genetisk diversitet trots låg andel sexuell rekombination. En unik artkaraktär för smaltången är en sexuell dimorfism där honornas befruktningstoppar är större än hanarnas.

# List of papers

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

- I. Schagerström E, Forslund H, Kautsky L, Pärnoja M, Kotta J, 2014 Does thalli complexity and biomass affect the associated flora and fauna of two co-occurring *Fucus* species in the Baltic Sea? *Estuarine, Coastal and Shelf Science* 149 pp. 187-193 (© Elsevier)
- II. Schagerström E., Kautsky L., Despite marine traits, the endemic brown algae *Fucus radicans* is restricted to the Baltic Sea *Submitted*
- III. Schagerström E., Saalo T. Temperature and light affect asexual reproduction in brown algae *Fucus radicans*. *Manuscript*
- IV. Ardehed A, Johansson D, Schagerström E, Kautsky L, Johannesson K, Pereyra R, 2015 Complex spatial clonal structure in the macroalgae *Fucus radicans* with both sexual and asexual recruitment.. *Manuscript*
- V. Schagerström E, Kautsky L, Macroscopic sexual dimorphism in *Fucus radicans* (Phaeophyceae) with implications for its reproductive ecology. *Manuscript*

My contribution to the papers was:

I performed most of the statistical analyses and wrote Paper I. For Paper II I planned and performed the experiments and studies, performed the statistical analyses and wrote the text. For Paper III I planned and performed the experiments and studies and wrote the text. For Paper IV I came up with the original idea of testing spatial depth distribution of clones and sexes, planned and performed the field studies and gave input on the ecological aspects of the paper. In Paper V I planned and performed the field samplings, statistical analyses and wrote the text.

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# Contents

Populärvetenskaplig sammanfattning .....	vii
Introduction .....	15
The Baltic Sea .....	15
A new species – <i>Fucus radicans</i> .....	18
Effects of climate change and eutrophication on <i>Fucus</i> populations .....	20
Aims of the thesis .....	23
The ecological role of <i>Fucus radicans</i> in the Baltic Sea ecosystem ....	25
Associated flora and fauna of <i>F. radicans</i> and <i>F. vesiculosus</i> (Paper I) .....	26
Sexual reproduction and the distribution limit .....	29
Effects of salinity on sexual reproductive success .....	29
<i>Fucus radicans</i> is not limited to the Bothnian Sea due to low-salinity adaptation (Paper II) .....	30
The effects of competition and herbivory on <i>Fucus</i> distribution .....	33
The southern distribution limit is set by biotic factors (Paper II) .....	34
Re-attachment of fragments – the asexual reproduction .....	37
Light and temperature affect re-attachment of fragments (Paper III) .....	38
Spatial distribution and evolution within and between <i>Fucus radicans</i> populations .....	43
Does the clonal apple fall far from the tree? (Paper IV) .....	44
Sexual dimorphism on a macroscopic scale .....	47
Morphological characters in <i>F. radicans</i> male and female thalli (Paper V) .....	47
Concluding remarks .....	49
The distribution of <i>F. radicans</i> in the Baltic Sea .....	49
Possible effects of predicted climate change on <i>F. radicans</i> .....	50
Considerations from a management perspective .....	51
Acknowledgements .....	52
References .....	53
Tack! .....	64

# Abbreviations and Glossary

Adventitious shoot- Shoot forming from the midrib anywhere on the thallus, used for asexual propagation. When separated from the mother thallus it is defined as a fragment.

Allee effect - the positive correlation between population density and individual fitness

Allopatric speciation – speciation by geographic isolation preventing gene flow

Antheridia – Packages containing 64 biflagellate sperms each.

Benthopleustophytic – Not attached to the substratum, free-living.

BP – Before Present, denotes years from present day.

Brackish – Here refers to a salinity between 1-15 PSU.

Circadian rhythm (endogenous rhythm) – Cyclic changes in organisms that are endogenously controlled, often on a 24 hr or monthly cycle.

Clone – see *Genet.*

Conceptacle – Cavity (on the receptacle) that contains the reproductive cells.

Dioecious – Having male and female gametes produced by separate individuals (unlike monoecious, where one individual produces both types of gametes).

DW – Dry weight biomass.

Ecotype – A grouping defined by genetically determined differences between populations within a species, reflecting local adaptation. Taxonomically called subspecies.

EDS - Energy Dispersive x-ray Spectroscopy.

Endemic – Peculiar to a particular region or area, not found in other places.

ESEM – Environment Scanning Electron Microscope (no pre-plating needed).

F.I. - Fertility Index, percentage reproductive apices of total number of apices.

Foundation species – a species that provides structure, increases habitat complexity and provides shelter and protection to the associated community from both abiotic and biotic factors.

Fragment – see *Adventitious branches.*

Fragmentation – Accidental or programmed breakage of a thallus into pieces that serve as asexual propagules which reattach and form new thalli.

Genet – One or several thalli with the same genotype, also called clones, sometimes referred to as a genetic individual.

Juvenile – Sexually recruited individual, not yet sexually mature.

Marine – Here refers to a salinity of more than 25 PSU.

MLG – Multi Locus Genotype.

MLL – Multi Locus Lineage.

Oogonium – Packages containing 8 egg cells each.

Parapatric speciation - speciation by geographic distance reducing gene flow

Perennial – Lives for more than two years and does not die back during winter.

Peripatric speciation – similar to *Allopatric speciation* but one population is much smaller than the other (i.e. on an island).

PSU – Practical salinity unit. SI unit for denoting salinity concentration in sea water based on the properties of sea water conductivity. It is equivalent to parts per thousand or (‰) or to g/kg.

Ramet – The alga equivalent of “branch”. One thallus can consist of one or several ramets, originating from one holdfast.

Receptacle – Fertile area of a thallus in which reproductive structures develop.

RA- Reproductive allocation, percentage biomass allocated to reproductive tissue.

Rhizoid – A single cell or multicellular filament, growing from the basal region of a thallus, that functions in attachment.

SEM – Scanning Electron Microscope, preps are pre-plated with thin layer of gold.

Sympatric - Occupying the same or overlapping geographic areas.

Sympatric speciation – Speciation without any geographical barriers.

Thallus (pl. thalli) – The alga equivalent of “plant”. The body of an alga, which is not differentiated into vascularized leaves, roots and stems. Here denoting a single unit of algae not attached to another i.e. mother thallus.

WW – Wet weight biomass.

Zygote – The product of gamete fusion (syngamy), a fertilized egg.



# Introduction

The Baltic Sea is unique, being atidal and having brackish water with a strong salinity gradient. The gradient entails decreasing species diversity as the salinity declines. The brown macroalgae belonging to the *Fucus* family are foundation species of this ecosystem, being perennial and providing structure and habitat for many species. As such, the *Fucus* species in the Baltic Sea are important target species for management.

Recently, a new species of *Fucus* was discovered, *Fucus radicans*, believed to be endemic to the Baltic Sea. Being so new, very little is known about its biology and ecology. We do however know that there are several threats to macroalgae in general and particularly *Fucus* in the Baltic Sea. Mainly from the ongoing eutrophication, but the predicted climate change also poses a threat in the near future. In order to effectively manage *F. radicans*, more knowledge of its function in the ecosystem, its dispersal ability, what restricts its distribution and its sensitivity to competition is needed.

It was discovered that *F. radicans* also reproduces asexually by fragmentation, with large spread of clones and skewed sexual ratios in populations as a result. Further insight in the genetic composition of populations on micro and macro scale and the asexual dispersal will help with management decisions as well as aid the understanding of speciation processes.

## The Baltic Sea

From a biological perspective, the Baltic Sea is a very young sea. Since the last glaciation ended 12 000 years BP, it has undergone a succession of alternating marine and freshwater stages (Ignatius 1980). The last greater inflow of marine waters occurred some 8000 years BP, known as the Littorina Stage. Since then, the salinity has gradually been diluted by freshwater runoff and the present Baltic Sea has had a fairly stable salinity for the last 3500 years (Björck 1995), from 12-15 psu at the Öresund and Danish Belts down to almost freshwater conditions (1 psu) in the innermost Bothnian Bay (Wallentinus 1978, Feistel et al. 2010) (Fig. 1).

The Baltic Sea is a semi-enclosed water body and its only connection to the North Sea and the Atlantic Ocean is through the shallow and narrow sills around Denmark (Öresund and the Danish Belt Seas) in the south, making it in this aspect comparable to a threshold fjord (Waern 1965). The connection

with the North Sea, is not large enough for the water body of the Baltic Sea to be affected by the gravitation of the moon, hence the Baltic Sea is atidal. Although it is subject to random meteorologically induced changes in water level, the annual fluctuations in salinity depend mainly on wind directions, saltwater inflow from the North Sea and precipitation (Feistel et al. 2010). The freshwater discharge from rivers is highest in May-June (Kullenberg 1981, Malm et al. 2001). On a local scale, freshwater discharge from the rivers may decrease salinity temporarily, particularly during spring flood when melting snow and ice may further reduce the already low salinities in the inner parts of the coastal areas. Increase in salinity can occur locally during dry periods as a result of evaporation in shallow enclosed bays (HELCOM 2013).

The surface current of the Baltic Sea moves counter clockwise, due to the Coriolis Effect. The surface waters enters by the Danish Straits and are transported eastwards along the German and Polish coastline. The current changes direction, heading north when it meets the Latvian coast. The fresh surface water from the Gulf of Finland flows eastwards and joins the northbound main gyre which moves on northwards up along the Finnish Bothnian Sea coast until the Quark. Here, it veers across from Finland to Sweden, where it then turns and moves south along the Swedish coast, and then exits through Öresund (Hällfors 1981, Lehmann & Hinrichsen 2000).

The seasonal sea surface temperature over the year varies from up to over +20°C during the late summer months at sheltered coastal sites to just above zero in the winter (Kullenberg 1981). The Gulf of Bothnia, consisting of the Bothnian Bay and the Bothnian Sea are covered with ice during the winter months for 130 – 200 days. The ice season usually begins in November and reaches the average maximum extent in March. The location of the southern ice edge is typically in the northern Baltic Proper (HELCOM 2013). Normally, in the inner archipelago the ice cover may reach a depth of 0.5-0.8 m and pack ice may extend to a depth of several meters (Waern 1952).

Compared to marine waters, the brackish Baltic Sea ecosystem has a low species diversity, even though it harbours species of both marine and freshwater origins. All organisms found in the Baltic Sea today are postglacial immigrants (Snoeijs 1999). Many of the species with marine origin that are present in the Baltic Sea today were introduced during the Littorina Stage and have since adapted to the marginal environment (Russell 1985). Several species have become genetically distinct ecotypes due to loss of genetic diversity (Johannesson & André 2006).

In temperate shore habitats the perennial macroalgae form important structures, providing substrate, shelter and food for associated flora and fauna. The lack of tide within the Baltic Sea creates a very different environment in the littoral zone compared to the tidal coasts, where many macroalgae are regularly exposed to air to some degree during low tides. Some species in the Baltic Sea can live submerged for most of the year yet be exposed to air for varying lengths of time due to weather conditions i.e. extended low water.



Very few perennial macroalgae have managed to adapt to the brackish environment in the Baltic Sea. There are ca. 170 macroalgae taxa present in the Baltic Sea (Nielsen et al. 1995, Snoeijs 1999). The species richness decreases gradually along the salinity gradient from the Öresund and Danish Belt Seas down to ca. 100 species in westernmost Baltic Proper (8-12 psu) and only ca. 70 species in the Baltic Proper (6-7 psu). In the Bothnian Sea, the steepest decrease occurs at around 5 psu and only 30 species are found in the low salinity (3 psu) of the Bothnian Bay.

The large brown kelps are not found in salinities lower than 15 psu (Lüning 1990), instead the shores of the Baltic Sea are characterized by the fucoids *Fucus vesiculosus* L. and *Fucus serratus* L., which have managed with differing success to remain in the Baltic Sea (Lüning 1990, Malm et al. 2001) (Fig. 1). The lack of competition from other large perennial brown algae provides the opportunity to expand all the way from the surface down to depths where light availability becomes limiting (Kautsky et al. 1986). *Fucus vesiculosus* is the dominant species and is generally recognized as the main foundation species of the Baltic Sea ecosystem (Dayton 1971, Wahl et al. 2011).

The upward distribution of *Fucus* species is limited by ice and ice scouring, sometimes clearing away the *Fucus* belt down to 5 meters depth after harsh winters (Waern 1952, Kiirikki 1996). However, the regeneration capacity from bits of remaining basal parts is considerable and enough of the basal parts often remains in cracks and crevices of bedrock and boulders to re-establish the population over time (Kiirikki & Ruuskanen 1996, Malm & Kautsky 2004).

Not only algae have difficulties in adapting to the brackish waters difficult, it also affects the fauna community in the Baltic *Fucus* belt, which differs from its marine counterparts. Due to the low salinity, many herbivores i.e. limpets, periwinkles and sea urchins are absent. Instead, common grazers are the marine isopod *Idotea* spp., several gammarids and the freshwater gastropods *Radix balthica* L., *Lymnea stagnalis* L. and *Theodoxus fluviatilis* L. (Malm et al. 1999, Hansen et al. 2008). *Idotea balthica* Pallas 1772 can occasionally occur in large numbers and is then able to severely graze down *F. vesiculosus* (Kangas et al. 1982, Rönnberg et al. 1985, Engkvist et al. 2000).

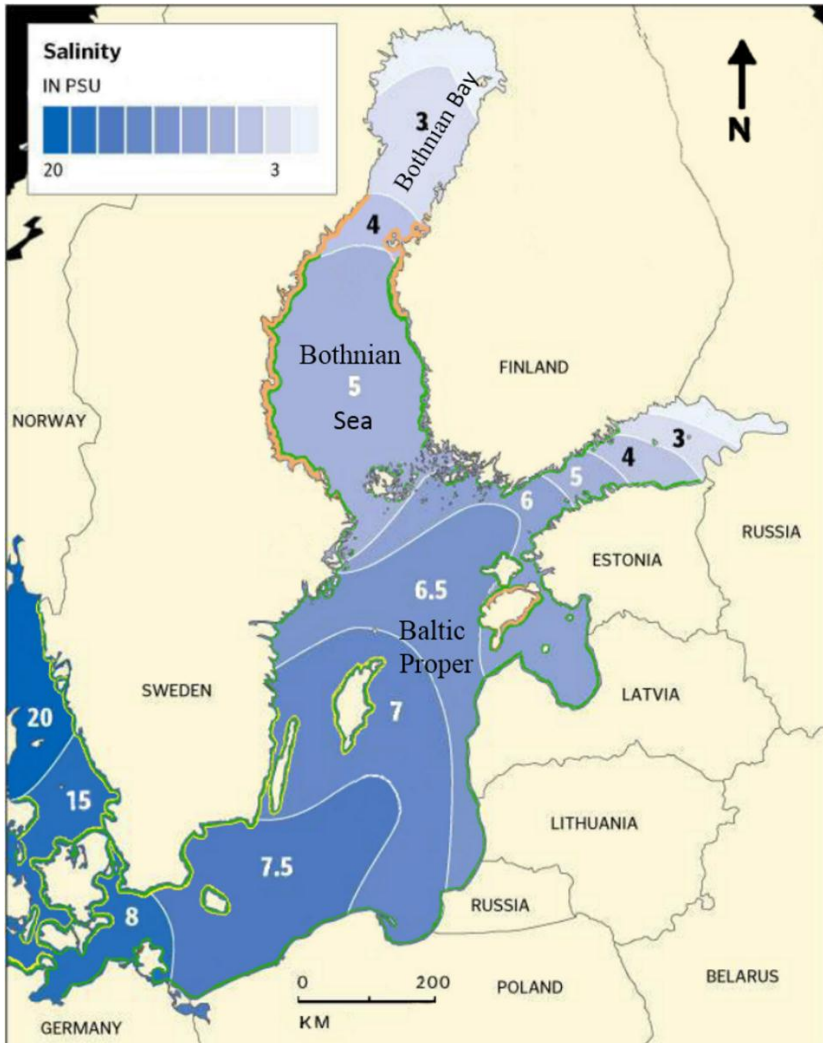


Figure 1. The Baltic Sea with the surface water salinity gradient and the distribution limits of *Fucus radicans* (orange), *F. serratus* (yellow) and *F. vesiculosus* (green). Substrate availability is not taken into account.

### A new species – *Fucus radicans*

*Fucus vesiculosus* is a species harbouring a wide morphological variation, which has been mainly attributed to variations in wave exposure (Jordan & Vadas 1972, Ruuskanen & Bäck 1999) and salinity (Kalvas & Kautsky 1993, 1998, Ruuskanen & Bäck 2002). In the past, *F. vesiculosus* has been described by Kjellman (1890) as among one of 24 different morphologies, grouped into 4 main forms. There are also several benthopleustophytic morphs of *F.*

*vesiculosus* (Svedelius 1901) which lack receptacles and are thus considered to be sterile. Waern (1952) documented that the low salinity of the Bothnian Sea reduced the thallus size of *F. vesiculosus*, a phenomenon known as brackish dwarf morphism. It was long assumed that low salinity was the main reason for the narrow, bushy appearance of the *F. vesiculosus* populations in the northern Baltic Sea (Bäck et al. 1991, Kalvas & Kautsky 1993, Ruuskanen & Bäck 1999). This morphological variety was also noted in the Gulf of Finland by Luther (1981), who suggested that this morph might even prove to be another species.

Studies on the genetic variation of the Bothnian Sea populations of *F. vesiculosus* gave not only the discovery of asexual reproduction, which was hitherto unknown to occur in fucoids (Tatarenkov et al. 2005), but also that the dwarf morph significantly differed genetically from the “common” morph. Thus, the bushy dwarf morph was separated from *F. vesiculosus* and described as a new species, *Fucus radicans* sp. nov. Bergström et al. (Bergström et al. 2005). *Fucus radicans* has so far been shown to be endemic to the Baltic Sea and can be found in the Bothnian Sea, where it is present all along the Swedish coast and on the northern parts of the Finnish coast, and in the Väinameri Archipelago Sea around the Estonian island Saaremaa (Fig. 1) (Bergström et al. 2005, Forslund et al. 2012).

*Fucus radicans* is dioecious with a gametic meiosis (Fig. 2). The reproductive structures, (receptacles) develop at the tips of the branches. In the dioecious *Fucus* species, antheridia (male) or oogonia (female) are formed within conceptacles, which can be seen as cavities of the receptacle surface. In *F. vesiculosus* as well as in *F. radicans*, each oogonia contain 8 haploid egg cells, each antheridia consists of 64 laterally biflagellate spermatozoa (Hoek et al. 1993). The eggs have negative buoyancy and sink to the sea floor once released. The fucoid sperm are negatively phototactic, thus they actively swim away from light (Brawley & Johnson 1992), finding the eggs by pheromones released by the eggs (Maier & Müller 1986; Brawley et al. 1999). This is also assumed to be the case for *F. radicans*, though studies confirming this are needed.

*Fucus radicans* receptacles mature during spring and the reproductive period extends from late May until September, depending on water temperature (Forslund & Kautsky 2013) in the Bothnian Sea. It is unknown how long it takes for a *F. radicans* thallus to reach sexual maturity. It might be that thalli formed from re-attached fragments reach maturity faster due to the relative older age of the tissue, compared to sexually recruited thalli. The estimated age for *F. vesiculosus* to reach maturity in the Baltic Proper is approximately 4-5 years (pers. obs.). Preliminary data from an ongoing experiment with seeded *F. radicans* placed in the field show a much lower growth rate (reaching about 5 cm in 5 years) compared to *F. vesiculosus* (about 20-25 cm in 5 years) and no receptacles have formed in *F. radicans* during this period (unpubl. data).

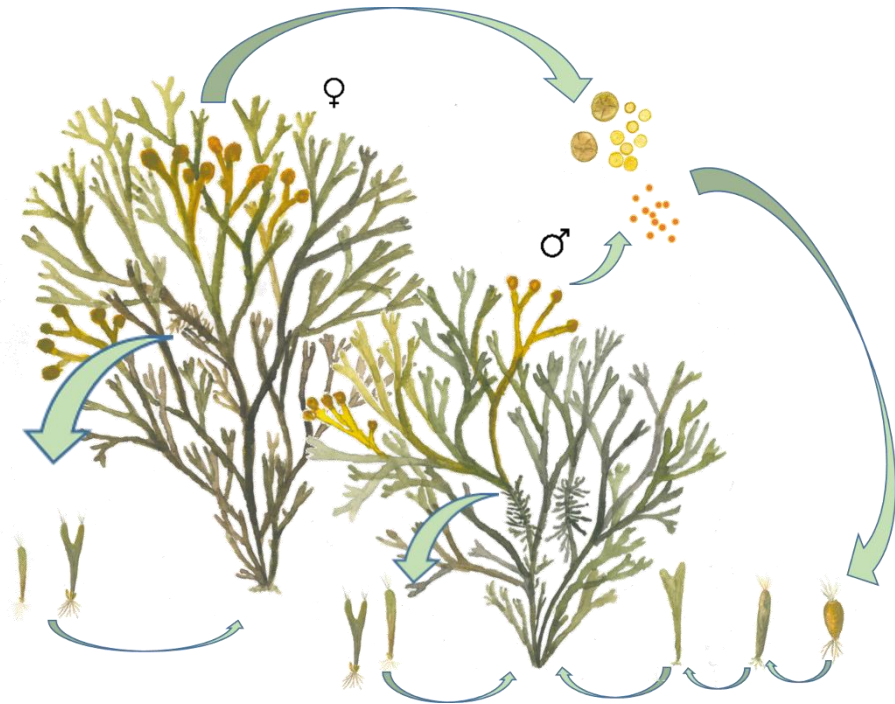


Figure 2. The dioecious life cycle of *F. radicans* has both sexual reproduction via eggs and sperm which form a zygote (right) and asexual reproduction through adventitious branches which fall off and re-attach to the substrate, forming new, clonal thalli (left). Illustration by L. Kautsky.

Each sexually mature thallus is also able to reproduce asexually simultaneously. The asexual reproduction is by way of fragmentation (Collado-Vides 2001), where adventitious branches formed on the stem or midrib of the lower and mid parts of the thalli supposedly break off and disperse before re-attaching to the substrate (Fig. 2) (Bergström et al. 2005, Tatarenkov et al. 2005). The dispersal and re-attachment of fragments might not be restricted to a certain season as the sexual reproduction is.

## Effects of climate change and eutrophication on *Fucus* populations

The effects of climate change on the Baltic Sea is a much discussed topic. Predictive models show both future reductions in average salinity and oxygen concentrations due to increased precipitation and increased winds (Meier 2006, Meier et al. 2012) or increased salinity due to decreasing precipitation (Hansson et al. 2010) both scenarios being caused by temperature rise.

A future increase in eutrophication has also been predicted by Meier et al. (2012). Another model suggests that although the water temperature will increase by approximately +3°C and that salinity will decrease with 1.5 psu until year 2100, the consequences of the current Baltic Sea Action Plan (BSAP) will have much stronger effects on the Baltic Sea ecosystem than predicted climate change, due to shifts from the present nitrogen limited system towards a phosphorus limited system (Friedland et al. 2012). Today, nearly the entire Baltic Sea area is affected by nutrient enrichment, most of which is anthropogenic, resulting in oxygen depletion and increased algal blooms. Since the late 1980s the overall nutrient inputs to the Baltic Sea have decreased, and the present levels of nutrient inputs equals those in the early 1960s (HELCOM 2014). The level of eutrophication varies between different sub-basins. Regarding the areas described in this thesis, the Baltic Proper as well as the Väinameri Archipelago Sea on the Estonian coast are both classified as affected by eutrophication (eutrophic), whereas the Bothnian Sea has previously been classified as unaffected (oligotrophic) until the latest report where data from 2007- 2011 showed an increase in nutrients, which changed its classification to mesotrophic (HELCOM 2014).

The increasing eutrophication of the Baltic Sea has led to an increase in primary production, decreased transparency (Secchi depth) in the water column and increasing amounts of drift-algal mats (Bonsdorff et al. 1997). The increasing amounts of phytoplankton and ensuing reduction in light reaching the sea floor has negatively affected the depth distribution of *F. vesiculosus* (e.g. Kautsky et al., 1986, Eriksson et al. 1998, Torn et al., 2006). Historical data from around the Baltic Sea coastlines show a maximum depth distribution of *F. vesiculosus* down to 10-11 meters during the 1940-60's. Re-inventories from the 1980's and onwards show both decreasing maximum depth (4.7-8.5 meter) and reduced densities (e.g. Kautsky et al. 1986, Schories et al. 2008). This trend has, however, turned in the 1990's and there has been recoveries on local scale (Albertsson 2014). It is estimated by Vogt & Schramm (1991) that the biomass (WW) of *F. vesiculosus* along the German coastline of the Baltic Sea has decreased by 94 to 95 %, although this is due to several causes, not only nutrient enrichment.

The *Fucus* cover on hard bottom substrate has been shown to decrease as a function of Secchi depth, which in turn is controlled to a large extent by the total amount of nitrogen in the water (Nielsen et al. 2002, Krause-Jensen et al. 2009) but recent models by Rinne et al. (2011) add that Secchi depth is not the single contributing factor to *Fucus* cover or distribution depth limit, as these are also affected by level of exposure, salinity and slope of the shore, thus supporting the earlier findings of Bäck & Ruuskanen (2000). A large increase in nutrient concentrations in the water has been shown to affect the growth and survival of adult *F. vesiculosus* thalli both by reducing the light penetration due to increased phytoplankton biomass and by the increasing

amount of epiphytic filamentous algae that will grow on and shade the host thalli (Rohde et al. 2008).

Increasing primary production has also increased sedimentation rates. When hard bottoms are covered by sediment, the recruitment of fucoids is reduced as the zygotes cannot penetrate the sediment layer and attach to the substratum. Råberg et al. (2005) showed a higher density and survival of juvenile *F. vesiculosus* when comparing areas cleared from sediment to areas with natural sediment conditions. The survival rate of *F. vesiculosus* zygotes was less than 1% on substrates covered with sediment (Berger et al. 2003). High levels of nitrate have also been shown to have a direct negative effect on *F. vesiculosus* by reducing the germination and attachment rate of the zygotes (Bergström et al. 2003).

Nutrient enrichment also favours the growth of filamentous algae. The increase in filamentous algal cover, attached on the substratum and free-floating drift-algae, similarly reduces the reproductive success in fucoids by physically preventing zygotes from reaching and attach to the substrate (Kraufvelin et al. 2007). Råberg et al. (2005) also showed that the actual physical presence of the brown filamentous algae *Pylaiella littoralis* (Linnaeus) Kjellman on the substratum reduced the settling success of *F. vesiculosus* germlings. Both germination and rhizoid formation in *F. vesiculosus* zygotes were negatively affected even by low concentrations of exudates coming from *P. littoralis* (Råberg et al. 2005).

A decline in *F. radicans* due to climate change i.e. reduced salinity and increased temperature and/or increased levels of nutrients could have effects on higher trophic levels as has been shown for *F. vesiculosus* (Wikström et al. 2006). Since *F. radicans* is already established as the dominant biotope-forming marine algae in the northern part of the Baltic Sea (Råberg and Kautsky 2007, Forslund et al. 2012), further knowledge of this new species biology and ecology, in particular the ability to further adaptation and mechanisms regulating reproduction and distribution, is needed for an effective management and preservation of the fucoid dominated shallow rocky bottom habitat in the Baltic Sea.

# Aims of the thesis

Since *Fucus radicans* has previously been treated as *F. vesiculosus*, much of its basic ecology and reproductive biology is unknown.

To address this knowledge gap, I performed a comparative study of the ecological roles of *F. radicans* and *F. vesiculosus*, both important foundation species of the Baltic Sea (**Paper I**). In order to improve predictions of future climate change on *F. radicans* and its community, as well as improve assessments of data to aid marine planning, more knowledge about the ecological role of this new species is needed. The aim of **Paper I** is to quantify the morphological complexity and compare the associated flora and fauna of *F. vesiculosus* and *F. radicans* collected both in the Bothnian Sea, the main distribution area, and around the Estonian island Saaremaa, in the Baltic Proper, the only site outside the Bothnian Sea where *F. radicans* is also present.

No studies have explained why *F. radicans* is limited to the Bothnian Sea and not found in the Baltic Proper, except for the outlier population around Saaremaa. In **Paper II**, three factors possibly restricting the dispersal and establishment of *F. radicans* further south into the Baltic Proper, are studied. To determine if *F. radicans* is adapted to the low salinity environment of the Bothnian Sea and has lost the ability to reproduce successfully at higher salinities, I first investigated the effect of salinity on the reproductive output and reproductive success of *F. radicans* (**Paper II**). Secondly, I performed a cage experiment in the field to test the limiting effects of shading by the dominant and larger *F. vesiculosus* and of grazing by the common herbivore *Idotea balthica* on *F. radicans*, either alone or in combination. The overall aim of **Paper II** is to determine if any, or a combination of these two biotic factors affects the present southern distribution limit of *F. radicans*.

Dispersal and competition are main factors in determining distribution, and *F. radicans* also has the ability to reproduce asexually through fragmentation. This is unique for *F. radicans* within the genus *Fucus*, though fairly common in other algae genera. Little is known about the mechanisms regulating asexual reproduction which is important for understanding the distribution of clones, especially which factors favour the re-attachment process. The aim in **Paper III** is to test if temperature and light affects re-attachment. Therefore an experiment was performed testing if this process has a specific window of opportunity, i.e. during calm dark winter conditions, cold but light spring

conditions or warm, light summer conditions, or if the fragments re-attach to the substrate throughout the year.

**Paper IV** addresses questions related to dispersal pattern of sexually and asexually recruited specimens on both micro- and macroscale, the age of different clones and the question “if the clonal apple falls far from the tree or not”. The aim is to determine if clonal patchiness occurs or if different clones are more randomly distributed throughout the population. Three sites were sampled and genotyped using microsatellites for investigating small scale patterns. This was then pooled with other data, making a large scale spatial analyses encompassing a total of 16 sites throughout the distribution range of *F. radicans* (**Paper IV**).

Finally, what seemed like an occurrence of sexual macroscopic dimorphism discovered during field sampling was investigated further in **Paper V**. Here, I compared the receptacle size and weight as well as thalli width and height of *F. radicans* males and females from the Bothnian Sea and Estonia from several sites, with the aim to discover if *F. radicans* males actually have smaller receptacles and narrower thalli than the females. This has never before been reported in *Fucus* spp. and **Paper V** also discusses what the implications of this might be for the reproductive ecology of *F. radicans*.

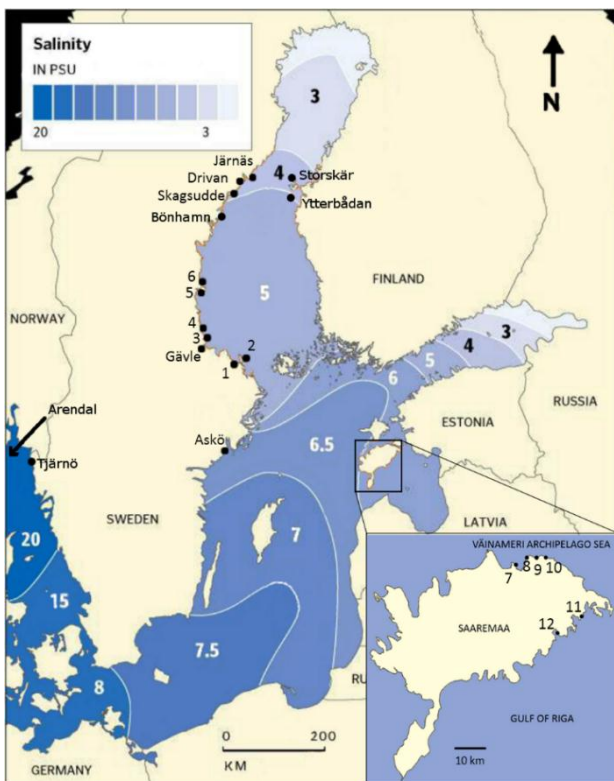


Figure 3. Map showing the location of sampling sites. Numbered sites were used in Paper I and V, named sites were used in Paper II-V.



# The ecological role of *Fucus radicans* in the Baltic Sea ecosystem

Rocky marine areas are some of the most species rich communities, with a wide range of macroalgal species. The most common inhabitants of temperate rocky shores are brown perennial macroalgae (Santelices et al. 2009). Size is a major component of habitat architecture and in these algal dominated habitats, the thallus size has been known to affect the active habitat-choice in invertebrates and thus the number of associated species and the animal abundance (Hauser et al. 2006). For example, a single kelp thallus can host more than 100 species and 90 000 specimens and fucoid beds can host hundreds of species with animal densities of 100 000 individuals m<sup>-2</sup> (Christie et al. 2009).

Not only size is important for high species richness in macroalgal communities, though. The differences in morphological complexity between macrophyte species have also been shown to affect macroinvertebrate abundance, favouring more structurally complex thalli (Kraufvelin & Salovius 2004, McAbendroth et al. 2005, Warfe and Barmuta 2006, Hansen et al. 2011). For example, invertebrate fauna are more affected by water motion on flat thalli, which have a larger surface exposed to water flow than more complex thalli (Tuya et al. 2008). An increased complexity of thalli also provides a better shelter for the macroinvertebrate community from predation (Diehl 1992, Warfe & Barmuta 2006). Measuring complexity however, is neither easy nor are there any standard methods available for comparing complexity across ecosystems and habitats (Kovalenko et al. 2012).

Studies on the importance of *F. radicans* to the associated faunal community compared to filamentous algae have shown that it might be as important as the larger *F. vesiculosus*, but also more sensitive to anthropogenic change (Råberg & Kautsky, 2007). *Fucus vesiculosus* and *F. radicans* are both considered to be foundation species in the Baltic Sea since they increase the habitat complexity and provide structures which in turn gives shelter and protection from both abiotic and biotic factors to the associated community (Wahl et al. 2011, Forslund 2012). As such, they are providing substratum for many epibionts (Wikström & Kautsky 2007) and are also considered safeguarding the faunal richness (Kautsky et al. 1992). Knowledge of the effects of a decrease or an increase in *F. radicans* stands/habitat on associated fauna and flora can be used to make more accurate predictions of

how future climate change might affect the Baltic Sea *Fucus* dominated ecosystems. Decreasing species richness of an already low-diversity system will reduce the resilience of its communities to disturbances or environmental stress (Lilley & Schiel 2006, Wahl et al. 2011) and large scale losses of fucoid canopies in the Baltic Sea might depress the stability of the whole coastal ecosystem (Bulleri et al. 2012).

Thus, a study comparing the associated flora and fauna as well as the structural complexity of *F. radicans* and *F. vesiculosus* from sympatric sites throughout *F. radicans* distribution range was conducted.

## Associated flora and fauna of *F. radicans* and *F. vesiculosus* (Paper I)

The collection of *F. radicans* and *F. vesiculosus* was carried out in 2007 at six sites along the Swedish coast of the Bothnian Sea and from five sites around the Estonian island Saaremaa located between the Gulf of Riga and Väinameri Archipelago Sea in the Baltic Proper (Fig. 3). The salinity of the Bothnian Sea region varied between 4-5 psu, while the Saaremaa region had a salinity between 6-7 psu. The *Fucus* thalli were collected in pairs consisting of one *F. radicans* and one *F. vesiculosus* growing less than 1 m apart to minimize variation in exposure and other abiotic factors (Råberg & Kautsky 2007, Kersen et al. 2011). Six pairs were collected at each site between 0.5-1.5 m, as this is the depth range of *F. radicans* around Saaremaa (Forslund & Kautsky 2013).

To collect the associated community of each thalli, a mesh bag (< 1 mm mesh size) was placed over each thallus and closed around the basal parts before the thallus was removed. Samples were frozen until sorting. At the laboratory the associated flora and fauna (> 1 mm) were sorted and identified. After the associated flora and fauna was removed from the sample, the *Fucus* thallus was dried and weighed. The thalli were later rehydrated and pressed. Due to the large size of the specimens from the Bothnian Sea, one ramet was randomly selected from each thallus of these. First, the area and perimeter of the pressed thalli was calculated, then the area was doubled to account for both sides of the thallus. The perimeter was then divided by total area, providing a coarse measure of thallus complexity (Gee & Warwick 1994, McAbendroth et al. 2005, Warfe et al. 2008).

Taxonomic richness (epiphytic algae and invertebrates), biomass (epiphytic algae and invertebrates) and abundance of invertebrates were all expressed as per 100 g DW *Fucus* to be comparable. These variables, as well as measures of *Fucus* DW and complexity index (C.I.) were compared between regions, *Fucus* species and sites (**Paper I Fig. 2**). Beyond that, multivariate analyses i.e. hierarchical PERMANOVA, nMDS and SIMPER were also used to explore the differences in composition of macrophyte and

invertebrate assemblages between regions, sites and *Fucus* species (**Paper I Fig. 3 & Table 3**). It was hypothesized that complexity would differ between *F. radicans* and *F. vesiculosus*, and that this difference would explain structure of the associated flora and fauna communities.

As predicted, the thallus complexity of *F. radicans* was higher than *F. vesiculosus* both for the Bothnian Sea and the Saaremaa region (Fig. 4, **Paper I**). It is worth noting that the *F. radicans* found around Saaremaa lacks the numerous adventitious branches that are often found on the thalli of *F. radicans* growing in the Bothnian Sea (Bergström et al., 2005; Forslund and Kautsky, 2013). However, both *Fucus* species from the Bothnian Sea had a more complex structure than their counterparts from the Saaremaa region. Contrary to other studies (e.g. Kostylev et al. 2005, Tuya et al. 2008, Cacabelos et al. 2010) the thallus complexity did not explain the structure of the associated flora and fauna. Instead, thallus size, here measured as biomass, explained more of the variability for both the epiphytic macrophytes and invertebrate communities than the complexity (**Paper I**). The expected effect of thallus complexity on the associated community was thus refuted.

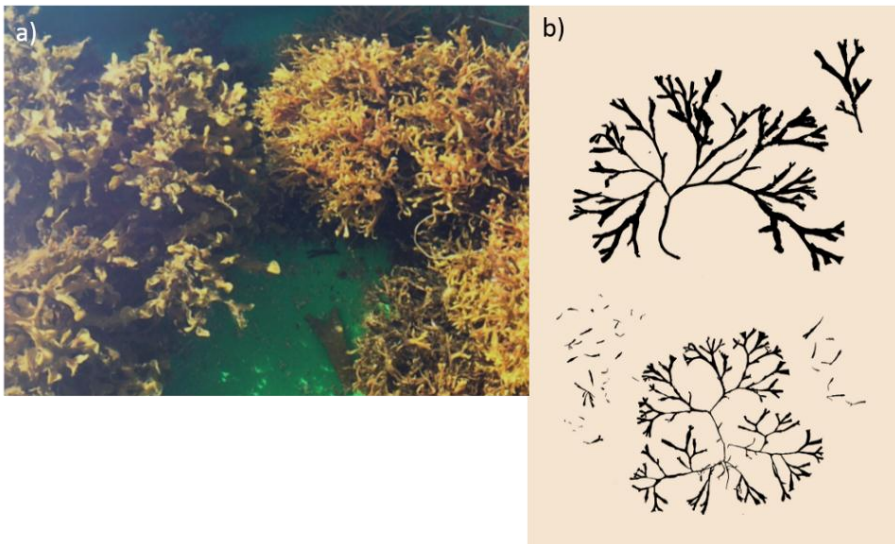


Figure 4. a) *Fucus vesiculosus* (left) and *F. radicans* (right) from the same site (Gävle) and b) pressed specimens of *F. vesiculosus* (top) and *F. radicans* (bottom) from Hölick (Number 5 Fig 3). Observe the numerous adventitious branches on the pressed specimen of *F. radicans*.

*Fucus vesiculosus* in the Bothnian Sea hosted a higher species richness and faunal abundance compared to the sympatric *F. radicans* as well as both *F. radicans* and *F. vesiculosus* from Saaremaa, due to the larger biomass (**Paper I**). Increased epiphytic diversity offers a more diverse habitat and diet for associated invertebrates and the epiphytic macroalgal assemblage have been

shown to be more diverse on large fucoids compared to smaller fucoids (Warfe and Barmuta, 2006; Cacabelos et al., 2010; Wernberg et al., 2013). Larger thalli also provides better protection for invertebrates from fish predation (Diehl 1992, Kotta et al. 2010).

A total of 41 taxa of associated flora and fauna associated to the two *Fucus* species were recorded. Similar total numbers were recorded for both regions and on both fucoids. In the Bothnian Sea as well as the Saaremaa region both fucoid species hosted around 30 taxa and some unique species were found only on either of the two fucoids (**Paper I**), which was in concordance with the previous results of Wikström & Kautsky (2007) and Råberg & Kautsky (2007).

The results in **Paper I** indicated a potentially large site-scale variability in community characteristics, which highlights that the knowledge from a survey cannot simply be extrapolated beyond the borders of the survey area. Other studies of broad patterns of *F. vesiculosus* and epiphytic species in the Baltic Sea also found that they were defined by regional differences (Kersen et al. 2011). Epiphyte biomass was overall higher in the Saaremaa region compared to the Bothnian Sea, and it is possible that the regional differences observed in the associated macroalgal and invertebrate communities were reflecting regional variation in some large scale stressor (Wahl et al. 2011) like nutrient enrichment or simply natural gradients (**Paper I**). In contrast to the mesotrophic Bothnian Sea (Rönnberg & Bonsdorff 2004, HELCOM 2014), Saaremaa is situated in the eutrophied Baltic Proper with the north coast in the shallow Väinameri Archipelago Sea and the south coast in the Gulf of Riga. The higher biomass of epiphytic algae and higher species diversity in the Saaremaa region might be explained by the increased nutrient concentrations (Kotta et al. 2008; Kraufvelin et al. 2010) or by natural gradients. Further studies might investigate if any genetic differences between *F. radicans* inhabiting the Bothnian Sea and the Saaremaa region (Johannesson et al., 2011) also affect the diversity and abundance of the associated community.

# Sexual reproduction and the distribution limit

The reproductive system in fucoids has evolved on marine intertidal coasts, where the release of gametes is synchronized by multiple cues, such as the daily tide (Brawley 1992, Pearson & Brawley 1997), light (Brawley & Johnson 1992), turbulence (Serrão et al. 1996b) and also a circadian lunar rhythm (Ladah et al. 2008). In the Baltic Sea however, *F. vesiculosus* lives almost constantly submerged. Mainly because the lack of diurnal tide but also since the absence of competition from other species enables *F. vesiculosus* to spread deeper, until limited by light (Kautsky et al. 1986). The synchronization of gamete release in the Baltic Sea ecotype of *F. vesiculosus* is instead controlled by an endogenous clock, with peaks around full and new moon during the reproductive season (Andersson et al. 1994).

On intertidal coasts, the gamete release is triggered by the tide through first a period of desiccation of the mature receptacles during low tide and then rehydration by high tide when the gametes are released (McLachlan et al. 1971, Berndt et al. 2002). In the Baltic Sea, shallow-growing thalli of *F. vesiculosus* can be exposed above water for more than a day during the occasional periods of meteorologically induced low water levels (Malm et al. 2001). These unpredictable desiccation periods of varying length might explain why the dry-triggered gamete release is not present in the Baltic Sea ecotype of *F. vesiculosus* (Andersson et al. 1992). It does, however, seem to still be present in *F. radicans*, since a pilot study showed a more than tenfold release of eggs from mature receptacles that had been desiccated for one hour and then rehydrated for another hour compared to receptacles kept in water (unpubl. result). This might imply that the deeper distribution range, beginning at 2 m depth (Bergström et al. 2005), has enabled *F. radicans* to keep this trait simply by lack of evolutionary pressure. It might also be that *F. radicans* is closer to the marine origin than the Baltic Sea ecotype of *F. vesiculosus* due to the clonal reproduction which leaves out the recombination of DNA that drives adaptation.

## Effects of salinity on sexual reproductive success

The most sensitive stage to low salinity for algae is during fertilization, as shown for the brown algae *Sargassum muticum* (Yendo) Fensholt (Steen 2004). The salinity requirements of the least tolerant life stage will create the

physiological barrier towards expanding into lower salinities. Several of the steps in *Fucus* spp. fertilization are affected by osmotic changes in the surrounding water. Sodium concentration, i.e. salinity plays a vital role during fertilization for both the egg and sperm. The polyspermy block is dependent on sodium ions (Brawley 1987, 1991), and a low sodium concentration will increase the level of polyspermy which is fatal to the zygote, thus reducing the reproductive success (Serrão et al. 1999). The fertilized egg is protected from lysis once the cell wall is formed, whereas unfertilized eggs from areas of 6 psu rapidly burst from lysis if placed in salinities of 3-4 psu (Serrão et al. 1999).

The sperm flagella that propel them through the water mass are also driven by sodium ions. The fertilization success in the laboratory for *F. vesiculosus* can reach 95% in salinities of 6 and 12 psu, but when measured in the field it varies between 5% up to nearly 100 % in the Bothnian Sea and central Baltic Proper (Serrão et al. 1996b, 1999). Lowering the salinity below 6 psu reduces the proportion of motile sperm and sperm velocity in *F. vesiculosus*, which makes the window of opportunity for successful reproduction in *F. vesiculosus* becomes quite narrow in low salinities (Serrão et al. 1999). This window might become even more restricted in the Bothnian Sea during periods of freshwater runoff.

To explore the effects of salinity on the reproduction, and thus the distribution of *F. radicans*, two experiments were setup to investigate the effect of salinity on egg production and fertilization success (**Paper II**). I also attempted to determine if thalli from sites with high degree of clonality in *F. radicans* were still capable of reproducing sexually (**Paper II**).

## *Fucus radicans* is not limited to the Bothnian Sea due to low-salinity adaptation (Paper II)

*Fucus radicans* and *F. vesiculosus* were collected at a sympatric site outside Gävle in the southern Bothnian Sea (Fig. 3) where the salinity varies from 3.9 to 5.7 psu. Additional reproductive *F. radicans* thalli were also gathered in the northern Bothnian Sea from Drivan, Järnäs and Skagsudde (Fig. 3). The collection was done during the reproductive period to enable sex determination of each thallus. The algae were kept at the Askö Laboratory (Fig. 3) in outdoor seawater flow through tanks with a salinity about 6 psu.

The experiment setup for egg release was created with the three salinities 4, 6 and 12 psu. The northern limit for both *F. vesiculosus* and *F. radicans* distribution is close to 4 psu. The salinity at *F. radicans* southern limit is approximately 6 psu (Forslund et al. 2012), and 12 psu (Öresund area, Fig. 1) is within the salinity range of 10-20 psu where the Baltic Sea ecotype of *F. vesiculosus* has its highest reproductive success (Serrão et al. 1996a). Female receptacles of *F. radicans* and *F. vesiculosus* were placed in water-filled test

tubes with a salinity of either 4, 6 or 12 psu and kept at field like conditions, placed in outdoor tanks with flow through seawater, for 50 days over the reproductive season (Fig. 5a). To relate numbers of eggs released to receptacle size, receptacles were dried at the end of the experiment at 70°C for 24 hours and the receptacle dry weight (DW) determined. To standardize conditions in this experiment artificially made saltwater was used (Andersson 1996).

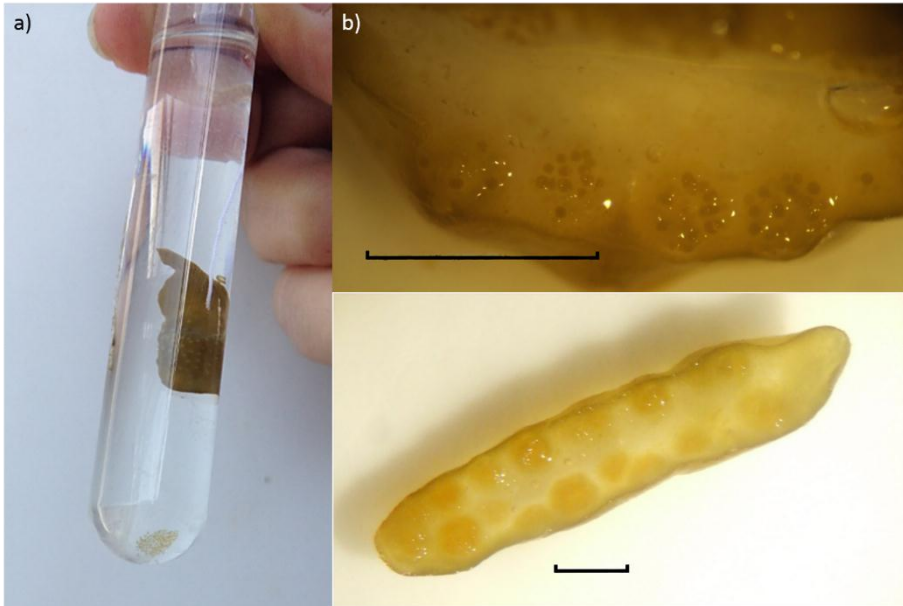


Figure 5. a) *Fucus radicans* receptacle suspended in test tube by a thread. Released eggs can be seen gathered at the bottom. b) Cross cuttings of female (top) and male (bottom) *F. radicans* receptacles. The oogonia are visible as small peas within the conceptacles (top), whereas the male conceptacles (bottom) are perceived as filled with orange (antheridia). Scale bars show 1mm.

The experiment to determine fertilization success was performed at Norrbyn, Umeå University. Sampling of *F. radicans* thalli was done during the reproductive period (July) just before a full moon. Thalli from all sites were first pooled and then separated into males and females by dividing a mature receptacle (Fig. 5b). Outside or in the beginning of the reproductive period, a microscope or similar is often needed. Here, however, the receptacles were mature enough to be distinguishable with the naked eye. The receptacles were then cut off and placed in glass beakers kept damp in dark and cold until about 18:00 h, when water was added, using 8 different salinities (3, 3.5, 4, 5, 6, 12, 24 and 30 psu) to one female and one male beaker each. Ambient seawater (3 psu) was used, the higher salinities made by adding sea salt (NaCl) dissolved in seawater. Gamete release was induced by placing the receptacles in light conditions at 16° C for about 4 hours during the peak in natural gamete

release, which occurs during evening (19:00-22:00 h) (McLachlan et al. 1971, Andersson et al. 1994, Forslund & Kautsky 2013).

Once the gametes were released, receptacles were removed. Each separate solution of eggs and sperm were cleaned of further debris by sieving (mesh size 100  $\mu\text{m}$ ), then sperm and eggs from each matching salinity were mixed for fertilization (Forslund & Kautsky 2013). After fertilization has occurred a cell wall forms around the egg. Fertilization success was calculated by adding Calcofluor White, a stain which makes the cell wall fluorescent under UV light (Brawley & Bell 1987), and then counting both number of fertilized eggs and total number of eggs under a UV microscope.

Increasing the salinity had a clear positive effect on egg production in *F. radicans*, which produced more eggs per DW receptacle in both 6 and 12 psu compared to the natural salinity of 4 psu after 50 days (**Paper II, Fig. 2a**). There was, however, no difference in egg production between the two higher salinities. The egg production in *F. vesiculosus* did not show any response to salinity (**Paper II, Fig. 2b**). Measured as total number of eggs produced during the 50 days of experiment, *F. vesiculosus* released more eggs than *F. radicans*, which was due to the receptacles being almost four times larger. The same positive trend to salinity was seen in the fertilization success, where it is evident that *F. radicans* has maintained an affinity for high salinity (**Paper II, Fig 3**). Even though there was a small dip at 12 psu, *F. radicans* generally performed better in high salinities and almost reached 100 % fertilization in the marine salinities of 24 and 30 psu. The two lowest salinities of 3 and 3.5 psu had to be left out of the analysis since the eggs underwent lysis and could not be counted.

Since the fertilization in fucoids is external, a high gamete concentration is critical for the reproductive success (Pearson & Brawley 1996, Berndt et al. 2002) and a tight synchronous release of gametes in order to minimize gamete dilution is essential to ensure high fertilization success (Pearson et al. 2004). The distribution limits of *F. serratus* (Malm et al. 2001) compared to *F. vesiculosus* can be explained by their adaptation in producing high enough concentration of viable gametes in low salinities (Serrão et al. 1996a, Serrão et al. 1999, Malm & Kautsky 2003). The distribution limit for *F. serratus* in the Baltic Sea is around 8 psu (Fig. 1). In this salinity, *F. serratus* produced less than a 10th of the eggs produced by *F. vesiculosus* from the same site (Malm & Kautsky 2003). The northern distribution of *F. vesiculosus* in the Baltic Sea is also considered to be limited by the low salinity since the distribution limit here correlates with the swimming ability of the male gametes (Serrão et al. 1996a) as well as the minimum sodium concentration needed for the polyspermy block to function properly (Brawley 1987, Serrão et al. 1999). My experiments show that low salinity is also the limiting factor for the sexual reproduction of *F. radicans* and the sexual reproduction limit in *F. radicans* should be around 3.5 – 4 psu. (**Paper II**).



The first part of **Paper II** clarifies that even the populations of *F. radicans* at the northernmost distribution limit with high levels of clonality are also fully capable of sexual reproduction. As an increase in salinity from 4 to 6 psu had positive effects on both egg production and fertilization success in *F. radicans*, the hypothesis presented that the reproduction in *F. radicans* is especially adapted to low salinities was refuted. Increasing gamete concentration and a clear increase in fertilization success in higher salinities show that increasing salinity is not a barrier for further expansion by *F. radicans* into the Baltic Proper. Instead, the hypothesis that the southern distribution limit for *F. radicans* is caused by biotic interactions, not salinity was formed (**Paper II**).

## The effects of competition and herbivory on *Fucus* distribution

Competition for light and substrate will limit the distribution of macroalgae (Pedersen et al. 2012). As a result of the low salinity of the Bothnian Sea the thallus size of *F. vesiculosus* decreases and the algal belt also become less dense (Waern 1952, Ruuskanen & Bäck 1999) so that the competition between thalli for both space and light is reduced. When the salinity increases in the south (Fig 1), the thalli size of *F. vesiculosus* increases from about 20 - 40 cm in the Bothnian Sea (Bäck et al. 1991, Ruuskanen & Bäck 1999, Björkman 2013) up to 110 cm in the Baltic Proper at sheltered sites (Kangas et al. 1982, Bäck 1993, Kalvas & Kautsky 1993). *Fucus radicans* reaches a size of 15-20 cm along the Swedish coast of the Bothnian Sea but only 12 cm in the Quark (Björkman 2013, Forslund & Kautsky 2013). According to Kautsky et al. (1986) and Wahl et al. (2011 and refs. therein) growth rate in *Fucus* spp. is determined by light availability. As the growth of juvenile *F. vesiculosus* is limited by shading from the taller adult canopy (Eriksson et al. 2006) in sympatric sites where *F. vesiculosus* grow taller than *F. radicans*, similar effects of shading might be expected on the growth rate of *F. radicans*.

In **Paper II** the effects of shading as a limiting factor was investigated together with the factor grazing. Grazing is a well-known limiting factor for macroalgal distribution (i.e. Worm & Lotze 2006, Sjøtun et al. 2007). The main herbivores in the Baltic Sea actually grazing on *Fucus*, not just its epiphytes, are isopods of the genus *Idotea* (Salemaa 1979), mainly *Idotea balthica*, which has even been shown to have a preference for *Fucus* species over other macroalgae (Jormalainen et al. 2001). *Idotea balthica* is a marine species and its distribution in the Baltic Sea is limited by salinity (Naylor 1955) reaching about half-way into the Bothnian Sea (Leidenberger et al. 2012). Events of severe reduction of *Fucus* belts during very high densities of *I. balthica* has been documented on both the Swedish (Engkvist et al. 2000,

Nilsson et al. 2004) and the Finnish (Kangas et al. 1982, Rönnerberg et al. 1985) coasts. According to Jormalainen & Ramsay (2009) a reduction of light availability through shading also increased the level of isopod grazing on *Fucus* as well as reducing the thallus growth.

The field experiment presented in **Paper II** combines the single effects of shading and grazing in order to explore if one or both factors, singularly or through additive effects, contribute to the present southern distribution limit of *F. radicans*.

## The southern distribution limit is set by biotic factors (Paper II)

The depth range of *F. radicans* in the Bothnian Sea reaches from 1.5 meters down to depths of 6-8 meters (Råberg & Kautsky 2007, own. obs.). The *F. radicans* thalli used were previously collected material from Järnäs, Drivan and Skagsudde (Fig. 3) that had been acclimatized to shallow depth light conditions at approximately 2 meters for two years prior to the experiment.

The experiment consisted of four treatments; 1) shading, 2) grazing, 3) grazing + shading and 4) control. The light attenuation caused by the mesh within the cages was equal to that of about 2 m depth (control). The shading treatment consisted of short *F. vesiculosus* branches with air bladders floating on top within the cage, thereby creating a light reduction of 85%. This amount of shading will affect the growth rate of *F. vesiculosus* (Middelboe et al. 2006). The adequate grazing pressure of reported normal natural densities of *I. balthica* (Engkvist et al., 2000, Orav-Kotta & Kotta, 2004) was calculated to one *I. balthica* per 5 g *F. radicans* biomass (WW), or 7 individuals per cage. The cages were placed at 0.5 m depth in a shallow bay outside the Askö Laboratory (Fig. 3) for seven weeks during late summer 2013. The fronds of *F. radicans* were weighed before and after the experiment, using the biomass production (g WW) as measure of effect.

The results indicated that both shading and grazing had a negative effect on biomass production in *F. radicans*. The biomass increase of 16% in the shaded treatment did not differ from the 22% increase of the control, which the grazed and the grazed+shaded treatment did (8.5% and 0.8% increase in biomass respectively). These two did however, not differ from each other and the combined treatment was the only showing a net loss in biomass for some replicates. The results in **Paper II** support the suggestion that the additive effect of shading and grazing might hamper the further distribution of *F. radicans* southwards into the Baltic Proper (Fig. 6).

An increase in salinity from 5 to 7 psu will have a positive effect on the growth of *F. vesiculosus* (Wallentinus 1979), which in turn might increase the level of shading of the smaller *F. radicans*. The abundance of the grazer *I.*

*balthica* is also favored by increasing salinity (Salemaa 1979, Leidenberger et al. 2012, **Paper I**). Observations by Kangas et al. (1982) as well as personal observations show that *I. balthica* prefer to graze on weakened (by shading or age) thalli growing in the lower part of the *Fucus* belt distribution. A further decrease in light attenuation due to reduced Secchi depth or epiphytic filamentous algae in the more nutrient enriched waters of the Baltic Proper compared to the Bothnian Sea might increase grazing on *F. radicans* enough to suppress further range expansion (Fig. 6). The response of *F. radicans* to these direct (i.e. salinity) and indirect effects (i.e. increased grazing pressure) of climate change will define its future realized niche.

In **Paper II**, the effects of salinity was only investigated on the formation of zygotes and the limiting effects of grazing and competition was only tested on adult thalli. Future experiments should address the effects of all three factors on survival rate of sexually recruited juveniles. Germling survival of *F. vesiculosus* showed that although a large number of zygotes settled during the season (up to 1 000/cm<sup>2</sup>), survival had dropped by at least one order of magnitude after two months (Serrão et al. 1997). Also, grazing preferences and consumption rates can differ between juvenile and adult tissue (Van Alstyne et al. 1999). It is possible that the effects of grazing on juvenile *F. radicans* by the gastropods *T. fluviatilis* and *Radix peregra* O. F. Müller, 1774 is similar to those for juvenile *F. vesiculosus* (Malm et al. 1999). It would, however, be of great interest to further explore if these gastropods show a preference of one *Fucus* species over the other, as seen in the isopod *I. balthica* (Forslund et al. 2012, **Paper I**). Any effect of *T. fluviatilis* or *R. peregra* grazing on *F. radicans* juveniles should be further investigated, as both species are present within the whole distribution range of *F. radicans* (**Paper I**) and may have an impact on the recruitment of new individuals.

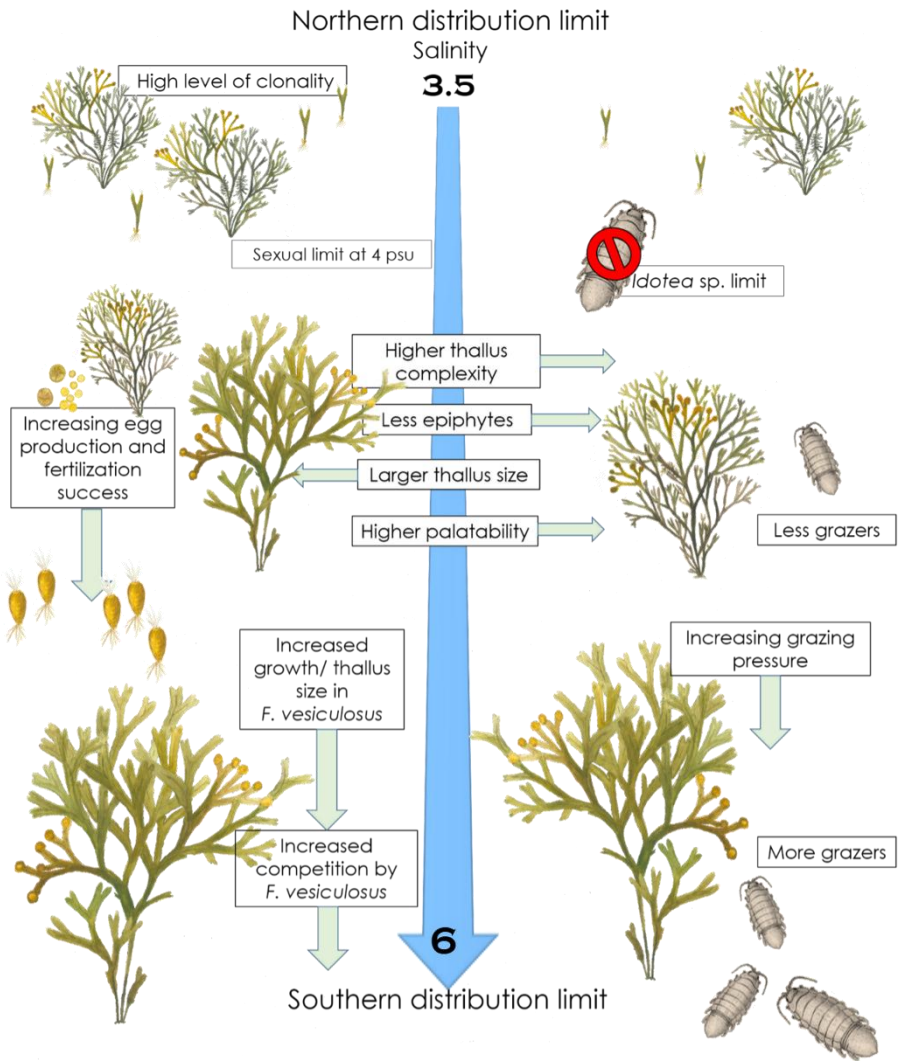


Figure 6. A model of the main findings on what factors are affecting the distribution of *F. radicans* presented in this thesis.

# Re-attachment of fragments – the asexual reproduction

Asexual reproduction through different forms of clonality is not uncommon in macroalgae (e.g. Santelices & Varela 1993, Collado-Vides 2001, Wright & Davis 2006) and is believed to have evolved independently at several occasions within and between clades (Lane et al. 2006). Studies on seagrass (*Zostera marina*) shows that clonal growth is more important for population maintenance at the species geographical distribution limit compared to core sites (Billingham et al. 2003). For macroalgal species living in marginal habitats it is not uncommon with a reduced sexual reproduction (Dorken & Eckert 2001). Several of the macroalgae species present in the Baltic Sea today have to some extent reduced the sexual stage in reproduction due to the low salinity (Hällfors 1985). Among the macroalgae species still present in the lower salinities (< 6 psu) of the Baltic Sea, have many adapted their life cycles by shifting towards asexual reproduction, e.g. *Ceramium tenuicorne* Kützing (Bergström et al. 2003) and *Furcellaria lumbricalis* (Hudson) J.V. Lamouroux (Kostamo 2008).

Asexual reproduction through fragmentation was not previously described as occurring in the *Fucus* family, although the opposite with adventive embryos forming from detached rhizoidal filaments was discovered by McLachlan & Chen (1971). The adventive branches that form on *Fucus* thalli in response to wounding, especially so in *F. vesiculosus*, do not reattach or regenerate a new holdfast (Moss 1961). However, clonality in *F. vesiculosus* was discovered by Tatarenkov et al. (2005) in the Baltic Sea, but only limited to within site at the northernmost distribution limit. Interestingly, this type of asexual reproduction seems to occur in *F. radicans*, where a high percentage of detached fragments developed new rhizoids and thus became new attached individuals (Tatarenkov et al. 2005).

Gene flow among populations depends largely on the dispersal range of the zygotes (Santelices 2002). The asexual reproduction in *F. radicans* has increased the species' ability for long-distance dispersal from a few meters (Serrão et al. 1997, Coleman & Brawley 2005) to almost 550 km, where one female clonal lineage has been present at several sites along the Swedish coast of the Bothnian Sea (Johannesson et al. 2011). Compared to the populations in Estonia, there is much less genetic differentiation between Bothnian Sea populations both on the Swedish and the Finnish coast (Pereyra et al. 2013)

which demonstrates how dispersal by fragments enables a gene flow between geographically distant populations within the Bothnian Sea.

In the Estonian populations of *F. radicans* (Fig 1, 3.), clonality has so far not been found and genetic analyses show a stronger affinity between Estonian *F. radicans* and *F. vesiculosus* than between *F. radicans* from Estonia compared to *F. radicans* from the Bothnian Sea (Johannesson et al. 2011, Pereyra et al. 2013). It is not yet resolved why the populations within the Bothnian Sea have such high levels of clonality or why the trait of clonal reproduction seems to be absent in the Estonian populations.

The trigger and mechanism behind the asexual part of *F. radicans* life cycle remains to be identified. It is possible that some abiotic factors are involved. To address this knowledge gap, an experiment was conducted to investigate the effects of light and temperature on the settling of *F. radicans* fragments (**Paper III**). This experiment was a first attempt at discovering if the asexual reproduction in *F. radicans* has a window of opportunity similar to that described for *F. vesiculosus* sexual reproduction (Serrão et al. 1999) and which might be determined by a certain time of the year or other factors. The small fragments are easily disturbed and even low water movement might lift them from the bottom up into the water column. Tatarenkov et al. (2005) suggested that low water movement might be essential for *F. radicans* fragments to successfully attach to the substrate. This would best correspond with winter when translated into seasonal conditions, as ice cover will create extended calm periods. The distribution range of *F. radicans* in the Bothnian Sea is within areas that are covered by ice during 2-4 months most winters (HELCOM 2013).

## Light and temperature affect re-attachment of fragments (Paper III)

Fifteen different thalli, both males and females, from 3 sites (Drivan, Järnäs and Skagsudde) (Fig. 3) were collected during summer and kept at Askö Laboratory in outdoor flow through tanks with ambient salinity (6 psu) for four months until transported to the experiment facilities in Roskilde, Denmark. Each thallus was packed separately to keep them from mixing. In the laboratory, a hundred or so fragments, 5-10 mm long, were removed from each thallus. Each replicate consisted of four randomly selected fragments on a small ceramic tile in a transparent plastic jar with lid. By using both male and female thalli from three sites we assumed that more than two genotypes were present in spite of the high levels of clonality documented at these sites (**Paper IV**) (Johannesson et al. 2011).

The setup used the factors light and temperature combined in four treatments to mimic the abiotic conditions of the four seasons, with warm

water temperature and high light conditions representing summer, cold water temperature and low light conditions representing winter and cold water temperature with high light conditions representing both spring and autumn. The warm water temperature and low light conditions treatment was not ecologically relevant but was needed to balance the experimental setup design. High and low temperatures ( $+14^{\circ}\text{C}$  and  $+4^{\circ}\text{C}$  respectively) correlate with the winter and summer temperatures of the Bothnian Sea (HELCOM 2013). The light levels were set at  $96\text{-}100\ \mu\text{mol m}^{-2}\ \text{s}^{-1}$  with a 16:8 light:dark regime for high light conditions, equal to a clear summer day at the sampled sites (Serrão et al. 1999) and  $30\text{-}33\ \mu\text{mol m}^{-2}\ \text{s}^{-1}$  with a 6:18 light:dark regime for low light conditions, equal to under ice conditions (Tulonon et al. 1994). The experiment used natural seawater from the North Sea diluted with tap water (with naturally high DIC levels) down to a salinity of 5 psu. The water in each replicate was replaced at halftime (3.5 weeks) in order to avoid nutrient depletion (McLachlan 1977, Bidwell & McLachlan 1985). The experiment ran for seven weeks, after which the number of settled fragments was counted and general appearance of fragments was observed.

The results of the experiment revealed that high temperature and high light conditions i.e. the summer-mimicking treatment resulted in the highest settling percentage ( $45.8 \pm 8.1\%$ ) (**Paper III, Fig. 1**). For spring/autumn  $15.6 \pm 6.5\%$  settled, and in winter  $14.6 \pm 4.2\%$  settled. The high temperature, low light –treatment showed the lowest settling, with only  $2.1 \pm 1.0\%$  success. The results showed an interaction between light and temperature. Light affected settling success in the high temperature, with higher settling in the high light than the low light, but not in the low temperature. Temperature, on the other hand, increased settling in high light but not in low light (**Paper III, Table 1**).

Due to the manipulation of both light intensity and day length between the two light treatments, distinguishing the individual effects of these two sub factors on the settling success of *F. radicans* fragments was not possible. Temperature has been shown to affect the level of sexual reproduction in a clonal kelp, whereas increased levels of nutrients instead favoured clonal growth (Demes & Graham 2011). There was, however, no interactions found between the two factors for kelp, but nutrient level would be a relevant factor for further experiments on fragment reattachment, as the nutrient levels in the Bothnian Sea seems to be increasing (HELCOM 2014).

Examinations of the fragments under a stereoscope and later under ESEM revealed how thick bunches of long hairs protruded from the cryptostomata openings of the fragments (Fig. 7). The function of hyaline hairs is similar to that of the apical hairs of zygotes and euryhaline hairs formed in some filamentous red algae (DeBoer & Whoriskey 1983) in assisting the uptake of nutrients (Chapman 1995 and refs. therein). The hyaline hairs that develop on *Fucus* both apically and from cryptostomata openings along the midrib have been shown to facilitate phosphate uptake (Hurd et al. 1993). It was also noted

by McLachlan (1977) that *Fucus zygotes* grown in nutrient poor medium developed several long apical hairs. A small fragment might thus be assisted in its growth by the formation of more cryptostomata hairs if the surrounding medium is low in some essential nutrient such as phosphate.

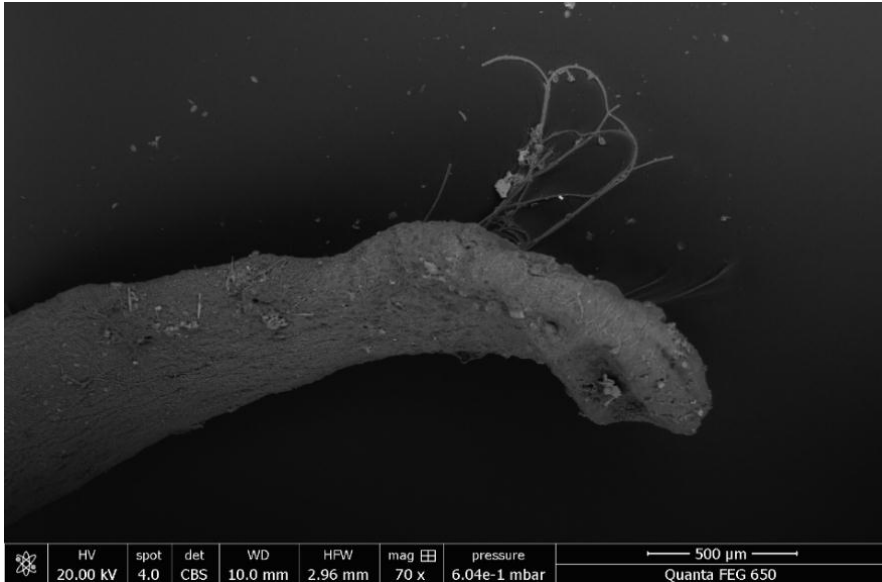


Figure 7. Environmental Scanning Electron Microscope (ESEM) image showing the apical part of a *Fucus radicans* fragment. Several cryptostomata openings are visible along the upper edge and long cryptostomata hairs protrude from each opening.

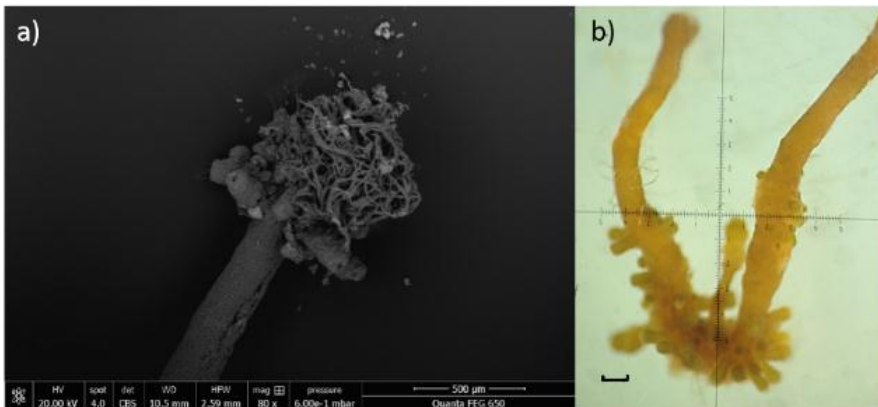


Figure 8. ESEM photography (a) showing the basal part of an unattached fragment where rhizoids have formed (scale bar shows 500  $\mu$ m) and also the rich formation of new shoots (stereoscope image, scale bar shows 1 mm) (b)

Basal rhizoid formation could be seen on several fragments under a stereoscope, with or without forming any attachment to the substrate (Fig. 8a). On most fragments apical growth was observed, but also the formation of one



or several new shoots from the basal part (Fig. 8b), which might be a response to the wounding of tissue, same as in *F. vesiculosus* (Moss 1961, Van Alstyne 1989).

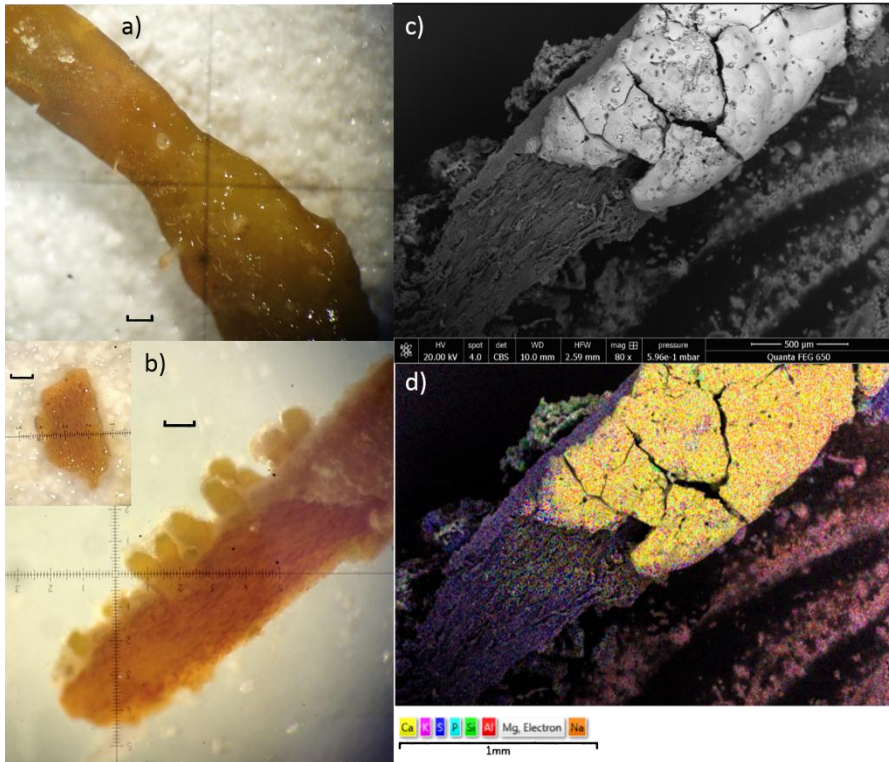


Figure 9. a) The side of the fragment facing the tile here upturned and viewed under a stereoscope, revealing an opaque substance, also visible in b) where the strength of attachment tore the fragment apart, leaving a piece still attached to the tile (inset). On the upper side of the fragment can be seen green new shoots forming. c) The same fragment viewed under ESEM and d) an element analysis using EDS (energy dispartative x-ray spectroscopy) showing the main chemical components, the opaque substance consisting mainly of calcium (Ca).

Several fragments had formed another type of strong attachment to the substrate by what seemed like secreting an opaque substance which glued the side of the fragment to the ceramic tile (Fig. 9a), leaving both the basal and apical ends more or less free. The strength of the attachment was thus so strong that part of the fragment cortex remained attached to the ceramic tile when the fragment was removed using tweezers (Fig. 9b). When the fragments that had secreted opaque substance were viewed under ESEM (Fig. 9c), the origin of substance from around the cryptostomata openings became clear (**Paper III, Fig 5**) although there was no determining whether it was secreted by the openings or merely initially formed around them. Through an EDS (energy dispartative x-ray spectroscopy) analysis of element composition it was

revealed that calcium (Ca) was the main compound of this secretion, with calcium carbonate (CaCO<sub>3</sub>) the most likely form, though this is not confirmed by the analysis (Fig. 9d). It is possible that some sort of ion precipitation was initiated or assisted by the presence of cryptostomata hair, either by creating a high Ca<sup>2+</sup> concentration around the fragment through selective uptake or merely by being a substratum for crystal growth.

A sexually produced *Fucus* zygote forms an extracellular adhesive mucilage in order to attach to the substratum (Hardy & Moss 1979). Observed gold-plated under SEM, the mucus was described as “small threads” (Moss 1981, and photos therein). No precipitations similar to those observed in **Paper III** are mentioned, nor do the photos of mucilage excretion by Hardy & Moss (1979) or Moss (1981) in any way resemble the here observed calcium excretion. It should however be of interest to perform an EDS analysis of the zygote adhesive mucilage for comparison of the element composition.

# Spatial distribution and evolution within and between *Fucus radicans* populations

The theory of sympatric speciation was first published by Darwin (Darwin 1859), and since then several attempts have been made to either prove or falsify it (e.g. Mallet 2008 and refs. therein). Classically, speciation has been divided into four separate models; allopatric, peripatric, parapatric and sympatric speciation (Coyne & Orr 2004). However, this rigid classification has since been abandoned, as it leaves little room for the changing nature and mechanisms involved in speciation (Mallet 2007). Butlin et al. (2008) suggest that it would perhaps be more correct from an ecological point of view to consider allopatric and sympatric speciation to be the endpoints of a less definable continuum of initial levels of gene flow between diverging populations.

The high level of clonality in *F. radicans* has been suggested as a possible mechanism behind the divergence between the two species *F. vesiculosus* and *F. radicans* (Pereyra et al. 2009). The Frozen Niche Variation hypothesis states that genotypes who reproduce clonally are “frozen” (Vrijenhoek 1979, 1984). Their particular adaptations to the ecological niche remains unchanged, since there is no genetic overlap in asexual reproduction, just “copy-paste”. The higher level of specialization this entails should therefore make clonal populations more successful within their potential distribution range than sexually recruited populations, whose offspring will be more randomly adapted (Vrijenhoek 1979). The genotypes of *F. radicans* with the best ability to reproduce clonally would thus be predicted to be present in higher abundance within low salinity environments. The genetic isolation caused by asexual reproduction might have caused the rapid speciation process documented by Pereyra et al. (2009).

The variations in salinity caused by upwelling or river runoff can result in spatial differentiation on local scales. An increase in salinity through upwelling of water with higher salinity during the reproductive period might enable sexual reproduction locally, which will create new genotypes within a population. Decreasing salinity from river runoff will instead favour the proportion of asexually recruited thalli.

Regarding seaweeds, there is a lack of knowledge concerning such questions as what the degree of genetic relatedness is between mates and possible effects of inbreeding or how changes in gene flow under different

field conditions affect the genetic variability between populations (Santelices 2002). The red macroalgae *Chondrus crispus* Stackhouse for example, shows a difference in the spatial distribution between gametophytes and tetrasporophytes (Mathieson 1982 in Scrosati et al. 1994, Kautsky & Ehn 1992), which might be caused by different ecophysiological properties between the two reproductive phases (Carrington et al. 2001).

In **Paper IV** it was therefore hypothesized that the relative age of separate clones would show a positive correlation to geographic distribution, i.e. that old clones would be more widely distributed, perhaps found further apart and at several sites, whereas young clones would only be found within one site, and at close distance from each other (cluster), similar to the pattern found in large seagrass clones (Reusch & Boström 2010).

Accordingly, clones differing from each other by only 1-2 mutations were assumed to be closely related and likely to be derived from each other, forming multi-clonal lineages (MLL's) (Arnaud-Haond et al. 2007). The aim of this analysis was to create a family tree of the dominant clones. Such patterns of genetic variation within and between populations and spatial distributions might give further insight in the process of speciation as well as provide more information on the extent of fragment dispersal both within and between populations. Hence, the study presented in **Paper IV** investigated if there were any differences in the spatial distribution between the sexes and/or the major dominant clones of *F. radicans*.

## Does the clonal apple fall far from the tree? (Paper IV)

The field sampling of this study was carried out at Järnäs (Swe P) and two different sites (Swe N and Swe O) at Drivan, northern Bothnian Sea, where the salinity ranges between 3.5 -4.5 psu (Fig. 1, 3). Both Järnäs and Drivan contain mainly *F. radicans*, and at Järnäs only female *F. radicans* thalli were found (n=95). It is interesting to note that the skewed sex ratio with 80 % females that was sampled at Drivan in 1995 and 1996 by Serrão et al. (1999) had since changed and the sex ratio found in 2010 was now close to 50/50, suggesting a rather fast increase of male thalli in the population. Returning to the Järnäs site during 2011 and 2012, sampling 25 and 100 thalli respectively, still only female thalli have been found (pers. obs.).

The sampling of thalli was done by placing 4 (at Järnäs only 3) 30 m measuring tapes along a gradient between 0.5 m 6.0 m depth with a common origo, beginning at the start of the *Fucus* belt and spreading deeper in a fan-shape, encompassing a total angle of 120 degrees at each site. SCUBA divers using a hand held measuring tape proceeded to sample thalli found within 1 m on each side of the measuring tape, thus creating a 2 m wide transect. The depth and location of each thalli relative to the transect (0-1 m) and on the transect (0-30 m) was noted, and a small piece of each thallus carrying both

receptacles and vegetative tips was placed in a pre-tagged bag. These were used to first determine the sex of the thallus and was afterwards stored individually in silica gel prior to the genetic microgeographic analyses. The genetic analyses were performed at the Sven Lovén Centre for Marine Sciences, Tjärnö, where the DNA from each sample was extracted and amplified before genotyped at nine microsatellite loci (L20, L38, L58, L85, L94: Engel et al. 2003; and Fsp1, Fsp2, Fsp3, Fsp4: Perrin et al. 2007) using a standard kit.

The analyses of the spatial coordinates together with the associated sexual and genetic data were used to create geographic maps of genotypes (MLGs), clonal lineages (MLLs) and sex for each of the three sampled sites (**Paper IV, Fig. 1 a-f**). Only a few large clones were found, and most thalli belonged to any of these. In all three sites a large female clone was dominant and another female clone was also relatively common. Two of the male clones found at the Drivan sites were however common enough to also be counted as dominant. The all-female Järnäs site showed two dominant clones. There were also thalli belonging to smaller clones on all three sites, as well as unique (i.e. only found once in the samples) genotypes. The analyses showed that the spatial aggregation of clones was low. However, the chances that two thalli belong to the same clone decreased with distance. A probability of 31-39% within 1 m was reduced to 8-24% at 30 m (**Paper IV, Fig. 2**), although most clones seemed to have a large spatial distribution even between sites. This differs from the clonal distribution pattern in the vascular plant *Z. marina* where most clones are found within one population or area (Reusch & Boström, 2010).

There was no correlation between clonal distribution and depth distribution nor between sex and depth distribution and the general pattern showed an intermingled genotype distribution, similar to patterns found in other fragment-dispersed organisms (Wulff 1991, Heinken 1999, Ceccherelli & Piazzini 2001)

A site where a few clones are dominant indicates a low sexual recruitment, either due to low salinity (**Paper II**), unknown favourable conditions for asexual re-attachment (**Paper III**) or only one sex is present. Successful sexual events at such sites are most likely very rare, as the pattern of variation is dependent on the number of sexually recruited individuals in each generation, and even one sexual event in three years will result in highly divergent genets (Bengtsson 2003). It is highly likely that one or two successful reproductions occurred at the site Drivan (Fig. 3) between 1996 and 2010 to cause the change in sex ratio previously mentioned.

To visualize the extent of gene flow on a larger scale by mapping the spatial distribution on a macrogeographic scale, we added data from three previously unpublished sites (Swe K – M) at Skagsudde (Fig 3) and 12 sites published by Johannesson et al (2011), compiling a total of 16 sites encompassing the entire distribution range of *F. radicans* in the Bothnian Sea and Baltic Proper (**Paper**

**IV, Table 1 and Fig 4)** (Forslund et al. 2012). The analysis show a clear pattern of asexual dominance in the lower salinities of the Bothnian Sea, both on MLG and MLL level (**Paper IV, Fig 4**). The more sexual populations on the Finnish coast might be explained by the slightly higher salinity due to the current direction (Feistel et al. 2012), which thus enables sexual reproduction (**Paper II**) same as for the populations in Estonia (Fig. 1).

The genetic relationship between clones on this macrogeographic scale was also analysed by measuring the differing number of somatic mutations and creating a network (family tree) of clonal lineages (MLLs) (**Paper IV, Fig 3**). The results show that “new” genotypes are formed via somatic mutations which in the clonal populations of *F. radicans* provides genetic diversity. Here, the dominant female also had the largest number of closely related genotypes (MLGs), showing a high level (>30) of total somatic mutations in the 9 microsatellite loci analysed. The dominance both in ramets and closely related genets suggests that the female clone has been present for a long time. Considering the young age of the species (Pereyra et al. 2013), this clone might have originated early on in the history of *F. radicans*. Another clone likely to be quite old was a large male clone, whose distribution ranged over 100 km (**Paper IV, Fig 4**). It is interesting to note that the more or less wholly sexually reproducing populations of *F. radicans* in Estonia hardly produce any adventitious branches, thus allocating all energy on sexual reproduction by producing numerous receptacles (Forslund & Kautsky 2013, **Paper I, Paper V**), whereas the clones in the Bothnian Sea produce both numerous adventitious branches and viable receptacles for sexual reproduction (Tatarenkov et al. 2005, **Paper II, Paper III**), which might make for better adaptation. Although the sexual recruitment within the Bothnian Sea populations is low even when both sexes are equally present, the low salinity during the reproductive period will determine the sexual success (**Paper II**).

# Sexual dimorphism on a macroscopic scale

It is often possible to tell the sexes apart in animals by their colour, shape or size. In Phaeophyceae, any sexual dimorphism is often on a microscopic scale commonly between male and female gametes, as is the case in the *Fucus* genera (Vernet & Harper 1980). Sexual dimorphism on a macroscopic scale in brown macroalgae is, however, rather uncommon (Kogame et al. 2005). **Paper V** presents evidence that there is such a macroscopic dimorphism between female and male thalli of *F. radicans*, which appears to be a species specific character.

## Morphological characters in *F. radicans* male and female thalli (Paper V)

It became apparent after several fieldtrips of sampling thalli that it was possible to distinguish between male and female *F. radicans* thalli simply by their size, both on the thalli itself and its receptacles. To test the accuracy of this notion, a sampling was made at Skagsudde (Fig. 3) of 40 thalli, of which a 50/50 sex ratio was wanted. 16 out of 20 thalli sampled as males and all 20 females turned out to be correct.

Thus I formed the hypotheses that there was a macroscopic sexual dimorphism present in *F. radicans*, that *F. radicans* males had smaller thallus and receptacles respectively than females and that this sexual dimorphism would only be found in *F. radicans*, not any other dioecious furoid. I also investigated if the apparent sexual dimorphism was a species characteristic or simply an artefact of clonality by sampling both the highly clonal populations of *F. radicans* in the Bothnian Sea (Fig.3, **Paper V Appendix**) and the Väinameri Archipelago Sea populations in Estonia (Fig 3, site 7, 9 and 10), where no clones have yet been found (Johannesson et al. 2011, **Paper IV**).

The sampling of *F. radicans* in the Bothnian Sea for this study was done using SCUBA by persons unaware of the aim of the study in order to get an unbiased sample of the two sexes. Several morphological characters used to differentiate between *Fucus* species (Bergström et al. 2005, Zardi et al. 2011) were measured in order to determine if sexual dimorphism actually occurs in *F. radicans*. On each individual thalli five mature receptacles were cut off and measured (height, length, width, WW, DW and DW/WW ratio) to give an

average measure. Additionally, the number of conceptacles per receptacle was counted. To compare the variation in receptacle size and weight, female and male receptacles were also collected from two other dioecious *Fucus* species, *F. serratus* and *F. vesiculosus*, which are both present in the Baltic Sea (Fig. 1).

**Paper V** shows that there is a macroscopic sexual dimorphism for several characters in *F. radicans*. Although not uncommon within Phaeophyceae, this has previously not been documented in fucoids.

The receptacles in male *F. radicans* are smaller than female receptacles (Fig. 10) and also have a higher DW/WW ratio than the female receptacles. The difference in DW/WW ratio shows that male receptacles have a lower water content, thus feeling more firm to the touch. The number of conceptacles per receptacle however, does only differ between the sexes in the Vänameri Archipelago Sea, not in the Bothnian Sea.

Male thalli had a higher Fertility Index (F.I) than female thalli (28% compared to 13%). Consistent with the findings regarding complexity in **Paper I**, thalli from the Bothnian Sea also had a higher number of total apices ( $p= 0.002$ ) than those from Vänameri Archipelago Sea. This combines to make male thalli in the Bothnian Sea very bushy, as many apices implies more dichotomies, if the thalli are of similar height.

The genetic diversity within *F. radicans* has been mapped in several sites throughout the whole distribution range (**Paper IV**). The ability for long-range dispersal through fragmentation provides a large gene flow over large distances. At the same time there is a high level of clonality, with a few dominant clones found in several locations. The possible effects of clonality on phenotype, however, does not explain the sexual dimorphism presented in **Paper V**. As the macroscopic sexual dimorphism was found both in the highly clonal Bothnian Sea populations of *F. radicans* and in the Vänameri Archipelago Sea populations, although slightly less pronounced, this suggests larger receptacles on female thalli to be a species specific trait in *F. radicans*.

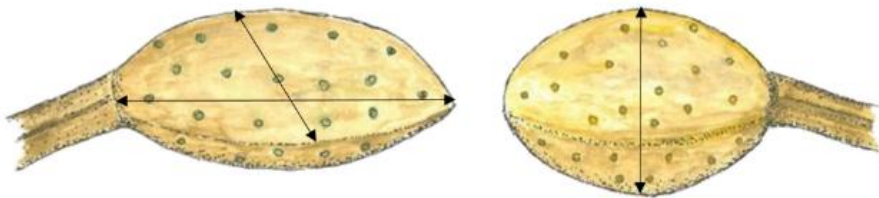


Figure 10. Receptacles of female (left) and male (right) *F. radicans* shown to scale with arrows illustrating length, width and height measurements (illustration by L. Kautsky).



# Concluding remarks

The main aim of this thesis has been to contribute to more biological and ecological knowledge about the perennial macroalgae *Fucus radicans*. Not only because so far little is known about this new species, but also to aid future management and preservation of an ecologically important foundation species endemic to the Baltic Sea.

## The distribution of *F. radicans* in the Baltic Sea

A summarized overview of my main findings regarding the distribution of *F. radicans* is schematically presented in figure 10.

**In Paper II** I both investigate if the present distribution of *F. radicans* is a result of competition by *F. vesiculosus* and/or grazing by *I. balthica* and speculate on the possibility of a marine origin of *F. radicans*. *Fucus radicans* in the Bothnian Sea is generally smaller than *F. vesiculosus* (**Paper I**). Not being able to compete for light in higher salinities and thereby become more palatable to grazers (**Paper II**) might have led to *F. radicans* being confined to the Bothnian Sea. This possible scenario would also explain the presence of the *F. radicans* population in the Väinameri Archipelago Sea around the island Saaremaa on the Estonian coast (Fig 1), but it is not supported by the phylogeographic findings of Pereyra et al. (2009). These show a close relationship between *F. radicans* and the Baltic Sea ecotype of *F. vesiculosus*. Instead, Pereyra et al. (2013) suggest that the origin of *F. radicans* occurred in parallel within the Baltic Sea, once in the Bothnian Sea, and once in the Väinameri Archipelago Sea.

Regardless of origin and how the present distribution came about, the question of future distribution remains. The predicted dilution of the Baltic Sea salinity by Meier et al. (2006) and Friedland et al. (2012) might enable *F. radicans* to enter the Baltic Proper in the future (**Paper II**). The scenario of endemic speciation and *F. radicans* young age as a species (Pereyra et al. 2009, 2013) might help explain the present distribution in the sense that if *F. radicans* evolved in the Bothnian Sea, it is actually spreading south, but has not reached further than the present range due to a combination of dispersal range and time. The widespread dispersal of one female clone (**Paper IV**), suggest that this clone has been around a long time in the species' history in order to establish over such a large geographic range. The scenario of endemic

speciation in the Bothnian Sea does not present a satisfactory explanation for the Estonian population, though, as this seemingly lacks the asexual dispersal, as is shown by both the morphological complexity studies in **Paper I** and the genetic mapping in **Paper IV**, but has the species specific sexual dimorphism (**Paper V**). Instead, it has been suggested that a parallel speciation has occurred, where *F. radicans* has evolved independently in two geographically separate locations (Pereyra et al. 2013). Further, in the Estonian populations the reproductive time differs between *F. radicans* and the sympatric *F. vesiculosus* (Forslund & Kautsky 2013), which might be the driving mechanism behind a speciation event there (Palumbi 1994).

## Possible effects of predicted climate change on *F. radicans*

The predicted changes in climate for the Baltic Sea are an increase in temperature by 3-4 degrees over the next 50-60 years which will lead to an increase in precipitation, and thus increased river runoff by 15-20 %. This would dilute the salinity of the Bothnian Sea by 1 psu and the Baltic Proper by 2 psu (Meier et al. 2012, HELCOM 2013). In my thesis, I have focused mainly on the direct effect of salinity on *F. radicans*, but also the indirect effects of increased grazing and competition (**Paper II**). Although the asexual dispersal seems to be less sensitive to salinity there is a limit to where adult thalli can survive, close to the limit for sexual reproduction. Even though *F. radicans* can disperse by fragments, adult thalli are only found a couple of kilometres further north of Drivan (Forslund et al. 2012).

The genetic mapping presented in **Paper IV** contributes more insight into the intriguing genetics of *F. radicans*. Little interdisciplinary research has been performed on how rear edge populations respond to changing climate (but see review by Hampe & Petit 2005), considering these populations may possess unique genetic traits that are not present in core populations. However, as *F. radicans* is only present in a marginal marine environment, seen as rear edge for many marine species, it is difficult to determine what populations are edge and core in *F. radicans*.

There is a remarkably low genetic diversity within *F. radicans*, with as many as seven loci out of nine being homozygous in the large female clone (Bergström et al. 2005, **Paper IV**). Such low genetic redundancy is considered to increase a species vulnerability (Frankham 2005). As *F. radicans* is dominated by a few widespread clones, any large scale change in climate conditions which would take out one of the clones would cause a massive loss in biomass and habitat in the Bothnian Sea. Such large-scale effects on the associated flora and fauna (**Paper I**) will in turn affect the whole ecosystem.

Predicted future increases in precipitation (Meier et al. 2012), for example, will quite possibly have an effect on the distribution of *F. radicans*. Already existing near its limit of sexual reproduction, even a slight decrease in salinity from 5 to 4 psu would cause a decrease in *F. radicans* sexual recruitment. However, this might not necessarily reduce the reproductive output of *F. radicans*, but instead the affected populations would be recruited from more or less wholly asexual dispersal (**Paper II, III & IV**), but maintaining the previously mentioned implications of low genetic redundancy.

Rear edge populations does not always have a lower genetic diversity (Eckert et al. 2008, Zardi et al. 2015). Loss of genetic diversity in a species contribute to the risk of extinction, either by extinction of locally adapted populations or by inbreeding. In order to conserve the genetic structure of rear edge populations the best strategy would be to find and preserve as many local populations as possible, according to Hampe & Petit (2005) as these may be genetically unique in their adaptations to their respective environment (Schmidt et al. 2008, Larsen et al. 2012, Neiva et al. 2012). A high genetic diversity may enhance the rate of recovery in a species after an extreme climate event and thus reduce the effects on the ecosystem functioning and the associated communities (Reusch et al. 2005), which are reported for *F. radicans* in **Paper I**. Genetic factors, such as the genetic diversity presented in **Paper IV**, should therefore be included in population stability assessments, as leaving them out may lead to an underestimation of the real threat, resulting in inadequate or poor management decisions (Frankham 2005).

## Considerations from a management perspective.

The National marine monitoring program uses the presence, percentage cover and depth distribution of *Fucus* spp. in the Baltic Sea as an indication of light penetration/turbidity and thus of the quality of water. Both macroinvertebrate abundance and taxon richness has also been shown in other studies to increase with increased macrophyte diversity (Parker et al., 2001; Hansen et al., 2008). Habitat complexity, created by *Fucus* in the Baltic Sea (**Paper I**), plays a critical role in determining species richness and rates of habitat recoveries (Kovalenko et al. 2012). This gives complexity a crucial influence on the relative performance of marine protected areas (MPAs) and it has been suggested by Miller et al. (2011) that environmental complexity should be incorporated when assessing the effectiveness of MPAs. It is therefore important to keep both *F. radicans* and *F. vesiculosus* viable in order to maintain the seascape scale diversity and promote the stability of the species-poor coastal ecosystem of the Baltic Sea (Wahl et al., 2011), which need to be considered in marine planning..

The results in **Paper I** shows that there is a need to separate *F. vesiculosus* from *F. radicans* instead of only noting presence of *Fucus* sp. during

monitoring and ecological assessment studies since it is not yet known if they respond differently to the parameters we today extrapolate water status from by the presence/absence of *F. vesiculosus*. In **Paper I** I also highlight the need for well-informed management of this new species, as loss of this foundation species would affect the whole ecosystem.

Another human-induced environmental impact on the Baltic Sea ecosystem is the nutrient enrichment, which would escalate with increasing precipitation as this would increase nutrient loads from land by river runoff (Viitasalo et al. 2015). The effects of increased nutrients and ensuing peaks of filamentous algae on the survival on sexually recruited *Fucus* juveniles are known (i.e. Bergström et al. 2003, Råberg et al. 2005), but future studies should also look into how nutrient enrichment affects the reattachment of *F. radicans* fragments (**Paper III**) as this might seriously hamper the recruitment in the future.

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# Tack!

*“Work is more fun than fun“ Noël Coward*

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*“An education was a bit like a communicable sexual disease. It made you unsuitable for a lot of jobs and then you had the urge to pass it on”*  
*T. Pratchett*