Population genetics of *Saccharina latissima* (sugar kelp) in Norway

Thesis for the degree of Master of Science

Marine Biology

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March 2019

Acknowledgements

There are many people I wish to show my gratitude for and to thank for their support over the course of my master's thesis. First, I would like to give a special thanks to my supervisor, Kjersti Sjøtun, for outlining the scope of this project, and for giving me valuable feedback, encouragement and support throughout the duration of this project. I will also give a special thanks to Pedro Ribeiro for giving me valuable input on how to interpret analyses used in population genetics, and for always being available to answer all my questions. I would like to thank my co-supervisor at IMR, Geir Dahle, for taking care of the microsatellite analyses and all that follows at IMR's DNA-laboratory in Bergen, and for giving me pointers on what analyses to run. Thanks to Francois Besnier for running the Lositan analysis. I want to thank David Rees for giving me useful instructions and guidance at the DNA-laboratory at the University of Bergen, and for teaching me the methods involved in extracting and processing of the genetic material. Also, thanks to Louise Lindblom for giving an educational introductory course to the DNA-lab at the University of Bergen, and for always being close by and eager to answer all my questions. I would like to thank Mari Heggernes Eilertsen for stepping in as substitute supervisor, and for supporting me when I thought everything was doomed. If it had not been for Kenneth Meland, I would not have figured out the errors in the data set, and for that I am forever grateful. Thanks to Kjersti Sjøtun (again), Pia Ve Dahlen and Stein Fredriksen for providing me with samples needed to conduct this project.

Finally, I would like to thank my family and friends for all their love and support.

Abstract

The interest in cultivating sugar kelp, *Saccharina latissima* in Norway is increasing and therefore knowledge about the population genetics of this species is important. A total of 345 samples were genotyped from sixteen sampling locations along the Norwegian coast, and with special emphasis on Norway's two biggest fjord systems; Hardangerfjord and Sognefjord. Microsatellite- and statistical analyses of sampled *S. latissima* populations demonstrated some genetic differentiation, and the result of population structure analyses suggested that the material could be separated into three different genetic groups. The overall pattern of the genetic structure indicated some restrictions on geneflow inward the fjords, while the gene flow along the coast is quite good. This was supported by the pair-wise F_{ST} values and a significant isolation-by-distance pattern. The result from the present study intended to give important knowledge about sugar kelp population genetics in Norway and be valuable for implementation of coastal regulations for kelp cultivation activity.

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1 Introduction

1.1 Ecological and economical importance

Kelp forests dominate shallow subtidal rocky coastlines in most temperate and cold-water marine environments worldwide (Dayton, 1985; Lüning, 1990; Steneck et al., 2002), including the coastline of Norway. Their high productivity, biomass and community structure role make kelps especially important (Dayton, 1985), and they considered to be both ecologically and economically important (Guzinski et al., 2016). They create habitat, shelter and nursery for many organisms living in lower intertidal and shallow subtidal environments such as marine mammals, fish, and invertebrates, as well as other algae and support therefore high biodiversity (Dayton, 1985; Bertness & Bruno, 2001; Lippert et al., 2001; Møller Nielsen et al., 2016). This underpins one important ecosystem function of kelps: providing a suitable habitat for a great variety of species. Species associated with kelp forests either serve as food for higher trophic levels or are consumers of their host or the associated assemblage (Bartsch *et al.*, 2008). With this high biodiversity kelp forests concentrate a source of nutrition for coastal marine ecosystems via food webs based on particulate organic matter (detritus) (Steneck *et al.*, 2002). The trophic connections suggest a complex and finely triggered interaction web among kelps and their associated fauna, flora and microorganisms (Bartsch *et al.*, 2008).

In addition to providing ecosystem services and being important as ecosystem species, kelps are also utilized by humans and are of great economic importance many places of the world. The major part of kelp is cultured, while harvesting from natural kelp populations constitute a minor fraction. The total world production (cultivation) of kelp in 2016 was just over 30 million tonnes fresh weight (FAO, 2018). In Europe and America, the most interesting kelp for cultivation have been sugar kelp, *Saccharina latissima* (Linnaeus) C. E. Lane, C. Mayes, Druehl & G. W. Saunders for human consumption, fish feed and potential biofuel (Paulino *et al.*, 2016), which gives this species an economic value.

Norway has a long and complex coastline extending over 100 000 km and has a well-established aquaculture sector offering suitable preconditions for developing large-scale cultivation of macroalgae biomass (Stévant et al., 2017).The prerequisites for industrializing cultivation of macroalgae are therefore very good in Norway (Skjermo et al., 2014). Industrial cultivation of kelp provides opportunities to produce a biomass which can be the basis for

many various products, and which can help Norway to become more self-sufficient to food, feed ingredients, and bioenergy. Kelps are primary producers that can be cultivated without the use of soil, fertilizer, fresh water, pesticides or antibiotics, and Norway has vast coastal areas that can be used (Skjermo, 2019). *Saccharina latissima* is one of the promising species for industrial cultivation in Norway (Handå, 2019) because of its potential for high biomass yields and valuable nutritional content (Stévant, Rebours and Chapman, 2017). In Norway there are have several companies that focus on commercial cultivation of *Saccharina latissima*. The Norwegian Directorate of fisheries keeps a register over all companies engaged in aquaculture. This register is continuously updated every week, and as of January 21st, 2019, there were 44 companies with a total of 83 concessions to cultivate *Saccharina latissima* in Norway.

1.2 Distribution of *Saccharina latissima*

Saccharina latissima is a perennial brown macroalgae formerly known as *Laminaria saccharina*, that belongs to the class *Phaeophyceae*, order *Laminariales* and family *Laminariaceae*. *S. latissima* has a circumpolar distribution (Bolton et al., 1983) and is native to the coastal regions of the Northern Hemisphere (Figure 1: Lüning, 1990). Kelps are cold-water organisms (Steneck *et al.*, 2002) and are generally found in areas where the summer temperature does not exceed 20˚C in the water and where there is sufficient nutrition present, at least in parts of the year. According to Lüning 1990, gametophytes of *S. latissima* have an upper survival limit at 22-23 ˚C, but for the kelp to be able to reproduce (produce gametes) the temperature should be below 18 ˚C. Optimum temperature for young sporophyte growth in sugar kelp is set to be around 10-15 ˚C (Lüning, 1990).

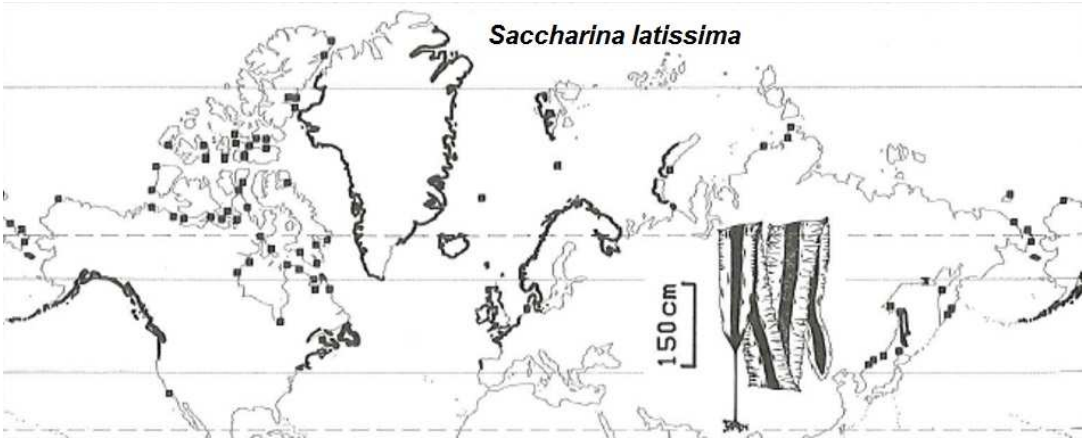

Figure 1: A map representing the coastal regions of the Northern Hemisphere native to *Saccharina latissima*. The map is slightly modified from Lüning (1990).

1.3 Life cycle of kelp

Saccharina latissima has, like all species in the order *Laminariales*, a complex heteromorphic diplohaplontic life cycle. The complete life cycle involves several steps; maturation, release of propagules, dispersal, arrival at the substrate, attachment and germination of the propagules, fertilization and development of new sporophytes. The mature diploid sporophyte releases haploid zoospores (propagules) into the water column most commonly during autumn and winter (Bartsch *et al.*, 2008). The proliferation stage for all kelp species is when the spores are released from the mature sporophyte. Once the spores are released, their dispersal is influenced by physical processes such as currents and water motion, and by survivorship in the water column and availability of suitable substrata (Dayton, 1985). The haploid spores are tiny and there are several variables that affect the dispersal range e.g. the dispersibility of the propagules, the concentration of propagules released, and most important the currents and water motion at the dispersal site (Dayton, 1985; Stévant et al., 2017). In seaweed biology, dispersal refers to the spreading of propagules in all directions from the mature diploid sporophyte (Fredriksen et al., 1995), and is a mechanism that promotes genetic diversity within populations and decreases it between them (Reed et al., 1992). The spreading range for the spores is generally no more than some tens of meters from the parental thalli, and their numbers fall exponentially with distance from the source (Reed et al., 1992; Fredriksen et al, 1995). The mortality of these spores is enormous, as sugar kelp only grows on a narrow belt along the rocky shores at certain depths and the chance for a spore to arrive in a suitable habitat is relatively small. If the zoospores eventually reach the bottom substrate, preferably rocky bottom, they will germinate and develop into microscopic male and female gametophytes only if the conditions allow them to. If the spores end up too deep, they will not be able to develop any further. *Saccharina latissima* has dioecious gametophytes meaning the zoospores of opposite sex must settle within a certain range of each other to fertilize. The egg produces an attractant (the pheromone lamoxirene) that guides the spermatozoids to the egg and make them emerge from their antheridia (Bartsch *et al.*, 2008). However, the range of the attraction of the egg does not exceed 1 mm, meaning the egg and the spermatozoids need to be very close to one another for a successful fertilization (Reed, 1990; Fredriksen et al., 1995). If the fertilization is successful, the male gametophyte and the female gametophyte will give rise to a new macroscopic diploid sporophyte generation. Sporophytes reach maturity when they are from 8 to 12 months old (Parke, 1948).

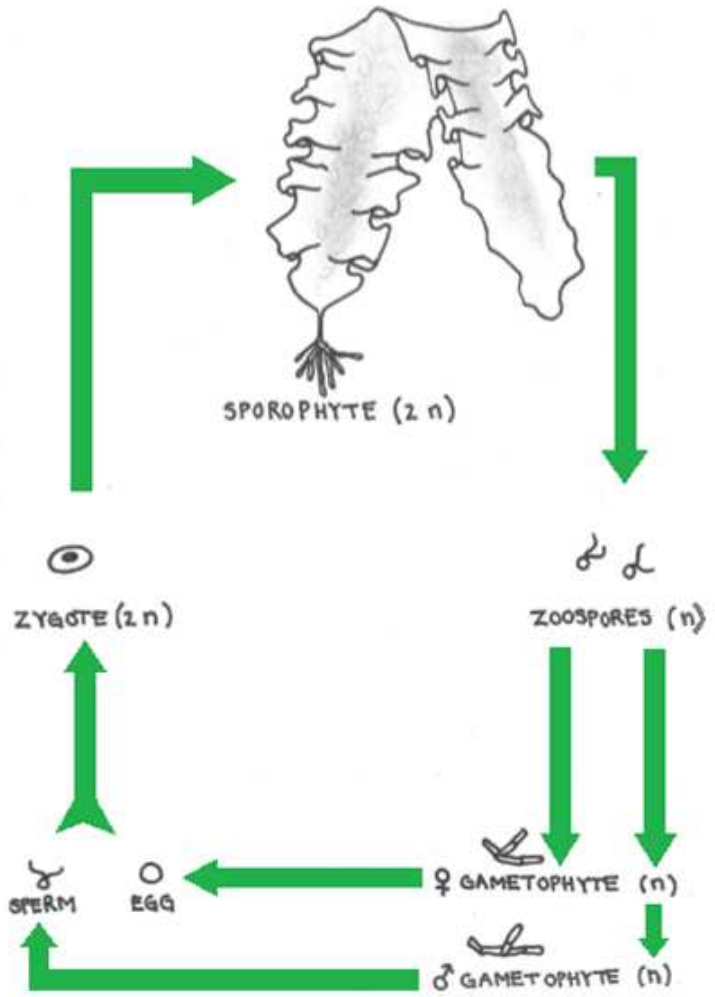

Figure 2: The drawing represents the life cycle of *Saccharina latissima* where the mature diploid sporophyte (2n) release haploid zoospores (n) which will eventually germinate into dioecious gametophytes when they reach a decent spot on the bottom substrate. The female gametophyte produces eggs (n) while the male gametophyte produces spermatozoids (n) which will through fertilization give rise to a diploid zygote (2n), which will germinate into a new diploid sporophyte (2n).

1.4 Gene flow and population genetics of *Saccharina latissima* in Norway

In population biology, dispersal refers to the general phenomenon of propagule displacements from one area to another regardless of scale (Valero et al., 2001). For kelps, in this case, *Saccharina latissima*, gene flow will be strongly influenced by spore dispersal. While the vast majority of spores are predicted to settle close to parental thalli, results from Brennan et al. (2014) revealed that there is potential for long-distance dispersal, although the general pattern is one of isolation-by-distance. As mentioned earlier, dispersal of haploid spores of *S. latissima* is affected by the currents and water motion at the dispersal site. In Norway one would assume that The Norwegian Coastal Current (NCC) can play an important role in geneflow of *S. latissima*, as this current flows northwards along the Norwegian coast (Sætre, 2007). Hardangerfjord and Sognefjord are the biggest fjord systems in Norway, and along with the rest of the fjords these are the main source of freshwater to the NCC (Aure et al., 2007).

The freshwater is forced by pressure out towards the coast, flowing in a brackish upper layer and can take spores from *S. latissima* from one area to another. Water exchange between fjord and coast is the sum of different components, such as tides, winds and atmospheric pressure. Tidal variations have a great influence on the coast/fjord water exchange due to rapid fluctuations and relatively large tidal differences. Fluctuations in density in the coastal water generate horizontal coast/fjord pressure differences, which induce in- or outflowing currents (Aure et al., 2007).

Studies on population genetics focus on the organization of genetic variability within and between populations of a species and can say something about the proliferation of genes or a degree of isolation of populations. The advances of molecular biology today offer a selection of several polymorphic DNA genetic markers where microsatellites are one of them (Valero *et al.*, 2001). Microsatellites or SSRs short for Simple Sequence Repeats, are widely used in plant genetics studies (Vieira et al., 2016) and are genetic markers that can be used to locate a specific segment of genetic material that has a known location on a chromosome. They represent a cost efficient and quick method of analysing gene exchange between populations, provided that polymorphic microsatellites in sufficient numbers have been developed for the species in question.

Cultivation of sugar kelp in Norway is in the initial phase, and little is known about the genetic variation along the Norwegian coast, except the recently published paper by Evankow et al. (2019). There are a few issues that needs to be addressed before scaling up the macroalgal production. One issue is the question about the risk of genetic interactions between cultivated crops and wild populations (Stévant, Rebours and Chapman, 2017). There is a risk for spreading of spores from fertile cultivated sporophytes if these get mature before the biomass is harvested. Also, there is a risk that the thallus or parts of the thallus of small sporophytes can be lost and continue to grow and get fertile outside the cultivated areas. Genetic interactions can thus be expected. Cross breeding between cultivated and wild kelp can be regarded as a possible negative interaction with the ecosystem and cultivation through breeding of strains for certain traits can thus represent a threat against the wild populations (Skjermo *et al.*, 2014).

The genetic structure and degrees of isolation between populations of species that are relevant for aquaculture in Norway needs to be researched more.

A recent article by Mooney et al. (2018) studied the pattern of gene flow in *Saccharina latissima* across the northern part of the Irish Sea, by combining population genetics (microsatellites) and hydrodynamic modelling approach. It was concluded by suggesting that geographical distance and proximity need to be taken into account when planning the siting of kelp farms with the aim of minimizing gene flow to and from natural populations (Mooney et al., 2018). Information about the genetic diversity within the different species to be cultivated is vital to establish a knowledge base for guidance of the authorities in development of the regulations for kelp cultivation (Skjermo *et al.*, 2014).

Evankow et al. (2019) provided an assessment of the genetic heterogeneity of two bioeconomically important kelp species, *Laminaria hyperborea* and *Saccharina latissima*, across the Norwegian coast, by applying microsatellite genotyping. The study was based on a master study done in 2015 (Evankow, 2015). The sampling sites took place from the Skagerrak region up to Greenland Sea, and suggested that there was significant genetic structure, differentiation and varying genetic diversity of *S. latissima* along the Norwegian coast. An IBD pattern was found and *S. latissima* was separated into three geographical clusters along the Norwegian coast.

Guzinski et al. (2016) did a genetic diversity study between European *S. latissima* populations in 2016. The chosen localities were distributed along the European Atlantic coast from Southern Brittany (France) to Spitzbergen (Norway). The study revealed low genetic diversity within and low connectivity between the populations.

Paulino et al. (2016) described and published 12 polymorphic microsatellites in 2016; SLN319, SLN32, SLN320, SLN34, SLN35, SLN36, SLN314, SLN510, SLN511, SLN54, SLN58 and SLN62. These microsatellite markers have been used in later studies focusing on genetic diversity and structure in different areas in the world. For example, a study of the genetic structure of *S. latissima* was done in eastern Maine, USA in 2017. This was done because of an interest to develop sugar kelp cultivation in this area. The purpose was to characterize the genetic structure by using the 12 microsatellite loci. Overall, *S. latissima* exhibited relatively low genetic diversity in this study area. It was detected that sugar kelp populations can be finely structured across small spatial scales, and that future management and cultivation efforts should aim to maintain genetic diversity and assess the culture potential of local populations (Breton et al., 2017).

Another genetic diversity study was done on *S. latissima* along a salinity gradient in the North Sea – Baltic Sea transition zone in 2016. Patterns of genetic diversity of *S. latissima* populations were evaluated along the salinity gradient area of Danish waters which were designated brackish and were compared to marine reference sites outside the gradient area, using the same 12 microsatellite markers. Results showed that the brackish populations were less diverse, more related, and showed increased differentiation over distance compared to the marine populations (Møller Nielsen et al., 2016).

This thesis will focus on the population genetics of *Saccharina latissima* in Norway, and especially Hardangerfjord and Sognefjord, Norway's two biggest fjord systems. To get a few more components in this study samples from a site in Oslofjord (East), Hafrsfjord (Southern Norway), Runde (Mid-Norway, West) and Sommarøy (Northern Norway) are included as well. The samples will be used in a study of degree of local population connection and gene flow in a fjord seascape. The reason for looking at the population genetics in these two fjord systems is because the populations here is potentially isolated, and the environment is different from the coastal environment.

1.5 Aim of the study

This Master thesis will focus on the kelp *Saccharina latissima*, where the main aim is to describe the population genetics of this kelp between and within two large fjord systems in Norway, and to identify possible genetically separated groups in the material. The large fjord systems in focus are Hardangerfjord and Sognefjord on the South-West coast of Norway. Two sub aims will also be included, where the first sub aim is to discuss the genetic structure and distribution of haplotypes in relation to the general coastal current patterns. The second sub aim is to find results which are useful for coastal management. The results will give an indication of degree of gene flow in *S. latissima* along the coast, and if isolated populations exist. Microsatellite DNA markers will be used to investigate patterns of genetic diversity, differentiation and structure, testing the null hypothesis (H_0): There are no genetic differences between or among sites, such that all sampled individuals are part of a single panmictic population unit, which implies there are no significant differences in allele frequencies or differences in heterozygote frequencies (F_{ST}=0) between sites.

1.5.1 Study questions

- Will the genetic structure gradually change along the coast (South to North)?
- Will the genetic structure gradually change from the outer to the innermost parts of the fjords?
- Will the result show greater isolation between the sites along the coast, or between sites located in the fjord and along the coast?
- How is the genetic structure in Hardangerfjord (HA) relative to Sognefjord (SO)?

2 Material and Methods

2.1 Study area

Samples of *Saccharina latissima* for this master thesis were collected from 16 sites along the Norwegian west coast from south to north, with special emphasis on two large fjord systems; Hardangerfjord and Sognefjord (Figure 4 and Table 1).

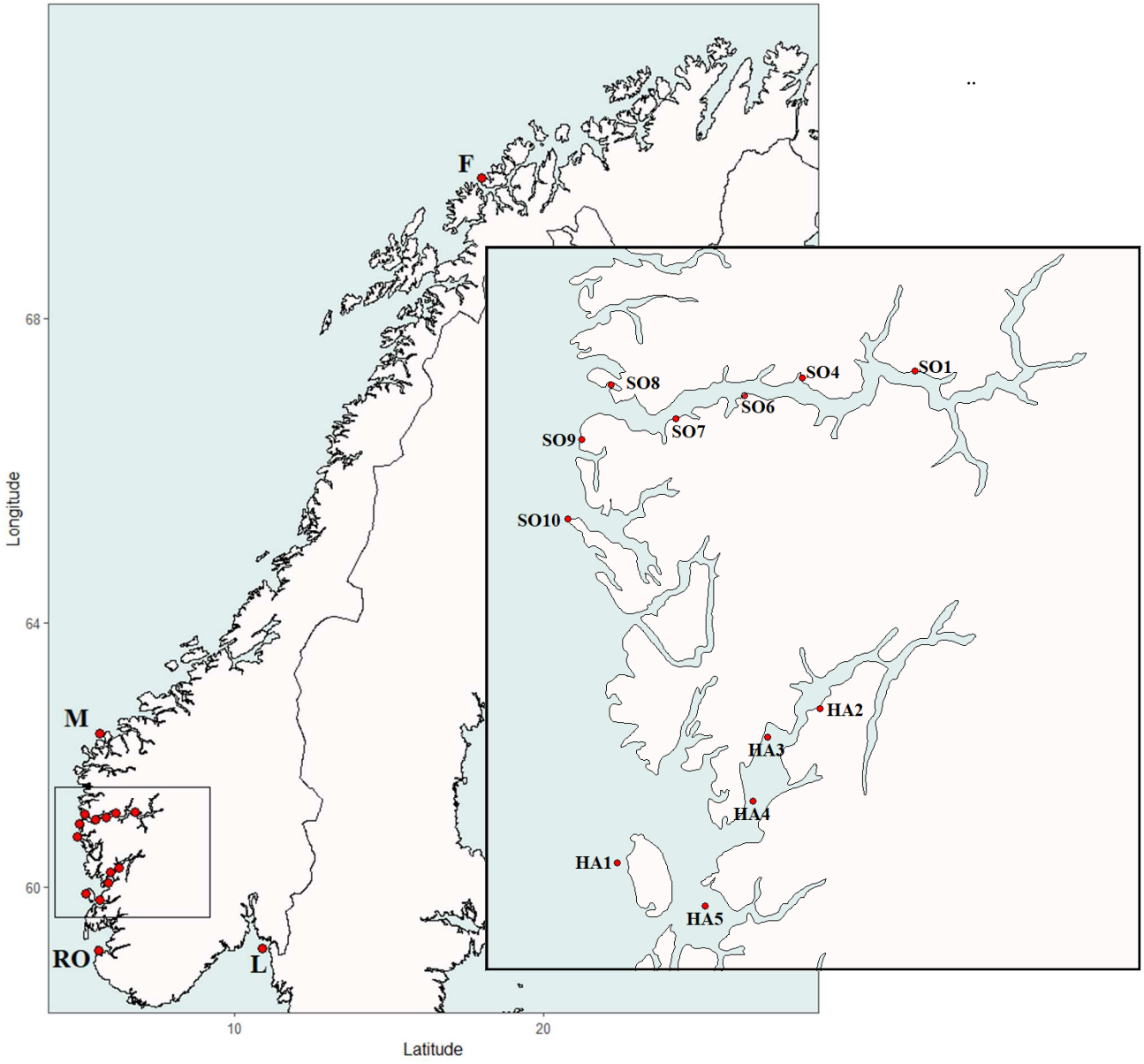

Figure 3: Map showing the locations of the 16 sampling sites of *Saccharina latissima*. For detailed information, see Table 1.

Samples from Hardangerfjord and Sognefjord were collected in a gradient from the innermost parts of the fjord (or the innermost site where *S. latissima* was found) to the outer coastal parts. Some extra samples were collected from Oslofjord (East), Hafrsfjord (Southern Norway), Runde (Mid-Norway, West) and Sommarøy (Northern Norway) as well. Initially samples were collected from 21 sites, but due to problems with DNA extractions and microsatellite analyses, some were omitted from further analyses. All samples were collected during 2016-2018 (Dates shown in Table 1).

Table 1: Overview of the 16 sites used in this study with coordinates, sampling date, how many samples of *Saccharina latissima* were collected at each site and who the samples were collected by. Localization of the sites is shown in Figure 3.

Site	Position	Date	Sample Site-ID	No. Samples	Collected by
Outer Oslofjord	58°59'19.46"N 10°55'25.8"E	20.08.17	L	30	Dalen, Fredriksen
Hafrsfjord	58°57'36.5"N 5°36'35.9"E	01.06.18	RO	30	Næss
Klosterfjord	59°46'53.0"N 5°40'13.4"E	10.11.17	HA5	29	Sjøtun, Næss
Solesnes, Jondal	60°18'19.6"N 6°16'49.5"E	08.11.17	HA2	27	Sjøtun
Skjerring	60°13'48.6"N 6°00'12.2"E	09.11.17	HA3	33	Sjøtun, Næss
Gjermundshamn	60°03'34.0"N 5°55'24.0"E	09.11.17	HA4	27	Sjøtun, Næss
Bårdholmen	59°53'45.6"N 5°12'09.6"E	08.08.17	HA1	28	Sjøtun
Kilstraumen	60°48'00.4"N 4°56'25.0"E	28.04.17	SO10	21	Sjøtun
Nyhamnarsundet	61°00'19.1"N 5°00'45.9"E	28.04.17	SO9	19	Sjøtun
Oppedalsvika	61°03'33.7"N 5°30'44.5"E	27.04.17	SO7	17	Sjøtun
Fuglsetfjorden	61°06'13.4"N 5°52'12.4"E	27.04.17	SO6	21	Sjøtun
Leikanger	61°10'55.9"N 6°47'05.3"E	25.04.17	SO1	23	Sjøtun
Lånefjorden	61°09'53.0"N 6°11'15.1"E	26.04.17	SO4	19	Sjøtun
Risnesstraumen	61°08'49.6"N 5°10'09.6"E	27.04.17	SO8	21	Sjøtun
Runde	62°23'52.9"N 5°39'42.3"E	21.03.18	M	24	Fredriksen
Sommarøy	69°38'21.2"N 18°01'4.5"E	13.08.16	F	12	Fredriksen

2.2 Collection of samples and preservation

The sampling was conducted in different ways, depending on the depth range of *S. latissima* at the site, local topography, and what equipment was available for sampling. The sampling method that was most commonly used was to drag a triangular formed scrape along the sea floor where the sugar kelp most likely would grow. Most of the fjord sites were very steep and *S. latissima* was most common shallower than 5 metres. At these stations the sampling was done from a small motorboat, using a small triangular scrape dragged along the sea bottom by hand. At some sites, samples were collected by hand or with a rake during low tide. When the samples were collected by hand it was done in a semi-random manner to avoid collection of adjacent individuals that might originate from the same gametophyte. When using a scrape this was difficult to control, but several dredgings were done at each site and kelp individuals picked randomly from the catch for sampling. The overall sampling depth was between 0 and 10 m with some sites sampled in the shallow part and some in the deeper part of this range, and samples were collected from a stretch along the shore for at least 30-50 minutes for most of the sites.

Clean pieces of blade tissue of 1-2 cm^2 were cut from the meristematic region of the blade on each kelp sample, added to screw-capped tubes and then covered with silica gel beads, to preserve and desiccate the *S. latissima* samples until DNA extractions were performed. See Appendix 1 to see how sampling was conducted in Hafrsfjord in June 2018.

2.3 Genetic analysis procedure

The DNA extractions, PCR (polymerase chain reactions) and dilutions were performed at the DNA laboratory at the Department of Biology, University of Bergen. The microsatellite analyses were performed at the IMR's (Institute of Marine Research) DNA-laboratory in Bergen (Nordnesgaten 50). Twelve polymorphic microsatellite markers have been characterized in the sugar kelp *Saccharina latissima*, and are listed in Table 2 (Paulino et al., 2016). Locus SLN 511 was omitted because of low success rate (no PCR fragments were detected after two rounds), and consequently 11 out of 12 microsatellite loci were used in this study.

Table 2: Characterization of 12 microsatellite loci in *Saccharina latissima* (Paulino et al., 2016), including locus name, GenBank accession number, primer sequences, repeat motif, annealing temperature (T_a) and magnesium concentration ($MgCl_2$) used in PCR, and size range (bp) of the alleles. Locus SLN511 (shown in italics) did not amplify for the samples and was omitted.

Locus name	**Genebank accession**	**Primer sequences (5′-3′)**	**Repeat motif**	**T_a (°C)**	**MgCl2 (mM)**	**Size range (bp)**
SLN314	KT723013	F: CTGTGTGTGTTGTCGTACATCG R: GGATTTCTTATTTGAGGGAGGG	$(TAC)_{11}$	58	2.0	235–302
SLN319	KT723014	F: CGAAGGAAGTGAATGACAACAA R: GGTAGTTACGGATTGCGACAAG	$(ACA)_{10}$	56	2.0	378–433
SLN32	KT723015	F: GAGAAAACATGCCCAGGTCTA R: GTATCGCTGTACCCTCCTCCT	$(CAG)_{11}$	57	2.0	222–280
SLN320	KT723016	F: TACGATGGTTTATGGGTTAGGG R: AGCGAACAACGAAGCAACTAAT	$(TGT)_{13}$	56	2.0	210–241
SLN34	KT723017	F: ACGAAGTGCTAATAATGTGCCG R: GAGATAGCCCGACCACTGC	$(AGC)_{10}$	56	2.0	183–319
SLN35	KT723018	F: GCGTATGAACAAAATGACCGTA R: TGTGAGTTCCTTTCTTGTGAGC	$(CTG)_{11}$	56	2.0	343–372
SLN36	KT723019	F: CGAGACTTTTGGGTAGATTTCG R: CGCCTGCCTCTTGTCTAAGTA	$(AGT)_{19}$	57	2.0	264–315
SLN510	KT723020	F: CCGTCTATGGCGAGAAAGAGAT R: ATCTTACCTGGGCACTTGCTTT	$(ACACA)_{13}$	58	2.0	242–339
SLN511	*KT723021*	*F: ATGTCCTGACCTGACCTACAGC R: AATTCTGTGAACATTCGGGAGT*	*$(ACCTT)_{19}$*	*54*	*2.5*	*366–400*
SLN54	KT723022	F: GTGGTTGCTGTTGTTGCTGT R: CGAATAAAGACAAATCGGCTG	$(ATATC)_{11}$	54.5	1.5	298–337
SLN58	KT723023	F: GCGAAGAAACGAGGGTTACAT R: CTGGGTTTGTCGAGTGTTGAT	$(GCAAG)_8$	55	2.0	153–173
SLN62	KT723024	F: ACAAAGCGTTCTCAACCGAT R: CGACACCCTACACAATACGAAA	$(TATACA)_6$	55	2.0	164–281

2.3.1 DNA extraction

Genomic DNA was extracted from a small piece of plant tissue, around 4 mm^2. The tissue was subsampled from the silica gel dried meristem blade fragments. Due to the high number of samples initially collected for this project it was decided that DNA ought to be extracted by using the Qiagen DNeasy® 96 Plant Kit. After a lot of trial and error the Macherey Nagel NucleoMag® Plant kit was eventually tested on the "problem samples" and others, and this kit proved to give clean DNA for most of the remaining samples.

Preparation before DNA extraction

When working with desiccated kelp tissue, the samples needed to be broken down to smaller pieces to prepare the tissue for DNA extraction, and this was done by using the Qiagen TissueLyser II. Use of the TissueLyser, for rapid and convenient disruption of plant tissue samples, was recommended for the most efficient processing in both DNeasy Plant procedures and NucleoMag Plant procedures. Complete and quick disruption of starting material was essential to ensure high DNA yields and to avoid DNA degradation. *Saccharina latissima* samples were placed into collection microtube racks, one sample in each tube along with a 3 mm tungsten carbide bead (Qiagen). This was done before using either the DNeasy® 96 Plant Kit or the NucleoMag® Plant Kit. The preparation of the kelp tissue varied a bit depending on what kit was used. When using the DNeasy® 96 Plant Kit, and before placing the collection microtube racks in the TissueLyser II, a working lysis solution was added to all the tubes. For 2 x 96 samples was 90 ml Buffer AP1 (preheated to 65˚C), 225µl RNase A and 225µl Reagent DX (anti foaming component) mixed to make a fresh working lysis solution. 400µl of working lysis solution was pipetted into each collection microtube along with the plant material. When using the NucleoMag® Plant Kit, the only components in the collection microtube racks were the plant tissue and the tungsten carbide bead. Also, only one 96 plate was extracted at a time, so an extra collection microtube rack was made to balance the TissueLyser, and later the centrifuge steps for optimal operation. The collection microtube racks were placed into adaptor sets, which were fixed into the clamps of the TissueLyser II and shaken vigorously for 1,5 minutes at 30 Hz (DNeasy® 96 Plant Kit) and 20 seconds at 20 Hz (NucleoMag® Plant Kit). This was done in two rounds, where the racks were reassembled so that the collection microtubes nearest the TissueLyser in round one was furthest from the TissueLyser II in round two. Rotating the racks of collection microtubes in this way ensured that all samples was thoroughly disrupted.

DNeasy® 96 Plant Kit

The DNeasy® 96 Plant Kit provided a fast and easy way to purify genomic DNA from plant tissue. DNA purification followed the description in DNeasy Plant procedures, where plant material was first mechanically disrupted (Qiagen TissueLyser II) and then lysed by addition of lysis buffer and incubation. RNase A in the lysis buffer digests the RNA in the sample. After lysis, proteins and polysaccharides were salt-precipitated. Cell debris and precipitates were removed by centrifugation. Binding buffer and ethanol were added to the cleared lysate

to promote binding of the DNA to the DNeasy membrane. The sample was thereafter applied to a DNeasy 96 plate and centrifuged. DNA bound to the membrane while contaminants such as proteins and polysaccharides were efficiently removed by two wash steps. Pure DNA was eluted in a small volume of water.

When using the DNeasy® 96 Plant Kit, the basic protocol was followed (See Appendix 2) with a few minor modifications. The protocol was for purifying DNA from 2 x 96 samples of dried plant tissue. The procedure included 22 steps, from desiccated plant tissue to purified genomic DNA. The first 8 steps are described above as a preparation for the DNA extraction itself. All the steps were followed thoroughly, but the centrifugation steps, especially step 12 and 20 had to be prolonged. In step 12 the protocol states 5 minutes at 6000 rpm, but as the centrifuge that was used (Thermo Scientific Heraeus® Multifuge® 3S-R Plus Centrifuge) had a maximum speed of 5650 rpm, the centrifugation was set to 10 minutes in the first round, and if needed, a second round of 10 minutes. According to the protocol step 20 included centrifuging for 15 minutes, but this did not dry the membranes properly, so 5 more minutes were added.

NucleoMag® Plant Kit

The procedure of the NucleoMag® 96 Plant Kit is based on reversible adsorption of nucleic acids to paramagnetic beads under appropriate buffer conditions. The DNA of *Saccharina latissima* was extracted with CTAB-Lysis Buffer MC1. The binding conditions to bind DNA to NucleoMag® C-Beads was made by mixing MC2 buffer and NucleoMag® C-Beads, which were paramagnetic beads. For 96 samples, 2880 µL of NucleoMag® C-Beads was mixed with 38,4 mL of MC2 buffer by vortexing. The premixing step allowed an easier homogenous distribution of the beads to the individual wells of the separation plate, which was recommended to keep the beads resuspended. The NucleoMag® C-Beads sank to the bottom so it was important to keep the mixture in motion while pipetting. The kit was designed for use with NucleoMag® SEP magnetic separator plate. This type of separator is recommended in combination with a suitable microplate shaker for optimal resuspension of the beads during the washing and elution steps, but the beads got easily resuspended in the buffer by pipetting up and down several times, so there was no need for the microplate shaker. After magnetic separation, which was carried out in a square-well block, and removal of supernatant, the paramagnetic beads were washed with wash buffers MC3, MC4, and 80 % ethanol to remove contaminants and salt.

There was no need for a drying step as ethanol from previous wash steps was removed by wash buffer MC5. Finally, highly purified DNA was eluted with low-salt elution buffer MC6 and was ready to be used for further analysis.

When using the NucleoMag® Plant Kit the manufacturer's instructions were followed (See Appendix 3), with modifications from Fort et al. (2018). A master mix for 100 samples (1 plate: 96 samples) comprised 50 ml MC1, 100 µl Proteinase K solution, 300 µl RNase A and 1,9 ml ddH_2O (double distilled water). 523 µl of the master mix were added into each sample.

Post DNA extraction

DNA extracts from both DNeasy® 96 Plant Kit and NucleoMag® Plant kit was diluted 1:10 (2 µl DNA + 18 µl ddH_2O) and stored in the dark in a 4˚C refrigerator.

2.3.2 Pre-PCR

Four loci were grouped together (multiplex); SLN 319, SLN 320, SLN 34 and SLN 32, and amplified in a single reaction. SLN 54, SLN 58 and SLN 62 could not be multiplexed, so each locus got its own PCR cocktail and was amplified individually (singleplex), before reactions for the three loci were combined into one plate after PCR. The last group, SLN 35, SLN 36, SLN 314 and SLN 510 was multiplexed and amplified in one single reaction. All eleven primers were available and at the DNA-laboratory at BIO. Stock solutions were prepared by diluting the primers to 100 µmol in TE (Tris-EDTA) buffer and stored in a -18˚C freezer.

Table 3: Reaction cocktail for one multiplex (4 primers) sample.

Reagents	Volume
ddH_2O	1.26 µl
Forward primer (x4) *	0.03 µl
Reverse primer (x4) *	0.03 µl
AmpliTaq 360 mix	2.5 µl
TOT	4 µl

* Multiplex SLN 319: Primers (forward and reverse): SLN319, SLN320, SLN34 and SLN32.
* Multiplex SLN 35: Primers (forward and reverse): SLN35, SLN36, SLN314 and SLN510.

When making a reaction cocktail for two plates (192 samples) the recipe was multiplied with 210 just to make sure that there was enough reaction cocktail for all the samples (See Appendix 4).

Table 4: Reaction cocktail for one single plex (1 primer) sample.

Reagents	Volume
ddH_2O	1.44 µl
Forward primer	0.03 µl
Reverse primer	0.03 µl
AmpliTaq 360 mix	2.5 µl
TOT	4 µl

When making a reaction cocktail for a single plex sample 0.03 µl of each forward and reverse primer was needed. The difference in multiplex and single plex was the number of primers used. In a multiplex there was 4 primers that equal a volume of 0.03×8 µl. In a single plex there was only one primer, forward and reverse, giving a volume of 0.03×2 µl. To get the total volume of the reaction cocktail to 4 µl in a single plex sample, the volume of water (ddH_2O) was increased accordingly.

Five reaction cocktails were used in this study based on the basic recipes described above. The exact recipes of these cocktails can be found in Appendix 4. 4 µl of reaction cocktail was pipetted into each tube using the Repet-Man, then 1 µl of 1:10 DNA added to the strips. After this was done the plate containing the DNA extracts was ready for PCR performed in Bio-Rad S1000™ and C1000™ Thermal Cyclers.

2.3.3 PCR (Polymerase Chain Reaction)

PCR amplifications were performed in Bio-Rad S1000™ and C1000™ Thermal Cyclers. The different multiplex had different programs where the basic setting was the same, but the annealing temperature varied. PCR amplifications were performed in 5 µl reaction mixtures containing 1 µl of 1:10 template DNA and 4 µl of reaction cocktail. The PCR programs used in this study can be found in Appendix 5 and 6.

The single plex SLN 54, SLN 62 and SLN 58 were combined into a new plate post-PCR. 2 µl SLN 54, 2 µl SLN 62 and 2 µl SLN 58 was added into each well of the 96 plate. Then all the PCR products were diluted prior to microsatellite analyses at IMR, by adding 20 µl H_2O. The diluted PCR products was stored in a 4˚C refrigerator for no longer than two days. If they needed to be stored longer, they needed to be frozen at -18˚C.

2.4 Microsatellite Analyses

Genetic variation was assessed at 11 polymorphic microsatellite loci as previously described (Paulino et al., 2016) and are listed in Table 2. The microsatellite reactions were analysed in ABI 3730 DNA Analyzer (Applied Biosystems) at IMR's DNA-laboratory in Bergen. This device uses capillary electrophoresis of fluorescent-labelled DNA-fragments and are therefore suitable for microsatellite analysis. Before the PCR products were analysed in the ABI machine, a mixture comprising of 8 µl volume of GeneScan™ 500 LIZ™ size standard and 800 µl of Formamide was prepared at IMR, and 8 µl of this mixture was transferred into ABI plates with 2 µl of the diluted PCR products.

Binning and allele scoring were performed manually using GeneMapper™ Software 5 (Applied Biosystems). The GeneScan™ 500 LIZ™ size standard contained 16 DNA fragments with known sizes (35-500 bp) where all of them were marked with LIZ fluorophore, which have another colour than the microsatellite fragments that were being studied. The standard appeared as orange fragments, while the microsatellites appeared as red, blue, green or yellow (VIC, NED, PET, FAM). GeneMapper used the standard curve to calculate the size of all the fragments in each well. For those samples that showed weak or no peaks, high background noise or were scored with uncertainty in GeneMapper, the PCR process were repeated. For individuals with low scoring success across all loci, the entire process was re-run using new DNA extracts from the new magnetic method, using the NucleoMag® Plant kit.

2.5 Statistical Analyses

2.5.1 Genetic diversity and Hardy-Weinberg Equilibrium

Genetic diversity estimates such as allele frequencies, observed number of alleles (N_a), observed (H_O) and expected heterozygosity (H_E) and inbreeding coefficient (F_{IS}) were computed per sampling site and locus using GENEPOP 4.2 (web version) (Rousset, 2008).

Allelic richness had to be standardized to cope with uneven sample sizes and was therefore estimated by rarefaction to the smallest sample size (Petit et al., 1998) using FSTAT 2.9.3.2. (Goudet, 1995). Allelic richness, also referred to as allelic diversity or mean number of alleles per locus, is one of the most commonly reported measures of genetic variation (Leberg, 2002).

Significant deviations from Hardy-Weinberg equilibrium (HWE) and genotypic linkage disequilibrium (LD) between pairs of loci were tested by measuring their inbreeding coefficients (F_{IS}) and associated P-values using a Hardy-Weinberg exact test implemented GENEPOP 4.2 (web version), using the default settings (Rousset, 2008). GENEPOP 4.2 (web version) was also used to perform exact tests for HWE by microsatellite loci (test multi-population) and by population (test multi-locus) and considered a heterozygote deficit as the alternative hypothesis. This global exact test was performed by the Markov Chain Algorithm (Guo and Thompson, 1992), and the default settings of the Markov Chain parameters were 1000 dememorizations, 100 batches with 1000 iterations per batch. To correct for type I errors that may arise from multiple comparisons, significance values were adjusted using the sequential Bonferroni correction procedure with $\alpha = 0.05$ (Rice, 1989).

Evidence for null alleles and scoring errors caused by large allele dropout and stuttering was studied with MICROCHECKER 2.2.3 (Van Oosterhout et al., 2004), a software for identifying and correcting genotyping errors in microsatellite data. LOSITAN-selection workbench (Beaumont & Nichols, 1996; Antao et al., 2008) was used to test the presence of candidate loci under positive or balancing selection, with the default parameters and 50.000 simulations. To test whether potentially problematic loci may influence the results, F_{ST} analyses and STRUCTURE analyses were performed with all eleven loci, and after removing two loci.

2.5.2 Population structure and genetic differentiation

The software program STRUCTURE version 2.3.4 (Pritchard et al., 2000) was used to investigate population structure and is one of the most widely used population analysis tools that allows researchers to assess patterns of genetic structure in a set of samples (Porras-Hurtado et al., 2013). A structure analysis is a way of showing potential genetic homogenic clusters and is a decent way to visually display any structure that might appear in the material (Evanno et al., 2005).

It can identify subsets of the whole sample by distinguishing allele frequency differences within the data and can assign individuals to those sub-populations based on analysis of likelihoods (Porras-Hurtado et al., 2013)The program was run using a Markov chain Monte Carlo length of 1,000,000 steps after a burn-in of 200,000. The best number of clusters (K, set from 2-6) was determined over 20 independent runs using the web-based program Structure Selector (Li and Liu, 2018) a web server to select and visualize genetic clusters based on Evanno method also known as ΔK method (Evanno, Regnaut and Goudet, 2005).

To test for population differentiation among all samples, an exact G-test was conducted with the software GENEPOP 4.2 (web version). First genic differentiation for all populations (G-test and default settings), then the test was applied for all pairs of populations. Population differentiation was also estimated by calculating F_{ST} for each population and locus, and pair-wise F_{ST} (Weir and Cockerham) between sites in GENEPOP 4.2 (web version). Genetic differentiation among sampling sites was tested using the Analysis of Molecular Variance (AMOVA) implemented in GenAlEx (Genetic Analysis in Excel version 6.5) (Peakall & Smouse, 2006; Peakall & Smouse, 2012).

Isolation-by-distance (IBD) was tested in GENEPOP 4.2 (web version) using a Mantel test (1000 permutations), which tests for a correlation between pair-wise genetic distance and geographic distance matrices between sites. To do an IBD analysis by populations, a semi matrix of F_{ST} estimates had to be made, as well as a semi matrix of geographical distances measured in kilometres. IBD was tested by correlating these matrices of geographical distance measured along the coast line in kilometres, and F_{ST} values using GENEPOP 4.2 (web version). The distances were measured directly in the map (www.norgeskart.no), and the shortest sea distance between the sites were noted in the matrix. The program ISOLDE in GENEPOP was run, and this program computed a regression of $F_{ST}/(1-F_{ST})$ estimates to the natural logarithm of the geographic distances. The results from this test was used as input file in Excel to plot the result of the analyses.

3 Results

The final dataset included 345 individuals from 16 sites genotyped at 11 out of 12 microsatellite markers (loci) (Appendix 7). One microsatellite locus, SLN511 were omitted before analyses due to problems with amplification of the locus.

3.1 Testing for linkage disequilibrium, null alleles and candidate loci under selection

When allele frequency for alleles of two or more loci are linked the loci are in a state of linkage disequilibrium (LD) (Selkoe and Toonen, 2006). A linkage disequilibrium analysis was used to test each pair loci in each population, looking at 55 pair-wise comparisons within samples. Looking at the P-value for each locus pair across all populations (Fisher's method), linkage disequilibrium was not detected after applying the sequential Bonferroni corrections ($P \leq 0.0009$) and all loci were treated as independent variables (See Appendix 10 for the P-values).

Another problem which may occur in microsatellite analyses is the presence of null alleles, which may be evident as an excess of homozygotes across several sampling sites. The occurrence of null alleles can have different origins, for example lack of amplification of certain alleles (Selkoe and Toonen, 2006). Analysis of null alleles was done by MICROCHECKER, and the result showed potential null alleles at two loci, SLN34 and SLN54. A Hardy-Weinberg test implemented in GENEPOP 4.2 (web-version) strengthened the result from MICROCHECKER by testing for heterozygote deficit, as heterozygote deficiency (also called homozygote excess) can result from the presence of null alleles. Results by each population indicated that SLN34 and SLN54 showed highly significant deviations from HWE in form of heterozygote deficiency in most of the populations. Statistical analyses were therefore done both including and excluding these two alleles, in order to test if they had any effect on the results.

One prerequisite for using microsatellites in population genetic studies is that they should be neutral (Vieira *et al.*, 2016), e.g. not under selection or associated with parts of the genome which are under selection. The presence of candidate loci under positive or balancing

selection was detected. The assessment was a function of the relationship between heterozygosity and the fixation index (F_{ST}) at each locus across all individuals.
Two loci, SLN35 and SLN54 (SLN54 was also identified with null alleles) were found to be candidates for positive selection, and one locus, SLN314, was found to be a candidate under balancing selection. Outliers are tagged with labels (Figure 4). SLN314 is very close to being neutral (Figure 4) and is for that reason not considered further.

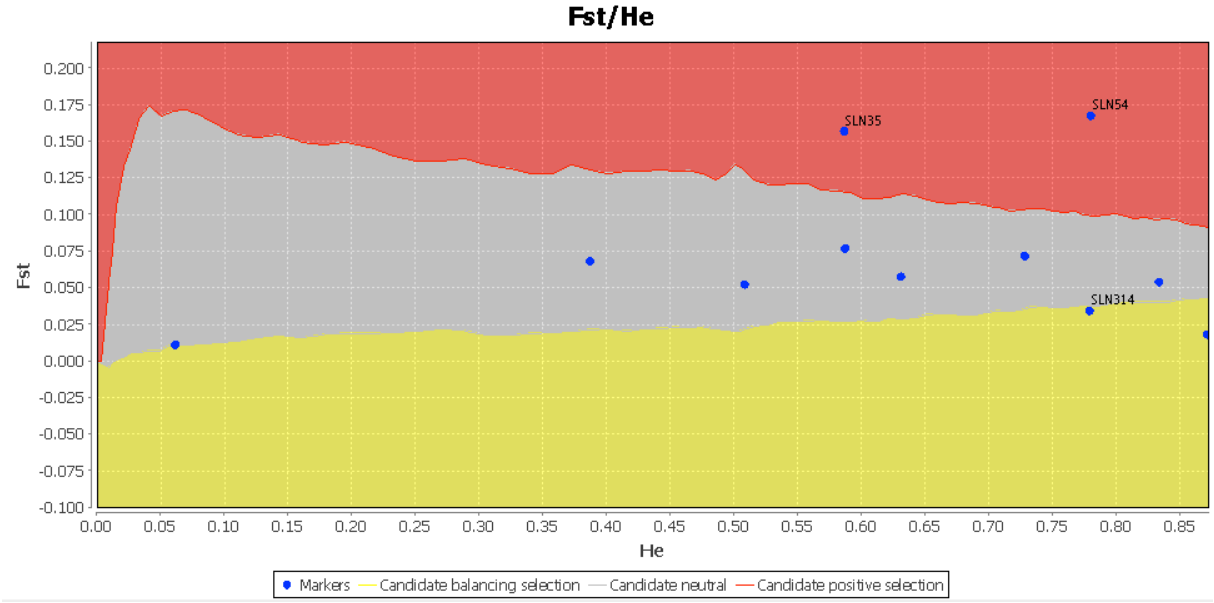

Figure 4. Two loci, SLN35 and SLN54 were candidates for positive selection (red area), and SLN314 were candidate for balancing selection (yellow area). Outliers are tagged with labels.

After detecting loci with null alleles and candidate loci under positive selection is was decided to make three different datasets: one including all eleven loci, one excluding the null alleles (SLN54 and SLN34), and one excluding all "problematic" loci (SLN54, SLN34 and SLN35) to run all the statistical analyses to compare with each other.

In addition, it was decided to examine pair-wise F_{ST}, isolation-by-distance and to do a STRUCTURE analysis on the neutral loci (excluding SLN54, SLN34 and SLN35) after excluding the populations sampled in Hardangerfjord and Sognefjord. This was done in order to see if there was any difference in the genetic structure revealed by the analysis when only the coastal populations (L, RO, HA1, SO10, SO9, M and F) were included.

3.2 Statistical Analyses

3.2.1 Genetic diversity and Hardy-Weinberg Equilibrium

Estimates of genetic diversity for the eleven microsatellite loci screened in the sugar kelp, *Saccharina latissima* can be found in Appendix 8, and summaries of this is listed in Table 6 and 7. The eleven microsatellite loci exhibited variable levels of polymorphism, as the total number of alleles observed across populations ranged from 5 at locus SLN62 to 26 at locus SLN36 with an average of 12.73 (Table 6). A measure to illustrate locus variability is allelic richness (A). Allelic richness across populations ranged from 1.226 at SLN58 to 5.156 at SLN36 and was based on the minimum sample size of 4 diploid individuals (or 8 genes). It had the highest mean value in the northernmost population at site F (3.434) and lowest at the innermost site in Sognefjord, SO1 (2.437). When looking at each locus allele richness was highest at SLN36 at site F (6.151) and lowest at SLN58 in population HA2, SO1, SO4 and SO8 (1.000) (Figure 5). The overall pattern shows a drop of allelic richness in the fjord populations, Hardangerfjord (HA) and Sognefjord (SO), which is most pronounced at the innermost site of Sognefjord (SO1) (Figure 5 and 6).

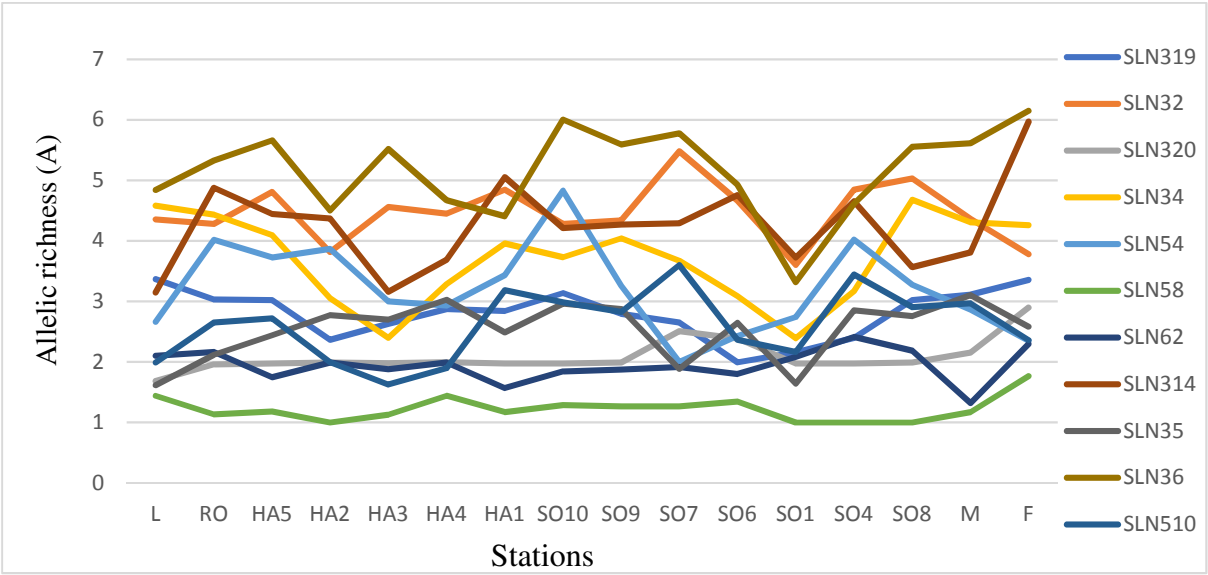

Figure 5: Allelic richness (A) at each locus at every population site.

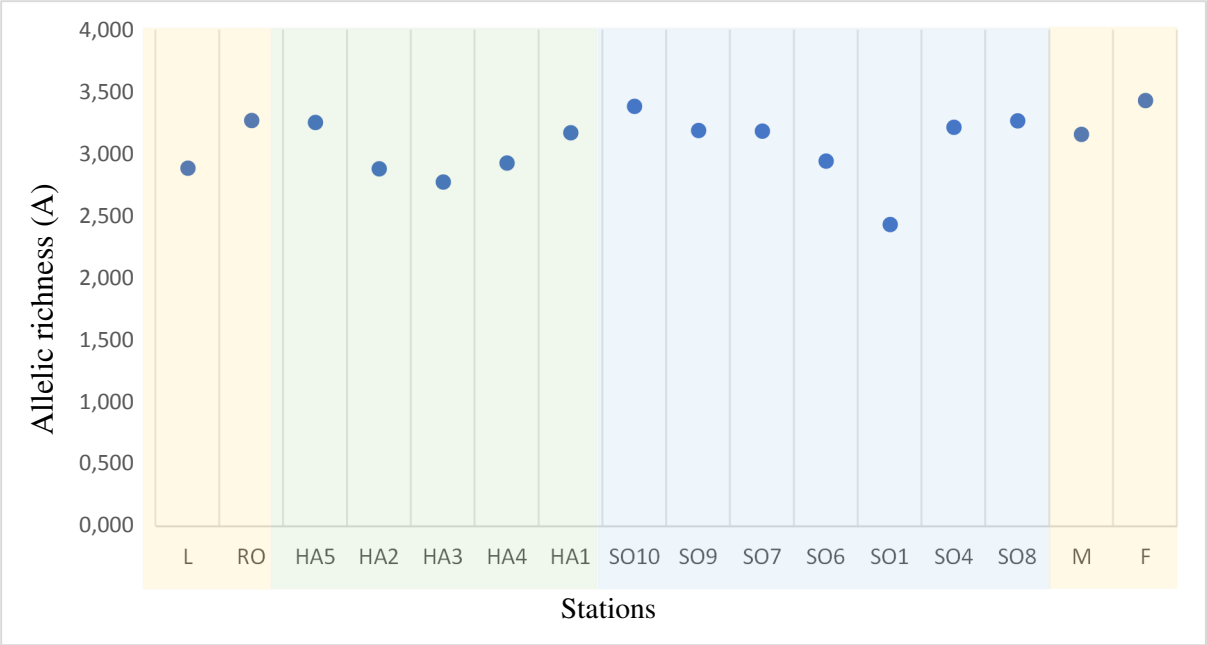

Figure 6: Mean allelic richness (A) at 11 loci per population.

Observed (H_O) and expected heterozygosity (H_E) across populations ranged from 0.036-0.800 and 0.053-0.854, respectively (Table 7). The number of observed heterozygotes was generally lower than expected number of heterozygotes across all populations. H_O had the lowest value across all populations at locus SLN58 (0.036) and the highest at SLN32 (0.800), while H_E had the lowest value across all populations at locus SLN58 (0.053) and the highest value at locus SLN36 (0.854) (Appendix 8 and Table 6). Average H_O and H_E value for all loci was 0.487 and 0.566, respectively.

Significant deviations from Hardy-Weinberg equilibrium indicated by F_{IS} (inbreeding coefficient) values were detected by 24 of the 176 exact tests across loci and samples after sequential Bonferroni correction ($P \leq 0.0045$) (Appendix 8) When testing for each locus across all populations, significant F_{IS} values were found for the loci SLN34, SLN54 and SLN314 (Table 6). Results of the exact test for HWE across loci and populations, considering a heterozygote deficit as the alternative hypothesis, are shown in Table 5. Populations SO10, SO9, M and F had non-significant p-values for the statistical test, while the rest of the populations showed heterozygote deficit from HWE. Heterozygote deficiencies occurs when there are more homozygotes than expected under Hardy-Weinberg equilibrium (Selkoe and Toonen, 2006). Multi-locus test across populations to assess deviations from HWE showed no significant p-values for the SLN319, SLN32, SLN62 and SLN510 markers.

Table 5. Exact test for Hardy-Weinberg Equilibrium across populations and loci. P-value for each population across all loci ($P \leq 0.0045$) and for each locus across all populations ($P \leq 0.0031$); significant values after Bonferroni correction are shown in bold.

Population	P-Value	S.E.	Locus	P-Value	S.E.
L	**0.0000**	0	SLN319	0.9492	0.0038
RO	**0.0000**	0	SLN32	0.2916	0.0210
HA5	**0.0000**	0	SLN320	0.0964	0.0025
HA2	**0.0000**	0	SLN34	**0.0000**	0
HA3	**0.0000**	0	SLN54	**0.0000**	0
HA4	**0.0003**	0.0001	SLN58	**0.0009**	0.0002
HA1	**0.0001**	0.0001	SLN62	0.5728	0.0052
SO10	0.1317	0.0105	SLN314	**0.0003**	0.0003
SO9	0.0284	0.0051	SLN35	**0.0017**	0.0003
SO7	**0.0000**	0	SLN36	**0.0000**	0
SO6	**0.0000**	0	SLN510	0.4886	0.0110
SO1	**0.0002**	0.0001			
SO4	**0.0000**	0			
SO8	**0.0000**	0			
M	0.0060	0.0029			
F	0.2759	0.0165			

Table 6: Summary statistics for each microsatellite locus across all populations for *Saccharina latissima*, indicating the number of alleles (N_a), allelic richness (A), observed (H_O) and expected (H_E) heterozygosity, inbreeding coefficient (F_{IS}) and associated P-values, and genetic differentiation among samples (F_{ST}). Significant values after Bonferroni correction are depicted in bold type ($P \leq 0.0031$).

Locus	N_a	A	H_O	H_E	F_{IS}	P-Value	F_{ST}
SLN319	8	2.798	0.641	0.593	-0.081	0.027	0.062
SLN32	20	4.470	0.800	0.789	-0.014	0.098	0.046
SLN320	6	2.090	0.435	0.472	0.078	0.739	0.056
SLN34	17	3.698	0.305	0.686	**0.555**	Highly sign.	0.055
SLN54	15	3.214	0.292	0.669	**0.563**	Highly sign.	0.155
SLN58	6	1.226	0.036	0.053	0.327	0.021	0.003
SLN62	5	1.948	0.399	0.357	-0.117	0.682	0.076
SLN314	15	4.251	0.650	0.740	**0.121**	0	0.035
SLN35	9	2.530	0.432	0.493	0.123	0.096	0.160
SLN36	26	5.156	0.773	0.854	0.095	0.004	0.016
SLN510	13	2.607	0.589	0.522	-0.129	0.745	0.091

Table 7: Summary statistics for 16 sampling sites of *Saccharina latissima*, showing the number of alleles (N_a), allelic richness (A), observed (H_O) and expected (H_E) heterozygosity and inbreeding coefficient (F_{IS}) across all loci.

Population	**N_a**	**A**	**H_O**	**H_E**	**F_{IS}**
L (Oslofjorden)	4.545	2.891	0.461	0.516	0.107
RO (Hafrsfjorden)	7.545	3.274	0.485	0.588	0.175
HA5 (Klosterfjorden)	6.091	3.258	0.472	0.604	0.219
HA2 (Solesnes, Jondal)	4.000	2.885	0.443	0.585	0.242
HA3 (Skjerring)	4.818	2.781	0.425	0.496	0.142
HA4 (Gjermundshamn)	4.545	2.933	0.525	0.577	0.090
HA1 (Bårdholmen)	5.909	3.176	0.502	0.575	0.126
SO10 (Kilstraumen)	5.727	3.388	0.599	0.602	0.005
SO9 (Nyhamnarsundet)	5.091	3.194	0.537	0.603	0.111
SO7 (Oppedalsvika)	4.909	3.188	0.460	0.574	0.199
SO6 (Fuglesetfjorden)	4.818	2.947	0.496	0.544	0.089
SO1 (Leikanger)	3.818	2.437	0.390	0.445	0.125
SO4 (Lånefjorden)	5.000	3.219	0.503	0.599	0.161
SO8 (Risnestraumen)	5.273	3.271	0.476	0.614	0.226
M (Runde)	5.455	3.163	0.518	0.568	0.088
F (Sommarøy)	5.182	3.434	0.614	0.635	0.033

3.2.2 Population structure and genetic differentiation

3.2.2.1 FST analyses

F_{ST} (fixation index) is commonly used as a measure of population differentiation due to genetic structure and describes the decrease in heterozygosity of sub-populations relative to the total heterozygosity across sites, or across both sites in pair-wise comparisons. In undifferentiated populations F_{ST} equals zero whereas in strongly differentiated populations F_{ST} tends to one. Pair-wise exact tests of genic differentiation revealed significant population structuring across all loci and populations. Pair-wise estimates of F_{ST} values between all populations showed significant genetic differentiation between most sites (103 of 120 pair-wise F_{ST} values; Table 9). In total all pair-wise comparisons of genic differentiation revealed significant differences between all but 19 pairs of sites, even after Bonferroni correction ($P \leq 0.00042$, Table 9). P-value for each population pair across all loci (Fisher's method) can be found in Appendix 9. To test whether null alleles or loci under selection may influence the results, F_{ST} analyses were performed several times, where three of the analyses are shown below: once with all eleven loci (Table 9), once after removing loci with potential null alleles, SLN34 and SLN54 (Table 10), and once after excluding all "problematic" loci, SLN54, SLN34 and SLN35 (Table 11).

The purpose was to test if large deviations from the pattern shown in Table 9 occurred. The results showed in general minor changes, but some more F_{ST} comparisons changed from significant to non-significant.

Table 9: Matrix of pair-wise F_{ST} values over all 11 loci for the 16 populations of *Saccharina latissima.* Significant values after Bonferroni correction are depicted with green background (P-values for the significance test can be found in Appendix 9).

	L	RO	HA5	HA2	HA3	HA4	HA1	SO10	SO9	SO7	SO6	SO1	SO4	SO8	M
RO	0.130														
HA5	0.087	0.029													
HA2	0.139	0.087	0.067												
HA3	0.218	0.090	0.073	0.091											
HA4	0.149	0.070	0.040	0.049	0.033										
HA1	0.121	0.043	0.005	0.069	0.079	0.052									
SO10	0.105	0.027	0.004	0.049	0.063	0.038	0.002								
SO9	0.101	0.014	0	0.046	0.067	0.036	0	0							
SO7	0.147	0.014	0.017	0.084	0.089	0.073	0.012	0.017	0						
SO6	0.157	0.046	0.045	0.083	0.085	0.085	0.061	0.038	0.029	0.046					
SO1	0.247	0.070	0.112	0.131	0.087	0.116	0.116	0.078	0.100	0.089	0.077				
SO4	0.132	0.029	0.018	0.053	0.058	0.036	0.033	0.012	0.016	0.034	0.038	0.069			
SO8	0.120	0.026	0.003	0.042	0.044	0.027	0.022	0.010	0	0.0002	0.059	0.088	0.016		
M	0.099	0.057	0.022	0.119	0.111	0.087	0.070	0.049	0.042	0.074	0.075	0.162	0.047	0.056	
F	0.139	0.103	0.051	0.095	0.139	0.094	0.072	0.084	0.059	0.080	0.108	0.215	0.106	0.065	0.104

Table 10: Matrix of pair-wise F_{ST} values over 9 loci (SLN54 and SLN34 removed, potential null alleles) for the 16 populations of *Saccharina latissima.* Significant values after Bonferroni correction are depicted with green background.

	L	RO	HA5	HA2	HA3	HA4	HA1	SO10	SO9	SO7	SO6	SO1	SO4	SO8	M
RO	0.147														
HA5	0.093	0.022													
HA2	0.144	0.081	0.072												
HA3	0.201	0.049	0.063	0.099											
HA4	0.134	0.042	0.040	0.053	0.036										
HA1	0.118	0.022	0.006	0.084	0.091	0.066									
SO10	0.099	0.006	0.000	0.061	0.063	0.042	0.000								
SO9	0.113	0.003	0.000	0.052	0.058	0.039	0.000	0.000							
SO7	0.164	0.008	0.027	0.085	0.079	0.074	0.014	0.048	0.000						
SO6	0.176	0.017	0.023	0.069	0.037	0.046	0.032	0.013	0.006	0.024					
SO1	0.257	0.026	0.088	0.138	0.064	0.097	0.097	0.078	0.071	0.053	0.051				
SO4	0.148	0.019	0.016	0.058	0.044	0.027	0.033	0.016	0.170	0.036	0.031	0.056			
SO8	0.116	0.007	0.006	0.042	0.034	0.025	0.029	0.000	0.000	0.007	0.018	0.051	0.004		
M	0.111	0.068	0.010	0.113	0.064	0.058	0.052	0.024	0.038	0.078	0.057	0.134	0.038	0.038	
F	0.121	0.087	0.053	0.080	0.131	0.096	0.070	0.057	0.058	0.081	0.060	0.181	0.092	0.068	0.088

Table 11: Matrix of pair-wise F_{ST} values over 8 loci (SLN54, SLN34 and SLN35 removed) for the 16 populations of *Saccharina latissima.* Significant values after Bonferroni correction are depicted with green background.

	L	**RO**	**HA5**	**HA2**	**HA3**	**HA4**	**HA1**	**SO10**	**SO9**	**SO7**	**SO6**	**SO1**	**SO4**	**SO8**	**M**
RO	0.070														
HA5	0.055	0.033													
HA2	0.113	0.068	0.068												
HA3	0.156	0.080	0.064	0.103											
HA4	0.122	0.061	0.045	0.063	0.029										
HA1	0.075	0.052	0.008	0.071	0.087	0.061									
SO10	0.061	0.027	0.008	0.045	0.067	0.045	0.008								
SO9	0.044	0.016	0.000	0.047	0.068	0.044	0.000	0.000							
SO7	0.051	0.021	0.016	0.052	0.077	0.061	0.012	0.008	0.000						
SO6	0.062	0.028	0.029	0.08	0.112	0.095	0.065	0.038	0.017	0.026					
SO1	0.158	0.068	0.108	0.096	0.082	0.108	0.133	0.081	0.100	0.092	0.079				
SO4	0.100	0.033	0.027	0.048	0.061	0.039	0.048	0.027	0.025	0.038	0.040	0.071			
SO8	0.076	0.038	0.009	0.032	0.029	0.019	0.029	0.011	0.000	0.001	0.056	0.082	0.022		
M	0.088	0.055	0.028	0.142	0.11	0.101	0.078	0.055	0.048	0.068	0.059	0.152	0.057	0.072	
F	0.119	0.081	0.044	0.108	0.132	0.105	0.048	0.072	0.044	0.032	0.093	0.189	0.103	0.053	0.128

Overall, when excluding the loci with null alleles, ten comparisons went from significant to non-significant compared to Table 9 where all the 11 loci are considered. When excluding all both candidate loci under positive selection and loci with null alleles the pattern did not change much from when these were included. Only three comparisons went from significant to non-significant in addition to those that already was non-significant (Table 9 and 11).

With all 11 loci included there was one pair-wise F_{ST} comparison (RO-SO7) with a non-significant value (Figure 7, left), and when excluding all the "problematic" loci (SLN34, SLN54 and SLN35) one more comparison (RO-SO9) became non-significant (Figure 7, middle). When removing loci with null alleles (SLN34 and SLN54), comparisons between RO and two sites in Hardangerfjord (HA5 and HA1) and three sites in Sognefjord (SO10, SO9 and SO7) became non-significant in addition. These five sites, HA5, HA1, SO10, SO9 and SO7 are in the outer parts of both fjords (Figure 7, right).

Figure 7: Non-significant F_{ST} values shown between site RO and tagged fjord sites when including all 11 loci (left), after removing SLN54, SLN34 and SLN35 ("Problematic" loci) (middle) and after removing loci with null alleles (SLN54 and SLN34) (right).

Site-wise F_{ST} comparisons with all loci showed insignificant values when comparing HA5 with the outermost site in Hardangerfjord HA1, and with the outer sites in Sognefjord, SO10, SO9, SO8 and SO7. Removing the "problematic" loci (SLN54, SLN34 and SLN35) showed the same outcome (Figure 8, left). After removing loci with null alleles, these comparisons were still non-significant. In addition, two more comparisons (between HA5 and SO4 and between HA5 and the coastal site north from Sognefjord, M) became non-significant (Figure 8, right).

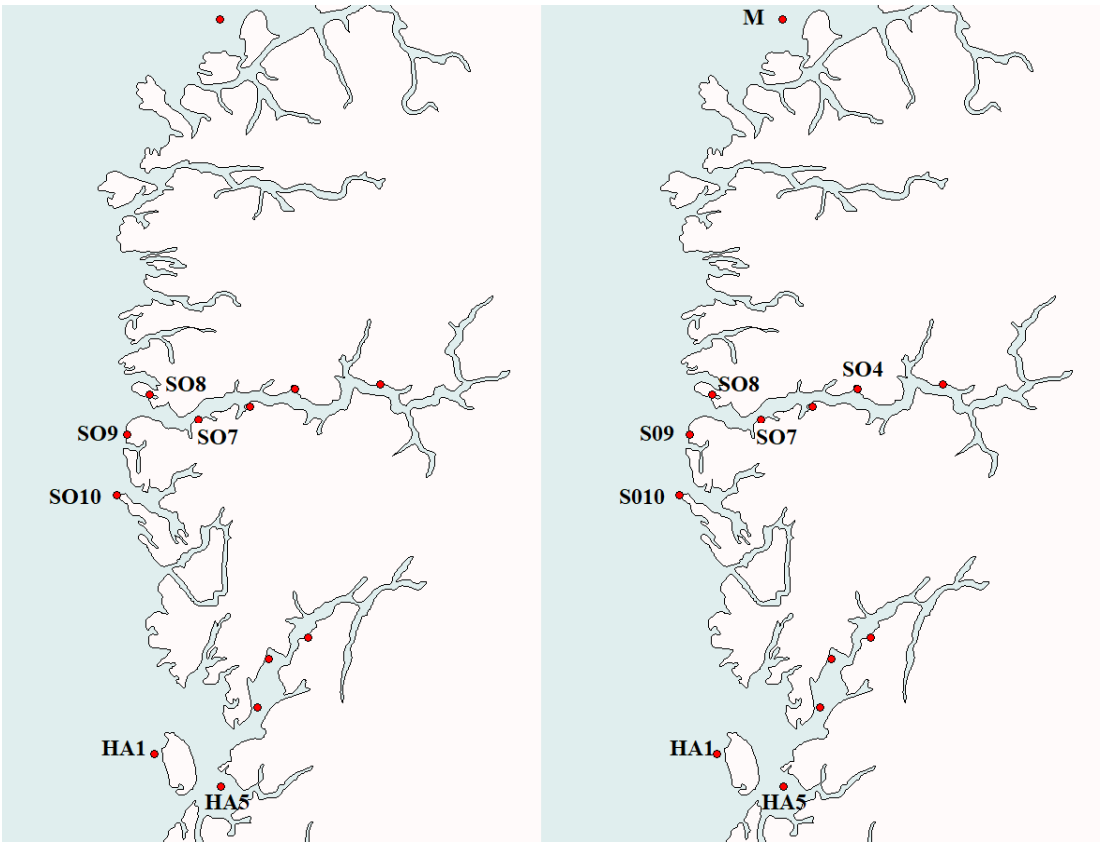

Figure 8: Non-significant F_{ST} values shown between site HA5 and tagged fjord sites when including all 11 loci and after removing all "problematic" loci (SLN54, SLN34 and SLN35) (left), and after only removing loci with null alleles (SLN54 and SLN34) (right).

The outermost site in Hardangerfjord HA1 compared to three sites in Sognefjord, SO10, SO9 and SO7 gave non-significant pair-wise F_{ST} values (Table 9), and the outcome was the same for all 11 loci, 8 loci (excluded SLN54, SLN34 and SLN35) and 9 loci (Excluding null alleles SLN54 and SLN34). When comparing the outermost site in Sognefjord SO10 with four sites further in in Sognefjord the F_{ST} analysis gave non-significant comparisons between SO10 and SO9, SO8, SO7 and SO4. The same outcome showed when excluding SLN54, SLN34 and SLN35 ("problematic" loci) (Figure 9, left). After removing loci with null alleles these pair-wise comparisons were still non-significant plus two more comparisons between SO10 and SO6, and SO10 and M. (Figure 9, right).

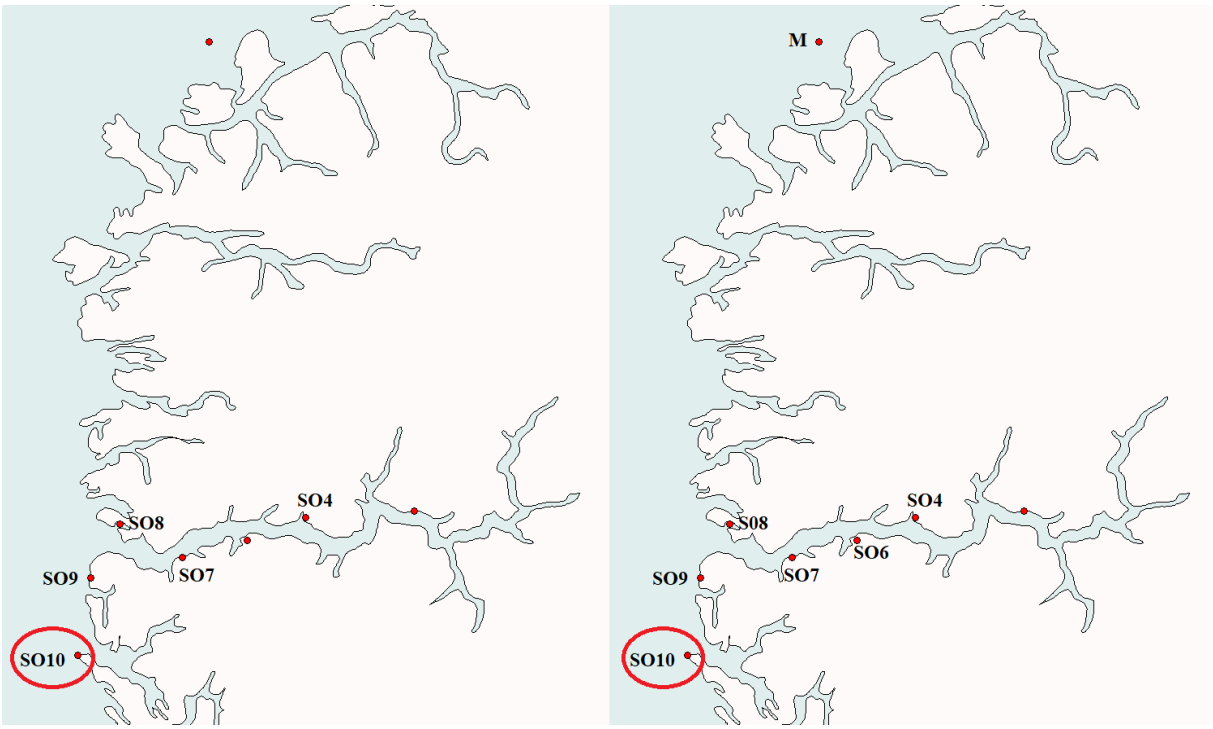

Figure 9: Non-significant F_{ST} values shown between site SO10 and tagged sites when including all 11 loci and after removing all "problematic" loci (SLN54, SLN34 and SLN35) (left), and after only removing loci with null alleles (SLN54 and SLN34) (right).

One of the outer sites in Sognefjord SO9 compared to SO8, SO7 and SO4 had a non-significant outcome after an F_{ST} analysis including all 11 loci (Figure 10, left). When removing the "problematic" loci and when removing the loci with null alleles four comparisons were non-significant, and all of them were in Sognefjord, SO8, SO7, SO6 and SO4 (Figure 10, right).

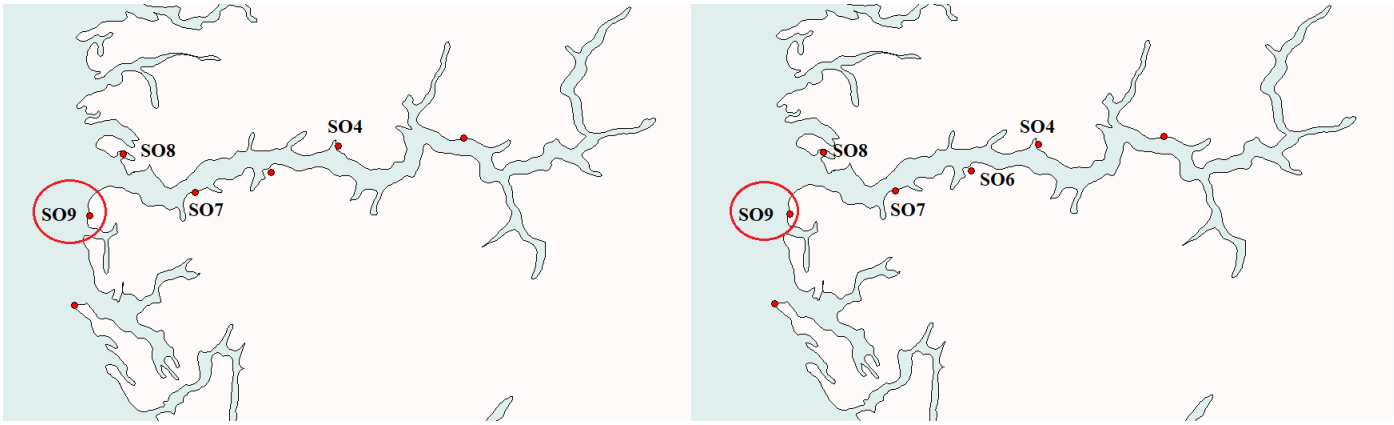

Figure 10: Non-significant F_{ST} values shown between site SO9 and tagged sites when including all 11 loci (left), and after removing all "problematic" loci (SLN54, SLN34 and SLN35), or only loci with null alleles (SLN54 and SLN34) (right).

When comparing SO7 to two other sites in Sognefjord, SO8 and SO4, this gave non-significant pair-wise F_{ST} values when all loci were included. The outcome was the same for all 11 loci, and after removing loci with potential null alleles. By removing all of the "problematic" loci (SLN54, SLN34 and SLN35) one comparison between SO7 and the northernmost site F became non-significant in addition.

Also, when comparing SO4 to SO8 this gave a non-significant pair-wise F_{ST} value, and the outcome was the same for all 11 loci, 8 loci (excluding SLN54, SLN34 and SLN35) and 9 loci (Excluding null alleles SLN54 and SLN34) (Table 9, 10 and11).

3.2.2.2 Analysis of molecular variance

An analysis of molecular variance (AMOVA) was conducted in GenAlEx three times: first for all populations in one region, thereafter for two and three regions. The analysis grouping all stations in one region showed that the molecular variance was highest within individuals (88%), then among individuals (7%) and small among populations (5%) (Figure 11). An attempt to seperate the sites into 2 and 3 regions (Coastal populations and fjord populations or coastal populations, Hardangerfjord and Sognefjord), could not detect any variation between the regions (Data not shown).

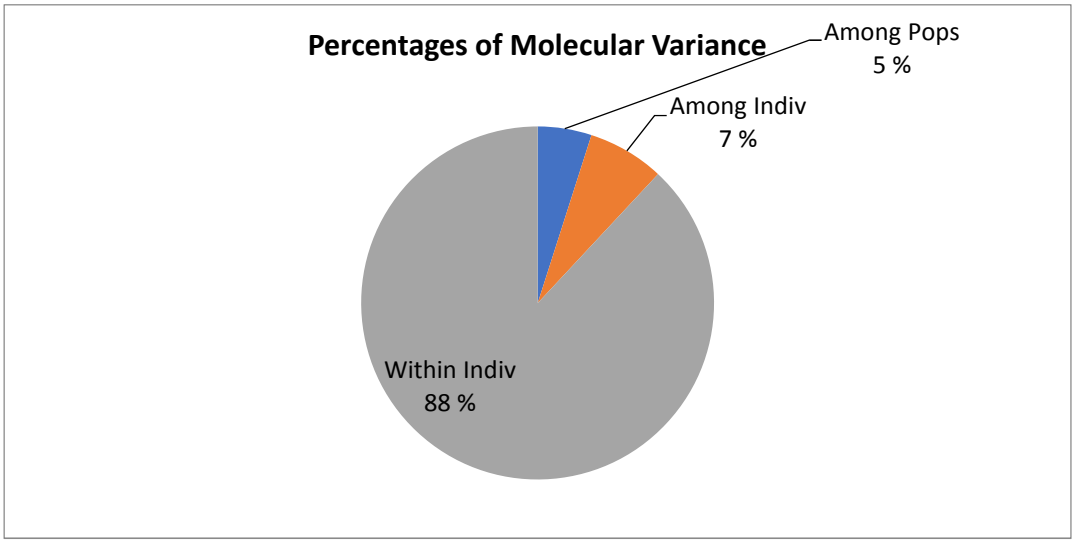

Figure 11: Pie chart summarizing the outcome of AMOVA, showing the partitioning of molecular variance within and among individuals and populations. "Problematic" loci were removed (SLN54, SLN34 and SLN35).

3.2.2.3 Isolation-by-distance

Isolation-by-distance (IBD) was tested using a Mantel test, which tests for a correlation between pair-wise genetic distance and geographic distance matrices between sites. Although there was a great spread in the plot, making the IBD unclear, results of the Mantel test showed a significant positive correlation between genetic differentiation ($F_{ST}/(1-F_{ST})$) and geographic distance between sites ($P \leq 0.001$, $R^2 = 0.24$, Figure 12).

Table 12: Matrix of pair-wise F_{ST} values over 8 loci (SLN54, SLN34 and SLN35 removed) for the 16 populations of *Saccharina latissima* (lower half) and geographic distances in km (upper half) between the 16 sites included in the study. Significant values after Bonferroni correction are depicted with grey background.

	L	**RO**	**HA5**	**HA2**	**HA3**	**HA4**	**HA1**	**SO10**	**SO9**	**SO7**	**SO6**	**SO1**	**SO4**	**SO8**	**M**	**F**
L	-	410	520	592	574	554	524	625	661	696	717	773	734	680	817	1832
RO	0.070	-	110	182	164	144	116	217	247	282	303	359	320	266	407	1422
HA5	0.055	0.033	-	72	54	34	30	125	155	190	211	267	228	175	328	1343
HA2	0.113	0.068	0.068	-	21	39	95	170	200	235	256	312	273	220	373	1388
HA3	0.156	0.080	0.064	0.103	-	18	77	148	178	213	234	290	251	198	343	1353
HA4	0.122	0.061	0.045	0.063	0.029	-	60	129	159	194	215	271	232	179	332	1347
HA1	0.075	0.052	0.008	0.071	0.087	0.061	-	101	131	166	187	243	204	151	304	1319
SO10	0.061	0.027	0.008	0.045	0.067	0.045	0.008	-	30	65	86	142	103	50	195	1210
SO9	0.044	0.016	0.000	0.047	0.068	0.044	0.000	0.000	-	35	56	112	73	19	173	1188
SO7	0.051	0.021	0.016	0.052	0.077	0.061	0.012	0.008	0.000	-	21	78	39	24	185	1200
SO6	0.062	0.028	0.029	0.08	0.112	0.095	0.065	0.038	0.017	0.026	-	61	22	44	205	1220
SO1	0.158	0.068	0.108	0.096	0.082	0.108	0.133	0.081	0.100	0.092	0.079	-	43	101	262	1277
SO4	0.100	0.033	0.027	0.048	0.061	0.039	0.048	0.027	0.025	0.038	0.040	0.071	-	63	224	1239
SO8	0.076	0.038	0.009	0.032	0.029	0.019	0.029	0.011	0.000	0.001	0.056	0.082	0.022	-	161	1176
M	0.088	0.055	0.028	0.142	0.11	0.101	0.078	0.055	0.048	0.068	0.059	0.152	0.057	0.072	-	1015
F	0.119	0.081	0.044	0.108	0.132	0.105	0.048	0.072	0.044	0.032	0.093	0.189	0.103	0.053	0.128	-

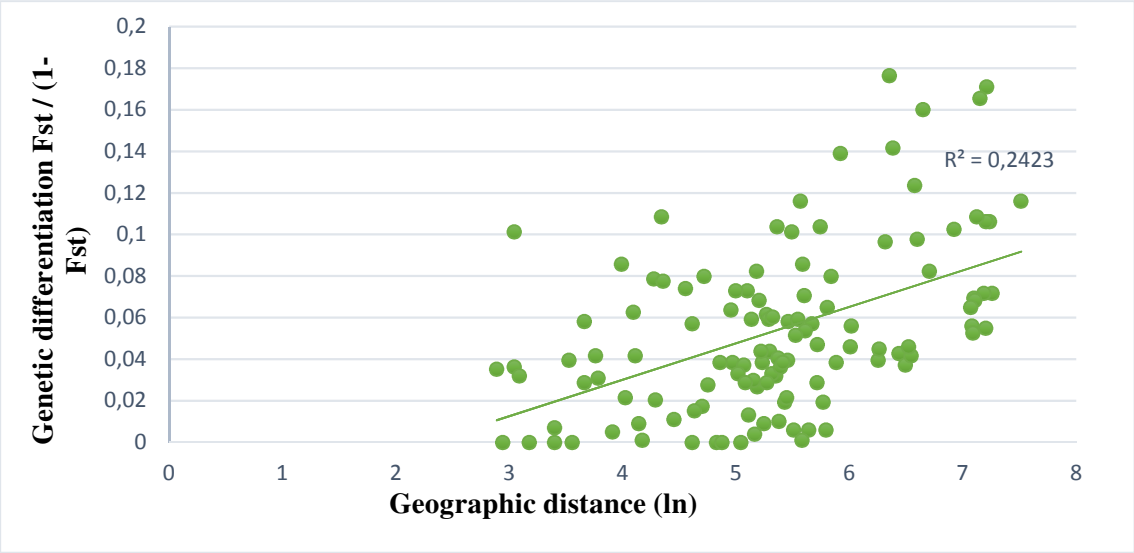

Figure 12: Isolation-by-distance correlation of genetic differentiation (F_{ST} / (1-F_{ST})) and log transformed geographic distances between 16 sites of *Saccharina latissima* based on allele frequency data from 8 microsatellite markers. "Problematic" loci were removed (SLN54, SLN34 and SLN35).

As mentioned earlier isolation-by-distance was also tested on just the open coastal populations (L, RO, HA1, SO10, SO9, M and F). The fjord populations HA5, HA2, HA3, HA4, SO7, SO6, SO1, SO4 and SO8 were excluded from the analysis. The results of the Mantel test showed a significant positive correlation between genetic differentiation (F_{ST}/(1-F_{ST})) and geographic distance between sites ($P \leq 0.001$, $R^2 = 0.51$, Figure 13).

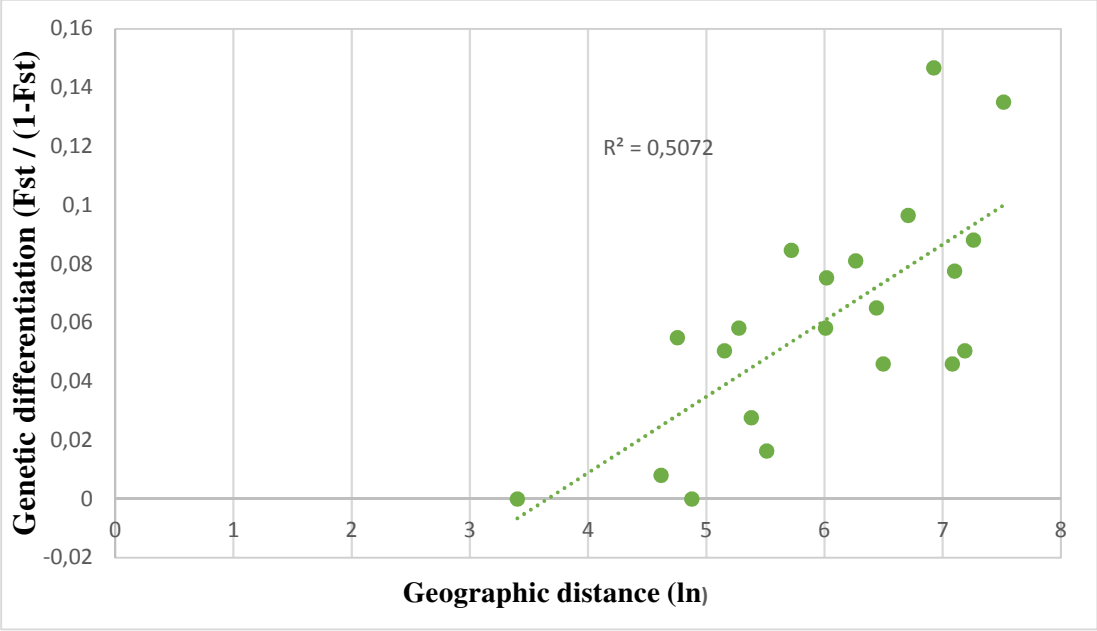

Figure 13: Isolation-by-distance correlation of genetic differentiation (F_{ST} / (1-F_{ST})) and log transformed geographic distances between 7 sites of *Saccharina latissima* based on allele frequency data from 8 microsatellite markers. "Problematic" loci were removed (SLN54, SLN34 and SLN35).

3.2.2.4 Structure analyses

The software program STRUCTURE version 2.3.4 (Pritchard, Stephens and Donnelly, 2000) was used to investigate population structure, and the web-based program Structure Selector determined the best number of clusters (K, set from 2-6) over 20 independent runs based on Evanno method (Evanno et al., 2005). STRUCTURE was run using a Markov chain Monte Carlo length of 1 000 000 steps after a burn-in of 500 000. Structure selector determined that five clusters (K=5) and three (K=3) would be best to show the structure in the material depending on what loci are included and excluded.

Figure 14 displays the estimated result both when including all eleven loci (A) and when omitting the two loci with null alleles (B). Structure selector determined that five genetic groups would be best to display the structure in the material for both of these analyses. When including all loci one can see that there is a genetic group that are most pronounced in the Oslofjord population, L. The rest of the coastal populations (RO, M and F) including the outermost populations in the fjord systems (HA5, HA1, SO10, SO9 and SO8), are quite similar, i.e. show little structure meaning there are little geographical separation of these 5 genetic groups. In Hardangerfjord (HA populations) there is one genetic group (blue) that dominates, while sites in Sognefjord (SO populations) show to some extent similarities with the coastal populations, except for the innermost population SO1. Here there is a genetic group (violet) that take up most of the genetic clustering.

When excluding the loci with null alleles (SLN54 and SLN34), one can see that the genetic group which dominated in Oslofjord when including all loci, is still present, but to a smaller degree. Overall the structure is quite homogenous throughout the whole material.

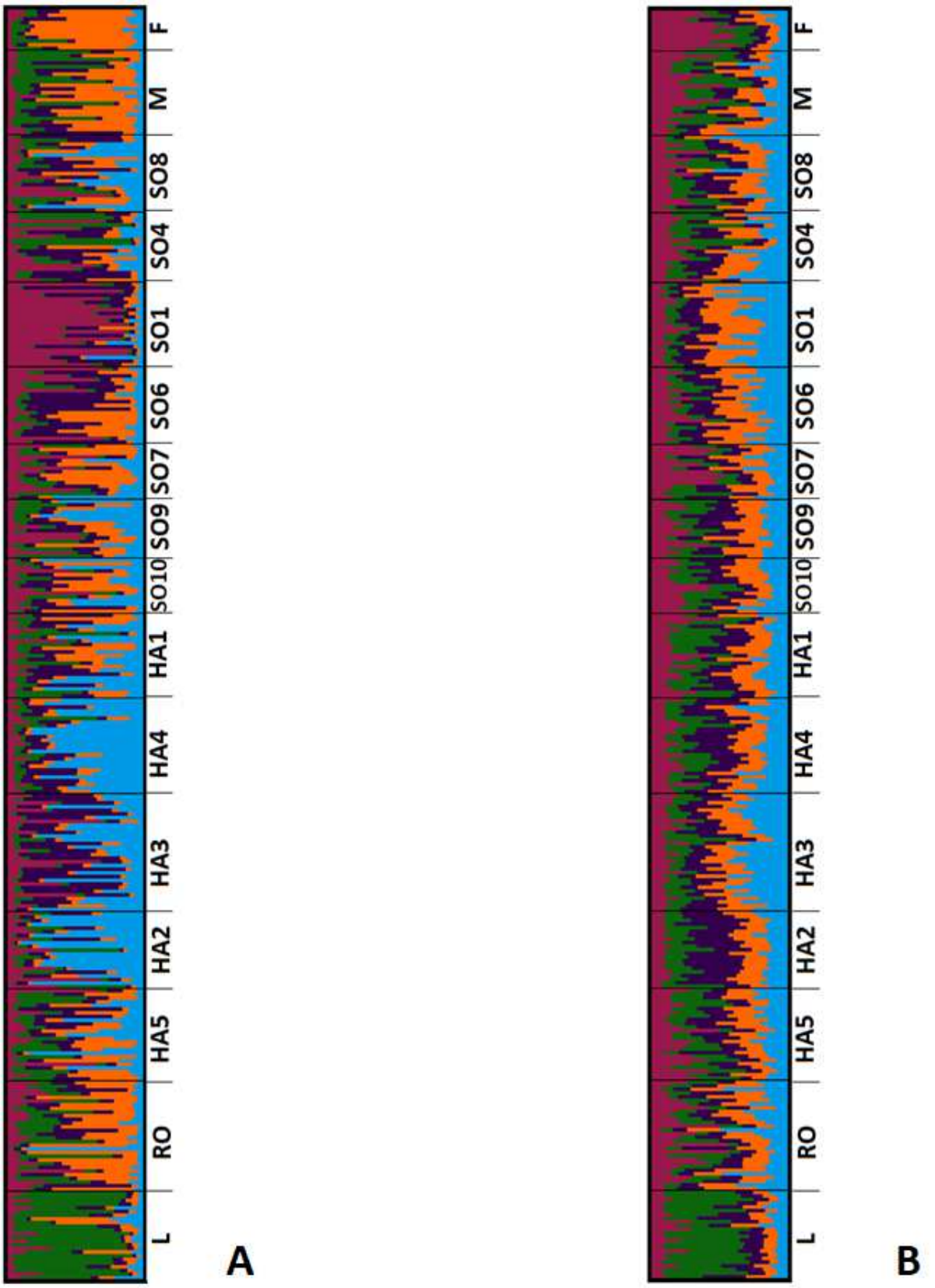

Figure 14: A: 11 loci and B: 9 loci (null alleles removed, SLN54 and SLN54).

Figure 15 displays the estimated result when all “problemtatic” loci such as null alleles and candidate loci under positive selection (SLN54, SLN34 and SLN 35) were excluded from the STRUCTURE analyses. These STRUCTURE graphs display the genetic structure most correctly, as they only include neutral loci. Figure 15 (left) display the overall results along the Norwegian coast and the two large fjord systems Hardangerfjord and Sognefjord. Structure selector determined that K= 3 would be best to display the structure in the material.

Figure 15 (B) shows the result with all the sites situated inside the fjords excluded (HA5, HA2, HA3, HA4, SO7, SO6, SO1, SO4, SO8). The purpose of this analysis was to show if there was a different structure when only coastal populations were considered. Seven populations are considered as coastal populations here, L, RO, HA1 (Outermost site belonging to Hardangerfjord), SO10, SO9 (Two of the outermost sites in Sognefjord), M and F. Also here Structure selector determined that three genetic groups would be best to display the structure in the material. The results of this analysis suggested that the geneticstructure along the coast is clearly homogenous.

The results also show that the genetic groupings shown in STRUCTURE are to some extent dependent on the populations included. Looking at figure 15 the only difference between A and B are the number of populations included in the analysis. Both have the same three genetic groups, but the proportion of the genetic groups shift. STRUCTURE is based on permutations and will make an estimate of the material, and will not represent the true reality.

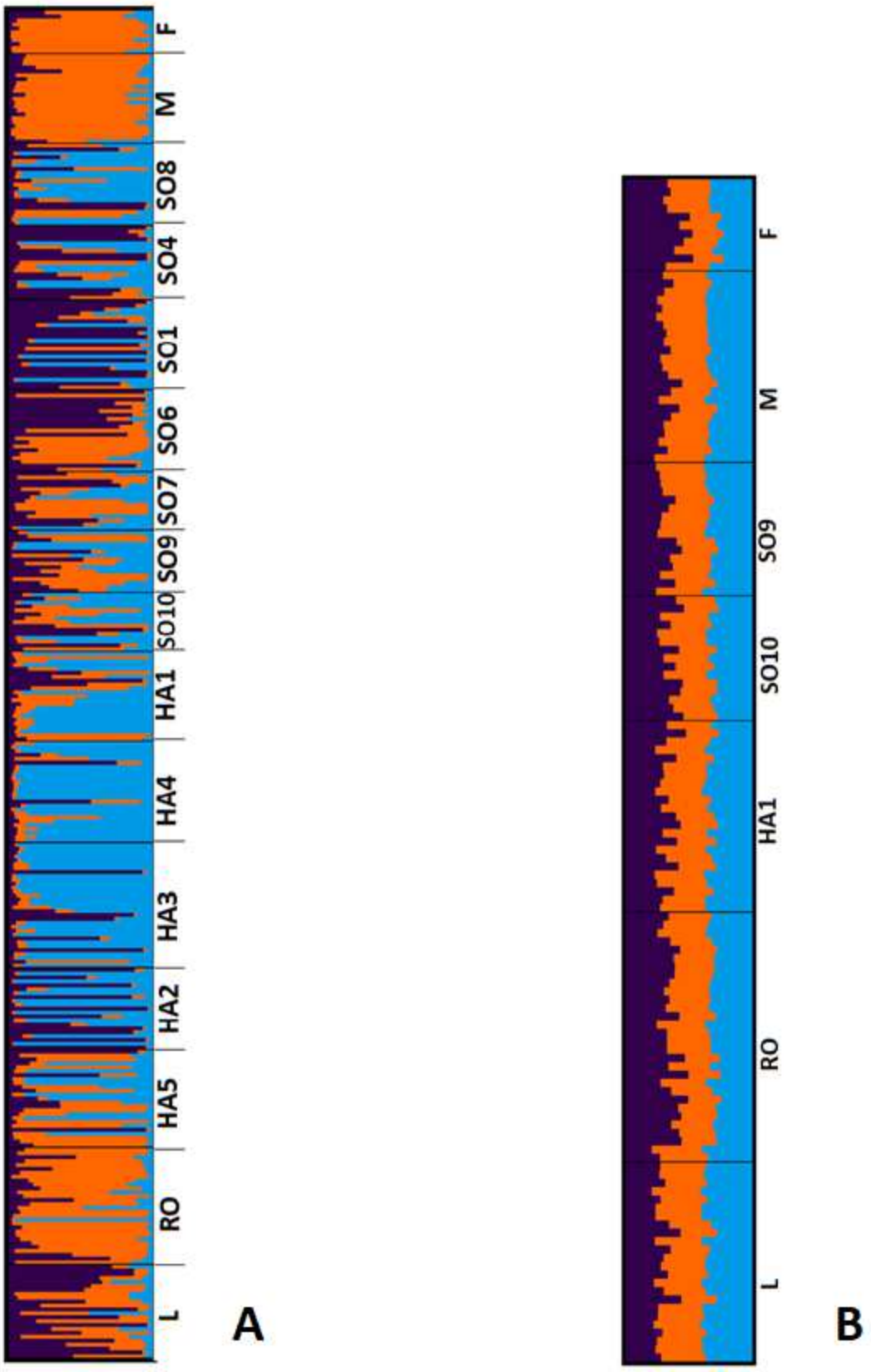

Figure 15: A: 8 loci (SLN54, SLN34 and SLN 35 excluded) and B: 8 loci tested on the coastal populations.

4 Discussion

The interest in cultivating sugar kelp, *Saccharina latissima* in Norway is increasing and therefore knowledge about the population genetics of this species is important. Except from the work of Evankow et al. (2019), little has been done on the population genetics of *S. latissima* in Norway. Consequently, this study was done to get a better understanding and to describe the population genetics of *S. latissima* along the Norwegian coast, especially outside and within two large fjord systems in Norway, Hardangerfjord and Sognefjord, and to identify possible genetically separated groups in the material.

4.1 Discussion of the material

Working with desiccated *S. latissima* tissue proved to be quite difficult when using a DNA extracting kit that did not seem to give clean DNA. This was the case when working with Qiagen DNeasy® 96 Plant Kit, which caused trouble when working with microsatellites. After a lot of trial and error the Macherey Nagel NucleoMag® Plant Kit was eventually tested on the "problem samples" and others, and this kit proved to give clean DNA for most of the remaining samples.

Initially, twelve polymorphic microsatellites markers were described by Paulino et al. (2016) in sugar kelp. Eleven of these was used in the present study. One loci (SLN511) had to be discarded because of amplification problems. This can be one drawback when using microsatellite markers (Selkoe and Toonen, 2006).

Two loci with null alleles and two candidate loci for positive selection were detected in the material. One locus, SLN54 proved to be a locus with both null alleles as well as a candidate locus for positive selection. The other locus with identified null alleles was SLN34, which also was detected in three other publications who used the same microsatellites, Paulino et al. (2016) who characterized the microsatellites, Møller Nielsen et al. (2016) and Breton et al. (2017). All of these studies kept this locus in the analyses because it did not influence the result. In present study however, this locus had, along with SLN54 and SLN35 (candidate for positive selection) an effect on the results.

It was decided that the focus would be on the analyses where the candidate loci for positive selection and loci with null alleles were removed. When removing these loci, the "correct" dataset comprised eight neutral loci, SLN314, SLN319, SLN32, SLN320, SLN36, SLN510, SLN58 and SLN62. The reason for that is because according to Selkoe & Toonen (2006), any locus that can be a candidate for selection should be excluded from analyses based on neutral assumptions, in this case, analyses based on F_{ST}. Also, any loci with strong evidence of null alleles should be excluded, which in present study have this kind of strong evidence from two different analyses done with MICROCHECKER 2.2.3 and an exact HWE test implemented in GENEPOP 4.2 (web version).

4.2 Discussion of the result

4.2.1 Genetic diversity

Paulino et al. (2016) characterized 12 polymorphic microsatellites markers in sugar kelp, and 11 of these was used in present study. Other studies done with the same microsatellite markers are along a salinity gradient in the North Sea – Baltic Sea transition zone (Møller Nielsen et al., 2016) and in Eastern Maine, USA (Breton et al., 2017). The studies from European waters (Møller Nielsen et al., 2016; Paulino et al., 2016) and on the eastern coast of Maine, USA (Breton et al., 2017), had similar expected heterozygosity (H_E) as in the present study. The two studies from Europe showed higher genetic diversity than the populations along the coast of Maine. The overall low diversity in the populations along the eastern coast of Maine could be explained by a relatively recent colonization event in the northwest Atlantic after the Last Glacial Maximum (Breton et al., 2017). Also, populations of *S. latissima* in western Greenland waters exhibited lower diversity than European populations (Paulino et al., 2016). In this study however, there is no sign of decrease in allelic richness and expected heterozygosity northwards, on the contrary the northern-most population (F) showed the highest value of allelic richness and expected heterozygosity. This may possibly be due to a northern transport of alleles along the coast by the unidirectional Norwegian Coastal Current.

Møller Nielsen et al. (2016) evaluated patterns of genetic diversity of *S. latissima* along the salinity gradient area of Danish waters and compared designated brackish and marine sites. The results showed that the populations located in the brackish environment were less diverse,

more related, and showed increased differentiation over distance compared to the marine populations.

The present study focused on the Norwegian coast with emphasis on Hardangerfjord and Sognefjord. The fjord systems have a brackish surface layer in an increasing gradient from the outermost to the innermost parts of fjords, and with a strong seasonal variation (Sætre, 2007). Møller Nielsen et al. (2016) found lower allelic richness and high degree of differentiation of the populations in the most brackish part of their investigation area. In the present study sites from Hardangerfjord and Sognefjord showed a local drop in allelic richness, and some degree of separation into genetic groups. This may suggest limited dispersal and reduced flow of alleles into the fjords, possibly in combination with some degree of local adaptation. Removing a candidate locus for selection (SLN35) had a large impact on the results of the STRUCTURE analysis, and especially in the fjords. However, the fjord populations from Hardangerfjord and Sognefjord did not give a clear indication on being less diverse than the coastal populations in this study, except the local drop in allelic richness.

4.2.2 Population structure and genetic differentiation

To answer the first study question, if the genetic structure gradually will change along the coast from south to north, the results of the STRUCTURE analyses suggest that there is not a gradually changing genetic gradient along the coast. The structure graphs suggest high degree of admixture and little genetic structure apart from the fjord areas. With that being said, the distance between the site north of Sognefjord, M, and the northern most sampled site F, is 1015 km. Ideally one would have sampled sites more evenly along the coast the get a more thoroughly estimate of the structure.

Regarding the fjord systems (Hardangerfjord and Sognefjord) some localities/populations show, at least to some extent, genetic groupings. One genetic group appear to be more dominant in Hardangerfjord, and another group in Sognefjord, but overall a gradually change in structure is not detected within the fjord systems. By looking at the overall structure pattern it may seem like there are some restrictions on gene flow inward in the fjords, while the gene flow along the coast is quite good. Low levels of structuring can be an indication of high levels of gene flow (Brennan et al., 2014). The Norwegian shoreline offers quite good conditions for *S. latissima* from South to North, with no obvious large barriers for gene flow, so a fairly extensive gene flow along the coast can thus be expected. This can also be supported by the pair-wise F_{ST} values. It is known that water movement and currents play an

important role in the dispersal range for kelps, and that gene flow is in turn strongly influenced by spore dispersal (Brennan *et al.*, 2014).
The Norwegian Coastal Current is a unidirectional current that flows northwards along the Norwegian coast and can function as a "highway" for spores when they are released from the sporophyte.

Freshwater in Hardangerfjord and Sognefjord mixes to a brackish layer, flowing out towards the coast specially during early summer – autumn, and can by that cause some level of restriction to gene flow. Gene flow is further influenced by topography and landscape quality between populations in addition to geographical distance (Kloareg et al., 2007; Brennan et al., 2014). Guzinski et al. (2017) discovered low genetic diversity within and low connectivity between European *Saccharina latissima* populations. This low connectivity might be because of gene flow barriers, for example large sand beaches along the coast from Belgium to Denmark. Such barriers would lead to differentiated populations.

The STRUCTURE analysis done on only the seven coastal populations revealed a genetic structure that was clearly homogenous i.e. little genetic structure. This indicates good gene flow between the coastal populations, which is strengthened by a significant IBD pattern. When comparing the graph that include all populations with the one excluding the fjord populations (both done when all "problematic" loci were removed), one can see that both are separated into three genetic groups, but how the different groups appear in the different populations varies. When excluding the "fjord effect" there are fewer interactions and the proportions of the genetic groups shift. The only possible explanation to this is that the STRUCTURE software only gives an estimate of the material and will "force" separation of the data into genetic clusters based on permutations and will not represent the true reality. The STRUCTURE analysis done on all sites along the coast showed that there is a genetic group in Oslofjord (L), but that is not very pronounced compared to the other coastal populations. This is thus difficult to evaluate if the genetic grouping displayed in Oslofjord represents a clear and separate group from the rest of the coastal stations. Between Oslofjord (L) and Rogaland (RO) there is however a long coastline of sand (Jæren) which is not a suitable habitat for sugar kelp. These populations were also quite differentiated (F_{ST} 0.070), indicating that this sandy coastline could to some degree serve as a barrier to gene flow.

The data suggest isolation by geographical distance to a certain extent. The genetic relationship among sampling sites as a function of geographic distance (i.e. isolation-by-distance) was statistically significant, which suggest that gene flow for sugar kelp along the Norwegian coast is, at least to some degree, related to geographic separation of populations. Generally, marine coastal ecosystems are assumed to be structured following an isolation-by-distance pattern, meaning the genetic differentiation between sites will increase as a function of distance (Wright, 1943; Evankow et al., 2019). This is, however, not always the case. Due to the overall stochastic nature of coastal marine currents and potential long-range dispersal, this IBD pattern is not always clear (Breton et al., 2017). Isolation-by-distance based on $F_{ST}/(1-F_{ST})$ has been found for *S. latissima* in the Irish Sea when looking at it in larger scales, but when dividing the populations into groups the evidence for an IBD pattern disappeared (Mooney et al., 2018). Studies of sugar kelp in European water have not found a clear IBD pattern based on $F_{ST}/(1-F_{ST})$ (Guzinzki et al., 2016), which is also true for a study done along the coast of Maine, USA (Breton et al., 2018). Evankow et al. (2019) did not identify IBD when using the traditional regression of $F_{ST}/(1-F_{ST})$ but showed strong signatures of IBD along the Norwegian coast when using chords distance D_{CE}. Breton et al. (2017) found that kelp populations within the study area exhibited significant differentiation. However, the greatest level of differentiation was detected between two populations which were geographically closer to each other than several other comparisons, thus contributed to an overall lack of evidence for a significant IBD model to the population structure. This implied that other geographical features such as local ocean currents also influence the population structure.

The pair-wise F_{ST} analysis was done to see if the populations included in present study differentiated from each other. Most of the 120 comparisons between all 16 populations was found to be significant, meaning that differentiation between the populations were detected. The overall pattern displays little to great genetic differentiation between the populations in present study, according to Hartl and Clark (1997). When looking at the two populations that are without a doubt furthest apart from each other (L (Outer Oslo fjord) and F (Sommarøy)) one would think, when having the isolation-by-distance in mind, that the F_{ST} value would be the highest in the pair-wise F_{ST}-matrices, but it is not. Comparisons between population L and one population/site in Sognefjord, SO1 and two populations/sites in Hardangerfjord, HA3 and

HA4 gives an indication of being more differentiated due to higher F_{ST} values. All these three sites are located well inside both fjords.
This suggest that there is reduced gene exchange between some fjord sites and coastal sites. This is also suggested by the drop in allelic richness in some of the fjord sites. To answer another study question, the results show greater isolation between sites located in the fjords compared to sites located in the coastal area, than between sites located along the coast. For example, comparing the F_{ST} values between the site south of Hardangerfjord (RO) compared to the two outermost sites in Hardangerfjord, HA1 (116 km) and HA5 (110 km), these are 0.052 and 0.033, respectively. Then by comparing the same two outermost sites in Hardangerfjord with the innermost sampled site, HA2 which is almost the same distance, one can see that the F_{ST} values are higher, 0.068 and 0.071, respectively. The same pattern can be seen when comparing the outermost site in Hardangerfjord (HA1) with the two outermost sites in Sognefjord (SO10 and SO9) which again is compared to the innermost site in Sognefjord. The F_{ST} values are lower and less differentiated between coastal sites, and higher and more differentiated when comparing a coastal site to a site located further in the fjords.

The genetic differentiation in Hardangerfjord lies between little and moderate, while in Sognefjord the F_{ST} values are smaller, which indicates that the populations are more genetically similar to each other (except for the innermost site, SO1). Also, the genetic structure in Hardangerfjord (HA) differ from the structure to Sognefjord (SO).

4.3 Implications for S. latissima cultivation in Norway

Breton et al. (2017) studied fine-scale population genetic structure of sugar kelp in eastern Maine, USA, and conveys that future management and cultivation efforts should aim to maintain genetic diversity and says it is crucial to assess the culture potential of local populations before choosing to start kelp cultivation.

Risks associated with kelp farming include the introduction of alien species, risk of genetic interactions between cultivated crops and wild populations, and thus the loss of natural genetic variation. The first task to protect natural variation is to document it (Luttikhuizen et al., 2018). Mooney et al. (2018) concluded after studying the pattern of gene flow in *Saccharina latissima* across the northern part of the Irish Sea, that geographical distance and

proximity need to be considered when planning the siting of kelp farms with the aim of minimizing gene flow to and from natural populations.

The result from the present study are intended give important knowledge about sugar kelp population genetics in Norway and be valuable for implementation of coastal regulations for kelp cultivation activity. When deciding sites that may be suitable for cultivation of sugar kelp, one must understand the distance over which gene flow can occur. In present study it has been suggested that there are quite good conditions for extensive gene flow along the Norwegian coast. This is also reflected in the pair-wise F_{ST} analyses, where the F_{ST} values between southern Norway (RO) up to the west coast SO10 and SO9 etc. seem to be non-significant, meaning little differentiation of the genetic variation in these populations, even though they are distributed far from each other. When looking at the populations in the fjord systems one can notice that there is more differentiation, meaning that the populations could be more isolated and not so receptive to connectivity and gene flow. This seems to be the case in Hardangerfjord, and not so much in Sognefjord, except the innermost site, SO1. With this information in mind, one could assume that sites along the coast can possible be suitable for kelp farms, without influencing or risking loss of natural genetic variation.

For future studies of population genetics of *S. latissima* in Norway additional samples should be genotyped from more sampling locations, and with higher genome coverage (i.e. more microsatellites) to identify areas of special concern for the development of kelp farms, minimizing gene flow to and from natural populations. Adding more material in the study is needed to obtain a full understanding of the genetic diversity of *S. latissima* along the Norwegian coast and to suggest and devise appropriate management strategies for future large-scale cultivation of this species.

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Appendices

Appendix 1

How sampling was conducted in Hafrsfjord in June 2018.

1: *Saccharina latissima* collected by hand in Hafrsfjord in June 2018. **2**: 1-2 cm^2 meristem blade fragments were cut from each macroalgae. **3**: The blade fragment was rolled together and put into tubes mixed with silica gel beads. **4**: The tube was shaken until the silica beads covered the whole blade fragment.

Appendix 2

DNeasy® 96 Plant Kit, Quick-Start Protocol

DNeasy® 96 Plant Kit

The DNeasy 96 Plant Kit (cat. no. 69181) can be stored at room temperature (15–25°C) for up to 1 year.

For more information, please refer to the *DNeasy Plant Handbook* and the *TissueLyser Handbook*, which can be found at www.qiagen.com/handbooks.

For technical assistance, please call toll-free 00800-22-44-6000, or find regional phone numbers at www.qiagen.com/contact.

Notes before starting

- This protocol is for purifying DNA from 2 x 96 samples of fresh plant tissue.
- Ensure that you are familiar with operating the TissueLyser and the QIAGEN® 96-Well-Plate Centrifugation System.
- Perform all centrifugation steps at room temperature (15–25°C).
- If necessary, redissolve any precipitates in Buffer AP1 and Buffer AW1 concentrates.
- Add ethanol to Buffer AW1 and Buffer AW2 concentrates.
- Preheat Buffer AP1 to 65°C.
- Prepare a fresh working lysis solution: For 2 x 96 samples, combine 90 ml Buffer AP1, 225 µl RNase A, and 225 µl Reagent DX.

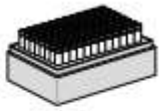
1. Place up to 50 mg leaves into each tube in 2 collection microtube racks.
2. Add 1 tungsten carbide bead to each collection microtube.
3. Pipet 400 µl working lysis solution into each collection microtube. Tightly seal the microtubes using the caps provided.
4. Assemble each rack of collection microtubes into the TissueLyser.
5. Grind the sample for 1.5 minutes at 30 Hz.

6. Reassemble the racks so that the collection microtubes nearest the TissueLyser in steps 4 and 5 are now furthest from the TissueLyser.
7. Grind the samples for another 1.5 min at 30 Hz.
8. Centrifuge to collect any solution from the caps.
9. Add 130 µl Buffer P3 to each collection microtube and reseal using new caps.
10. Place a clear cover over each rack and shake vigorously up and down for 15 s. Centrifuge to collect any solution from the caps.
11. Incubate the collection-microtube racks for 10 min at –20°C.
12. Centrifuge the collection-microtube racks for 5 min at 3800 x g (6000 rpm).
13. Transfer 400 µl of each supernatant to a new collection microtube.
14. Add 600 µl of Buffer AW1 to each sample. Close microtubes with new caps.
15. Place a clear cover over each rack and shake vigorously up and down for 15 s. Centrifuge to collect any solution from the caps.
16. Place 2 DNeasy 96 plates on top of S-Blocks. Mark the DNeasy 96 plates for later sample identification.
17. Transfer 1 ml of each sample to each well of the DNeasy 96 plates.
18. Seal each DNeasy 96 plate with an AirPore Tape Sheet. Centrifuge for 4 min at 3800 x g. If lysate remains in the DNeasy 96 plates after centrifugation, centrifuge for another 4 min.
19. Remove the tape. Add 800 µl Buffer AW2 to each sample.
20. Centrifuge for 15 min at 3800 x g without tape to dry the membranes.
21. Place each DNeasy 96 plate on a new Elution Microtubes RS rack.
22. Add 100 µl Buffer AE and seal with new AirPore Tape Sheets. Incubate for 1 min at room temperature (15–25°C). Centrifuge for 2 min at 3800 x g.
23. Repeat step 22. Seal the Elution Microtubes RS with new caps to store DNA.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual.

Appendix 3

NucleoMag® Plant Kit, Protocol-at-a-glance

1	**Homogenize and lyse** plant sample material (20–50 mg)	**500 µL MC1** **Mix** **56 °C, 30 min**	
2	**Clear lysates** by centrifugation, transfer 400 µL of cleared lysate to a Square-well Block for further processing	**5,600 x *g*, 20 min** **400 µL cleared lysate**	 
3	**Bind** DNA to NucleoMag® C-Beads	**30 µL NucleoMag® C-Beads** **400 µL MC2**	
		Mix by shaking for 5 min at RT *(Optional: Mix by pipetting up and down)*	
		Remove supernatant after 2 min separation	
4	**Wash** with MC3	Remove Square-well Block from NucleoMag® SEP **600 µL MC3**	

		Resuspend: Shake 5 min at RT *(Optional: Mix by pipetting up and down)*	⟷
		Remove supernatant after 2 min separation	
5	**Wash** with MC4	Remove Square-well Block from NucleoMag® SEP **600 µL MC4**	
		Resuspend: Shake 5 min at RT *(Optional: Mix by pipetting up and down)*	⟷
		Remove supernatant after 2 min separation	
6	**Wash** with 80 % ethanol	Remove Square-well Block from NucleoMag® SEP **600 µL 80 % ethanol**	
		Resuspend: Shake 5 min at RT *(Optional: Mix by pipetting up and down)*	⟷
		Remove supernatant	
7	**Wash** with MC5	Leave Square-well Block on NucleoMag® SEP **600 µL MC5** **Incubate for 45–60 s** *Note: Do not resuspend the beads in Buffer MC5!*	
		Remove supernatant	
8	**Elute** DNA	Remove Square-well Block from NucleoMag® SEP **50–200 µL MC6** *(Optional: Elute at 55 °C)*	
		Shake 5 min at RT *(Optional: Mix by pipetting up and down)*	⟷
		Separate 2 min and transfer DNA into elution plate/tubes	

Appendix 4

Five reaction cocktails used in this study. Four loci were grouped together (multiplex); SLN319 (name of the multiplex 1), SLN320, SLN34 and SLN32, and amplified in a single reaction. The same goes for SLN35 (name of the multiplex 2), SLN36, SLN314 and SLN510. SLN54, SLN58 and SLN62 could not be multiplexed, so each locus got its own PCR cocktail and was amplified individually, before the three loci were combined into one plate after PCR. When single plex 1, 2 and 3 were combined the plate was named SLN54.

	RECIPE OF REAGENTS		**PRIMERS (microsatellite loci)**
Multiplex 1 SLN 319	ddH_2O Forward primer (x4) Reverse primer (x4) AmpliTaq 360 mix DNA TOT	264.6 µl 6.3 µl 6.3 µl 525 µl 1 µl per sample 840 µl	SLN 319 (VIC) SLN 320 (PET) SLN 34 (NED) SLN 32 (FAM)
Multiplex 2 SLN 35	ddH_2O Forward primer (x4) Reverse primer (x4) AmpliTaq 360 mix DNA TOT	264.6 µl 6.3 µl 6.3 µl 525 µl 1 µl per sample 840 µl	SLN 35 (VIC) SLN 36 (FAM) SLN 314 (NED) SLN 510 (PET)
Single plex 1 SLN 54	ddH_2O Forward primer Reverse primer AmpliTaq 360 mix DNA 1:10 TOT	302,4 µl 6.3 µl 6.3 µl 525 µl 1 µl per sample 840 µl	SLN 54 (VIC)
Single plex 2 SLN 62	ddH_2O Forward primer Reverse primer AmpliTaq 360 mix DNA 1:10 TOT	302,4 µl 6.3 µl 6.3 µl 525 µl 1 µl per sample 840 µl	SLN 62 (FAM)
Single plex 3 SLN 58	ddH_2O forward primer reverse primer AmpliTaq 360 mix DNA 1:10 TOT	302,4 µl 6.3 µl 6.3 µl 525 µl 1 µl per sample 840 µl	SLN 58 (PET)

Appendix 5

5 PCR programs used in this study, corresponding to master mixes 1-5 in Appendix 2. SA *Saccharina latissima*, first number is the annealing temperature and the second number is the number of cycles.

	PCR PROGRAMME
Multiplex SLN 319	[SA 57 35]
Multiplex SLN 35	[SA 58 35]
Single plex SLN 54	[SA 545 35]
Single plex SLN 62	[SA 55 35]
Single plex SLN 58	[SA 55 35]

Appendix 6

5 PCR programmes used in this study, corresponding to master mixes 1-5 in Appendix 2. Repeats refer to number of times to repeat steps 2-4 (number of cycles). Forever refers to a setting that remain until the samples are removed from the PCR machine.

[SA 57 35]

Step	1	2	3	4	Repeats	6	7
Temperature (˚C)	95	95	57	72		72	12
Time (Seconds)	300	30	30	40		20	Forever
					←34		

[SA 58 35]

Step	1	2	3	4	Repeats	6	7
Temperature (˚C)	95	95	58	72		72	12
Time (Seconds)	300	30	30	40		20	Forever
					←34		

[SA 545 35]

Step	1	2	3	4	Repeats	6	7
Temperature (˚C)	95	95	55.5	72		72	12
Time (Seconds)	300	30	30	40		20	Forever
					←34		

[SA 55 35]

Step	1	2	3	4	Repeats	6	7
Temperature (˚C)	95	95	55	72		72	12
Time (Seconds)	300	30	30	40		20	Forever
					←34		

Appendix 7

Raw data file for this master thesis

	SLN319	SLN32	SLN320	SLN34	SLN54	SLN58	SLN62	SLN314	SLN35	SLN36	SLN510
L-02	422431	250258	210212	000000	316316	169169	000000	293296	360360	288294	266271
L-03	422422	247250	210210	203212	310310	169169	158158	278278	360360	288291	271271
L-04	422431	247253	210210	197200	316316	169169	153158	278278	360360	285297	266271
L-05	422431	261270	210212	200200	316316	169169	158158	278296	360360	294294	266271
L-06	422431	261261	210210	194200	316316	169169	153158	278278	360360	288300	266271
L-07	419434	247247	210210	200209	000000	169169	158158	278278	357360	288291	271271
L-08	422431	247253	210210	197203	316316	169169	158158	275278	360360	288303	266271
L-09	428434	258267	210210	200206	000000	169169	153158	278278	360360	291294	271271
L-10	422431	253261	210210	200200	310310	169169	153158	275278	360360	291297	266266
L-11	419422	247247	210210	209209	316316	169169	158158	000000	360360	288297	271271
L-12	431431	247261	210212	203203	310310	157157	158158	278296	360360	294297	266271
L-13	000000	247264	210210	203203	000000	169169	153158	278278	360360	288288	271271
L-14	431431	247250	210212	197197	310310	169169	153158	280293	360360	288297	271271
L-15	422431	247250	210210	200203	316316	157169	158163	275278	360360	294303	266271
L-16	422431	247253	210210	203203	316316	169169	153158	278293	354354	291297	266266
L-17	428428	247258	210210	203206	318348	169169	153158	278296	360360	291294	266271
L-18	419422	247247	210212	230230	348348	169169	153158	293296	360360	285291	266271
L-19	431431	247253	210212	200209	316316	169169	158158	278296	357360	294300	266266
L-20	419431	258261	210210	200200	316316	169169	158158	278278	360360	291300	271271
L-22	422431	250250	210210	200200	000000	169169	153158	278278	360360	285294	266266
L-23	419422	247261	210210	200209	316316	169169	158158	278278	360360	291294	266271
L-25	431431	247253	210210	197212	310310	169169	158158	278290	360360	300303	271271
L-26	422431	253261	210210	206209	316316	169169	153158	275278	360360	294297	266271
L-27	422431	250250	210210	197197	000000	000000	153158	278278	360360	291303	266271
RO-01	419419	250250	210210	206218	318348	169169	158158	269293	357357	294294	266271
RO-02	419422	250250	210210	200200	316316	169169	158158	278278	357357	288294	266266
RO-03	422434	250261	000000	200200	316344	169169	153158	269280	357357	285294	266266
RO-04	422422	247247	210210	209209	316344	169169	153158	272279	357360	288297	246266
RO-05	422422	244250	212212	212215	316348	169169	000000	278284	357357	282294	266266
RO-06	422422	247250	210212	197200	344352	169169	153158	269269	357357	297297	266271
RO-07	422434	250253	210212	200200	348352	169169	158158	278278	360360	285294	246266
RO-08	422425	241250	210212	197209	344348	169169	153158	269287	357360	294297	266271
RO-09	422422	244247	210212	000000	348348	169169	158158	281287	359360	288297	266271
RO-10	419422	247253	210212	197197	344344	169169	158158	278278	357360	288291	271271
RO-11	419422	219247	210210	200212	348348	169169	153158	281290	357357	294296	261271
RO-12	422422	253261	212212	215215	324324	169169	153158	293293	357357	300300	266266
RO-13	419422	250250	210212	191212	318318	169169	163163	269278	357357	264291	271276
RO-14	419431	250250	210210	209209	344344	169169	158158	287293	357360	288291	271271
RO-15	419422	250253	210212	197197	316318	169169	158158	290293	357357	291297	246271
RO-16	419419	232250	210212	200209	316316	169169	158158	266278	357357	297300	266266
RO-17	419419	244250	210210	209209	316316	169169	158158	278278	357357	294297	266266

RO-18	422431	250250	210210	209209	316344	169175	158158	278293	357360	287287	266271
RO-19	419431	247247	210210	209209	318344	169169	158158	278281	357357	288302	266266
RO-20	422431	250264	210212	200209	316348	169169	153153	278281	357357	291303	266271
RO-21	422422	250258	210210	212212	318348	169169	158158	278284	357357	288288	266266
RO-22	419434	253264	210210	197209	316344	169169	153158	278278	357357	291294	266266
RO-23	422422	247264	210210	200200	316316	169169	158158	293293	357360	293293	266271
RO-24	422422	250250	212212	200203	348348	169169	153158	281299	354357	300303	266266
RO-25	422422	247250	210210	200200	316344	169169	153158	281287	360360	294297	246266
RO-26	419422	250258	210212	224224	322348	169169	158158	279293	357357	297303	266271
RO-27	422422	258264	210212	197197	316348	169169	153158	281293	357360	291291	266271
RO-28	419419	250250	210212	200200	316344	169169	153158	278278	357357	291297	266266
RO-29	419431	247250	210210	000000	316344	169169	158158	279293	357357	288294	266266
RO-30	422431	227250	210210	200200	348348	169169	153158	278278	357357	291294	266271
HA5-01	431431	244256	210210	200200	316318	169169	158158	275278	357363	264297	271271
HA5-02	419431	250258	210210	200203	316348	169169	000000	000000	357357	293297	266271
HA5-03	431434	241253	210210	203203	316318	169169	158158	281290	357360	288300	266271
HA5-04	419422	247247	212212	000000	318318	169169	158158	000000	360360	300300	266271
HA5-05	422422	247250	212212	200209	310310	169175	153158	281287	357360	291300	266266
HA5-07	419422	253261	210210	200200	324348	169169	158158	278293	357360	308315	271271
HA5-08	419419	247250	210212	206209	344344	000000	158158	266281	360360	291305	266271
HA5-09	419422	247261	212212	203203	318318	169169	153158	293293	357357	294294	271271
HA5-10	419425	244261	210210	203203	318318	169169	158158	275296	360360	294294	266271
HA5-11	419419	244247	210210	206209	316316	169169	153158	278293	357360	288288	266276
HA5-12	422431	244250	210212	000000	316316	169169	158158	281281	357357	281291	266266
HA5-13	419422	247261	212212	197206	316318	169169	153158	278287	357360	294297	271271
HA5-14	419419	261267	210210	203203	316324	169169	158158	278281	340357	294300	266266
HA5-15	419422	232247	210210	200200	318324	169169	153158	281296	357357	279299	266271
HA5-16	422422	247250	212212	200200	316316	000000	158158	281296	360363	291306	271291
HA5-17	419419	247253	210210	000000	316318	169169	158158	281293	357357	294294	266271
HA5-18	419422	253258	212212	209209	318318	169169	158158	293296	360360	297297	266271
HA5-19	419419	247250	210210	200200	318318	169169	158158	278278	357360	291291	246266
HA5-21	422431	247261	210212	206206	000000	169169	158158	281281	357357	294303	266271
HA5-22	419422	253253	210212	200200	318318	169169	158158	278278	357357	297300	266271
HA5-23	419422	247247	210210	215215	316318	169169	158158	278278	357360	288315	266266
HA5-24	419422	247247	210212	209209	324324	169169	158158	278280	360360	294297	246266
HA5-25	419419	247250	210210	209212	316316	169169	158158	278278	357357	291291	246271
HA5-28	419422	244250	210210	209209	316318	000000	153158	278281	357360	291306	271271
HA5-29	422431	250256	210210	200200	310310	169169	153158	278287	357357	300305	266266
HA2-02	422422	250261	210210	209209	310310	169169	153158	278278	360360	294297	266271
HA2-03	419419	250261	212212	200200	318324	169169	153158	296296	340360	291294	266266
HA2-04	419422	258267	210212	000000	310310	169169	158158	281281	357360	294297	271271
HA2-06	422422	261267	210212	200200	318326	169169	153153	281293	357357	285303	266266
HA2-08	419422	261267	210212	200200	000000	169169	153158	280284	357360	294297	266271
HA2-09	419419	258261	210210	191191	310310	169169	153153	281281	360360	285288	266266
HA2-10	422422	250261	212212	191191	316324	169169	153158	000000	340360	294294	271271
HA2-11	419434	258261	212212	200200	318318	169169	153158	296296	357357	285297	271271

HA2-12	422422	250250	210212	191191	316316	169169	153158	278296	360360	285285	266271
HA2-13	419419	250250	210212	200200	324324	169169	000000	293296	357360	291291	266271
HA2-14	422422	250261	210212	203203	310310	169169	153153	275281	360360	291297	266271
HA2-16	422422	253258	210210	203203	324324	169169	158158	278293	340357	285288	271271
HA2-17	419419	247261	212212	191191	324324	169169	153153	281293	357360	285288	266271
HA2-18	422422	261267	210210	200200	316316	169169	158158	278281	340357	297297	266271
HA2-19	419419	250250	212212	191191	318318	169169	153158	278278	360360	285294	266271
HA2-21	422422	250261	212212	000000	324324	169169	153158	279281	357360	288294	271271
HA2-23	422431	253261	210212	200200	000000	169169	153153	281284	360360	291297	266266
HA2-24	419422	000000	210212	200200	318324	169169	153153	293296	340357	285294	266266
HA2-25	422422	261261	210212	200200	000000	169169	153153	281281	340360	288300	266271
HA2-26	419422	250261	212212	000000	318318	169169	153158	278281	357360	285288	266266
HA2-27	419422	258261	210212	000000	316316	169169	153158	278278	340357	294297	266266
HA3-1	419422	247261	212212	191191	318318	169169	158158	278293	357357	294297	266266
HA3-2	419419	250258	210212	200200	322322	169169	153158	279281	340360	297300	266271
HA3-3	419437	250250	212212	000000	318318	169169	153158	278278	360360	294294	266271
HA3-4	431437	250253	210210	000000	318318	169169	158158	278293	357357	288297	266266
HA3-5	419422	250250	212212	200200	310310	169169	158158	278278	357360	288303	266266
HA3-6	422422	247253	212212	000000	318318	169169	153158	278278	357360	297303	266266
HA3-7	419422	250256	212212	000000	318318	169169	158158	278278	357360	288294	266266
HA3-8	419422	247250	210212	200200	310318	169169	158158	278278	357360	294297	266266
HA3-9	419422	261267	212212	203203	318318	169169	158158	278278	357360	297302	266266
HA3-10	422422	244247	212212	200200	318318	169169	158158	278278	357357	291297	266266
HA3-11	419422	264264	212212	200200	324324	169169	158158	278290	357357	288300	266266
HA3-12	419422	250253	210212	200200	318318	169169	158158	278281	357357	294300	266266
HA3-13	419419	250253	210212	000000	310310	169169	153158	278284	357360	285302	266266
HA3-14	419437	250256	210212	000000	310310	169175	153158	278281	357357	291291	266266
HA3-15	419422	256261	212212	200200	316324	169169	158158	278278	357357	297303	266271
HA3-16	419419	250250	212212	200200	318318	169169	153158	278293	357357	290294	266271
HA3-17	419431	247253	212212	197200	318324	169169	158158	278278	357357	291291	266266
HA3-18	419419	250256	210212	191191	318318	000000	158158	278284	360360	291291	266266
HA3-19	419422	261267	210212	200200	318318	169169	158158	278278	357357	303308	266266
HA3-20	419422	247261	210210	203203	318324	169169	158158	278278	357360	288300	266271
HA3-21	419419	261261	210212	000000	318318	169169	153158	278293	357360	288300	266266
HA3-23	419422	250253	212212	200200	324324	169169	153158	278281	357357	285297	266266
HA3-24	419422	247253	210212	200200	318318	169169	158158	290293	357357	291302	266266
HA3-25	419422	250256	210210	000000	318318	169169	153158	293293	357360	294302	266266
HA3-26	419422	250250	210210	200200	310310	169169	158158	281290	339357	294303	266266
HA3-27	419419	250261	210212	191200	324324	169169	158158	278278	354354	288306	266266
HA3-28	419419	250253	210212	200200	318318	169169	158158	278278	357357	300303	266271
HA3-29	419422	250250	210212	000000	324324	169169	153158	278278	354357	288303	266266
HA3-30	419437	250261	210212	000000	318318	169169	153153	278284	340357	285294	266266
HA3-31	419419	250256	210210	200200	324324	169169	153158	281290	354360	291294	266266
HA3-32	422422	261267	212212	200200	318324	169169	158158	278278	357360	303303	266271
HA3-33	419422	261267	210212	200200	324324	169169	153158	278284	357357	291314	266266
HA4-01	419422	244250	210212	206206	318318	169169	153158	278284	340357	291299	266266

HA4-02	419422	247247	210210	203203	318318	169169	158158	000000	000000	291294	266266
HA4-03	419422	253264	210212	200200	324324	163169	153158	278278	357357	297299	266266
HA4-04	419419	244264	212212	200200	316324	169169	153158	278296	357360	288294	266266
HA4-05	422422	247261	210212	200203	318318	169169	153158	278293	357357	288291	266271
HA4-06	419437	244250	210210	000000	316318	169169	158158	278293	340357	291291	266266
HA4-07	419422	250250	210212	200200	318318	157169	158158	278293	340357	288303	266271
HA4-08	419422	250250	210212	191191	318318	169169	158158	278296	357359	288291	271271
HA4-09	419419	247247	210212	200200	318324	169169	153158	278293	360360	288299	266266
HA4-10	419422	247250	210210	200200	324324	169169	153158	278284	340360	291291	266271
HA4-11	419431	247253	210212	200200	316316	163169	153158	278296	340357	291294	266271
HA4-12	419422	247261	210212	200203	324324	169169	153158	293296	357357	291291	266266
HA4-13	419419	247264	210210	000000	318318	169169	153153	278278	340357	291291	266266
HA4-14	419422	247258	210212	200200	318324	169169	153153	278290	357360	291302	266266
HA4-15	419422	253258	210212	200200	318318	169169	153158	296296	357360	288297	266266
HA4-16	422422	250250	210212	200200	318324	169169	158158	293293	340360	294297	266271
HA4-17	419422	250250	210212	203203	318318	169169	153158	278293	357360	285309	266271
HA4-18	419419	258261	210212	000000	324324	169169	153158	278296	340357	291309	271271
HA4-19	431437	247261	210210	203203	324324	169169	000000	278278	357360	303309	266266
HA4-20	419437	247261	210212	200200	324324	169169	158158	281281	357360	291303	266266
HA4-21	419431	250253	212212	212212	310316	169169	153158	278293	360360	288288	266271
HA4-22	422419	250250	210212	200200	318322	169169	158158	293293	340340	288291	266266
HA4-23	419431	244261	212212	203203	316324	169169	153158	278296	340357	291291	266266
HA4-24	419419	250250	210212	000000	318318	169169	153158	000000	357360	291294	266266
HA4-25	419422	247264	210212	206206	318324	169169	153158	293296	360360	285291	266271
HA4-27	422422	247250	212212	206206	318318	169169	153158	290293	340360	297305	266266
HA1-02	422431	224261	210212	200200	316318	169169	158158	000000	000000	291291	266271
HA1-03	431431	247270	210210	200203	316316	169169	158158	269293	340357	288288	266271
HA1-04	422431	250261	212212	191200	324324	169169	153158	281293	357357	285288	246266
HA1-07	419422	250261	210210	200200	318318	169169	158158	000000	360360	294294	266271
HA1-08	422422	247261	210212	191209	318318	169169	158158	278293	357357	291294	271271
HA1-09	419431	244247	212212	200203	318324	169169	153158	290290	357360	291294	266271
HA1-11	419422	250250	210210	200200	318318	169169	158158	278281	357357	294314	246266
HA1-12	419419	250261	210212	200200	318318	169169	158158	000000	000000	291294	271271
HA1-14	419422	261267	210212	197200	318318	169169	158158	281281	357357	291294	246272
HA1-15	419431	261264	210210	203203	318318	169169	158158	275278	357357	288291	266271
HA1-16	419422	244250	210212	203203	324324	169169	158158	278290	357357	294306	246261
HA1-17	422431	216250	210210	209209	324324	169169	158158	278299	357357	288288	266266
HA1-18	422422	250253	210212	194200	318324	169169	158158	278293	357360	000000	266271
HA1-19	419422	250261	210212	209245	316328	169169	158158	281281	357357	288288	246271
HA1-20	422422	253261	210210	200245	316316	169169	158158	000000	357357	288291	271271
HA1-21	422422	247261	210210	200215	310310	169169	153158	278281	340357	291300	271271
HA1-22	422431	247261	212212	191191	316318	169169	158158	284296	360360	285291	271271
HA1-23	419431	247250	210212	200203	316316	169169	158158	269280	360360	297297	266271
HA1-25	419431	247256	210212	200200	324324	169169	158158	287296	357360	288288	266271
HA1-26	419422	250261	210212	200209	318318	169169	000000	278293	357357	288297	266271
HA1-27	422422	235250	210210	200200	318318	169169	153158	278278	354360	291300	271271

HA1-28	419422	238253	210210	203245	302316	157169	158158	000000	000000	288291	271281
HA1-29	419422	247250	210210	200200	318318	169169	158158	278281	357357	294314	246266
SO10-01	419434	247256	210212	209236	316324	169169	158158	281284	357357	285303	261271
SO10-02	419422	250256	210212	200200	000000	169175	158158	278278	354360	294303	266271
SO10-03	419419	247247	210212	200200	316324	169169	153158	000000	357357	288309	266271
SO10-06	422419	247267	210212	203206	324324	169169	153158	278281	357357	300305	266266
SO10-07	422431	250258	210212	200200	320324	169169	153158	278278	360360	285291	256271
SO10-11	422431	250261	210210	200200	310310	169169	000000	276278	357357	294297	271271
SO10-12	422425	247250	210210	200242	310314	169169	158158	278281	360366	288291	261266
SO10-13	419422	247250	210212	200200	318318	169169	158158	278278	357366	300317	271271
SO10-14	419422	253267	210210	000000	318328	169169	158158	000000	357360	285294	266271
SO10-15	419422	247250	210212	200200	000000	169169	158158	284293	360360	297297	271271
SO10-17	422422	247250	210212	185200	316318	169169	158158	281293	357360	288306	266271
SO10-18	422431	250258	210210	200200	324324	169169	158158	266278	357357	288291	266266
SO10-19	419422	250250	210212	200203	324324	169169	158158	281293	357360	291291	266271
SO10-20	419422	250261	210210	209209	330330	169169	153158	278284	357363	285291	246271
SO10-21	419422	250253	210212	203215	000000	000000	153158	281296	357357	300302	266271
SO9-01	419431	247253	210212	203206	000000	169169	153158	278278	357360	291291	266271
SO9-02	419422	250250	212212	200209	000000	169169	153158	269281	357357	294299	271271
SO9-04	419422	250250	210210	203203	000000	169169	158158	278284	357360	297306	271271
SO9-05	422431	241247	212212	200203	000000	169169	158158	278293	340357	296300	266271
SO9-06	000000	247258	210210	200200	316316	169169	153158	281293	357360	291297	271276
SO9-07	422422	250256	210210	200200	308324	000000	158158	279281	357357	297297	266271
SO9-13	422422	250250	210210	197200	000000	169169	153158	276296	357357	291300	271291
SO9-14	419422	253261	212212	200200	000000	169169	158158	278278	351357	288306	271271
SO9-15	422422	247250	210212	200209	000000	169169	158158	278281	340357	297303	266271
SO9-16	419422	250250	210210	000000	318318	169169	158158	293293	354357	291312	266271
SO9-17	422431	253267	212212	191209	000000	169169	158158	278281	357360	285288	266266
SO9-18	422422	261261	210212	209209	318318	169169	153158	296296	357360	285294	271291
SO9-19	419422	250253	210212	203203	318318	169169	000000	278278	360360	291297	266291
SO9-20	419431	247253	210212	191191	316316	169169	153158	278281	357357	282291	266271
SO9-21	419419	247250	210210	200209	316316	157169	158158	281293	357360	297300	266266
SO9-22	419431	250261	210210	191203	318324	169169	158158	278278	357357	288297	266271
SO7-02	419422	253264	212212	203203	318318	169169	158158	278281	357363	285285	266266
SO7-03	422422	250261	210212	000000	000000	169169	153158	278278	360360	288297	271271
SO7-04	422422	258261	210212	200200	000000	169169	158158	000000	357357	291291	271271
SO7-06	422422	247264	210210	200200	316318	169169	153158	000000	357357	297297	266271
SO7-08	422431	229250	210212	200203	000000	169169	153158	269280	357357	291308	271271
SO7-10	422422	250264	210210	209209	000000	169169	158158	278287	357360	294296	271296
SO7-11	419422	241241	212212	200209	000000	169169	153158	278278	357357	288288	256296
SO7-12	419422	250258	210210	200200	000000	169169	158158	284299	357357	285299	261266
SO7-14	419422	247256	210210	000000	000000	169169	158158	278278	357357	291294	291291
SO7-15	422419	247253	210250	203203	000000	169169	158158	278281	357357	297303	271291
SO7-16	422422	247250	210212	200215	318318	169169	158158	278287	357357	288300	266271
SO7-17	419422	253258	210212	197197	000000	169169	153158	281284	357357	291294	266266
SO7-18	422431	250261	206212	209209	316316	163169	158158	278281	357357	297303	266266

SO7-19	422422	247253	210210	209209	000000	169169	153158	269281	357357	296296	271271
SO7-20	419434	258258	210210	209209	000000	169169	153158	278280	357357	288299	266266
SO6-01	419422	253258	212212	200200	316318	169169	158158	278278	357357	294297	266271
SO6-02	422422	250250	210210	200200	310310	169169	158158	290296	357357	288297	266271
SO6-03	419422	244244	210212	200245	316316	169169	158158	293293	354357	294297	266271
SO6-04	419419	250250	210210	212212	318318	169169	158158	279281	357357	300306	266271
SO6-05	419422	253261	210212	200200	000000	169169	158158	279281	357360	300306	266271
SO6-06	422422	250258	210212	200209	316316	169169	158158	278293	354357	297306	266266
SO6-07	419422	247250	200212	200200	316316	157157	158158	279293	360360	294306	266271
SO6-08	419422	247250	210212	182182	316316	169169	158158	272281	357357	291303	266271
SO6-09	419422	258258	210212	000000	316316	169169	158158	279293	357357	291306	266296
SO6-10	422422	250253	210212	200200	316316	169169	153158	278284	354357	294300	266271
SO6-11	419422	250253	212212	000000	316316	169169	158158	272293	354357	291294	266271
SO6-12	419422	250253	212212	200200	316316	169169	153158	278284	357357	294300	266271
SO6-13	422419	250267	212248	200200	316316	169169	158158	284284	360360	288297	266266
SO6-14	419422	247256	212212	200200	316316	169169	153158	278290	357357	285297	266266
SO6-15	419419	247247	212212	000000	310310	169169	158158	278281	354357	291294	271276
SO6-16	419422	247250	210210	200200	316316	169169	153158	278278	357360	288294	266271
SO6-17	000000	253256	000000	194200	316316	169169	153158	281281	354360	291294	266271
SO6-18	419422	247258	212212	200200	310310	169169	153158	279293	357357	291291	271271
SO6-19	422422	250267	212212	200200	310316	169169	158158	278281	354357	291294	266266
SO6-20	419422	250270	210212	197200	000000	169169	158158	278278	354357	306306	266271
SO6-21	422422	247250	210210	206209	310310	169169	153158	278281	357357	285294	266266
SO1-01	419434	250258	210212	000000	000000	169169	158158	269278	357357	294294	266266
SO1-02	422422	250256	210210	200200	000000	169169	158158	278284	357357	291297	266266
SO1-04	419422	250256	212212	000000	324324	169169	153158	278296	357357	294294	266291
SO1-05	422422	247258	210210	197200	310310	169169	158158	278281	357357	294294	266266
SO1-06	422422	250250	210210	200200	310310	169169	153158	281281	357357	294294	266271
SO1-07	419422	250256	212212	200200	000000	169169	000000	278284	357357	294297	266266
SO1-08	419422	250261	210210	206206	324324	169169	153158	269278	357357	294294	266291
SO1-09	422419	250258	210212	206206	310310	169169	158158	278281	357357	291291	266266
SO1-10	419422	250256	210212	200200	324324	169169	158158	278278	357357	291297	266271
SO1-11	419422	250258	210212	200200	310310	169169	153158	281287	357363	291297	266271
SO1-12	419419	241250	210212	200200	000000	169169	158168	281281	357357	294297	266266
SO1-13	419422	250258	210210	200200	310310	169169	153158	269281	000000	000000	266271
SO1-14	419422	258258	210210	000000	324324	169169	158158	278281	357357	297297	266266
SO1-16	422422	250258	210212	000000	310310	169169	158158	278287	354357	291297	266266
SO1-17	419422	244253	210210	000000	310310	169169	153158	278281	357357	291294	266266
SO1-18	422422	250258	210212	200200	310310	169169	153158	278278	357357	291294	266266
SO1-19	419422	247250	210212	197197	000000	169169	158158	278278	357357	297297	266291
SO1-20	422422	250256	210210	200200	000000	169169	158158	281284	357357	294294	266276
SO1-21	422422	250258	210210	200200	000000	169169	158158	269269	354357	288288	266266
SO1-22	419422	250250	212212	200200	000000	169169	158158	278278	357357	291294	266266
SO1-23	419419	250250	210210	197197	000000	169169	153158	278287	357357	294297	266266
SO1-24	419419	250256	210212	200200	316316	169169	153158	278284	354357	294294	266266
SO1-25	422422	250250	210212	200200	310320	169169	153158	278281	357357	297303	266266

SO4-01	419422	258261	210210	200200	316316	169169	158158	278287	357357	288303	266306
SO4-02	419419	250256	210212	200200	316316	169169	153158	278278	357360	288303	266266
SO4-03	419422	253256	210212	200200	316316	169169	153158	278278	357360	291303	266271
SO4-04	422419	250250	212212	197197	318324	169169	158158	278281	357357	288294	271271
SO4-05	419422	250250	210212	197209	324324	169169	153158	281281	357363	291291	271291
SO4-06	419422	247250	210212	209209	316316	169169	153158	281296	357357	291297	266291
SO4-07	419422	221244	210210	200200	000000	169169	158158	275278	357357	291297	266266
SO4-08	419434	247256	210212	200200	318318	169169	000000	281293	354357	285297	266271
SO4-09	419419	244256	212212	200200	318318	169169	153158	278281	357360	294294	266301
SO4-10	419422	229247	210210	203203	000000	169169	158158	269293	354357	285297	266266
SO4-11	419422	250261	210210	000000	320320	169169	153158	280280	360360	291291	266286
SO4-12	419422	250261	210210	000000	320320	169169	148148	280280	360360	291291	266286
SO4-13	419422	250261	210212	200200	000000	169169	158158	279281	357360	291294	266271
SO4-14	419419	253264	210212	200200	000000	169169	158158	278281	357360	294300	266271
SO4-15	419422	250258	210210	200200	324324	169169	153158	278278	357357	297300	266271
SO4-16	419422	250261	210210	000000	320320	169169	153158	280280	360360	291291	266286
SO4-17	419422	250256	210212	000000	310310	169169	000000	287293	357363	288294	266266
SO4-18	419419	250261	210210	209209	316316	169169	000000	280280	357357	288297	286291
SO4-19	419431	250256	210212	203203	320320	169169	000000	275280	354357	288291	266266
SO8-01	419422	250256	210210	000000	318318	169169	153153	278278	360363	300300	271271
SO8-02	419422	247250	210212	215215	318318	169169	153158	293293	357357	297297	266291
SO8-03	419431	241250	210212	209209	318318	169169	158158	278278	357360	288305	266271
SO8-04	419419	261264	210210	212212	316316	169169	158168	293296	357360	288291	271271
SO8-05	419422	247258	210210	191197	310310	169169	158158	278278	357363	294294	266266
SO8-06	422422	247258	210210	203203	310310	169169	153158	281284	357360	288291	266271
SO8-07	000000	247250	210210	197197	316318	169169	158158	281281	357360	285294	266291
SO8-08	000000	250253	000000	200200	318318	169169	153158	278278	357357	285288	266271
SO8-09	419422	247261	210212	209209	318324	169169	153153	278281	357360	297303	266291
SO8-10	419419	258258	212212	200200	324324	169169	158158	278281	357360	288306	266266
SO8-11	425422	253258	212212	200209	318318	169169	153153	281293	357357	270294	291291
SO8-12	422425	244256	212212	200200	316318	169169	158158	278278	357357	291303	266266
SO8-13	419431	250267	210210	209209	318318	169169	000000	278278	357360	279297	266271
SO8-14	422422	250256	210210	000000	318318	169169	153153	278278	360363	300300	271271
SO8-15	419422	247250	210210	200212	316316	169169	158158	281281	357359	294303	271271
SO8-16	419422	247250	210212	215215	324324	169169	153158	293293	357357	297297	266291
SO8-17	419422	247247	210210	191209	318324	169169	153158	278293	357359	297300	266281
SO8-18	419419	250261	212212	200200	324324	169169	153158	275280	357360	294297	266271
SO8-19	422422	250261	210212	209209	318318	169169	153158	278278	357357	279297	266271
SO8-20	422431	250256	210212	200206	316318	169169	158158	278280	357360	291294	266266
SO8-21	419425	244256	212212	200203	316318	169169	158158	278278	357357	291303	266266
M-01	419419	250253	210212	000000	316318	169169	158158	278281	357363	294297	266266
M-02	419419	244250	210210	197203	348348	169169	158158	278278	357363	291305	266266
M-03	419419	247250	210212	200200	316348	169169	158158	293293	357357	306306	266271
M-04	422419	247253	212212	209209	348348	169169	158158	278293	360360	297306	261271
M-05	419422	247261	210210	000000	348348	169169	158158	266278	360360	288297	266271
M-06	419419	247253	212212	000000	324348	169169	158158	278281	357360	294303	261261

M-08	419419	256264	210210	000000	348348	169169	158158	279281	357357	306306	266271
M-09	419419	247247	210212	194200	316348	169169	153158	278284	354357	288288	266271
M-10	416434	247261	210210	197197	316316	169169	158158	278281	360360	291303	261266
M-11	419419	229253	210212	000000	348348	169173	153158	278278	357363	291297	271276
M-12	419422	241247	210210	000000	318318	169169	158158	278287	357357	291297	266271
M-13	419431	247247	210212	206209	348348	169169	158158	278281	357357	294300	261266
M-14	419419	247261	210212	000000	316318	169169	158158	278281	357360	282300	266266
M-15	416425	247250	206210	200209	316348	169169	158158	278281	360363	288300	266271
M-16	419422	247261	210210	203209	316318	169169	158158	278278	360360	291312	261271
M-17	419422	241261	210212	000000	316316	169169	158158	278281	360360	300300	261271
M-18	419431	229250	210212	206206	316316	169169	158158	278278	357363	282288	266266
M-19	419422	250250	210212	200209	318348	169169	158158	278281	354360	291306	266271
M-20	419422	250261	210212	203209	316316	169169	158158	266278	360360	285297	266271
M-21	419419	247247	212212	200200	316348	169169	158158	278284	360363	291294	266266
M-22	419434	247261	210210	197197	316316	169169	158158	278281	360360	291303	261266
M-23	419422	247261	210210	197197	316316	169169	158158	272272	357363	303303	266266
M-24	431422	247250	210212	200200	316316	169169	158158	278280	360363	291306	261271
F-12	419434	224247	210212	197206	318318	169169	158158	278281	357360	291300	266271
F-13	428422	224253	212212	203206	318318	169169	158158	269290	354357	287293	266266
F-14	422431	224224	206212	200203	000000	169169	000000	278280	360360	288306	266271
F-15	422431	224229	206210	200200	318318	000000	158158	000000	360360	303309	266271
F-16	419422	224250	212212	200209	318348	154154	153163	272293	360360	288303	266271
F-17	419422	247247	210210	191206	318318	000000	158158	272299	360360	290297	271271
F-18	419422	224247	206210	206212	316318	169169	153158	272287	360360	290294	271286
F-19	422431	224224	206212	200206	316318	169169	158158	293293	357357	285294	271271
F-20	422422	224253	212212	200206	316318	000000	158158	281293	357357	294300	266271
F-22	422422	253258	206210	197206	316318	169169	000000	276281	357360	300300	266271
F-23	422422	224247	210212	206206	316318	169169	153158	276278	354360	294294	266266

Appendix 8

Estimates of genetic diversity for eleven microsatellite loci screened in the sugar kelp, *Saccharina latissima*. N, number of individuals; N_a, number of alleles; A, allelic richness; H_O, observed heterozygosity; H_E, expected heterozygosity; F_{IS}, inbreeding coefficient and associated P-values. Significant values after Bonferroni correction ($P \leq 0.0045$) are depicted in bold type.

Location	SLN319	SLN32	SLN320	SLN34	SLN54	SLN58	SLN62	SLN314	SLN35	SLN36	SLN510	All loci
L Outer Oslofjord												
N	23	24	24	23	19	23	23	23	24	24	24	30
N_a	5	8	2	8	4	2	3	6	3	7	2	4.545
A	3.370	4.354	1.687	4.583	2.666	1.444	2.104	3.148	1.617	4.841	1.991	2.891
H_O	0.739	0.750	0.250	0.522	0.053	0.043	0.565	0.565	0.083	0.917	0.500	0.461
H_E	0.686	0.792	0.223	0.827	0.553	0.127	0.423	0.553	0.161	0.846	0.496	0.516
P-value	0.005	0.301	1	0.008	0	0.071	0.252	0.212	0.045	0.999	1	
F_{IS}	**-0.078**	0.053	-0.122	0.365	**0.905**	0.656	-0.336	-0.021	0.483	-0.084	-0.007	0.107
RO Hafrsfjord												
N	30	30	29	28	30	30	29	30	30	30	30	30
N_a	5	11	2	10	7	2	12	12	4	13	5	7.545
A	3.034	4.281	1.960	4.435	4.020	1.133	2.166	4.881	2.119	5.327	2.655	3.274
H_O	0.533	0.700	0.414	0.357	0.600	0.033	0.414	0.667	0.300	0.767	0.533	0.485
H_E	0.625	0.743	0.436	0.806	0.778	0.033	0.424	0.826	0.379	0.874	0.544	0.588
P-value	0.668	0.920	1	0	0.042	-	0.018	0.018	0.142	0.373	0.575	
F_{IS}	0.146	0.058	0.051	**0.557**	0.228	0	0.023	0.193	0.209	0.123	0.019	0.175
HA5 Klosterfjord												
N	25	25	25	22	24	22	24	23	25	25	25	29
N_a	5	10	2	7	6	2	2	9	4	15	5	6.091
A	3.021	4.809	1.974	4.098	3.724	1.182	1.747	4.448	2.442	5.664	2.724	3.258
H_O	0.640	0.840	0.200	0.273	0.417	0.046	0.292	0.696	0.440	0.680	0.600	0.472
H_E	0.647	0.825	0.463	0.788	0.733	0.046	0.254	0.798	0.553	0.897	0.603	0.604
P-value	0.189	0.325	0.007	0	0.001	-	1	0.661	0.603	0.009	1	
F_{IS}	0.010	-0.018	0.568	**0.654**	**0.431**	0	-0.150	0.129	0.205	0.242	0.004	0.219
HA2 Solesnes, Jondal												
N	21	20	21	17	18	21	20	20	21	21	21	27
N_a	4	6	2	4	5	1	2	8	3	7	2	4
A	2.367	3.815	1.993	3.057	3.869	1.000	1.99	4.373	2.773	4.502	1.995	2.885
H_O	0.333	0.800	0.476	0	0.222	0	0.500	0.600	0.619	0.81	0.429	0.443
H_E	0.545	0.738	0.502	0.654	0.794	0	0.492	0.807	0.626	0.825	0.510	0.585
P-value	0.061	0.275	1	0	0.000	-	1	0.103	0.758	0.244	0.662	
F_{IS}	0.389	-0.084	0.052	**1**	**0.720**	-	-0.016	0.256	0.011	0.019	0.159	0.242

HA3 Skjerring												
N	32	32	32	22	32	31	32	32	32	32	32	33
N_a	4	9	2	4	5	2	2	6	5	12	2	4.818
A	2.631	4.563	1.982	2.401	3.000	1.129	1.879	3.162	2.698	5.517	1.627	2.781
H_O	0.656	0.781	0.438	0.091	0.156	0.032	0.375	0.500	0.469	0.844	0.219	0.425
H_E	0.568	0.801	0.477	0.397	0.617	0.032	0.347	0.549	0.531	0.890	0.198	0.496
P-value	0.150	0.004	0.713	0	0.000	-	1	0.113	0.341	0.630	1	
F_{IS}	-0.155	**0.025**	0.083	**0.771**	**0.747**	0	-0.081	0.091	0.118	0.052	-0.107	0.142
HA4 Gjermundshamn												
N	26	26	26	22	26	26	25	24	25	26	26	27
N_a	4	7	2	5	5	3	2	6	4	10	2	4.545
A	2.874	4.453	1.996	3.289	2.935	1.44	1.989	3.692	3.027	4.674	1.898	2.933
H_O	0.692	0.692	0.654	0.091	0.326	0.115	0.640	0.708	0.720	0.769	0.308	0.525
H_E	0.612	0.804	0.506	0.656	0.634	0.112	0.487	0.728	0.676	0.795	0.363	0.577
P-value	0.270	0.041	0.233	0	0.005	1	0.205	0.412	0.854	0.688	0.580	
F_{IS}	-0.132	0.139	-0.292	**0.861**	**0.454**	-0.027	-0.315	0.027	-0.065	0.033	0.153	0.090
HA1 Bårdholmen												
N	23	23	23	23	23	23	22	18	20	22	23	28
N_a	3	13	2	8	6	2	2	11	4	8	6	5.909
A	2.841	4.846	1.978	3.958	3.434	1.174	1.566	5.053	2.488	4.408	3.185	3.176
H_O	0.696	0.957	0.435	0.522	0.261	0.044	0.182	0.778	0.300	0.682	0.696	0.502
H_E	0.644	0.827	0.464	0.714	0.699	0.044	0.169	0.846	0.496	0.806	0.652	0.575
P-value	0.889	0.893	1	0.275	0	-	1	0.140	0.035	0.036	0.102	
F_{IS}	-0.080	-0.157	0.064	0.270	**0.627**	0	-0.077	0.081	0.395	0.154	-0.067	0.126
SO10 Kilstraumen												
N	15	15	15	14	12	14	14	13	15	15	15	21
N_a	5	7	2	8	8	2	2	7	5	12	5	5.727
A	3.139	4.283	1.978	3.733	4.832	1.286	1.842	4.211	2.967	6.005	2.988	3.388
H_O	0.867	0.867	0.667	0.429	0.5	0.071	0.357	0.769	0.467	0.867	0.667	0.599
H_E	0.650	0.769	0.452	0.632	0.837	0.071	0.302	0.766	0.607	0.917	0.629	0.602
P-value	0.159	0.857	0.112	0.035	0.007	-	1	0.762	0.437	0.195	1	
F_{IS}	-0.333	-0.127	-0.474	0.322	0.403	0	-0.182	-0.004	0.231	0.055	-0.061	0.005
SO9 Nyhamnarsundet												
N	15	16	16	15	8	15	15	16	16	16	16	19
N_a	3	8	2	6	4	2	2	8	5	12	4	5.091
A	2.793	4.338	1.993	4.044	3.262	1.267	1.874	4.271	2.874	5.592	2.829	3.194
H_O	0.667	0.688	0.313	0.533	0.250	0.067	0.400	0.625	0.625	0.875	0.688	0.537
H_E	0.631	0.783	0.504	0.786	0.723	0.067	0.329	0.779	0.556	0.883	0.617	0.603
P-value	0.785	0.285	0.290	0.103	0.002	-	1	0.074	1	0.887	1	
F_{IS}	-0.057	0.122	0.380	0.321	**0.654**	0	-0.217	0.198	-0.124	0.009	-0.115	0.111

SO7 Oppedalsvika												
N	15	15	15	13	4	15	15	13	15	15	15	17
N_a	4	9	4	5	2	2	2	7	3	10	6	4.909
A	2.652	5.482	2.512	3.676	2	1.267	1.916	4.292	1.887	5.779	3.603	3.188
H_O	0.600	0.867	0.467	0.231	0.250	0.067	0.467	0.769	0.133	0.667	0.400	0.460
H_E	0.510	0.891	0.548	0.766	0.583	0.067	0.367	0.753	0.250	0.913	0.724	0.574
P-value	0.352	0.568	0.525	0.001	0.429	-	0.529	0.337	0.198	0.012	0.013	
F_{IS}	-0.178	0.027	0.148	**0.699**	0.571	0	-0.273	-0.021	0.467	0.271	0.447	0.199
SO6 Fuglsetfjorden												
N	20	21	20	18	19	21	21	21	21	21	21	21
N_a	2	9	4	8	3	2	2	8	3	8	4	4.818
A	1.993	4.67	2.386	3.089	2.431	1.348	1.801	4.756	2.647	4.927	2.367	2.947
H_O	0.650	0.762	0.450	0.278	0.105	0	0.333	0.714	0.476	0.905	0.714	0.496
H_E	0.497	0.814	0.543	0.485	0.493	0.095	0.283	0.835	0.537	0.849	0.536	0.544
P-value	0.354	0.361	0.777	0.010	0	0.026	1	0.072	0.066	0.541	0.147	
F_{IS}	-0.307	0.064	0.172	0.428	**0.786**	1	-0.177	0.144	0.113	-0.066	-0.333	0.089
SO1 Leikanger												
N	23	23	23	18	14	23	22	23	22	22	23	23
N_a	3	8	2	3	4	1	3	6	3	5	4	3.818
A	2.162	3.604	1.978	2.392	2.742	1	2.079	3.726	1.643	3.32	2.166	2.437
H_O	0.522	0.783	0.435	0.056	0.071	0	0.500	0.696	0.182	0.500	0.348	0.390
H_E	0.513	0.672	0.465	0.428	0.582	0	0.392	0.715	0.172	0.695	0.311	0.445
P-value	0.620	0.329	1	0	0	-	0.460	0.965	1	0.031	1	
F_{IS}	-0.017	-0.165	0.064	**0.870**	**0.877**	-	-0.276	0.028	-0.057	0.280	-0.118	0.125
SO4 Lånefjorden												
N	19	19	19	15	15	19	15	19	19	19	19	19
N_a	4	10	2	4	5	1	3	9	4	7	6	5
A	2.399	4.849	1.978	3.169	4.023	1	2.414	4.66	2.854	4.614	3.448	3.219
H_O	0.790	0.895	0.474	0.067	0.067	0	0.533	0.579	0.526	0.727	0.684	0.503
H_E	0.522	0.817	0.462	0.624	0.810	0	0.495	0.830	0.589	0.820	0.6477	0.599
P-value	0.032	0.159	1	0	0	-	0.025	0.005	0.506	0.162	0.913	
F_{IS}	-0.513	-0.095	-0.025	**0.893**	**0.918**	-	-0.077	**0.303**	0.107	0.102	-0.056	0.161
SO8 Risnesstraumen												
N	19	21	20	19	21	21	20	21	21	21	21	21
N_a	4	10	2	8	4	1	3	7	4	11	4	5.273
A	3.024	5.033	1.990	4.681	3.277	1	2.186	3.566	2.76	5.556	2.906	3.271
H_O	0.684	0.905	0.300	0.316	0.286	0	0.400	0.381	0.667	0.762	0.523	0.476
H_E	0.649	0.848	0.497	0.833	0.669	0	0.515	0.681	0.551	0.893	0.636	0.614
P-value	1	0.186	0.159	0	0	-	0.464	0	0.343	0.049	0.189	
F_{IS}	-0.054	-0.067	0.397	**0.621**	**0.573**	-	0.223	**0.441**	-0.210	0.147	0.176	0.226

M Runde												
N	23	23	23	15	23	23	24	23	23	23	23	24
N_a	6	9	3	6	4	2	2	9	4	11	4	5.455
A	3.112	4.364	2.157	4.306	2.863	1.174	1.321	3.811	3.105	5.613	2.971	3.163
H_O	0.609	0.826	0.522	0.467	0.391	0.044	0.087	0.739	0.522	0.783	0.696	0.518
H_E	0.567	0.782	0.503	0.819	0.640	0.044	0.085	0.683	0.676	0.897	0.644	0.568
P-value	0.092	0.391	1	0.001	0.053	-	1	0.104	0.059	0.220	0.254	
F_{IS}	-0.073	-0.057	-0.037	**0.430**	0.389	0	-0.023	-0.083	0.228	0.128	-0.080	0.088
F Sommarøy												
N	11	11	11	11	10	8	9	10	11	11	11	12
N_a	5	6	3	7	3	2	3	10	3	12	3	5.182
A	3.354	3.778	2.902	4.259	2.349	1.767	2.297	5.972	2.586	6.151	2.362	3.434
H_O	0.727	0.727	0.636	0.818	0.600	0	0.333	0.900	0.364	0.818	0.636	0.614
H_E	0.618	0.705	0.673	0.768	0.461	0.250	0.389	0.917	0.577	0.923	0.564	0.635
P-value	0.583	0.843	0.750	0.970	1	0.066	0.362	0.827	0.297	0.475	1	
F_{IS}	-0.177	-0.032	0.054	-0.065	-0.301	1	0.143	0.018	0.370	0.113	-0.129	0.033
All locations												
N	340	344	342	295	298	335	329	329	340	343	345	345
N_a	8	20	6	17	15	6	5	15	9	26	13	13
A	2.798	4.470	2.090	3.698	3.214	1.226	1.948	4.251	2.530	5.156	2.607	3.090
H_O	0.641	0.800	0.435	0.305	0.292	0.036	0.399	0.650	0.432	0.773	0.589	0.487
H_E	0.593	0.789	0.472	0.686	0.668	0.053	0.357	0.740	0.493	0.854	0.522	0.566
P-value	0.027	0.098	0.739	High. Sign.	High. Sign.	0.021	0.682	0	0.096	0.004	0.745	
F_{IS}	-0.081	-0.014	0.078	0.555	0.563	0.327	-0.117	0.121	0.123	0.095	-0.129	0.138

Appendix 9

Genic differentiation for each population pair (Fisher's exact Probability test)

Number of populations : 16
Number of loci : 11

Markov chain parameters
Dememorisation : 1000
Batches : 100
Iterations per batch : 1000

P-value for each population pair across all loci (Fisher's method)

Population pair	Chi2	df	P-Value
L & RO	Infinity	22	Highly sign.
L & HA5	Infinity	22	Highly sign.
RO & HA5	Infinity	22	Highly sign.
L & HA2	Infinity	22	Highly sign.
RO & HA2	Infinity	22	Highly sign.
HA5 & HA2	Infinity	22	Highly sign.
L & HA3	Infinity	22	Highly sign.
RO & HA3	Infinity	22	Highly sign.
HA5 & HA3	Infinity	22	Highly sign.
HA2 & HA3	Infinity	22	Highly sign.
L & HA4	Infinity	22	Highly sign.
RO & HA4	Infinity	22	Highly sign.
HA5 & HA4	95.4388	22	0.0000
HA2 & HA4	Infinity	22	Highly sign.
HA3 & HA4	90.7889	22	0.0000
L & HA1	Infinity	22	Highly sign.
RO & HA1	Infinity	22	Highly sign.
HA5 & HA1	34.0583	22	0.0484
HA2 & HA1	83.6327	22	0.0000
HA3 & HA1	Infinity	22	Highly sign.
HA4 & HA1	Infinity	22	Highly sign.
L & SO10	Infinity	22	Highly sign.
RO & SO10	Infinity	22	Highly sign.
HA5 & SO10	29.4347	22	0.1329
HA2 & SO10	73.7462	22	0.0000
HA3 & SO10	Infinity	22	Highly sign.
HA4 & SO10	78.8224	22	0.0000
HA1 & SO10	29.8317	22	0.1226
L & SO9	Infinity	22	Highly sign.
RO & SO9	53.7768	22	0.0002

HA5 & SO9	18.9712	22	0.6471
HA2 & SO9	64.3779	22	0.0000
HA3 & SO9	Infinity	22	Highly sign.
HA4 & SO9	66.0496	22	0.0000
HA1 & SO9	21.7050	22	0.4776
SO10 & SO9	18.6694	22	0.6656
L & SO7	Infinity	22	Highly sign.
RO & SO7	41.6449	22	0.0069
HA5 & SO7	44.1114	22	0.0034
HA2 & SO7	Infinity	22	Highly sign.
HA3 & SO7	Infinity	22	Highly sign.
HA4 & SO7	Infinity	22	Highly sign.
HA1 & SO7	46.3394	22	0.0018
SO10 & SO7	29.6099	22	0.1283
SO9 & SO7	20.8493	22	0.5301
L & SO6	Infinity	22	Highly sign.
RO & SO6	Infinity	22	Highly sign.
HA5 & SO6	87.8193	22	0.0000
HA2 & SO6	Infinity	22	Highly sign.
HA3 & SO6	Infinity	22	Highly sign.
HA4 & SO6	Infinity	22	Highly sign.
HA1 & SO6	Infinity	22	Highly sign.
SO10 & SO6	Infinity	22	Highly sign.
SO9 & SO6	59.8231	22	0.0000
SO7 & SO6	82.5495	22	0.0000
L & SO1	Infinity	22	Highly sign.
RO & SO1	Infinity	22	Highly sign.
HA5 & SO1	Infinity	22	Highly sign.
HA2 & SO1	Infinity	20	Highly sign.
HA3 & SO1	Infinity	22	Highly sign.
HA4 & SO1	Infinity	22	Highly sign.
HA1 & SO1	Infinity	22	Highly sign.
SO10 & SO1	Infinity	22	Highly sign.
SO9 & SO1	Infinity	22	Highly sign.
SO7 & SO1	Infinity	22	Highly sign.
SO6 & SO1	Infinity	22	Highly sign.
L & SO4	Infinity	22	Highly sign.
RO & SO4	Infinity	22	Highly sign.
HA5 & SO4	54.5106	22	0.0001
HA2 & SO4	96.4863	20	0.0000
HA3 & SO4	Infinity	22	Highly sign.
HA4 & SO4	Infinity	22	Highly sign.
HA1 & SO4	Infinity	22	Highly sign.
SO10 & SO4	39.9300	22	0.0110
SO9 & SO4	43.5727	22	0.0041
SO7 & SO4	45.2705	22	0.0025

SO6 & SO4	Infinity	22	Highly sign.
SO1 & SO4	Infinity	20	Highly sign.
L & SO8	Infinity	22	Highly sign.
RO & SO8	Infinity	22	Highly sign.
HA5 & SO8	30.9811	22	0.0965
HA2 & SO8	82.4999	20	0.0000
HA3 & SO8	Infinity	22	Highly sign.
HA4 & SO8	Infinity	22	Highly sign.
HA1 & SO8	59.3735	22	0.0000
SO10 & SO8	35.9463	22	0.0308
SO9 & SO8	24.6483	22	0.3142
SO7 & SO8	31.1574	22	0.0929
SO6 & SO8	Infinity	22	Highly sign.
SO1 & SO8	Infinity	20	Highly sign.
SO4 & SO8	44.9516	20	0.0011
L & M	Infinity	22	Highly sign.
RO & M	Infinity	22	Highly sign.
HA5 & M	Infinity	22	Highly sign.
HA2 & M	Infinity	22	Highly sign.
HA3 & M	Infinity	22	Highly sign.
HA4 & M	Infinity	22	Highly sign.
HA1 & M	Infinity	22	Highly sign.
SO10 & M	Infinity	22	Highly sign.
SO9 & M	69.3541	22	0.0000
SO7 & M	84.3934	22	0.0000
SO6 & M	Infinity	22	Highly sign.
SO1 & M	Infinity	22	Highly sign.
SO4 & M	Infinity	22	Highly sign.
SO8 & M	Infinity	22	Highly sign.
L & F	Infinity	22	Highly sign.
RO & F	Infinity	22	Highly sign.
HA5 & F	Infinity	22	Highly sign.
HA2 & F	Infinity	22	Highly sign.
HA3 & F	Infinity	22	Highly sign.
HA4 & F	Infinity	22	Highly sign.
HA1 & F	Infinity	22	Highly sign.
SO10 & F	Infinity	22	Highly sign.
SO9 & F	Infinity	22	Highly sign.
SO7 & F	Infinity	22	Highly sign.
SO6 & F	Infinity	22	Highly sign.
SO1 & F	Infinity	22	Highly sign.
SO4 & F	Infinity	22	Highly sign.
SO8 & F	Infinity	22	Highly sign.
M & F	Infinity	22	Highly sign.

Appendix 10

Genotypic linkage disequilibrium

Number of populations : 16
Number of loci detected : 11

Markov chain parameters
Dememorisation : 10000
Batches : 1000
Iterations per batch : 10000

P-value for each locus pair across all populations (Fisher's method)

Locus pair	Chi2	df	P-Value
SLN319 & SLN32	33.274911	32	0.4050
SLN319 & SLN320	32.896257	32	0.4230
SLN32 & SLN320	37.487290	32	0.2319
SLN319 & SLN34	25.465381	32	0.7868
SLN32 & SLN34	28.603682	32	0.6392
SLN320 & SLN34	36.289108	32	0.2754
SLN319 & SLN54	41.005581	32	0.1321
SLN32 & SLN54	27.788863	28	0.4757
SLN320 & SLN54	23.694519	30	0.7857
SLN34 & SLN54	29.136730	30	0.5104
SLN319 & SLN58	16.981530	24	0.8494
SLN32 & SLN58	11.598820	24	0.9841
SLN320 & SLN58	12.356180	24	0.9756
SLN34 & SLN58	6.021158	20	0.9989
SLN54 & SLN58	17.423703	22	0.7396
SLN319 & SLN62	19.171667	32	0.9642
SLN32 & SLN62	25.718847	32	0.7759
SLN320 & SLN62	22.717177	32	0.8870
SLN34 & SLN62	17.183255	32	0.9849
SLN54 & SLN62	28.127671	32	0.6630
SLN58 & SLN62	20.747574	24	0.6536
SLN319 & SLN314	23.019309	30	0.8145
SLN32 & SLN314	24.004702	30	0.7718
SLN320 & SLN314	52.287680	30	0.0071
SLN34 & SLN314	33.765791	30	0.2903
SLN54 & SLN314	22.219309	30	0.8459
SLN58 & SLN314	8.875334	20	0.9843
SLN62 & SLN314	29.055575	30	0.5147
SLN319 & SLN35	29.556289	32	0.5908

SLN32 & SLN35	15.363968	32	0.9943
SLN320 & SLN35	29.693152	32	0.5838
SLN34 & SLN35	36.852762	32	0.2544
SLN54 & SLN35	42.262519	32	0.1060
SLN58 & SLN35	9.812133	22	0.9879
SLN62 & SLN35	25.823932	32	0.7713
SLN314 & SLN35	31.702808	30	0.3815
SLN319 & SLN36	15.090796	28	0.9775
SLN32 & SLN36	31.106638	30	0.4102
SLN320 & SLN36	29.191426	30	0.5076
SLN34 & SLN36	17.166314	30	0.9705
SLN54 & SLN36	24.094151	28	0.6766
SLN58 & SLN36	8.437757	22	0.9958
SLN62 & SLN36	24.407962	28	0.6598
SLN314 & SLN36	45.778223	30	0.0326
SLN35 & SLN36	31.706857	30	0.3813
SLN319 & SLN510	42.431714	32	0.1028
SLN32 & SLN510	37.156264	32	0.2435
SLN320 & SLN510	19.335435	32	0.9619
SLN34 & SLN510	33.645611	32	0.3877
SLN54 & SLN510	35.723693	32	0.2976
SLN58 & SLN510	13.092129	24	0.9646
SLN62 & SLN510	30.283459	32	0.5536
SLN314 & SLN510	33.911182	30	0.2844
SLN35 & SLN510	49.884755	32	0.0229
SLN36 & SLN510	32.660848	30	0.3374