Genetic complexity in the marine environment:

Population genomics of saithe (*Pollachius virens*), Greenland halibut (*Reinhardtius hippoglossoides*), beaked and golden redfish (*Sebastes mentella* and *S. norvegicus*) in the North Atlantic

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This PhD thesis is dedicated

to my sisters
(Nila Saha and Shila Saha)

and

to my parents
(Thakurdas Saha and Ranu Saha).

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Paper 2 Westgaard, J-I., Saha, A., Kent, M., Hansen, H. H., Knutsen, H., Hauser, L., Cadrin, S. X., Albert, O. T., and Johansen. T. SNP markers from RAD sequences reveal management relevant genetic patterns in Greenland halibut (*Reinhardtius hippoglossoides*). Canadian Journal of Fisheries and Aquatic Sciences, In review.

Paper 3 Saha, A., Johansen, T., Hedeholm, R., Nielsen, E. E., Westgaard, J-E., Hauser, L., Planque, B., Cadrin, S. X., Boye, J. Geographic extent of introgression in *Sebastes mentella* and its effect on genetic population structure. Evolutionary Applications, In review.

Paper 4 Saha, A., Hauser, L., Planque, B., Fevolden, S-E., Hedeholm, R., and Johansen, T. Cryptic *Sebastes norvegicus* species in Greenland waters revealed by microsatellites. ICES Journal of Marine Science, In review.

Appendix II Excerpts of manuscript in preparation: Genetic structuring of *S. mentella* based on genome-wide SNP data (unpublished data)

Contributions

	Paper 1	**Paper 2**	**Paper 3**	**Paper 4**
Concept and idea	TJ, AS, LH	TJ, JIW, AS	AS, TJ, JB	AS, TJ
Sampling design and fish collection	TJ, FN, IH, EH	TJ	RH, JB, TJ	RH, TJ
SNP/ microsatellite genotyping	MK, TK, SL	MK, HH	JIW	
Analytical methods	AS	JIW, AS	AS	AS
Data analysis and interpretation	AS	JIW, AS	AS	AS
Manuscript writing	AS	JIW, AS	AS	AS
Manuscript editing	SEF,FN, BP, LH, MK, IH, EH, TJ, AS	HK, LH, AS, MK, SC, OA, TJ, JIW	EN, LH, BP, TJ, RH, JIW, SC, JB, AS	SEF, LH, BP, RH, TJ, AS

AS = Atal Saha, TJ = Torild Johansen, LH = Lorenz Hauser, SEF = Svein Erik Fevolden, BP = Benjamin Planque, MK = Matthew Kent, JIW = Jon-Ivar Westgaard, SC = Steven X. Cadrin, HK = Halvor Knutsen, JB = Jesper Boje, RH = Rasmus Hedeholm, EN = Einar E. Nielsen, FN = Francis Neat, IH = Irene Huse, EH = Eydna Homrum, SL = Sigbjørn Lien, TK = Tina Kirubakaran, HH = Hanne Hellerud Hansen, OA = Ole Tomas Albert

Summary

The preservation of populations' resilience and evolutionary potential of exploited species rely on their sustainable management. Genetic diversity within species and populations may serve to define management units that are biologically meaningful. Exploration of genetic complexity in marine species has been difficult because of the apparent lack of barriers to migration, resulting in low levels of differentiation. Recent developments in genetic methods provide improved capabilities to identify biologically distinct populations and thereby to explore genetic complexity. In this work, I investigated genetic complexity in four commercially exploited species from the North Atlantic: saithe (*Pollachius virens* L.), Greenland halibut (*Reinhardtius hippoglossoides*), and beaked and golden redfish (*Sebastes mentella* and *S. norvegicus*). The results were used 1) to assess the consistency between current management units and units identified by results based on genetic data for each species, and 2) to assess the efficiency of SNP data compared to conventional markers in studying population genomics. Panels of nuclear genomic markers, including single nucleotide polymorphisms (SNPs) and microsatellites, derived from modern genomic approaches, and data on species life history traits, were analyzed to explore genetic complexity within these highly migratory and continuously distributed species.

The investigation reveals biologically distinct populations within each of these species. Four genetic clusters of saithe and two clusters of Greenland halibut were found in the North Atlantic. For beaked redfish, results using both the genome-wide SNP and microsatellite data supported one group ('shallow') throughout the North Atlantic and a second group ('deep') in the central North Atlantic and Canadian waters. A localized group ('slope') of beaked redfish was identified in Greenland and Icelandic waters. Microsatellite DNA supported three unrecognized cryptic species of golden redfish in Greenland and nearby waters. Genetic isolation in golden and beaked redfish was greater than in saithe and Greenland halibut, which is possibly associated with unique life history features of redfishes. Greenland waters exhibited a richer redfish biocomplexity than other waters. The results indicate a correlation between genetic differentiation and life history differences in the studied species. These findings imply that distinct genetic heterogeneity can exist in different marine species and may be influenced by abiotic factors like geographic distance, seascape and hydro-dynamics, water depth, and time since divergence. Furthermore, different biotic factors such as natal homing, sex biased migration, introgression, and sexual selection may contribute to shaping species' genetic patterns. The results highlight that in most cases the current management units of these species are comprised of multiple biological populations. The new definition of gene pools may serve to define biologically meaningful management units to ensure their sustainable exploitation and preserve evolutionary legacies.

This study provides the first SNP-based population genomic investigation in saithe, Greenland halibut and beaked redfish. Comparative analyses of SNP and conventional marker systems demonstrate a higher resolution for SNP markers. Results from genome-wide SNP data identified the three genetic groups of beaked redfish from a much smaller sample set, and the estimated genetic differentiation was much greater than that found by other marker. Both in beaked redfish and Greenland halibut a sub-set of outlier SNPs were identified, implying possible signals of selection in these loci or nearby genomic sites. These outliers may provide increased power in population assignment of the species. The present work illustrates outstanding opportunities of SNP marker system for investigating population genomics of non-model organisms.

1. Introduction

Aquatic species represent the major human food source harvested from the natural environments and therefore special attention is required to ensure sustainable use of this biological diversity (Ryman *et al.* 1995). Identification of genetic heterogeneity and subsequent use of the results in management regimes are essential for sustainable management of living resources (Ryman *et al.* 1995; Reiss *et al.* 2009). Failure in identification of genetic populations (Waples & Gaggiotti 2006) or cryptic species (Bickford *et al.* 2007) within exploited species, and management of populations or species as single homogenous units when discrete reproductive units exist, can lead to overexploitation and depletion of the populations or species most easily captured (Allendorf *et al.* 2008). The severe decline of many exploited marine species has raised concern on the sustainable use of aquatic biodiversity. For example, loss of genetic diversity may be the reason why Greenland halibut, redfish and Norwegian coastal cod are depleted in the Northeast Atlantic (ICES 2007). Data on genetic population and species structure should be available for exploited species to define biologically meaningful management units (Reiss *et al.* 2009).

Biocomplexity refers to the existence of different biological populations within a single species, which display various biological and ecological traits (Michener *et al.* 2001). In the marine environment, biocomplexity can play a significant role in increasing stability and resilience of living resources (Hilborn *et al.* 2003). Exploration of marine biocomplexity is hence essential to support the management and conservation of exploited species. Biological populations, defined as reproductively isolated groups of individuals of the same species at a given time and space (Waples & Gaggiotti 2006), are the primary units of evolutionary change (Hauser & Carvalho 2008). Genetic diversity within and between populations may provide the capacity to adapt to a range of environmental conditions (Bonin *et al.* 2007). It is therefore vital to protect individual populations and the genetic diversity within them, across their geographical and environmental ranges, to preserve 'the evolutionary legacy and future evolutionary potential' of marine species (Hauser & Carvalho 2008). A single population, within a multiple population complex, that might be a minor component at one time may become a major component during another time. For example, Hilborn et al. (2003) have shown that the contribution of different populations of sockeye salmon (*Oncorhynchus nerka*) to the exploited stock in Bristol Bay have greatly varied with time, likely in response to climate variations. Furthermore, the loss of genetic diversity due to overexploitation of a local population is unlikely to be replaced through immigration (Hauser & Carvalho 2008). Therefore, preservation of the genetic diversity of each single population within a complex is needed to ensure sustainable exploitation of the complex as a whole.

Commercially exploited marine species often display weak population genetic structure due to large population sizes, wide distributions, absence of physical barriers to

migration, relatively recent separation, and highly dispersive life stages (Waples 1998; DeWoody 2000). These constraints hinder exploration of biocomplexity in the marine environment. As a consequence, the presence of multiple biological populations within a single management unit may remain undetected. Nevertheless, advancements in molecular genetic approaches have resulted into notable successes for the studies of demographic and evolutionary dynamics of wild marine populations (for review see Hauser & Carvalho 2008; Salmenkova 2011; Gagnaire *et al.* 2015). Two particular DNA marker systems, microsatellites (tandemly repeated genomic sequences) and SNPs (single nucleotide polymorphisms: variations in single nucleotides), have been proven to be efficient tools for extensive molecular ecology investigations (Chistiakov *et al.* 2006; Helyar *et al.* 2011; Abdul-Muneer 2014). Microsatellites have been used to identify barriers to gene flow in the forms of oceanic currents (Spies 2012), bathymetry (Knutsen *et al.* 2009), and salinity (Nielsen *et al.* 2003). Although microsatellites usually exhibit greater neutral allelic diversity per locus, SNPs are more abundant throughout both the neutral and selective regions of genomes (Morin *et al.* 2004; Helyar *et al.* 2011). Modern sequencing techniques have made it feasible to identify and genotype thousands of SNPs in hundreds of individuals at increasingly lower prices (Hemmer-Hansen *et al.* 2014). As a result, the studies of genomic variation across the genetic populations, i.e. population genomics, have become more accessible even for non-model organisms (Therkildsen *et al.* 2013; Larson *et al.* 2014b). Compared to microsatellites, analyses of SNPs are more comparable between laboratories (Helyar *et al.* 2011; Seeb *et al.* 2011). SNPs are increasingly being applied to explore neutral and adaptive divergence in diverse marine species (e.g. Bourret *et al.* 2013; Hess *et al.* 2013; Larson *et al.* 2014a; Milano *et al.* 2014). Population genomic data are therefore expected to bring better resolution in the investigations on commercially exploited marine species that have previously been reported to have weak or no genetic structure (Hemmer-Hansen *et al.* 2014).

In this work, I have studied genetic complexity in saithe (*Pollachius virens* L.), Greenland halibut (*Reinhardtius hippoglossoides*), beaked and golden redfish (*Sebastes mentella* and *S. norvegicus*) using SNP data developed through modern molecular genomic approaches, and a conventional marker system, microsatellites. The selected species represent commercially exploited marine species across the North Atlantic with overlapping distribution range. They are migratory, and deep-water species (except saithe). Compared to oviparous (i.e. egg spawner) saithe and Greenland halibut, ovoviviparous (i.e. live bearer) redfish is assumed to possess more mechanism for reproductive isolation. For instance, reproduction biology of redfish includes mate recognition, mate choice and courtship behaviour (Johns & Avise 1998; Love *et al.* 2002). Followed by internal fertilization, redfish releases planktonic larvae. The age of redfish in the North Atlantic has been recorded up to 75 years (Campana *et al.* 2011), whereas both saithe and Greenland halibut can live for around 30 years (Albert *et al.* 2009; Mehl *et al.* 2011). The long lifespan of redfish may promote adaptation to different environments (Mangel *et al.* 2007). Therefore, more genetic isolation may exist within beaked and golden redfish than in saithe and Greenland halibut. The main knowledge gaps and hypotheses dealt with for the four species are briefly described below:

1.1 Saithe (*Pollachius virens)*

In the Northeast Atlantic, there are four saithe management units within the advisory framework of ICES (Fig. 1, paper 1): 1) Icelandic, 2) Faroese, 3) Northeast Arctic, and 4) North Sea, Skagerrak, and West of Scotland/ Rockall (ICES 2014a, 2014b, 2014c, 2014d). Tagging experiments (e.g. Armannsson *et al.* 2007; Homrum *et al.* 2013) have supported significant saithe migration between the geographical locations, and some initial genetic studies (e.g. Eiríksson & Árnason 2014; Behrmann *et al.* 2015) have indicated some structure within the species. Whether the migration observed by tagging experiments reflects cross ocean dispersal or just feeding migration has not yet been confirmed due to insufficient genetic data. The species, with its extensive distribution throughout the North Atlantic (Fig. 1 shows the distribution of saithe in the Northeast Atlantic), encounters diverse environmental conditions. As a consequence, the extent of gene flow may vary among the locations. Seascape genetics, along with SNP data, may provide an efficient approach in exploring genetic complexity within saithe across its distribution range and to assess the consistency between the management and biological units of the species.

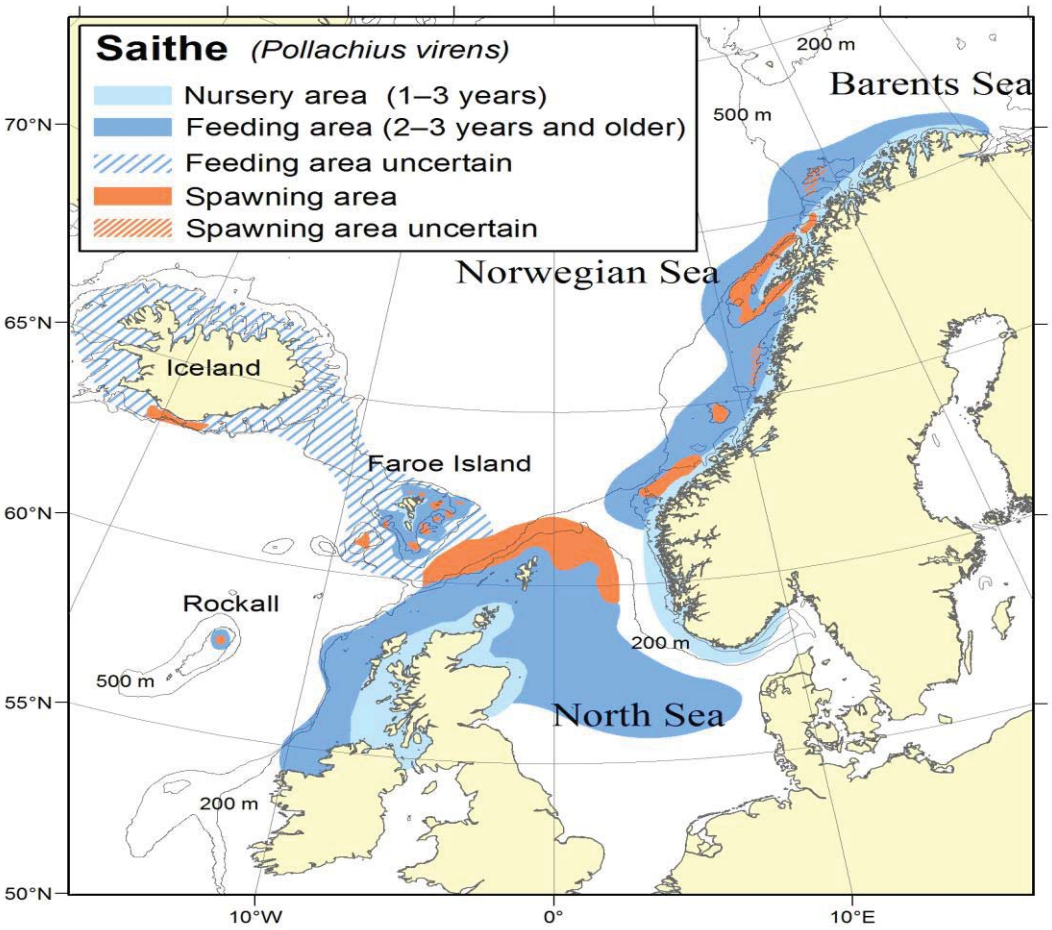

Fig.1: The distribution of saithe in the Northeast Atlantic. The spawning, nursery and feeding areas of the species are shown.

1.2 Greenland halibut (*Reinhardtius hippoglossoides)*

In the North Atlantic, Greenland halibut is presently perceived as consisting of three major management units (Fig. 2): 1) the Northeast Arctic unit in the Barents Sea, 2) the West Nordic unit in the Iceland, East Greenland and Faroe Islands waters, and 3) the Newfoundland-Baffin Bay-West Greenland complex (Høines & Gundersen 2008). Previous genetic studies have reported contradicting results as some studies identified no structure within the North Atlantic (e.g. Vis *et al.* 1997), while others found structure from a particular area (e.g. Riget *et al.* 1992; Knutsen *et al.* 2007). The investigation by Knutsen et al. (2007) found partially isolated populations in the Northeast Atlantic with evidence of isolation by distance. Roy et al. (2014) in their study using microsatellite DNA have described a common gene pool within the Northwest Atlantic, whereas the sample from west Svalbard was significantly different from others. Tagging experiments (Albert & Vollen 2014) have suggested that the West Nordic and Northeast Arctic units exploit a common nursery ground in the waters off Svalbard, as tagged juveniles were recaptured in both areas at comparable rates. These authors reported no recapture from southwest of Iceland and Greenland waters. Hence, additional investigations are required to estimate the number of gene pools and their connectivity within the species complex, and to assess the consistency between the management and biological units of the species.

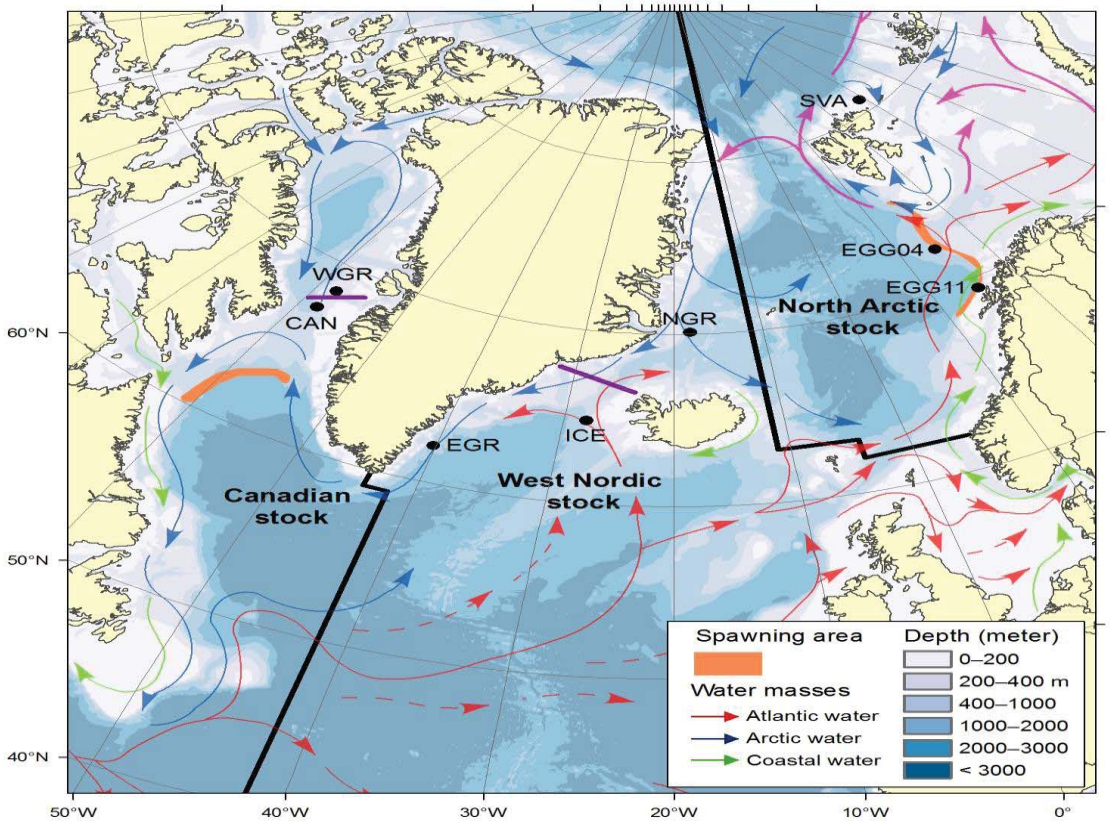

Fig. 2: Sampling of Greenland halibut in the present study. The existing three management units and spawning areas of the species in the Northeast Arctic and Canadian waters are illustrated (for details see paper 2).

1.3 Beaked redfish (*S. mentella)*

The genus *Sebastes* is represented by four known species in the North Atlantic. Among them, *S. mentella* is commercially the most important species. The numbers of genetic groups, their distribution (Fig. 3) and connectivity within *S. mentella* have long been a matter of dispute (see Cadrin *et al.* 2010; Cadrin *et al.* 2011; Makhrov *et al.* 2011). Based on the available genetic evidences, data on life history traits and morphologies, Cadrin *et al.* (2010) suggested four genetic groups of beaked redfish in the North Atlantic. The 'shallow pelagic' (= 'shallow') group is described between 200 and 500 m depth across the North Atlantic, while the 'deep pelagic' (= 'deep') group has mainly been reported between 550 and 800 m depth in the Irminger Sea. These two groups also exhibit distinct morphological characteristics (Magnusson & Magnusson 1995; Stefánsson *et al.* 2009b). A third group, 'slope', has been mentioned along the continental slope of the Icelandic Shelf (Stefansson *et al.* 2009a), although its identity has not been fully established. Finally, a 'western group' has been described in West Atlantic waters. The present management units of beaked redfish recognize these biological units, but questions remain on the distribution boundaries among these groups (Cadrin *et al.* 2010). For example, the 'slope' group in the East Greenland and Iceland waters are assessed separately by the International Council for the Exploration of the Sea (ICES 2015) although it is known that the stock identity is uncertain. Despite the ambiguity about its genetic identity, beaked redfish in the Northeast Arctic waters are managed separately. Like Greenland halibut, subsequent investigations are required to estimate the number of gene pools and their connectivity within the *S. mentella* complex, and to assess the consistency between the management and biological units of the species.

Redfish, particularly *S. mentella* and *S. norvegicus*, represent illustrative examples of biocomplexity operating in the marine environment (Schmidt 2005; Cadrin *et al.* 2010). This is believed to result from particular life history traits, such as discrete larval extrusion in both time and space (Magnusson & Johannesson 1995), mate recognition and courtship behavior (Helvey 1982). Despite the existence of these isolating mechanisms, introgression has been reported across the species throughout the North Atlantic (see Roques *et al.* 2001; Pampoulie & Daníelsdóttir 2008; Artamonova *et al.* 2013). This is possibly linked with *Sebastes*'s recent evolutionary history in the North Atlantic (Barton & Hewitt 1989; Briggs 1995). Interestingly, introgession has been assumed to influence the gene pool dynamics within *S. mentella* in the West Atlantic (Roques *et al.* 2001) and Irminger Sea waters (Artamonova *et al.* 2013). Hence, it is vital to estimate the geographic extent of introgression within the species complex and to assess its impact on the genetic population structure of *S. mentella*.

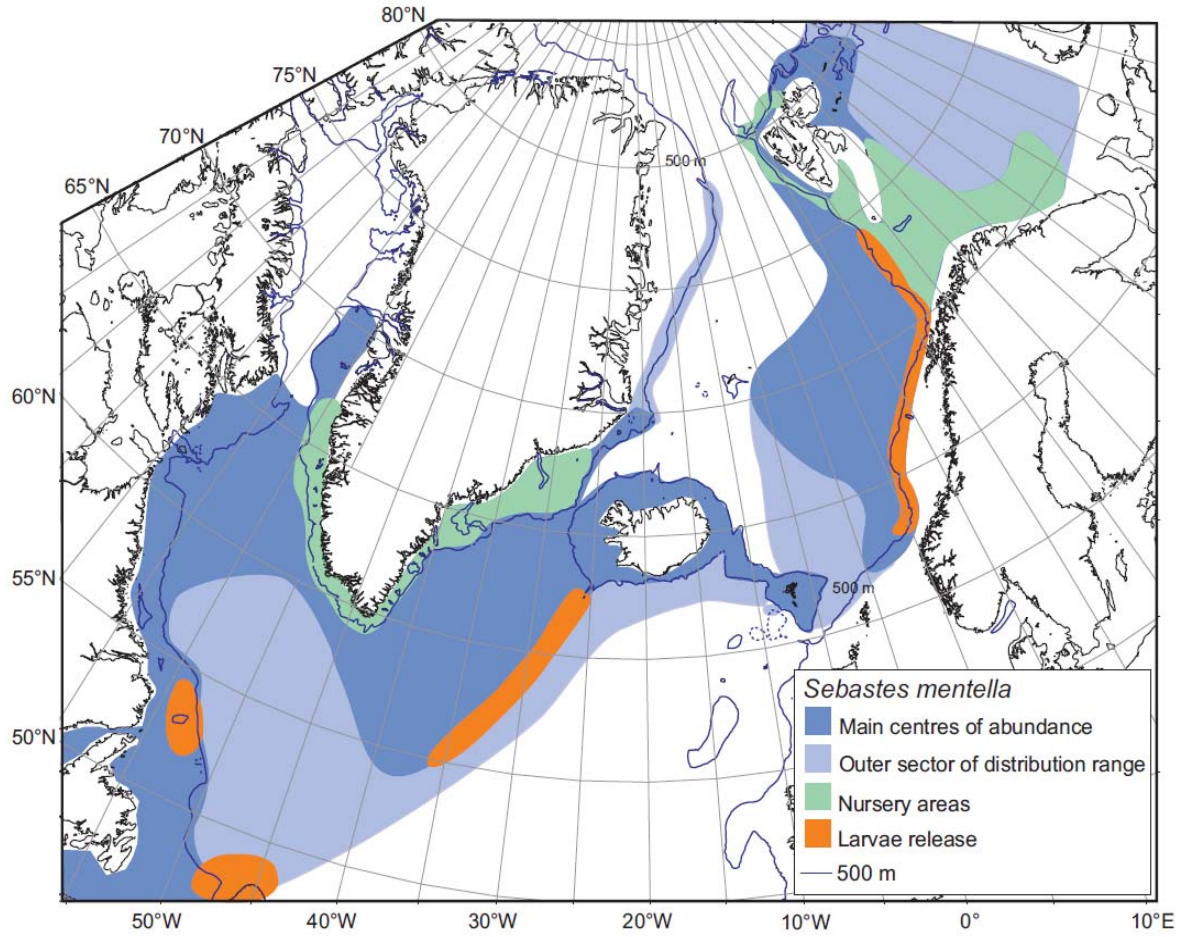

Fig. 3: The distribution of *S. mentella* across the North Atlantic. Adapted from Planque *et al*. (2013).

1.4 Golden redfish (*S. norvegicus)*

Redfish in the North Atlantic are morphologically very similar, leading to persistent difficulties in species identification (Johansen 2003; Schmidt 2005; Pampoulie & Daníelsdóttir 2008). This morphological similarity has motivated hypotheses of recent speciation within the genus, which may be linked to separation by depth (Barsukov *et al.* 1984). Compared to *S. mentella*, much less attention has been paid to *S. norvegicus* due to its relatively less commercial importance. Nevertheless, distinct biological units have been proposed for golden redfish (e.g. Johansen *et al.* 2000b; Schmidt 2005; Pampoulie *et al.* 2009), which are not reflected in the present management units (ICES 2014a). The so called 'giants' group of the species has been claimed as a species of its own, although debate persists (Johansen *et al.* 2000b; Schmidt 2005; Artamonova *et al.* 2013). The taxonomic status of the 'giants' remains unresolved and further investigation is required to clarify its connectivity with the co-existing gene pools. Marine habitats are believed to be rich with cryptic speciation (Miglietta *et al.* 2011), and cryptic *Sebastes* species have been documented in the Pacific (e.g. Kai *et al.* 2002; Gharrett *et al.* 2005). Therefore, existence of currently unrecognized cryptic *Sebastes* species in the North Atlantic may be hypothesized, which can be tested with subsequent investigations applying modern molecular genetic markers.

1.5 Objectives

The main objectives of this study are:

i) to identify the gene pools of saithe, Greenland halibut, beaked and golden redfish, and estimate the extent of interaction/ connectivity among their gene pools

ii) to explore the underlying biotic and abiotic factors driving their differentiation

iii) to assess the consistencies between the management and biological units of the species

iv) to assess the efficiency of SNP data vs traditional marker systems to study population genomics.

Paper specific (main) aims were:

Paper 1: to investigate the genetic structuring of saithe across the North Atlantic using seascape genetics of SNPs

Paper 2: a) to develop species specific SNP markers for Greenland halibut and apply a selection of SNPs to study the population genetic structure of the species
b) to compare the genetic structure of Greenland halibut with the existing knowledge on the population genetic structure of the species, and to discuss potential management implications

Paper 3: a) to examine the genetic structure of *S. mentella* in Greenland, Iceland and Irminger Sea waters

b) to investigate the extent of introgression among co-occurring *Sebastes* species/ gene pools

c) to assess if introgression may drive apparent species and population structure in the region.

Paper 4: to investigate the species structure of *Sebastes* in Greenland waters, paying special attention to the possible existence of cryptic species within the *S. norvegicus* complex

2. Materials and methods

Samples of saithe, Greenland halibut, beaked and golden redfish were collected from selected locations across the North Atlantic (Appendix I Table 1). The adult and juvenile fish were caught, during both spawning and non-spawning seasons, at known spawning and non-spawning grounds. The fish were caught by commercial and research vessels using trawl. Depths of sampling, sex, body length and/ or age of fish were recorded. For beaked redfish, an extensive sampling (35 samples consisting of 2562 fish) was carried out mainly focusing on Greenland waters (paper 3). DNA was isolated from ethanol preserved gill and muscle tissues using the E-Z 96 Tissue DNA kit following the manufacturer's instructions (Omega Bio-Tek, Inc, USA). SNP markers were developed for saithe (paper 1), Greenland halibut (paper 2) and beaked redfish (Appendix five). A total of 13 microsatellites were analyzed for the studies in redfish (for details see papers 3, 4).

SNP detection: RAD and ddRAD sequencing

For the SNP detection, Restriction site-Associated DNA (RAD) sequencing (Baird *et al.* 2008) approach was applied in studying saithe (paper 1) and Greenland halibut (paper 2). RAD sequencing samples at reduced complexity across target genomes (Davey & Blaxter 2010), meaning that it sequences the homologous regions across the genome. The method can deliver many markers across genomes for any organism at decent price. In saithe, SNPs were identified from two pools of individuals collected from the North Sea. The STACKs (Catchen *et al.* 2013) bioinformatic pipeline was used for analyzing millions of reads and screening of loci. To achieve high precision in SNP detection, the RAD sequences were aligned against the cod genome (Star *et al.* 2011) and only those loci were selected which were scored with higher confidence. The Sequenom Mass array platform was used for genotyping the samples with the selected SNPs.

For Greenland halibut, the analytical pipeline developed for saithe was used except for two aspects: 1) pools for SNP detection was composed of individuals from three locations (Barents Sea, East Greenland, and West Greenland) to minimize the possible ascertainment bias (Helyar *et al.* 2011), and 2) the sequences were not possible to align against any reference genome as was done in saithe study.

For studying population genomics of beaked redfish (Appendix five), a genotyping by sequencing (GBS) approach coupled with double digest RAD seq (ddRAD) was applied. The ddRAD protocol provides an efficient approach for SNP detection and genotyping in model and non-model species (Peterson *et al.* 2012). The method allows us to resequence a comparable genomic sub-fraction (tags) across individuals belonging to the same species and the application of two restriction enzymes may provide a more precise fragment selection for sequencing (Peterson *et al.* 2012). Therefore, it is expected that ddRAD will be able to generate more data with higher precision at comparatively lower price. For the projects' DNA sequencing collaborator (CIGENE), it was the first time testing ddRAD protocol in any species.

Population genomic analyses

To test for the neutrality of loci, island models allowing (Foll & Gaggiotti 2008) and non-allowing (Antao *et al.* 2008) differences in the population sizes were applied. For the identified outliers, the associated tag sequences were blasted in the NCBI database using the 'BLASTN' window to explore their possible association with any functional parts of the genome. Two seascape genetic approaches (e.g. Manni *et al.* 2004; Crida & Manel 2007) were used for detecting possible barriers to gene flow in saithe and Greenland halibut (papers 1, 2). Unlike Wombling method (Manni *et al.* 2004), Monmonier algorithm (Crida & Manel 2007) cannot estimate the number of barriers to gene flow, but identifies the location as per rank of importance. Both model based (e.g. Pritchard *et al.* 2000; Corander & Marttinen 2006) and non model-based (e.g. Jombart *et al.* 2010) clustering approaches were used in the study to identify genetic clusters of beaked and golden redfish (papers 3, 4). Since DAPC (Jombart *et al.* 2010) does not rely on assumptions of Hardy-Weinberg and linkage equilibrium, the complete SNP panel was used to identify the number of clusters in samples of beaked redfish (Appendix five). Finally, to quantify gene flow among the identified genetic clusters, isolation-with-migration (IM, Hey 2009) model was used in beaked and golden redfish, whereas migrate-n (Beerli & Palczewski 2010) was used in saithe. In contrast to migrate-n, IM model assumes no recombination within loci and the program has no SNP model implemented.

3. Abstracts of the papers

3.1 Seascape genetics of saithe (*Pollachius virens*) across the North Atlantic using single nucleotide polymorphisms (Paper 1): ICES Journal of Marine Science (2015), 72 (9), 2732-2741.

The identification of isolated populations in widely distributed marine species is often impeded by low levels of genetic differentiation. However, modern genetic approaches now allow for the efficient detection of potentially large numbers of novel genetic variations, thereby improving the power to identify biologically meaningful management units. To investigate the genetic structuring of saithe (*Pollachius virens L.*), we applied seascape genetic approaches to 131 single nucleotide polymorphism (SNP) loci genotyped in 584 fish collected from eleven locations across the North Atlantic. Wombling analysis and the Monmonier algorithm revealed four genetic clusters (Barents Sea, Central Northeast Atlantic, Rockall and Canada) across the species distribution range. These results imply that genetic structuring in saithe may be influenced by abiotic factors such as geographical distance, and bathymetry as well as biotic factors such as sex-biased migration, and natal homing. The results suggest a potential mismatch between management and biological units across the Northeast Atlantic, which may have implications for sustainable exploitation of the species.

3.2 SNP markers from RAD sequences reveal management relevant genetic patterns in Greenland halibut (*Reinhardtius hippoglossoides*) (Paper 2): Can. J. Fish. & Aqua. Sci. In review

Exploited marine resources need to be managed the best possible way. A new powerful tool for fisheries management are novel genetic markers allowing identification of genetic population (stock) structure, thus providing biologically sound management units. Single Nucleotide Polymorphisms (SNP) markers derived from Restriction site Associated DNA (RAD) sequencing were developed, and their performance was evaluated to investigate the stock structure of Greenland halibut (*Reinhardtius hippoglossoides)*. A total of 200 SNPs and 384 individuals from eight locations across the Atlantic were analyzed, resulting in 96 SNPs that could be reliably scored to infer the population genetic structure. Our results suggest a division of Greenland halibut into at least two populations, an eastern Atlantic population and a western Atlantic population, with a larger generic divergence in comparisons across the Denmark Strait. We found an isolation by distance pattern in the Eastern Atlantic, but not across the Atlantic. In general, Greenland halibut display weak but significant population structure (overall F_{ST} =0.003; p<0.001), which requires a combination of genetic markers to achieve a high statistical power. The weak population structure observed can be explained by connectivity among populations due to the migratory behavior and/or egg and larval drift of Greenland halibut.

3.3 Geographic extent of introgression in *Sebastes mentella* and its effect on genetic population structure (Paper 3): **Evolutionary Applications. In review**

Hybridization within the genus *Sebastes* has been described but the geographic extent of introgression in *S. mentella* and its role in genetic population structure in large parts of the North Atlantic remains unresolved. Here, we identify the genetic population structure of *S. mentella* and investigate possible introgression within the genus by analyzing 13 microsatellites in 2562 redfish specimens sampled throughout the North Atlantic. The data supports a historical divergence between the 'shallow' and 'deep' groups, even outside the Irminger Sea where they were described previously. A third group, 'slope', has an extended distribution on the East Greenland Shelf, in addition to earlier findings on the Icelandic Slope. Furthermore, *S. mentella* from the Northeast Arctic and Northwest Atlantic waters are genetically different populations. In both areas, interspecific introgression may play a significant role in driving allele frequency differences. Evidence of introgression was found for almost all the identified *Sebastes* gene pools, but to a much lower extent than suggested earlier. Greenland waters appear to be a mixing zone for many of the genetically independent *Sebastes* groups. However, this study illustrates that the identified groups maintain their genetic integrity in this region despite introgression.

3.4 Cryptic *Sebastes norvegicus* species in Greenland waters revealed by microsatellites (Paper 4): **ICES Journal of Marine Science. In review**

Identification of cryptic species can have profound implications in management, conservation and biodiversity contexts. In the North Atlantic, the genus *Sebastes* is currently represented by four species, although cryptic species have been suspected. The connectivity of the gene-pools within the genus in Greenland waters, in particular, remains largely unexplored. Using a panel of ten microsatellite markers for 594 fish we explored the species complex of *Sebastes norvegicus* in Greenland waters. Genetic analyses provided evidence for three cryptic species in samples that were morphologically identified as *S. norvegicus*. They were termed *S. norvegicus*-A, *S. norvegicus*-B, and *S. norvegicus* giants. A few phenotypic features exist to identify adult *S. norvegicus* giants, but no such characteristics have been identified for the two other cryptic species. The proposed cryptic species should be recognized in the management regime to ensure sustainable exploitation and conservation of *Sebastes* species in Greenland waters.

3.5 Excerpts of manuscript in preparation: Genetic structuring of *S. mentella* based on genome-wide SNP data (Appendix II unpublished data)

Genotyping by sequencing (GBS) offers a simultaneous opportunity to identify and genotype genome-wide SNPs in large number of individuals, and therefore improving the power in resolving complicated genetic population structure. Here, we applied double digest restriction-site associated DNA (ddRAD) sequencing in *S. mentella*, a marine species for which genetic structure has long been disputed. The main objectives of the investigation are to test genetic structure of *S. mentella* using genome-wide SNP data and to compare the results with that of microsatellite DNA. A total of 33 539 SNPs were genotyped in eight samples of which 1065 loci were finally selected allowing a maximum of 20 % missing genotypes per

locus per sample. The present data identified three genetic clusters in the samples representing the ‘shallow’ (50–500 m water depth), ‘deep’ (550–800 m) and ‘slope’ (around Greenland waters) groups. The significant neutral genomic differentiation (1058 loci, maximum F_{ST} = 0.04) observed between the ‘shallow’ and ‘deep’ groups implies high extent of reproductive isolation between them, whereas the substantial differentiation (maximum F_{ST} = 0.46) at seven outlier loci may suggest adaptive radiation in *S. mentella*. Within the ‘deep’ group, the samples from the Faroe Islands and Irminger Sea were different from each other. Likewise, the samples from Northeast Arctic and central North Atlantic were different from each other within the ‘shallow’ group. These findings were consistent with results analyzing microsatellites and other marker systems but the genome-wide SNPs revealed genetic structure of the species at a finer scale. Population assignment of the fish into the three genetic groups was in agreement with results using microsatellites. This work demonstrates that genome-wide SNPs obtained through the GBS approach can be a good tool in delineating complicated population genetics of marine species.

4. General discussion

This study represents the first SNP-based investigation in saithe, Greenland halibut and beaked redfish. The applied population genomic methods were successful in dealing with designated research questions, illustrating significant progress in population genomics of non-model organisms. This work demonstrates the existence of distinct gene pools (for explanation see Appendix III Box 1) in saithe, Greenland halibut, beaked and golden redfish in the North Atlantic. Genetic structuring was observed not only across wide species distribution ranges, but also on local scales. The results suggest more complex patterns of intraspecific and interspecific diversity for these species than was previously recognized. Results based on population genomic and life history data indicate that the observed genetic complexity is possibly influenced by several factors which include geographic distance, seascape and hydro-dynamics, water depth, natal homing, introgression, sexual selection, and time since divergence. A mismatch between the management and biological units was revealed, emphasizing that the current results could contribute to defining management units that are biologically meaningful.

The population genetic patterns of the studied species identified by SNPs, were in line with those based on microsatellite data. The applied SNP panels had high detection power even in the cases of low levels of genetic differentiation. The genome-wide SNPs derived from the genotyping by sequencing (GBS) approach, provided higher resolution for studying population genomics of *S. mentella*. The present work shows that SNPs can be successfully used to investigate population genomics of wild marine fish species. In the following sections, I will discuss the major findings of this dissertation under some key questions.

4.1 Genetic diversity in marine species: heterogeneity in homogenous environments?

The present study demonstrates genetic structuring in four widely distributed migratory species throughout the North Atlantic. The results imply that genetic isolation of varied extent can arise within different species, despite an apparently high potential for gene flow. Historically, marine species have been thought to display no or low genetic structuring due to the apparent lack of barriers to migration in the surrounding environments (Waples 1998; DeWoody 2000). Evidences for genetic structure within many marine species have increased during the last decades (for review see Hauser & Carvalho 2008; Salmenkova 2011). With the availability of increasing number of high resolution molecular markers and improvements in analytical tools, it is expected that more such evidences will amass in foreseeable future (Gagnaire *et al.* 2015). Although a publication bias cannot be denied for studies reporting genetic structuring (Hauser & Carvalho 2008), genetic investigations

applying modern molecular tools should be encouraged for species allegedly believed to have no or shallow genetic heterogeneity.

4.2 What drives genetic isolation within marine species?

The present study reports different factors contributing to the observed genetic heterogeneity in the four selected species, which are in consistent with several other studies (e.g. Roques *et al.* 2002; Shaw *et al.* 2004; Ruzzante *et al.* 2006; Knutsen *et al.* 2009). The results indicate a correlation between the genetic and life history differentiation in the four species. Seven processes have been identified, which can potentially explain the genetic differentiation. These are detailed in the sections below.

4.2.1 Geographic distance

Geographic distance seems to be an important driver to the observed genetic differentiation. Since species distribution ranges often exceed individual dispersal capability, an isolation by distance (IBD) may exist in many species (Slatkin 1993). The investigation of saithe revealed strong IBD in spawning fish from the Northeast Atlantic (paper 1). The analyses of Greenland halibut genotypes revealed IBD for samples within the Northeast Atlantic, but not across the North Atlantic (paper 2). For the 'shallow' group of beaked redfish, the samples from the Northeast Arctic, Irminger Sea, and Canadian waters were different from one another (paper 3). These results support geographic distance as a possible driver of genetic differentiation. Isolation by distance may be observed for neutral markers in species with continuous distribution and limited dispersal (Hauser & Carvalho 2008). Such patterns are expected to reflect true biological significance of the results rather than sampling noise (Palumbi 2003), and therefore IBD in saithe and Greenland halibut samples provide support to the estimated low differentiation.

4.2.2 Seascape and hydro-dynamics

The seascape genetic analyses of saithe and Greenland halibut samples revealed reduced gene flow in some regions despite the occurrence of IBD within the Northeast Atlantic waters. As to saithe, a bathymetric barrier to gene flow seems evident for the Rockall sample. For Greenland halibut, a barrier to gene flow seems to exist between Northeast Greenland and waters south of Iceland. The differentiation of the 'deep' group samples of beaked redfish from the Faroe Islands and Irminger Sea (Table 2, Appendix II) also suggests that a bathymetric barrier to gene flow exists in this region: a suggestion consistent with that of Shum et al. (2015). Since samples of all four species were not collected from the same locations, an assessment of biogeographic discontinuity across species cannot be made. Nevertheless, the discontinuities observed in the present study are consistent with the conclusions of previous studies in the same geographical regions (e.g. Shaw *et al.* 1999; Knutsen *et al.* 2009).

4.2.3 Water depth

Water depth can drive genetic heterogeneity of marine species, which is comparatively less explored than many other factors such as geographic distance (Stefansson *et al.* 2009a; Shum *et al.* 2015). For the cryptic species within golden redfish, the 'giants' were found in deeper waters (521 – 786 m) than Norvegicus-A and B (188 – 417 m). In Greenland halibut, the present analyses indicated a genetic barrier between the two samples from west of Greenland collected at different depths (963 m, and 292 – 561 m, Fig. 1, paper 2). Although statistically not significant, there appears to be a difference between emigration and immigration rates of saithe around the shallower Rockall waters and in the rest of the Northeast Atlantic.

Both the GBS and microsatellite data supported the two genetic groups of *S. mentella* associated with different water depths (i.e. 'shallow' ≤ 550 m and 'deep' ≥ 550 m). Shum et al. (2015) have estimated a historical divergence between these groups using mitochondrial DNA, suggesting that the observed segregation by depth possibly have resulted from climatic and oceanographic processes. In the present investigation, the preferred depth range for the 'slope' group around east Greenland and Iceland waters was 227 – 375 m. The 'shallow' group fish were detected in deeper waters in the Northeast Arctic (mean depth = 467 m, maximum depth = 575 m) compared to the 'shallow' group in the Irminger Sea (mean depth = 240 m). In the Irminger Sea, the 'shallow' group *S. mentella* can occur in water as shallow as 50 m. This may be possible because of the much warmer oceanic current flows in these waters (Magnusson & Magnusson 1995; Johansen 2003) which could be reflected in food availability.

4.2.4 Natal homing and sex biased migration

In saithe, the Rockall spawning sample was genetically different from the spawning sample of the Faroe Islands but not from the autumn sample (juveniles). Given the hydrodynamics of the region (paper 1), natal homing was hypothesized for saithe spawning in Rockall waters. These findings imply that two distinct saithe populations, i.e. originating from Rockall and the Faroe Islands, may exploit the same nursery ground around the Faroe Islands. Furthermore, IBD was registered in spawning samples of saithe but not in non-spawning samples, suggesting that natal homing followed by feeding migration may be a general pattern to some of the saithe populations. Interestingly, data on sampling distances and fish sex information revealed that migration in saithe is likely to be male-mediated.

In *S. mentella* (paper 3 and Appendix II), the phenotypically defined reference 'shallow' and 'deep' groups were supported by genetic analyses in samples caught from the Irminger Sea and Faroe Islands. This result implies that the phenotypic distinction between 'shallow' and 'deep' *S. mentella* is genetically rooted. Although morphological identification of these groups is difficult for juveniles, GBS and microsatellite data revealed that juveniles from the 'deep', 'shallow' and 'slope' groups co-exist in Greenland waters during autumn. In contrast, the adult samples from Greenland waters caught during the larval extrusion period

(spring) comprised very few fish from the 'deep' and 'shallow' groups. These findings provide genetic support to the suggested hypothesis of a common *Sebastes* nursing zone in Greenland waters (Magnusson & Johannesson 1995). The results herein demonstrate first and foremost that there exist genetically and morphologically diverse groups of *S. mentella* in the Northeast Atlantic waters despite seasonal mixing, indicating natal homing. Natal homing has been reported for a series of other fish species like Pacific salmon (e.g. Stewart *et al.* 2003; Neville *et al.* 2006), weakfish (Thorrold *et al.* 2001), Atlantic herring (Ruzzante *et al.* 2006) and also suggested for Atlantic cod (Wright *et al.* 2006). The present results show that natal homing is likely to occur in saithe and beaked redfish but not in Greenland halibut or golden redfish.

For the 'shallow' group beaked redfish, the F_{ST} estimate using GBS data between samples from the Faroe Islands and Norway was larger in males than females. This observation was congruent with the 'deep' group specimens from Greenland and the Faroe Islands, both for neutral and outlier SNPs. These findings suggest that migration in beaked redfish is mainly mediated by females, while males are more philopatric. The interpretation is in contrast with Shum *et al*. (2015) who suggested female philopatry in beaked redfish. Migratory behaviour in females is supported by their migration to larval extrusion grounds between March and May (Magnusson & Magnusson 1995), which is also supported by the present analyses of adult fish.

4.2.5 Introgression

The present investigation of introgression in redfish (paper 3) revealed greater extent of cross-species gene flow, mainly towards *S. mentella* from *S. norvegicus* and *S. viviparus*, in the Northeast Arctic than other waters. Given the differentiation of the Northeast Arctic sample, it is possible that introgression plays a role in driving such allele frequency differences. A consistent explanation has been given by Roques et al. (2002) for the 'Western component' of beaked redfish. In contrast, introgression observed within the *S. norvegicus* complex was much lower. The genetic analyses supported a more ancient divergence between the identified *S. norvegicus* clusters, suggesting possible occurrence of cryptic species (paper 4). The observed low to moderate extent of gene flow across *Sebastes* spp. implies incomplete reproductive isolation for these species possibly due to their close evolutionary relationship.

4.2.6 Sexual selection

Differentiation within golden and beaked redfish was much greater than within saithe and Greenland halibut. Compared to egg spawners like saithe and Greenland halibut, live bearers like redfish possess life history characteristics that can provide an effective mechanism towards genetic isolation (Cadrin *et al.* 2010). It is possible that ovoviviparity combined with selective mating and courtship behaviour (see Helvey 1982; Kendall 1991) are contributing to genetic isolation in *S. norvegicus* and *S. mentella*.

4.2.7 Time since divergence

The results herein show that the extent of genetic isolation in Greenland halibut and saithe are of the same order. As to redfish, the differentiation between the cryptic species of golden redfish is greater than that of the groups of beaked redfish. These observations might be correlated with the time since divergence of different gene pools, which is supported by the IM estimates (paper 3 and 4). Although the divergence between the *S. norvegicus* 'giants' and 'Norvegicus-B' is more recent than that between 'shallow' and 'deep' groups of *S. mentella*, the former pair exhibits more differentiation. This may be explained by the much smaller estimate of effective population size parameter for the 'giants'.

4.3 *Sebastes*: a genus illustrating rich genetic complexity?

This work identifies different *Sebastes* gene pools and their diverse levels of connectivity in different habitats throughout the North Atlantic. As to golden redfish, *S. norvegicus*, three formerly unrecognized cryptic species were described, implying greater redfish diversity than previously reported. The study of *S. mentella* revealed three distinct genetic groups: the 'shallow', 'deep' and 'slope'. Sub-structuring was evident within both the 'shallow' and 'deep' groups. For example, the 'shallow' fish from the Northeast Arctic, Irminger Sea, and Canadian waters appeared genetically different (paper 3), and the 'deep' fish from the Irminger Sea, Faroe Islands, and Canadian waters were different from one another (Appendix II). Population genetic structure of *S. mentella* has long been subjected to debate (see Cadrin *et al.* 2010; Cadrin *et al.* 2011; Makhrov *et al.* 2011). Given the observation of hybridization between *S. mentella* and *S. viviparus*, Artamonova et al. (2013) hypothesized that the 'deep pelagic' group is likely to be hybrids of contemporary redfish. Although the present findings recognize low to moderate extent of gene flow across the species, it is evident that all the identified gene pools (including the 'deep pelagic' group, and all sub-groups) maintain their genetic integrity. Hence, along with the 'slope' group, three genetic groups within *S. mentella* seem valid around the Irminger Sea, Greenland and Iceland waters.

The results based on GBS data for *S. mentella* indicated that selective forces may contribute to the species complex. The clustering of the reference 'shallow' and 'deep' samples by seven identified outliers is fully in line with the results from 1058 neutral SNPs and morphological data (Appendix II). The blasting of these outlier sequences revealed that three SNPs are possibly associated with the part of genome facilitating immunological responses. These findings may imply a depth associated local adaptation. Other studies of *S. mentella* (e.g. Magnusson & Magnusson 1995; Cadrin *et al.* 2010) have reported different rates of parasitic infestation for the 'deep' and 'shallow' groups. It is possible that, being subjected to selective forces, these two groups have different immunological response to parasites. The blasting of the outlier sequences disclosed annotation only for three outliers, where a low sequence similarity was observed. Nevertheless, the role of selection at those or

nearby loci cannot be ruled out. The differentiation observed between the 'shallow' and 'deep' groups of *S. mentella* is supported by a deep evolutionary divergence (paper 3) together with indications of local adaptation (Appendix II). These findings are in line with the results based on mitochondrial DNA (Shum *et al.* 2014; Shum *et al.* 2015). Therefore, the present findings may form the basis to recognize the 'deep' and 'shallow' groups of *S. mentella* as two evolutionarily significant units (ESU, Waples 1991). This claim is similar to that of Shum et al. (2015).

The present genetic study of beaked and golden redfish illustrates how individuals of the same species exploiting the same nursery ground can still be assigned to distinct biological populations, or even cryptic species. The observed intraspecific and interspecific diversity within the genus might have resulted from the combined effect of cryptic speciation, introgression, and local adaptation. It is possibly the unique life history of *Sebastes* spp. (Cadrin *et al.* 2010) and their recent evolutionary divergence in the North Atlantic (Sundt *et al.* 1998) that facilitates such a complex structure.

4.4 Greenland waters: a habitat where a rich genetic complexity of redfish is observed?

The present study unveils genetic complexity within the genus *Sebastes* in Greenland waters, which has long been unclear. First, the present results based on microsatellite and GBS data provide genetic evidence that supports the hypothesis of a mixing zone in this region. Interestingly, the results revealed that the commercial fleet operating in these waters mainly catches adult 'slope' *S. mentella* in the spawning season. Second, all the three proposed cryptic *S. norvegicus* species are found in these waters (paper 4). Finally, significant introgression is observed in Greenland waters (paper 3). The level of introgression was lower than that in the Northeast Arctic waters (paper 3) and that of Canadian waters (Roques *et al.* 2001). There is evidence that all the identified gene pools, including the three cryptic *S. norvegicus* species, maintain their genetic integrity despite the presence of some but low introgression. Greenland waters represent an important habitat for redfish since it provides nursing grounds for all groups of *S. mentella* and *S. norvegicus* (Magnusson & Johannesson 1995, present study). Morphological identification of juvenile redfish is generally difficult throughout the North Atlantic and tends to be even more problematic in Greenland waters (Johansen 2003). Johansen (2003) suggested that the species identification problems in Greenland waters may be linked to unrecognized hybridization. The present results suggest that introgression and cryptic speciation contribute to the difficulty in species identification in this region.

4.5 Are the management units of the studied species in line with their biological units?

The present investigation reveals disagreement between the management and biological units in all four selected species. First, multiple biological populations are prevailing within single management units (Papers 1, 3, 4). For instance, saithe from Halten Bank and the Barents Sea currently belong to the Northeast Arctic management unit (paper 1). The Rockall saithe, which appeared as a significant unit of its own, is managed as part of the North Sea stock. For beaked redfish (paper 3), both the 'shallow' and 'deep' groups were identified in Canadian waters which are not recognized in the management practice. As to golden redfish (paper 4), the possibility of cryptic species is ignored and the species is managed as a single population (ICES 2014b). Ignoring these distinct gene pools can cause overexploitation of the weaker component and such practice may reduce diversity and evolutionary potential of the species (Hauser & Carvalho 2008).

Second, there is evidence that single biological populations are managed as different stocks. For example, saithe from the North Sea, Faroe Islands and Iceland waters were not found to be genetically different from one another but they are managed separately. In *S. mentella*, the 'slope' group is found around both Iceland and Greenland waters, but are managed separately. Greenland halibut is today managed as three stocks, but only two populations are suggested by genetic studies (paper 2, Roy *et al.* 2014). The existence of a separate 'West Nordic' stock of Greenland halibut is not supported (paper 2, Roy *et al.* 2014). Management schemes that operate on more management units than actual populations may not have negative impact on populations, but such practices can reduce economic benefits (Waples 1998). However, the management boundaries are often based on political decisions which may not reflect agreement with biological populations, although the latter should be encouraged to ensure sustainable resource management. For *Sebastes*, a mechanical mixing zone around Greenland waters is evident. It was also apparent that the Rockall saithe may mix with the Faroe population as juveniles.

The present results provide a basis to revise the spatial management units of beaked redfish and Greenland halibut so that these better reflect the ecological and evolutionary units of the studied species. For saithe and golden redfish, it will be important to test the temporal stability of the observed genetic pattern before revision of the existing management units.

4.6 SNP: an efficient marker system for the population genomics of diverse species?

This work demonstrates the potential for SNP marker to investigate complex genetic patterns in wild marine species. Investigations in saithe, Greenland halibut, and beaked redfish show that a desired number of informative SNP loci can be detected and genotyped efficiently in diverse species. In this project, I applied restriction-site associated DNA (RAD) sequencing (Baird *et al.* 2008) to detect SNPs in saithe and Greenland halibut. For genotyping these fish in the selected SNP loci, the Sequenom Mass array platform was used. RAD

sequencing employs restriction enzymes to obtain the desired number of DNA fragments. The barcodes, amplification and sequencing primers are subsequently ligated to the fragments. The sequencing of these fragments is done following the sequencing protocol. The method provides an opportunity to detect large numbers of SNP loci, rapidly and cost-effectively, across the genome even in non-model organisms where reference genomes are absent (Baird *et al.* 2008). Sequencing technologies are rapidly evolving and the technique of genotyping by sequencing (GBS) represents a recent example of advancement in this field. GBS provides a simultaneous opportunity to identify and genotype a large number of loci across many individuals. Application of the GBS coupled with ddRAD sequencing (Peterson *et al.* 2012) in 277 *S. mentella* specimens resulted into a total of 1058 neutral and seven outlier SNPs, even with stringent selection criteria of a maximum of 20 % missing data per sample and one locus per sequence. The number of loci identified in beaked redfish was greater than for saithe and Greenland halibut. It is to mention that more than 40 000 SNPs are already available to analyze the cryptic speciation within the *S. norvegicus* complex for this project. The rapid and cost effective large-scale marker developments of SNP makes it preferred over conventional marker systems.

Genetic differentiation estimated in this study using SNP panels was always greater than that estimated using conventional marker systems. In saithe, the applied SNP panels identified four genetic clusters across the North Atlantic whereas neither mitochondrial DNA (Eiríksson & Árnason 2014) nor microsatellite and RAPD markers (Behrmann *et al.* 2015) could detect notable genetic patterns. In Greenland halibut, the F_{ST} differentiation observed was greater with SNPs than that quantified by microsatellites (Knutsen *et al.* 2007). Likewise, the F_{ST} differentiation quantified within *S. mentella* samples using SNP data was higher than that measured by conventional marker systems. However, the microsatellites used for saithe (Behrmann *et al.* 2015) and Greenland halibut (Knutsen *et al.* 2007) were not originally developed for those species. The F_{ST} differentiation estimated by microsatellites developed for Greenland halibut (Roy *et al.* 2014) was comparable with that of the present investigation. Given the internal mathematical dependence of F_{ST} on the number of alleles and their frequency and heterozygosity, higher estimates by SNP marker system may not be surprising (Jakobsson *et al.* 2013).

The investigation of *S. mentella* using both microsatellite and GBS data provided a direct opportunity to test for the resolution of two marker systems, and the results show higher resolution using genome-wide SNPs than microsatellites to explore genetic isolation in the species. The applied SNP panels accurately separated the reference samples from each other, and identified three genetic groups within the mixing region, i.e. Greenland waters (Magnusson & Johannesson 1995). The existence of the ‘shallow’ and ‘deep’ groups are in agreement with previous findings based on hemoglobin and allozyme marker systems (Johansen *et al.* 2000a), microsatellites (paper 3; Roques *et al.* 2002; Stefansson *et al.* 2009a; Shum *et al.* 2014), mitochondrial DNA (Shum *et al.* 2014; Shum *et al.* 2015) and morphological data (Magnusson & Magnusson 1995). Identification of the ‘slope’ group is also consistent with previous results based on microsatellite DNA (paper 3, Stefansson *et al.*

2009a). However, these genetic groups were better discriminated using GBS data than using any other marker system. This is most likely linked with high resolution power of the genome-wide SNPs. In addition, the results of fish population assignments using neutral SNPs and microsatellite data were highly congruent, with discrepancies in only 4 % of the individual fish analyzed. A portion of these discrepancies may be attributed to differences in the clustering methods used rather than marker systems, given that clustering using GBS data is based on DAPC approach whereas microsatellite data (paper 3) were analyzed with model based Bayesian methods.

The search for candidate SNPs under selection in beaked redfish and Greenland halibut revealed a number of outliers, but not in saithe. As mentioned earlier, it is possible that adaptive forces are involved in these loci or nearby sites. Loci linked to selection may provide an additional tool in the species' population genetic investigation. Adaptive loci can identify locally adapted populations which may be vulnerable to environmental changes. Thus, genetic patterns estimated by outliers should be considered carefully (Hauser & Carvalho 2008). Particularly, in *S. mentella*, assignment of individuals into the 'shallow' and 'deep' groups based on seven outliers was consistent with the assignment based on 1058 neutral SNPs (Appendix II). In line with adaptive divergence, the genetic differentiation quantified using these outliers was elevated in both Greenland halibut and beaked redfish. These loci may prove highly effective at assigning individuals to their population of origin (Nielsen *et al.* 2009), particularly in areas with high levels of mixing (e.g. Greenland waters). The ability to identify both neutral and adaptive loci using SNPs highlights the utility to this marker system in comparison with conventional neutral markers such as microsatellites (Helyar *et al.* 2011).

The applied SNP panels were effective at detecting low levels of genetic differentiation. Both in saithe and Greenland halibut, the SNP panels identified very low levels of genetic divergence for the given sample sizes. Nevertheless, the possibility of undetected population structure cannot be completely rejected. For example, the sample sizes for studying sex-biased migration in saithe were small which resulted into reduced detectability of genetic structure. Increased sample sizes may bring greater resolution to population genetic investigations, as suggested by Morin et al. (2009). In contrast, Willing et al. (2012) suggested increasing the number of loci is more effective at identifying population structure. Either way, it is important to ensure enough power for the evaluation of empirical data in population genomic studies (Ryman *et al.* 2006). Both in saithe and Greenland halibut, it is evident that χ^2 tests bring higher power than Fisher's method in estimating genetic differentiation, which is expected for the bi-allelic SNP marker system (Ryman *et al.* 2006). No power analysis was performed for the genome-wide SNP set.

5.1 Conclusions

This work introduces SNP-based investigation of saithe, Greenland halibut and beaked redfish. The applied population genomic methods efficiently dealt with the designated research questions, illustrating convincing progress in population genomics of non-model organisms. The study reveals previously unrecognized genetic patterns in four (golden redfish in addition to the aforementioned three) commercially exploited marine species which have continuous distribution throughout the North Atlantic. The results demonstrate that the identified gene pools of the species maintain distinct biological and/or ecological traits in different habitats despite a high potential for gene flow. The observed genetic complexity of the four species may be caused by an array of factors and adds support to the growing evidence that wild marine species are genetically more structured than previously believed.

The observed mismatch between management and biological units in all four species raises concern over their effective management and sustainable exploitation. In particular, beaked and golden redfish may be vulnerable to commercial exploitation since they display late maturation and slow growth rates. Hence, data like the present ought to be incorporated into management regimes to define management unit boundaries that are biologically meaningful. Management schemes that recognize each of these distinct gene pools may be more effective in preserving the evolutionary potential of the species.

The present investigation shows that SNP marker system efficiently delineates genetic population structure in saithe, Greenland halibut and beaked redfish. In the latter, both the neutral and adaptive SNP panels recognized the phenotypic distinction between the ‘shallow’ and ‘deep’ groups. Moreover, the results based on SNP data also identified both the adult and juvenile ‘slope’ group of beaked redfish in east Greenland waters, signifying this region as an important fishing area for this genetic distinct group. As a general conclusion, the results based on SNP data revealed genetic patterns of three species across the distribution ranges and on local scales. The present estimates of the genetic differentiation by SNP loci are greater than that by any other markers. The observed genetic complexity explored by SNPs supports promising opportunities for the marker system to investigate population genomics in other non-model organisms.

5.2 Perspectives

During the course of this study a substantial number of new SNP markers were developed but only a fraction of them were used for analyses of a limited number of samples. As to the studies based on microsatellite, more than 2500 fish collected from different years were analyzed. Future investigations of these four species should aim at achieving a rational sampling strategy in terms of detecting both spatial and possible temporal variation.

The present study of saithe comprises samples covering a relatively short time span and an overall low genetic differentiation is displayed. Since some species can exhibit different biological patterns at different times (Hellberg *et al.* 2002), it is important to investigate temporal stability of the genetic pattern for saithe. Thus, future sampling should be more extensive in order to possibly identify the exact location of genetic barriers. Sampling of spawning fish diminishes the problems connected with mechanical mixing of different populations, which seems evident in the present non-spawning samples. Regarding the SNP development in saithe, samples from distant locations may be included to minimize possible ascertainment bias (the present process is only based on the North Sea saithe). Within the frame of this project, SNPs have already been developed from the Barents Sea sample ready to be included in new analyses.

Also for Greenland halibut, sampling should be improved, such as inclusion of more spatial and temporal replicates of spawning fish. Unlike saithe, samples from three distant locations are included for the SNP development in Greenland halibut, which is likely to minimize ascertainment bias. Although the applied SNP panels show good power in revealing genetic patterns both in saithe and Greenland halibut, more SNPs can be included. Inclusion of more loci, in particular selective loci, may provide improved power to fish population assignment studies (Helyar *et al.* 2011).

For Beaked redfish, an extensive sampling has been carried out for microsatellite studies around Greenland waters. The genetic patterns identified using microsatellites are supported by results based on SNP markers genotyped for a limited number of the same fish. More samples should be taken from the neighbouring regions and more fish from individual samples should be genotyped for SNPs. Also from Canadian waters, more samples will be required to draw sound conclusions on beaked redfish structure in that area.

For golden redfish, GBS data are already available and will be analyzed within the frame of this research project. However, an improved sampling strategy may be required also for this species to assess temporal stability of the present findings. To define the distribution boundaries of the proposed cryptic species, more spatial samples should be included across the North Atlantic. Moreover, intensified studies of morphological (phenotypic) structuring, plus mating time and space, should be encouraged to provide a holistic knowledge of these cryptic species.

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Appendix I

Table 1 Details of the samples analyzed in the present study. Sampling locations, time and depth (meter) are presented. Fish length is measured in centimeter; sex ratio is given as % female and life stages as % adult. Juvenile *Sebastes* (Seb) were collected from both East and West Greenland. The *S. mentella* samples (M) from Greenland waters were collected both on surveys (R) and by commercial (C) vessels over two years. Q refers to sampling zones around Greenland (cf. Fig. 1, paper 3). *N* = sample sizes, N_{micro} = Number of fish analyzed using microsatellites (in paper 3) and NA = data not available. Sample codes in bold indicate that these samples have been analyzed both in paper 3 and 4. ^{S} = spawning sample, ^{A} = samples were pooled as no differentiation was found, ^{B} = sample consists of three sub-samples.

Species	Code	Location	Time	Sample Size (N)	Depth (m)	Length (cm)	Female (%)	Adult (%)
Pollachius virens (**paper 1**)	BS	Barents Sea	Aug, 2012	47	NA	53	NA	80
	HB^{S}	Halten Bank	Feb, 2013	48	140	NA	NA	100
	TAM^{S}	Tampen	Feb, 2012	48	NA	53	57	80
	VB^{S}	Viking Bank	Feb, 2011	48	NA	63	55	75
	NS	North Sea^{B}	July, 2010/2011	60	NA	54	41	80
	WS^{S}	West of Scotland	April, 2012	48	165	NA	NA	100
	ROC^{S}	Rockall	April, 2010/2011	48	150	98	57	100
	ICL	Iceland	Aug, 2005	50	NA	NA	51	NA
	FI	Faroe Island	Aug, 2011	48	185	NA	52	30
	FI^{S}	Faroe Island	Feb, 2012	48	179	NA	60	100
	CAN	Canada	July, 2006	31	NA	NA	53	NA
Paper 2								
Reinhardtius hippoglossoides	CAN	Canada	Aug/nov, 2010	39	963	29-82	NA	NA
	WGR	West Greenland	July, 2011	44	292-561	21-49	61	NA
	EGR	East Greenland	Aug, 2011	46	1330	38-98	26	NA
	ICE	Iceland	Oct, 2006	45	318-346	48-82	24	13
	NGR	North East Greenland	Sep, 2006	46	322-749	19-73	52	7

Table 1 contd.

Species	Code	Location	Time	Sample Size (N)	Depth (m)	Length (cm)	Female (%)	Adult (%)
Reinhardtius hippoglossoides	EGG04	Norwegian Slope 04	Nov, 2004	40	909	32-62	100	5
	EGG11	Norwegian Slope 11	Nov, 2011	39	690	42-65	0	8
	SVA	Svalbard	Sep, 2011	43	564	12-19	53	0
Paper 3								
S. mentella	M-Nor 1	Northeast Arctic	Oct, 2006	91	340	34	56	100
	M-Nor 2		2006, 2009[A]	155	445	34	59	100
	M-Nor 3		Mar, 2009	76	508	38	83	100
	M-Nor 4		Nov, 2011	91	575	38	60	100
	M-Oc	Irminger Sea	1995, 2001[A]	80	240	37	36	100
	M-Deep		2001	73	845	43	35	100
	M-ICL	Icelandic Shelf	Oct, 2001	59	651	37	50	92
	MC11Q2Q3	East Greenland	Mar, 2011	137	372	36	56	64
	MC11Q2		Mar, 2011	108	367	37	58	100
	MR11Q1Q2		Aug, 2011	80	348	31	48	31
	MR11Q3		Aug, 2011	69	462	32	38	71
	MR11Q5		Aug, 2011	49	430	31	45	61
	MR11Q5Q6		Aug, 2011	48	452	30	42	56
	MU11		Nov, 2011	26	NA	36	64	76
	M1C12Q2		May, 2012	49	375	34	45	99
	M2C12Q2		Feb, 2012	83	375	37	NA	100
	M3C12Q2		April, 2012	96	375	35	34	100
	MC12Q3		Feb, 2012	45	375	37	NA	100
	MSeb12WGL	West Greenland	Jun, 2012	91	325	13	NA	NA
	MR12Q2_6	East Greenland	Jun, 2012	93	227	32	48	28
	MSeb12Q2		Aug, 2012	92	561	12	NA	NA

Table 1 contd.

Species	Code	Location	Time	Sample Size (N)	Depth (m)	Length (cm)	Female (%)	Adult (%)	
S. mentella	MR12Q6		Aug, 2012	28	437	33	NA	98	
	MR12WGL	West Greenland	Jun, 2012	89	478	25	NA	33	
	MR12Q3	East Greenland	Aug, 2012	89	580	36	47	69	
	MR12Q4		Aug, 2012	81	471	29	60	84	
	MR12Q5		Aug, 2012	80	448	31	50	89	
	M-FC	Flemish Cap	July, 2001	95	495	28	55	35	
Paper 4									
S. norvegicus	**Nor-Nor**	Northeast Arctic	Oct, 2001	41	195–417 (258)	29–54 (36)	15	100	
	Nor-GL A/ EGN	East Greenland	Feb, 2011	108	365–370 (367)	26–57 (38)	58	87	
	Nor-GL B/ EGS		Aug, 2011	70	188–332 (239)	17–49 (28)	44	29	
	Nor-WGL	West Greenland	Jun, 2012	49	521 (521)	43–62 (54)	65	100	
	Nor-Giant	East Greenland	Aug, 1996	17	594–786 (692)	71–84 (79)	82	100	
Reference samples									
S. viviparus	**VV-Ice**	Iceland	Mar, 2001	53	155–305 (175)	11–27 (18)	47	45	
	VV-Nor	Northeast Arctic	1992, 2001[A]	26	75–170 (137)	14–24 (20)	NA	100	
S. fasciatus	**Fasc**	Flemish Cap	Oct, 2001	45	240–322 (295)	15–31 (21)	62	36	
Appendix five (GBS data)									N_{micro}
S. mentella	Nor A[A]	Northeast Arctic	Mar, 2009	18	508	33–42 (38)	61	100	17
	Nor B[A]		Nov, 2011	20	575	35–42 (40)	75	100	20
	FI-shallow	Faroe Islands	Sep, 2002	38	415	36–49 (44)	71	100	-
	FI-deep		Sep, 2002	28	572	43–52 (49)	14	100	-
	IS-deep	Irminger Sea	July, 2001	26	830–850 (840)	36–47 (42)	NA	100	23
	GL A	East Greenland	Aug, 2011	26	355–455 (425)	19–45 (30)	46	27	26
	GL B		Aug, 2011	25	423–430 (429)	20–40 (32)	52	49	25
	GL C		Aug, 2012	96	473–869 (580)	28–38 (34)	43	77	85

Appendix II

Excerpts of manuscript in preparation: Genetic structuring of *S. mentella* based on genome-wide SNP data (unpublished data)

Background

S. mentella is a commercially exploited marine species across the North Atlantic. The number of genetic groups, their distribution and connectivity within the species has long been a matter of dispute (see Cadrin *et al.* 2010; Cadrin *et al.* 2011; Makhrov *et al.* 2011). Based on the available genetic evidences, data on life history traits and morphologies, three genetic groups have been reviewed within the Northeast Atlantic by Cadrin *et al.* (2010). The 'shallow' group is described between 200 and 500 m depth across the North Atlantic, while the 'deep' group has mainly been reported between 550 and 800 m depth in the Irminger Sea. These two groups also exhibit distinct morphological characteristics. A third group, 'slope', has been mentioned along the continental slope of Icelandic Shelf (Cadrin *et al.* 2010), although its identity has not been established due to insufficient genetic studies. The genetic connectivity of these three groups with *S. mentella* across the Northeast Atlantic is not fully resolved.

Population genetic structure of *S. mentella* has sometimes been contradictory for different molecular marker systems (for review see Cadrin *et al.* 2010). In this regard, genome-wide SNP data may bring higher resolution, as evident in other studies (e.g. Larson *et al.* 2014a; Larson *et al.* 2014b), to disclose the level of reproductive isolation among different genetic groups and to determine the effect of natural selection. Genotyping by sequencing (GBS) provides a novel approach to identify and genotype a large number of SNP loci throughout the genome (Narum *et al.* 2013), and therefore it may serve as an effective tool to delineate biocomplexity within the species. The main objectives of this investigation are to test genetic structure of *S. mentella* using genome-wide SNP data and to compare the results with that of microsatellite DNA (paper 3).

Principal materials and methods

A total of 277 *S. mentella* specimens were collected from eight locations across the Northeast Atlantic (Fig.1, Table 1). Samples of 'deep pelagic' (= 'deep') *S. mentella* group were collected from the Irminger Sea and the Faroe Islands. The 'shallow pelagic' (= 'shallow') group specimens were caught above 500 m water depth from the Faroe Islands. Samples from east Greenland and Northeast Arctic (Norwegian) waters were included from

two different years. The adults (≥ 29 cm) and juveniles (4–28 cm) were defined according to length (Drevetnyak & Nedreaas 2009). DNA was extracted from the gill filaments using an E-Z 96 Tissue omega DNA Kit (Omega Bio-Teck Inc.) protocol. DNA quantity was assessed using a broad range double-strand DNA reagent kit (Life Technologies Corp.), and quality was checked by agarose gel electrophoresis. A total of 196 of these fish were analyzed and clustered into 'shallow', 'deep' and 'slope' groups using 13 microsatellites (paper 3).

Table 1 Details of the *S. mentella* samples analyzed using GBS data. The sex ratio as % female and life stages are presented as % adult (≥ 28 cm). Sampling depths and fish lengths (mean in brackets) are provided. Code = Sample code, *N* = sample sizes, N_{micro} = number of fish analyzed using 13 microsatellites (paper 3), NA = Data not available (cf. Fig. 1).

Location	Code	Lat/Long	Time	*N*	N_{micro}	Depth (m)	Length (cm)	Female (%)	Adult (%)
Northeast Arctic	Nor A*	66.93/ 8.17	Mar, 2009	18	17	508	33–42 (38)	61	100
	Nor B*	69.38/ 15.14	Nov, 2011	20	20	575	35–42 (40)	75	100
Faroe Islands	FI-shallow	62.78/ -6.62	Sep, 2002	38	-	415	36–49 (44)	71	100
	FI-deep	60.17/ -7.84	Sep, 2002	28	-	572	43–52 (49)	14	100
Irminger Sea	IS-deep	62.05/ -27.08	July, 2001	26	23	830–850 (840)	36–47 (42)	NA	100
East Greenland	GL A	61.14/ -41.46	Aug, 2011	26	26	355–455 (425)	19–45 (30)	46	27
	GL B	64.28/ -35.70	Aug, 2011	25	25	423–430 (429)	20–40 (32)	52	49
	GL C	62.2/ -40.67	Aug, 2012	96	85	473–869 (580)	28– 38 (34)	43	77

*The samples were pooled for downstream analyses, since no differentiation was found.

As a genotyping by sequencing (GBS) approach, ddRAD sequencing (Peterson *et al.* 2012) was applied for this investigation. After preparation of the library, sequencing was performed using a MiSeq (Illumina, California, USA) and 500 cycles MiSeq Reagent Kit v2. The forward reads were subsequently analyzed using STACKs pipeline. To test for the neutrality of the loci, island models allowing differences in the population sizes (Foll & Gaggiotti 2008) were applied. The tag sequences containing outlier SNPs were blasted in the NCBI database using the 'BLASTN' window to explore their possible association with any functional parts of the genome. Both model based (Pritchard et al. 2000) and non model-based (Jombart *et al.* 2010) clustering approaches were used for the clustering of individuals in the study.

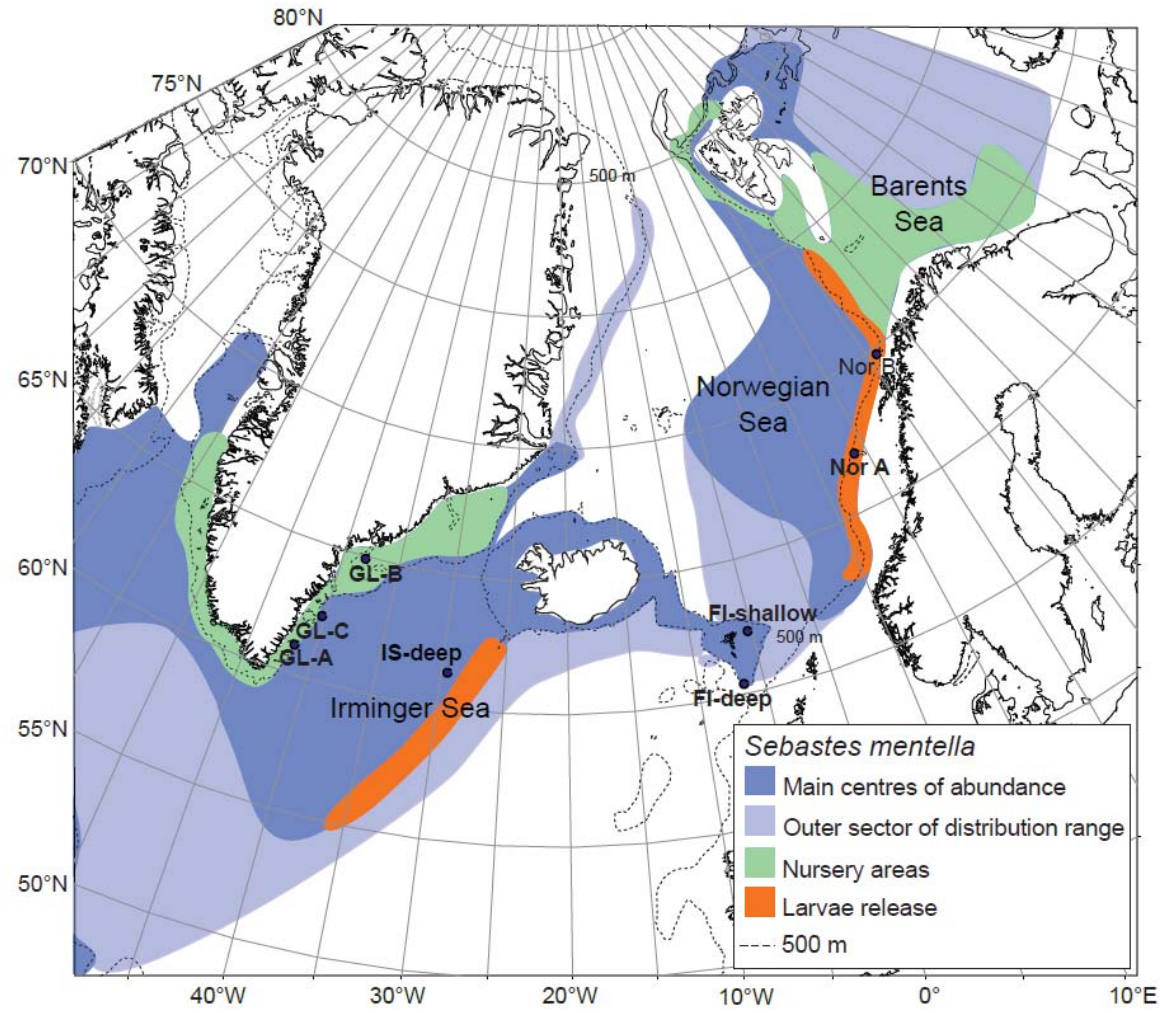


Fig. 1: Sampling of *S. mentella* across the Northeast Atlantic. The distribution pattern of the species is illustrated, whereas the sampling locations for the study are shown with black circles. The map has been modified from Planque *et al*. (2013).

Results

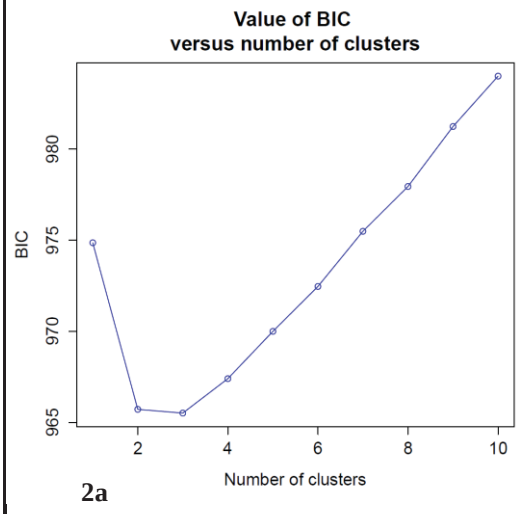
More than 35 000 SNPs were initially identified in 277 *S. mentella* specimens using the GBS approach. A total of 5047 loci were retained allowing a maximum of 20 % missing data per sample. Finally, a total of 1065 SNPs were selected after excluding all but one locus per sequence tag (descriptive statistics on these loci are available on request). The genome scan approach identified seven of these loci as outliers. When blasted in the NCBI database, three outliers were found associated with genes involved in immunological response in rock bream (*Oplegnathus fasciatus*). Consistent with the microsatellite study, neutral SNP data identified three genetic groups of *S. mentella* (Fig. 2). All the three genetic groups were

present in Greenland waters. In contrast, the seven outliers suggested a total of two genetic groups for the sample set (Fig. 3). The F_{ST} differentiations estimated between the 'shallow' and 'deep' groups by the seven outliers were much greater than those by neutral loci (greatest F_{ST} = 0.46, Table 2). Although the clustering analyses clustered fish from different waters into single genetic group, the F_{ST} estimates suggested significant differentiation between them (e.g. fish from Greenland and the Faroe Island waters). Such sub-structuring was estimated within both the 'shallow' and 'deep' groups, supported by both the neutral and outlier SNPs (Table 2).

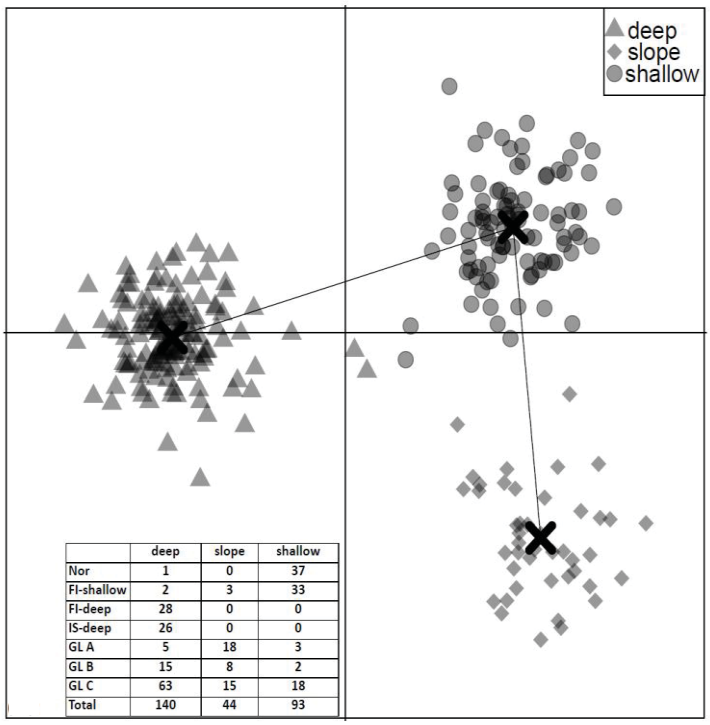
Table 2 Pair-wise F_{ST} (Weir & Cockerham 1984) values between different *S. mentella* sample pairs after clustering by DAPC using 1058 neutral SNPs. The 'shallow', 'deep' and 'slope' groups identified from different sampling locations (cf. Table 1) are compared. Below the diagonal, the F_{ST} estimates using 1058 neutral SNPs are presented. Above the diagonal, the F_{ST} estimates using seven outlier SNPs are presented. All F_{ST} estimates, except those in bold, are significant (P = 0.05) even after FDR (= 0.05) control. N = sample sizes.

	N	**Nor-shallow**	**FI-shallow**	**FI-deep**	**IS-deep**	**GL-shallow**	**GL-deep**	**GL-slope**
Nor-shallow	37	-	**-0.006**	0.395	0.462	0.053	0.427	0.235
FI-shallow	33	**-0.002**	-	0.387	0.455	0.050	0.415	0.220
FI-deep	30	0.041	0.034	-	**0.008**	0.299	0.014	0.348
IS-deep	26	0.043	0.034	0.008	-	0.372	**0.002**	0.386
GL-shallow	23	0.005	0.006	0.044	0.045	-	0.311	0.086
GL-deep	83	0.037	0.028	**-0.001**	**0.002**	0.040	-	0.321
GL-slope	41	0.034	0.029	0.056	0.060	0.035	0.055	-

Population assignment of the 196 fish using SNP data were consistent with results based on microsatellites. When the DAPC clustering results using 1058 neutral SNPs (Fig. 2b) were compared with the Bayesian clustering outputs using 13 microsatellites, there were full agreement in 178 cases (91 %). Partial agreement, meaning that SNP data suggest pure individual while microsatellites suggest admixed individual, was observed in eleven cases (6 %). Results from the two marker systems disagreed for only seven fish (4 %). The clustering of the morphologically defined reference samples using the neutral SNP panel, outlier SNP panel and microsatellite DNA was in agreement.



2a



	deep	slope	shallow
Nor	1	0	37
FI-shallow	2	3	33
FI-deep	28	0	0
IS-deep	26	0	0
GL A	5	18	3
GL B	15	8	2
GL C	63	15	18
Total	140	44	93

2b

Fig. 2: DAPC analysis (Jombart *et al*. 2010) of the *S. mentella* samples using 1058 neutral loci. BIC value (2a) supported three clusters (2b) for the given dataset. Population assignments of the number of fish from different samples are presented (Fig. 2b). Number of PCA used = 92, Number of discriminant functions retained = 2, Variance conserved = 71 %.

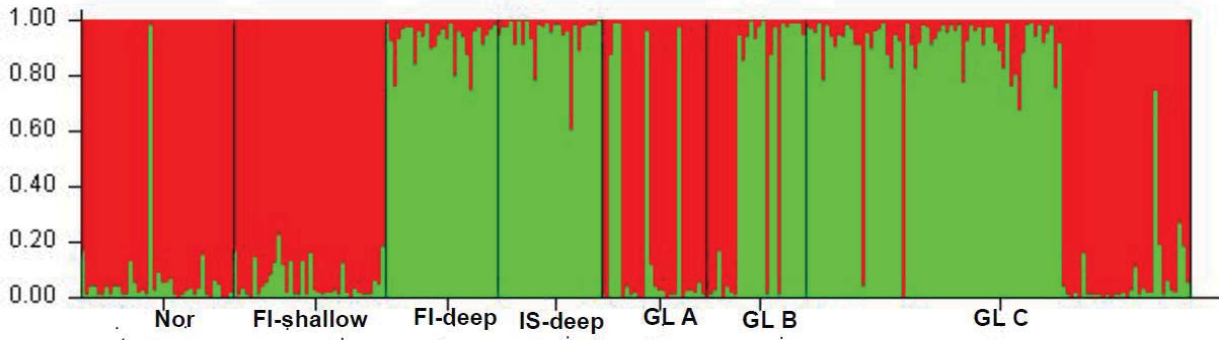


Fig. 3: Bayesian population clustering (Pritchard et al. 2000) of *S. mentella* samples using seven outliers. The analysis suggested a total of two genetic groups in the dataset, as indicated by red and green colors. The clustering of the reference samples (FI-shallow, FI-deep and IS-deep) was consistent with results using the neutral SNPs and microsatellites (cf. Table 1).

Appendix III

Box 1 Definition of Gene pools

In this work, different terms have been used to recognize the gene pools within species depending on the estimated genetic connectivity or differentiation. The purpose of this task is not to establish terminologies, rather to express diverse extent of connectivity of different gene pools.

- **Population/ Biological population/ genetic population/ biological unit/ component** has been defined as reproductively isolated groups of individuals of the same species at a given time and space (Waples & Gaggiotti 2006). For example, the identified four saithe populations and two Greenland halibut populations.

- The term '**group/ genetic group**' has been used to refer to the 'shallow pelagic' and 'deep pelagic' beaked redfish identified from the Irminger Sea and adjacent waters (Stefánsson *et al.* 2009b, Shum *et al*. 2014, Shum *et al.* 2015). Here, 'groups/ genetic groups' imply more differentiated gene pools than 'populations'. For instance, the 'shallow' group beaked redfish (paper 3) is composed of three populations: the Northeast Arctic, Irminger Sea, and Canadian populations. Likewise, the 'deep' group beaked redfish from the Irminger Sea, Faroe Islands, and Canadian waters represent three distinct populations. 'Group' has also been used to refer to the 'slope' beaked redfish identified in Greenland and Iceland waters.

- For the recognition of **Evolutionary Significant Unit (ESU)**, it has been considered necessary to identify reproductive and historical isolation, and adaptive distinctness as suggested by Waples (1991). For example, the 'shallow pelagic' and 'deep pelagic' groups of beaked redfish have been claimed as two ESUs.

- **Cryptic species** are two or more species that are categorized as a single species as no apparent morphological distinction has been established (Bickford *et al.* 2007). In this work, the gene pools have been defined as possible 'cryptic species' if their extent of reproductive isolation, quantified through gene flow and genetic distances, was larger than or comparable with that between the established species (see De Queiroz 2007).

Paper 1

Seascape genetics of saithe (*Pollachius virens*) across the North Atlantic using single nucleotide polymorphisms

Saha, A., Hauser, L., Kent, M., Planque, B., Neat, F., Kirubakaran, Tina Graceline, Huse, I., Homrum, E.I´., Fevolden, S-E., Lien, S., and Johansen, T

ICES Journal of Marine Science, 72: 2732–2741

Paper 2

SNP markers from RAD sequences reveal management relevant genetic patterns in Greenland halibut (*Reinhardtius hippoglossoides*)

Westgaard, J-I., Saha, A., Kent, M., Hansen, H. H., Knutsen, H., Hauser, L., Cadrin, S. X., Albert, O. T., and Johansen. T

Canadian Journal of Fisheries and Aquatic Sciences, In review

Paper 3

Geographic extent of introgression in *Sebastes mentella* and its effect on genetic population structure

Saha, A., Johansen, T., Hedeholm, R., Nielsen, E. E., Westgaard, J-E., Hauser, L., Planque, B., Cadrin, S. X., Boye, J

Evolutionary Applications, In review

Paper 4

Cryptic *Sebastes norvegicus* species in Greenland waters revealed by microsatellites

Saha, A., Hauser, L., Planque, B., Fevolden, S-E., Hedeholm, R., and Johansen, T

ICES Journal of Marine Science, In review