Species and population differentiation in the North Atlantic *Sebastes*. A study of mtDNA variation

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A Dissertation submitted in partial satisfaction of the requirements for the MS degree in population genetics

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by

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I declare that this dissertation is based on my own observations, that it is written by myself, and that it has not previously been submitted in part or in whole for a higher degree.

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Abstract

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Sequence variation of a 420 base pair fragment of the mitochondrial cytochrome *b* gene was analyzed in *Sebastes marinus* and *Sebastes mentella*. In addition, sequence variation of a 567 base pair fragment of the same gene was analyzed in *Sebastes viviparus*. The majority of haplotypes are shared between *S. mentella* and *S. marinus* but none are shared with *S. viviparus*. Certain forms or types of *S. mentella* found in the Irminger Sea are argued by some to be separate stock units defined as "oceanic" and "pelagic" types. Strong controversies exist over whether these types represent more than one population. Here, differentiation was observed between these two types and between them and the main or "demersal" form of *S. mentella*. Differences were also observed between the main species *S. mentella* and *S. marinus*. The species share a number of haplotypes and observed differences are based on differences in haplotype frequencies. Incorrect classification can cause the observed polyphyly. Alternatively, and more likely, the high observed polyphyletic pattern suggest ancient lineages that still are retained in present day populations. The non-sharing of haplotypes found within *S. viviparus* defines it as a monophyletic group and clearly separates it from the other two species, with estimated time since divergence $700,000$ years. Based on the observed polyphyly for *S. mentella* and *S. marinus*, incomplete lineage sorting of ancient polymorphism is strongly supported with very recent divergence times, approximately $19,000$ years based on assumptions of neutral theory the estimated divergence times among the "demersal" form of *S. mentella* and the subgroups in the Irminger Sea is very recent, or $4,200$ years.

Professor Einar Árnason
Chair, Committee in charge

Útdráttur

Tegundir og aðskilnaður stofna *Sebastes* í Norður Atlantshafi. Rannsókn á erfðabreytileika í hvatberaerfðaefni

eftir

Svövu Ingimarsdóttur
MS í Stofnerfðafræði

Háskóli Íslands

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Erfðabreytileiki DNA hvatbera (mtDNA) innan *S. marinus* og *S. mentella* var metinn með raðgreiningu. Svæði innan cytochrome *b* gensins var raðgreint, alls 420 basapör. Auk þess var erfðabreytileiki fyrir sama gen kannaður hjá *S. viviparus* með raðgreiningu á 567 basapörum. *S. mentella* og *S. marinus* deila með sér flestum setgerðunum sem fundust en engin af þessum setgerðum finnast hjá *S. viviparus*. Sérstökum formum eða stofnum innan *S. mentella* sem finnast í Irmingerhafi, hefur verið lýst sem aðskildum stofnum. Það eru úthafskarfi og djúp-úthafskarfi. Miklar deilur eru um hvort þessir stofnar séu aðgreindir eða hluti af einum og sama stofninum. Niðurstöður sýna aðgreiningu á milli þessara stofna og á milli þeirra og *S. mentella*. Aðgreining finnst einnig á milli *S. mentella* og *S. marinus*. Hóparnir deila mörgum setgerðum og sú aðgreining sem finnst byggist á mismunandi tíðni setgerða í hópunum. Há tíðni á fjölætta setgerðum gæti bent til rangrar flokkunar til tegunda. Önnur og mun líklegri ástæða fyrir slíku mynstri bendir til fornra setgerða sem enn finnast í stofnum þessara fiska í dag. *S. viviparus* aðgreinist greinilega frá hinum og breytileiki innan hans er því einætta. Aðskilnaður *S. viviparus* frá hinum tveimur er metinn um 700 þúsund ár. Miðað við þann fjölætta strúktúr sem finnst hjá *S. marinus* og *S. mentella* er ófullgerð aðgreining setgerða talin helsta skýringin. Aðskilnaður þeirra er talinn vera mjög nýlegur, eða um 19 þúsund ár. Aðskilnaður á milli *S. mentella* og undirhópa í Irminger hafi er einnig mjög nýlegur, eða um 4 þúsund ár.

Professor Einar Árnason
Formaður umsjónarnefndar

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Introduction

Many species concepts have been proposed during the past decades (COYNE and ORR, 2004). Among the widely adopted are concepts involving reproductive isolation or phenotypic distinctness of populations, such as the biological species concept (BSC) (MAYR, 1963; FUTUYMA, 1998). Another concept, the phylogenetic species concept (PSC), aims to resolve the phylogenetic history of organisms (FUTUYMA, 1998). Related to the PSC, is the genealogical species concept (GS) proposed by BAUM and SHAW (1995). This concept defines species as a basal, exclusive group of organisms, meaning that each individual in a GS is more related to individuals belonging to the same GS (HUDSON and COYNE, 2002). By this criteria, two groups are recognized as distinct when they are reciprocally monophyletic (HUDSON and COYNE, 2002).

The large issues regarding mechanisms of speciation involve the importance of geographical isolation and the impact of selection and genetic drift. In addition, important factors such as gene flow and hybridization events can have great effects (COYNE and ORR, 2004). Allopatric speciation is probably the most common mode of speciation in which gene flow between populations is reduced by geographic or habitat barriers (FUTUYMA, 1998). Another major mode of allopatry called peripatric speciation or founder effect, describes a small subpopulation that settles in a new habitat along the periphery of a species range (MAYR, 1963; FUTUYMA, 1998). When divergence is initiated without geographical barriers to gene flow and proceeds along a spatial scale, reproductive isolation emerges. This has been defined as parapatry (FUTUYMA, 1998). Sympatric speciation occurs if a biological barrier to gene exchange arises within a population. This mode of speciation have been questioned by some or most authors which have argued that spatial segregation is necessary for speciation (FUTUYMA, 1998).

It is apparent that various modes of speciation mechanisms exists and organisms with very different life history traits are likely to speciate in very different ways (PALUMBI, 1992). Many terrestrial animals are characterized by relatively small population sizes and low dispersal, and in many cases models of natural selection and gene flow correspond reasonably

well with divergence patterns observed in natural populations (PALUMBI, 1992). However, speciation in the marine environment is likely to be different than on land because there are few absolute barriers to gene flow in the sea (PALUMBI, 1994). In addition, allopatric speciation is challenged in many marine taxa, especially those with high fecundity and larvae that drift long distances (PALUMBI, 1994).

High dispersal species in the marine environment are generally characterized by large population sizes, rapid gene flow, and by being distributed over large areas (PALUMBI, 1992). These characters tend to limit genetic differentiation and to slow down the speciation process in the sea due to fewer barriers to gene flow (e.g. PALUMBI, 1992; DAWSON and JACOBS, 2001). However, molecular studies have revealed cryptic species in many marine taxa (e.g. ROCHA-OLIVARES *et al.*, 1999; DAWSON and JACOBS, 2001), suggesting high biodiversity in the marine environment. Thus opportunities for speciation have been more frequent than generally recognized (DAWSON and JACOBS, 2001). In the case of recent speciation events, difficulties can arise in investigating speciation at the molecular level because criteria of reciprocal monophyly do not hold (WANG *et al.*, 2008). In this case, insufficient time has passed for putative species to develop distinctive lineage differences between them (FUNK and OMLAND, 2003).

Documentation of species-level polyphyly suggests this phenomenon to be far more widespread among closely related taxa than previously thought (FUNK and OMLAND, 2003). In this case, some DNA sequence alleles for a given species, may be more closely related to alleles of another species. Upon studying the mitochondrial genomes (mtDNA), one may expect to encounter this phenomenon. It can be described as showing one of three general phylogenetic patterns (HEDRICK, 2005). First, reciprocal monophyly, where two populations have been separated without gene flow for some time, in which a higher within-population than between-populations similarity is expected for all sequences. Second, a state called polyphyly, in which some lineages within a population are more closely related to lineages in another population than to lineages in their own population. This could result if the two populations have recently separated and there has not been enough evolutionary time for the lineages to sort independently and alternative alleles to become fixed in different populations. This can also be observed in case of recent or ongoing hybridization between populations. The third category is paraphyly, generally thought of as the transitional state between reciprocal monophyly and polyphyly. In this case, lineages in one of the population form a monophyletic group and are nested within the broader phylogenetic group (the other population) which contains all of the lineages in both present-day populations. In some cases, however, observed polyphyly is

regarded as a taxonomical error and frequently is dismissed. In these instances, individual specimens do not fit the reciprocal monophyly at the study locus. When using mtDNA to study population differentiation among closely related species, polyphyletic patterns can be observed due to recent speciation events (FUNK and OMLAND, 2003). This situation becomes highly likely when ancestral lineages are polymorphic and random sampling of the same or similar mtDNA haplotypes predate speciation event (MEYER, 1994).

The project "The Barcode of Life Initiative" (BOLI) proposes species discrimination based on very short gene sequences (Barcode of Life Initiative). The method is based on the retrieval of a short DNA sequence from the mtDNA cytochrome *C* oxidase I (COI) (e.g. HEBERT *et al.*, 2003; HAJIBABAEI *et al.*, 2007; MEYER and GUSTAV, 2005). Documentation of high accuracy of genetic barcoding using mitochondrial markers have been put forward on well defined phylogenetic groups (e.g. HAJIBABAEI *et al.*, 2007; WARD *et al.*, 2005). However, closely related species can show high levels of polyphyly and therefore, might not be differentiable by barcoding techniques (FUNK and OMLAND, 2003).

Fisheries scientists have long been concerned about the dynamics of population structure of exploited fish with the purpose of sustaining the resource (CARVALHO and HAUSER, 1994). Different management strategies could affect the population of a species being exploited and failure to recognize population structure can lead to over exploitation of some stocks and under exploitation of other stocks (CARVALHO and HAUSER, 1994). Determination of population structure and stock components of any fish species is a complex task and numerous methods have been applied in order to gain insight into this pattern. Among the methods used are data on geographic and temporal distributions, meristic and morphometric characters, life history traits, and infestation of parasites (SCHMIDT, 2005). In addition, molecular methods have proven their usefulness in species identification and systematics and fisheries biologists have increasingly used genetic approaches to investigate the inter- and intraspecific structure of commercially exploited fish species (SHAKLEE and CURRENS, 2003).

The redfish species (genus *Sebastes*) are members of a most species rich family Scorpaenidae, with close to 110 species worldwide, with most of the species residing in the North Pacific (NELSON, 1984). The *Sebastes* species have attracted attention because of species richness and ecological diversity in form and function (HYDE and VETTER, 2007). They can be found in shallow waters around 100 m to depths in excess of 1400 m. This highly speciose group also shows great diversity in body shape, size, and head spination (HYDE and VETTER, 2007). The forms range from elongated to deep bodies, strong or heavily reduced head spination, and often striking color patterns (HYDE and VETTER, 2007). For such a highly speciose

genus, intermediates are common between these morphological extremes. At least 70 species are found throughout the Northeast Pacific (LOVE *et al.*, 2002), and a wide array of recently evolved sibling species has been documented (ORR and BLACKBURN, 2004). The genus *Sebastes* is believed to have originated in the Northwest Pacific in the middle Miocene some 15 million years ago (WOURMS, 1991; HYDE and VETTER, 2007) with substantial diversification in the late Miocene (5 million years ago) (HYDE and VETTER, 2007). These well defined paleogeographic colonization events can serve as calibration points for molecular clocks (HYDE and VETTER, 2007).

The Trans-Arctic interchange during the Late Pliocene allowed marine species in the Pacific and the Atlantic to interchange with the opening of the Bering Strait approximately 4–3.5 million years ago (VERMEIJ, 1991). Access to the Arctic basin was allowed during this high latitude warming and numerous Pacific taxa are documented to have made the journey into the North Atlantic (HYDE and VETTER, 2007). Among them was the ancestor of the *Sebastes alutus* clade which successfully colonized the North Atlantic, eventually forming the four closely related North Atlantic *Sebastes* species (HYDE and VETTER, 2007).

The species currently found in the North Atlantic consist of a complex of four taxa: *S. mentella*, *S. marinus*, *S. fasciatus* and *S. viviparus*. They are widely distributed throughout the North Atlantic with the exception of *S. fasciatus* and *S. viviparus* which are essentially restricted to the Northwest Atlantic and to the Northeast Atlantic respectively (BARSUKOV *et al.*, 1991). *S. mentella* is usually found at depths between 500–800 m, but it has been found both at shallower (200 meters) and at deeper waters (around 1000 m; MAGNÚSSON, 2000a). *S. marinus* is mostly found between 100–400 m but it too has been found at greater depths (MAGNÚSSON, 2000b). The distributions of the two most common species, *S. mentella* and *S. marinus*, thus are overlapping in large geographic areas and in sympatry their populations contribute to mixed fisheries (ROQUES *et al.*, 1999). *S. viviparus*, the smallest of the redfish species, is usually found at shallower depth range than *S. mentella* and *S. marinus* (JOHANSEN *et al.*, 2002). All redfish species in the North Atlantic are commercially important except *S. viviparus* because of its small size (JOHANSEN *et al.*, 2000b).

The genus *Sebastes* are generally characterized by long lifespans and slow growth rate compared to other bony fishes (GASCON, 2003). Redfishes have internal fertilization with the developing embryo receiving nourishment from the female and are thus considered viviparous (LOVE *et al.*, 2002). Mating takes place in autumn and the female retains the sperm for several months until the eggs are fertilized. Larvae develop within mother from February to April (MAGNÚSSON and MAGNÚSSON, 1995). Extrusion of larvae, 5–7 mm in size for *S.*

marinus and 7–8 mm for *S. mentella*, occurs from April to June. Each female extrudes between 40 and 400 thousand individual larvae (MAGNÚSSON and MAGNÚSSON, 1995). Redfishes are slow growing and generally mature at an age of 14–17 years in the North Atlantic. However, even later maturation ages, or 18–20 years, have been documented in the waters of the Faroe Islands (REINERT, 1998). These biological features make redfish considerably different from most other commercially exploited fish.

A large controversy, and an ongoing debate, exists concerning the stock structure of *S. mentella* in the Irminger Sea and adjacent waters. Different hypothesis have been put forward as to whether it is composed of one, two, or even three stocks (e.g. ICES C.M, 1998; SABORIDO-REY *et al.*, 2005; SCHMIDT, 2005). The exploitation of *S. mentella* in the North Atlantic was initially carried out on the continental shelves and banks of the Faroe Islands, Iceland, and East Greenland. It was believed to constitute of a single stock unit (deep-sea *S. mentella*) (SABORIDO-REY *et al.*, 2005). To avoid confusion, the "deep-sea" *S. mentella* will be referred to as the "demersal" type of *S. mentella* in this thesis. The existence of a second stock, the "oceanic" redfish was first described by MAGNÚSSON (1972) but it was not commercially exploited until 1982 when a new pelagic fishery started in the Irminger Sea (MAGNÚSSON and MAGNÚSSON, 1995). The "oceanic" stock is distributed over wide depth ranges, but mostly found at depths less than 400 m (MAGNÚSSON and MAGNÚSSON, 1995). The "oceanic" stock differs from the "demersal" type of *S. mentella* by several morphological characters. It has been distinguished from other *S. mentella* by darker complexion, dark and/or red-orange patches on the skin, and heavy infestation of parasites (MAGNÚSSON and MAGNÚSSON, 1995).

The redfish fishery moved to deeper waters (below 500 m) in the Irminger sea in the early 1990s and following that the existence of a possible third stock unit was suggested by some researchers (e.g. ICES C.M, 1998; SABORIDO-REY *et al.*, 2005). These fishes were considered to be different from those living above 400 m and to resemble more the "demersal" type living on the continental shelves (SABORIDO-REY *et al.*, 2005). This new type has been called the "pelagic" *S. mentella* to distinguish it from the "demersal" type found on the edges of the continental shelves (e.g. ICES C.M, 1998; SABORIDO-REY *et al.*, 2005). Separating these three types based on morphological and biological characteristics requires considerable experience. Currently only Icelandic scientists claim such experience (JOHANSEN *et al.*, 2000a). Apart from difficulties in distinguishing between the types of *S. mentella*, difficulties have also risen in separating the two species, *S. mentella* and *S. marinus*. A high morphological similarity between these two species has been observed in overlapping areas and a combination of

characters also is needed to separate them (MAGNÚSSON and MAGNÚSSON, 1995). Nonetheless, species identification based on phenotypic characters is apparently not straightforward and many individual fish have thus been classified as unknown *Sebastes* species under both field research and commercial exploitation.

A new form of *Sebastes* has been documented by some authors (e.g. JOHANSEN *et al.*, 2000b; SCHMIDT, 2005). These fishes have an average total length of 60 cm and are referred to as "giants". They resemble *S. marinus* in morphology and fisheries for this group started in 1996 along the Reykjanes Ridge below 500 m (JOHANSEN *et al.*, 2000b). An indication that "giants" represent a genetically differentiated group is suggested by JOHANSEN *et al.* (2000b) as they differ in hemoglobin patterns from those normally found in *S. marinus* and *S. mentella*.

Given the complexities in identifying the groups, the possibility of mis-classification into species cannot be ruled out. In addition, possible hybridization between redfish species complicates species discrimination even further (SCHMIDT, 2005). The potential for mis-classification complicates genetic analysis, especially for small number of data. This could partly be avoided by large number of sampling which could allow a statistical evaluation of the possibility. However, species identification based on morphology remains a difficult task due to overlapping characters (PAMPOULIE and DANÍELSDÓTTIR, 2008) for many species of fish and also for some of the *Sebastes* species.

Because of the interesting life-history characteristics of the *Sebastes* species, they have been the focus of much research. Molecular based studies have been used for the past decade to investigate taxonomic status and population structure of redfish in the North Atlantic (SCHMIDT, 2005). Heterogeneity has been observed between the "pelagic" and the "oceanic" types of *S. mentella* in the Irminger Sea based on hemoglobin and allozyme variants, suggesting two separate gene pools (JOHANSEN *et al.*, 2000a). Sequence analysis was conducted of mitochondrial 16S rRNA, which neither revealed differentiation between the two types in the Irminger Sea nor between *S. mentella* and *S. marinus* (SUNDT and JOHANSEN, 1998). Microsatellite markers have been applied to discriminate the four redfish taxa in the North Atlantic (ROQUES *et al.*, 1999). Based on shared microsatellite alleles, ROQUES *et al.* (1999) concluded that *S. marinus* represents the most basal lineage from which the other North Atlantic *Sebastes* arose. Analysis of tandem repeat polymorphism in the control region of mtDNA was examined by BENTZEN *et al.* (1998) which indicated no significant variation in number of repeats or in frequency of heteroplasmy among *S. mentella*, *S. marinus*, and *S. fasciatus*. A, study on the mtDNA ND3 gene and of microsatellite loci suggested that a cryptic group may exist within *S.*

marinus (SCHMIDT, 2005). Furthermore, recent findings of PAMPOULIE and DANÍELSDÓTTIR (2008) suggest discrimination of the North Atlantic *Sebastes* species and groups by using five microsatellite loci.

Population structure of *S. viviparus* has not been studied to the same extent as in *S. marinus* and *S. mentella*. Low levels of genetic variation have though been reported for *S. viviparus*, but hemoglobin patterns indicate substructuring of *S. viviparus* in Norwegian waters (JOHANSEN *et al.*, 2002). The same study suggests different gene pools for Icelandic and Norwegian waters.

Here I analyze mtDNA variation to study population structure and divergence between the closely related redfish species *S. marinus*, *S. mentella* and *S. viviparus*. I also study variation within *S. marinus* and of putative cryptic groups within *S. mentella* in the Irminger Sea. The aim is to test the hypothesis related to species delineation and to discriminate between the effects of incomplete lineage sorting and possible taxonomic mis-classification of species.

Materials and Methods

Sampling

North Atlantic redfish species, *S. mentella*, *S. marinus*, and *S. viviparus* were sampled by different scientists from marine research institutes in Iceland, the Faroe Islands, and Greenland during research surveys in the North Atlantic from 2005–2007 (Table 1). Samples of *S. mentella* were divided into three groups, two in the Irminger Sea, the "oceanic" type found above 400 m and the "pelagic" type found below 500 m. The third group is the "demersal" type found on the continental shelves of the East and West coast of Greenland and Iceland and around the Faroe Islands. A schematic of the relationships of the various taxa and continental slope and depth is presented in Figure 1.

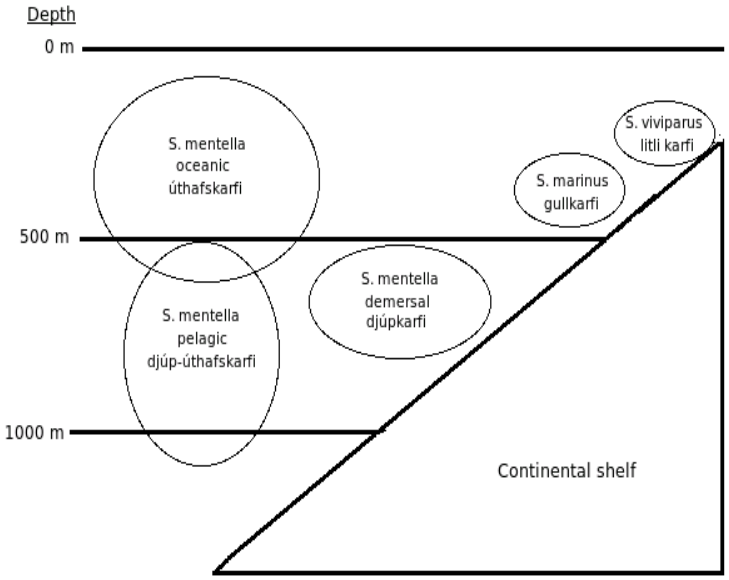

Figure 1: A schematic of the relationships of the various taxa and continental slope and depth. Icelandic names are also given. Modified from SCHMIDT (2005).

Sampling locations in the North Atlantic are presented in Figure 2 for *S. marinus* and *S. mentella* and its subgroups sampled from the Irminger Sea. Sampling locations for *S. viviparus*, which were collected around the Faroe Islands in two separate surveys, in March and during mating season in September 2006, are presented in Figure 3. Classification of redfish (genus *Sebastes*) to species was done in the field using general taxonomic characters (JÓNSSON, 1983). When species identification could not be conducted unambiguously using standard characters, individual fish were classified as unknown or *Sebastes* sp. Samples classified as unknown *Sebastes* were all collected on the East and West coast of Greenland.

Gill samples were collected directly on board the vessels in all surveys and preserved in 96% ethanol. In total 4766 individuals of the genus *Sebastes* (1030 *S. marinus*, 2931 *S. mentella*, 519 unidentified *Sebastes* sp., and 286 *S. viviparus*) were collected from different locations across the North Atlantic. Each sample was given a unique barcode to allow easy storage and retrieval of tissue from individual fish and to minimize errors. Barcodes and associated biological data were maintained in a PostgreSQL database. The database holds information for every survey, such as location and depth, and various individual measurements such as weight, length, and sex.

Table 1: Number of individuals sampled of various taxa. Location, year, month, and sex.

Species	Type	Location	Year	Month	Male	Female	Unknown	Total
S. mentella	demersal	Reykjanes Ridge	2005	October	72	50	-	122
S. mentella	demersal	E-Iceland	2005	October	117	87	-	204
S. mentella	demersal	Reykjanes Ridge	2006	October	44	48	-	92
S. mentella	demersal	SE-Iceland	2006	October	46	52	-	98
S. mentella	oceanic	Irminger Sea	2006	August	151	113	-	264
S. mentella	oceanic	Irminger Sea	2007	June	56	34	-	90
S. mentella	oceanic	Irminger Sea	2007	July	462	291	-	753
S. mentella	pelagic	Irminger Sea	2007	June	91	49	-	140
S. mentella	pelagic	Irminger Sea	2007	July	118	84	-	202
S. mentella	demersal	Faroe Islands	2006	March	10	3	-	13
S. mentella	demersal	Faroe Islands	2006	May	32	21	6	59
S. mentella	demersal	Faroe Islands	2006	September	321	391	-	712
S. mentella	demersal	E-Greenland	2005	June	103	78	1	182
S. mentella	Total				1623	1301	7	2931
S. marinus		E-Iceland	2005	October	29	15	-	44
S. marinus		Reykjanes Ridge	2005	October	40	24	-	64
S. marinus		Faroe Islands	2006	March	20	12	-	32
S. marinus		Faroe Islands	2006	May	140	101	15	256
S. marinus		E-Greenland	2005	June	3	4	4	11
S. marinus		Faroe Islands	2006	September	352	230	36	618
S. marinus		W-Greenland	2005	July	-	-	5	5
S. marinus	Total				584	386	60	1030
Sebastes sp.		W-Greenland	2005	June-July	35	24	389	448
Sebastes sp.		E-Greenland	2005	June-July	28	41	2	71
Sebastes sp.	Total				63	65	391	519
S. viviparus		Faroe Islands	2006	March	7	14	68	89
S. viviparus		Faroe Islands	2006	September	59	137	1	197
S. viviparus	Total				66	151	69	286
Grand Total					2336	1903	527	4766

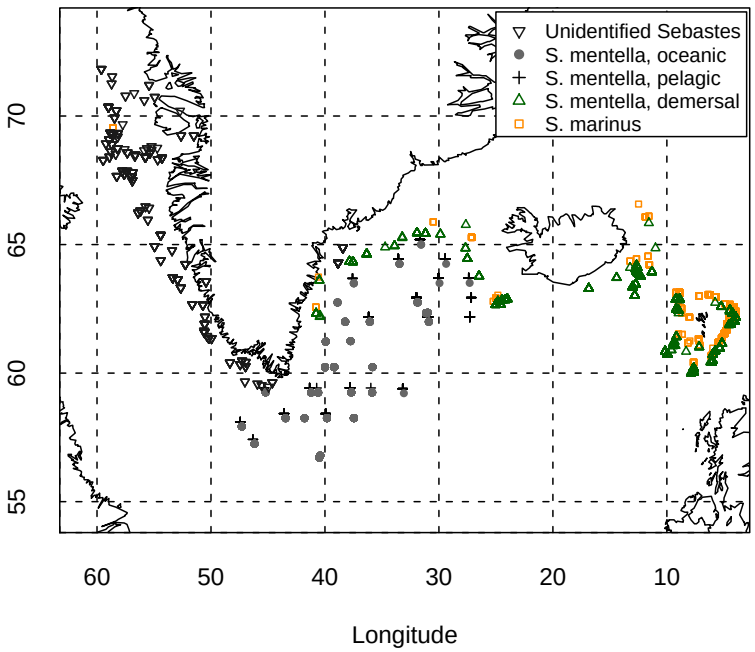

Figure 2: Sampling localities among *S. marinus* and *S. mentella* together with subgroups of *S. mentella* in the Irminger Sea. Each group is color coded, see upper right corner of the figure.

Preparation of molecular work

All molecular work, from DNA extraction to sequencing was done using 96 well plates. DNA was isolated in a dedicated DNA isolation room separate from the main laboratory. Before DNA isolation, three blank controls were randomly chosen and entered in a spreadsheet formatted to represent a 96 well plate. The barcode on each tube was read with a barcode reader directly into the spreadsheet. This facilitated checking that the correct specimen was in its place in the tray. Eight or twelve channel multipipettes were used for all subsequent procedures involving the plates to avoid or minimize errors of pipetting

DNA extraction, amplification and sequencing

Genomic DNA was extracted from the gill tissue in Chelex following WALSH *et al.* (1991). The extract was then boiled for five minutes, centrifuged, and diluted 1:19. A cyto-

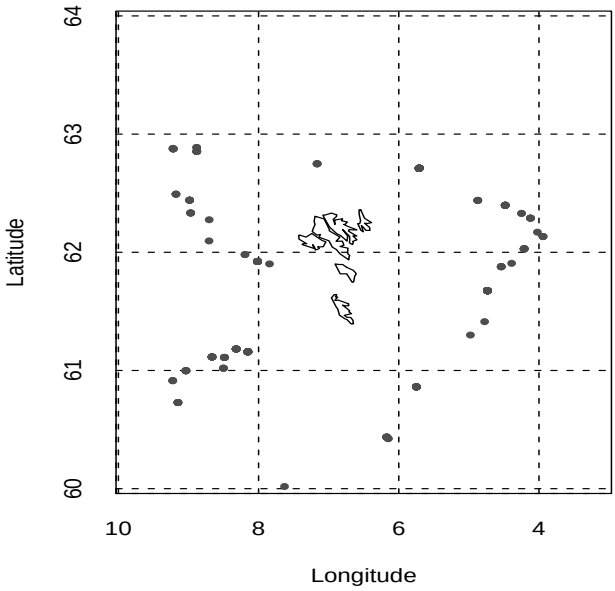

Figure 3: Sampling locations of *S. viviparus* around the Faroe Islands.

chrome *b* (cyt *b*) region of the mtDNA was amplified by the polymerase chain reaction (PCR) using primers 5′-TTA TTC AAC TAC AAG AAC C-3′ and 5′-ATA TCA TTC TGG CTT AAT GTG-3′. Amplifications were done with the following step cycle profiles: 3 min at 94°C followed by 35 cycles of denaturation (1 min at 94°C), annealing (30 sec at 50°C), and extension (1 min at 72°C) and with a final extension step of 7 min at 72°C. Prior to sequencing, nucleotides and excess primers were enzymatically removed from the PCR product using a combination of Exonuclease I and Antarctic Phosphatase (NEB). Sequencing was done with BigDye v3.1 chain termination sequencing kit (Applied Biosystems) according to manufacturer's specifications except that the amount of BigDye was reduced. The reaction conditions were performed at 96°C for 10 sec (initial denaturing cycle), followed by 25 cycles of 96°C for 10 sec (denaturing), 50°C for 5 sec (annealing), 60°C for 2 min (extension). Products of the sequencing reactions were purified with ethanol precipitation and run on an ABI 3100 capillary sequencer (Applied Biosystems).

Data analysis

The Phred/Phrap/Consed software (e.g. KWOK *et al.*, 1994; NICKERSON *et al.*, 1994; EWING *et al.*, 1998; GORDON *et al.*, 1998; EWING and GREEN, 1998) was used to read the

trace files of DNA sequencing. The software calls bases and assigns a quality value to each base. Quality values, or Phred quality scores, exceeded 35 in all cases (with most scores in the range of 60). This score gives a probability of 1 in 5000 that a wrong base is called, or 99.95% accuracy of the base call.

Sequences were aligned using Muscle (ROBERT, 2004) and ClustalW (THOMPSON *et al.*, 1994) and inspected by eye. Sequences were compared and haplotype names were assigned to identical sequences for subsequent analysis using R (R DEVELOPMENT CORE TEAM, 2006). Nucleotide divergence or the average number of net nucleotide substitutions per site between two species were estimated by $d_a = d_{xy} - (d_x + d_y)/2$ where d_{xy} is the average distance between taxa x and y, and d_x and d_y are the mean within-taxon distances (SAITOU and NEI, 1987). Calculations for these values were conducted in R (R DEVELOPMENT CORE TEAM, 2006) using the APE package (PARADIS *et al.*, 2004). DNA Maximum Likelihood from the Phylip package FELSENSTEIN (1991) was used for constructing a phylogenetic tree (FELSENSTEIN and CHURCHILL, 1996). *S. alutus* (accession number DQ678416 HYDE and VETTER, 2007) was used as an outgroup in the phylogenetic tree. With known approximate time values and divergence, substitution rate μ can be estimated as $\mu = d_a/2t$ were d_a is the net nucleotide divergence between two populations and t the time since divergence. Estimation of divergence time was based on calibration point from HYDE and VETTER (2007). Their tree was age calibrated by setting the most recent common ancestor to *S. alutus* and *S. marinus* at three million years ago. For estimation of divergence time this calibration point was obtained by direct measurement of tree branches in Figure 5 from HYDE and VETTER (2007). Substitution rate μ was subsequently estimated based on time evaluations from HYDE and VETTER (2007) and d_{xy} values and gave a substitution rate of $\hat{\mu}$ = 8.87e-09/site/year. This substitution rate was used in estimating divergence times among *S. marinus*, *S. mentella*, and *S. viviparus* and among *S. mentella* subgroups in the Irminger Sea.

Haplotype diversity $\hat{h}$ and nucleotide diversity $\hat{\pi}$ were estimated within each taxon and subgroups using DNAsp (ROZAS and ROZAS, 1999) and MEGA (KUMAR *et al.*, 1993). A median-joining network was constructed by using Network (BANDELT *et al.*, 1999). Exact *G*-test for genic differentiation between populations and estimation of F_{ST} were conducted in GENEPOP (RAYMOND and ROUSSET, 1995).

Results

Inter-specific variation

Variation of a 420 base pair fragment within the mitochondrial cyt *b* gene was studied among 4480 North Atlantic specimens of *S. mentella* and *S. marinus*. In addition, variation of a 567 base pair fragment of the same gene was studied among 286 individual *S. viviparus*.

A total of 90 haplotypes within the cyt *b* gene were found for *S. mentella* and *S. marinus* combined. A complete haplotype alignment of segregating sites for *S. mentella* and *S. marinus* is presented in Table 11 in the Appendix. A total of 15 haplotypes were found within *S. viviparus* with 14 substitution and 14 polymorphic sites. Haplotype alignment of segregating sites for *S. viviparus* is presented in Table 12 in the Appendix.

The 90 haplotypes within *S. marinus* and *S. mentella* showed 74 polymorphic sites and 79 substitutions. Most of the substitutions were synonymous transitions at third codon position, or 75%. The transition/transversion ratio was approximately 4:1 and purine:pyrimidine ratio was 1.2:1. Out of the 90 haplotypes 22 were formed by nonsynonymous changes of which 17 were singletons. The other five represented two to four individuals each. All amino acid changes showed high relative rates of acceptance according to DAYHOFF (1972), with most amino acid replacements being Isoleucine for Valine or Valine for Isoleucine.

Nucleotide substitutions among *S. viviparus* consisted only of transitions among the 15 haplotypes. Purine to pyrimidine ratio was 2.5:1. Nine singletons were found of which one was a nonsynonymous change at site 10 of the amino acid Isoleucine for Valine.

Haplotype diversity $\hat{h}$, nucleotide diversity $\hat{\pi}$, and number of haplotypes n_h found for each group are presented in Table 2. The data are presented as belonging to *S. marinus*, *S. viviparus* and the three types of *S. mentella*, the "demersal" type on the continental shelf and the "oceanic" and the "pelagic" types found in the Irminger Sea. The low diversity within *S. mentella* groups was due to the high frequency of a single haplotype. Haplotype diversities of the three subgroups within *S. mentella* were quite similar and the lowest within the total data. Haplotype diversity was highest within *S. marinus*, more than twice as high as within

the *S. mentella*, "demersal" type. This was due to the higher evenness in frequencies of the common haplotypes within *S. marinus*. Haplotype diversity for *S. viviparus* was intermediate, $\hat{h}$ = 0.402. Nucleotide diversity ($\hat{\pi}$) in general was very low. The lowest nucleotide diversities were observed within the three subgroups of *S. mentella*. *S. marinus* revealed the highest nucleotide diversity, again due to high evenness in haplotype frequencies. Nucleotide diversity for *S. viviparus* was in between that of *S. marinus* and the three groups of *S. mentella*.

Table 2: Haplotype and nucleotide diversity among taxa. Number of individuals analyzed per group (n), number of haplotypes (n_h), haplotype diversity ($\hat{h}$), nucleotide diversity ($\hat{\pi}$) and standard errors of $\hat{\pi}$ (SE)

Species or subgroup		n	n_h	$\hat{h}$	$\hat{\pi}$	SE
S. marinus	total data	1030	31	0.543	0.00145	0.00067
S. mentella "pelagic"	Irminger Sea	342	21	0.136	0.00033	0.00007
S. mentella "oceanic"	Irminger Sea	1107	35	0.177	0.00049	0.00011
S. mentella "demersal"	total data	1482	50	0.245	0.00066	0.00015
Sebastes species	Greenland	519	18	0.202	0.00053	0.00006
S. viviparus	Faroe Islands	286	15	0.402	0.00080	0.00018

A median joining network (Figure 4) shows a complex relationship among the various haplotypes within the cyt *b* region.

The presence of a haplotype found in both *S. marinus* and *S. mentella* together with its subgroups is indicated with colors. For greater visual clarity singletons were excluded from the network to reduce complexities due to homoplasy. Homoplasy was nevertheless considerable, even without the singletons. This was expected, because the number of haplotypes was considerably higher than the number of segregating sites. The sizes of the circles are all equal and do not show relative frequency among haplotypes. This is presented in this way because of sampling being unequal among taxa. The frequency differences are considered further below. Individuals of *S. marinus* and *S. mentella* and its subgroups showed high sharing of haplotypes. Because of the high haplotype sharing among the groups, species and groups are represented by different color codes, and groups that share a haplotype are located within the same circle. The median joining network indicated a very shallow genetic separation between the two valid species and among subgroups. Most of the haplotypes differed from their nearest neighbour by one substitution. A rare haplotype, *C22*, was represented by three individuals which were classified to three different groups, "oceanic" type located South in the Irminger Sea, "demersal" type found on the East coast of Iceland, and one unidentified *Sebastes* found South of Greenland. This haplotype differs from the rest in the median joining network by being connected via a median vector (mv3). A median vector is a hypothesized sequence, which may be ancestral, and is required to connect existing sequences within the network (BANDELT *et al.*, 1999).

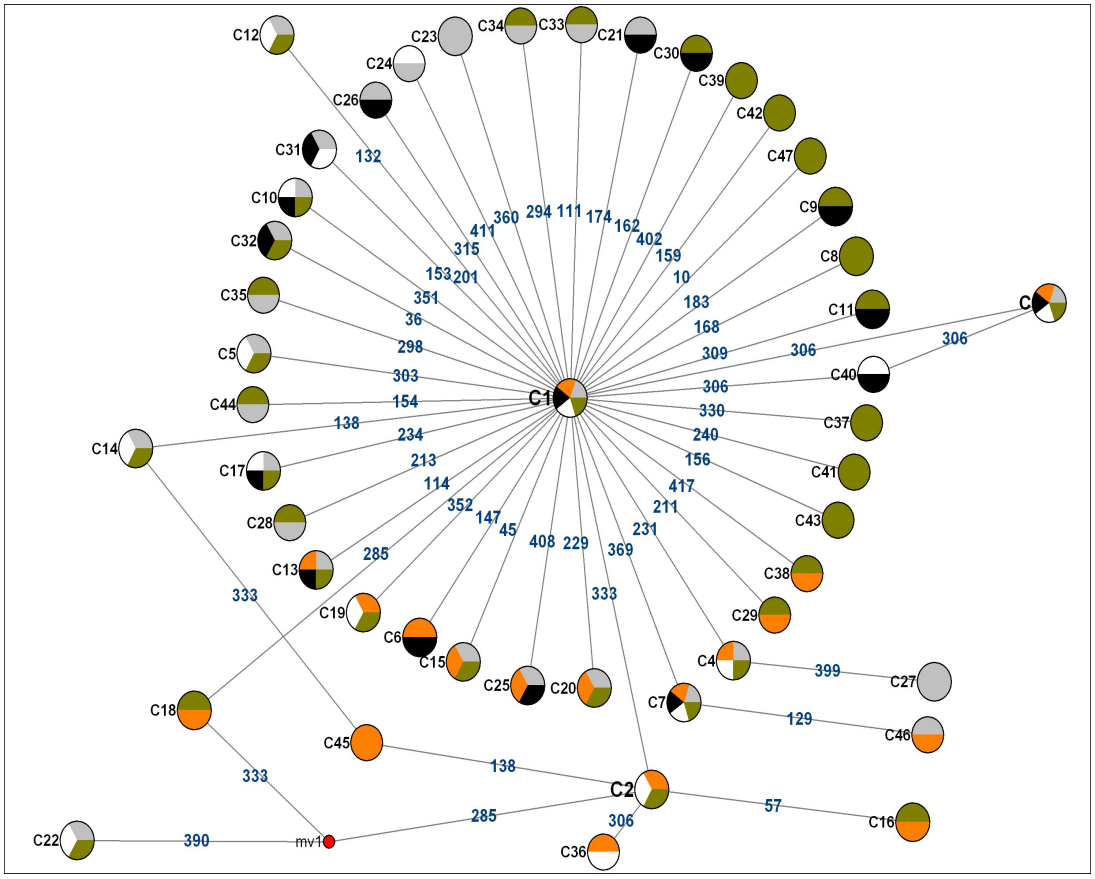

Figure 4: A median joining network of mitochondrial cyt *b* haplotypes among 4480 individuals of *Sebastes* species. Color abbreviations: *S. marinus*●, *S. mentella* "demersal"●, *S. mentella* "oceanic"●, *S. mentella* "pelagic"●, unclassified *Sebastes*○. Numbers in blue between circles indicate the segregating site between haplotypes. Singletons were excluded. The size of the pie sectors only reflects number of taxa sharing that haplotype, and does not represent frequency.

GenBank database (BENSON *et al.*, 2008) was scanned for sequence similarities for haplotype *C22* and sequence with accession number DQ678444 from HYDE and VETTER (2007) showed *S. fasciatus* as the closest match.

Distribution of haplotypes within *S. marinus* and *S. mentella* and subgroups for each locality are presented in Table 3. Relative frequencies of the three most common haplotypes are presented in Figure 5. The most common haplotype *C1* had the highest frequency within both *S. mentella* and *S. marinus* and was found in 3654 out of 4480 individuals or 82%. However, its frequency differed between the two species. Also the frequency of *C1* differed among subgroups of *S. mentella* in the Irminger Sea. Thus, the haplotype was found at 57% within all *S. marinus* collected, 86.5% within the "demersal" *S. mentella*, 90% within unidentified *Sebastes*, 91% within the "oceanic" type, and 93% within the "pelagic" type. A significant

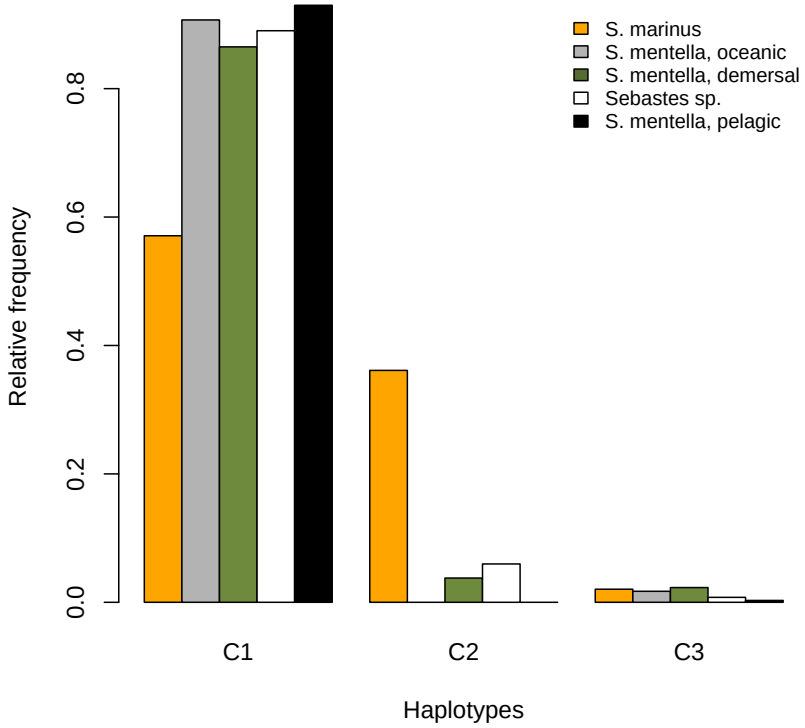

Figure 5: Relative frequency of the three most common haplotypes among *S. marinus* and *S. mentella* and its subgroups.

difference considering haplotype *C1* versus the rest of the haplotypes was observed between the "demersal" and the "oceanic" types ($X^2 = 10.4,\ df = 1,\ p = 0.0013$) and between the "demersal" and the "pelagic" types ($X^2 = 10.2,\ df = 1,\ p = 0.0014$). The difference between the "oceanic" and the "pelagic" types was not significantly different ($X^2 = 1.4,\ df = 1,\ p = 0.231$). In addition, the difference between unidentified *Sebastes* and any of the three groups of *S. mentella* was not significant. Unidentified *Sebastes* were all collected East and West of Greenland. These individuals resemble *S. mentella* and its subgroups with respect to frequency of the most common haplotype.

The second most common haplotype *C2*, $n = 459$, was also shared between *S. mentella* and *S. marinus*. However, its frequency also differed considerably between them. The *C2* haplotype was found at 45% within all *S. marinus* collected, but it was unevenly distributed among regions. Thus, it comprised only 9% in samples East of Iceland but 37% West of Iceland. It was found at 30% East of the Faroe Islands and at nearly 40% West of the Faroe

Islands. Only a few individuals of *S. mentella* shared haplotype *C2* with *S. marinus*, or three individuals around Iceland and 26 individuals from the Faroe Islands, mainly from the East of the Faroe Islands. Thus its overall frequency in the "demersal" type of *S. mentella* was just below 4%. It was found at 15% among *S. mentella* collected East of Greenland and at 6% in unidentified *Sebastes* sp. Furthermore, this haplotype was not found within the "oceanic" or the "pelagic" types of *S. mentella* in the Irminger Sea.

The third most common haplotype, *C3*, $n = 79$, was more evenly distributed, or at $\sim$2% within the three largest groups: *S. marinus* and the "demersal" and "oceanic" types of *S. mentella*. It was found at less than 1% in the unidentified *Sebastes* group and only in one individual or less than 1% (1/142) in the "pelagic" type of *S. mentella*.

Frequencies of eight haplotypes *C4–C11*, represented by ten to thirty five individuals each, are presented in Figure 6. These eight haplotypes are defined as medium-rare to distinguish them from common and rare haplotypes respectively. The Figure shows the difference in haplotype frequencies among the five groups: *S. marinus*, *Sebastes* species, and the three groups of *S. mentella*. The haplotypes were distributed unevenly among the groups. The "demersal" type was found carrying seven of the eight haplotypes and thus it shared haplotypes with the rest of the groups except for haplotype *C6*. In contrast, *S. marinus* was found carrying this haplotype in relatively high frequency and was only found carrying three haplotypes among the eight. One haplotype, *C8*, was exclusively found within the "demersal" type. Thus it can be called a private haplotype for that group. The "oceanic" and the "pelagic" types were found carrying four and five haplotypes respectively. However, they only shared two of them, *C7* and *C10*. The unidentified *Sebastes* species was found carrying the same four haplotypes as the "oceanic" type. All groups carried haplotype *C7*. The statistical significance of these differences is studied formally below.

A total of 36 rare haplotypes were observed, represented by two to seven individuals each. Nine of them were unique to a group, six within the "demersal" type, two within the "oceanic" type, and one within *S. marinus*. Thus they may be called private haplotypes on a global scale. Five of the ten (the tenth being haplotype *C8* mentioned above) private haplotypes were found exclusively within a geographic region and thus are private within those regions: two within the "oceanic" type in the Irminger Sea, two within the "demersal" type, one of which was on the East and another on the West coast of the Faroe Islands, and the last one within *S. marinus* around the Faroe Islands. However, on a smaller scale they cannot be considered private as they were found in different areas within each region. The other five private haplotypes were all found within the "demersal" type, but not within a particular geo-

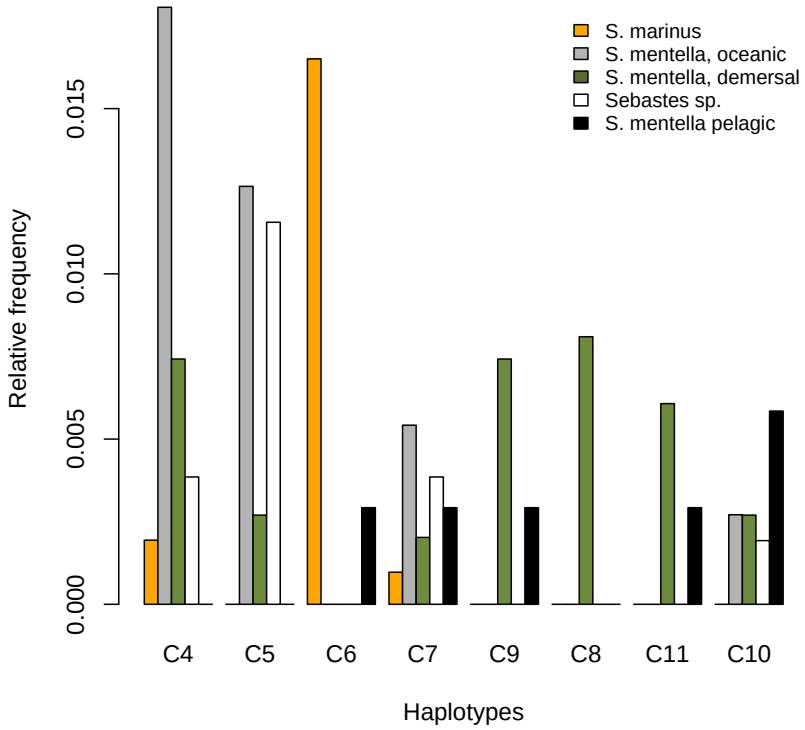

Figure 6: Relative within-taxon frequency of haplotypes *C4–C11* among *S. marinus* and *S. mentella* and subgroups.

graphic region or area. Thus, again, they were not private to a specific locality. Altogether 37 haplotypes were shared among *S. marinus*, *S. mentella*, and subgroups of *S. mentella*. A total of 43 haplotypes were singletons in the sample. Singletons were distributed relatively evenly among the taxa and among all sampling localities, whether they represented synonymous or nonsynonymous substitutions.

The most common haplotype within *S. viviparus*, *V1*, was found in 219 samples out of 286 individuals, or 77%. This haplotype was found among all sample locations around the Faroe Islands. The second most common haplotype, *V2*, was found in 33 individuals, or 11% and was restricted to the West and primarily Southwest of the Faroe Islands. The third most common haplotype *V3*, was found in 19 individuals, or 7% and was found all around the Faroe Islands except for West and Southwest of the Islands where haplotype *V2* occurred. None of the haplotypes found within *S. viviparus* were shared with either *S. mentella* or *S. marinus*. They thus represent a monophyletic group of lineages.

Table 3: Distribution of haplotypes within the cyt *b* gene of *Sebastes* of 13 subsamples within four geographic regions: Greenland, Irminger Sea, Iceland and the Faroe Islands. GR = Greenland, IS = Iceland, F = Faroe Islands, IR = Irminger Sea, me = "demersal" type of *S. mentella* in all localities, except for Irminger Sea, ma = *S. marinus*, sp = *Sebastes* species, IR.me.oceanic = "oceanic" type and IR.me.pelagic = "pelagic" type.

	Greenland			Irminger Sea		Iceland				Faroe Islands				
Haplotype	GR.me	GR.sp	GR.ma	IR.me.oceanic	IR.me.pelagic	IS.me.East	IS.me.West	IS.ma.East	IS.ma.West	F.me.East	F.me.West	F.ma.East	F.ma.West	Sum
C1	141	462	15	1004	318	276	197	30	22	296	372	329	192	3654
C2	27	31				2	2	10	40	4	22	158	164	459
C3		4		19	1	1			1	30	3	17	3	79
C4		2		20		1				8	2	2		35
C5		6		14		1				3				24
C6					1			1				12	4	18
C7		2		6	1					2	1	1		13
C8	1					6				1	4			12
C9	2				1	4				1	4			12
C10		1		3	2					4				10
C11	3				1	1	3				2			10
C12	1	1		5										7
C13	1			1	2			1			1		1	7
C14	1	1		2							2			6
C15					2	1		1		1				5
C16									1		1	1	1	4
C17		1		1	1						1			4
C18			1				3							4
C19		1					1			1		1		4
C20				1		1					1	1		4
C21				1	2									3
C22		1		1		1								3
C23				3										3
C24		1		2										3
C25				1	1							1		3
C26				2	1									3
C27				3										3
C28				2	1									3
C29						1				1			1	3
C30	2				1									3
C31		1		1	1									3
C32				1	1		1							3
C33				1							1			2
C34				1							1			2
C35				1							1			2

Continued on next page

Table 3: Distribution of haplotypes within the cyt *b* gene of *Sebastes* of 13 subsamples within four geographic regions: Greenland, Irminger Sea, Iceland and the Faroe Islands. GR = Greenland, IS = Iceland, F = Faroe Islands, IR = Irminger Sea, me = "demersal" type of *S. mentella* in all localities, except for Irminger Sea, ma = *S. marinus*, sp = *Sebastes* species, IR.me.oceanic = "oceanic" type and IR.me.pelagic = "pelagic" type.

	Greenland			Irminger Sea		Iceland				Faroe Islands				
Haplotype	GR.me	GR.sp	GR.ma	IR.me.oceanic	IR.me.pelagic	IS.me.East	IS.me.West	IS.ma.East	IS.ma.West	F.me.East	F.me.West	F.ma.East	F.ma.West	Sum
C36		1											1	2
C37										2				2
C38						1						1		2
C39						1					1			2
C40		1			1									2
C41	1					1								2
C42	1					1								2
C43							2							2
C44				1			1							2
C45												1	1	2
C46				1									1	2
C47							1				1			2
C48											1			1
C49											1			1
C50	1													1
C51						1								1
C52					1									1
C53		1												1
C54							1							1
C55												1		1
C56				1										1
C57					1									1
C58		1												1
C59				1										1
C60													1	1
C61													1	1
C62											1			1
C63				1										1
C64										1				1
C65													1	1
C66				1										1
C67				1										1
C68							1							1
C69							1							1
C70												1		1

Continued on next page

Table 3: Distribution of haplotypes within the cyt *b* gene of *Sebastes* of 13 subsamples within four geographic regions: Greenland, Irminger Sea, Iceland and the Faroe Islands. GR = Greenland, IS = Iceland, F = Faroe Islands, IR = Irminger Sea, me = "demersal" type of *S. mentella* in all localities, except for Irminger Sea, ma = *S. marinus*, sp = *Sebastes* species, IR.me.oceanic = "oceanic" type and IR.me.pelagic = "pelagic" type.

	Greenland			Irminger Sea		Iceland				Faroe Islands				
Haplotype	GR.me	GR.sp	GR.ma	IR.me.oceanic	IR.me.pelagic	IS.me.East	IS.me.West	IS.ma.East	IS.ma.West	F.me.East	F.me.West	F.ma.East	F.ma.West	Sum
C71					1									1
C72										1				1
C73				1										1
C74													1	1
C75												1		1
C76										1				1
C77													1	1
C78				1										1
C79												1		1
C80				1										1
C81													1	1
C82							1							1
C83				1										1
C84						1								1
C85												1		1
C86										1				1
C87										1				1
C88												1		1
C89											1			1
C90								1						1
Sum	182	519	16	1107	342	302	214	44	64	359	425	531	375	4480

Table 4: Segregating sites and frequency among five haplotypes from *S. mentella*, *S. marinus*, and *S. viviparus*.

Haplotype	Segregating sites								Frequency
	2	2	3	3	3	3	3	4	
	8	8	0	0	3	4	9	0	
	5	8	6	9	3	8	0	2	
C1	A	A	G	T	C	T	C	G	3654
C2	.	.	.	.	T	.	.	.	459
C22	G	.	.	.	T	.	T	.	3
V1	.	G	.	C	T	C	T	A	219
V3	G	G	.	C	T	C	T	A	19

To further explore the relationship between haplotypes, selected haplotypes were aligned together (Table 4). These haplotypes were: *C1*, the most common haplotype found among samples within all types of *S. mentella* and *S. marinus*, haplotype *C2*, found at high frequencies within *S. marinus* and very low frequencies in *S. mentella*, haplotype *C22*, found in three different groups and which was related to *S. fasciatus* as mentioned above, and haplotypes *V1* and *V3* found among *S. viviparus* samples.

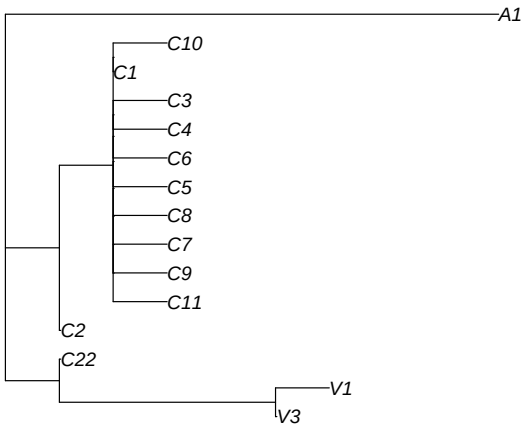

Figure 7: Phylogenetic maximum likelihood tree showing the most common haplotypes found, within *S. marinus* and *S. mentella*, *C1-C11*. Two haplotypes from *S. viviparus*, *V1* and *V3*, one from *S. alutus*, *A1* and haplotype, *C22*.

A phylogenetic tree based on maximum likelihood approach was constructed for the most common haplotypes within *S. marinus* and *S. mentella* and presented in Figure 7.

Two haplotypes found within *S. viviparus* and haplotype *C22* are also in the tree. For the construction of the tree *S. alutus* was set as an outgroup. Haplotype *C22*, which showed the highest similarity to *S. fasciatus*, forms a monophyletic lineage with *S. viviparus*. They share a T at site 390 and *C22* shares a G with *V3* at site 285 (Table 4). *S. marinus* and *S. mentella* subgroups within *S. mentella* represent haplotypes *C1* to *C11* shown in the phylogram. They form a monophyletic group with haplotype *C2* representing an earlier split.

Intra-specific variation

Samples of *S. marinus* were divided into five groups based on geographic region: Greenland, West and East of Iceland, and West and East of the Faroe Islands. Four different exact G-tests of population differentiation were conducted for all population pairs among these localities. Since the test is based on frequency of haplotypes (not on sequence variation differences), haplotypes can be lumped in different ways depending on what hypothesis is being tested in each instance (described further below). Significant values for the tests are presented in Table 5. The table is divided into two parts, a and b. The p-values in the upper triangle in

Table 5: Genic differentiation among localities for each population pair of *S. marinus*. Greenland (GR.ma), West of Iceland (IS.ma.West), East of Iceland (IS.ma.East), East of the Faroe Islands (F.ma.East), West of the Faroe Islands (F.ma.West). a: Upper triangle: p-values for overall differentiation. Lower triangle: Significance values resulting from testing for differentiation due to haplotype *C1* only. *b*: Upper triangle: p-values for differentiation due to the three most common haplotypes *C1–C3* only. Lower triangle: Significance values for differentiation due to medium-rare haplotypes only. $***p < 0.001$, $**p < 0.01$, $*p < 0.05$ and NS for $p > 0.05$.

a Localities	GR.ma	IS.ma.West	IS.ma.East	F.ma.East	F.ma.West
GR.ma	-	0.000	0.130	0.025	0.007
IS.ma.West	*	-	0.000	0.003	0.433
IS.ma.East	***	**	-	0.077	0.060
F.ma.East	*	***	NS	-	0.001
F.ma.West	**	*	*	**	-
b Localities	GR.ma	IS.ma.West	IS.ma.East	F.ma.East	F.ma.West
GR.ma	-	0.000	0.096	0.022	0.002
IS.ma.West	NS	-	0.000	0.000	0.028
IS.ma.East	NS	NS	-	0.249	0.023
F.ma.East	NS	NS	NS	-	0.000
F.ma.West	NS	NS	NS	NS	-

Table 5a resulted from testing the overall genic differentiation based on haplotype frequencies within each locality. Standard Errors (not shown) were from 0.001–0.008. Subsequent tests will be compared to these significant values in order to estimate the most likely cause for the observed differentiation. Six comparisons were significantly different out of ten for the over-

all genic differentiation test. The second test is testing whether the observed and significant difference in the upper triangle was due to differences in frequency of the most common haplotype only. This was done by lumping all haplotypes together except for the most common one, *C1*, thus testing *C1* versus the rest. Significant values for this test are presented in the lower triangle of Table 5a. The p-values in the upper triangle in Table 5b (third test) resulted from testing whether the observed and significant difference in the upper triangle of 5a was due to differences in frequency of the three most common haplotypes only, *C1–C3*. This was done by lumping all haplotypes together except for the three most common ones, thus testing differences among them and the rest. The fourth test is testing whether the observed and significant difference in the upper triangle of 5a was due to differences in frequency of the medium-rare haplotypes. This was done by lumping all haplotypes except those representing *C4–C11*. Two additional tests were conducted (data not shown). First a test was done whether the observed difference in the upper triangle of Table 5a was due only to singletons in the sample. As described above this test was done by lumping of haplotypes, but this time singletons were left as they were and the rest of the haplotypes were lumped together. None of these comparisons were significant. Second, a test was done whether the observed difference in the upper triangle of Table 5a was due only to frequencies of rare haplotypes in the sample (*C12–C47*). In this case singletons were lumped with common and medium-rare haplotypes and thus all of them act as one common haplotype found within the samples. None of these comparisons were significant. Hereafter, groups of haplotypes will be referred to as haplogroups: All haplotypes (*C1–C90*), the most common haplotype (*C1*), common haplotypes (*C1–C3*), medium-rare haplotypes (*C4–C11*), rare haplotypes (*C12–C47*), and singletons. Most of the observed significance among *S. marinus* were significant due the most common haplotype or the three most common haplotypes. Medium-rare haplotypes had no effect on the observed differentiation.

Exact G-tests of population differentiation were also done for all population pairs among localities among samples of *S. mentella*. Samples were divided into seven groups based on geographic region: Five of them represent the "demersal" type, East of Greenland (none were classified as *S. mentella* West of Greenland), West and East of Iceland, and West and East of the Faroe Islands, and two of them represent the "pelagic" and "oceanic" types in the Irminger Sea respectively. The same methods for testing were applied here as described for *S. marinus* above. Significant values are presented in Table 6. All comparisons showed overall genic differentiation except on one occasion, between West of the Faroe Islands and East of Iceland. (Table 6*a*, upper triangle) The observed differentiation for Greenland compared to all

Table 6: Genic differentiation among localities for each population pair of *S. mentella*, "demersal" from West of Greenland (Gr.me.East), West of Iceland (IS.me.West), East of Iceland (IS.me.East), East of the Faroe Islands (F.me.East), West of the Faroe Islands (F.me.West) and for the two subgroups of *S. mentella*: Irminger "pelagic" (IR.me.pelagic), Irminger "oceanic" (IR.me.oceanic). *a*: Upper triangle: p-values for overall differentiation. Lower triangle: Significance values resulting from testing for differentiation due to haplotype *C1* only. *b*: Upper triangle: p-values for differentiation due to the three most common haplotypes *C1–C3* only. Lower triangle: Significance values for differentiation due to medium-rare haplotypes only. ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$ and NS for $p > 0.05$.

a Localities	GR.me.East	IS.me.West	IS.me.East	F.me.East	F.me.West	IR.me.pelagic	IR.me.oceanic
GR.me.East	-	0.000	0.000	0.000	0.011	0.000	0.000
IS.me.West	***	-	0.000	0.000	0.000	0.000	0.000
IS.me.East	***	NS	-	0.000	0.072	0.019	0.000
F.me.East	NS	**	**	-	0.000	0.000	0.000
F.me.West	**	**	NS	NS	-	0.000	0.000
IR.me.pelagic	***	NS	NS	***	*	-	0.000
IR.me.oceanic	***	NS	NS	***	NS	NS	-
b Localities	GR.me.East	IS.me.West	IS.me.East	F.me.East	F.me.West	IR.me.pelagic	IR.me.oceanic
GR.me.East	-	0.000	0.000	0.000	0.001	0.000	0.000
IS.me.West	NS	-	1.000	0.000	0.010	0.244	0.003
IS.me.East	NS	*	-	0.000	0.003	0.559	0.020
F.me.East	**	***	**	-	0.000	0.000	0.000
F.me.West	NS	NS	NS	**	-	0.000	0.000
IR.me.pelagic	NS	NS	*	*	NS	-	0.064
IR.me.oceanic	**	***	***	NS	***	***	-

Table 7: Pairwise F_{ST} among localities within *S. marinus*

Localities	GR.ma	IS.ma.West	IS.ma.East	F.ma.East	F.ma.West
GR.ma	-	0.432	0.077	0.120	0.227
IS.ma.West		-	0.205	0.143	0.048
IS.ma.East			-	-0.0014	0.053
F.ma.East				-	0.027
F.ma.West					-

other localities was mostly due to the most common or the three most common haplotypes. The exception being comparison to East of the Faroe Islands were it differed from Greenland due to the medium-rare haplogroup. The observed difference of the "oceanic" type in the Irminger Sea from all other localities was primarily due to the frequency difference of the medium-rare haplogroup. Same pattern was observed for East of the Faroe Islands when compared to other localities. However, the observed difference for these two groups, the "oceanic" and samples from East of the Faroe Islands were due to the frequency difference of the most common and the three most common haplotypes. The observed difference of the "pelagic" type in the Irminger Sea compared to other localities was due to a joint effects of three tests, the frequency difference of the common, three most common and the medium-rare haplotypes. However, compared to West of Iceland it was due to rare haplotypes only ($p < 0.05$). Overall, the observed differences were mostly due to frequency difference of the most common haplotypes when localities are further apart, while mostly due to frequency difference of the medium-rare haplogroup for localities closer together.

The tests have been post-hoc multiple comparisons. For each comparison I have made five tests by applying Bonferroni and Dunn-Šidák approaches to obtain an experiment-wise error rate α at a critical probability α = 0.01. Therefore the significance values in Tables 5 and 6 represented by a single star or p > 0.01 are no longer significant experimentwise. This makes the observed differences above more clear cut.

Pairwise F_{ST} estimates for the between localities differentiation among samples of *S. marinus* are presented in Table 7. The high F_{ST} value for Greenland compared to other localities is probably due to the high frequency of the most common haplotype *C1* within them or 15/16 individuals. A low F_{ST} value with a negative sign was observed between East of Faroe Islands and East of Iceland, which means that the difference between them is nil. Differences between West of Iceland compared to other localities was relatively high and lower for comparisons between West of the Faroe Island to other localities.

Pairwise F_{ST} estimates for the between localities differentiation among samples of *S. mentella* are presented in Table 8. F_{ST} values for Greenland compared to all other localities

Table 8: Pairwise F_{ST} among localities within subgroups of *S. mentella*

Localities	GR.me.East	IS.me.West	IS.me.East	F.me.East	F.me.West	IR.me.pelagic	IR.me.oceanic
GR.me.East	-	0.049	0.072	0.039	0.031	0.093	0.088
IS.me.West		-	0.003	0.020	0.004	0.006	0.004
IS.me.East			-	0.028	0.007	0.001	0.002
F.me.East				-	0.017	0.037	0.025
F.me.West					-	0.013	0.010
IR.me.pelagic						-	0.002
IR.me.oceanic							-

are relatively high. This is also the case for East of the Faroe Islands compared to all other localities. West of the Faroe Islands showed lower F_{ST} compared to other localities than East of the Faroe Islands. The lowest values were observed for comparisons between Iceland and the Irminger Sea and also within those localities. Overall, the highest differentiation was observed for Greenland compared to other localities and lowest between the "pelagic" type and East of Iceland.

Divergence times and genetic distance

The ancestor of the Pacific species *S. alutus* is believed to be the closest relative of the four North Atlantic *Sebastes* species (HYDE and VETTER, 2007). The time since divergence has been estimated at approximately 3 million years (HYDE and VETTER, 2007). Time since divergence of the groups and substitution rate $\hat{\mu}$ = 8.87e-09/site/year was estimated based on a calibration point from HYDE and VETTER (2007). Estimated times of divergence among subgroups of *S. mentella* were estimated from the d_a values from Table 9, as $t = d_a/2\mu$. The estimated times of divergence were very recent for all three groups. The earliest divergence was estimated 4,200 years between the "demersal" and "pelagic" types and the most recent, or 3,100 years, was found between "demersal" and "oceanic" types of *S. mentella*. The split between "oceanic" and "pelagic" was estimated at 3,400 years. Divergence times were also

Table 9: Distance estimates between the three subgroups of *S. mentella*. Upper triangle: average number of net nucleotide substitutions per site d_a. Diagonal: intrapopulational diversity d_x. Lower triangle: average number of nucleotide substitutions per site d_{xy} between x and y.

Group	*S. mentella* "pelagic"	*S. mentella* "oceanic"	*S. mentella* "demersal"
S. mentella "pelagic"	0.00044	6×10^{-5}	7.5×10^{-5}
S. mentella "oceanic"	0.00052	0.00048	5.5×10^{-5}
S. mentella "demersal"	0.00061	0.00061	0.00063

estimated among *S. marinus*, *S. mentella* ("demersal" only), and *S. viviparus* based on d_a values from Table 10. The estimated times show a very recent divergence between *S. mentella* and

S. marinus or approximately $19,000$ years ago. The divergence between *S. viviparus* and *S. mentella* was estimated at $700,000$ years and the almost equidistant divergence between *S. viviparus* and *S. marinus* was estimated at $720,000$ years.

Table 10: Distance estimates between *S. marinus*, *S. mentella* "demersal", and *S. viviparus*. Upper triangle: average number of net nucleotide substitutions per site d_a. Diagonal: intrapopulational difference d_x. Lower triangle: average number of nucleotide substitutions per site d_{xy} between x and y.

Group	*S. marinus*	*S. viviparus*	*S. mentella*, "demersal"
S. marinus	0.00135	0.01181	0.00034
S. viviparus	0.01381	0.00066	0.01244
S. mentella, "demersal"	0.00133	0.01374	0.00064

Biological differences among *S. marinus* and *S. mentella* during mating season

Depth and length distribution for *S. marinus* and *S. mentella*, collected in the Faroe Islands during mating season, is presented in Figure 8. The Figure presents samples that share

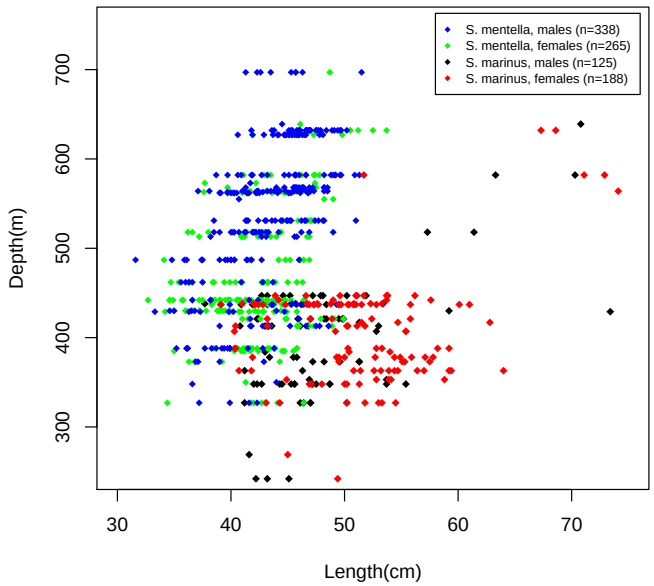

Figure 8: Length and depth distribution among *S. mentella* and *S. marinus* carrying haplotype *C1*. Samples were collected around the Faroe Islands during mating season in September 2006.

the most common haplotype, *C1*. The species form two distinctive clusters with *S. mentella* found on a broad depth range, ~350–700 meters, while the majority of *S. marinus* were found in a narrower depth range or ~300–450 meters. Individuals of haplotype *C2*, which was found

at very low frequencies within *S. mentella* and at relatively high frequencies in *S. marinus*, clustered together within the overlapping depth ranges and lengths of *S. mentella* and *S. marinus*. However, the observed difference was that larger fish were classified as *S. marinus* and smaller ones as *S. mentella* (data not shown). *S. mentella* were relatively smaller in size than *S. marinus* for individuals carrying the *C1* haplotype 8. In fact, less than 2% of *S. mentella* exceeded the length of 50 cm while 20% of *S. marinus* were over 50 cm in length. Samples representing haplotype *C1* were further divided into males and females of both species found around the Faroe Islands during mating season (Figure 9). The figure shows difference in depth range be-

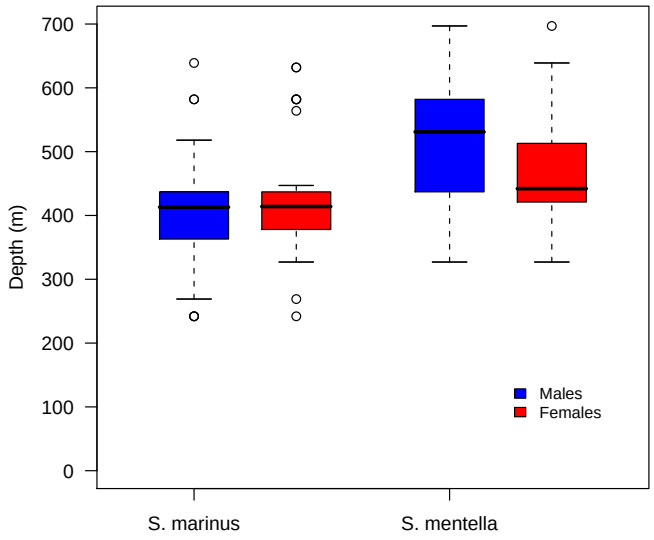

Figure 9: Depth distribution among males and females within *S. mentella* and *S. marinus* representing haplotype *C1*. Samples were collected around the Faroe Islands during mating season in September 2006.

tween males and females within *S. mentella*. If indeed they were mating at this time a similar depth range would have been expected for both sexes during the mating season. Therefore, mating either had not completely started or it was partly over. In contrast, individuals among males and females within *S. marinus* revealed similar depth ranges as would be expected if they were mating. As mentioned above, a considerable difference was observed in size between *S. mentella* and *S. marinus*, and a similar difference was also detected between males and females within each species (Figure 10). Females were significantly larger than males within *S. marinus*, ($t = -4.5,\ df = 309.6,\ p < 0.001$) while males were significantly larger than females

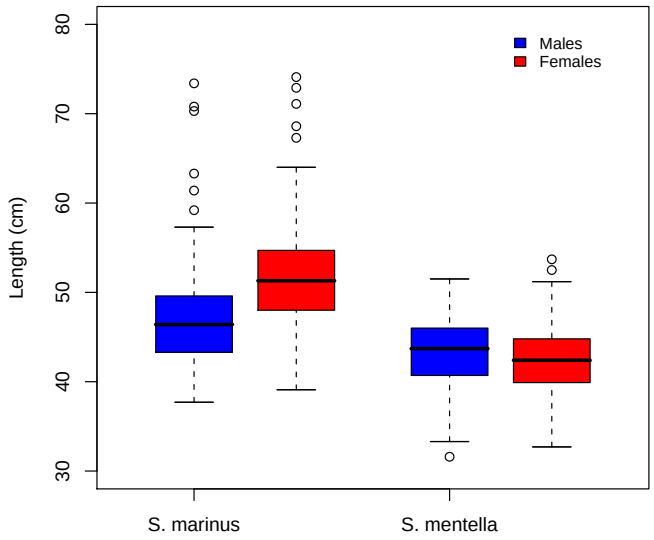

Figure 10: Length distribution among males and females within *S. mentella* and *S. marinus* representing haplotype *C1*. Samples were collected around the Faroe Islands during mating season in September 2006.

within *S. mentella*, ($t = 2.9,\ df = 602.4,\ p < 0.002$). In addition, the sex ratio was very different between the species. The female:male ratio was 44:56 within *S. mentella* whereas it was 60:40 within *S. marinus*. The difference in sex ratios between the two species was highly significant ($X^2 = 20.8,\ df = 1,\ p < 0.001$). A similar pattern was observed for both species that shared haplotype *C2* (data not shown).

Length distribution – *S. mentella* and subgroups

Length distribution for the three subgroups of *S. mentella* are presented in Figure 11. The "oceanic" type was relatively smaller in size compared to the other two groups, "pelagic" and "demersal", while the latter two were of similar length. A highly significant difference was found between the mean length of the "oceanic" and the "pelagic" types ($t = -19.7,\ df = 561.8,\ p < 0.001$). A highly significant difference was also found between the mean length of the "oceanic" and the "demersal" types ($t = -24.7,\ df = 2405.4,\ p < 0.001$) but not between the "demersal" and the "pelagic" types ($t = -0.34,\ df = 772.6,\ p = 0.73$).

The female:male ratio was similar for both groups in the Irminger Sea the "oceanic" and "pelagic" types. The female:male ratio for both groups was approximately 40:60. No

difference was observed in mean length of males and females within the groups (data not shown).

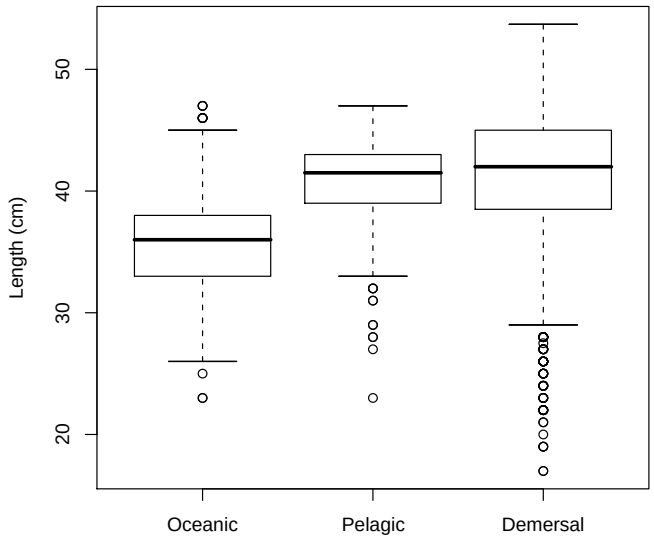

Figure 11: Length distribution among the three subgroups of *S. mentella*. The "oceanic" and the "pelagic" types from the Irminger Sea and the "demersal" type on the continental shelves for all countries.

Depth and geographical distribution of various haplotypes among *S. viviparus*

The three most common haplotypes within *S. viviparus* were found at different depths (Figure 12). Individuals of *S. viviparus* carrying the most common haplotype *V1* were found at a broad depth range from 100–600 meters. Individuals carrying haplotype *V2* were all found at a narrow and shallow depth range and never below 150 meters. However, *V3*, were all collected at relatively great depths ranging from 350–600 meters. As mentioned above, *S. viviparus* was collected in two surveys of the Faroe Islands. The survey in March was restricted to the West and Southwest of the Islands while the survey in September covered most parts around the Islands. Haplotype *V1* was detected in individuals from both surveys while haplotype *V2* was only found in March and haplotype *V3* only in September. Three rare haplotypes were found. Two of them *V5* and *V6*, found in two individuals each and the third, *V4*, found in three individuals. Both *V4* and *V6* were only found in the September survey and only East of the Islands. The two individuals carrying haplotype *V5* were collected in the two separate surveys.

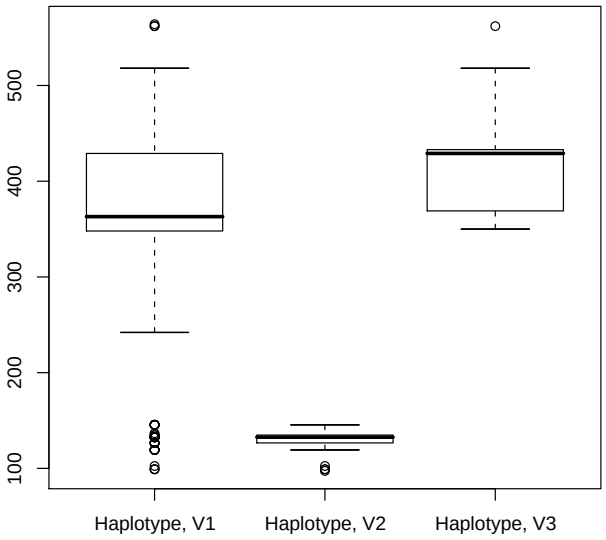

Figure 12: Depth distributions among the three most common haplotypes within *S. viviparus*. Samples were collected around the Faroe Islands.

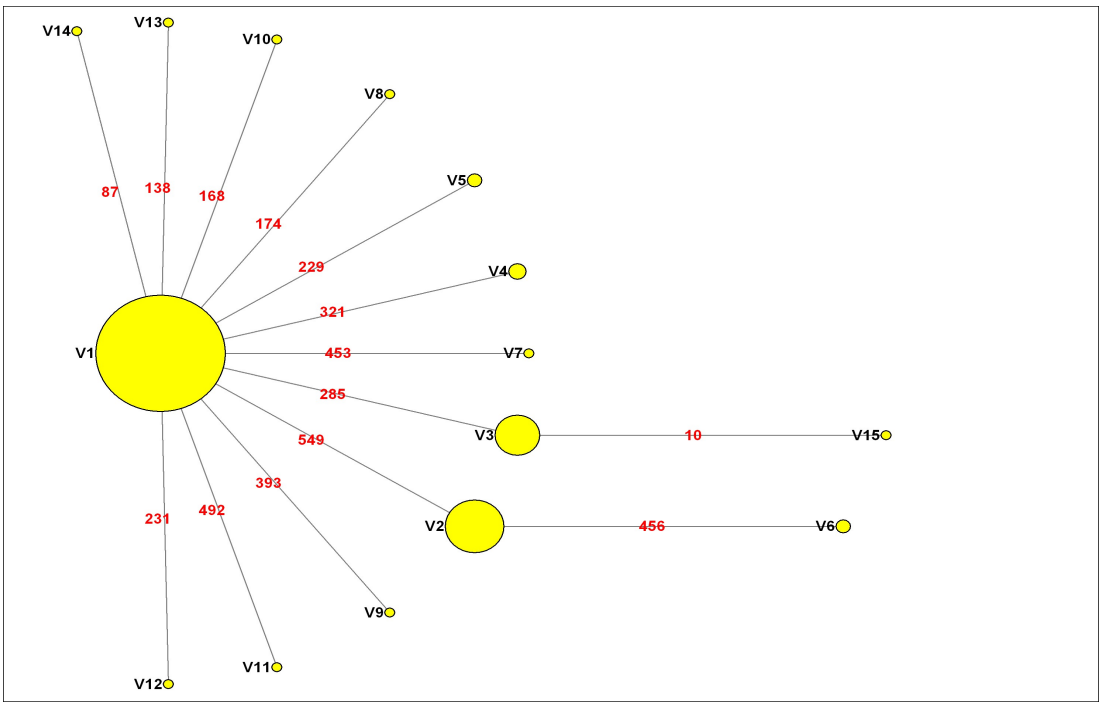

Figure 13: A median joining network of mitochondrial cyt *b* haplotypes among 286 individuals of *S. viviparus*.

One was found North and the other West of the Islands. The nine singletons observed were relatively evenly distributed to the East and the West of the Islands.

A median joining network (Figure 13) shows the relationship among the haplotypes within the cyt *b* region for *S. viviparus*. Most of the haplotypes differed from their nearest neighbour by one substitution.

Discussion

The North Atlantic *Sebastes* species can show high similarities in morphological characters in areas where they overlap. In particular this is the case for characters used in discriminating among subgroups of *S. mentella*. Furthermore, *S. marinus* and *S. mentella* sometimes reveal overlapping morphological characters which makes distinguishing between them difficult. The third species, *S. viviparus*, is usually well separated morphologically from both *S. marinus* and *S. mentella*.

This study provides mtDNA sequence data on a very large sample of both *S. mentella* and *S. marinus*. In addition a smaller sample of *S. viviparus* was studied. Previous studies of mtDNA variation using more limited sampling (e.g. BENTZEN *et al.*, 1998; SCHMIDT, 2005) have revealed low levels of genetic differentiation. Whereas BENTZEN *et al.* (1998) finds no difference between *S. marinus* and *S. mentella*, SCHMIDT (2005), however, found differences between *S. marinus* and *S. mentella*. She also found indication of potential cryptic group within *S. marinus* related to "giants" which also have been studied by others (e.g. JOHANSEN *et al.*, 2000b; PAMPOULIE and DANÍELSDÓTTIR, 2008).

By doing a more intensive sampling I hoped that I might be able to resolve some of the issues concerning species differences and cryptic groups within North Atlantic *Sebastes*. In particular, a larger sample would provide greater statistical confidence in the result.

The overlapping morphological characters means that there is great potential for incorrect classification. PAMPOULIE and DANÍELSDÓTTIR (2008) have stressed the difficulty of species identification due to overlapping of meristic and morphological characters. Incorrect classification of species to groups or taxa is a potential explanation of the observation that various taxa show complicated patterns of haplotype sharing. I can put forth the hypothesis that haplotype sharing is primarily due to incorrect classification. This hypothesis of incorrect classification makes certain predictions. For example, incorrect classification should not be haplotype-specific, and therefore all haplotypes should be shared in relation to their frequencies. This kind of haplotype sharing is not, however, observed in this study. Certain haplotypes

were private to a group, which is contrary to this expectations. Also if we assumed that haplotypes *C1* and *C2* represented good species it would mean that more than one half of *S. marinus* were incorrectly classified. A second hypothesis and an alternative explanation is incomplete lineage sorting or polyphyly (FUNK and OMLAND, 2003). Incomplete lineage sorting of ancestrally polymorphic mtDNA lineages has the potential of affecting a gene tree of any taxon (FUNK and OMLAND, 2003). Lineages of a given locus have their own history with some lineages sharing more recent, and other more ancient coalescent events (PAMILO and NEI, 1988). Based on the structure of haplotype sharing in this study, I regard lineage sorting as the most probable cause of the observed polyphyletic pattern between *S. marinus* and *S. mentella*, and among *S. mentella* subgroups. FUNK and OMLAND (2003) have suggested that species-level polyphyly is high in fishes or almost 25%, indicating this phenomenon to be much more important in the marine environment than previously thought.

S. viviparus does not share any haplotypes with *S. marinus* and *S. mentella* and thus represents a monophyletic group. This correlates well with its well separated morphology from *S. marinus* and *S. mentella*. However, within *S. viviparus* substructuring might be possible as evident by the distribution of haplotypes East and West of the Faroe Islands. Indication of substructuring among *S. viviparus* is that these haplotypes, found East and West respectively, were found at very different and non-overlapping depths. Nonetheless, the observed structure could be seasonal as one of the haplotypes defining a potential substructure was only found in the March survey and another haplotype only during the mating season in September. To further explore the possibility of a cryptic group within *S. viviparus*, comparisons with other geographical regions are necessary.

Analysis of mtDNA sequences allows marine fishes to be categorized based on different combinations of small and large values of nucleotide and haplotype diversities (GRANT and BOWEN, 1998). Based on this the low haplotype and nucleotide diversities observed within *S. mentella* are an indication of a recent population bottleneck or founder effect by a single mtDNA lineage and probably followed by a rapid expansion. Low nucleotide diversities and intermediate to high haplotype diversities as observed for *S. marinus* is often an indication of a population bottleneck followed by rapid population growth (GRANT and BOWEN, 1998). The median joining network further supports these conclusions. It shows the one prevalent haplotype embedded by haplotypes in low frequencies, mostly one substitution away from the central haplotype.

A single mutation at site 333 separates the major haplotypes *C1* and *C2*. Thymine seems to be highly conserved at this site as it is shared by all haplotypes within *S. viviparus* and

is also found within the highly polymorphic species *Helicolenus dactylopterus*, a close relative of the *Sebastes* species. In addition, individuals carrying haplotype *C22*, which showed the closest match to *S. fasciatus*, which also has Thymine at this site. This pattern could be an indication of Thymine being an ancestral state and Cytosine a derived one for this site. Thus, haplotype *C2* is an ancestral haplotype, representative of the state of the ancestor of *S. marinus* and *S. mentella*. *S. marinus* retains this state at a high frequency in its present day populations whereas *S. mentella* has all but lost it. On this basis *S. mentella* possesses a derived character in its present day populations.

Haplotype *C22* which showed the closest match to *S. fasciatus* was found in three individuals which were classified to three different groups (two of which were groups of *S. mentella* and one unidentified *Sebastes* sp.). These individuals might actually be *S. fasciatus* misidentified as *S. mentella*. That would imply the presence of *S. fasciatus* at a low density in the Eastern North Atlantic. Perhaps *S. fasciatus* has expanded its distributional range in response to global warming as has been suggested for various fish taxa (PERRY *et al.*, 2005). Alternatively, these specimens might be of hybrid origin. Introgressive hybridization between *S. fasciatus* and *S. mentella* has been suggested by ROQUES *et al.* (2001) in the Northwest Atlantic. In this event *S. fasciatus* mitochondrial types might be found far east of the location of the hybrid zone (ROQUES *et al.*, 2001). In contrast, SCHMIDT (2005) finds indication of ancient introgressive hybridization events between *S. marinus* and *S. fasciatus*. At this stage I have no way to differentiate between these hypothesis.

No clear structure of unique haplotypes defines the difference between *S. marinus*, *S. mentella* and *S. mentella* subgroups. Instead their differences are due to uneven frequencies of various haplogroups among them. Combination of haplogroups within each locality were most of the times significantly different, either because of haplotypes in high or low frequency, or both. The high sharing of haplotypes among the two main species, *S. marinus* and *S. mentella*, could indicate early stages of polyphyly whereas some lineages that are more representative of *S. marinus* are more closely related to lineages within *S. mentella* and vice verse. The polyphyletic pattern indicates a widespread incomplete sorting of ancestral polymorphism in both species.

The observed difference among localities for variation within *S. marinus* was not caused by any particular haplogroup. Rather, the observed difference was caused by the joint effects of the most common and the three most common haplotypes. A different pattern was observed for *S. mentella* and its subgroups. The observed differences among geographically close regions mostly seem to be due to frequency differences of medium-rare and rare hap-

lotypes. However, for regions lying further apart, the most common or the three most common haplotypes are the main cause for the observed frequency differences. KIMURA and OHTA (1973) showed that the expected age of neutral allele is directly related to its frequency (and see SLATKIN and RANNALA, 2000). Therefore the observed pattern may reflect different ages of the haplotypes with haplotypes having increased in frequency relative to their age (e.g. KIMURA and OHTA, 1973; SLATKIN and RANNALA, 2000). Thus the medium-rare and rare haplotypes might represent younger alleles in the populations and therefore more localized than the common haplotypes.

Rare haplotypes have greater information content about population structure, gene flow, and potential isolation than universal haplotypes (ÁRNASON *et al.*, 2000). The concept of private alleles has been advanced by SLATKIN (1985) as a powerful way to analyze gene flow or lack of gene flow. Haplotypes that are considered private in this study are those found in a single locality. They can, however, only be considered private on a global scale as they were not found in a single area within a given locality. The private haplotypes were in the haplogroups that were defined as the medium-rare and rare haplotypes. As mentioned above the medium-rare haplogroup was the main cause of the observed differentiation between the "oceanic" and "pelagic" types in the Irminger Sea. However, the "demersal" type found East and West of Iceland seems to more closely related to the "pelagic" type than to the "oceanic" type based on haplogroup differences. Similarly JOHANSEN *et al.* (2000a) found closer relatedness of the "pelagic" in the Irminger Sea and the "demersal" on the Icelandic shelf area.

Singletons comprised almost half of the haplotypes and were distributed relatively evenly among the samples. Although in high numbers, their effect on genic differentiation seems minimal and were all non-significant. Singletons in a sample are found at a single locality and cannot be considered private alleles because no test is possible of their privateness. For all we know they may be very rare but widespread (ÁRNASON *et al.*, 2000). Here high amount of singletons were observed in the sample as would be expected because of large sample size. Whether they represent private haplotypes cannot be evaluated.

The unidentified *Sebastes* individuals were all collected on the East and West coast of Greenland. Some of the individuals that were collected in the Greenland surveys were, however, classified to either *S. marinus* or *S. mentella* ("demersal" type). Because of very small sample size of *S. marinus* in Greenland, interpreting of any difference between them and samples in other localities is not valid. However, samples among the "demersal" type of *S. mentella* from Greenland, are numerous enough to allow a comparison to other localities. The relatively high F_{ST} observed between Greenland samples and other localities is most likely due

to misidentified specimens and that these individuals represent both *S. mentella* and *S. marinus*.

The main difficulties in distinguishing between *S. marinus* and *S. mentella* in the Greenland surveys was due to small size of the specimens. They were found as small as 8 cm, with majority, nearly 70%, smaller than 25 cm and non exceeded 50 cm. Some of these specimens were heavily infested with the parasite copepod *Sphyrion lumpi*, which also is quite common in the "oceanic" type of *S. mentella* (MAGNÚSSON and MAGNÚSSON, 1995). It is interesting in this respect that individuals of the unidentified specimens in Greenland and the "oceanic" type of *S. mentella* carried the same medium-rare haplotypes and some of the rare ones. Thus some of the *Sebastes* from the Greenland survey might have been of the "oceanic" type of *S. mentella*. This could also be one of the difficulties in discriminating between specimens as expertise in distinguishing the "oceanic" type from other *Sebastes* was not available in the field during the Greenland surveys.

Further support for the hypothesis that classification of *S. marinus* and *S. mentella* is correct (excluding Greenland) and that haplotype sharing represents incomplete lineage sorting can be obtained from biological and environmental factors. Therefore, I compared those sharing the most common haplotype *C1* with respect to depth, size, and sex. The observed biological differences were all significant and suggest that mating season could be at different times for the species. The unevenness of males and females within *S. mentella* could partly be explained by their broader depth range compared to *S. marinus*. However, more evenness in depth would be expected for both sexes if they were indeed mating. Thus mating may have been completed recently or that it had not started completely. Both the sex ratio differences and partially non-overlapping sizes and depth range indicate that they are separate biological entities. This supports the notion that their sharing of various haplotypes is due to incomplete lineage sorting, most likely because of their recent speciation.

For construction of a gene tree monophyly of a species is assumed for alleles at a study locus (FUNK and OMLAND, 2003). However, a phylogenetic tree or gene tree which is constructed from DNA sequences for a given locus does not necessarily agree with the tree that represents the evolutionary pathway of the species involved, or species tree (PAMILO and NEI, 1988). A recent speciation event may show such incongruities between gene trees and species trees, especially when ancestral polymorphism is retained in present day populations. This phenomenon is likely to be the main reason for the observed genetic structure of *S. marinus* and *S. mentella*. The diagram in Figure 14 (redrawn based on NEI, 1987) shows that gene splitting is usually earlier than population splitting. To estimate the population splitting time T, the average nucleotide differences within a group at the time of population splitting is subtracted

from d_{xy}, the gross difference between groups. The average nucleotide difference is estimated by $(d_x + d_y)/2$ under the assumption that expected value of d_x or d_y is the same for the evolutionary process (NEI, 1987).

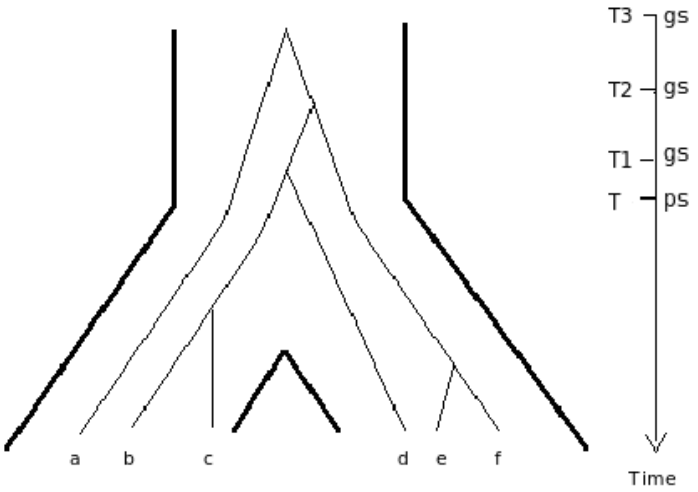

Figure 14: Time of gene splitting and population splitting. Redrawn after Figure 10.4 in NEI (1987). T1-T3, times of gene splitting (gs), T, time of population splitting (ps), and letters a, b, c, d, e and f represent haplotypes.

In light of history of the genus, *Sebastes* species readily diversify and subsequently speciate. Past colonization events are most likely correlated with the high latitude cooling in the North Pacific late in Miocene approximately 8 million years ago (e.g. WOURMS, 1991; HYDE and VETTER, 2007). This event led to the formation of productive upwelling systems in the North Pacific which allowed substantial diversification and dispersal of the *Sebastes* species (HYDE and VETTER, 2007). The dispersal event following the opening of the Bering Straits allowed the opportunity of calibrating a molecular clock model of genetic evolution (HYDE and VETTER, 2007). Following this calibration point, the split between *S. marinus* and *S. mentella* is fairly recent, or approximately 19.000 years. However, this time might be overestimated due to ancestral polymorphism that seems to be high in present day population within *S. marinus*. The time of this estimated divergence corresponds to the last glacial maximum (LGM), ~20.000 years ago. The LGM was followed by a deglaciation time until ~10.000 years ago (INGOLFSSON and NORDDAHL, 2001). The estimated divergence time among the *S. mentella* types in the Irminger Sea and the demersal type on the shelves was ~3000–4000 years. Detection of ancestral polymorphism was low for *S. mentella*. The short estimated time since the split of *S. marinus* and *S. mentella* raises questions of the effect of the LGM. It has

been suggested that this historical event may have been responsible for reduction of and even extermination of populations of marine organisms in the North Atlantic (ABOIM *et al.*, 2005). LGM, might thus have played a major role in the genetic structure observed in the present day species *S. mentella* and *S. marinus*. Genetic techniques are becoming more and more advanced. Consequently more marine species will be examined in greater detail in the future. I predict that we will likely find incidences of more cryptic groups within the genus.

As evident from the data *S. marinus* and *S. mentella* do not possess unique haplotypes between them within the cytochrome *b* gene. Several *Sebastes* species in the Pacific show color morphs that are not reciprocally monophyletic at the examined mitochondrial genes. Nonetheless, they show evidence of assortative mating when examined using faster evolving nuclear markers (KAI *et al.*, 2002). A species complex such as this could be likely in early stages of speciation with continuous divergence of the species in the future (HYDE and VETTER, 2007). The indication of sex ratio and possible difference in timing of reproduction in my study referred to above may possibly indicate assortative mating.

Populations in the marine environment are potentially affected by numerous selective pressures. Molecular markers can be applied to measure the impact of selection by a combination of polymorphism that are under selection and those that are not. To further study the species complex of the North Atlantic *Sebastes* species, several approaches could be applied such as developing and analyzing sequence variation of several nuclear genes. They may show genome wide effects as well as showing locus specific effects of selection, important for functional differentiation within and among populations (LUIKART *et al.*, 2003). Genes showing locus specific effects can give important information on functional factors important in the ecology of the organism.

The general idea of most marine species is lack of local adaptation on a small geographic scale which has been supported by low levels of genetic diversity (CONOVER *et al.*, 2006). Recently this view has been challenged due to evidence of geographically structured local adaptation in physiological and morphological traits (CONOVER *et al.*, 2006). Furthermore there is evidence of selection being the evolutionary force capable of sustaining adaptive divergence on contemporary time scales (CONOVER *et al.*, 2006). This combines the phenotype and the geography, that is the relationship between the geography of phenotypic variation and the geography of lineages in marine species (CONOVER *et al.*, 2006). Applying these concepts to *Sebastes* by searching for genes showing locus specific effect may significantly enhance our understanding of the ecology and evolution of this important group.

Appendix

Segregating sites among *S. mentella* and *S. marinus* are presented in Table 11. The data represent a 420 base pair mtDNA fragment from the cyt *b* region. Segregating sites among 286 individuals of *S. viviparus* are presented in Table 12. The data represent a 567 base pair mtDNA fragment from the cyt*b* region.

Table 11: Segregating sites of a 420 mtDNA fragment from the cyt *b* among 4480 individuals of *S. marinus* and *S. mentella*.

Segregating sites 10–229:

Haplotype	10	18	30	36	42	45	57	63	66	80	87	106	111	112	114	127	129	132	138	147	153	154	156	159	162	168	174	183	186	187	189	201	205	211	213	222	229
C1	G	A	A	T	G	G	C	C	A	T	G	T	C	A	G	G	T	T	G	T	T	G	T	T	T	A	A	G	A	T	C	T	G	G	T	C	G
C40	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
C3	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
C65	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
C36	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
C86	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
C15	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
C85	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
C2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
C61	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
C60	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
C70	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
C74	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
C77	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.
C56	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.
C79	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.
C6	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
C45	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
C14	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
C16	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
C9	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.
C89	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.
C32	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
C88	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
C90	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
C47	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
C22	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
C18	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
C72	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
C4	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
C27	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
C73	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
C64	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
C8	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.
C54	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.
C33	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
C52	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
C7	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
C46	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
C87	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
C26	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
C50	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
C51	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
C39	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
C38	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
C24	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
C25	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
C63	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
C23	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
C62	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
C19	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.

Segregating sites 230–417 and frequency:

Haplotype	230	231	234	237	240	241	246	248	255	259	285	288	291	294	298	299	303	306	309	312	315	322	330	333	351	352	357	360	369	372	390	399	402	408	409	411	417	Frequency
C1	T	G	C	C	T	G	C	T	C	C	A	A	A	A	A	T	A	G	T	A	T	G	A	C	A	T	C	A	G	C	C	C	G	T	G	A	C	3654
C40	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	2
C3	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	79
C65	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	1
C36	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	2
C86	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1
C15	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	5
C85	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	1
C2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	460
C61	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	A	.	.	1
C60	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	1
C70	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	1
C74	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	1
C77	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	1
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Continued on next page

Table 11: Segregating sites of a 420 mtDNA fragment from the cyt *b* among 4480 individuals of *S. marinus* and *S. mentella*.

Haplotype	Segregating sites																																																																										Frequency
	10	18	30	36	42	45	57	63	66	80	87	106	111	112	124	127	129	132	138	147	153	154	156	159	162	168	174	183	186	187	189	201	205	211	213	222	229	230	231	234	237	240	241	246	248	255	259	285	288	291	294	298	299	303	306	309	312	315	322	330	333	351	352	357	360	369	372	390	399	402	408	409	411	417	
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Table 12: Segregating sites of a 567 base pair mtDNA fragment from the cytochrome *b* among 14 haplotypes from 286 individuals of *S. viviparus*

Haplotype	Segregating sites															Frequency
			1	1	1	2	2	2	3	3	4	4	4	5		
	1	8	3	6	7	2	3	8	2	9	5	5	9	4		
	0	7	8	8	4	9	1	5	1	3	3	6	2	9		
V1	G	G	G	A	A	G	G	A	A	C	T	A	T	T		218
V2	.	.	.	.	.	.	.	.	.	.	.	.	.	C		33
V6	.	.	.	.	.	.	.	.	.	.	.	G	.	C		2
V3	.	.	.	.	.	.	.	G	.	.	.	.	.	.		19
V15	A	.	.	.	.	.	.	G	.	.	.	.	.	.		1
V4	.	.	.	.	.	.	.	.	G	.	.	.	.			3
V9	.	.	.	.	.	.	.	.	.	T	.	.	.	.		1
V5	.	.	.	.	.	A	.	.	.	.	.	.	.	.		2
V7	.	.	.	.	.	.	.	.	.	.	C	.	.	.		1
V8	.	.	.	.	G	.	.	.	.	.	.	.	.	.		1
V10	.	.	.	G	.	.	.	.	.	.	.	.	.	.		1
V11	.	.	.	.	.	.	.	.	.	.	.	.	C	.		1
V12	.	.	.	.	.	.	A	.	.	.	.	.	.	.		1
V13	.	.	A	.	.	.	.	.	.	.	.	.	.	.		1
V14	.	A	.	.	.	.	.	.	.	.	.	.	.	.		1

Bibliography

ABOIM, M. A., G. M. MENEZES, T. SCHLITT and A. ROGERS, 2005. Genetic structure and history of populations of the deep-sea fish *Helicolenus dactylopterus* (Delaroche, 1809) inferred from mtDNA sequence ananlysis. *Molecular Ecology* **14**: 1343–1354.

ÁRNASON, E., P. H. PETERSEN, K. KRISTINSSON, H. SIGURGÍSLASON and S. PÁLSSON, 2000. Mitochondrial cytochrome *b* DNA sequence variation of Atlantic cod from Iceland and Greenland. *Journal of Fish Biology* **56**: 409–430.

BANDELT, H.-J., P. FORSTER and A. RÖHL, 1999. Median-joining networks for inferring intraspecific phylogenies. *Molecular Biology and Evolution* **16**: 37–48.

Barcode of Life Initiative, 2003. `http://www.dnabarcodes.org`.

BARSUKOV, V. V., I. A. OGANIN and A. I. PAVLOV, 1991. Morphological and ecological differences between *Sebastes fasciatus* and *S. mentella* on the Newfoundland shelf and Flemish Cap Bank. *Journal of Ichthyology* **31**: 1–17.

BAUM, D. A. and K. L. SHAW, 1995. Genealogical perspectives on the species problem. *In Molecular and experimental approaches to plant biosystematics*, edited by HOCH, C. and A. G. STEPHENSON, pp. 289–303. Missouri Botanical Garden, St. louis.

BENSON, D. A., I. KARSCH-MIZRACHI, D. J. LIPMAN, J. OSTELL and D. L. WHEELER, 2008. Genbank. *Nucleic Acids Research* **36**: D25–D30.

BENTZEN, P., J. M. WRIGHT, L. T. BRYDEN, M. SARGENT and K. C. T. ZWANENBURG, 1998. Tandem repeat polymorphism and heteroplasmy in the mitochondrial control region of redfishes (*Sebastes*: Scorpaenidae). *Journal of Heredity* **89**: 1–7.

CARVALHO, G. R. and L. HAUSER, 1994. Molecular genetics and the stock concept in fisheries. *Reviews in Fish Biology and Fisheries* **4**: 326–350.

CONOVER, D. O., L. M. CLARKE, S. B. MUNCH and G. N. WAGNER, 2006. Spatial and temporal scales of adaptive divergence in marine fishes and the implications for conservation. *Journal of Fish Biology* **69**: 21–47.

COYNE, J. A. and H. A. ORR, 2004. *Speciation*. Sinauer Associates, Inc, Sunderland, Massachusetts.

DAWSON, M. and D. JACOBS, 2001. Molecular evidence for cryptic species of *Aurelia aurita* (Cnidaria, Scyphozoa). *Biological Bulletin* **200**: 92–96.

DAYHOFF, M. O., 1972. *Atlas of protein sequence and structure 1972*, volume 5. National Biomedical Research Foundation, Washington, D.C.

EWING, B. and P. GREEN, 1998. Basecalling of automated sequencer traces using Phred. II. Error probabilities. *Genome Research* **8**: 186–194.

EWING, B., L. HILLIER, M. C. WENDL and P. GREEN, 1998. Base-calling of automated sequencer traces using Phred. I. Accuracy assessment. *Genome Research* **8**: 175–185.

FELSENSTEIN, J., 1991. PHYLIP (phylogeny inference package) version 3.6a3. Distributed by the author. Department of Genome Sciences, University of Washington, Seattle.

FELSENSTEIN, J. and G. A. CHURCHILL, 1996. A hidden Markov model approach to variation among sites in rates of evolution. *Molecular Biology and Evolution* **13**: 93–104.

FUNK, D. J. and K. E. OMLAND, 2003. Species-level paraphyly and polyphyly: Frequency, causes and consequences, with insights from animal mitochondrial DNA. *Annual Review of Ecology, Evolution, and Systematics* **34**: 397–423.

FUTUYMA, D. J., 1998. *Evolutionary Biology*. Sinauer Associates, Inc. Publishers, Massachusetts.

GASCON, D. E., 2003. Redfish multidisciplinary research zonal program (1995–1998): Final report. *Technical Report 2462*, Canadian Technical Report of Fisheries and Aquatic Sciences.

GORDON, D., C. ABAJIAN and P. GREEN, 1998. Consed: A graphical tool for sequence finishing. *Genome Research* **8**: 195–202.

GRANT, W. S. and B. W. BOWEN, 1998. Shallow population histories in deep evolutionary lineages of marine fishes: Insights from sardines and anchovies and lessons for conservation. *Journal of Heredity* **89**: 415–426.

HAJIBABAEI, M., G. A. C. SINGER, P. D. N. HEBERT and D. A. HICKEY, 2007. DNA barcoding: how it complements taxonomy, molecular phylogenetics and population genetics. *Trends in Genetics* **23**: 167–172.

HEBERT, P. D. N., A. CYWINSKA, S. L. BALL and J. R. DEWAARD, 2003. Biological identifications through DNA barcodes. *Proceedings of the Royal Society, Series B.* **270**: 313–321.

HEDRICK, P. W., 2005. *Genetics of Populations*. Jones and Bartlett Publishers, Sudbury Massachusetts, 3rd edition.

HUDSON, R. R. and J. A. COYNE, 2002. Mathematical consequences of the genealogical species concept. *Evolution* **56**: 1557–1565.

HYDE, J. and R. VETTER, 2007. The origin, evolution, and diversification of rockfishes of the genus *Sebastes* (Cuvier). *Molecular Phylogenetics and Evolution* **44**: 790–811.

ICES C.M, I., 1998. Report of the study group on redfish stocks. *Technical Report G:3*, ICES.

INGOLFSSON, O. and H. NORDDAHL, 2001. High relative sea level during the Bolling interstadial in western Iceland: A reflection of ice-sheet collapse and extremely rapid glacial unloading. *Arctic, Antarctic, and Alpine Research* **33**: 231–243.

JOHANSEN, T., A. K. DANÍELSDÓTTIR, K. MELAND and G. NÆVDAL, 2000a. Studies of the genetic relationship between deep-sea and oceanic *Sebastes mentella* in the Irminger sea. *Fisheries Research* **49**: 179–192.

JOHANSEN, T., A. K. DANÍELSDÓTTIR and G. NÆVDAL, 2002. Genetic variation of *Sebastes viviparus* Krøyer in the North Atlantic. *Journal of Applied Ichthyology* **18**: 177–180.

JOHANSEN, T., G. NÆVDAL, A. K. DANÍELSDÓTTIR and N. R. HAREIDE, 2000b. Genetic characterisation of giant *Sebastes* in the deep water slopes in the Irminger sea. *Fisheries Research* **45**: 207–216.

JÓNSSON, G., 1983. *Íslenskir fiskar*. Fjölvaútgáfan, Reykjavík.

KAI, Y., K. NAKAYAMA and T. NAKABO, 2002. Genetic differences among three colour morphotypes of the black rockfish, *Sebastes inermis*, inferred from mtDNA and AFLP analyses. *Molecualar Ecology* **11**: 2591–2598.

KIMURA, M. and T. OHTA, 1973. The age of a neutral mutant persisting in a finite population. *Genetics* **75**: 199–212.

KUMAR, S., K. TAMURA and M. NEI., 1993. MEGA molecular evolutionary genetics analysis. *Technical report*, The Pennsylvania State University, University Park, PA 16802.

KWOK, P. Y., C. CARLSON, T. D. YAGER, W. ANKENAR and D. A. NICKERSON, 1994. Comparative analysis of human DNA variation by fluorescence-based sequencing of PCR products. *Genomics* **25**: 615–622.

LOVE, M., M. YOKLAVICH and L. THORSTEINSSON, 2002. The rockfishes of the North Pacific. *University of California Press, Berkeley, CA, USA* p. 405 pp.

LUIKART, G., P. R. ENGLAND, D. TALLMON, S. JORDAN and P. TABERLET, 2003. The power and promise of population genomics:From genotyping to genome typing. *Nature Reviews Genetics* **4**: 981–994.

MAGNÚSSON, J., 1972. Tilraunir til karfaveiða með miðsjávarvörpu í úthafinu. *Ægir* **65**: 430–432.

MAGNÚSSON, J., 2000a. Djúpkarfi og Úthafskarfi. *In Lífríki sjávar*, p. 7 pp. Námsgagnastofnun — Hafrannsóknastofnunin, Reykjavík.

MAGNÚSSON, J., 2000b. Djúpkarfi og Úthafskarfi. *In Lífríki sjávar*, p. 8 pp. Námsgagnastofnun — Hafrannsóknastofnunin, Reykjavík.

MAGNÚSSON, J. and J. V. MAGNÚSSON, 1995. Oceanic redfish (*Sebastes mentella*) in the Irminger Sea and adjacent waters. *Scientia Marina* **59**: 241–254.

MAYR, E., 1963. *Animal Species and Evolution*. The Belknap Press of Harvard University Press, Cambridge, Massachusetts.

MEYER, A., 1994. DNA technology and phylogeny of fish. *In Genetics and Evolution of Aquatic Organisms*, edited by BEAUMONT, A. R., pp. 220–290. Chapman & Hall, London.

MEYER, C. P. and P. GUSTAV, 2005. DNA barcoding: Error rates based on comprehensive sampling. *Plos Biology* **3**: e422.

NEI, M., 1987. *Molecular Evolutionary Genetics*. Columbia University Press, New York.

NELSON, J., 1984. *Fishes of the World*. John Wiley & Sons, New York.

NICKERSON, D. A., V. O. TOBE and S. L. TAYLOR, 1994. Polyphred: automating the detection and genotyping of single nucleotide substitutions using fluorescence-based resequencing. *Nucleic Acid Research* **25**: 2745–2751.

ORR, J. W. and J. E. BLACKBURN, 2004. The dusky rockfishes (Teleostei: Scorpaeniformes) of the North Pacific Ocean: resurrection of *Sebastes variabilis* (Pallas, 1814) and a redescription of *Sebastes ciliatus* (Tilesius, 1813). *Fishery Bulletin* .

PALUMBI, S. R., 1992. Marine speciation on a small planet. *Trends in Ecology & Evolution* **7**: 114–118.

PALUMBI, S. R., 1994. Genetic divergence, reproductive isolation, and marine speciation. *Annual Reviews of Ecology and Systematics* **25**: 547–572.

PAMILO, P. and M. NEI, 1988. Relationships between gene trees and species trees. *Molecular Biology and Evolution* **5**: 568–583.

PAMPOULIE, C. and A. K. DANÍELSDÓTTIR, 2008. Resolving species identification problems in the genus *Sebastes* using nuclear genetic markers. *Fisheries Research* **93**: 54–63.

PARADIS, E., J. CLAUDE and K. STRIMMER, 2004. Ape: Analyses of phylogenetics and evolution in R language. *Bioinformatics* **20**: 289–290.

PERRY, A. L., P. J. LOW, J. R. ELLIS and J. D. REYNOLDS, 2005. Climate change and distribution shifts in marine fishes. *Science* **308**: 1912–1915.

R DEVELOPMENT CORE TEAM, 2006. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria. URL `http://www.R-project.org`. ISBN 3-900051-07-0.

RAYMOND, R. and F. ROUSSET, 1995. Genepop (version 1.2): Population genetics software for exact tests and ecumenicism. *Journal of Heredity* **86**: 248–249.

REINERT, J., 1998. Redfish. fish-stocks and environment. *In School-Book Foundation of the Faroes*, edited by OLSEN, D., pp. 48–51. Thorshaven, The Faroe Islands. (In Faroese with English abstract).

ROBERT, E. C., 2004. Muscle: Multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research* **32**: 1792–1797.

ROCHA-OLIVARES, A., R. H. ROSENBLATT and R. D. VETTER, 1999. Cryptic species of rockfishes (*Sebastes*: Scorpaenidae) in the South hemisphere inferred from mitochondrial lineages. *Journal of Heredity* **90**: 404–411.

ROQUES, S., P. DUCHESNE and L. BERNATCHEZ, 1999. Potential of microsatellites for individual assignment: the North Atlantic redfish (genus *Sebastes*) species complex as a case study. *Technical report*, Université Laval, Ste-Foy, Québec, Canada.

ROQUES, S., J.-M. SÉVICNY and L. BERNATCHEZ, 2001. Evidence for broadscale introgressive hybridization between two redfish (genus *Sebastes*) in the North-west Atlantic: a rare marine example. *Molecular Ecology* **10**: 149–165.

ROZAS, J. and R. ROZAS, 1999. DNAsp version 3: an integrated program for molecular population genetics and molecular evolution analysis. *Bioinformatics* **15**: 174–175.

SABORIDO-REY, F., D. GARABANA, C. STRANSKY, S. MELNIKOV and V. SHIBANOV, 2005. Review of the population structure and ecology of *S. mentella* in the Irminger sea and adjacent waters. *Reviews in Fish Biology and Fisheries* **14**: 455–479.

SAITOU, N. and M. NEI, 1987. The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution* **4**: 406–425.

SCHMIDT, C., 2005. *Molecular Genetic Studies on Species and Population Structure of North Atlantic Redfish (Genus* Sebastes; *Cuvier 1829)*. Ph.D. thesis, University of Hamburg.

SHAKLEE, J. B. and K. P. CURRENS, 2003. *Population Genetics: Principles and Applications for Fisheries Scientists*, chapter Genetic Stock Identification and Risk Assessment. American Fisheries Society Publication, Herndon, VA 20172.

SLATKIN, M., 1985. Rare alleles as indicators of gene flow. *Evolution* **39**: 53–65.

SLATKIN, M. and B. RANNALA, 2000. Estimating allele age. *Annual Review of Genomics and Human Genetics* **1**: 225–249.

SUNDT, R. C. and T. JOHANSEN, 1998. Low levels og interspecific DNA sequence variation of the mitochondrial 16s rRNA in North Atlantic redfish *Sebastes* (Pisces, Scorpaenidae). *Sarsia* **83**: 449–452.

THOMPSON, J., D. HIGGINS and T. GIBSON, 1994. Clustal W: Improving the sensitivity of progressive multible sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research* **22**: 4673–4680.

VERMEIJ, G., 1991. Anatomy of an invasion: the trans-Arctic interchange. *Paleobiology* **17**: 281–307.

WALSH, P. S., D. A. METZGER and R. HIGUCHI, 1991. Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. *BioTechniques* **10**: 506–513.

WANG, J. Y., T. R. FRASIER, S. C. YANG and B. N. WHITE, 2008. Detecting recent speciation events: the case of the finless porpoise (genus Neophocaena). *Heredity* **101**: 145–155.

WARD, R. D., T. S. ZEMLAK, B. H. INNES, P. R. LAST and P. D. N. HEBERT, 2005. DNA barcoding Australia's fish species. *Philosophical Transactions of The Royal Society* **360**: 1847–1857.

WOURMS, J. P., 1991. Reproduction and development of *Sebastes* in the context of the evolution of piscine viviparity. *Environmental Biology of Fishes* **30**: 111–126.