WALTER AND ANDRÉE DE NOTTBECK FOUNDATION

No. 33

The life cycle and genetic structure of the red alga *Furcellaria lumbricalis* on a salinity gradient

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Academic dissertation in Ecology,
to be presented, with the permission of
the Faculty of Biosciences of the University of Helsinki,
for public criticism in the Auditorium 1041,
Biocenter 2, University of Helsinki, Viikinkaari 5, Helsinki,
on January 25th, 2008, at 12 noon.

HELSINKI 2008

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

I. Kostamo, K. & Mäkinen, A. 2006: Observations on the mode and seasonality of reproduction in *Furcellaria lumbricalis* (Gigartinales, Rhodophyta) populations in the northern Baltic Sea. – Bot. Mar. 49: 304-309.

II Korpelainen, H., Kostamo, K. & Virtanen, V. 2007: Microsatellite marker identification using genome screening and restriction-ligation. – BioTechniques 42: 479-486.

III Kostamo, K., Korpelainen, H., Maggs, C. A. & Provan, J. 2007: Genetic variation among populations of the red alga *Furcellaria lumbricalis* in northern Europe. – Manuscript.

IV Kostamo, K. & Korpelainen, H. 2007: Clonality and small-scale genetic diversity within populations of the red alga *Furcellaria lumbricalis* (Rhodophyta) in Ireland and in the northern Baltic Sea. – Manuscript.

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Contributions

The following table shows the main contributions of authors to the original articles or manuscripts.

	I	**II**	**III**	**IV**
Original idea	KK, AM	HK	KK	KK, HK
Sampling	KK	VV, KK	KK, CM	KK
Molecular data	-	KK	KK	KK
Analyses	KK	HK, KK	KK, HK	KK
MS preparation	KK, AM	HK, KK	KK, HK, CM, JP	KK, HK

Initials refer to authors of the paper in question: AM = Anita Mäkinen, CM = Christine Maggs, HK = Helena Korpelainen, JP = Jim Provan, KK = Kirsi Kostamo, VV = Viivi Virtanen.

The life cycle and genetic structure of the red alga *Furcellaria lumbricalis* on a salinity gradient

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The life cycle and genetic structure of the red alga *Furcellaria lumbricalis* on a salinity gradient. – W. & A. de Nottbeck Foundation Sci. Rep. 33: 1-34. ISBN 978-952-99673-4-6 (paperback), ISBN 978-952-10-4472-4 (PDF).

The life cycle and genetic diversity of the red alga *Furcellaria lumbricalis* (Hudson) Lamouroux were investigated in 15 populations in northern Europe. The occurrence of different life cycle phases and seasonality of reproduction were studied in four brackish populations in the northern Baltic Sea. Furthermore, a new method, based on genome screening with ISSR markers combined with a restriction-ligation method, was developed to discover microsatellite markers for population genetic analyses. The mitochondrial DNA *cox2-3* spacer sequence and four microsatellite markers were used to examine the genetic diversity and differentiation of red algal populations in northern Europe. In addition, clonality and small-scale genetic structure of one Irish and four Baltic Sea populations were studied with microsatellite markers. It was discovered that at the low salinities of the northern Baltic Sea, only tetrasporophytes and males were present in the populations of *F. lumbricalis* and that winter was the main season for tetrasporangial production. Furthermore, the population occurring at the lowest salinity (3.6 practical salinity units, psu) did not produce spores. The size of the tetraspores was smaller in the Baltic Sea populations than that in the Irish population, and there were more deformed spores in the Baltic Sea populations than in the Irish populations. Studies with microsatellite markers indicated that clonality is a common phenomenon in the Baltic Sea populations of *F. lumbricalis*, although the proportion of clonal individuals varied among populations. Some genetic divergence occurred within locations both in Ireland and in the northern Baltic Sea. Even though no carpogonia were detected in the field samples during the study, the microsatellite data indicated that sexual reproduction occurs at least occasionally in the northern Baltic Sea. The genetic diversity of *F. lumbricalis* was highest in Brittany, France. Since no variation was discovered in the mtDNA *cox2-3* spacer sequence, which is generally regarded as an informative phylogeographic marker in red algae, it can be assumed that the studied populations probably share the same origin.

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CONTENTS

1. INTRODUCTION

1.1. Plant life-histories and trade-offs

An alternation between haploid and diploid nuclear phases is a necessary consequence of eukaryotic sexuality. Variation in the relative timing of meiosis and syngamy allows organisms to vary widely in size at each phase and in the duration of these two phases. In the diplonts (higher plants, vertebrates), the haploid phase is limited to one or a few cells, and it undergoes little or no development. In the haplonts (bryophytes), the diploid phase is limited, and vegetative growth occurs primarily during the haploid phase. However, many algae, ferns, bryophytes, and fungi have a biphasic or triphasic life history, during which both the diploid and haploid phases undergo substantial development (Bell 1994).

Diplohaploidy has probably developed to reduce the cost of sex: If the duration of the life cycle phases is equal, then the diplohaplonts are required to produce sexual structures half as often as either haplonts or diplonts (Richerd et al. 1993, 1994). Recent studies support the concept that diplohaplontic organisms may exploit their environment more efficiently through habitat differentiation of different ploidy phases, although this is less evident in the case of isomorphic life histories (Kain 1984, DeWreede & Green 1990, Bolton & Joska 1993, Phillips 1994, Dyck & DeWreede 1995, Gonzáles & Meneses 1996, Lindgren & Åberg 1996, Piriz 1996, Hughes & Otto 1999). However, wherever the cost of sex is high, it can be assumed that the evolution of asexual reproduction is favoured (Mabel & Otto 1998).

Most plant species have the capacity for vegetative reproduction in addition to sexual reproduction (Salisbury 1942, Klimeš et al. 1997). In some habitats, individual plants reproducing asexually may have higher fitness values than do sexually producing individuals, although this may vary over time (Bengtsson & Ceplitis 2000). The balance between the two modes of population regeneration is one of the most important life-history characteristics of plants due to its effects on the demography (Abrahamson 1980, Eriksson 1986), population genetic structure (McLellan et al. 1997, Chung & Epperson 1999, Ceplitis 2001), dispersal (Stöcklin 1999, Winkler & Fischer 2001) and meta-population processes (Piquot et al. 1998, Gabriel & Bürger 2000, Stöcklin & Winkler 2004).

1.2. Life histories and reproduction of red algae

The red algae (Rhodophyta) are a distinct eukaryotic lineage, which lacks chlorophyll *b* and *c* but contain allophycocyanin, phycocyanin and phycoerythrin in the form of phycobilisomes on unstacked thylakoids. Furthermore, their plastids are bound by two membranes and they produce floridean starch that is deposited in the cytoplasm. All red algae lack flagella and centrioles at all stages of their life history (Gabrielson et al. 1990, Graham and Wilcox 2000). It has been estimated that 27 % of all known species of marine macrophytes are red algae (Dring 1982). Although a number of unresolved issues remain in red algal taxonomy, a phylogenetic consensus at and above the ordinal level has started to emerge after the use of molecular methods became more common in phycological studies (Saunders & Hommersand 2004, Yoon et al. 2006).

The life histories of the red algae are interpreted as biphasic or triphasic (Hommersand & Fredericq 1990). In general, the sex-

ual system of the red algae consists of three phases (*Polysiphonia*-type): a haploid sexual phase (the gametophyte), a diploid phase that develops directly on the female thallus (the carposporophyte) and a free-living diploid phase bearing meiosporangia (the tetrasporophyte) (Fig. 1). Most modified life histories encountered in the field and laboratory are thought to be derived secondarily from the triphasic life history. However, the recruitment of each stage depends on the survival, fertility and migration success, as well as on the efficiency of selection during the previous life history phases.

It has been hypothesised that selection has favoured the evolution of a triphasic life history in red algae as a compensation for

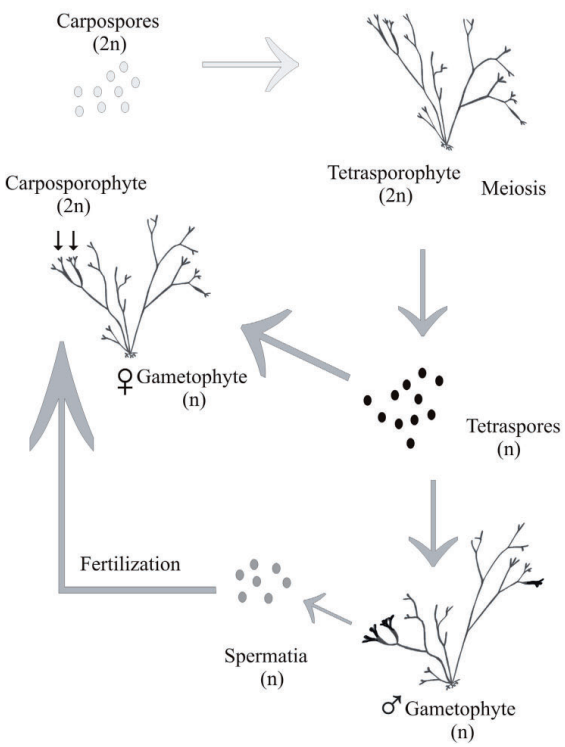


Figure 1. The sexual life cycle of the red alga *Furcellaria lumbricalis* consists of three phases: the tetrasporophyte, the gametophyte and the carposporophyte.

an inefficient fertilization in the absence of motile gametes (Hommersand & Fredericq 1990). The retention of the carposporophyte and its nourishment by the gametophyte are essential components of this adaptation, which enables the carposporophyte to utilize the nutrients provided by the gametophyte. Furthermore, there is a trend toward zygote amplification (Hawkes 1990), corresponding to the splitting of one biparental embryo into many genetically identical embryos that share the same genotype. Thus, a mating between male and female gametophytes descending from one or more tetrasporophytic individuals sharing the same genotype is analogous to selfing. Furthermore, in studies on the red alga *Gracilaria gracilis* (Stackh.) Steentoft, Irvine & Farnham (syn. *Gracilaria verrucosa* (Huds.) Papenf.), it has been discovered that approximately 5 % of individuals displayed ”rare sexual phenotypes”, which share the common feature that they effectively allow the population to skip the haploid macroscopic phase of the algal life cycle to some degree (Destombe et al. 1989). Similar phenomena have been observed in other red algal species as well (Tokida & Yamamoto 1965, Bird et al. 1977, West et al. 2001), although the germination of male and/or female spores and the development of haploid reproductive organs on the diploid plant were not stable characteristics of an individual over time. However, it has been discovered that in the red alga *Gracilaria gracilis* the survival of the gametophyte and tetrasporophyte stages was more important for population persistence and growth than for the fertility aspects (Engel et al. 2004), which indicates that the survival of adults is much more important for population dynamics than is reproductive success.

Clonal propagation is quite common in the Bangiophycidae, occurring via ramets

or thallus fragments, and it has the advantage of allowing continuous occupation of space (Hawkes 1990). Asexual reproduction and exclusively asexual algal populations are often assumed to be found in marginal environments (Wærn 1952, Bierzychudek 1985, Lewis 1985, Destombe et al. 1989, 1993, Wallentinus 1991, Karlsson et al. 1992, Gaggiotti 1994, Bergström et al. 2005, Johannesson & André 2006), where the reduced resource distribution and ability to produce clonal descendants adapted to a particular resource array allow the asexually reproducing populations to exist (Case & Taper 1986). The occurrence of asexual reproduction has been discovered in several macroalgal species in the hyposaline Baltic Sea, including the brown alga *Fucus vesiculosus* L. (Tatarenkov et al. 2005, Bergström et al. 2005) and the red algae *Ceramium tenuicorne* (Kützing) Wærn (Gabrielsen et al. 2002, Bergström et al. 2003). The asexual population regeneration of red algae can also occur through tetraspore-to-tetraspore-cycling, where the diploid tetrasporophyte produces diploid spores, thus avoiding the haploid part of the life history (West 1970, Maggs 1988, 1998) (Fig. 2). Some red algae are also capable of asexual reproduction via monospores or other dispersal propagules, although this phenomenon is more common in the primitive groups (Hawkes 1990).

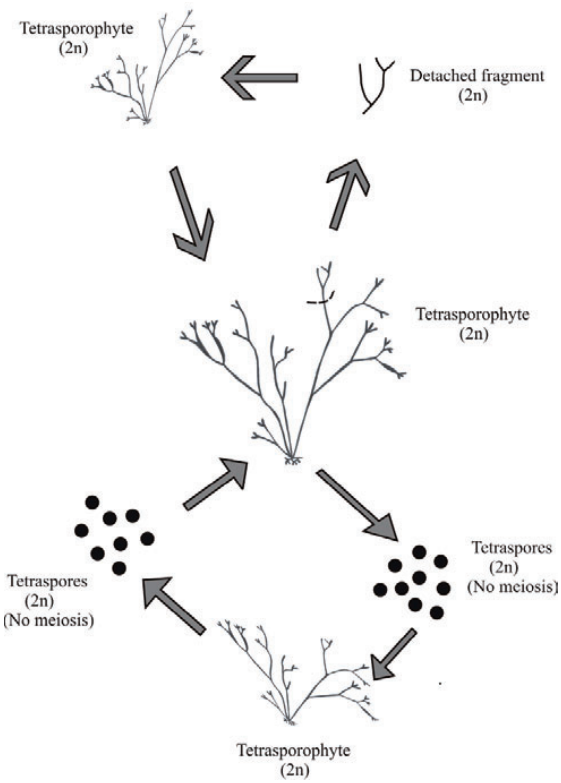


Figure 2. The asexual population regeneration of red algae can occur, e.g., through thallus fragmentation and reattachment or tetraspore-to-tetraspore cycling.

Also, spore and germling coalescence may influence the demography and genetic structure of red algal populations (Maggs & Cheney 1990, Santelices et al. 1996, 1999, 2003). The coalescence of two or more spores or germlings produces chimeric individuals which subsequently develop into single organisms. Furthermore, since it is not known to what degree coalescence occurs and because secondary pit connections are usually formed through the cell walls of two coalescing individuals, it is possible that genomic material or compounds influencing gene expression can be exchanged between the different parts of the chimeric individual.

1.3. The Baltic Sea

Different factors, including the characteristics of the physical environment (salinity, light quality and availability, sedimentation rate), and, to some degree also interactions with other organisms, influence the individuals and populations inhabiting the Baltic Sea. The genetic structure of populations

is also influenced by the unique history of the area, which provides the basis for all ecological and population genetic processes. Despite the unique character of the present Baltic Sea, many characteristics of its flora and fauna are still unknown. Many species occurring along the Baltic Sea salinity gradient show at least some degree of physiological adaptation to the brackish water conditions (Smith 1964, 1977, Rietema 1991, 1993, 1995, Kristiansen et al. 1994, Düwel 2001). In addition, a number of species show marked genetic differentiation between the Baltic and North Sea populations (Väinölä & Hvilsom 1991, Luttikhuizen et al. 2003, Olsen et al. 2004, Johannesson & André 2006), although, in many cases the differences may be introduced through the invasion of separate evolutionary lineages rather than being the result of local adaptation (Röhner et al. 1997, Väinölä 2003, Nikula et al. 2007).

During the last glacial maximum, the whole Baltic Sea area was covered by a continental ice sheet (Andersen & Borns 1994). The melting of the ice sheet was followed by several freshwater and marine phases. The present marine connection through the Danish straits opened up about 8 000 years ago, providing a colonization route to marine organisms occurring in the Atlantic Ocean (Björck 1995). The rapid decrease in salinity in the narrow and shallow Danish straits has had a great influence on the flora and fauna of the Baltic Sea. The overall salinity of the water basin is influenced by infrequent inflows of water from the Atlantic Ocean as well as by freshwater river run-off, the latter influencing salinity on a more local scale (Backhaus 1996, Gustafsson 2001).

All marine species exhibit strong hypoosmotic stress at low salinity. Macroalgae respond to hypoosmotic stress by passively tolerating an increased cell volume or by reducing the concentration of osmotically active solutes in the cell (Kirst 1990, Lobban & Harrison 1994). As a result, algae may suffer from decreased performance due to inefficient cellular metabolism, changes in cellular ultra structure, or ion metabolite deficiency in the cell (Kirst 1990). Impairment of the reproductive system due to reduced performance of gametes or unsuccessful fertilization may also influence the distribution of algal species at low salinities (Raven 1999, Serrão et al. 1999). However, the effects of low salinity are probably strongest along the salinity gradient, causing the exclusion of genotypes that are not able to acclimate to low prevailing seawater salinity.

Another factor influencing all organisms inhabiting the Baltic Sea is eutrophication. In the photic part of the benthos, its effects can be seen as increased levels of nutrients that are available for all photosynthetic organisms. This often results in an increased amount of phytoplankton in the water column (Niemi 1975, Hällfors et al. 1981), reduction in the amount of light in the water column, and a consequential influence on the distribution of species and their life histories. In red algae, adaptation to the changing irradiation environment occurs through changes in the photosynthetic pigmentation (Kirk 1983, Ramus 1983). Solar radiation influences the amount of photosynthetic pigmentation, with short-term changes induced by UV light (Figueroa et al. 1997). Photosynthetic properties, such as compensation and saturating irradiances, have been suggested to regulate the zonation of macroalgae on rocky shores (Lüning 1981) (Fig. 3), so that deeper-growing algae are generally more sensitive to light (due to lower compensation irradiances), which is considered to be an adaptation to low light

levels at greater depths. Furthermore, this adaptation does not disappear after a long period of cultivation in the laboratory, which means that it is most likely of a genetic origin both for the upper light limit (Hanelt et al. 1997) and for the lower light limit (Kirst and Wiencke 1995). In the red alga *Chondrus crispus* Stackhouse, sensitivity to photoinhibition correspons to the zonation of the algae on the shore, with deeper-growing algae showing a greater depression of fluorescence yield and slower recovery (Sagert et al. 1997). The maximum penetration of UV light in the water column varies from a few cm to more than 20 m (Karentz & Lutze 1990, Kirk 1994), depending on the particular wavelength and on the concentration of dissolved organic material in water. Sensitivity to UV light stress is one of the factors influencing the zonation patterns of algae on rocky shores (Maegewa et al. 1993, Bischof et al. 1998, 2000).

Along with reduced light availability, an eutrophication-induced increase in the

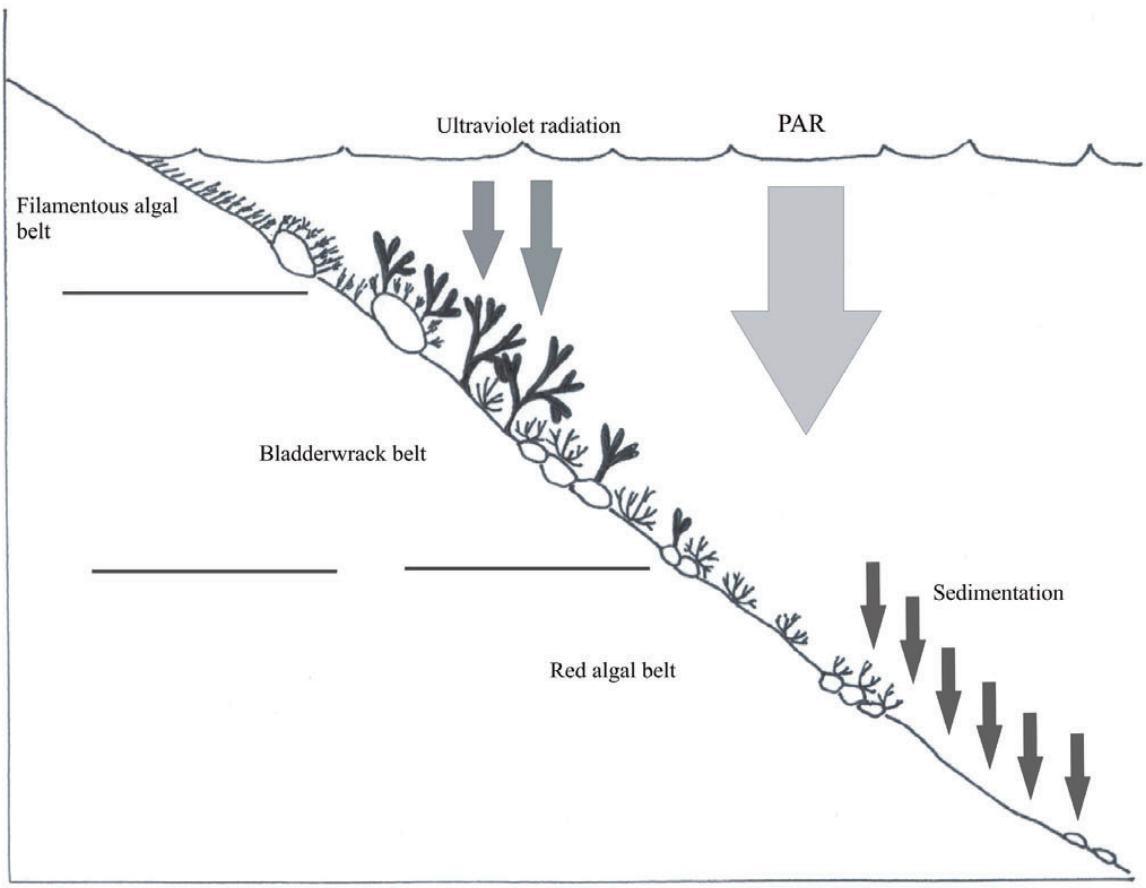


Figure 3. Underwater vegetation zonation in the northern Baltic Sea. Along with prevailing seawater salinity, the amount of photosynthetically active radiation (PAR), the amount of UV light and sedimentation rate influence the occurrence of algal belts on each shore.

sedimentation rate can change macroalgal distribution in the littoral zone of the Baltic Sea. It has been discovered in an earlier study (Eriksson & Johansson 2005) that species that depend on short periods of spore dispersal are significantly favoured by experimental sediment removal, while species that mainly disperse by fragmentation or have long continuous periods of spore release are more tolerant to sedimentation. Dispersal by fragments probably increases the likelihood of finding suitable patches of substrate in a temporally unstable sediment environment, because loose thalli fragments survive in the water for longer periods than do free spores and the release of fragments will not be confined to one short period of time (Eriksson & Johansson 2005). Thus, sedimentation has the potential to select against dependence on spores for population regeneration in a temporally unpredictable sediment environment in the deep end of the red algal belt (Eriksson & Johansson 2005). In colonization experiments, it has been discovered that population regeneration of the *Furcellaria lumbricalis* (Huds.) Lamour. in the northern Baltic Sea occurs only by thallus fragments that attach to the substrate with secondary rhizoids (Johansson 2002) (Fig. 2) and that colonization is a relatively uncommon event when compared to other algal species.

The increase in the amount of nutrients also promotes the growth of filamentous algae that can overgrow and overshadow even larger perennial algal species (Kangas et al. 1982, Wallentinus 1984, Rönnberg et al. 1992). Nutrient enrichment can also change the interactions between macroalgae and other organisms. For example, a change in the nutritional quality of algae can make them more suitable for herbivores (Hemmi & Jormalainen 2004), while a change in the predation pressure on herbivores may result in habitat choices overriding feeding preferences (Hay et al. 1987, 1989). Thus, changes in one trophic level can have an impact on all other trophic levels within the littoral community, including the species composition and biomass of the algal flora.

1.4. Aims of this study

The aim of my thesis was to study the life cycle and genetic structure of the populations of the red alga *Furcellaria lumbricalis* in the Baltic Sea. In paper **I**, we studied the reproduction mode of *F. lumbricalis* in four populations along the coast of Finland in order to discover whether asexual reproduction becomes more prominent in marginal environments. Furthermore, some unpublished data on ploidy and spore production in two marine populations and two brackish water populations are included in section 3.2. in order to further test the hypothesis that the role of asexuality is more pronounced in the hyposaline Baltic Sea. In the second paper, we developed a new method to discover microsatellite markers, and then we utilized the new methodology to develop marker sets for a number of bryophyte and algal species, including *F. lumbricalis*. In paper **III,** we studied the genetic structure of red algal populations in northern Europe with mtDNA sequencing and microsatellites, and in paper **IV,** we studied the degree of clonality and the fine-scale genetic structure within one marine and four brackish populations of *F. lumbricalis* with microsatellite markers.

2. MATERIAL AND METHODS

2.1. Study species

Furcellaria lumbricalis is a perennial red alga, occurring in cold waters of the North Atlantic and Arctic Oceans (Holmsgaard et al. 1981, Bird et al. 1983). The species is monotypic. The distribution of *F. lumbricalis* ranges in Europe from northern Spain to the Arctic, including the British Isles, the Faeroe Islands and the Baltic Sea. In North America, the major population of *F. lumbricalis* grows in the southern Gulf of St. Lawrence, and it has spread sparingly to adjacent outer coasts since the 1930's (Bell & MacFarlane 1933). It is likely that the populations in southern Europe and North America are introduced, because they are disjunct and limited (Bird et al. 1991).

Furcellaria lumbricalis is a subtidal algal species that grows in sheltered to moderately exposed habitats (Schwenke 1971). It grows in marine habitats in tidal pools from the upper eulittoral zone all the way down to depths of 28 m. In the Baltic Sea, *F. lumbricalis* grows in the bladder wrack belt and even in shady spots under *Fucus vesiculosus*. Below the bladder wrack belt, *F. lumbricalis* forms the red algal belt at depths of 2-10 m depending on water turbidity, competition and suitable substratum (Rosenvinge 1917, Wærn 1952, Taylor 1975, Kornfeldt 1979, Holmsgaard et al. 1981, Mäkinen et al. 1988) (Fig. 3). The individuals of *F. lumbricalis* form dense tufts on rocky surfaces and provide growing surface and shelter for many different invertebrates.

Furcellaria lumbricalis has a complex haploid-diploid life cycle typical of red seaweeds, including two free-living stages of different ploidy levels (a diploid stage, the tetrasporophyte and a haploid stage, the female and the male gametophyte) (Austin 1960 a, b). The third life cycle stage, the diploid carposporophyte, resides on the female gametophyte (Fig. 1).

F. lumbricalis is well known among the larger red algae for its tolerance of low salinity (Bird et al. 1991). The four practical salinity unit (4 psu) isohaline in the Gulf of Bothnia and Gulf of Finland marks the innermost distribution of *F. lumbricalis* in the Baltic Sea (Zenkevitch 1963, Bergström & Bergström 1999). However, an experimental assessment on the effects of salinity on growth showed an increase in biomass to be maximal at 20 psu under favourable conditions of light and temperature (Bird et al. 1979). Fertile *Furcellaria* individuals have not be detected from the lowest salinities, but they can be found from the southern part of the Baltic Sea, on the south-east coast of Sweden and around the island of

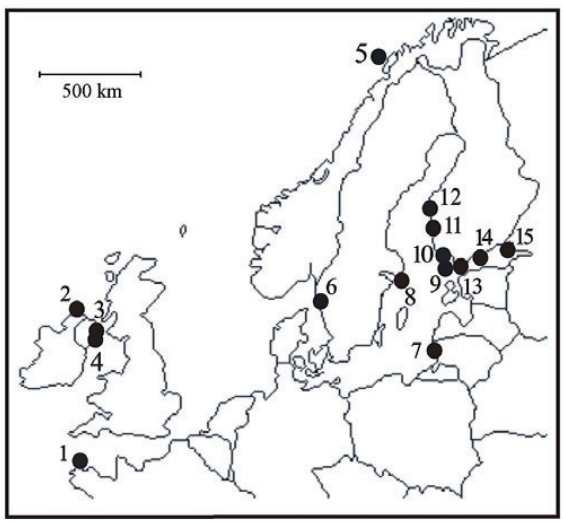


Figure 4. Fifteen *Furcellaria lumbricalis* populations from the Baltic Sea and the Atlantic Ocean were included in the study. The seawater salinity in the Atlantic Ocean is 35 practical salinity units (psu), while the Baltic Sea populations inhabit salinities between 3.6-30 psu.

Gotland (Svedelius 1901, Levring 1940), where the salinity is above 7 psu (Kautsky & Kautsky 1989).

Altogether 15 marine and brackish water populations of the red alga *F. lumbricalis* were investigated in this study (Table 1, Fig. 4). Marine populations, which grew in saline water of 35 psu, include populations from Roscoff (France), Donegal (Republic of Ireland), Castle Island and Dorn (Northern Ireland) and Lofoten (Norway). Sampling the marine populations was carried out in tidal pools because no *Furcellaria* were discovered from the deeper parts of the sublittoral, despite an extensive search. The *Furcellaria* individuals discovered from the tidal pools grew only in locations where they stayed permanently submerged and formed often mixed cultures with the red alga *Polyides rotundus* (Huds.) Grev. The population in Tjärnö, on the west coast of Sweden, was found in somewhat lower salinity (15-30 psu). The sampled Baltic Sea populations occur in brackish water environments where salinities range from 3.6-8 psu. Samples from the Baltic Sea populations were collected from bladder wrack belt and from red algal belt using SCUBA.

Table 1. Sampling sites of the *Furcellaria lumbricalis* populations, seawater salinity, sample sizes, and the numbers of different genotypes detected by a microsatellite analysis. Haploid individuals were excluded from the genetic analyses.

Locality	Geographic region	Seawater salinity (psu)	Sampled location
1. Roscoff	Brittany, France	35	Tidal pool
2. Fanad Head	Co. Donegal, Republic of Ireland	35	Subtidal Tidal pools
3. Dorn	Strangford Lough, Northern Ireland	35	Tidal inlet
4. Castle Island	Strangford Lough, Northern Ireland	35	Large tidal pool
5. Andenes	North Sea, Norway	35	Subtidal
6. Tjärnö	Skagerrak Sea, Sweden	15-30	Subtidal
7. Palanga	Baltic proper, Lithuania	8	Subtidal
8. Askö	Baltic proper, Sweden	6	Subtidal
9. Utö	Archipelago Sea, Finland	6	Subtidal
10. Seili	Archipelago Sea, Finland	5.4	Subtidal
11. Pori	Gulf of Bothnia, Finland	6	Subtidal
12. Närpiö	Gulf of Bothnia, Finland	4.9	Subtidal
13. Tvärminne	Gulf of Finland, Finland	5.3	Subtidal
14. Helsinki	Gulf of Finland, Finland	4.6	Subtidal
15. Ulko-Tammio	Gulf of Finland, Finland	3.6	Subtidal

2.2. Description of the studies

2.2.1. Life cycle of Furcellaria lumbricalis in the northern part of the Baltic Sea (Paper I)

The life cycle of *Furcellaria lumbricalis* was studied by monthly sampling (n = 30/ sampling site) for one year along the coast of Finland at four locations spanning 500 km along a salinity gradient from 3.6 to 5.4 psu (Table 1). Sampled individuals were studied with a light microscope to identify the life cycle phase. A two-way ANOVA was used to determine whether there were differences in the number of reproducing individuals among sampling sites and months. Tukey's HSD test was used as a *post hoc* test for multiple pairwise comparisons. A logistic regression analysis was used to study whether environmental factors influence the production of spores in populations inhabiting different environments. Further, in order to investigate the effects of environmental factors (salinity, seawater temperature, water transparency, day length) on the intensity of reproduction, a logit transformation was first conducted for the variable describing reproductive intensity and then a linear regression analysis was performed for the dataset. Furthermore, principal component analyses (PCA) were used to study whether there are some combinations of environmental variables that explain the environmental variability across the sampling sites and times. Axes which explained a significant proportion of the environmental variability were then correlated with reproduction intensity. Finally, because Component 1 of the PCA correlated to the reproduction intensity, the relationships between the regression slopes of individual sampling sites were examined with ANCOVA.

2.2.2. The ploidy and spore production of marine and brackish water population of F. lumbricalis

The ploidy of individuals inhabiting marine and brackish water habitats were studied based on morphological characters, i.e., the presence and absence of reproductive structures. The spore production was studied by measuring the size and shape of tetraspores in two marine populations and two brackish water populations of *F. lumbricalis*. Frequencies of different spore size classes were calculated and plotted. A logarithmic transformation was performed for the dataset and a one-way ANOVA was used to determine whether there were differences between the Irish and Baltic Sea populations in spore sizes. Finally, a linear regression analysis was used to determine whether the shape of the Baltic Sea spores influenced the size of the spores. Unfortunately, the growing experiments failed despite the fact that they were attempted several times with, e.g., different growing media and light conditions. Therefore, the viability of the marine versus Baltic Sea spores could not be estimated.

2.2.3. Development of microsatellite markers utilising restriction-ligation method (Paper II)

A new method based on genome screening with intersequence simple repeat (ISSR) markers and restriction-ligation was developed to find microsatellite sites from an organism's genome. Traditional methods for microsatellite development include pooling the DNA, DNA digestion and ligation with different restriction and ligation enzymes, the forming of single-stranded DNA library and the cloning of double stranded DNA (e.g., Zane et al.

2002). The major disadvantages of the traditional methods are that they are both time-consuming and expensive (Zane et al. 2002, Squirrel et al. 2003). The new method developed in this study includes genome screening for microsatellite regions using ISSR primers, cloning the acquired DNA, which has a microsatellite region in both ends of the sequence, and sequencing the DNA sequences acquired from positive bacterial colonies. The first specific primer for each microsatellite locus was then developed based on the information obtained from the first sequencing. Then a two-stranded DNA sequence (adapter) with a known sequence was ligated on restricted DNA. After this, a PCR amplification was performed using the specific microsatellite primer and a primer designed based on the adapter sequence. The PCR amplification product was sequenced, and the second specific microsatellite primer was designed based on the information obtained from the second sequencing. The new method is simpler than the traditional method, and it reduces the costs and laboratory requirements for marker development. Altogether, 21 species of bryophytes, three species of algae and racoon dog were used as material for this study when developing methodology and marker sets.

2.2.4. Genetic structure of Furcellaria lumbricalis populations in Northern Europe (Paper III)

The phylogeography and genetic diversity were studied in 15 populations of the red alga *Furcellaria lumbricalis* in northern Europe using the mitochondrial *cox2-3* spacer and four microsatellite markers. The mtDNA *cox2-3* spacer has previously been used in several red algal studies (Zuccarello et al. 1999, Gabrielsen et al. 2002, Provan et al. 2005), resulting in the identification of marine glacial refugia in the Atlantic Ocean (Provan et al. 2005) and distinguishing the level of genetic differentiation among populations inhabiting the Baltic Sea and the Atlantic Ocean (Gabrielsen et al. 2002). Genetic fingerprinting with four polymorphic microsatellite markers was used to obtain information about the genetic population structure among algal populations in the Atlantic Ocean and the Baltic Sea. Descriptive statistics of the within- and between-locality genetic diversity (Nei 1978), the estimator F_{ST}, deviations from Hardy-Weinberg equilibrium, presence of linkage disequilibrium, polymorphism information content (PIC) value (Botstein et al. 1980), analyses of molecular variance (AMOVA), assignment tests and the isolation by distance were calculated from the microsatellite dataset.

2.2.5. Clonality and small-scale genetic diversity within populations of Furcellaria lumbricalis in Ireland and northern Baltic Sea (Paper IV)

The degree of clonality and small-scale genetic structure of *Furcellaria lumbricalis* were studied within one Irish and in four Baltic Sea populations with four microsatellite markers. In the Irish population, algal samples were collected from one subtidal subpopulation (12 individuals) and three small tidal pool subpopulations (10 individuals from each tidal pool). In the Baltic Sea, altogether 30 individuals were collected from each of four locations, including 10 algal individuals inhabiting the maximum growing depth (red algal belt), 10 algal individuals inhabiting the optimum growing depth (red algal belt) and 10 algal individuals inhabiting the minimum growing depth (bladder wrack

belt) at each location. Descriptive statistics of the within-locality genetic diversity (Nei 1978), the estimator F_{ST}, deviations from the Hardy-Weinberg equilibrium, presence of linkage disequilibrium, degree of clonality, occurrence of rare alleles, assignment of individuals to different populations and analyses of molecular variance (AMOVA) were calculated.

3. RESULTS AND DISCUSSION

3.1. Development of microsatellite markers

Microsatellite repeat sequences are known to be ubiquitous in prokaryotic and eukaryotic genomes and present both in coding and noncoding regions. However, the distribution of microsatellites is not homogeneous within a single genome, because of different constraints of coding vs. noncoding sequences, historical processes (Arcot et al. 1995, Wilder & Hollocher 2001), and the possible different functional roles of different repeats (Valle 1993, Hammock & Young 2004). The frequency of microsatellite sequences also varies across taxa, in terms of both absolute numbers of microsatellite loci and repeat preference (Hancock 1999). In Bangiophycidae, microsatellite marker sets have earlier been developed for *Gracilaria gracilis* (Luo et al. 1999) and *G. chilensis* Bird, McLachlan & Oliveira (Guillemin et al. 2005).

It has previously been estimated that as many as 83 % of the loci originally included in the process of microsatellite development are lost during the different phases of the process (Squirrel et al. 2003). However, when utilising the new protocol, on average 54 % of ISSR sequences resulted directly in microsatellite development after the first sequencing, and further 41 % after sequence walking (**II**). The new methodology utilises sequences containing microsatellite repeat motifs on both ends of the studied sequence, which obviously increases the likelihood of successful marker development. Additionally, the new methodology makes it also possible to estimate the frequency of microsatellites in the genome of the studied species already at an early stage of the marker development, which gives valuable information during the first few steps of the marker development. The total number of microsatellites developed while testing the new methodology equalled 191 markers, of which 95 % were found to be polymorphic (**II**) (Korpelainen et al. 2007, Kostamo et al. 2007). Altogether eight microsatellite loci were originally discovered from the genome of *Furcellaria lumbricalis*. However, one of the microsatellite loci (F110R) was linked to another microsatellite locus (F110L), and only the more variable locus (F110L) was used in this study. Furthermore, three microsatellite loci were monomorphic.

Sequencing of microsatellite-rich areas in red algae never resulted in finding microsatellites within the sequenced ISSR amplification product between two microsatellite repeat sequences (**II**). A similar phenomenon was also observed in the green alga *Ulva intestinalis* L., in which all developed markers resulted from sequence walking, although only four markers were developed for this species (**II**) (Kostamo et al. 2007). This was not the case in bryophytes, where microsatellite-rich areas were often discovered, and 81 % of microsatellite markers were developed directly after the sequencing of the ISSR-amplification bands (**II**). It is thus possible that the great difficulties associated earlier with the development of microsatellite markers for red algae and

other seaweeds, are caused by the fewer microsatellite regions present in the red algal genome.

It has previously been discovered that AG/CT repeats are the most common repeat type in plants, (Morgante et al. 2002). Therefore, the use of ISSR primers that contain an AG/CT or AC/TG repeat in the screening of the genome probably results in more ISSR bands for sequencing and increases the likelihood of discovering suitable markers when compared to screening with other types of ISSR primers. Therefore, the AG/CT repeat was the most common ISSR repeat type used in our study (**II**). Overall, AG/CT repeats outnumbered the other repeat types discovered on several algal and moss species (29.2 %) (**II**).The second most common repeat type was AC/TG (11.8 %) (**II**). In the red alga *Furcellaria lumbricalis*, six out of eight microsatellite repeat sequences were AG/CT repeats and the remaining two microsatellite sequences were AC/TG repeats (**III**).

Typically, the increase in the number of alleles per microsatellite locus translates into an increase in the polymorphism information content (PIC) value (Botstein et al. 1980). PIC values are commonly used in studies of cultured vascular plants (Dávila et al. 1999, Richards et al. 2004, Oliveira et al. 2005, Palmieri et al. 2005) and occasionally of wild plants (Erickson et al. 2004). In an earlier microsatellite marker set developed for the red alga *Gracilaria chilensis*, six developed microsatellite markers had 2-4 alleles at each locus (Guillemin et al. 2005), which means that the PIC-values were lower than those we found in *F. lumbricalis,* although also the allele frequencies within a locus influence the PIC values. However, the dataset for *G. chilensis* was tested with only 40 individuals from different populations along the Chilean coast, while the dataset for *F. lumbricalis* consisted of 185 individuals from 15 populations in Northern Europe. An increase in the sample size and an increase in the geographic range of sampling most likely also increased the number of discovered alleles per locus, and thus affected the PIC values.

When comparing the acquired sequence data to GenBank databases, only four out of the 191 microsatellite loci (0.02 %) appeared to be physically linked to a known gene area (Table 2) (Korpelainen et al. 2007). All four linked loci were found in bryophyte species. One of the microsatellites was located in the middle of the coding area, while in the remainder of the cases the region was located just before or after a gene. All of the linked microsatellite loci were polymorphic. Furthermore, it is likely that even a higher number of the microsatellite loci discovered are linked with some gene, but it is not possible to associate sequences containing microsatellites to a specific genomic region due to the lack of sequence data in GenBank.

Table 2. Microsatellite linkage to active gene areas was discovered in four bryophyte markers out of the 191 microsatellite markers developed for 21 bryophyte species, 3 algal species and the racoon dog. The sequences of the developed microsatellite markers were compared to the GenBank database in order to discover whether the markers were physically close to a genomic area containing a gene.

Species	Locus	Polymorphic	Linkage area in genome	Species (GenBank)	GenBank Accession number
Hylocomium splendens	HYSP1 $[CA]_6$	Yes	Located in the IGS area before the 5S gene	Bryophyta (several species)	X80212
Polytrichum juniperinum	POJU5 $[AG]_6$	Yes	Large subunit rDNA gene in the chloroplast	Bryophyta (several species)	DQ629273
Rhizomnium punctatum	RHPU1 $[TG]_7$	Yes	Rac-like GTP binding protein (rac3) precursor RNA	Bryophyta (*Physcomitrella patens*)	AF146341
Rhytidiadelphus squarrosus	RHSQ8 $[CAA]_6$	Yes	mRNA for putative peroxidase (pod gene)	Asteraceae (*Zinnia elegans*)	AJ880395

3.2. Life cycle

There was a difference between the marine and brackish water populations in the number of different life cycle phases (Table 3). In three populations studied in the northern Baltic Sea, only tetrasporophytes and spermatophytes were discovered (**I**). In the fourth population, occurring at the lowest salinity (3.6 psu), all algae examined were vegetative. Austin (1960 a, b) has earlier postulated that the life cycle phase ratio of *F. lumbricalis* in the northern Atlantic Ocean is 2:1:1 (tetrasporophytes : male gametophytes : female gametophytes). However, this was not the case in either the Irish or the Baltic Sea populations studied. Furthermore, all the populations were strongly tetrasporophyte-biased in the Baltic Sea. Numerical dominance of gametophytes is common on a whole-habitat basis and/or on an annual basis in red algae (Mathieson & Burns 1975, Craigie & Pringle 1978, Mathieson 1982, Bhattacharya 1985, Dyck et al. 1985, Hannach & Santelices 1985, May 1986, Lazo et al. 1989, DeWreede & Green 1990, Bolton & Joska 1993, Scrosati et al. 1994, Dyck & DeWreede 1995, González & Meneses 1996, Lindgren & Åberg 1996, Piriz 1996, Zamorano & Westermeier 1996, Scrosati 1998), while the numerical dominace of tetrasporophytes seems to be restricted to smaller spatial or temporal scales and has been reported only for a few species (Hansen & Doyle 1976, Dyck et al. 1985, Lazo et al. 1989, DeWreede & Green 1990, Bolton & Joska 1993,

Phillips 1994, Dyck & DeWreede 1995). It has been estimated that in some other long-lived perennial red algal species even slight changes in population dynamic processes, such as survival, reproduction or recruitment rates, can influence the ploidy frequencies of populations (Engel et al. 2001, Engel et al. 2004, Fierst et al. 2005).

Table 3. Numbers of different life cycle phases in the marine and Baltic Sea populations of *Furcellaria lumbricalis*. N = number of sampled individuals, 2n = diploid individuals, n = haploid individuals, I = Ireland, NI = Northern Ireland, FI = Finland.

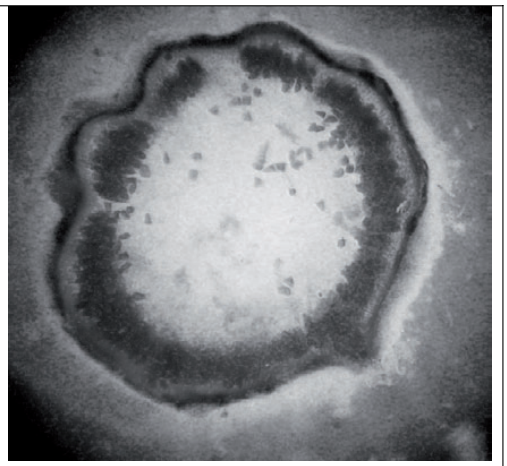
Location	N	2n	n	n	Vegetative individuals
Donegal (I)	89	8	10	2	69
Giant's Causeway (NI)	16	10	0	0	6
Castle Island (NI)	28	15	4	2	7
Dorn (NI)	43	5	7	10	21
Pori (FI)	456	112	17	0	327
Hanko (FI)	518	40	23	0	455
Helsinki (FI)	520	80	12	0	428
Kotka (FI)	388	0	0	0	388

A large portion of the algae in both habitats did not reproduce at all during the study period. In a nine-year study spanning all seasons, reproductive individuals of *Chondracanthus pectinatus* (Daw.) L. Aguilar & R. Aguilar were absent from the population only on two occasions (Pacheco-Ruíz & Zertuche-González 1999), although the intensity of reproductive effort fluctuated. In an earlier study Bird (1976) discovered that in the red alga *Gracilaria*, the ploidy of vegetative individuals reflects the ploidy frequencies of reproducing haploid and diploid individuals. Thus, most of the vegetative individuals would be tetrasporophytes in the northern Baltic Sea.

Winter months are the main season for tetrasporangial production in the Northern Baltic Sea, when sori with masses of sporangia are present. Similar results on the seasonality of tetrasporangial discharge of spores have been reported from other parts of Northern Europe (Austin 1960b) and North America (Bird 1977). However, smaller sori with only a few tetrasporangia were observed during spring at higher salinities, and some individual sporangia were detected in the population at 5 psu salinity even during the summer months. It appears that the timing and intensity of reproduction in the northern Baltic Sea might be regulated by a combination of factors, including seawater salinity, seawater transparency, temperature and photoperiod.

The tetraspores are formed in tetrads where there are two semi-circle-shaped spores and two square-shaped spores developing simultaneously (Fig.5). In the marine populations, the spores attain a round or slightly oval shape quickly after they are

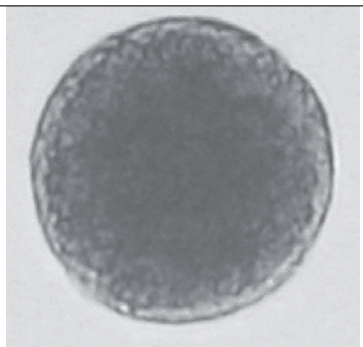
A.



B.

Figure 5 A-D. – A. Maturing tetrasporophytic sorus of *Furcellaria lumbricalis*. – B. Cross-section of thallus consisting of medulla filaments, tetrasporophytic sori and cortex. – C. Released tetrasporangia consisting of four tetraspores. – D. An individual tetraspore from a marine population.

C.



D.

released into seawater. However, the Baltic Sea populations produced tetraspores with different shapes (round, oval, square, triangular, semicircle, indefinite), and, therefore, instead of spore size, the area of all tetraspores were calculated for the analyses. It was discovered that the Baltic Sea tetraspores were significantly smaller in size than the Irish tetraspores ($MS = 44.2$, $F = 1649.4$, $df = 1$, $p<0.000$) (Fig. 6). Furthermore, there were significant differences among individuals within the Baltic Sea

populations of *F. lumbricalis* in spore sizes (Table 4). It also appears that the shape of the spore significantly affects its size ($B = -1.7\times10^{-8}$, $R^2=0.049$, $p<0.000$), round tetraspores being larger in size. Macroalgae respond to hypoosmotic stress by passively increasing the cell volume or by reducing the concentration of osmotically active solutes in the cell (Kirst 1990, Lobban & Harrison 1994). As a result, the changes in the cellular ultrastructure or ion metabolite deficiency in the cell may decrease the performance of an individual reproductive cell or an algal individual (Kirst 1990). In the Baltic Sea populations of the brown alga *Fucus vesiculosus*, low osmolalities (70-125 mmol/kg; 125 mmol/kg equals ≈ 4.3 ‰) cause an increase in the volume of both the eggs and sperm (Wrigth & Reed 1990, Serrão et al. 1996). Most unfertilised eggs burst at low salinities soon after their release. Furthermore, the sperm have a rounder shape in brackish water, and this appears to cause the displacement of the sperm's eyespot, accounting possibly for the lack of negative phototaxis of sperm in brackish water. However, a similar increase in the size of the tetraspores was not observed in *F. lumbricalis* populations in the northern Baltic Sea. Thus, the irregu-

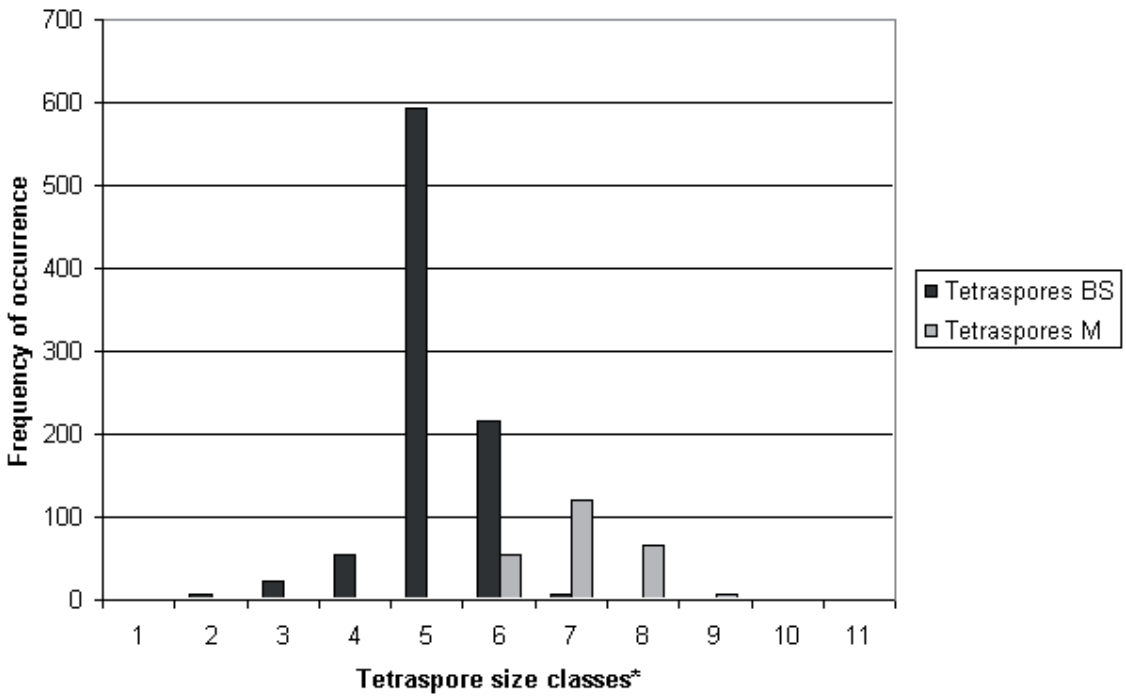


Figure 6. Tetraspore size class frequencies* in the Irish (M) and Baltic Sea (BS) populations. The Baltic Sea spores were significantly smaller than the spores originating from the marine populations.

*Size classes were $< 40 \times 10^{-9}$ m^2, $< 60 \times 10^{-9}$ m^2, $< 80 \times 10^{-9}$ m^2, $< 100 \times 10^{-9}$ m^2, $< 200 \times 10^{-9}$ m^2, $< 400 \times 10^{-9}$ m^2, $< 600 \times 10^{-9}$ m^2, $< 800 \times 10^{-9}$ m^2, $< 1 \times 10^{-6}$ m^2, $< 2 \times 10^{-6}$ m^2 , $< 4 \times 10^{-6}$ m^2 .

lar shape and smaller size of tetraspores in the Baltic Sea populations compared to the Irish populations might indicate that the role of spore production is not as important for populations inhabiting low salinities as for populations inhabiting higher salinities.

Table 4. One-way ANOVA on the effects of population, individual and population × individual on the size of tetraspores in *Furcellaria lumbricalis*. A logarithmic transformation was performed for the dataset.

Factor	df	MS	F	p
Model	22	0.28	12.93	<0.000
Population	1	1.79	84.22	<0.000
Individual	15	0.25	11.82	<0.000
Population × Individual	6	0.10	4.77	<0.000
Error term	880	0.02		

Furthermore, some clonality was observed in the Baltic Sea populations of *F. lumbricalis* in Lithuania and Finland (**III, IV**). The degree of clonality was about 13.5 % in four of the studied Baltic Sea populations (n = 30/population) (**IV**). However, only two clonal individuals were discovered from the six marine populations in the Atlantic Ocean (**III, IV**), even though sampling in one of the locations included also different subpopulations inhabiting the subtidal and small tidal pools (**IV**).

The hypothesis that asexual reproduction via thallus fragmentation and/or tetraspore-to-tetraspore cycling is more important in the population regeneration of *F. lumbricalis* in the brackish water than in a marine environment is highly likely, based on the following collected data: ploidy ratios are biased towards tetrasporophyte dominance, the size and shape of spores are different compared to those in marine populations, and there also exists a higher proportion of clonal individuals within populations. The occurrence of asexual reproduction has been discovered in several macroalgal species in the hyposaline waters of the Baltic Sea, including the brown alga *Fucus vesiculosus* (Tatarenkov et al. 2005, Bergström et al. 2005) and the red algae *Ceramium tenuicorne* (Gabrielsen et al. 2002, Bergström et al. 2003). In *F. vesiculosus*, reproduction may be limited at times in the brackish populations by a low rate of gamete release, low fertilization success and polyspermy (Serrão et al. 1996, 1999), and some of the northernmost populations are able to regenerate through adventitious branches that reattach to the substratum (Tatarenkov et al. 2005). In the red alga *C. tenuicorne*, population regeneration occurs in the northern Baltic mainly by vegetative reproduction, such as fragmentation and rhizoidal reattachment, although sexual reproduction is at least occasionally possible down to salinities of around 4 psu.

It can thus be hypothesised that populations occurring at low salinities (4.9-5.4 psu) in the northern Baltic Sea regenerate by thallus fragmentation and possibly by asexual tetraspore-to-tetraspore cycling without meiosis. Furthermore, the population occurring at a salinity of 3.6 psu probably survives solely by thallus fragmenta-

tion and reattachment. This hypothesis is also supported by observations made by Johansson (2002) in colonisation experiments, which show that the regeneration of the red algal species *F. lumbricalis*, *Polysiphonia fucoides* (Huds.) Grev. and *Rhodomela confervoides* (Huds.) Silva occurs in the northern Baltic Sea mainly by fragments, which are attached to the substratum by the byssus threads of the mussel *Mytilus trossulus* L.

3.3. Genetic structure of populations

Based on microsatellite markers, it was discovered that there is no genetic differentiation among the *Furcellaria lumbricalis* populations in Northern Europe (**III**). However, even the Baltic Sea populations growing in extremely low salinities showed a fair amount of genetic variability (**III**). These results agree with an earlier study (Valatka et al. 2000) using the RAPD method, which reported a high genetic diversity within the species in the Baltic Sea. Asexual populations often harbour a wealth of genetic variation (Ellstrand & Roose 1987, Herbert 1987, Suomalainen et al. 1987, Asker & Jerling 1992, Bengtsson 2003), coming from new mutations, as well as from remnant sexuality and/or multiple origins. Only a few sexual events per generation are sufficient to prevent the formation of an asexual genomic pattern (typically monomorphisms at several loci), which would demonstrate the presence of a predominantly asexual lifecycle (Bengtsson 2003). Thus, populations with a predominantly asexual mode of population regeneration can display almost any pattern of genotypic variation.

In red algae, a combination of sexual and asexual modes of reproduction is common, and asexual reproduction tends to be prevalent in the marginal parts of the species' distributions (Dixon 1965, Hawkes 1990, Maggs 1998). As mentioned previously, the occurrence of asexual reproduction has been discovered in several macroalgal species in the hyposaline waters of the Baltic Sea (Gabrielsen et al. 2002, Bergström et al. 2003, 2005, Tatarenkov et al. 2005). However, when studying the small-scale genetic structure of one Irish and four Baltic Sea populations of *F. lumbricalis*, we discovered that most populations and subpopulations are in or close to the Hardy-Weinberg equilibrium. This means that sexual reproduction occurs both in Ireland and in the Baltic Sea populations, despite the fact that female gametophytes have not been discovered in the northern Baltic Sea. However, it is possible that females exist but they do not annually produce carposporangia. Since the lifespan of algae is several years, sudden environmental changes, e.g., irregularly occurring salinity pulses, may change the environment to be more suitable for carpospore production. The excess of heterozygosity in the Baltic Sea populations revealed by the means of fixation indices suggests that a mild effect of heterozygosity advantage may be present. Selection for high levels of heterozygosity at the geographic limit has been discovered in the aquatic phanerogam *Zostera marina* (Billingham et al. 2003, Hämmerli & Reusch 2003) but also in common garden experiments and reciprocal transplant experiments (Backman 1991, Hämmerli & Reusch 2002).

A further study showed that in the Atlantic Ocean, there were only slight differences in subpopulation pairwise F_{ST}-values within the studied algal population consisting of one subtidal and four tidal pool populations (**IV**). Some subpopulation struc-

turing, based on F_{ST}-values, dendrogram and AMOVA, was discovered in the four *F. lumbricalis* populations studied in the Baltic Sea as well (**IV**). It is possible that environmental factors, such as eutrophication in the form of, e.g., an increased sedimentation rate, can inhibit the regeneration of species or populations that depend on short periods of spore dispersal, while species or populations that mainly disperse by fragmentation or have long continuous periods of spore release may gain an advantage (Eriksson & Johansson 2005).

3.4. Phylogeography

When examining the mitochondrial *cox*2-3 spacer in *F. lumbricalis* in Northern Europe, no variation in the spacer sequence among different geographic populations was discovered (**III**). Previously, Provan et al. (2005) found 13 polymorphic sites in the *cox*2-3 sequence when studying the phylogeography of the red alga *Palmaria palmata* in the North Atlantic. Furthermore, Gabrielsen et al. (2002) discovered that in the red alga *Ceramium tenuicorne*, the proportion of intraspecific variation in the *cox2-3* spacer sequence was up to 4 % in populations inhabiting the Baltic Sea and the southern coast of Norway. However, in the brown alga *Fucus distichus* (L.) Powell., only one mtDNA haplotype was found throughout a 2 800-km distance in the northeast Atlantic (Coyer et al. 2006), possibly indicating a recent bottleneck or a founder effect (Grant & Bowen 1998). Furthermore, in the lagoon cockle, *Cerastoderma glaucum* Poiret, all of the Baltic Sea populations were monomorphic based on the mtDNA COI gene and allozymes, thus indicating either a relatively small post-glacial population size or an extreme colonisation-expansion scenario (Nikula & Väinölä 2003).

The genetic divergence based on microsatellite markers was relatively low, especially considering that some of the populations included in the study are from extreme habitats at the edge of the species' distribution range (**III**). The highest genetic diversity was discovered from Brittany, France (**III**). Furthermore, based on the pairwise F_{ST}-values, the Norwegian population was clearly differentiated from the rest of the European populations, resulting from isolation during the LGM, a recent populations bottleneck (Väinölä 1992) or an isolation-by-distance effect (Watts & Thorpe 2006, Gómez et al. 2007). Earlier, it has been suggested that marine glacial refugia have been present in Southern France (van Oppen et al. 1995a, b, Stam et al. 2000, Coyer et al. 2003, Gysels et al. 2004, Provan et al. 2005, Hoarau et al. 2007) and in northern Scandinavia (van Oppen et al. 1995a, b). Our results clearly support these findings. Furthermore, linkage disequilibrium was more common in the Baltic Sea subpopulations than in the Atlantic Ocean subpopulations (**IV**). This may be due to random factors influencing the molecular markers used. However, high levels of linkage disequilibrium are also expected in recently bottlenecked and expanded populations, appearing, e.g., in areas glaciated during the last ice age (Hansson & Westerberg 2002).). It has been earlier suggested that *F. lumbricalis* originates from Europe, since its populations in Northern America are disjunct and limited (Bird et al. 1991). The lack of differentiation in the mtDNA sequence along with a lack of structure in the dendrogram based on the microsatellite data describing population differentiation clearly suggests that the species has spread into its modern

day distribution area from a single or very few locations, the Roscoff region in France being the most probable area of origin. It is possible that only a limited amount of divergence has occurred after the last glaciation, during which the species survived in one or two glacial refugia. Furthermore, the high salinity tolerance and capability of asexual population regeneration may prevent genotype exclusion even in marginal populations.

4. CONCLUSIONS

The populations of *Furcellaria lumbricalis* in the brackish Baltic Sea are facing difficult environmental conditions, including low prevailing seawater salinity, along with different factors related to eutrophication, i.e., reduced amount of light in the water column, increased sedimentation rate and changes in the biological interactions between different functional faunal and floral groups. Thus, it is likely that algae respond to the stress caused by the unfavourable environment by altering their life history parameters, provided that there is enough genetic variability in the genome. The increase in the level of within-population clonality and the lack of some life cycle phases observed in the Baltic Sea populations, when compared to the Atlantic Ocean populations, indicate that the overall role of asexual population regeneration through thallus fragmentation and reattachment and/or tetraspore-to-tetraspore cycling is more important in the brackish water populations than in the marine populations.

There were no differences in the mtDNA cox 2-3 spacer sequence among the studied populations, indicating that the populations probably share the same origin. However, a fair amount of genetic variability was discovered in all studied populations of *F. lumbricalis* with microsatellite markers. When quantifying the amount of genetic variability, the highest amount of genetic diversity was discovered in Brittany, France, which may result from historical factors, i.e., the species probably resided in the area during the last glaciation. The population in northern Norway was genetically differentiated from all other populations in Northern Europe, probably due to isolation during the last glacial maximum, a recent population bottleneck or isolation-by-distance effect. However, neutral molecular markers probably do not reveal a realistic picture of the functional genetic diversity within the species and therefore studies on the diversity of functional genes and the magnitude of variability in their functions are needed.

5. FUTURE WORK

We have investigated so far the amount of neutral genetic variation among and within red algal populations. However, neutral genetic variation does not present a valid picture of the adaptive capabilities of algae in a changing environment. Therefore, we have recently initiated a study on the genetic variation of genes involved in salinity stress tolerance in the red alga *Furcellaria lumbricalis* and in pH tolerance of an angiosperm water weed, *Elodea canadensis*, in a project funded by the Academy of Finland 2007-2010. The general aim of the project is to link the adaptive variation found within the populations with an environmental factor. The objectives of this project are:

1) To identify stress-responsive genes.
2) To analyse population genetic variation in stress-responsive genes.
3) To investigate and compare the evolution of neutral and adaptive genomic regions.
4) To test the relative importance of phenotypic plasticity and contemporary evolution in plants exposed to changing environments.
5) To develop faster and more cost-effective methods to identify and analyse genes involved in specific, adaptive characteristics.
6) To develop prognostic tools for monitoring populations.

At the moment, the genomic regions containing stress-responsive genes are under search. Thereafter, we will identify the genes and study variation within these genes. We are using an approach involving the exposure of a brackish water algal individual to marine water. After this, the resulting transcribed RNA will be extracted, and complementary DNA will be produced for the extracted RNA. The Amplified Fragment Length Polymorphism method (AFLP) will then be used to analyse the differences between a control sample, incubated in plain brackish water, and an algal sample exposed to marine water treatment. Differences in AFLP profiles will be used as indicators of genomic regions that are activated during the exposure of brackish water algae to marine water. After sequencing these regions, the full-lengths of the genes from the transcription-derived fragments will be reconstructed, and specific primers will be developed for the characterised genes to allow direct sequencing and population genetic sequence analysis to discover single nuclear polymorphisms (SNP) or other sequence polymorphisms.

6. ACKNOWLEDGEMENTS

I would never have managed without the help and support of several people. First of all, I would like to thank my supervisors Dr. Helena Korpelainen and Dr. Anita Mäkinen, for their support during the work. Helena, the discussions we have had and the fact that you have always found time to listen to my problems – both work related and other – have been really important to me during the years we have worked together. Furthermore, your ideas about the new methodology have provided me with challenging tasks in the laboratory and I am very happy that we can continue to work together in the future, too. Nitsu, you gave me the idea for this work and I have to say it has been really interesting – all the places I have seen and all the people I have met.

I would also like to thank Professor Christine Maggs and Dr. Jim Provan at the Queen's University of Belfast for their cooperation. Chris, it was really exciting to come to your group and discuss algal matters with you – for once I did not have to explain what red algae are or do. Jim, working with you gave me some valuable experience which I, hopefully, can use in the future.

I would like to acknowledge Juhani Vaittinen, the Finnish Coast Guard, the Helsinki Environment Centre, the Tvärminne Zoological Station and the Archipelago Research Institute staff for their assistance in sampling, which in some occasions occurred in very strange places and in very unfavourable conditions. I would also like to thank the Jenny and Antti Wihuri Foundation, Kone Foundation and all the other funding bodies that gave me support during this Ph.D. work.

The girls in our research group – Hanna, Maria Po, Maria Pi, Johanna and Tea, sim-

ply were there and were ready to take me to coffee breaks even if I was sometimes (or most of the time?) a little bit light-headed or moody. Special thanks to Viivi, who made me realize that it was time to finish the thesis and listened to me during the whole finishing process. Special thanks also to Marjo who has helped me in the lab and been a good friend. Anne M., thanks for collecting some of the samples. I would like to thank Kent Tankersley for help in prepairing the manuscript.

I would like to thank my friends for their support during the years I have been working on this project – I know that my mind sometimes tends to wander and it can be difficult to keep up with me during those moments. But it has been really good to have friends who take me out for bar-hopping or to other activities and help me to forget the stress I have been under.

And finally, thanks to my family for their support and help during the work. Cold winter days can be really cold when you have to stand next to the frozen Baltic Sea, it is snowing, you can barely see the bubbles and you know it is going to take another hour until you can get back to the car.

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