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Genetic population structure of the northern shrimp (*Pandalus borealis*) along the Norwegian coast

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Preface

In cooperation with the population genetics group at the Norwegian Institute of Marine Research (IMR). The project was initiated as a collaboration between IMR and the University of Tromsø. The genetic work was done at the genetic laboratory at IMR division in Tromsø under supervision by Tanja Hanebrekke.

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Given that I never have had any population genetics courses at the university my learning curve has been steep working with this Master's thesis. I have learned a lot about a range of subjects/themes, including population genetics, laboratory work, genetical and statistical analysis, genetic software's, and so on. Everything I have learned during the process of writhing this thesis will be very useful when I start my career as a biologist.

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Agneta Hansen

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Abstract

The coastal shrimp (*Pandalus borealis*) represent an important income for a small-scale fishery. The more economical important fishery of shrimp is located offshore. There is sparse information and literature regarding the coastal shrimp, and it is a lot we do not know about the species that inhabits our coast and fjords. Genetic research will provide more information on the species in these areas.

Genetic investigation using microsatellite markers revealed three divisions or clusters of shrimps among the samples included in this study: the Barents Sea, Troms-Trøndelag and Vancouver (reference sample). Finnmark fjords showed to be influenced with both the Barents Sea group and the coastal Troms-Trøndelag group. A clear border, however, was found between the Barents Sea and Troms-Trøndelag. The Finnmark fjords samples showed varying degrees of genetic divergence from the Barents Sea, some being significant others not. The general trend showed that the inner eastern Finnmark locations and all fjords from Laksefjorden and south was significant different from the Barents Sea. Genetic population structure between fjords did not display any general trend, despite the difference between coastal populations in Finnmark and Troms-Trøndelag. The only fjords that displayed significant different genetic structure within, was the Varangerfjord in eastern Finnmark.

The finding of genetic different coastal shrimps in Troms-Trøndelag indicate that they should be considered as a stock of their own. The Finnmark fjords also differ in some degree from the Barents Sea, and there should therefore also be considered further if this population should be considered as a separate stock.

Abbreviations

Abbreviated locality names are found in Table 1.

AC = Atlantic Current

AW = Atlantic Water

DA = Discriminant Analysis

DAPC = Discriminant Analysis of Principal Components

DFO = Department of fisheries and Oceans Canada

dH_2O = Distilled water

DNA = Deoxyribonucleic acid

FDR = False discovery rate

g = G-force

H_e = Expected heterozygosity

H_o = Observed heterozygosity

HW = Hardy-Weinberg

HWE = Hardy-Weinberg Equilibrium

ICES = The International Council of the Exploration of the Sea

IMR = Institute of Marine Research

Loc(us/i) = Marker(s)

MCMC = Markov Chain Monte Carlo

NCC = Norwegian Coastal Current

NCW = Norwegian Coastal Water

NWAC = Norwegian Atlantic Current

PCR = Polymerase Chain Reaction

RAPD = Random Amplification of Polymorphic DNA

SNP = Single-nucleotide polymorphisms

STR = Short tandem repeats

TAC = Total allowable catches

URL = Uniform Resource Locator

1. Introduction

The traditional way of thinking of population genetic variation in the ocean was that with wide distribution, extensive larval and adult dispersal, and large population sizes, opportunities for local adaptions would be constrained by high migration and exposure to a breadth of environments (Hauser and Carvalho, 2008). However, genetic studies have challenged such views by displaying population subdivision in marine fishes on a limited geographical scale, ranging from tens to a few hundred kilometers (Hauser and Carvalho, 2008, and references therein). Mutation, genetic drift due to finite population size, and natural selection favoring adaptions to local environmental conditions lead to genetic differentiation of local populations, while gene flow will oppose that differentiation (Slatkin, 1987). Indeed, gene flow has been suggested to be the major causing factor directing genetic homogeneity in marine fishes (Ward et al., 1994), where gene flow is a collective term that includes all mechanisms resulting in the movement of genes between populations (Slatkin, 1985). High dispersal capacity, however, does not automatically indicate elevated rates of gene flow (Palumbi, 1994). Behavioral mechanisms, selection against immigrants, complex oceanographic circulation processes and barriers may all counteract gene flow and panmixia (Drengstig et al., 2000). Gene flow may either constrain evolution by preventing adaptions to local conditions or promote evolution by spreading new genes and combinations of genes throughout a species' range (Slatkin, 1987).

The northern shrimp, *Pandalus borealis*, is by far the most abundant and important commercial shrimp species in the North-East Atlantic (Shumway et al., 1985; Garcia, 2007). Along the Norwegian coast, shrimp represent an important source of income even for small-scale fishery. The two economically most important stocks of shrimp in Norwegian waters are found in the Barents Sea, and the Norwegian Deep and Skagerrak (see chapter 1.1.4). While these offshore stocks are annually monitored and assessed, the patchily distributed populations along the coast have received little scientific attention. The present distribution, abundance and structure of fjord populations are to a large extent unknown. Genetic studies on shrimp in these areas will provide more information. Local adaption is highly relevant to fisheries management. Preservation of genetic resources is critical for ensuring perpetuation of stocks (Hauser and Carvalho, 2008). Coastal shrimp has recently started to receive attention from management and the general public, particularly in connection with the vulnerability of shrimp to chemical sea

lice controlling agents in connection with aquaculture (e.g. Bechmann et al., 2017; Bjørkan and Rybråten, 2019; Bechmann et al., 2020).

P. borealis is a species with wide distribution and interesting and very varied biology over large areas. A summary of what we know about the variation seen in for instance the life history can help in explaining the genetic results. Therefore, a short description of the distribution, life history, fishery, and management will be given first. A summary of the previous genetic research on *P. borealis* in North Atlantic and Pacific waters will thereafter be presented.

1.1 The Biology of *Pandalus borealis*

1.1.1 Geographic Distribution

P. borealis is a discontinuous circumpolar species common in boreal waters (Shumway et al., 1985). Its geographical distribution ranges from southern, warmer areas to northern, colder areas, with temperatures and latitudes ranging from -1°C to 12°C, and from 40 to 82 °N respectively (Nilssen and Hopkins, 1991). There are two subspecies of northern shrimp: *Pandalus borealis borealis* Krøyer 1938 in the Atlantic and *P. borealis eous* Makarov 1935 in the Pacific (Garcia, 2007; Rasmussen and Aschan, 2011). *Pandalus borealis eous* was raised to species level as *Pandalus eous* by Squires (1992), although of ongoing reluctance to accept this division (Bergström, 2000; Garcia, 2007). The two subspecies have apparent differences in morphology, probably due to important environmental adaptions (Rasmussen and Aschan, 2011). Temperature, substratum, salinity, and depth are all factors influencing distribution patterns (Shumway et al., 1985).

1.1.2 Lifecycle

P. borealis is a protandric hermaphrodite; each individual matures and functions first as a male, before they pass through a transitional (intersexual) phase and then become female (Shumway et al., 1985). The age of sex change is related to individual body size (Nilssen and Hopkins, 1991). In general, age at maturity increases with decreasing temperature (Shumway et al., 1985). Age at maturity for males ranges from 1.5 years to 3.5 years and for females from 1.5

years to 5.5 years. The spawning period varies from July to December, and the hatching period varies from February to June, depending on the location (Bergström, 2000).

As shrimp need to molt to grow, they lack suitable hard structures from which age can be determined. Age and growth rates have therefore been estimated using length-frequency data. These methods present some difficulties due to considerable overlap in size between the larger age groups classes (Shumway et al., 1985). Growth rates vary between regions, and between sexes and year classes (Shumway et al., 1985).

Sex ratio in *P. borealis* populations is a function of natural mortality, fishing mortality, and recruitment. Since migration occurs in certain populations, the sex ratio is also a function of location and season of sampling (Shumway et al., 1985). The age composition is affected by the same determinants as sex ratio. Age and size distribution in catches, age at first capture, and density of different age groups are all affected by recruitment, natural mortality as well as selectivity of the fishing gear and intensity of fishing (Shumway et al., 1985). Maximum age and size are greater towards the Arctic with Iceland and Spitsbergen showing 11+ and 8+ years respectively. In most areas studied, *P. borealis* lives for 4+ to 5+ years (Shumway et al., 1985).

Female *P. borealis* carry their fertilized eggs on their pleopods from the time of extrusion until hatching and release of the larvae, the period and duration of this process varies with temperature (Shumway et al., 1985). *P. borealis* has five pelagic larval stages which drift with ocean currents before settling on the bottom (Ouellet and Allard, 2006; Rasmussen and Aschan, 2011). The pelagic larval stage is relatively long with a potential for extensive dispersal (Drengstig et al., 2000). It is assumed that the transport processes during the pelagic larval stage influence recruitment, both directly by advectional losses of larvae and indirectly through temperature, food availability and predator-prey interactions (Pedersen et al., 2003, and references therein). Pedersen et al. (2003) found that temporal and spatial variations in the hydrodynamics of the Barents Sea seem to govern the pattern of larval settlement of *P. borealis.*

There is sparse information about the shrimp populations along the Norwegian coast when it comes to factors as life expectancy, age at sex change, maturity and size. However, they are probably located between shrimps in Skagerrak/North Sea and the Barents Sea in regards of such factors.

1.1.3 Distribution by size and sex

The distribution of adult *P. borealis* depends on size, age, sex, and season, and over broader time frames in many populations, these bounds are described by temperature, salinity, and depth tolerances (Shumway et al., 1985). Distributional differences occur due to horizontal and vertical movement and due to a tendency to segregate by size in a mixed population (Shumway et al., 1985). Aschan (2000) found that depth was the main environmental factor explaining spatial size distribution of shrimp in the Barents Sea, despite relatively little depth variation in the area. Small and medium sized shrimp were common in shallow areas and the periphery, while medium and large shrimp were frequently found associated with the Hopen Deep and the Bear Island Trench. Annual differences in distribution of adults occur with changes in abundance. During years of great abundance, not only is the shrimp density greater but also the total area of distribution can be greatly increased (Shumway et al., 1985). Seasonal distribution changes occur primarily due to migratory impulses expressed by various sex/age classes (Shumway et al., 1985).

Environmental determinants of the distribution of *P. borealis* include substratum, currents, depth, light, salinity, and temperature (Shumway et al., 1985). Temperature has been correlated most closely with changes in abundance of *P. borealis* (Shumway et al., 1985). The optimal temperature range seems to be between -1.6°C and 8°C, although they are most common in waters above 0°C, and die at temperatures below -1.6°C (Garcia, 2007). Jan Mayen has an extreme environment with low temperatures and infrequent, but abrupt temperature changes, shrimps in the Jan Mayen area are therefore considered to be on the edge of the species' distribution (Nilssen and Aschan, 2009). Shrimp in southern warmer areas have higher grow rates than shrimp in colder northern areas. Temperature also affect the longevity of shrimp, respectively with the longest lifespan in colder and shortest in warmer regions (Teigsmark, 1983; Nilssen and Hopkins, 1991; Aschan, 2000; Nilssen and Aschan, 2009). Salinity preferences for shrimp range from 33 to 35‰, but there are records of *P. borealis* found in areas with salinity as low as 23.4‰ (Garcia, 2007).

1.1.4 Fisheries and management

The shrimp fisheries in Norway commenced at the turn of the 20th century and in the mid 1950's, an economically significant fishery took place along the whole Norwegian coast (Teigsmark, 1983). Today the species is commercially harvested along the coast, in Skagerrak and the Norwegian Deep, and in the Barents Sea, including the Svalbard area (Hvingel and Søvik, 2019; NAFO and ICES, 2019). Coastal and fjord shrimp are fished by a coastal fishing fleet consisting mainly of vessels <15 m (Hvingel and Søvik, 2019). Shrimps in some fjords are assumed partly isolated from shrimps in the open ocean, but have not been considered separate stocks (Hvingel and Søvik, 2019). The coastal and fjord shrimp south of 62°N are managed as part of the stock in Skagerrak and the Norwegian Deep (Hvingel and Søvik, 2019). All shrimp north of 62°N belong to the same Norwegian management unit (stock). ICES, however, consider only coastal shrimp north of 70°N as part of the Barents Sea stock (Hvingel and Søvik, 2019).

There are quotas for the Skagerrak/Norwegian Deep stock (including coastal shrimp), but no quotas are set for the shrimp stock north of 62°N, including the Barents Sea. The smallest mesh size for catching shrimp is 35 mm (URL #1: Directorate of Fisheries). The coastal shrimp fishery is further managed by a minimum landing size (carapace length of 15 mm) and closing of areas with excessive numbers of juvenile fish and shrimp in catches (URL #1: Directorate of Fisheries). As opposed to in the Barents Sea there are no fishing licenses or number of effective fishing days for shrimp fishers along the coast (Garcia, 2007). Shrimp trawlers in Norway operate both inside fjords and sheltered waters as well as in offshore areas (Knutsen et al., 2015). Catches from fjords are small compared to those taken in the open sea (Knutsen et al., 2015).

P. borealis in the Barents Sea and in the Svalbard fishery protection zone is considered as one stock (NAFO and ICES, 2019). Using biological data, Berenboim (1982) proposed that the Barents Sea shrimp consisted of only one super-population (Drengstig et al., 2000). Norwegian and Russian vessels exploit the stock in the entire area, while vessels from other nations are restricted to the Svalbard fishery zone and the "Loop Hole" (NAFO and ICES, 2019). The Russian zone are the only area where there are established total allowable catches (TAC) (NAFO and ICES, 2019). Landings in the recent 10-year period have varied between 20 000 and 45 000 tonnes/year by Norwegian vessels, this amounts to 25-75 percentage (%)

of the total landings (NAFO and ICES, 2018). The rest of the landings are by vessels from Russia, Iceland, Greenland, Faroes and the European Union (NAFO and ICES, 2019).

1.2 Previous genetic studies on *Pandalus*

Earlier genetic analyses of the population genetic structure of *P. borealis* in the North-East Atlantic did not find any distinct sub-populations in the open sea and the genetic variance between individuals in a location was high (Rasmussen et al., 1993; Martinez et al., 1997; Drengstig et al., 2000; Martinez et al., 2006). However, gradients of changes in genetic material between areas related to geographic distance and sea currents was found (Pedersen et al., 2003). Shrimps in the North-East Atlantic were analyzed for allozymic variation (Drengstig et al., 2000), they found genetic variation between Norwegian fjords and the Barents Sea, and among fjords. Both allozymes and the DNA (deoxyribonucleic acid) based method RAPD (Random Amplification of Polymorphic DNA) found no differentiation between shrimp from the Barents Sea and Svalbard area (Drengstig et al., 2000; Martinez et al., 2006). Although, there may be some subpopulation structure in environmentally extreme areas. Furthermore, their findings confirmed that shrimp from the Barents Sea and Svalbard areas differ from shrimp in the Norwegian fjords and around Jan Mayen. They concluded that a characteristic of *P. borealis* in the Northeast Atlantic is large genetic variability at an individual level.

In the Pacific Ocean, Kartavtsev et al. (1993) genetic electrophoretic studies of enzymes (allozyme) on *P. borealis* in the Sea of Japan, the Sea of Okhotsk and the Bering Sea showed that the loci allele frequencies within any sea were rather similar, but that they greatly differed between the sea basins. They assumed that shrimp inhabiting the same basin were genetically homogeneous.

Microsatellites has been developed for *P. borealis* in recent years (Pereyra et al., 2012). Two microsatellite studies have been conducted on *P. borealis*, one in Skagerrak and the North Sea, and one across the North Atlantic (Jorde et al., 2015; Knutsen et al., 2015). Genetic structure among oceanic *P. borealis* samples of Skagerrak and the eastern North Sea was found to be weak and non-significant, in accordance with the current management regime of one single stock. However, populations in Skagerrak fjords generally displayed elevated levels of genetic differentiation (Knutsen et al., 2015).

In the large-scale population study of the genetic structure of northern shrimp across the North Atlantic it was found that differences in bottom temperature among localities correlated well with the large-scale genetic divergence pattern (Jorde et al., 2015). Larval drift was to a lesser extent found to explain the pattern observed. In Norwegian waters, they found small and insignificant genetic differences between shrimp in the Norwegian Deep and along the coast of Trøndelag, but profound and significant differences between this coastal sample and shrimp in the Barents Sea region (Jorde et al., 2015). However, the population genetic structure of shrimp in fjords and coastal areas between Trøndelag and Varanger is still unknown.

1.3 Objectives

To follow up the work by Jorde et al. (2015), genetic samples have been collected in the years 2010 to 2018 in preparation for further work on the genetic stock structure of Norwegian coastal shrimp. The genetic stock structure of fjord and coastal shrimp populations is largely unknown and has not been investigated using more up-to-date methods. The objective of the present study is therefore to test the population genetic structure of northern shrimp along the Norwegian coast using microsatellites, emphasizing the following research questions: (i) where is the border between coastal shrimp and the Barents Sea shrimp?, (ii) is there genetic population structure between fjords? and (iii) is there genetic population structure between inner and outer parts of the fjords? By using the same set of microsatellite markers as in Jorde et al. (2015), results in this thesis are directly comparable with their results. The results from this master project is highly relevant for the management of northern shrimp along the Norwegian coast.

2. Materials and methods

2.1 Study area

The coastal areas and fjords of North Norway are under influence of the northward flowing Norwegian Coastal Current (NCC) containing Norwegian Coastal Water (NCW) (Figure 1) that has its origin in the Baltic Ocean and Skagerrak (Eilertsen and Skarðhamar, 2006). As a consequence of this the northern coastal waters are influenced by processes happening further south, but also by interactions with the outer laying Atlantic Water (AW) (Figure 1) in the Norwegian Atlantic Current (NWAC) (Eilertsen and Skarðhamar, 2006).

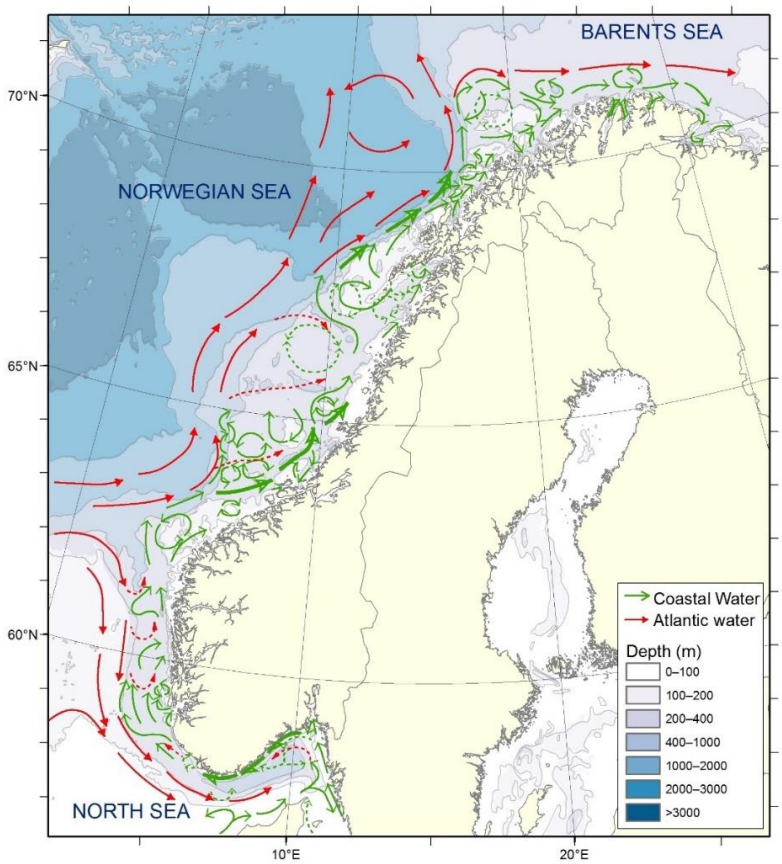

Figure 1. Map showing Norwegian Coastal Water (NCW), Atlantic Water (NWAC), and the Bathymetry along the Norwegian coast. Map by Karen Gjertsen and Roald Sætre (IMR).

Fjords in the Trøndelag and Nordland counties are characterized by steep mountains and deep basins with sills, which generally characterize Norwegian fjords (Myksvoll et al., 2013). Follafjord is a very narrow fjord, measuring only approximately 625 meters (m) in width and with a 40 m deep sill at the entrance area, with a maximum depth of approx. 165 m (URL #2:

Kartverket, Follafjorden). Ranfjorden has a sill depth of 40-60 m at the entrance area, and maximum depth of approx. 550 m (URL #3: Kartverket, Ranfjorden). Folla is a fjord system consisting of two fjords with a joint opening, both of the fjords have deep basins down to 574 m, with sill depths of 265-225 m (Myksvoll et al., 2013).

The length, depth, width and sill depth vary considerably between the larger fjords in the Troms and Finnmark county (Wassmann et al., 1996). Although Troms and Finnmark now belong to the same county (from January 2020), they will hereafter be referred to as two separate regions/areas because of their differences in topography and bathymetry. Except for Malangen, most fjords in the former Troms part of the county are narrow and have relatively shallow sills with maximum depths of less than 200 m. Some of the fjords are connected with the NCW through narrow inlets (Wassmann et al., 1996). Fjords in Troms are sill-fjords, although with varying sill-depths. Sills in narrow fjords are found at the entrance area (Wassmann et al., 1996).

All the main fjords of Finnmark, except from the Altafjord, are broad. The main fjords are 80-100 kilometers (km) long, with a maximum width of 10-20 km (Wassmann et al., 1996). Porsangerfjorden is divided into three parts, the inner part that is separated from the middle part by a 30 m deep sill, the middle part that is separated from the outer part by an island, and the outer part that has a deep sill of 180 meters (Myksvoll et al., 2012). The outer part of Porsangerfjorden is well connected with the coastal water masses (Myksvoll et al., 2012). Circulation in the Varangerfjord are structurally different from the other fjords) due to its wide entrance and similarity to a bay (Pedersen et al., 2009). Except from the Altafjord, all the other fjords in Finnmark (Tanafjorden, Laksefjorden) have unhindered contact with the NCC and the Barents Sea (Wassmann et al., 1996).

The Barents Sea is a shelf sea covering approximately 1.4 million km^2 with an average depth of 230 m (Pedersen et al., 2003). The circulation is dominated by the Atlantic Current (AC) flowing northwards along the western shelf break of Norway. South of this Atlantic inflow, the NCC continues along the Finnmark and Kola coast. A branch of the AC also enters the Barents Sea through the Bear Island Trench (Pedersen et al., 2003). In the northern and eastern parts of the Barents Sea, Arctic water flows south-westwards near the surface. The Atlantic inflow continues in a northeast direction below this layer (Pedersen et al., 2003).

2.2 Sampling

Shrimp samples for genetic analyses were collected in the coastal and fjord areas from Trøndelag county to Varanger, and in the southern part of the Barents Sea during the period 2010-2018 (Table 1, Figure 2). Samples were collected during research cruises by the Norwegian Institute of Marine Research (IMR) and by local fishers. One reference sample was collected off Vancouver Island in Pacific Canada in 2015 (Table 1) by scientists at the Department of Fisheries and Oceans Canada (DFO). Only female shrimps were collected, they could consist of several year classes, thereby providing a more representative sample of the total population. At sea, the tissue samples were collected and conserved in 70 % ethanol. Samples were stored at 4 °C until DNA extraction at IMR's facilities in Tromsø. Fishermen froze the shrimp samples for later tissue and DNA sampling at IMR. All the samples were collected using bottom/shrimp trawl.

Table 1. Sampling location, sampling years, and number of genotyped individuals (n) of Pandalus borealis. t= total number analyzed. Abbr.= the abbreviated locality name. †: Samples also analyzed by Jorde et al. (2015).

	Locality	*Abbr.*	*Year*	*Position*	*n/t*
Barents Sea	Barents Sea south	BSS1†	2010	71°15`N 28°48`E	19/20
	Barents Sea south	BSS1†	2010	71°17`N 30°28`E	18/20
	Barents Sea south	BSS1†	2010	71°16`N 32°15`E	18/20
	Barents Sea south	BSS1†	2010	71°52`N 30°17`E	19/20
	Barents Sea south	BSS1†	2010	71°49`N 28°39`E	11/11
	Barents Sea south	BSS2	2016	72°26`N 34°19`E	91/94
	Barents Sea south	BSS3	2016	72°16`N 20°57`E	50/51
	Barents Sea south	BSS4	2016	71°10`N 22°01`E	40/43
Troms and	Varangerfjorden outer	VARO	2017	69°52`N 30°47`E	92/94
Finnmark	Varangerfjorden middle	VARM	2017	70°01`N 30°02`E	90/94
	Outside Vardø	VAR	2016	70°30`N 31°36`E	92/94
	Tanafjorden outer	TANO	2017	70°52`N 28°35`E	91/94
	Tanafjorden middle	TANM	2017	70°41`N 28°24`E	92/94
	Laksefjorden middle	LAKM	2017	70°42`N 26°56`E	90/94
	Laksefjorden inner	LAKI	2017	70°27`N 26°41`E	92/94
	Porsangerfjorden outer	PORO	2016	70°58`N 26°26`E	89/94
	Porsangerfjorden middle	PORM	2017	70°25`N 25°18`E	75/94
	Porsangerfjorden inner	PORI	2018	70°11`N 25°15`E	92/94
	Kvænangen	KVN	2018	69°53`N 21°42`E	91/94
	Reisafjorden	REI	2018	69°54`N 21°07`E	92/94
	Lyngen	LYN	2017	69°25`N 20°13`E	91/94
	Malangen	MAL	2011	69°30`N 18°05`E	91/96
Nordland	Folla	FO2011	2011	67°35`N 14°49`E	96/96
	Ranfjorden	RAN	2017	66°09`N 12°59`E	92/94
Trøndelag	Follafjord inner	FOFI	2010	64°56`N 12°16`E	94/96
	Tviberg	NOM†	2010	64°45`N 11°05`E	96/96
Canada	Vancouver	VANC	2015	49°20`N 123°27`E	85/96

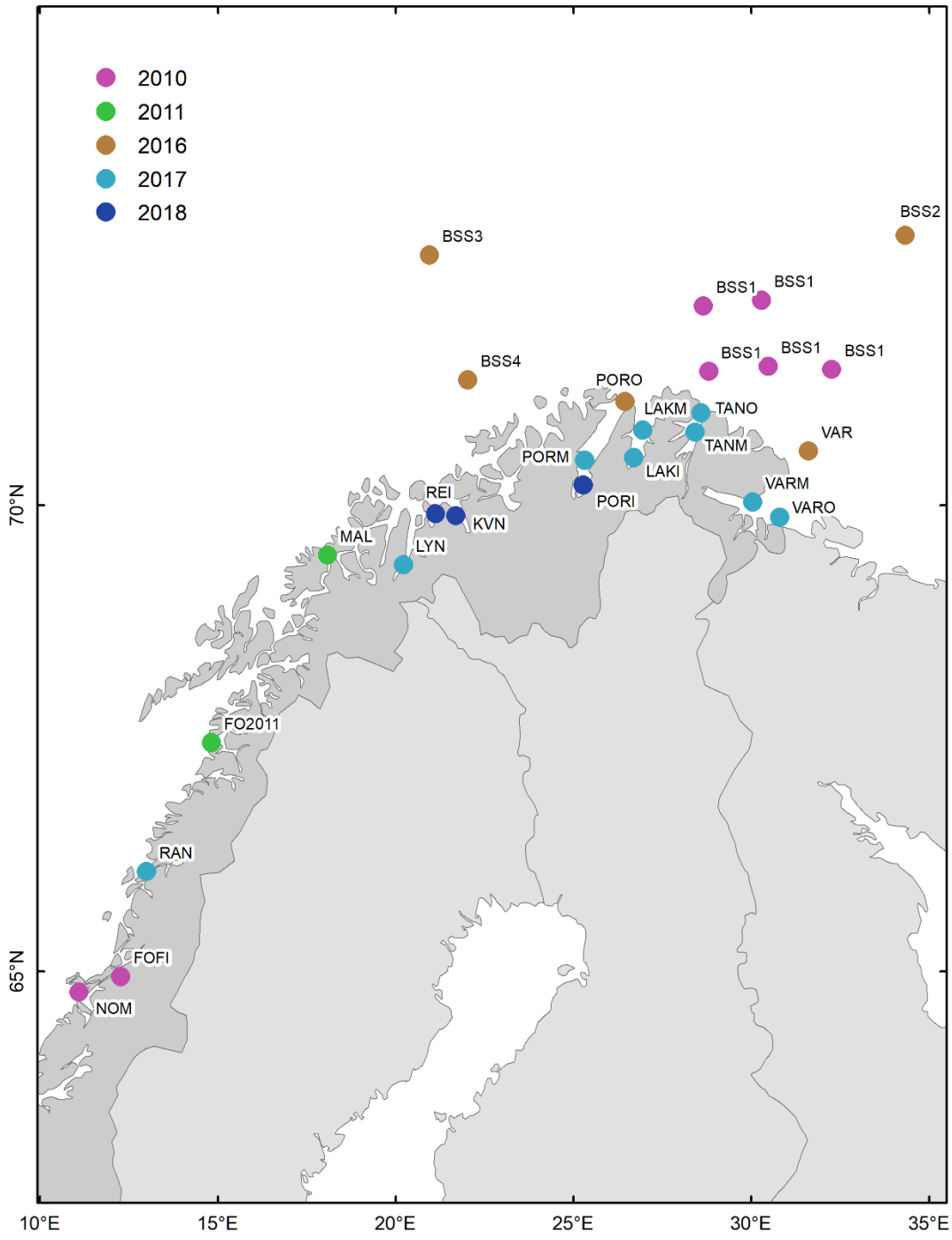

Figure 2. Map showing the study and sampling area of Pandalus borealis. Sampling localities given in abbreviated names; the abbreviations are found in Table 1. Colors indicate the sampling year. The sample from Vancouver Island is not shown in this map.

2.3 Microsatellite analysis

Microsatellite markers (loci), also known as short tandem repeats (STRs), are polymorphic DNA loci consisting of a repeated nucleotide sequence. Each repeat unit can be 2 to 7 nucleotides in length, and alleles differ by the number of repeats (Life Technologies, 2014). In a typical microsatellite analysis, loci are amplified by polymerase chain reaction (PCR) using fluorescently labeled forward primers and unlabeled reverse primers. The PCR amplicons are separated by size using electrophoresis (Life Technologies, 2014). The shrimp microsatellites were first developed by Pereyra et al. (2012), and the twelve microsatellites selected for this present study was first analyzed by Jorde et al. (2015) and Knutsen et al. (2015).

Microsatellites are powerful loci for population genetic analysis because the number of alleles at a microsatellite is often very large (20 or more), they have a high mutation rate, are very abundant in most genomes (Griffiths et al., 2015). For this study, molecular genetic analyses (10 microsatellite DNA loci) were done based on a selection of samples from the Norwegian coast. The genetic data were analysed in standard population genetic software for detecting possible population structure. DNA from some stations were already extracted (FOFI, MAL and VANC) and some were analysed and genotyped in advance (BSS1, FO2011 and NOM). Some of the samples from the Barents Sea (BSS1) and Tviberg (NOM) (Table 1) has also been analysed by the same set of microsatellites in Jorde et al. (2015).

2.4 DNA isolation

For the DNA isolation an Omega E-Z 96 Tissue DNA Kit (Omega Bio-Tek Inc.) was used. The procedure for the DNA isolation was done according to the Omega E-Z 96 Tissue DNA manual (Appendix I). The lab work for each isolation was conducted over two days, with tissue sampling and lysis the first day, and fixation of the eluted DNA the next day. For each isolation there was produced two 96 plates with DNA. Two negative controls (distilled water, dH_2O) were randomly positioned for each plate to have a reference and control later in the process. This resulted in 94 samples per plate.

2.5 PCR and genetic analysis

The PCR was performed in 5 microliter (µl) reaction volume and organized in three different multiplexes using a total of 12 microsatellite loci developed by (Pereyra et al., 2012) as this is a follow-up study from Jorde et al. (2015), an identical approach were used in this study (for details see Appendix II). The separation of the alleles was done by electrophoresis using an ABI3500 Genetic Analyzer (Life Technologies).

2.6 GeneMapper and genotyping

GeneMapper software 6.0 (Thermo Fisher Scientific) was used for quality check and genotyping of the samples. Some of the samples used in this study were already analyzed and genotyped beforehand (Table 1) making it important to be consistent with the new samples. For the genotyping all samples for each locus were checked through in GeneMapper, correcting eventual errors. After genotyping, the dataset generated in GeneMapper was subsequently exported to Excel.

2.7 Statistical analysis

The dataset was organized in Excel and converted by the add-in GenAlEx 6.5 (Peakall and Smouse, 2006) to the different softwares used in the statistical analysis. Departure from Hardy-Weinberg Equilibrium (HWE) was tested in each sample separately, locus by locus. This was performed in R (R core Team, 2012) using the Genepop 1.1.4 package (Rousset, 2008). Correction for multiple testing, false discovery rate (FDR), was done in an online tool (URL #4: FDR-tool). All corrections for multiple testing was performed according to the Benjamini-Hochberg procedure, with a q-value of 0.05 as a threshold for significance (Benjamini and Hochberg, 1995). In evolutionary terms, HWE says that for a population meeting certain conditions, the genotype frequencies of a genetic locus can be expressed in terms of the allele frequencies (Hao and Storey, 2019). Tests for HWE in practice usually involve verifying the Binomial distribution of the genotypes in terms of allele frequencies (Hao and Storey, 2019). Because HWE is expected to occur for most large, randomly mating populations, departures from HWE are often interpreted as genotype errors and are often removed from analyses (Schaid et al., 2006).

Observed and expected heterozygosity (H_o and H_e) within each sample and in each locus was calculated in Genepop. Weighted average F_{ST} values between all pairwise samples were calculated in ARLEQUIN 3.5 (Excoffier and Lischer, 2010), using 10.000 permutations, and corrected for multiple testing. The parameters F_{ST} and F_{IS} offer a convenient means of summarizing population structure (Weir and Cockerham, 1984). The parameter F is the inbreeding coefficient, and it gives the departure from the amount of homozygosis under random mating towards complete homozygosis (Wright, 1951). F_{ST} is the correlation of genes of different individuals in the same population, and F_{IS} the correlation of genes within individuals within population (Weir and Cockerham, 1984).

The software STRUCTURE 2.3.4 (Pritchard et al., 2000) analyses differences in the distribution of genetic variants among populations with a Bayesian interactive algorithm by placing samples into groups whose members share similar patterns of variation (Porras-Hurtado et al., 2013). STRUCTURE uses a systematic Bayesian clustering approach applying Markov Chain Monte Carlo (MCMC) estimation (Porras-Hurtado et al., 2013). The MCMC process begins by randomly assigning individuals to a pre-determined number of groups, then variant frequencies are estimated in each group and individuals re-assigned based on those frequency estimates (Porras-Hurtado et al., 2013).

Correlated allele frequency and admixture model with the locprior option in STRUCTURE was used to identify major clusters using the data for the dataset, performing six independent runs and five repetitions for each value of K (clusters) with a burn in period of 10.000 followed by 100.000 MCMC iterations. It was performed on all sampled stations, and on all the Norwegian coast samples alone. Delta K and the best K-value (Appendix figure I, Appendix figure II) for the dataset created in STRUCTURE was identified with the online web page: STRUCTURE HARVESTER (Dent A. and vonHoldt, 2012), using the Evanno method (Evanno et al., 2005). A Clumpp infile file with the appropriate K was downloaded from the web page. Clumpp v1.1.2 (Jakobsen and Rosenberg, 2007) was used to generate a permuted outfile. A STRUCTURE bar plot, based on the outfile created with Clumpp, was generated in R (Appendix figure III). Pie-charts on a map, based on the STRUCTURE bar plot, was constructed with the R package LEA v2.4.0 (Frichot and François, 2015) using the add.pie functions in the R package “mapplots”.

Discriminant Analysis of Principal Components (DAPC) is a multivariate method designed to identify and describe clusters of genetically related individuals (Jombart et al., 2010). The contributions of alleles to the structures identified by DAPC can allow for identifying regions of the genome driving genetic divergence among groups (Jombart et al., 2010). The R package Adegenet 2.1.1 (Jombart and Ahmed, 2011) was used to perform DAPC on the full dataset, and on all stations except from the VANC station.

3. Results

In total 12 microsatellites were analyzed for 1989 individuals. One of the locus, PbA108, was removed from the dataset after genotyping because of low quality (only amplified randomly). When comparing loci frequencies in the HWE test, the locus Pba104a, was significantly out of HWE after FDR corrections in 8 of 23 samples due to elevated levels of heterozygote deficit (data not shown) and removed from further analysis. For the remaining 10 loci 16 departures (of 230 tests) from HWE were found randomly distributed across samples/loci (Appendix table II). Observed and expected heterozygosity showed an overall small, but not significant heterozygote deficit in most of the locations investigated (Table 2). Heterozygote excess was found in the samples from BSS4 and PORI. Overall heterozygote deficit was also found for all loci except from, PbC105 and SD3-62 (Appendix table II).

The shrimp reference sample from VANC (Canada) was found to be highly significant different from all the other samples by pairwise F_{ST} (Table 3). Pairwise F_{ST} between samples showed significant difference also between the Barents Sea (BSS1, BSS2, BSS3 and BSS4) and every sample south and west of TANO, as well as the inner TANM sample and VARM, which also were significantly different from the Barents Sea (Table 3). KVN showed significant difference from all the Finnmark-fjords, and the general trend showed that also samples south of KVN differed significantly from the fjords in Finnmark, with only six pairwise F_{ST}-values that were not significant (Table 3). The inner located VARM and LAKI were the only Finnmark-fjords that showed no significance to a few of the fjords located between Troms and Trøndelag (p-values ranging between 0.051 and 0.2). Genetic differentiation within fjords was only found in Varanger (VARO - VARM; F_{ST}=0.003, p=0.025).

Table 2. Mean total observed heterozygosity (H_o), expected heterozygosity (H_e), and $F_{is,}$-values for all loci per station. A positive Fis-value indicates heterozygote deficit, a negative F_{is}-value indicates heterozygote excess.

Station	H_o	H_e	F_{is}
BSS1	0.746	0.761	0.020
BSS2	0.726	0.753	0.036
BSS3	0.746	0.753	0.009
BSS4	0.775	0.768	-0.010
VARO	0.736	0.757	0.028
VARM	0.721	0.742	0.028
VAR	0.740	0.758	0.023
TANO	0.717	0.750	0.044
TANM	0.734	0.747	0.018
LAKM	0.710	0.731	0.029
LAKI	0.733	0.742	0.013
PORO	0.719	0.732	0.018
PORM	0.731	0.744	0.017
PORI	0.744	0.743	-0.001
KVN	0.680	0.702	0.032
REI	0.726	0.729	0.005
LYN	0.692	0.709	0.025
MAL	0.699	0.716	0.024
FO2011	0.634	0.705	0.101
RAN	0.692	0.722	0.040
FOFI	0.699	0.713	0.019
NOM	0.668	0.732	0.088
VANC	0.765	0.812	0.058

Table 3. Pairwise genetic distances, F_{ST}-table, with p-values on top and F_{ST}-values underneath. Significant values are given in bold text. P-values are FDR (False Discovery Rate) corrected. Color coded in accordance with the STRUCTURE plot (Figure 2).

	BSS1	BSS2	BSS3	BSS4	VARO	VARM	VAR	TANO	TANM	LAKM	LAKI	PORO	PORM	PORI	KVN	REI	LYN	MAL	FO2011	RAN	FOFI	NOM	VANC
BSS1		0.179	0.064	0.072	0.121	**<0.001**	0.086	0.161	**0.015**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
BSS2	0.001		0.705	0.170	0.179	**<0.001**	0.161	0.406	**0.003**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
BSS3	0.003	-0.001		0.304	**0.025**	**<0.001**	0.120	0.170	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
BSS4	0.003	0.002	0.001		0.135	**<0.001**	0.304	0.453	**0.010**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
VARO	0.002	0.001	0.004	0.003		**0.024**	0.357	0.517	0.775	**<0.001**	**0.008**	**0.030**	0.111	0.137	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
VARM	0.011	0.010	0.015	0.010	0.003		**<0.001**	**0.006**	0.400	0.658	0.754	0.866	0.651	0.714	**0.018**	0.097	**0.007**	**0.021**	**0.034**	0.105	**0.008**	**0.004**	**<0.001**
VAR	0.002	0.001	0.002	0.001	0.001	0.007		0.567	**0.027**	**<0.001**	**<0.001**	**<0.001**	**0.001**	**0.002**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
TANO	0.002	0.001	0.002	0.000	0.000	0.004	0.000		0.237	**<0.001**	**<0.001**	**0.004**	**0.001**	**0.011**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
TANM	0.003	0.005	0.009	0.005	-0.001	0.000	0.003	0.001		**0.007**	0.176	0.434	0.348	0.796	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
LAKM	0.013	0.017	0.020	0.016	0.007	0.000	0.011	0.007	0.004		0.315	0.199	**0.010**	**0.046**	**0.043**	**0.021**	**0.008**	**0.006**	**0.017**	**0.027**	**0.023**	**0.018**	**<0.001**
LAKI	0.010	0.012	0.015	0.011	0.004	-0.001	0.007	0.007	0.001	0.001		0.520	0.472	0.686	**0.002**	0.124	**0.003**	**0.007**	**0.004**	0.051	0.065	0.208	**<0.001**
PORO	0.008	0.010	0.015	0.010	0.003	-0.001	0.008	0.004	0.000	0.001	0.000		0.318	0.481	**0.003**	**0.011**	**<0.001**	**<0.001**	**<0.001**	**0.013**	**0.002**	**0.05**	**<0.001**
PORM	0.009	0.008	0.013	0.009	0.002	0.000	0.005	0.006	0.001	0.004	0.000	0.001		0.341	**<0.001**	**0.002**	**0.001**	**<0.001**	**<0.001**	**0.002**	**<0.001**	**<0.001**	**<0.001**
PORI	0.006	0.006	0.011	0.009	0.001	-0.001	0.004	0.003	-0.001	0.002	-0.001	0.000	0.001		**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.002**	**<0.001**
KVN	0.024	0.029	0.035	0.028	0.015	0.003	0.022	0.017	0.009	0.003	0.005	0.004	0.007	0.007		0.652	0.810	0.642	0.843	0.359	0.756	0.171	**<0.001**
REI	0.022	0.025	0.029	0.022	0.013	0.002	0.018	0.015	0.007	0.003	0.002	0.003	0.005	0.005	0.000		0.523	0.622	0.566	0.243	0.768	0.317	**<0.001**
LYN	0.027	0.030	0.036	0.028	0.016	0.004	0.023	0.019	0.010	0.004	0.004	0.006	0.006	0.008	-0.001	0.000		0.349	0.400	0.774	0.555	0.065	**<0.001**
MAL	0.024	0.028	0.035	0.027	0.015	0.003	0.022	0.017	0.010	0.004	0.004	0.005	0.007	0.006	0.000	0.000	0.001		0.520	0.063	0.520	0.191	**<0.001**
FO2011	0.026	0.030	0.038	0.030	0.016	0.003	0.023	0.021	0.011	0.004	0.004	0.006	0.008	0.007	-0.001	0.000	0.001	0.000		0.097	0.358	**0.017**	**<0.001**
RAN	0.021	0.024	0.030	0.022	0.012	0.002	0.019	0.015	0.006	0.003	0.002	0.003	0.005	0.006	0.001	0.001	-0.001	0.002	0.002		0.297	0.454	**<0.001**
FOFI	0.025	0.030	0.034	0.028	0.016	0.004	0.023	0.019	0.010	0.003	0.002	0.005	0.006	0.007	-0.001	-0.001	0.000	0.000	0.001	0.001		0.157	**<0.001**
NOM	0.016	0.022	0.027	0.023	0.010	0.004	0.016	0.014	0.006	0.004	0.001	0.003	0.006	0.004	0.002	0.001	0.002	0.001	0.004	0.000	0.002		**<0.001**
VANC	0.054	0.055	0.059	0.054	0.044	0.041	0.049	0.052	0.045	0.047	0.042	0.048	0.044	0.045	0.057	0.046	0.052	0.052	0.054	0.048	0.051	0.050	

The STRUCTURE bar plot (Figure 3) showed the same trend as given by the F_{ST} – test and divided the samples into three main clusters (Appendix figure I). STRUCTURE plot showing only the coastal stations (Figure 4) was divided into two clusters (Appendix figure II). The VANC sample (yellow) and the Barents Sea samples (orange) were completely different from the other samples. The Barents Sea samples showed a homogenous trend, where the orange color indicated the samples as almost completely belonging to one group. The third cluster (in blue) encompassed the fjord samples from Kvænangen (KVN) and southwards, while the Finnmarks fjords showed a more mixed pattern. A close resemblance was found between VAR and the Barents Sea. Similarly, the outer VARO and TANO showed high proportions of shrimp that belonged to the orange group. The remaining Finnmark fjords showed a tendency of being more mixed. When only including the coastal and fjord areas in the analysis (Figure 4) the same trend appeared as for all the sampled locations. However, the outer VARO, VAR and TANO stations showed almost equal proportions of belonging to the orange and blue group. The remaining Finnmark fjords are resembling the blue group more than the orange. The STRUCTURE plot (Figure 3) is visualized in pie-charts on a map (Figure 5) and shows the geographic distribution of the sampling locations and clusters. The VANC sample is not shown in this map but would appear almost completely yellow as in the STRUCTURE plot (Figure 3).

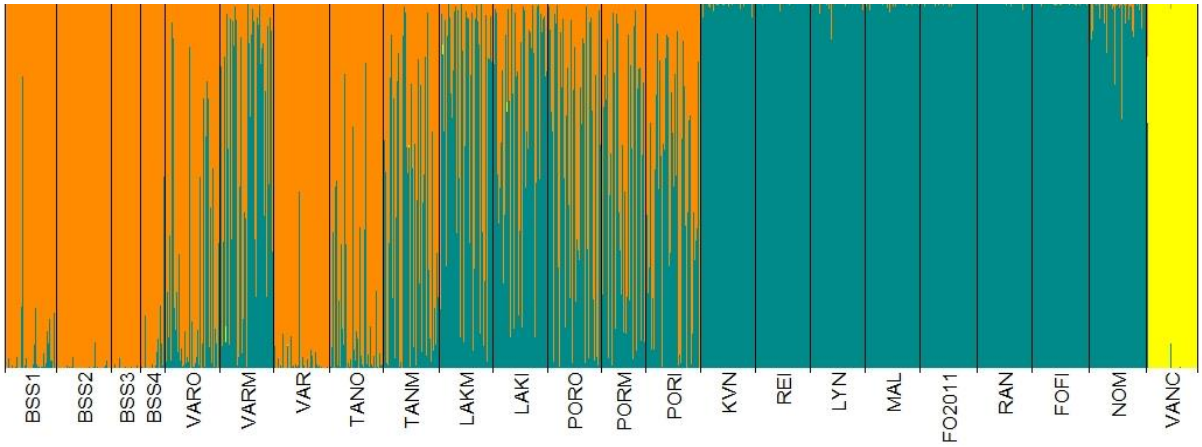

Figure 3. STRUCTURE plot for all sampled stations, visualizing estimated probability of individual shrimp (Pandalus borealis) assigned to the different groups. Samples are distributed into three clusters/groups. Each vertical colored line (orange, blue, yellow or mixed) shows an individual.

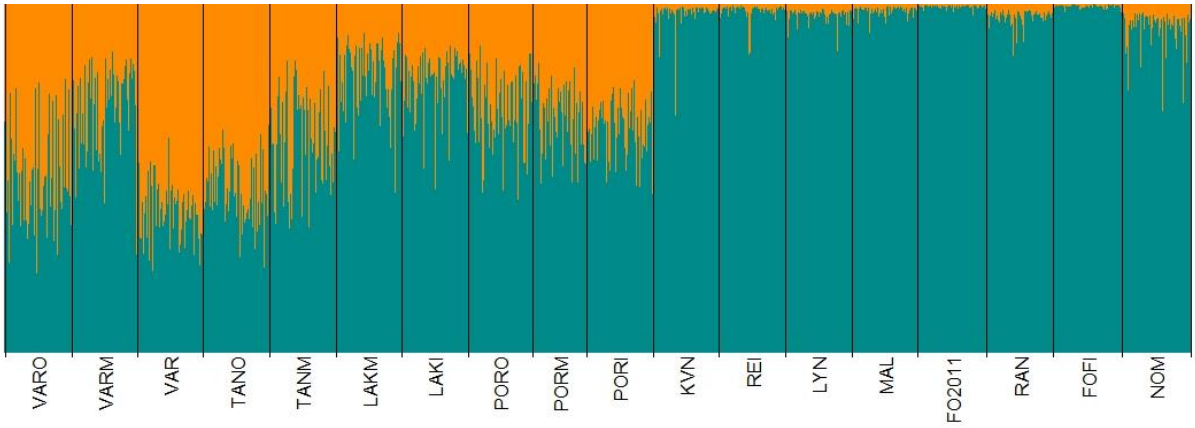

Figure 4. STRUCTURE plot includes only the shrimp that was sampled from the fjord- and coastal areas. It shows a visualization of estimated probability of individual shrimp (Pandalus borealis) to different groups. Samples are distributed into two clusters/groups. Each vertical colored line (orange, blue or mixed) shows an individual.

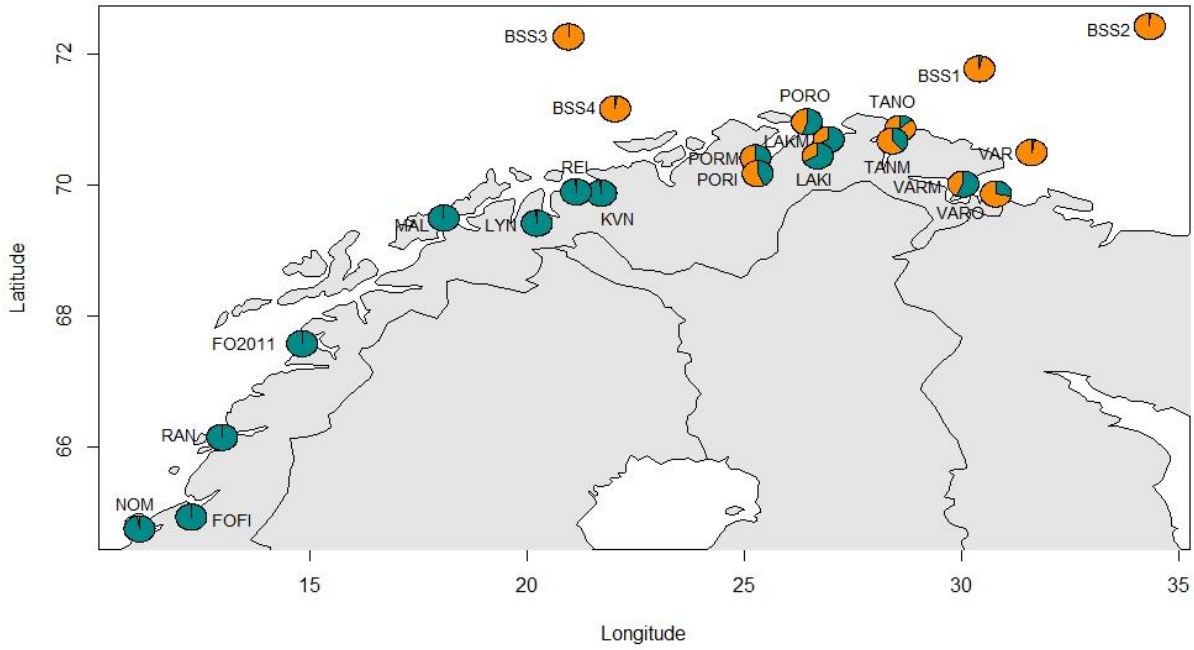

Figure 5. STRUCTURE plot visualized in pie charts on a map on the approximate geographic location to each station. Only the sampled locations in Norway are displayed in the map.

A similar trend as observed in the earlier plots can be seen in the DAPC plots (Figure 6). Vancouver is quite different from the rest of the samples (Figure 6A) with almost no overlaps. There are, however, overlaps between the rest of the samples. The Barents Sea samples and the samples from Troms to Trøndelag differ from each other, with the Finnmark fjords intermixing between (Figure 6B). The coastal samples isolated (Appendix figure IV) are even more segregated with the outer eastern Finnmark samples (VARO, VAR and TANO) showing a divergence from the Troms-Trøndelag samples.

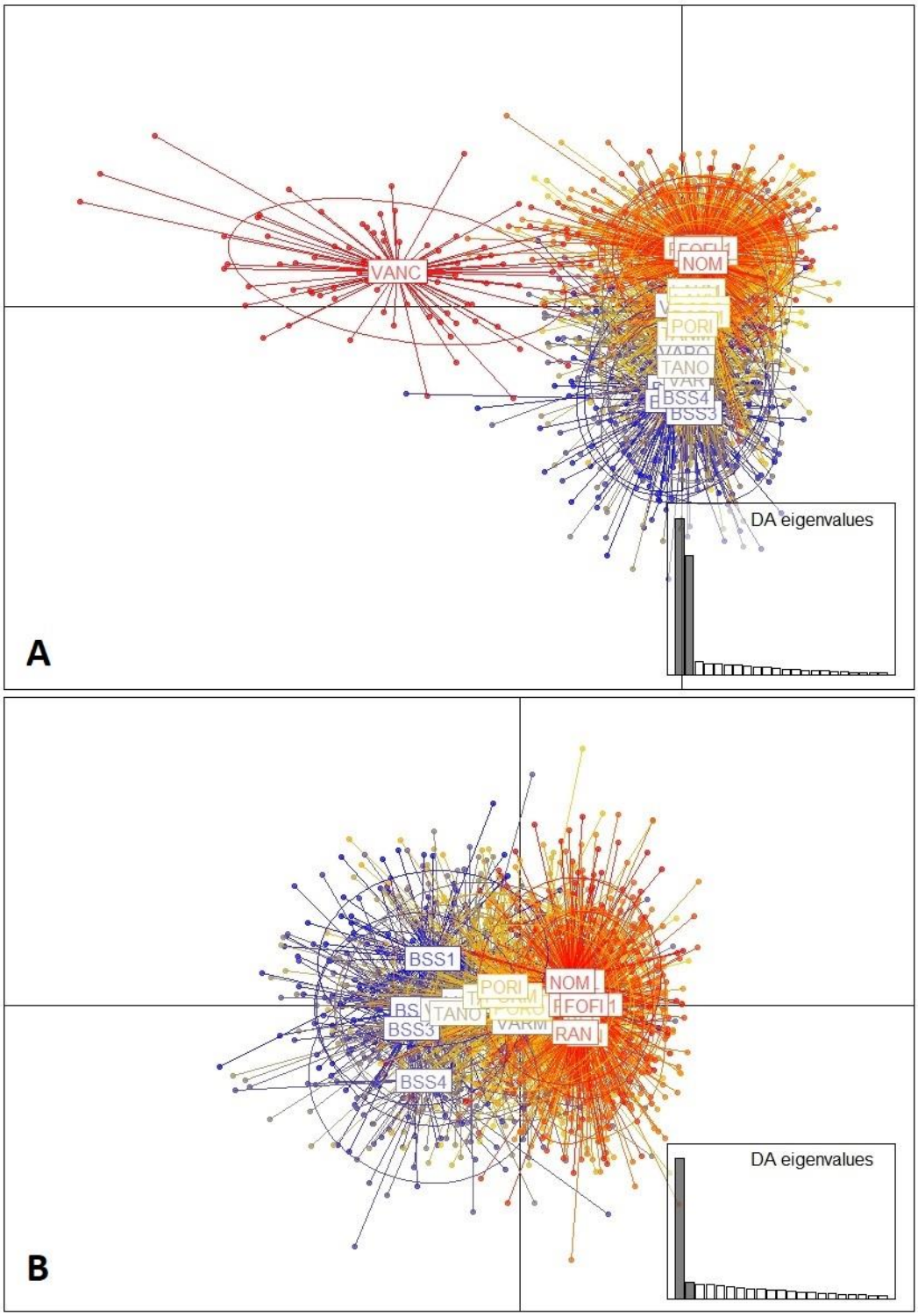

Figure 6. A) Discriminant analysis of principal components (DAPC) plot with all sampled station. B) DACP plot without the Vancouver station. Discriminant Analysis (DA) eigenvalues display the number of discriminant functions retained.

4. Discussion

The main findings in this thesis was the division of shrimp into three groups, the Barents Sea group, the Troms-Trøndelag group, and the Vancouver Island group (Figure 3, Figure 5, Figure 6). The Finnmark fjords showed a mixture of both belonging to the Troms-Trøndelag and the Barents Sea group. All the inner fjord samples from eastern Finnmark differed significantly from the Barents Sea. In addition, all fjords from Laksefjorden, and southwards, differed from the Barents Sea. The Vancouver Island sample was significantly different from all the other samples and was placed in a group/cluster of its own.

4.1 Border between the coastal and the Barents Sea shrimp

The results indicate that the border between the Barents Sea and the coastal shrimp lays somewhere between Kvænangen (KVN) and Porsangerfjorden (PORI, PORM, PORO). The Finnmark fjords were more influenced by the Barents Sea shrimp compared with the fjords further south, as shown by the STRUCTURE plots (Figure 3, Figure 4). However, comparisons of pairwise F_{ST}-values showed that the Varangerfjorden middle (VARM), Tanafjorden middle (TANM) and every station west and south of Tanafjorden differed significantly from the Barents Sea samples (Table 3). The fact that the outer fjord samples in eastern Finnmark (VARO and TANO) in addition to the sample laying close to Vardø (VAR) showed no significance (Table 3) with the Barents Sea, suggests that border is close to the coast in this area. However, in Drengstig et al. (2000) allozyme study they found that shrimps from fjords in eastern Finnmark were genetically indistinguishable from the Barents Sea shrimp. Conflicting with the finding in this study, they found that the Tanafjord sample, that was sampled in the inner part of the fjord, grouped together with samples taken in the middle of the Barents Sea.

The study by Drengstig et al. (2000) had overlaps with the study area in this present microsatellite study. Their samples also included the Barents Sea, Varangerfjord, outside Vardø, Tanafjord, Porsangerfjord, Lyngen (Ullsfjord), and Malangen. In addition, they also sampled fjords that were close to the fjords sampled in this study. Similar to the finding in this study they found a genetic subdivision into three groups, 1) the Troms-Nordland region plus

inshore Iceland, 2) western Norway (Værøya/Trondheim and Romsdalsfjord), and 3) the Barents Sea, Svalbard, Jan Mayen, offshore Iceland and Finnmark. They also found inshore outshore differences in Iceland, suggesting that there are coastal separate genetic units in Iceland, as found between coastal and fjord areas and the open sea in this study.

Norwegian fjord populations in Troms (Malangen and Balsfjord) also differed significantly from the Barents Sea and Svalbard in Martinez et al. (2006), supporting the findings in this study. Furthermore, genetic structuring at fjord level were also found further south in Skagerrak fjords, using nine of the same microsatellite loci as used in this thesis (Knutsen et al., 2015). Where they found genetic differences between the fjords and the open Skagerrak sea.

Populations in coastal areas that are genetically distinct from populations in the open ocean/sea have also been found for fish species like Norwegian coastal cod (Knutsen et al., 2003; Skarstein et al., 2007; Westgaard and Fevolden, 2007), and Norwegian fjord populations of European sprat (Quintela et al., 2020).

The Barents Sea locations that were sampled in in this thesis belonged to the southern part of the Barents Sea. However, in Jorde et al. (2015) the southern area of the Barents Sea clustered together with the northern part and Svalbard. Genetical homogeneity in the Barents Sea population has also been confirmed in earlier allozymic and RAPD studies (Drengstig et al., 2000; Martinez et al., 2006). Therefore, samples taken in the northern part of the Barents Sea or close to Svalbard would most likely not lead to a different observed genetic pattern. In other words, the genetic difference between shrimp's further north in the Barents Sea and between the fjords would not lead to other genetic patterns.

4.2 Genetic population structure between fjords

Genetic structure between fjords was found between the Finnmark fjords and the fjords south of Porsangerfjorden (Table 3, Figure 3, Figure 4). The results do not reveal any genetic population structure between neighboring fjords. However, all the inner sampled localities in the Finnmark fjords differed significantly from the Barents Sea, but the outer samples did not. Laksefjorden differed significantly from the outer eastern Finnmark fjord samples (VARO, VAR, TANO) (Table 3). On the other hand, shrimp in Laksefjorden had similar proportions of

belonging to the Barents Sea group as shrimp in the middle of Varengerfjorden (VARM), despite of having no sill or being enclosed. Laksefjorden and Porsangerfjorden are located right next to each other, however, Laksefjorden generally displayed lower proportions of Barents Sea shrimp.

All the fjords from Troms to Trøndelag showed a homogenous trend, and none of these showed any significant divergence from each other. Similar to the findings in this thesis, Troms and Nordland grouped together in the study by Drengstig et al. (2000). Furthermore, evidence of genetic divergence on a smaller geographical scale in the Troms/Nordland region was also found in their study. However, genetic divergence on a smaller geographical scale in Troms/Nordland was not found in this study. In addition, their western Norway sample grouped alone. However, the Tviberg (NOM) sample grouped together with Skagerrak and Norskerenna in Jorde et al. (2015), and together with Troms-Trøndelag in this study. Therefore, intermediate discrete genetic units in western Norway should be investigated more accurately with DNA-based methods.

By using microsatellites, the investigated Skagerrak fjords, Grønnsfjord, Topdalsfjord, Oslofjord, Kosterfjord, and Gullmarsfjord, showed a pattern of being generally divergent from each other (Knutsen et al., 2015). Only two of the fjords (Håøya and Stolsfjord) deviated from this pattern. The finding of genetic differences between fjords are quite different from what can be observed in the study area of this current study.

4.3 Genetic structure within fjords

Varangerfjorden was the only fjord in this study that displayed genetic variation within the fjord. None of the other fjords displayed any significant internal genetic structure. However, despite of no significant difference between TANM and TANO, and similar to what can be observed in the Varangerfjord, the outer sample (TANO) showed higher proportions of similarity to the Barents Sea group (Figure 3, Figure 4, Figure 5). Shrimps sampled in the inner part of Follafjord (FOFI) were not significantly different from shrimp sampled at the outer laying Tviberg locality (NOM), this is a bit surprising, considering the narrow nature of the fjord (see chapter 2.1).

The inner part of Porsangerfjorden has low connectivity to the coastal waters and is subjected to strong cooling during a large part of the year (Myksvoll et al., 2012). The environment in the inner part of Porsangerfjorden is very different from the rest of the fjord and holds a unique arctic ecosystem (Myksvoll et al., 2012, and references therein). Despite of this, results could not identify any significant genetic difference between the inner and outer parts of Porsangerfjorden, however, higher probability of belonging to the Barents Sea group (orange) is found in the inner part (Figure 4, Figure 5).

4.4 Oceanographic features/physical properties and larval drift

Pelagic larvae, juveniles and adults are likely to respond differently to various isolation factors. Life-stages with different mobility could experience different breakdowns in gene flow and in their potential for evolutionary diversification (Jorde et al., 2015). Dispersal and gene flow are higher in species with planktonic larvae than in species with non-planktonic larvae (Hedgecock, 1986), the long planktonic larval stages (up to 4 months) of shrimp could counteract the process of structuring into seperate genetic units. However, pelagic larvae may face unknown barriers to dispersal and even when they do reach distant populations their contributions to those populations may be minimized by reduced viability or fecundity (Hedgecock, 1986). Although factors such as temperature, salinity, current speed, and food probably influence the development of body form in marine crustaceans, the operation of natural selection and the stochastic effect of random genetic drift cause genetic differentiation between populations where gene flow, mediated by larval dispersal or adult migration, is restricted (Beaumont and Croucher, 2006).

It was not possible to obtain long term environmental data locally in the investigated fjords, and therefore the effect of environmental variables for the detected population structure could not be analyzed statistically. However, Jorde et al. (2015) found a positive relationship between temperature and population structure, and to a lesser extent larval drift on a large-scale genetic differentiation pattern.

The Norwegian coast is experiencing different retention regimes, there is often large retention in the fjords, medium at the coast and no retention off shore (Myksvoll et al., 2014). This implicates that particles and planktonic organisms are more often advected northwards

with NCC and NWAC when advected from coastal and offshore areas. Larval drift by the strong NCC promote genetic homogeneity, but appears ineffective across large temperature gradients (Jorde et al., 2015). From a genetic perspective, there are indications of transportation of shrimp from the coast from Trøndelag to Troms area into the Barents Sea, shown by some proportions of coastal shrimp in the Barents Sea samples (Figure 3, Figure 4, Figure 5) but not at quantities that affect the genetic structure found in the Barents Sea. Hence, both the food availability during the transport and the environment that larvae drift to can be unfavorable (Palumbi, 1994).

Pedersen et al. (2003) found that temporal and spatial variations in the hydrodynamics of the Barents Sea seem to govern the pattern of larval settlement of *P. borealis.* In all their conducted study years (1996-1998) they found that the main area of settlement was in the northern Barents Sea in the area of the Polar Front. From this, it seems that larvae that hatch in the open Barents Sea generally are transported northwards, and to a lesser extent into fjords and coastal areas. However, a study on drift patterns of capelin larvae showed that larvae that hatched further west (in Troms and Finnmark) was more rapidly transported offshore compared to an eastward situation where larvae were transported downstream along the shelf and brought into the Varangerfjord (Pedersen et al., 2009). This scenario could be likely for shrimp larvae too, as they are carried by the same currents. In addition, eddies in the Varangerfjord area act as retention fields and may retain some of the larvae (Pedersen et al., 2009). The process of retention may be one of the explanatory factors for the discovered difference between outer and inner parts of Varangerfjorden.

The sampled locations in this study vary greatly in regards to bathymetry and surrounding topography (see chapter 2.1). Variability of the studied fjords range from semi-enclosed fjords with sills to open fjords that appear more bay-like. In fact, there is a rich and wide variety of fluid dynamic processes that occur in fjords, the interplay between geomorphology and environmental forcing defines the relative importance of differing physical fluid processes within a given fjord (Inall and Gillibrand, 2010). Topographic barriers include a relatively shallow sill and a relative narrowing of the coastline at the entrance area, both affect the flow in different and various ways (Inall and Gillibrand, 2010). The coastline of Troms and Nordland consists of a high number of islands and fjords, which influence and complicate the current patterns in this area. It is plausible that local oceanographic features may constrict gene flow,

and thus allow for genetic differences between shrimps in fjords and those of the coast (Drengstig et al., 2000). The difference observed between Finnmark and Troms-Trøndelag could be explained by the more open nature of Finnmark fjords, as discussed in Drengstig et al. (2000), compared to the generally more narrow sill-fjords in Troms-Trøndelag.

In general there is great variation between fjords with regards to topography, climatology and hydrodynamics (Svendsen, 1995). The climatic conditions vary greatly from year to year, especially in fjords. Fjords north of Tromsø are found to correlate well regarding variations in air and sea temperatures, if there is a cold year there is cold everywhere, and if there is a warm year this is true for the entire area from Tromsø and northwards (Eilertsen and Skarðhamar, 2006). This variability in sea temperature could lead to very different experienced climatic conditions from year to year, and possibly different dispersal pattern.

4.5 Outgroup sample

The Vancouver sample differed significantly from all the other samples from the Barents Sea and, Norwegian fjords and coast. Earlier allozyme studies has shown that local populations of *P. borealis* in the Barents Sea, Bering Sea and Sea of Japan are self-reproducing and appear to be isolated panmictic units (Kartavtsev et al., 1991). Similar F_{ST} values (approx. 0.05) as seen between VANC and all the other stations in this current study (Table 3) can be seen between the Barents Sea and the Sea of Japan (in the Pacific) in Kartavtsev et al. (1991). The Bering Sea and Barents Sea displayed a smaller pairwise F_{ST} value (<0.01). Following studies also revealed that the neighboring Bering Sea, Sea of Okhotsk, and Sea of Japan genetically differed from each other (Kartavtsev et al., 1993). The difference between the Barents Sea and the Sea of Japan supports the finding of the genetic divergence from VANC and all the other samples in this study.

The microsatellites used in this study was tested on other pandalid shrimp species, but did not perform well on them (Pereyra et al., 2012), the fact that the microsatellites worked well on the pacific sample, the presumed subspecies *Pandalus borealis eous*, imply that shrimps in the Pacific and in the Atlantic are closely related.

Findings of genetic population structure has also been discovered in other shrimp species. A study on another shrimp species, the Brown shrimp (*Crangon crangon),* showed morphologic differentiation that likely could be the cause of random genetic drift and selection in populations with restricted gene flow (Beaumont and Croucher, 2006). In the area were the shrimp species showed morphologic differentiation they also differed genetically. Likewise, morphological differences has been documented between larval stages of the two subspecies *P. borealis eous* and *P. borealis borealis* (Rasmussen and Aschan, 2011).

4.6 Current and potential future management

P. borealis north of 62°N is presently considered as one management unit. The result in this thesis show, however, that the population structure in fjords and coastal areas is different from the population structure in the Barents Sea. The eastern Finnmark fjords resemble the Barents Sea the most, but there is still not fully homogeneity between them. As mentioned, all coastal samples west of Tana middle (TANM), except of Tana outer (TANO), showed a significant divergence from the Barents Sea (Table 3). This indicates that shrimp in this area constitute a distinct genetic population and that they should be considered as a separate management unit of their own. Likewise, Drengstig et al. (2000) concluded that from a management point of view their results suggested that shrimp from coastal areas, where appropriate, should conservatively be treated as separate harvest units. The finding of local Skagerrak fjord populations in Knutsen et al. (2015) study also contradict the current management regulations, where shrimp in the entire study area is treated as a single stock.

Catches from the coastal areas are small compared to the catches in the Barents Sea. Total landings along the whole Norwegian coast in 2019 was 4 014 tonnes, and from 62°N to Varanger 1 134 tonnes (Data from the Norwegian Fisheries Directorate), while catches in the recent 10-year period in the Barents Sea and Svalbard zone alone have varied between 20 000 and 45 000 tonnes/year by Norwegian vessels alone (NAFO and ICES, 2018). Fjord populations are of less economic value than the Norwegian Deep and Skagerrak stocks, but are important for the coastal fishery and not least for maintaining genetic variability and biocomplexity of the species in Norwegian waters (Knutsen et al., 2015), it would therefore be interesting to manage and consider the fjord populations as separate stocks.

4.7 Evaluation of methods and data

Genetic markers have the advantage of being intimately linked to reproduction, allowing for detection of dispersal and intermixing at all life stages, including eggs and larvae, which typically are inaccessible to commonly applied ecological methods (Knutsen et al., 2015). Earlier genetic studies on shrimp in the same area used allozyme and RAPD analysis (Drengstig et al., 2000; Martinez et al., 2006). Nevertheless, large differences at particular loci may question allozyme studies neutrality for population genetic studies. Furthermore, RAPD markers dominance and both their reproducibility and homology issues also raise concerns about their suitability for population genetics studies (Pereyra et al., 2012). Microsatellites were used to identify population structure in this study. However, there is an increasing effort to adopt single-nucleotide polymorphism (SNP) markers as a marker of choice because of the numerous advantages that SNPs hold over microsatellites, including processing efficiency, ease in both scoring and standardizing genotypes among laboratories, and the high density in which they are observed across most genomes (Hess et al., 2011). Yet, microsatellites has higher mutation rates than SNPs, 10^{-3}- 10^{-4} as compared to 10^{-8}-10^{-9} mutations per locus (Griffiths et al., 2015). The ratio of SNP to microsatellites that is required to achieve equivalent power varies across applications, the average number of random SNP markers required to equal information content of random microsatellites have been estimated in some terrestrial animal species to vary from >2.5 times more (Hess et al., 2011, and references therein).

Different analytic techniques used on the same data set may lead to different conclusions about the existence and strength of genetic structure, reliable interpretation of the results from different methods depends on the efficiency and reliability of different statistical methods (Blair et al., 2012). In Blair et al. (2012) study, the Bayesian clustering method perform best overall, both in terms of highest success rates and the lowest time to barrier detection. Unfortunately, the reliance of Bayesian clustering methods on explicit models also comes at a cost. Model based approaches rely on assumptions such as the type of population subdivision, which are often difficult to verify and can restrict their applicability (Jombart et al., 2010). Both a Bayesian clustering method (STRUCTURE) and a multivariate method (DAPC) were conducted in this thesis. All the statistical analysis conducted showed the same trend, strengthening the conclusions of the detected genetic population structure.

All the locations in this study were sampled once and in different years. Ideally, temporal sampling in all or some of the investigated fjords could have improved the strength of the observed genetic structure, and would have prevented having only a snapshot of the population structure at one specific time and place. However, temporal replicates from other parts of the species distributional range showed little or no genetic differentiation over time (Jorde et al., 2015). The environmental variability is greater in fjords compared to the open ocean (see chapter 4.4) and could affect the population dynamic in fjords. Nevertheless, regardless of different sampling years for the different localities, the fjords displayed the same tendency across years (Figure 5). In addition, the BSS1 sample was sampled in 2010, and the rest of the Barents Sea samples were sampled in 2016 (Figure 1). Despite of the six year difference in sampling years the genetic structure appeared to be the same (Figure 3, Figure 5).

Almost all the transects inside the fjords were conducted in the Finnmark region (except from NOM and FOFI), and none of the fjord transects were done in Troms and Nordland fjords. In addition, ten samples in total came from Finnmark, compared to eight in Troms, Nordland and Trøndelag combined (Figure 2 and Table 1). One of the objectives in this thesis was: (iii) is there genetic population structure between the inner and the outer parts of the fjords? A better conclusion could have been made if there were more stations to compare, and if these stations were spread evenly in the study area, especially after discovering the border between coastal and Barents Sea shrimp starting in Troms, Kvænangen. This gives a poor understanding of the structure of coastal and fjord shrimp inside the fjords in this area. In addition, since Altafjord is the only fjord in Finnmark that is narrow and have hindered contact with the outside water masses (see chapter 2.1), it would also be interesting to investigate this fjord, particularly because of the clear contrast/border between Finnmark and Troms (Figure 3, Figure 4, Figure 5).

5. Conclusion

The main findings included divisions of shrimp into three groups: the Barents Sea, Troms-Trøndelag and Vancouver. Finnmarks fjords showed to be influenced with both the Barents Sea group and the coastal Troms-Trøndelag group. A clear border, however, was found between the Barents Sea and Troms-Trøndelag, while the Finnmark fjords samples showed varying degrees of genetic divergence from the Barents Sea, some being significant, others not. The general trend showed that the inner eastern Finnmark locations and all fjords from Laksefjorden and south was significant different from the Barents Sea. Genetic population structure between fjords did not display any general trend, despite the difference between coastal populations in Finnmark and Troms-Trøndelag. The only fjords that displayed significant different genetic structure within was the Varangerfjord in eastern Finnmark.

In future studies it would be interesting to investigate whether environmental explaining variables could explain the genetic population structure observed. Further, would it be interesting to investigate and analyze SNP on shrimps. SNP investigations could perhaps reveal more profound results or effects on the genetic population structure. It would also make sense to have more transects within fjords between Troms and Trøndelag. Given the clear border that can be observed between Porsangerfjorden and Kvænangen, samples in the more enclosed Altfjorden would be interesting to investigate.

The finding of genetic different coastal shrimps in Troms-Trøndelag indicate that they should be considered as a stock of their own. The Finnmark fjords also differ in some degree from the Barents Sea, and there should therefore also be considered further if this population should be considered as a separate stock.

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URL #4: FDR-tool (retrieved 2/7/2020).
https://tools.carbocation.com/FDR

Appendix

Appendix I: DNA isolation

Day 1- Tissue samples was cut in 1-2 mm^2 pieces and placed into a Lysis plate, one sample per well. TL buffer (44 milliliter (ml)) and Proteinase K (5,5 ml) were mixed together in a 50 ml vortex tube. 225 ul of the solution was added to each well with the use of an 8-channel Pipette. The lysis plates were sealed with a silicon mat and vortexed briefly (up to 3000 rounds per minute (rpm)). The lysis plates with the samples were incubated at 60 (degrees Celsius) °C overnight for the samples to be completely lysed.

Day 2- The plates were shaken vigorously from side to side. Important to check if the lysate was completely lysed after the shaking. The plates were vortexed briefly. 450 µl BL buffer/ethanol mix was added to each well, a new silicon mat was placed over the plates. The plates were shaken vigorously from side to side in approximately one minute (important to mix the phases properly to ensure that the biding of DNA to the column) and were afterwards centrifuged briefly again.

An E-Z 96™ DNA plate were placed on top of a 96-well Square-well Plate. The lysate (600 ul from each well) was transferred to the E-Z 96™ DNA plate. The E-Z 96™ DNA plates was sealed with an AeraSeal film and afterwards centrifuged at 4000×g (G-force) in 10 minutes. Important to check if all the solution had passed through the filter. The AeraSeal film was removed, and 500 µl HBC buffer was added to each well, a new AeraSeal was placed over the plate. The plate was again centrifuged at 4000×g in 5 minutes. The AreaSeal was removed and 600 µl Wash Buffer was added to each well, before a new AeraSeal was placed on again. The plate was centrifuged at 4000×g in 5 minutes. The last process with the DNA Wash Buffer was repeated, and for each spin the waste collected in the 96-well Square-well Plate was removed. After the repeated DNA wash buffer step and the remove of waste, the plates were centrifuged in 15 minutes at 4000×g. The last centrifugation was important to dry the membrane in the E-Z 96™ DNA plate sufficiently.

The E-Z 96™ DNA plate were placed up on a "96-well Racked Microtube" plate. 200 µl Elution buffer (70°C) was added to each well and placed in room temperature before a new

centifugation (at 4000×g in 5 minutes). An aliquote of 50 µl eluted DNA was added from the "96-well Racked Microtube" plate to a VWR plate. The VWR plate was sealed with Microamp clear adhesive film and the "96-well Racked Microtube" plate was corked with "Caps for Racked Microtubes". Both plates with eluted DNA was stored at -20°C.

Appendix II: PCR and genetic analysis

Three types of multiplexes were used, all of them contained Qiagen Multiplex Mastermix (2x), 2 ul per sample, and distilled water. The multiplexes contained different primers and reaction volumes (Appendix table I). The reaction volumes were multiplied by 1.1 to correct for volumes lost when pipetting.

DNA and Multiplex were added to a MicroAmp Optical 96-Well Reaction Plate. For each well, 4 ul of Multiplex and 1 ul of DNA was added. The plate was sealed with MicroAmp clear adhesive film and centrifuged briefly (up to 3000rpm). The plate was then placed in the Thermal cycler, with a PCR profile of: 95°C in 15 minutes, then 25 cycles (28 cycles for Multiplex 3) of 95°C for 30 seconds, 56°C in 90 seconds, and 72°C in 1 minute. The thermal cycle was finished up by 60°C in 30 minutes, before it cooled down to 4°C (infinitely). The same process was repeated for all multiplexes.

After the PCR, the plate was centrifuged briefly. In a fume hood, 12 µl of a solution of Hi-DI formamide and GeneScan 500 LIZ Size Standard was added to each well, a 3500 Series Septa 96-well mat was placed over the plate (and then aluminum foil over for safe handling). The plate was centrifuged briefly, and then put into the thermal cycler for denaturation. The thermal profile for the denaturation was: 95°C in 5 minutes, 4°C for 7 minutes, and 4°C infinitely. The plate was then centrifuged briefly and put into the ABI 3500 Genetic Analyzer (Life Technologies) for separation of the alleles by electrophoresis.

Appendix III: Tables and figures

Appendix table I. Overview of the multiplexes used, content, and reaction volumes.

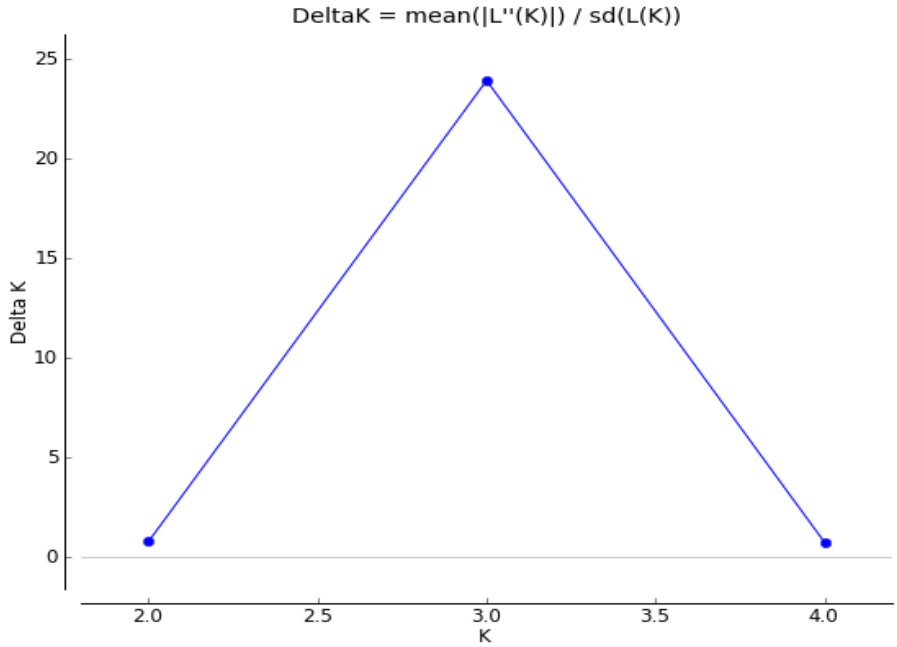
Multiplex		**Content**	**Reaction volume (µl) per sample**
1		Mastermix (2x)	2
		H_2O	0.55
	Primers	**PbC8 (10 µM)**	0.59
		PbA104a (10 µM)	0.4
		SD2-14 (10 µM)	0.06
		PbC105 (10 µM)	0.4
2		Mastermix (2x)	2
		H_2O	0.2
	Primers	**PbC109 (10 µM)**	0.8
		PbA110 (10 µM)	0.4
		PbA108 (10 µM)	0.4
		PbD9 (10 µM)	0.2
3		Mastermix (2x)	2
		H_2O	0.87
	Primers	**SD1-41 (10 µM)**	0.35
		SD3-62 (10 µM)	0.1
		PbA1 (10 µM)	0.1
		SD2-68 (10 µM)	0.58

*Appendix table II. Observed heterozygosity (H_o), expected heterozygosity (H_e), F_{is}-values, and Hardy-Weinberg test P-values (HW) per loci and station. P-values in the HW-test are FDR (False Discovery Rate) corrected. NS=non-significant, *=<0.05, **=<0.01, ***=<0.001. A positive Fis-value indicates heterozygote deficit, a negative Fis-value indicates heterozygote excess.*

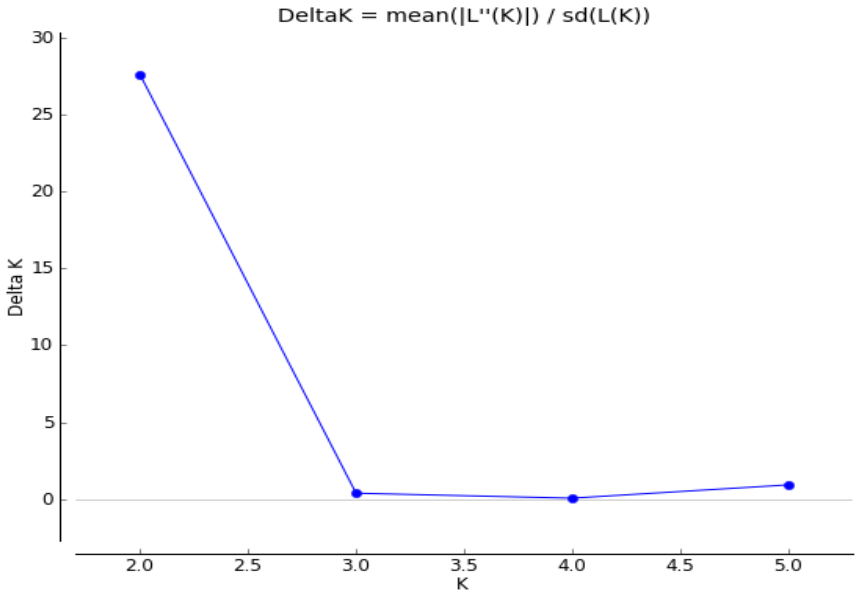
		PbC105	***PbC8***	***SD2-14***	***PbA110***	***PbC109***	***PbD9***	***PbA1***	***SD1-41***	***SD2-68***	***SD3-62***
BSS1	H_o	0.85	0.68	0.85	0.79	0.87	0.72	0.75	0.87	0.75	0.33
	H_e	0.85	0.74	0.90	0.73	0.92	0.78	0.75	0.84	0.74	0.36
	F_{is}	0.00	0.08	0.06	-0.07	0.06	0.09	0.00	-0.04	-0.02	0.07
	HW	NS	NS	NS	NS	***	NS	NS	NS	NS	NS
BSS2	H_o	0.82	0.73	0.82	0.69	0.96	0.77	0.64	0.84	0.74	0.26
	H_e	0.87	0.77	0.89	0.69	0.94	0.79	0.76	0.83	0.74	0.25
	F_{is}	0.06	0.06	0.07	0.00	-0.02	0.03	0.17	-0.01	0.01	-0.07
	HW	NS	NS	NS	NS	NS	NS	*	NS	NS	NS
BSS3	H_o	0.86	0.78	0.96	0.72	0.96	0.72	0.72	0.78	0.68	0.28
	H_e	0.85	0.76	0.92	0.76	0.94	0.82	0.77	0.76	0.72	0.25
	F_{is}	-0.01	-0.03	-0.04	0.05	-0.03	0.12	0.06	-0.02	0.05	-0.14
	HW	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
BSS4	H_o	0.90	0.73	0.98	0.75	0.95	0.85	0.78	0.78	0.70	0.35
	H_e	0.85	0.78	0.90	0.79	0.94	0.77	0.75	0.78	0.74	0.36
	F_{is}	-0.05	0.07	-0.08	0.05	-0.01	-0.10	-0.03	0.01	0.06	0.02
	HW	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
VARO	H_o	0.85	0.83	0.86	0.66	0.89	0.85	0.72	0.73	0.69	0.28
	H_e	0.84	0.80	0.89	0.67	0.93	0.80	0.78	0.86	0.73	0.26
	F_{is}	-0.01	-0.04	0.04	0.02	0.04	-0.06	0.09	0.16	0.05	-0.08
	HW	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
VARM	H_o	0.84	0.78	0.87	0.66	0.92	0.77	0.68	0.82	0.70	0.18
	H_e	0.85	0.79	0.86	0.67	0.94	0.80	0.78	0.87	0.70	0.16
	F_{is}	0.01	0.01	-0.01	0.02	0.01	0.04	0.13	0.06	0.01	-0.08
	HW	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
VAR	H_o	0.90	0.85	0.86	0.71	0.91	0.76	0.63	0.82	0.65	0.32
	H_e	0.84	0.81	0.89	0.72	0.94	0.80	0.72	0.83	0.73	0.29
	F_{is}	-0.07	-0.04	0.04	0.02	0.02	0.05	0.12	0.02	0.11	-0.09
	HW	NS	NS	NS	NS	NS	NS	**	NS	NS	NS
TANO	H_o	0.87	0.66	0.88	0.70	0.88	0.77	0.73	0.78	0.62	0.29
	H_e	0.85	0.74	0.89	0.72	0.94	0.80	0.75	0.85	0.71	0.25
	F_{is}	-0.02	0.11	0.01	0.02	0.06	0.03	0.03	0.08	0.14	-0.13
	HW	NS	*	NS	NS	***	NS	NS	NS	NS	NS
TANM	H_o	0.88	0.68	0.87	0.66	0.97	0.77	0.68	0.88	0.72	0.22
	H_e	0.84	0.77	0.89	0.67	0.93	0.80	0.77	0.85	0.73	0.22

		PbC105	PbC8	SD2-14	PbA110	PbC109	PbD9	PbA1	SD1-41	SD2-68	SD3-62
	F_{is}	-0.05	0.11	0.02	0.02	-0.05	0.03	0.11	-0.03	0.02	0.00
	HW	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
LAKM	H_o	0.77	0.81	0.81	0.66	0.91	0.76	0.74	0.85	0.62	0.17
	H_e	0.84	0.74	0.86	0.64	0.93	0.82	0.79	0.85	0.65	0.20
	F_{is}	0.09	-0.10	0.06	-0.02	0.02	0.07	0.05	0.00	0.04	0.15
	HW	NS	NS	*	NS	NS	NS	NS	NS	NS	NS
LAKI	H_o	0.90	0.82	0.82	0.75	0.91	0.85	0.65	0.88	0.63	0.12
	H_e	0.85	0.79	0.84	0.73	0.94	0.78	0.80	0.85	0.72	0.11
	F_{is}	-0.06	-0.03	0.03	-0.03	0.03	-0.08	0.18	-0.04	0.12	-0.05
	HW	NS	NS	NS	NS	NS	NS	*	NS	NS	NS
PORO	H_o	0.85	0.76	0.84	0.69	0.96	0.71	0.67	0.84	0.66	0.20
	H_e	0.85	0.75	0.87	0.62	0.94	0.78	0.75	0.87	0.71	0.19
	F_{is}	-0.01	-0.02	0.04	-0.10	-0.02	0.09	0.10	0.03	0.07	-0.08
	HW	NS	NS	NS	NS	*	NS	NS	NS	NS	NS
PORM	H_o	0.81	0.81	0.89	0.72	0.96	0.73	0.67	0.85	0.74	0.15
	H_e	0.86	0.81	0.87	0.71	0.94	0.77	0.79	0.87	0.69	0.17
	F_{is}	0.06	0.00	-0.03	-0.02	-0.03	0.04	0.15	0.02	-0.07	0.16
	HW	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
PORI	H_o	0.82	0.71	0.86	0.67	0.96	0.83	0.73	0.87	0.80	0.21
	H_e	0.84	0.74	0.87	0.67	0.95	0.80	0.75	0.88	0.74	0.20
	F_{is}	0.03	0.04	0.01	-0.01	-0.01	-0.04	0.03	0.02	-0.09	-0.03
	HW	NS	NS	NS	NS	NS	NS	*	NS	NS	NS
KVN	H_o	0.86	0.73	0.76	0.56	0.93	0.74	0.67	0.81	0.63	0.11
	H_e	0.86	0.73	0.79	0.63	0.92	0.79	0.74	0.83	0.63	0.11
	F_{is}	0.00	0.00	0.04	0.11	-0.02	0.05	0.09	0.02	0.01	-0.04
	HW	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
REI	H_o	0.90	0.77	0.84	0.68	0.93	0.79	0.70	0.85	0.65	0.14
	H_e	0.85	0.80	0.82	0.71	0.93	0.80	0.75	0.85	0.66	0.13
	F_{is}	-0.06	0.03	-0.03	0.03	0.00	0.01	0.07	0.00	0.01	-0.06
	HW	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
LYN	H_o	0.85	0.76	0.77	0.68	0.90	0.66	0.77	0.82	0.59	0.12
	H_e	0.85	0.76	0.79	0.70	0.91	0.77	0.76	0.85	0.58	0.12
	F_{is}	0.00	0.01	0.03	0.02	0.01	0.14	-0.01	0.04	-0.01	-0.05
	HW	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
MAL	H_o	0.86	0.75	0.73	0.65	0.91	0.80	0.69	0.88	0.60	0.12
	H_e	0.85	0.74	0.75	0.69	0.93	0.82	0.75	0.87	0.61	0.15
	F_{is}	-0.01	-0.01	0.04	0.07	0.02	0.02	0.08	-0.01	0.02	0.17

		PbC105	PbC8	SD2-14	PbA110	PbC109	PbD9	PbA1	SD1-41	SD2-68	SD3-62
	HW	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
FO2011	H_o	0.84	0.72	0.70	0.63	0.90	0.71	0.59	0.72	0.44	0.09
	H_e	0.85	0.79	0.76	0.65	0.92	0.80	0.75	0.84	0.60	0.08
	F_{is}	0.02	0.08	0.07	0.03	0.02	0.12	0.22	0.14	0.28	-0.03
	HW	NS	*	NS	NS	NS	NS	***	***	***	NS
RAN	H_o	0.83	0.68	0.79	0.71	0.87	0.78	0.64	0.89	0.65	0.08
	H_e	0.85	0.76	0.81	0.74	0.91	0.78	0.77	0.87	0.65	0.07
	F_{is}	0.03	0.10	0.02	0.04	0.04	0.00	0.17	-0.02	0.00	-0.02
	HW	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
FOFI	H_o	0.86	0.71	0.79	0.68	0.98	0.84	0.65	0.78	0.64	0.07
	H_e	0.85	0.73	0.80	0.72	0.94	0.80	0.78	0.86	0.59	0.07
	F_{is}	-0.02	0.02	0.01	0.06	-0.04	-0.05	0.16	0.10	-0.09	-0.03
	HW	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
NOM	H_o	0.84	0.67	0.77	0.62	0.92	0.58	0.62	0.81	0.68	0.17
	H_e	0.84	0.75	0.80	0.73	0.93	0.81	0.74	0.86	0.68	0.18
	F_{is}	-0.01	0.10	0.04	0.15	0.02	0.29	0.16	0.06	-0.01	0.05
	HW	NS	NS	NS	*	NS	***	NS	NS	NS	NS
VANC	H_o	0.81	0.77	0.92	0.81	0.91	0.82	0.80	0.82	0.68	0.32
	H_e	0.86	0.90	0.94	0.80	0.96	0.87	0.86	0.94	0.68	0.35
	F_{is}	0.06	0.14	0.02	-0.02	0.06	0.06	0.07	0.12	-0.01	0.08
	HW	NS	NS	NS	NS	*	NS	NS	NS	NS	NS
Total	H_o	0.85	0.75	0.83	0.69	0.92	0.76	0.70	0.83	0.66	0.19
	H_e	0.85	0.77	0.85	0.70	0.93	0.80	0.77	0.85	0.69	0.19
	F_{is}	-0.5	0.03	0.03	0.02	0.01	0.4	0.1	0.03	0.03	-0.01



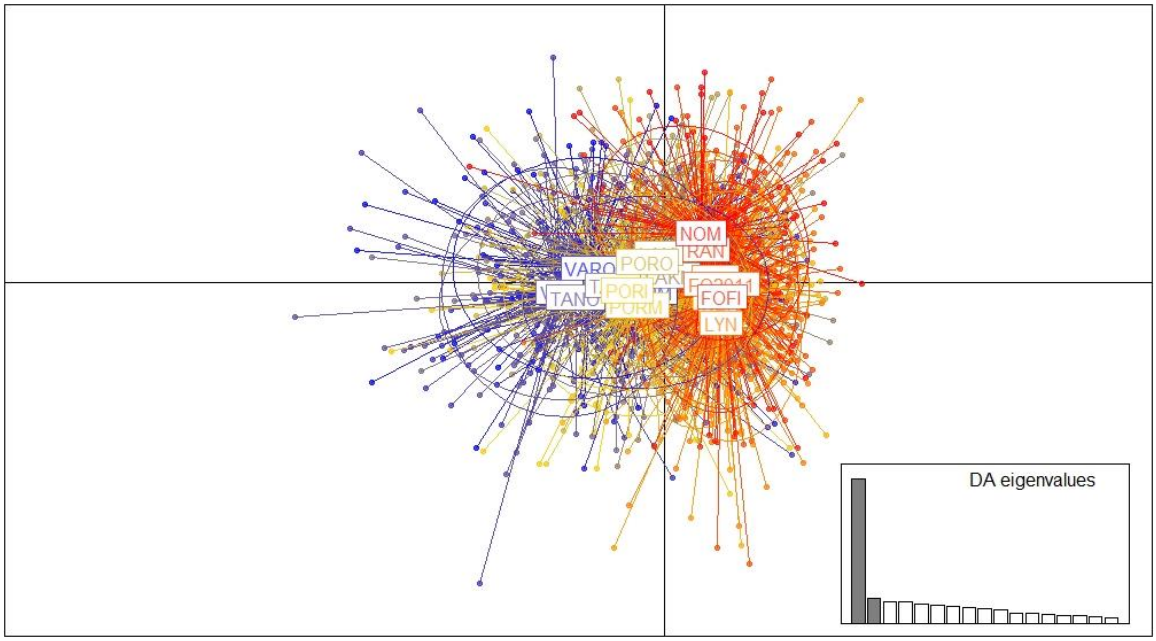
Appendix figure I. Showing how many K (clusters) assigned to the sample by STRUCTURE HARVESTER using the Evanno method. This shows the best fitted clusters (K=3) for all samples. Source: Dent A. and vonHoldt (2012).



Appendix figure II. Showing how many K (clusters) assigned to the sample by STRUCTURE HARVESTER using the Evanno method. This shows the best fitted clusters (K=2) for the coastal samples. Source: Dent A. and vonHoldt (2012).

```
# Structure Plot in R, by Matthew G. Johnson. September 1, 2011.
# This script will plot the familiar Structure barplot (each bar is an
individual).
# Each bar will be an individual with colors corresponding to the inferred
cluster.
# The input file is to be taken directly from CLUMPP-- a whitespace-delimited
text # file with NO HEADER.
# The putative (original) populations should be in column 4, and the Q-matrix
# should start in column 6.
# You should specify your original population names, and colors for clusters.
# The only thing below the dashed line that might need adjustment is the text
line, # if your original populations have names that are too big.
# You need to change the following lines:
filename = "Reke_K3.outfile" #Outfile from CLUMPP
k=3
barcolors = c("cyan4","yellow","darkorange")
poplabels =
c("BSS1","BSS2","BSS3","BSS4","VARO","VARM","VAR","TANO","TANM","LAKM","LAKI","P
ORO","PORM","PORI","KVN","REI","LYN","MAL","FO2011","RAN","FOFI","NOM","VANC")
#-------------------------------------------
# You shouldn't need to change these lines
mydata = read.table(filename,header=FALSE)
klast = k + 5
par(mar=c(5.5,0,0,0))
bp = barplot(t(mydata[,6:klast]),
             col=barcolors,
             space = 0,
             axes=F, border=NA)
# Automatically grab beginning and end of each population for plotting of lines
and # labels
popsizes = tapply(mydata$V1,mydata$V4,length)
numpops = length(popsizes)
poploc = 0
popbegins = rep(NA,k)
popends = rep(NA,k)
for(x in 1:numpops){
  popbegins[x] = poploc
  popends[x] = poploc + popsizes[x]
  poploc = poploc + popsizes[x]}
popmidpoints = (popbegins+popends)/2
# Puts a dark line between each original population
abline(v=c(0,popends),lwd=1,xpd=F)
# Label the populations underneath, at the midpoint of each population.
for(x in 1:numpops){
  mtext(poplabels[x],side=1,at=popmidpoints[x],padj=0.5,cex=1,font=1,las=3)}
```

Appendix figure III. Shows R-script used to make the STRUCTURE plots.



Appendix figure IV. DAPC plot showing only the 18 coastal samplings. Discriminant Analysis (DA) eigenvalues display the number of discriminant functions retained.