UiT The Arctic University of Norway

Faculty of Biosciences, Fisheries and Economics, Department of Arctic Marine Biology

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Ingrid Marie Bruvold

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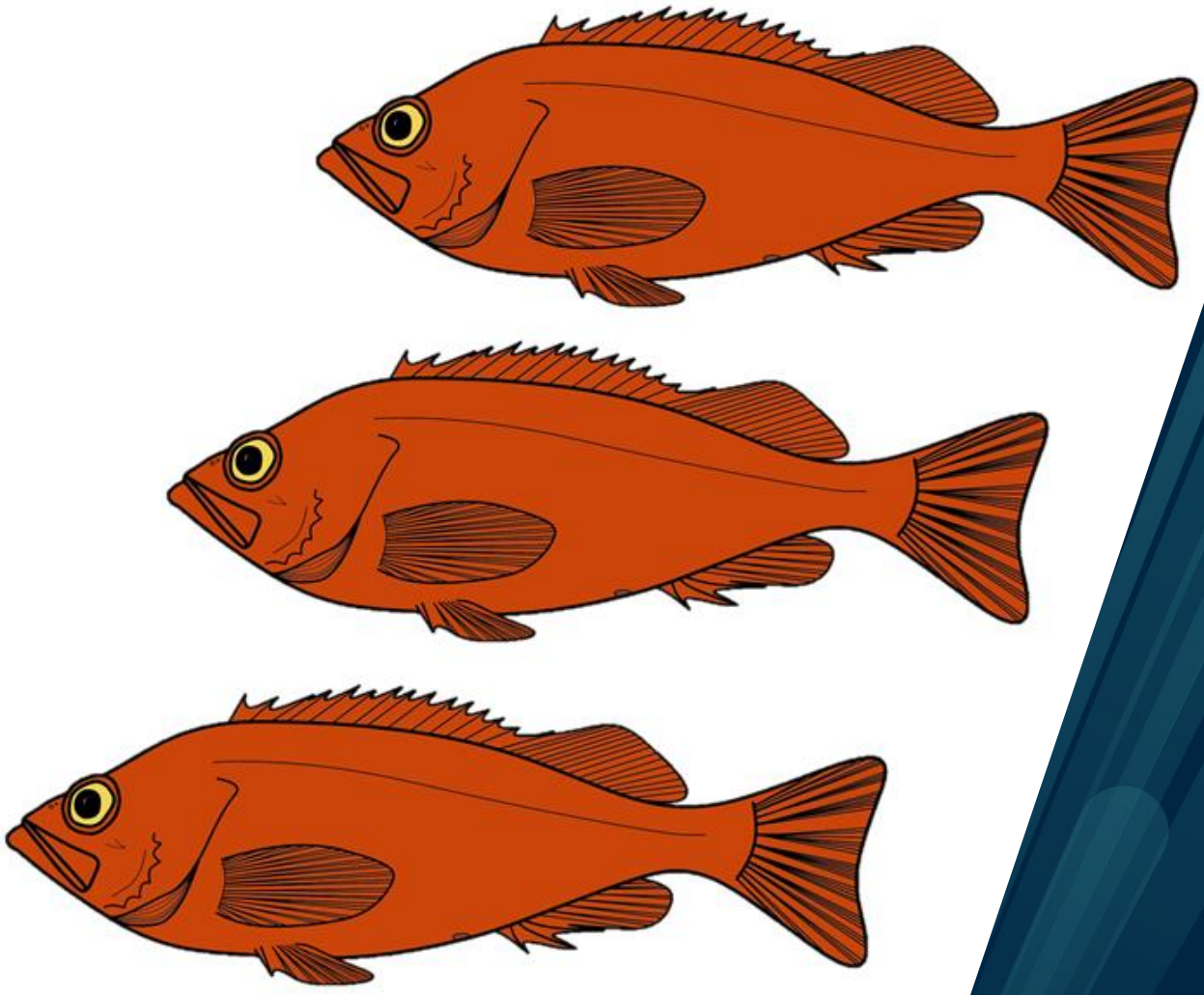

Morphological variation in the redfish (*Sebastes* spp.) complex in Norwegian waters

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Master of Science in Biology – Marine Ecology and Resource Biology

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Supervisors

Arve Lynghammar: UiT – The Arctic University of Norway

Torild Johansen: The Institute of Marine Research

Hannes Höffle: The Institute of Marine Research

Cover illustration: Golden redfish (*Sebastes norvegicus*) by Ingrid Marie Bruvold.

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Preface

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Abstract

The golden redfish *Sebastes norvegicus* is a long-lived commercial species of redfish in the North Atlantic. Excessive harvest through decades has led to a decline in the mature population in Norwegian waters, which is currently considered to be severely depleted. Accumulating genetic evidence suggest a more complex structure within the *Sebastes* genus in the North Atlantic, which has recently formed a hypothesis of a cryptic species complex among *S. norvegicus* represented by the two types A and B. Despite apparent genetic divergence between the types, they have yet to be described morphologically. The external morphology of genetically identified whole fish from Norwegian and East Greenland waters was investigated using traditional morphometric methods to identify descriptive characters for delimitation, applying linear discriminant analysis and random forest classification procedures for extraction of shape information. Combined with non-parametric meristic analysis, the results suggest that features such as beak length and eye orbit size provide good discrimination between the proposed cryptic species as well as separating them from *S. mentella* and *S. viviparus*. Analyzing growth patterns of the proposed cryptic species did not explain previous findings of two distinct growth trajectories among *S. norvegicus* in Norwegian waters, but indicated that they do not possess equal potential for maximum growth. These findings support the hypothesized existence of a species complex in Norwegian waters which can be distinguished both with morphological and genetic analysis, and should be considered in development of monitoring and management strategies further on.

Scientific terms and abbreviations

Term	*Description*
Morphology	The study of shapes
Introgressive hybridization	Exchange of genes between populations through hybridization between a hybrid and its parent species
Cryptic species	Multiple species superficially recognized as a single species based on externally similar appearances
Ecotype	A variant or type, not distinct enough (genetically, phenotypically) to be classified as a species
Ovoviviparity	Internal fertilization with development of eggs inside the female until the larvae hatch, before they are released
Lecithotrophy	Only obtaining embryonic nutrition from egg yolk
Fish stock	Delineated group of fish of commercial interest
Reproductive barrier	Mechanisms preventing reproduction between individuals
LDA	Linear Discriminant analysis, multivariate method for analysis of morphometric data

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1 Introduction

The genus *Sebastes* (Cuvier, 1829) is a diverse group of redfish represented by over 110 species in the North Pacific, South Pacific, and North Atlantic. Only four recognized species of redfishes are found in the North Atlantic (Figure 1): the golden redfish *Sebastes norvegicus* (Ascanius, 1772) and the beaked redfish *S. mentella* Travin, 1951 are distributed across the ocean from the east coast of North America to Novaya Zemlya, while the Norway redfish *S. viviparus* Krøyer, 1845 and the Acadian redfish *S. fasciatus* Storer, 1854 are mainly found in the eastern and western areas of the North Atlantic, respectively (Mecklenburg et al. 2018).

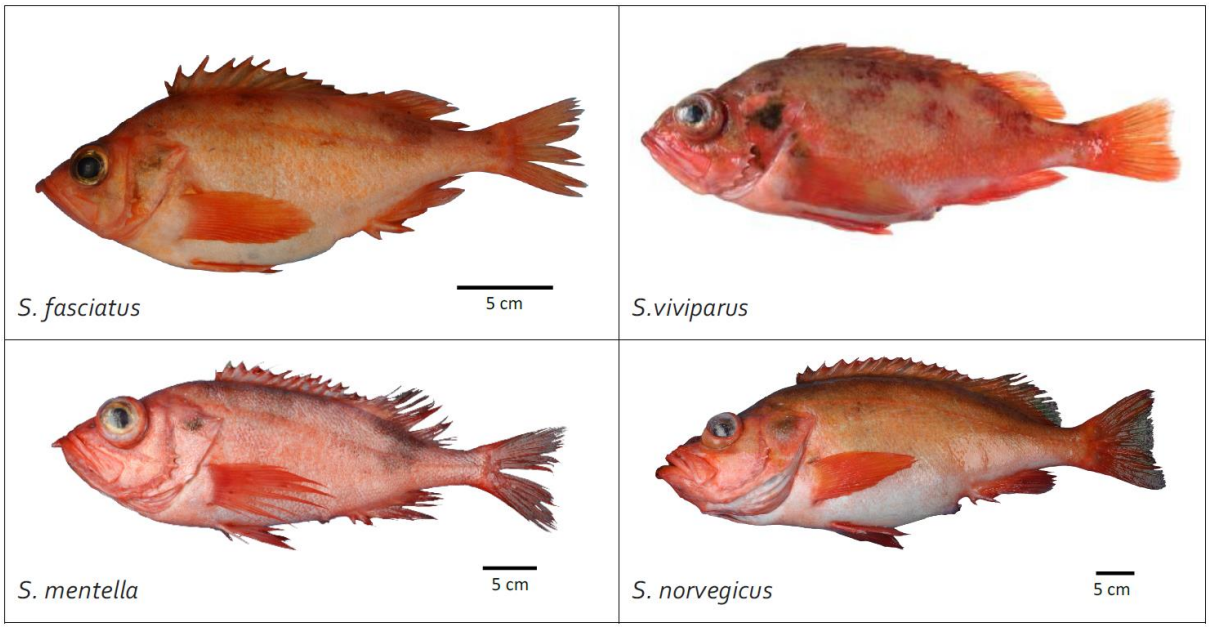


Figure 1. The four species of Sebastes *found in the North Atlantic. Photographs -* S. fasciatus*: Dolores Garabana,* S. viviparus*: Thomas de Lange Wenneck,* S. mentella *and* S. norvegicus*: Alf Harbitz. Figure assembled by Natalia Monferrer (Monferrer and Planque 2019).*

The evolutionary origin of the *Sebastes* species in the North Atlantic has been a topic of great interest among biologists due to the large diversity in morphology and life history within the genus (Kendall 2000). Phylogenetic studies indicate that the North Atlantic populations of redfish were likely established after the introduction of Pacific ancestors around 4 – 3.5 million

years ago at a time when sea levels rose (Hyde and Vetter 2007). The change in bathymetry opened up the Bering Strait passage, allowing for biotic exchange between the Pacific Ocean and the Atlantic Ocean, in an event termed the 'Trans-Arctic interchange' (Vermeij 1991). From this event, Hyde and Vetter (2007) developed a molecular clock which estimated that the speciation of redfish in the North Atlantic from the ancestral population into the current species began approximately one million years ago. This speciation is a relatively recent event on the geological time scale, with *S. viviparus* branching from the basal lineage first, followed by *S. fasciatus* and *S. norvegicus* (previously known as *S. marinus*). *Sebastes norvegicus, S. mentella, S. viviparus,* and *S. fasciatus* are considered separate species, but evidence of relatively recent introgressive hybridization (Roques et al. 2001, Schmidt 2005) suggests that they might not be entirely reproductively isolated. The phylogenetic relationship between the species has been problematic to establish because they share highly similar external morphology, and the classification of *S. mentella* and *S. norvegicus* as separate species was still a topic of discussion in the 1960's (Kotthaus 1961). Currently, the species structure is still extensively researched with a focus on stock discrimination (Garabana 2005, Trella et al. 2013), ecomorphs (Johansen et al. 2000a, Danıelsdóttir et al. 2008), and genetic variation (Johansen et al. 2000a, Johansen et al. 2000b, Garabana 2005, Schmidt 2005, Danıelsdóttir et al. 2008, Stefánsson et al. 2009, Trella et al. 2013, Saha et al. 2016, Saha et al. 2017, Monferrer and Planque 2019) within and among populations of *S. norvegicus* and *S. mentella* in the North Atlantic.

1.1 Life history characteristics of *Sebastes*

The three *Sebastes* species in the Norwegian waters are long-lived, slow growing fish which mature relatively late (reviewed in Wienerroither et al. 2011, Mecklenburg et al. 2018). *Sebastes norvegicus* commonly display indeterminate growth, where the fish continue to increase in size after maturity has been reached (Monferrer and Planque 2019). They have been reported to reach a length up to 122 cm (Drevetnyak et al. 2011), although frequently found to stop growing after maturation at 12 years of age (ICES 2010) and ~35 cm in length (Monferrer and Planque 2019). While lifespans of over 60 years have been recorded in *S. norvegicus* (Planque and Nedreaas 2014b), *S. mentella* can live for over 70 years (Campana et al. 1990) but rarely exceeds a length of 47 cm in Norwegian waters (Planque and Nedreaas 2014a). *Sebastes viviparus* is the smallest species of the three, typically reaching a length of 36 cm and

a maximum age of 40 years (Wienerroither et al. 2011). Common to all the species is an ovoviviparous reproductive mode with internal fertilization (Wienerroither et al. 2011). This is a lecithotrophic viviparous reproductive pattern where fertilized eggs develop into larvae within the female until hatch, only receiving embryonic nutrition from the egg yolk (Wourms 1991). This allows for a trade-off between fecundity and offspring mortality, where the number of eggs produced is lower than in oviparous fish, but larger sized larvae may have increased survival rate from the point of extrusion compared to smaller eggs (Wourms 1991).

1.2 Commercial fishery and management

The fishery of *Sebastes* species has a long history in the North Atlantic. Historically, the fishery of both *S. norvegicus* and *S. mentella* has been of great commercial importance to nations such as Greenland, Norway, Germany, Russia, and Iceland, with the latter performing the majority of redfish harvest in the North Atlantic (ICES 2019). *Sebastes norvegicus* has been harvested from Icelandic waters since the 1920's, peaking at 140 thousand tonnes landed in 1951. Population declines promoted the implementation of protective measures such as temporarily or permanently closing areas where juveniles are present, either based on known nursery grounds or through size-based monitoring of catches temporarily restraining the fishery in areas where juvenile presence is high (>33% of redfish catches; ICES (2019)). As the late maturation of the *Sebastes* species generally means that the generation time is longer than a decade (Wienerroither et al. 2011), these species are vulnerable to excessive harvest of juveniles and the mature population. Direct fishery for *S. norvegicus* in East Greenland waters decreased after population collapse in the late 1980's, and although fishery resumed in 2009, catches continued to be relatively sparse and mainly focused on *S. mentella* (ICES 2019). Following a population decline over several decades in the Norwegian waters (ICES 2018a), restrictions for trawl fishery of *S. norvegicus* were implemented in 2003 (ICES 2018b). In 2006, the Norwegian stock of *S. norvegicus* was listed as *vulnerable* in the Norwegian red list for species, but was given the status *endangered* in 2010 as the abundance in juvenile and young fish continued to decrease (Kålås et al. 2010). Further restrictions on the fishery were gradually put in place until 2015, when all direct fishing with conventional gear was prohibited (ICES 2018b). This stock is currently considered severely depleted (ICES 2018a).

1.3 A species complex

Rapid speciation events and genetic divergence have been observed in multiple redfish lineages, thought to be enabled by a combination of life history characteristics (Hyde and Vetter 2007, Cadrin et al. 2010). This broad diversification has brought attention to distinct features of redfish biology and life history enabling establishment of reproductive barriers (Wourms 1991, Cadrin et al. 2010). Firstly, the reproductive mode of redfish involving internal fertilization requires mate recognition, mate selection, and courtship rituals (Wourms 1991, St-Pierre and De Lafontaine 1995, Johns and Avise 1998, Cadrin et al. 2010). Assortative mating may involve non-visual communication, opening up for other specific behaviors or mechanisms to evolve, e.g. chemical signaling or sound production (Hallacher 1974, Cadrin et al. 2010). In addition, the duration of the redfish larval stage after extrusion is relatively long compared to species with similar reproductive modes (Hyde and Vetter 2007) increasing the time frame for larval dispersal with ocean currents and the opportunity to expand the range of available habitats (Waples 1998). Secondly, habitat preference and spatial barriers act as additional mechanisms for speciation. Redfish species in the Pacific Ocean have largely geographically overlapping distributions, and establish narrow niches based on habitat and depth preferences. Here, ontogenetic shifts in the respective preferences have been observed (Hyde and Vetter 2007). Geographical barriers in the marine environment are typically less obvious than in terrestrial landscapes, and the mechanisms behind speciation events in these marine areas of high connectivity with potential for long distance dispersion are therefore obscure (Waples 1998). Among others, depth has been investigated as a potentially important factor in redfish species divergence (Rocha-Olivares et al. 1999, Hyde et al. 2008, Hess et al. 2014) by providing barriers to overcome that require physiological, behavioral and biological adaptations (Bakay and Mel'nikov 2008). With changes in depth follow changes in light intensity, available prey, salinity, and temperature, promoting additional mechanisms for divergence as seen in *S. mentella* (Stefánsson et al. 2009, Shum et al. 2015) through spatial restrictions on reproduction. Combined, these factors likely provide a basis for adaptive radiation in redfish.

Cryptic species can be defined as two or more species that are classified as a single species based on superficially indistinguishable morphology (Bickford et al. 2007). Naturally, these are likely more prevalent in marine environments than previously anticipated (Janzen et al. 2017). With the increase in efforts to identify cryptic species using continuously advancing methods over the last two decades, new species are frequently uncovered across marine, freshwater, and

terrestrial habitats (Poulin and Pérez-Ponce de León 2017). Highly similar external morphology among North Atlantic redfish and consequently frequent misclassification (Pampoulie and Daníelsdóttir 2008) has led to hypotheses of recent speciation, in congruence with the life history and biology of redfish which allow for reproductive barriers to develop relatively quickly (Cadrin et al. 2010). However, morphological similarities are not necessarily indicative of recent speciation where divergence in external traits has yet to evolve (Bickford et al. 2007). Although this is true in some cases (Rowe et al. 2011), this common assumption has been contested by deeply divergent species exhibiting low morphological variation (Colborn et al. 2001) and/or convergent phenotypes caused by subjection to similar selection pressures (Nedreaas and Nævdal 1991, Nedreaas et al. 1994, Johansen et al. 2000b, Schmidt 2005, Bunke et al. 2013, Shum et al. 2015). Research focused on the diversity within the *Sebastes* genus has led to the discovery of multiple cryptic species in the Pacific Ocean (Rocha-Olivares et al. 1999, Hyde et al. 2008, Hess et al. 2014), as well as a potential species complex in the North Atlantic within *S. norvegicus* (Schmidt 2005, Saha et al. 2017). A giant type of *Sebastes norvegicus* was hypothesized in the 1960's (Kotthaus 1961), and later identified with molecular and morphological studies in Icelandic waters (Johansen et al. 2000b). Furthermore, two additional cryptic species were recently proposed to be part of the complex and named *S. norvegicus* type A and type B revealed by genetic methods (Schmidt 2005, Saha et al. 2017). The two types have not yet been described morphologically.

1.3.1 Investigating morphological variation

Morphology has been, and still is, a fundamental tool in taxonomy (Ward et al. 2009). Morphological analyses for classification of redfish have been extensively used in redfish stock and species delineation in the last few decades (Power and Ni 1985, Nedreaas et al. 1994, Saborido-Rey 1994, Kendall 2000, Saborido-Rey and Nedreaas 2000, Garabana 2005, Trella et al. 2013). With the introduction of statistical procedures for quantifying morphological traits, Power and Ni (1985) first identified the beak length, eye orbit width, interorbital width, body depth, and pectoral fin base width as good discriminators between S. norvegicus and "beaked redfishes" (*S. mentella* and *S. fasciatus* grouped together) in the Labrador-Newfoundland region. When comparing morphological characters between *S. mentella*, *S. norvegicus*, and *S. viviparus* collected in Norwegian waters, Saborido-Rey (1994) found that the distance from the

spiny dorsal fin to the anal fin in combination with the distance from the snout to the spiny dorsal fin and the pelvic fin to the anal fin provided very good classification of the three species. In addition, the number of gill rakers was a good discriminator between *S. mentella* and *S. viviparus*, with an intermediate number of gill rakers in *S. norvegicus* overlapping with the two other species. However, the characters found by Power and Ni (1985) were not highly ranked in their analysis (Saborido-Rey 1994). Focusing on *S. mentella*, *S. norvegicus*, and *S. viviparus* in the Norway, Greenland, Iceland and Faroe Islands area, Garabana (2005) was successful in applying both traditional and geometric morphometric methods on a dataset consisting of over 3000 fish, achieving varying classification accuracy between different regions across the North Atlantic reflecting a morphological gradient across the spatial range. Their findings highlighted a lower morphological variation among species in the Greenland area compared to the specimens in Norwegian waters, but previous studies on morphological variations in redfish have not taken into account the potential cryptic speciation across the North Atlantic.

Morphological methods are typically divided into morphometrics, aiming to describe the shape of the fish, and meristics, describing quantitative features of the fish which can be counted or otherwise characterized. Morphometrics are further separated into traditional (linear) morphometrics and geometric morphometrics (Klingenberg 1996). Traditional morphometrics is conducted by defining specific landmarks on a fish and measuring the distance between these landmarks (Power and Ni 1985). Geometric morphometrics is an image analysis based method relying on software to interpret the shape of a fish by predetermined landmarks as well as the general outline of the fish (Klingenberg 2016), based on the Truss network of distances (Strauss and Bookstein 1982). Both methods produce a number of continuous measurements, which are commonly analyzed using multivariate methods developed specifically for extracting and evaluating information from many variables simultaneously (Claude 2008).

Traditional morphometric measurements can be used to classify data into different groups. A variety of classification procedures can be used, and linear discriminant analysis (LDA) is regularly used (Fisher 1936, Doyle et al. 2018). LDA is a supervised classification and dimensionality reducing procedure that generates linear combinations of continuous variables based on known group membership. These linear combinations maximize the between-group variation while minimizing the within-group variation, optimizing the separation between groups, and can be used for pattern recognition to identify which variables contribute to

separating groups (Lachenbruch and Goldstein 1979). The analysis is optimal for datasets that confine to the assumptions of multivariate normality, homogeneity of variance and low dimensionality where the number of observations in the smallest group exceeds the number of variables (Fisher 1936). However, morphometric data often violate these assumptions (Reyment 1971), especially at low sample sizes (Van Bocxlaer and Schultheiß 2010). The high-dimensional low-sample-size problem of LDA refers to the increasing risk of overfitting when the ratio between number of variables and number of observations increases, which can affect the model's ability to correctly identify explanatory variables and lower the prediction accuracy (Subramanian and Simon 2013). Violations of the model assumptions for normality and equality of variance can affect prediction accuracy, although the model can perform well despite violations (Li et al. 2006, Doyle et al. 2018). The results of this analysis should therefore be interpreted with the uncertainty of the model robustness in mind if the data are found to violate assumtions. The performance of LDA under assumption violations can be assessed with non-parametric classification procedures such as Random Forest as suggested by Doyle et al. (2018). This is a supervised algorithm that assembles "trees" from randomly chosen variables, which are used to identify patterns in the data allowing for prediction of new cases. The algorithm makes no assumptions about the underlying distribution or variance in the data (Breiman 2001), and may therefore be suitable regardless of non-normality or heterogeneity of variances in the dataset. Assumption violations are often disregarded in morphometric studies, and supplementary analysis can strengthen the results of traditional methods (Feldesman 2002, Doyle et al. 2018).

1.3.2 Genetic studies

The technological advancement of molecular tools has allowed exploration of genetic variation among the *Sebastes* species in the North Atlantic. This effort has largely been directed at quantifying and resolving species classification issues due to external similarities (Pampoulie and Daníelsdóttir 2008), as well as characterizing the commercially important *S. mentella* stock (Johansen et al. 2000a, Cadrin et al. 2010). Research on the genetic variation among *S. mentella* eventually identified three separate management units of *S. mentella* ecomorphs, delineated by depth and geographic location into a 'deep pelagic', a 'shallow pelagic' and a 'slope' morph (Stefánsson et al. 2009, Saha et al. 2016). The genetic structure of *S. norvegicus* was similarly

extensively researched, and with the continuous improvement and development of tools for identifying variations, it was found to be more diverse than previously assumed (Nedreaas et al. 1992, Johansen et al. 2000b, Schmidt 2005, Pampoulie and Daníelsdóttir 2008, Bunke et al. 2013) eventually revealing the aforementioned species complex (Schmidt 2005, Saha et al. 2017). Molecular genetic methods for species identification have included among others analysis of haemoglobin and tissue enzymes (Nedreaas and Nævdal 1991, Nedreaas et al. 1994, Johansen et al. 2000a), mitochondrial DNA (Roques et al. 1999, Schmidt 2005, Bunke et al. 2013, Shum et al. 2015), and nuclear genetic (microsatellite) markers (Pampoulie and Daníelsdóttir 2008, Saha et al. 2016, Saha et al. 2017). In recent years, Single Nucleotide Polymorphisms (SNPs) have been developed as a tool for genotyping with improved efficiency and error rate compared to microsatellites (Seeb et al. 2011). This method relies on identifying alleles at a specific loci, which can be highly useful if regions of high genetic divergence can be identified in the genome (Nosil et al. 2009) and provide good discrimination with relatively few markers (Smith et al. 2007, Garvin et al. 2011, Xu et al. 2019). A combination of SNP markers developed at the Institute of Marine Research in Norway have proved successful in genetically identifying the proposed cryptic species as well as *S. mentella* and *S. viviparus* (T. Johansen, personal communication, May 18th 2021).

1.3.3 Growth patterns

Further examinations of length-at-age data from *S. norvegicus* in Norwegian waters has highlighted two main trajectories of growth (Monferrer and Planque 2019). Monferrer and Planque (2019) investigated length-at-age data from over 13,000 specimens of morphologically identified *S. norvegicus* caught in Norwegian waters. They found that the growth curve for juveniles was nearly linear until maturation, when different patterns of growth appeared. Two main growth patterns were described; fast growth, where the fish grows until it reaches maturation at 30-40 cm, and slow growth, where individuals continue to grow past maturation up to 70-80 cm (Monferrer and Planque 2019). Furthermore, Monferrer and Planque (2019) found that larger specimens of *S. norvegicus* were typically associated with deeper habitats, agreeing with the observations of habitat preference usually displayed by ectotherms where larger, mature fish are more frequently found in deeper, colder waters (Saborido-Rey 1994, Black et al. 2021). The authors suggested supplementing the analysis with genetic methods to

determine if the two observed growth patterns can be explained by cryptic speciation among *S. norvegicus*, which has previously been unexplored. Findings of the two potential cryptic species of *S. norvegicus* types A and B in Greenland waters (Schmidt 2005, Saha et al. 2017) further increases the interest in determining whether the species complex is present in Norwegian waters as well.

Research questions

The present study builds on the observations of two different growth patterns among S. norvegicus in Norwegian waters (Monferrer and Planque 2019) and the hypothesized *S. norvegicus* species complex found in Greenland waters (Schmidt 2005, Saha et al. 2017). Using a combination of morphometric and meristic methods, the study aims to reveal diagnostic characters useful for identifying the hypothesized *S. norvegicus* types A and B in Norwegian waters, as well as separate them from *S. mentella* and *S. viviparus*. Prior to analysis, the specimens are genetically assigned to species or types using three Single Nucleotide Polymorphism markers developed at IMR (T. Johansen, personal communication, May 18th 2021). The study focuses on the following research questions: i) are the two S. norvegicus types present in Norwegian waters, ii) can they be identified by external morphology, and iii) do they exhibit distinct growth patterns? For reference, the samples collected in Norwegian waters are compared with specimens of *Sebastes* spp. from East Greenland and Icelandic waters.

2 Materials and methods

2.1 Samples and study area

A total of 1,010 redfish were used in the present study, including both archived tissue samples (n = 843) collected by the Institute of Marine Research (IMR) as well as whole fish for morphological analysis (n = 167). The main focus of the study was on *Sebastes* sp. in Norwegian waters, although reference samples from East Greenland waters (n = 129) and Icelandic waters (n = 66) were included. The samples were collected by research and commercial vessels in the three North Atlantic regions (Figure 2) across 14 surveys (Table 1). The majority of samples were collected along the shelf edge 'Eggakanten' in the Norwegian Sea as well as on the Barents Sea shelf in spring (March to May) and autumn (September to October) from 2016 to 2020. Reference samples were collected in Icelandic waters in March 2017 and in East Greenland waters in April 2020 by commercial vessels. Whole fish for morphological analysis were only available for the Norwegian and East Greenland waters.

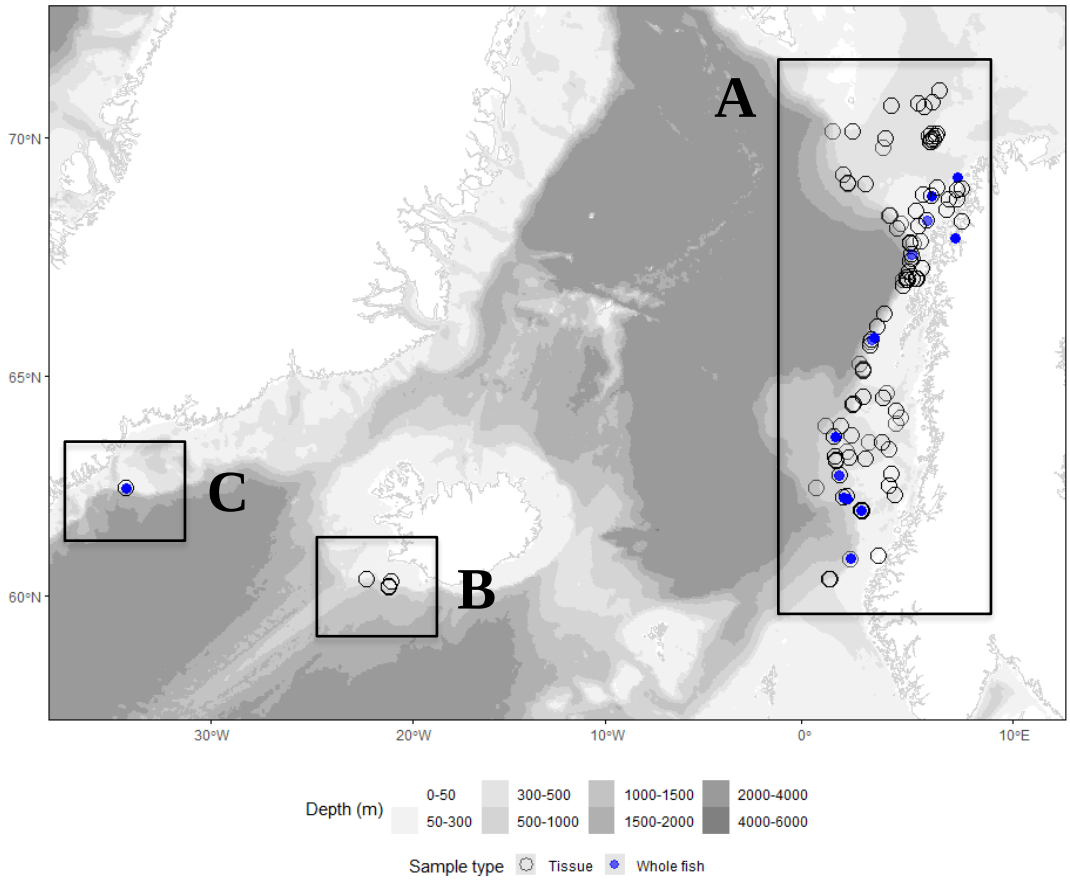


Figure 2. Collection locations of individual *Sebastes* sp. specimens used in the study within the three regions (A: Norwegian waters, B: Icelandic waters, C: East Greenland waters.) Blue points: Whole fish for morphometric analysis and tissue samples for genetic analysis. Black circles: Tissue samples for genetic analysis only.

The fish were morphologically classified to species at sea, with the exception of 28 fish with undetermined species assignment. Information about age, length, sex, maturity, and catch location was available for all archived samples collected by the IMR. In cases where only the standard length of a fish was given, the total length was estimated using a conversion factor (Appendix 1). Total length was rounded to the next cm below for comparison of length-at-age data.

Table 1. Overview of survey data and biological data for all specimens included in the study.

** = Total length estimated from standard length using conversion formula (Appendix 1). n = number of fish.*

						Catch data				Biological data						
						Depth range (m)				*Age range (years)*		*Length range (mm)*		*Sex*		
Year	**Region**	**Vessel type**	**Surveys**	**Species**	**n**	**Min**	**Max**	**Sampling month(s)**	**Gear**	**Min**	**Max**	**Min**	**Max**	**F**	**M**	**na**
2016	Norway	Research	1	*S. norvegicus*	42	296	695	Mar - Apr	Bottom trawls	9	50	265	730	24	18	0
				S. mentella	116	240	1036	Mar - Apr	Bottom trawls, pelagic trawls	8	55	240	475	76	40	0
2017	Norway	Commercial	2	*S. norvegicus*	49	70	205	Mar, Apr	Bottom trawls, gillnets	16	33	390	530	19	29	1
2018	Norway	Research	1	*S. norvegicus*	50	241	1193	Mar - Apr	Bottom trawls	9	41	340	760	33	16	1
2019	Norway	Research	1	*S. norvegicus*	9	198	201	Mar	Bottom trawls	11	49	364	433	1	8	0
		Commercial	3	*S. norvegicus*	106	30	300	Sept, Sept - Nov	Gillnets	7	47	220	512	16	9	81
				Undetermined	28	80	450	Mar, Sept - Oct	Bottom trawls, gillnets	19	44	356*	480	5	5	11
2020	Norway	Commercial	2	*S. norvegicus*	95	100	335	May	Gillnet, autoline	8	45	330	660	13	26	56
		Research	2	*S. norvegicus*	273	63	544	Mar - Apr, Oct	Bottom trawls	2	63	115	700	129	131	13
				S. mentella	26	667	379	Mar - Apr	Bottom trawls	29	60	359	495	15	15	0
				S. viviparus	21	399	390	Mar	Bottom trawls	8	38	207	268	13	13	0
2020	Greenland	Commercial	1	*S. norvegicus*	95	600	225	Apr	Autoline	12	45	289	635	34	37	24
				S. mentella	34	600	225	Apr	Autoline	11	26	262	366	1	1	32
2017	Iceland	Commercial	1	*S. norvegicus*	39	510	247	Mar	Autoline	13	45	250	460	20	20	0
				S. mentella	27	510	247	Mar	Autoline	15	43	260	400	10	17	0

2.2 Genetic analysis

All whole fish and tissue samples in the study were genetically assigned using Single Nucleotide Polymorphism (SNP) markers. Three SNP markers were used for assignment into the proposed cryptic species *S. norvegicus*-B and *S. norvegicus*-A suggested by Saha et al. (2017) based on specimens analyzed in Greenland waters, as well as *S. mentella* and *S. viviparus* (Table 2). SNP1 and SNP2 identify *S. viviparus* and *S. mentella* respectively, while SNP3 separates *S. norvegicus*-A from *S. norvegicus*-B. These SNP primers are unpublished primers from the IMR (T. Johansen, personal communication, May 18th 2021) routinely used in the genetic assignment of *Sebastes* specimens in their laboratory. A detailed description of this method and background for genetic assignment is not part of the present thesis.

Table 2. Three SNP markers are currently used in the laboratory at the Institute of Marine Research to genetically assign Sebastes *specimens. A combination of the SNP markers is needed for genetic assignment, of which the SNP3 marker is known from analysis of Greenland specimens to separate* S. norvegicus*-A and* S. norvegicus*-B (T. Johansen, personal communication, May 18th 2021).*

	SNP 1		SNP 2		SNP 3	
Genetic assignment	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2
S. norvegicus-A	C	C	T	T	A	A
S. norvegicus-B	C	C	T	T	G	G
S. mentella	C	C	C	C	G	G
S. viviparus	T	T	T	T/C	G	G

2.3 Morphology

Morphological data collection was divided into traditional morphometric measurements and meristic counts, and all specimens were processed by the author. All statistical analyses were performed in R using version 4.0.4 (R Core Team 2021).

2.3.1 Individuals in the analysis

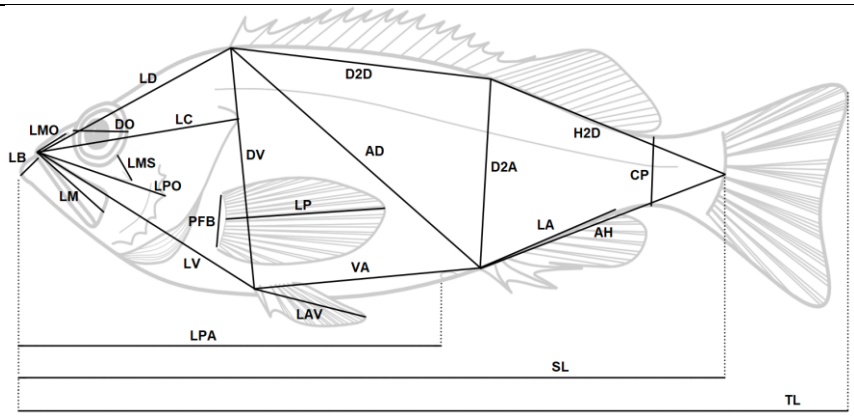
A total of 167 whole redfish specimens from Norwegian waters and East Greenland waters (Figure 2) were frozen upon collection and stored for a period of three to 16 months until use in the morphological study. The Norwegian specimens (n = 111) were collected in the Barents Sea and the Norwegian sea in 2019 and 2020, of which 18 were sent to the IMR by a commercial vessel with undetermined species assignment due to ambiguous morphology (Table 1). These undetermined specimens were caught in the Lofoten area in March, 2019. The East Greenland reference specimens (n = 56) were collected in 2020. No specimens were available for morphological analysis from Icelandic waters.

2.3.2 Preparation of frozen specimens

Prior to morphological examination, the fish were submerged in freshwater and defrosted overnight. All specimens were photographed and weighed to the nearest gram before morphometric measurements were taken, including total and standard length, and the meristic counts and characters were recorded. Following completion of morphological examination, the information on sex and maturity stage as well as otoliths for subsequent age estimation were collected from all specimens. Tissue samples were taken from the pelvic fin and stored in 2 ml screwcap tubes filled with 1 ml 96% ethanol for genetic analysis. The fish were processed in batches over multiple sessions.

2.3.3 Morphometrics

Following previous morphometric studies by Kelly et al. (1961), Power and Ni (1985) and Garabana (2005) on redfish in the North Atlantic, 23 linear interlandmark distances (Figure *3*) were defined as variables for traditional morphometrics and measured on the defrosted fish. In case of the eye orbit diameter (abbreviated as DO, also referred to as eye orbit width in the aforementioned literature), this was measured as the horizontal distance of the inside of the bony ring as specified by Phillips (1957). The author further recommended to place the measuring device within the notch that is often apparent at the anterior part of the orbit in rockfishes, which was practiced for the current study.



	Abbreviation	Character
1	AN	Width between opercula
2	LB	Distance from tip of the beak to inside of lower jaw
3	LMS	Distance from snout to second nasal opening
4	LM	Length of maxilla
5	DO	Eye orbit diameter
6	LMO	Distance from posterior part of eye orbit to post-ocular spine
7	LD	Distance from snout to first dorsal fin
8	LC	Distance from snout to edge of operculum
9	LPO	Distance from snout to preopercular spine
10	LV	Distance from snout to pelvic fin
11	LAV	Length of pelvic fin
12	VA	Distance from pelvic fin to anal fin
13	DV	Distance from first dorsal spine to base of first pelvic spine
14	AD	Distance from base of first dorsal spine to anal fin
15	LP	Length of pectoral fin
16	PFB	Width of pectoral fin base
17	D2D	Length of first dorsal fin base
18	H2D	Distance from base of first soft dorsal fin ray to hypural
19	D2A	Distance from base of first soft dorsal fin ray to anal fin
20	AH	Distance from anal fin to hypural
21	LA	Length of anal fin base.
22	LPA	Pre-anal length
23	CP	Minimum depth of caudal peduncle

Figure 3. Interlandmark distances measured in traditional morphometrics. Not displayed: Width between opercula (AN) which was measured on the dorsal side. SL = Standard length, TL = Total length.

Data acquisition and preparation

The linear interlandmark distances (Figure *3*) were measured directly on 167 fish using a Mitutoyo 150 mm digital caliper, or with a ruler in cases where the distance exceeded the capacity of the caliper. The neck width (AN) was measured between opercula on the dorsal side of the fish, while the remaining dimensions were recorded on the left side of the fish when possible.

The dataset consisting of fish from Norwegian waters (n = 111) was screened for outliers. Extreme values can affect the outcome of multivariate analyses, especially in data sets with small sample sizes, and may be difficult to detect in high-dimensional multivariate data (Leys et al. 2018). Potential outliers were identified in plots of residuals from regressions between the measured variables and standard length (Appendix 2), and the original data was examined to evaluate if abnormal values could be illegitimate. Three fish with outlier values for measurements were subsequently removed from the dataset of 111 fish. Another three individuals were removed due to inconclusive genetic species assignment, along with six fish with incomplete observations.

With the inclusion of the East Greenland specimens (n = 56), no additional outliers were detected in the residuals. Three specimens with inconclusive genetic species assignment and 23 fish with incomplete observations were removed from the dataset.

Size correction

In traditional morphometric studies, separating shape from size is challenging. Redfish typically exhibit allometric growth (Ingram 2015), which means that the shape of the fish changes with size (Mosimann and James 1979). This often causes morphometric measurements to be strongly correlated with size (Klingenberg 2016). When these variables are measured on specimens of different sizes, they should therefore be transformed to obtain a component with reduced size dependency for the comparison and identification of shape variation within and among groups (Bookstein et al. 1981). In the present study, ratios were used to represent the shape variation as the high dimensionality and low sample size of the data prevented the calculation of allometric vectors or representative regression coefficients. Although not an ideal method for removing size dependencies in morphometric data, ratios have been widely used in

morphometric studies (Albrecht et al. 1993, Doyle et al. 2018) and have been found to produce the same conclusions from analysis as regression- and multivariate approaches (Garabana 2005).

Statistical analysis

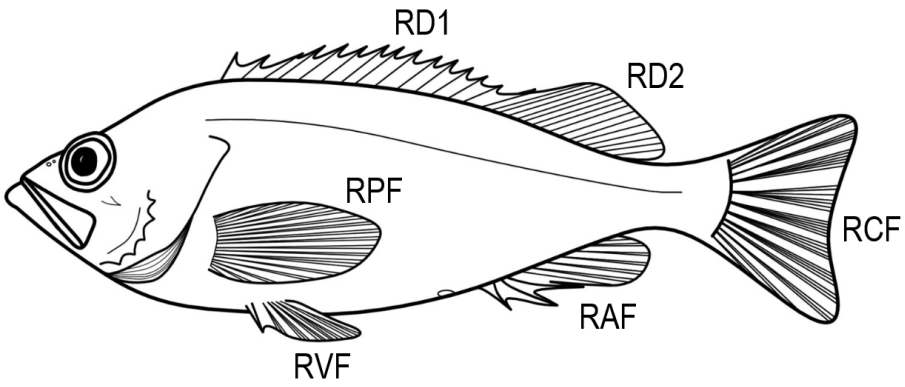
The samples were grouped by genetic assignment prior to all statistical analysis. To assess the suitability of the data to statistical classification procedures, distribution and covariance matrices were examined in the ratio dataset. Mardia's skewness and kurtosis were computed for assessment of multivariate normality in the dataset, and quantile-quantile plots were produced using the package *MVN* in R Studio (Korkmaz et al. 2014) to assess multivariate normality in the data. Homogeneity of variance was tested using Levene's test in addition to Fligner-Killeen's test in the *car* package (Fox and Weisberg 2019) which is a non-parametric test considered to be more robust to deviations from normality.

In the present study, two multivariate morphometric methods were used. First, linear discriminant analysis (LDA) was applied to the dataset with estimation of accuracy and Cohen's Kappa. Then, Random Forest classification procedure was used as a supplementary analysis (Doyle et al. 2018). Both analyses were followed by repeated k-fold cross-validation for estimation of model prediction accuracy of group membership. This cross-validation method splits the dataset into k equal parts, partitioned as k-1 training sets and one test set. Prediction accuracy can be averaged when the cross-validation is repeated multiple times (Kohavi 1995). For the present analysis, a 10-fold cross-validation was performed with 5 repeats. In addition, the Random Forest algorithm computes an out-of-bag estimate of model classification error which was also presents, as this is calculated differently from cross-validation. For every classification tree the algorithm builds, one third of the samples are held out from the analysis which can later be used as a testing dataset in error estimation (Breiman 2001). Finally, the Random Forest algorithm was used with permutation to estimate overall relative variable importance and pairwise between-group variable importance by the mean decrease in accuracy index. This index describes the classification accuracy reduction if a given variable is removed from the analysis (Hong et al. 2016). The classification procedures were first performed on the dataset containing only the specimens collected in Norwegian waters, and then on the complete dataset including fish from both Norwegian and East Greenland waters.

2.3.4 Meristics

Nine meristic variables were defined including countable features and angles (Figure 4) following previous morphological studies analyzing meristics in redfish (Kelly et al. 1961, Garabana 2005). The number of fin rays in the dorsal fins, the pectoral fin, the pelvic fin, the anal fin and the caudal fin were recorded. Due to the consistency in the number of spines in the pelvic fin and anal fin, the total number of spiny rays and soft rays were recorded for each fin. The base of short fin rays was closely examined to prevent counting a single branched fin ray as multiple fin rays. Following the method described by Garabana (2005), the angles of the third and fifth preopercular spines were recorded as codes (Appendix 3). All counts and angles were recorded on the left side of the fish when possible. Finally, the anterior branchial arch on the left side of the fish was removed with scissors and the number of gill rakers were counted.

For the fish collected in East Greenland waters, only the number of fin rays in the pectoral fin (RPF), number of fin rays in the anal fin (RAF), the number of gill rakers (GR), the angle of the third preopercular spine (PS3) and the angle of the fifth preopercular spine (PS5) were recorded due to time constraints. These were selected based on showing the greatest amount of variation between species in previous meristic studies (Garabana 2005).



	Abbreviation	Character
1	RD1	Number of rays in the spiny dorsal fin
2	RD2	Number of rays in the soft dorsal fin
3	RPF	Number of soft fin rays in the pectoral fin
4	RVF	Total number of fin rays in the pelvic fin
5	RAF	Total number of fin rays in the anal fin
6	RCF	Number of soft fin rays in the caudal fin
7	GR	Number of gill rakers
8	PS3	Angle of the 3rd preopercular spine
9	PS5	Angle of the 5th preopercular spine

Figure 4. Quantifiable features included in meristic analysis. Categories of preopercular spine angles (PS3, PS5) recorded following Garabana (2005) described in detail in Appendix 3.

2.3.5 Data preparation and statistical analysis

The fish were grouped by genetic assignment prior to statistical analysis of the meristic features. Among the 111 fish from Norwegian waters, no outliers were detected in plots of residuals. Three specimens with heterozygous genetic results were removed, and all remaining fish with incomplete measurements (n = 23) were removed. Three fish from the East Greenland waters (n = 56) presented inconclusive genetic results and were excluded from further analysis.

Because of the ranked nature of count data, the non-parametric multivariate Kruskal-Wallis H test was applied to the meristic dataset (Valentin et al. 2002). The Kruskal-Wallis H test is used to evaluate whether mean values are equal across groups. Using the *dunn.test* package (Dinno 2017), a non-parametric post-hoc Dunn Multiple Comparison test using p-values adjusted with

the Benjamini-Hochberg method (Dinno 2015) was performed to identify differences in group means. The Benjamin-Hochberg method attempts to reduce the false discovery rate when comparing multiple groups (Dinno 2015).

2.4 Growth patterns

To determine whether average length-at-age size differed significantly between groups, *S. norvegicus*-A, *S. norvegicus*-B and *S. mentella* from Iceland, East Greenland and Norwegian waters were grouped into age intervals. As the age ranges differed between the three groups in the dataset, the interval used in the analysis was restricted to the narrowest age range available. The lower and upper ages of the interval were set to 11 and 45+ years, respectively, and fish with higher age estimates were included in the final group. The normality and variance of the data were checked before the non-parametric Kruskal-Wallis H-test was used to compare the means of the groups, followed with a post-hoc Dunn test for multiple comparisons (Valentin et al. 2002). Von Bertalanffy growth curves were approximated using the FSA package (Ogle et al. 2021).

3 Results

3.1 Genetic assignment

3.1.1 Genetic results for the morphological dataset

The SNP markers assigned the fish into one of the four predetermined groups: *S. norvegicus*-A, *S. norvegicus*-B, *S. mentella*, and *S. viviparus*. In both the morphometric and meristic dataset, all four groups were represented among the fish collected Norwegian waters, while only *S. norvegicus*-A and *S. norvegicus*-B were present in the material collected from East Greenland waters (Table 4). In total, six fish were heterozygous for the third Single Nucleotide Polymorphism (SNP) marker that was used to distinguish between *S. norvegicus*-A and *S. norvegicus*-B; three in the morphometric dataset, and three in the meristic dataset.

3.1.2 Genetic results for the total dataset

The classification of the 815 whole fish (n = 157) and tissue samples (n = 658) from Norwegian waters showed that 561 (68.2%) and 46 (5.6%) fish were assigned by SNP markers to the proposed cryptic species *S. norvegicus*-B and *S. norvegicus*-A respectively (Table 3). This included the 18 undetermined fish with ambiguous morphology sent from a commercial vessel to the IMR, of which 16 were genetically assigned to *S. norvegicus*-A. Most specimens classified as *S. norvegicus* at sea were of the type B, making this is the most common type while type A is rare in Norwegian waters according to the available data. Nearly all of the genetically assigned *S. norvegicus* type B collected in Norwegian waters were classified at sea as *S. norvegicus*, while specimens of type A were initially classified as *S. mentella*, *S. norvegicus*, or not determined at sea. While the absolute number of type A fish classified as *S. norvegicus* was high, they were proportionally more often classified as *S. mentella* at sea. None of the specimens classified as *S. mentella* at sea were genetically assigned to *S. norvegicus*-B, while 6 fish identified as *S. norvegicus* at sea were genetically classified as *S. mentella*. Lastly, 26 specimens (3.2%) were heterozygous for the SNP3 marker separating *S. norvegicus*-A and *S. norvegicus*-B. One individual was heterozygous for the SNP marker separating *S. mentella* and *S. viviparus*.

Because *S. norvegicus*-A and heterozygous fish are not recognized species and therefore cannot be “correctly” classified at sea, they were not included in the calculation of classification rates.

Assuming that *S. norvegicus* is represented by the majority of the cryptic specimens which is *S. norvegicus*-B, this is considered the correct classification for the purpose of calculating the classification rate. Among the remaining specimens genetically assigned to *S. norvegicus*-B, *S. mentella*, and *S. viviparus* (total = 736), only 5.4% were incorrectly classified at sea (research vessels = 6.3%, commercial vessels = 3.7%).

Among the tissue samples from Icelandic waters (n = 66) and whole fish and tissue samples collected in East Greenland (n = 129), nearly all specimens which were classified as *S. mentella* at sea were genetically assigned to *S. norvegicus*-A, with the exception of one correctly classified fish as well as one *S. norvegicus*-B and one *S. viviparus* (Table 3). Of the 134 fish identified as *S. norvegicus* at sea in Icelandic and East Greenland waters, 128 fish were genetically classified as type B while only four specimens were classified as type A, with no specimens genetically identified as *S. mentella*. The majority of fish genetically assigned to *S. norvegicus*-B and *S. mentella* had originally been correctly classified at sea.

Table 3. Classification matrix depicting morphological classification at sea (rows) and genetic assignment (columns) for the total dataset consisting of 1,010 fish divided by area (Norway, East Greenland, and Iceland) and vessel type (research or commercial vessel). Specimens with 'undetermined' classification at sea include 18 whole fish with ambiguous morphology sent to the IMR, as well as ten archived samples of unspecified species assignment at sea. Classification accuracy was calculated for the specimens genetically classified as S. norvegicus-*B,* S. mentella *and* S. viviparus*.*

		Genetic classification				
Classification at sea	Total (n)	*S. norvegicus*-A (n)	*S. norvegicus*-B (n)	*S. mentella* (n)	*S. viviparus* (n)	Heterozygous (n)
		Norwegian waters – Research vessels				
S. norvegicus	374	18	312	6	25	13
S. mentella	142	12	0	126	0	4
S. viviparus	21	0	0	0	21	0
Total	**537**	**30**	**312**	**132**	**46**	**17**
		Norwegian waters – Commercial vessels				
S. norvegicus	250	1	237	0	9	3
Undetermined	28	15	12	0	0	1
Total	**278**	**16**	**249**	**0**	**9**	**4**
		East Greenland waters – Commercial vessels				
S. norvegicus	95	0	95	0	0	0
S. mentella	34	31	0	1	0	2
Total	**129**	**31**	**95**	**1**	**0**	**2**
		Icelandic waters – Commercial vessels				
S. norvegicus	39	4	33	0	1	1
S. mentella	27	23	1	0	1	2
Total	**66**	**27**	**34**	**0**	**2**	**3**

3.2 Morphology

3.2.1 Morphometrics

Individuals included in the analysis

Because of the low frequency of heterozygous specimens in the analysis (n = 3) and uncertainty regarding their biological origin as well as genetic and morphological relations to the four predetermined groups, the three heterozygous fish were excluded from the traditional morphometric analysis. The total dataset used in the analysis consisted of 129 specimens from Norwegian waters (n = 99) and East Greenland waters (n = 30). The fish from Norwegian waters measured from 174 to 380 mm in standard length within an age range from 8 to 60 years, while the fish from East Greenland waters ranged from 282 to 428 mm in standard length and 13 to 29 years of age (Table 4). Due to the low sample size and underrepresentation of female fish in the dataset for *S. norvegicus*-A and *S. norvegicus*-B, it was considered inappropriate to separate the dataset by sex to assess the influence of sexual dimorphism on shape.

Table 4. Data on length, age and sex of Sebastes *spp. included in morphometric analysis divided by collection area. The sex of the* S. norvegicus*-A specimens from East Greenland waters was incorrectly registered and this information was therefore not available (NA) for the dataset.*

Genetic species assignment	*Sample size (n)*	*Length range (standard length, mm)*	*Mean ± SD (mm)*	*Age range (years)*	*Male (n)*	*Female (n)*
Norwegian waters						
S. norvegicus-A	14	308 – 360	339.9 ± 14.1	29 – 60	11	3
S. norvegicus-B	38	262 – 370	320.3 ± 25.9	19 – 44	27	11
S. mentella	25	303 – 380	339.2 ± 20.6	9 – 49	12	13
S. viviparus	22	174 – 228	205.6 ± 15.5	8 – 38	8	14
East Greenland waters						
S. norvegicus-A	22	282 – 355	309.0 ± 17.5	13 – 25	NA	NA
S. norvegicus-B	8	335 - 428	394.1 ± 33.7	17 – 29	2	4

Data transformation

Before conducting classification analysis of the morphometric data, the variables were transformed to reduce the effect of size-dependency. Transformation of the raw data to ratios between variables and the standard length reduced the correlation between variables (Appendix 4). Mardia's skewness and kurtosis showed that the data grouped by species assignment did not adhere to a multivariate normal distribution. Levene's test and Fligner-Killeen's test determined that the groups did not have equal variances for all variables included in the analysis, indicating that the assumptions for the linear discriminant analysis (LDA) were violated. Transformations of the data using square root, log, and Box Cox were unsuccessful in producing multivariate normally distributed data and homogeneity of variances, so the untransformed ratios were retained in further analysis. LDA was applied to the dataset and classification accuracy was compared against the results of the Random Forest to determine the reliability of the LDA under assumption violations.

Linear discriminant analysis

Linear discriminant analysis (LDA) was performed to evaluate the separation yielded by the morphometric variables for the four predetermined groups. For the Norwegian samples, the first two linear discriminants composed by the LDA explained 94% of the total variation between groups (Figure 5). These two discriminants provided complete separation of *S. mentella* and *S. viviparus*, while *S. norvegicus*-A and *S. norvegicus*-B partially overlapped as indicated by the 95% confidence interval ellipses. In the dataset containing samples from both Norwegian and East Greenland waters, the overlap between the two *S. norvegicus* types was more extensive. The reduced discrimination between the types was reflected in the relatively lower total variation explained by the first two discriminants (91%). In both datasets, the beak length (LB) contributed the most explanatory power to the first discriminant (LD1) and varied to the greatest degree between groups, as indicated by arrow length. The second discriminant (LD2) was most strongly influenced by caudal peduncle height (CP), while eye orbit diameter (DO) and distance from snout to upper nostril (LMO) influenced both axes. However, LMO had a greater influence on discrimination among the Norwegian samples.

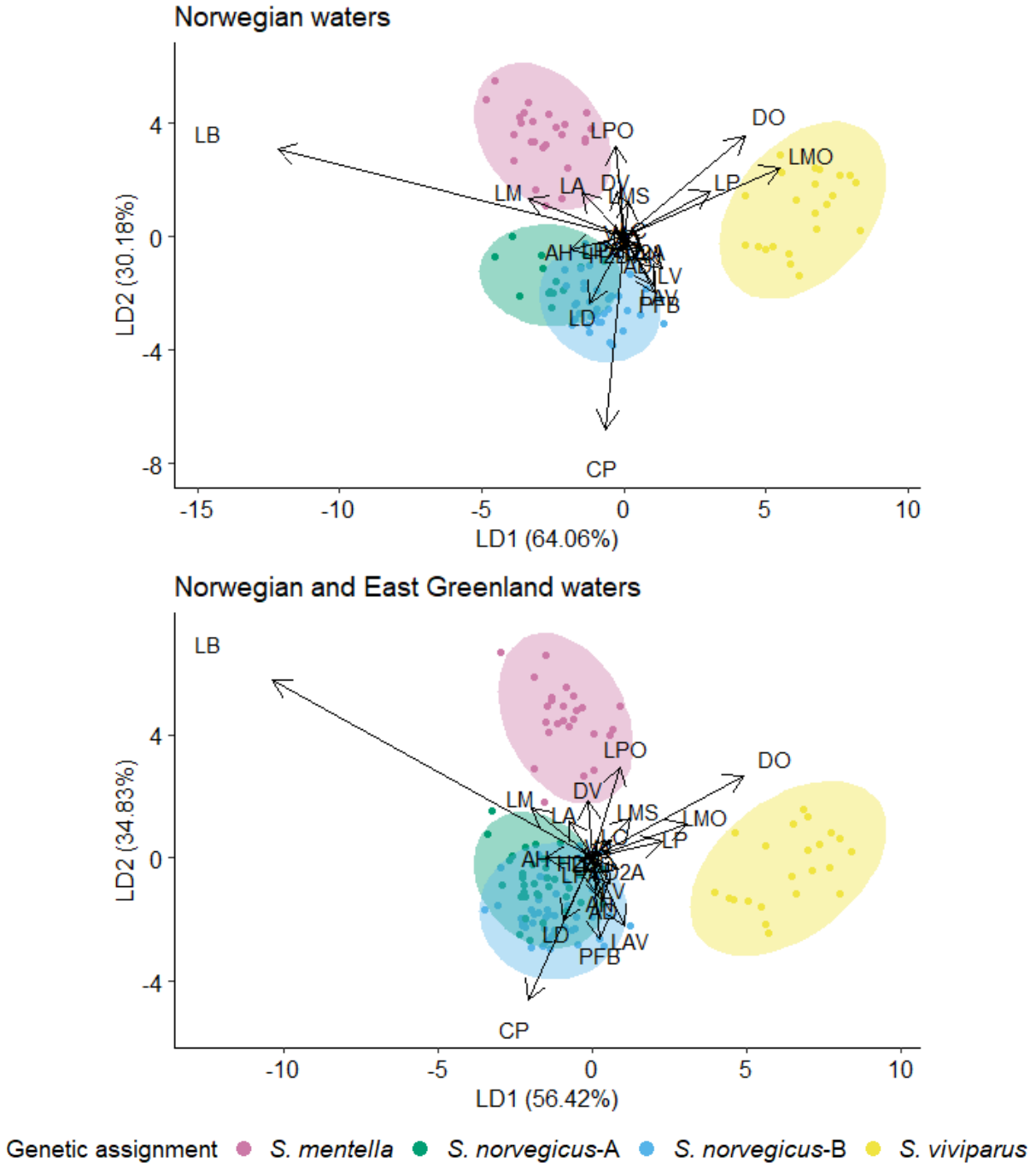


Figure 5. Ordination plots from results of linear discriminant analysis on morphometric variables. Values in brackets indicate the proportional separation between groups provided by the linear discriminants (LD1 and LD2). Results presented for the morphometric dataset with fish from Norwegian waters (top, n = 99) and dataset with all individuals including both Norwegian and East Greenland specimens (bottom, n = 129). The arrow lengths and directions indicate vector loadings, showing how variables influence the linear discriminants and to what extent. Ellipses show 95% confidence interval for each group.

The classification accuracy of *Sebastes* spp. by the LDA model was assessed with 10-fold cross-validation with 5 repeats. Linear discriminant analysis applied to the dataset with specimens from Norwegian waters achieved a cross-validated accuracy of 0.91 (Cohen's Kappa = 0.88). The classification matrix for the cross-validated LDA (Table 5) shows that the model most frequently confused *S. norvegicus*-B and *S. norvegicus*-A, primarily in the direction of type B specimens being incorrectly classified as type A *S. norvegicus*. A few specimens of *S. mentella* were assigned to *S. norvegicus*-A and *S. norvegicus*-B by the model. All *S. viviparus* were correctly classified. With the inclusion of East Greenland samples, the model classified the specimens similarly to the Norwegian dataset, with a slight increase in correct classification of *S. norvegicus*-A as well as instances of incorrect classification of *S. viviparus* as *S. norvegicus*-B. Cross-validated accuracy and Cohen's Kappa were marginally reduced to 0.89 and 0.85, respectively when East Greenland samples were included.

Table 5. Classification matrix for the repeated 10-fold cross-validated linear discriminant analysis on samples from Norwegian waters (top, n = 85) and all samples from Norwegian and East Greenland waters (bottom, n = 138). Cell values show percentual average cell counts across resamples. Predicted class is model prediction of group membership, while actual class shows genetic assignment. The model performs the prediction on a subset of the data.

Actual class

Norwegian waters

Predicted class	*S. norvegicus*-A	*S. norvegicus*-B	*S. mentella*	*S. viviparus*
S. norvegicus-A	12.5	5.1	1.0	0.0
S. norvegicus-B	1.6	32.3	1.0	0.0
S. mentella	0.0	1.0	23.2	0.0
S. viviparus	0.0	0.0	0.0	22.2

Norwegian and East Greenland waters

Predicted class	*S. norvegicus*-A	*S. norvegicus*-B	*S. mentella*	*S. viviparus*
S. norvegicus-A	13.1	5.1	1.0	0.0
S. norvegicus-B	1.0	32.3	1.0	1.0
S. mentella	0.0	1.0	23.2	0.0
S. viviparus	0.0	0.0	0.0	21.2

Random Forest classification

Random Forest classification was applied to the data as a supplementary analysis for evaluating the performance of LDA under assumption violations. A 10-fold cross-validation repeated 5 times gave an accuracy of 1 (Cohen's Kappa = 1.0) for the dataset containing Norwegian specimens, perfectly classifying all individuals. With the inclusion of East Greenland specimens, the accuracy dropped to 0.92 (Cohen's Kappa = 0.89). Here, two *S. norvegicus*-B were incorrectly classified as *S. norvegicus*-A and one *S. norvegicus*-A was classified as *S. mentella* while the remaining samples were correctly classified.

The Random Forest algorithm also computes an out-of-bag estimate of classification error, which showed different results (Appendix 6) from the cross-validation. Fish from Norwegian waters were classified with an out-of-bag error of 4.04%, in which the model incorrectly assigned specimens of *S. norvegicus*-A to *S. mentella* and *S. viviparus*. The proposed cryptic species type A had the highest class error (0.14), while all *S. norvegicus*-B were correctly classified. The inclusion of East Greenland specimens produced an out-of-bag error rate of 10.08%, increasing the model's confusion between *S. norvegicus*-A and *S. norvegicus*-B and raising the class error of the latter. The classification accuracy stayed high for *S. mentella* and *S. viviparus* in the model with only one specimen classified incorrectly in each group.

Table 6. Classification matrix for the Random Forest classifier showing number of correctly and incorrectly classified fish as well as class error. Predicted class shows model prediction, while actual class shows genetic assignment. The model performs the prediction on a subset of the data. Top: Specimens from Norwegian waters (n = 99). Bottom: Samples from Norwegian and East Greenland waters (n = 129).

Actual class

Norwegian waters

Predicted class	*S. norvegicus*-A	*S. norvegicus*-B	*S. mentella*	*S. viviparus*
S. norvegicus-A	12	0	0	1
S. norvegicus-B	0	38	1	0
S. mentella	0	0	24	0
S. viviparus	1	0	0	6
Class error	0.14	0.0	0.04	0.05

Norwegian and East Greenland waters

Predicted class	*S. norvegicus*-A	*S. norvegicus*-B	*S. mentella*	*S. viviparus*
S. norvegicus-A	33	6	1	0
S. norvegicus-B	2	39	1	1
S. mentella	1	0	23	0
S. viviparus	1	1	0	21
Class error	0.08	0.15	0.08	0.05

Overall variable importance was assessed through Random Forest with permutation which ranked the variables from most important to least important (Figure 6) by mean decrease in accuracy. The mean decrease in accuracy index describes the loss of classification accuracy caused by the removal of a given variable. Eye orbit diameter (DO) was ranked as the most important variable, followed by beak length (LB), pelvic fin length (LAV), pectoral fin length (LP) and caudal peduncle height (CP) for the dataset composed of Norwegian specimens. The inclusion of East Greenland samples in the analysis provided similar results (Appendix 5). In the interest of finding descriptive traits for identification of the potential cryptic species in Norwegian waters, only the Norwegian samples are discussed further in pairwise variable importance.

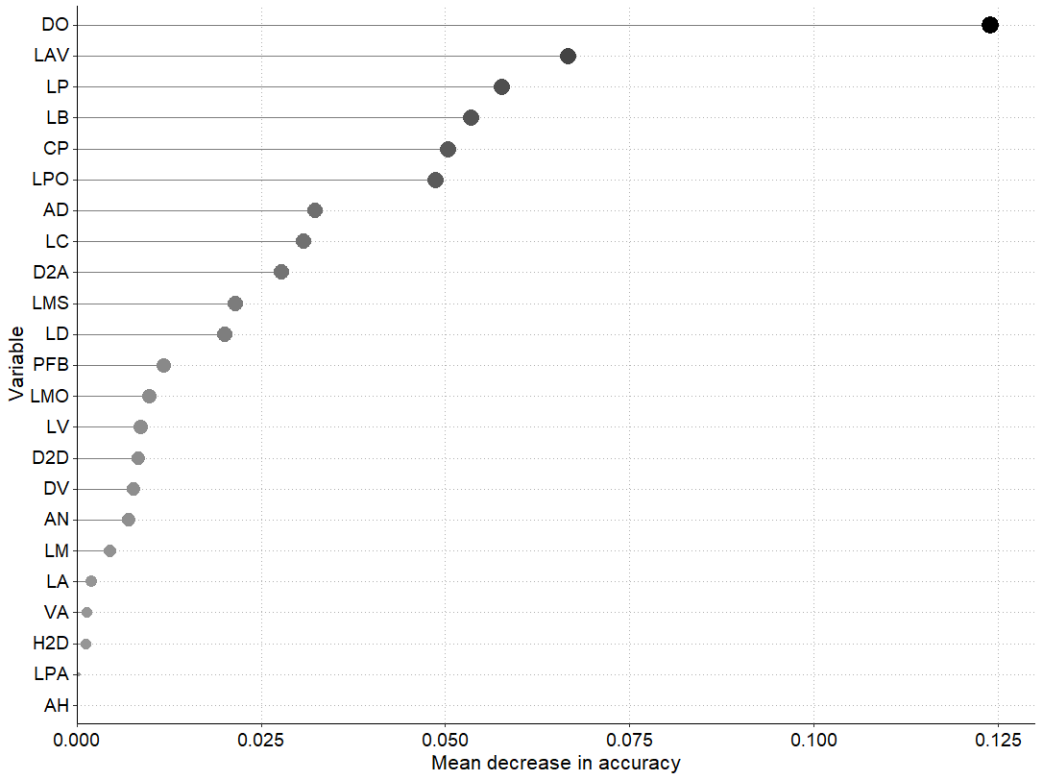


Figure 6. Mean decrease in accuracy indicating relative variable importance ranked by Random Forest for the Norwegian samples in the morphometric dataset. Higher values indicate greater variable importance. Top ten linear distance variables: Eye orbit diameter (DO), pelvic fin length (LAV), pectoral fin length (LP), beak length (LB), caudal peduncle height (CP), length from snout to preopercular spine (LPO), length from first dorsal fin to anal fin (AD), length from snout to edge of operculum (LC), length from second dorsal fin to anal fin (D2A), length from snout to upper nostril (LMS). Increased point size and color intensity imply greater variable importance.

Pairwise comparisons of variable importance (Figure 7) showed that a few variables were consistently highly ranked across comparisons. Between the two proposed cryptic *S. norvegicus* types A and B, the variables eye orbit diameter (DO), snout to preopercular spine (LPO), and beak length (LB) were ranked highest in variable importance suggesting that these three characters provided the most discrimination. The caudal peduncle height (CP) was the third most important character for separating *S. norvegicus*-A and *S. mentella* after eye orbit diameter and beak length. Eye orbit diameter was generally ranked high in variable importance across all comparisons. Compared with *S. viviparus*, pectoral fin length (LP) and pelvic fin length (LAV) were also important characters for identifying *S. norvegicus*-A. In the comparisons including *S. mentella*, the model frequently ranked caudal peduncle height as an important variable, while consistently attributing high variable importance to the pectoral and pelvic fin length when *S. viviparus* was part of a comparison.

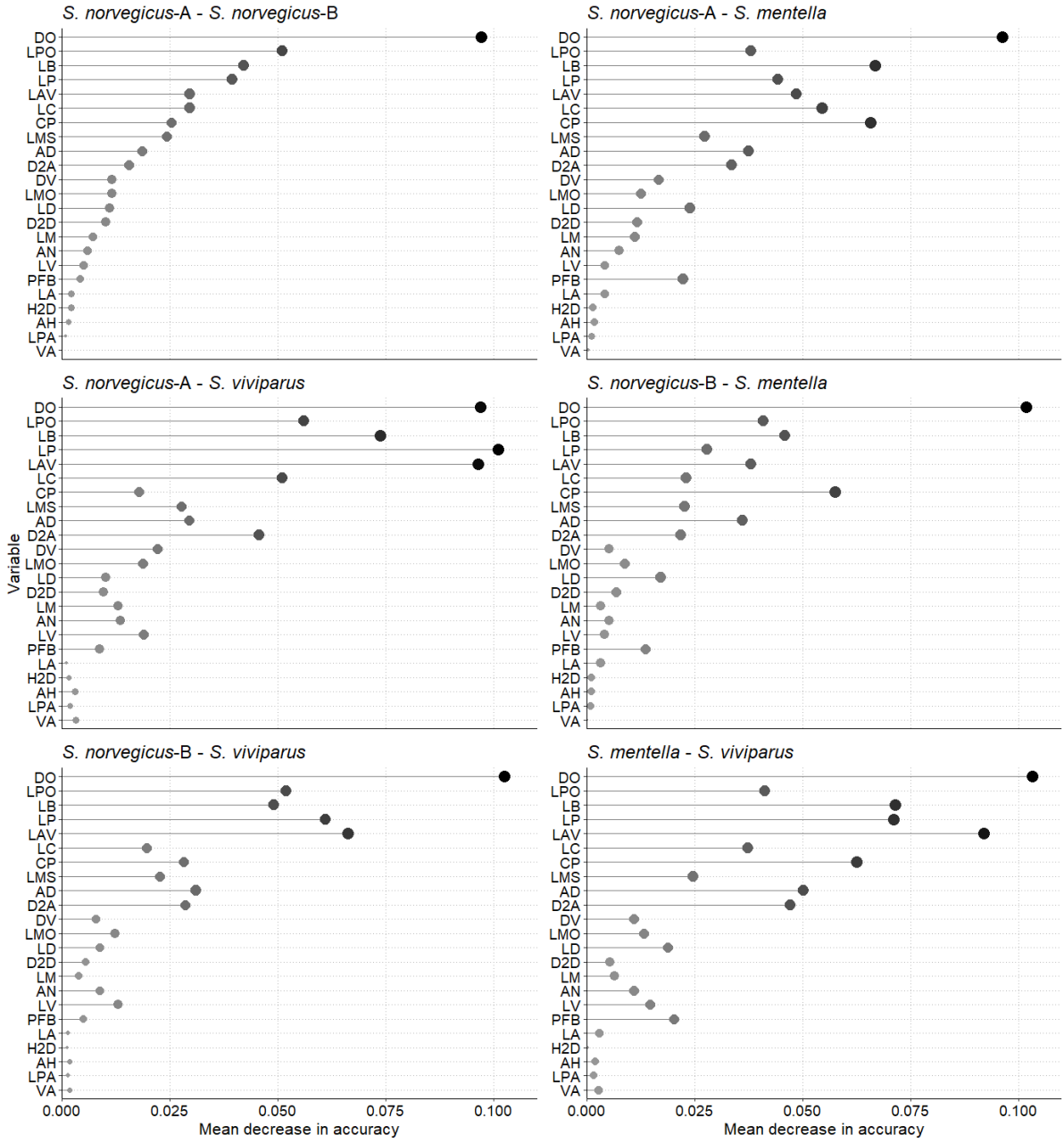


Figure 7. Pairwise plots showing relative morphometric variable importance between Sebastes *spp. ranked by Random Forest permutation. Mean decrease accuracy describes the loss of classification accuracy with the removal of a given variable. Morphometric variables: Eye orbit diameter (DO), snout to preopercular spine (LPO), beak length (LB), pectoral fin length (LP), pelvic fin length (LAV), snout to edge of operculum (LC), caudal peduncle height (CP), snout to upper nostril (LMS), first dorsal fin to anal fin (AD), second dorsal fin to anal fin (D2A), first dorsal fin to pelvic fin (DV), eye to post-ocular spine (LMO), snout to first dorsal fin (LD), length of first dorsal fin base (D2D), maxilla length (LM), neck width (AN), snout to pelvic fin (LV), pectoral fin base width (PFB), anal fin base length (LA), second dorsal fin to hypural (H2D), anal fin to hypural (AH), pre-anal length (LPA), pelvic fin to anal fin (VA). Increased point size and color intensity imply greater variable importance.*

3.2.2 Meristics

The final dataset in meristic analysis consisted of 138 fish (Table 7). In Norwegian waters (n = 85), all four groups of *Sebastes* spp. were represented in the meristic dataset. Only specimens of *S. norvegicus*-A and *S. norvegicus*-B were available from East Greenland waters (n = 53). Length and ranges varied between groups, with generally older and smaller *S. norvegicus*-A from Norwegian waters than *S. norvegicus*-A from East Greenland waters. *Sebastes norvegicus*-B specimens collected in Norwegian waters were also smaller on average than the East Greenland samples, but represented a greater range in age.

Table 7. Specimens of Sebastes *spp. included in meristic analysis divided by collection area (Norwegian waters and East Greenland waters. Information about sex was not available (NA) for 33 specimens from East Greenland waters due to incorrect registration.*

Genetic species assignment	*Sample size (n)*	*Length range (standard length, mm)*	*Mean ± SD (mm)*	*Age range (years)*	*Females (n)*	*Males (n)*	*NA (n)*
Norwegian waters							
S. norvegicus-A	7	320 - 360	342.1 ± 12.5	23 - 44	2	5	-
S. norvegicus-B	33	262 - 530	333.7 ± 61.2	9 - 49	12	21	-
S. mentella	23	303 - 380	341.8 ± 20.1	29 - 57	13	10	-
S. viviparus	22	174 - 228	205.6 ± 15.5	8 - 38	14	8	-
East Greenland waters							
S. norvegicus-A	26	275 - 355	307.0 ± 17.4	12 – 25	-	-	26
S. norvegicus-B	27	335 - 635	496.2 ± 100.0	17 - 36	9	11	7

The non-parametric Kruskal-Wallis H test was used to assess the differences between species in mean ranks of the meristic data. The results showed that with the exceptions of the number of fin rays in the first dorsal fin (RD1, p = 0.09) and the number of fin rays in the pelvic fin (RVF, p = NA), the test was significant for the remaining variables (Table 8) suggesting that mean variable ranks were not equal between groups. The fin ray count for the pelvic fin (RVF) was constant at 6 total soft and spiny fin rays for all observations in Norwegian waters.

Table 8. Summary of results from Kruskal-Wallis H test and multiple comparison Dunn test on meristic variables including no. of pectoral fin rays (RPF), no. of fin rays in the first dorsal fin (RD1), no. of fin rays in the second dorsal fin (RD2), no. of caudal fin rays (RCF), no. of anal fin rays (RAF), no. of pelvic fin rays (RVF), no. of gill rakers (GR), angle of the 3rd preopercular spine (PS3), angle of the 5th preopercular spine (PS5) for samples from Norwegian waters. Significance levels: ns = not significant, asterisk indicates significance. For the Dunn test, ranked means between groups are significantly different if they do not share a letter for a given variable.

Kruskal-Wallis H test					Dunn test			
Norwegian waters								
Variable	H	df	p-value		*S. norvegicus*-A	*S. norvegicus*-B	*S. mentella*	*S. viviparus*
RPF	41.17	3	< 0.001	*	a	a	a	b
RD1	6.62	3	0.09	ns	-	-	-	-
RD2	23.87	3	< 0.001	*	ab	a	a	b
RCF	17.06	3	< 0.001	*	ab	a	a	b
RAF	31.54	3	< 0.001	*	b	c	a	c
RVF	NA	NA	NA	NA	-	-	-	-
GR	31.16	3	< 0.001	*	b	ab	a	c
PS3	60.54	3	< 0.001	*	b	b	a	b
PS5	91.49	3	< 0.001	*	b	b	a	c
Norwegian and East Greenland waters								
RPF	52.35	3	< 0.001	*	a	a	a	b
RAF	65.85	3	< 0.001	*	a	b	c	c
GR	42.19	3	< 0.001	*	a	b	a	b
PS3	88.17	3	< 0.001	*	a	b	b	b
PS5	114.36	3	< 0.001	*	a	b	b	c

In order to determine which groups had different mean ranks for a given variable, the groups were compared pairwise in a multiple comparison post-hoc Dunn test (Table 8). The ranked means for RD1 and RVF respectively were equal across all groups according to the test, confirming the findings of the Kruskal-Wallis H test. The two *S. norvegicus* types A and B only differed significantly in anal fin ray count (RAF), which was generally higher in type B than type A (Appendix 9). *Sebastes norvegicus*-A had more gill rakers than *S. mentella* on average, although the ranges overlapped. The number of gill rakers was typically highest in *S. viviparus.* The preopercular spine angles were similar in *S. norvegicus*-A and *S. norvegicus*-B, usually exhibiting a down-backwards pointed angle of the third preopercular spine (PS3) and a downwards pointing angle of the fifth preopercular spine (PS5, angles described in detail in Appendix 3). *Sebastes mentella* varied in the combination of angles for PS3, but usually had a forward pointing PS5. All specimens of *S. viviparus* consistently displayed backwards pointing preopercular spines. Specimens of *S. norvegicus*-A and *S. norvegicus*-B collected in East

Greenland waters were found to follow the same pattern of preopercular spine angles as the specimens collected in Norwegian waters, but deviated in the mean gill raker count. These were typically fewer in *S. norvegicus*-B, and *S. norvegicus*-A spanned a greater range in gill rakers among the East Greenland samples. There was no obvious distinction in meristic features between *S. norvegicus*-A and *S. norvegicus*-B allowing for immediate identification.

3.3 Growth pattern

The mean length-at-age for *S. norvegicus*-A (n = 104), *S. norvegicus*-B (n = 565), and *S. mentella* (n = 123) from Icelandic, East Greenland, and Norwegian waters grouped by age classes of five years (Figure 8) were assessed with a Kruskal-Wallis H-test. *Sebastes viviparus* was not included in the analysis due to a lack of data. The Kruskal-Wallis H-test for comparison of means was significant for all year groups. The following post-hoc Dunn test showed that the average ranking of *S. norvegicus*-B was not equal to *S. norvegicus*-A and *S. mentella* respectively in the first 6 age intervals. In the final age interval (41+ years), only *S. mentella* and *S. norvegicus*-B were significantly different. No significant differences were found between *S. norvegicus*-A and *S. mentella* in any age groups. The distribution of *S. mentella* and *S. norvegicus*-A largely overlapped with the lower part of the length rage for *S. norvegicus*-B. The average length-at-age generally increased for all groups with age, with the exception of the average length for *S. norvegicus*-B in the two last groups (Figure 8).

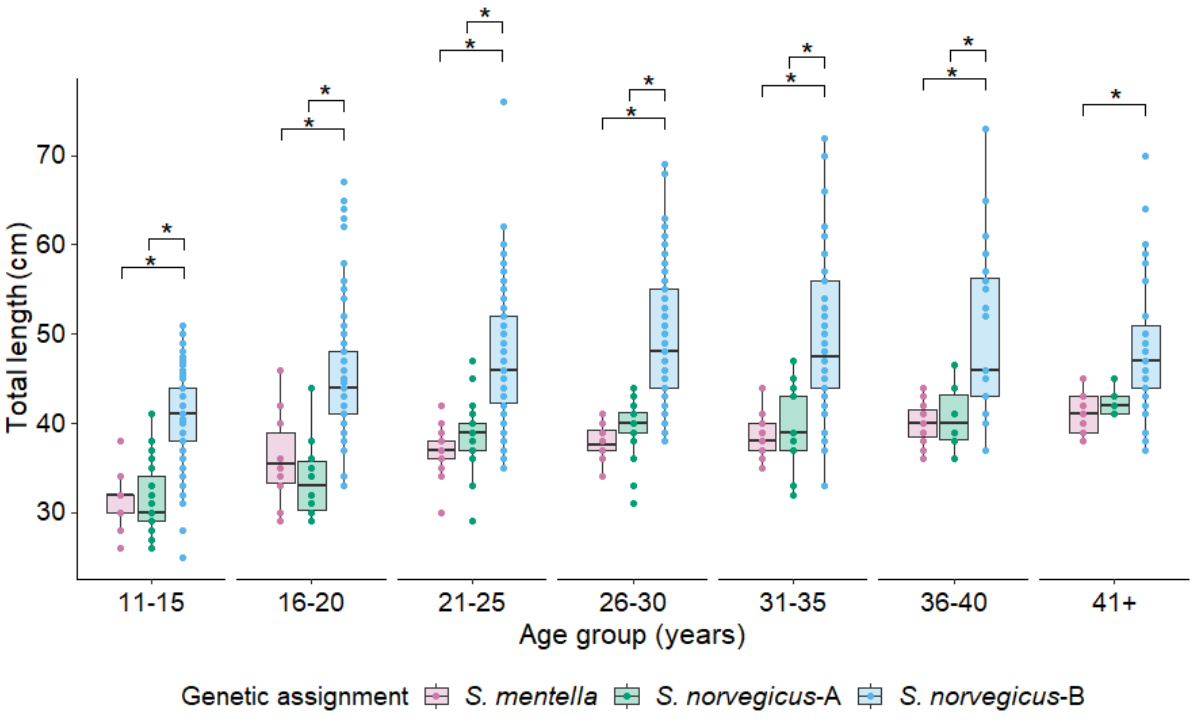


Figure 8. Length-at-age shown as age intervals of 792 redfish collected in Icelandic, East Greenland, and Norwegian waters grouped by year class. Length and age data collected from the IMR databank and the morphological study for S. norvegicus*-A (n = 104),* S. norvegicus*-B (n = 565) and* S. mentella *(n = 123). Heterozygous individuals and* Sebastes viviparus *were not included in the analysis due to a lack of data. Asterisk indicates significant differences in group means for pairwise comparisons.*

Von Bertalanffy growth curves were fitted for *S. norvegicus*-A, *S. norvegicus*-B, *S. mentella*, and *S. viviparus* (Figure 9) to investigate the potential differences in growth trajectories. The growth patterns of *S. norvegicus*-A and *S. norvegicus*-B could not be separated due to overlapping, but the size range of adult specimens of *S. norvegicus*-B was greater than in type A, reaching a maximum length of 76 cm. *Sebastes mentella* and *S. norvegicus*-A largely overlapped in growth patterns, with maximum lengths of up to 47 and 46 cm respectively. With the exception from overlap in juvenile growth, *S. viviparus* displayed a distinct growth pattern with generally small individuals up to 31 cm in total length.

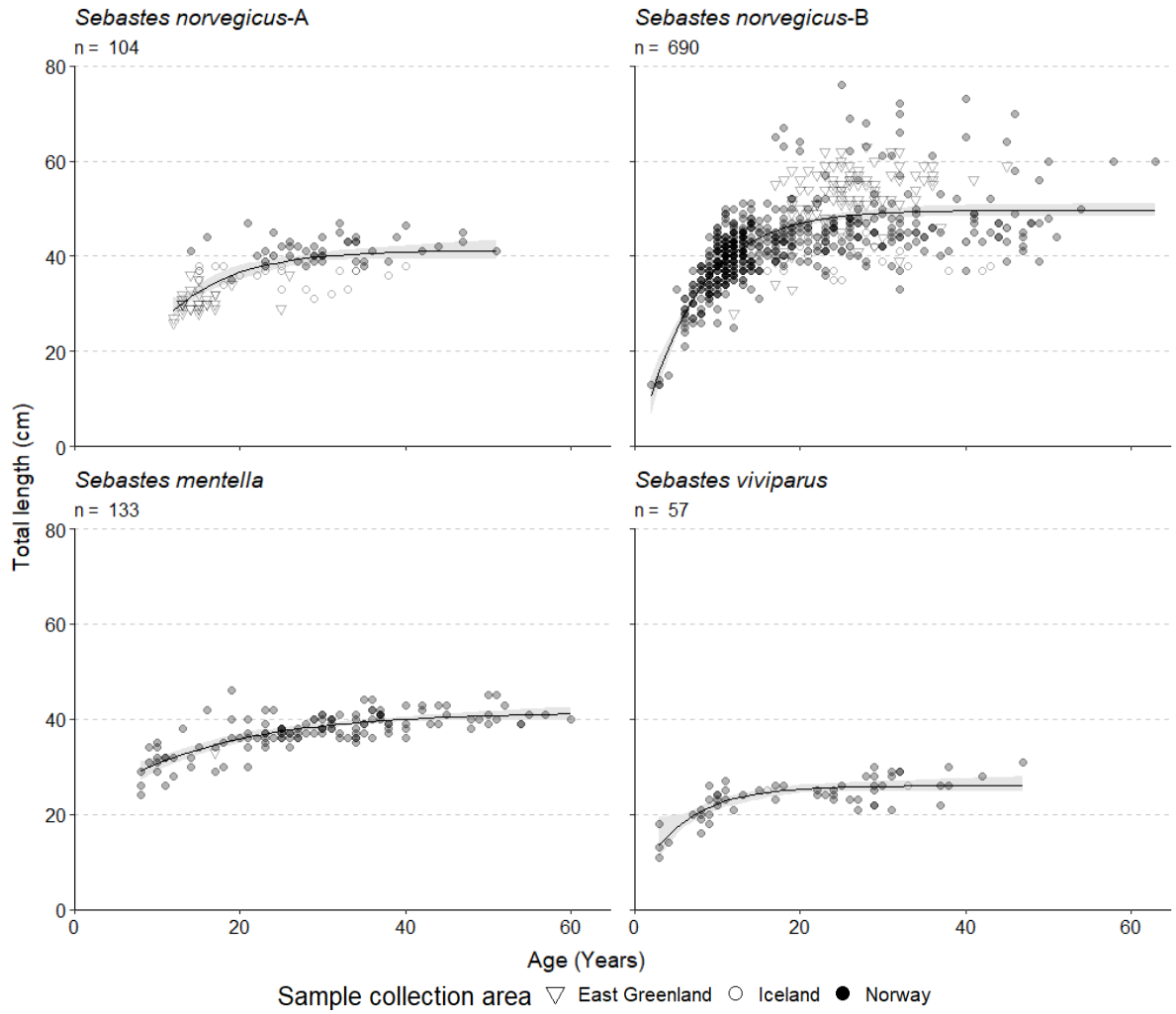


Figure 9. Fitted von Bertalanffy growth models for S. norvegicus-*A (n = 104),* S. norvegicus-*B (n = 690),* S. mentella *(n = 133), and* S. viviparus *(n = 57). Heterozygous individuals were not included in the analysis due to lack of data. Filled circles: Norwegian specimens. Open triangles: East Greenland specimens. Open circles: Iceland specimens. Total length was rounded to the nearest cm below.*

4 Discussion

This is the first study to identify external morphological characters useful in separation of the two proposed cryptic species *S. norvegicus*-A and *S. norvegicus*-B in Norwegian waters. The findings of this study show that the proposed species complex can be delimitated using external morphometric traits, with emphasis on eye orbit diameter, beak length, and the distance from the snout to the upper nostril as the most important physical attributes in traditional morphological identification. The classification accuracy in multivariate analysis of *S. norvegicus*-A and *S. norvegicus*-B using morphometric characters was comparable to the classification accuracy between the recognized species *S. mentella* and *S. viviparus*. In regards to growth patterns, the analysis revealed that there was a marked difference in the growth potential between the two proposed cryptic species, with *S. norvegicus*-B reaching a greater maximum length than *S. norvegicus*-A. Furthermore, growth trajectories varied to a greater extent among *S. norvegicus*-B than *S. norvegicus*-A in the available data, with a greater maximum length achieved by *S. norvegicus*-B. The two proposed cryptic species had overlapping growth trajectories, and could not be distinguished by growth patterns.

4.1 Genetics

The genetic analysis using Single Nucleotide Polymorphism (SNP) markers confirmed that the proposed cryptic species complex of *Sebastes norvegicus* was present in the samples collected for this study represented by *S. norvegicus*-A and *S. norvegicus*-B as described by Saha et al. (2017), both among whole fish for morphological analysis as well as in archived samples. The abundances of *S. norvegicus*-A and *S. norvegicus*-B were drastically different in the material, with the type B being more common in Norwegian waters according to the available data, and the type A likely correlating to the rare specimens described first by Schmidt (2005). Classification of these two types at sea revealed potential patterns between areas, as well as varying classification accuracy among the recognized species; fish collected by a commercial vessel in Icelandic and East Greenland waters almost exclusively classified *S. norvegicus*-A as *S. mentella* at sea, while a greater proportion of specimens were classified as S. norvegicus-B in Norwegian waters (see Appendix 7 for further details). In addition to the proposed cryptic species complex, the genetic results revealed heterozygotes present in the dataset, which were

excluded from analysis of morphology and growth patterns due to low abundances in the available data.

4.2 Morphology

4.2.1 Morphometrics

Analyses of morphometric characters were highly successful in identifying characters useful in the separation of the proposed cryptic species, as well as separation from *S. mentella* and *S. viviparus*. The morphometric characters suggested by both linear discriminant analysis (LDA) and Random Forest analysis to explain the most variation among the fish were largely overlapping. Beak length, eye orbit diameter, caudal peduncle height, and distance from snout to upper nostril were emphasized by LDA as the variables driving separation between species to the greatest degree, with small variations between the two datasets (Norwegian samples vs. East Greenland samples). Random Forest ranked the overall variable importance of variables starting with eye orbit diameter, and listed pectoral and pelvic fin length, beak length, and caudal peduncle height as the top five traits in decreasing order of importance. This was not consistent with what (Saborido-Rey 1994) found in Norwegian waters, which highlighted other traits such as body depth at the pectoral fins and the distance from the pelvic fin to the anal fin. However, the results of this study were in agreement with the findings of Power and Ni (1985). They concluded that the most important and reliable variables for morphometric species discrimination between the redfish were the beak length, eye orbit diameter, body depth at the pectoral fins (equal to the variable DV in the present study) as well as the height of the caudal peduncle. In the present study, eye orbit diameter and beak length were found to be at an intermediate size in *S. norvegicus*-A between *S. norvegicus*-B and *S. mentella* (Appendix 8). Saborido-Rey and Nedreaas (2000) conducted a morphometric analysis on *S. mentella* in the Northeast Arctic, finding evidence for morphological variation between sampling sites. The authors found that neck width provided the most discriminatory power followed by length from the snout to the preopercular spines and eye orbit diameter. In the present study, the signal from neck width is negligible in all pairwise comparisons. Potential causes for the differences in results may be attributed to differences in methodology, but other factors such as morphological disfiguration during storage and freezing. The ecogeographical gradient in morphology found by Garabana (2005) highlights the influence of environment on phenotypic traits, which is

difficult to take into account without detailed records of ontogenetic habitat and environmental fluctuations.

High classification accuracies were reported by two independent models, highlighting a set of descriptive traits which can be used further in practical applications to increase the detection of the potential cryptic species. The morphometric analyses in congruence with the genetic results showed that the groups could be separated well based on the ratio-transformed variables. The linear discriminant analysis (LDA) was highly successful in correctly classifying the species with a cross-validated Cohen's Kappa value of 0.91, which describes the classification accuracy of the model in light of the expected classification accuracy by random assignment. Although there are no set limits for interpretation of the Kappa coefficient, values exceeding 0.80 are generally regarded as providing a high degree of agreement (Landis and Koch 1977). Greater inaccuracy in classification of the proposed cryptic species compared to the recognized species suggests that the morphometric shape variation may be lower among more closely related fish, which is to be expected considering the shorter time frame of morphological divergence (Hyde and Vetter 2007) and typically higher degree of geneflow (Saha et al. 2017). Both the LDA and the Random Forest models consistently classified specimens of *S. mentella* and *S. viviparus* with high accuracy, while the models showed increased confusion between the proposed cryptic species, classifying *S. norvegicus*-A mainly as *S. norvegicus*-B and vice versa. Compared to LDA, the Random Forest algorithm performed slightly better. This was not the same observation (Doyle et al. 2018) made, which was that Random Forest provided a more conservative estimate of classification accuracy. The Random Forest classifier provided a good separation of the groups based on the ratio-transformed variables, correctly classifying the groups with an estimated error rate at around 4% for the Norwegian specimens.

When comparing the classification accuracy of *Sebastes* specimens in Norwegian waters to that of the combined dataset with East Greenland specimens, the accuracy dropped for both the LDA and the Random Forest analysis. In the ordination plots from LDA, this was evident for the *S. norvegicus*-A and *S. norvegicus*-B in a greater overlap between the 95% confidence ellipses. There are multiple potential causes for the observed decline in accuracy. Firstly, the fish from the East Greenland waters were on average larger than the fish from Norwegian waters, potentially affecting the shape if the ratio transformation was not effective in removing enough shape variation from the variables. Secondly, morphological traits in fish are known to

be affected by epigenetics and environmental factors in addition to the genetic component, exhibiting plasticity in morphological expression under different conditions (Bonini-Campos et al. 2019). As information on environmental conditions was not available, this effect cannot be ruled out. Thirdly, studies have shown that redfish morphology can vary to a great extent between and among species in different areas, which Garabana (2005) found when comparing morphological variation within and between populations. Despite the lower classification accuracy, the outcome of the analyses were consistent when specimens from both areas were included. Both methods recorded a similar variation in the dataset, which strengthens the reliability of the results given by the LDA and indicates that the analysis is relatively robust to the assumption violations of normality, heterogeneity of variances, and unequal sample sizes when applied to the dataset used in the study.

Genetic assignment of the fish showed that some specimens were heterozygous for the third SNP marker separating *S. norvegicus*-A and *S. norvegicus*-B, found in samples from all three areas. One fish in the genetic analysis also displayed heterozygosity for the SNP marker separating *S. viviparus* and *S. mentella.* The appearance of heterozygous individuals in the genetic analysis could have multiple explanations. Firstly, this may indicate that there are genetic variations within the types causing a small fraction of fish to display heterozygous alleles for the SNP3 marker, either randomly or through genetic divergence within ecotypes, suggesting that the markers are imperfect in distinguishing all specimens. Consequently, this method of genetic assignment may require additional markers to capture the full genetic variation. Secondly, heterozygous individuals can also indicate persistence of geneflow between *S. norvegicus*-A and *S. norvegicus*-B. Both hybridization and introgressive hybridization, defined as the exchange of genetic material between species and exchange of genetic material between hybrid offspring and parent species respectively, are well documented within the *Sebastes* genus (Rehbein 2013, Schwenke et al. 2018), particularly between *S. mentella* and *S. fasciatus* (Roques et al. 2001) and between *S. mentella* and *S. viviparus* (Artamonova et al. 2013) in the West Atlantic. The extent of interspecific hybridization and its effect on genetic population structure is suspected to be extensive (Artamonova et al. 2013, Saha et al. 2016), but is currently largely unexplored in the North Atlantic, further complicated by the limited knowledge of *Sebastes* migration patterns and reproductive habits.

4.2.2 Meristics

The meristic analysis revealed differences in the ranges of counts and angles between species for the majority of the variables with the exception of the first dorsal fin ray count and the pelvic fin ray count. Between *S. norvegicus*-A and *S. norvegicus*-B in Norwegian waters, most variables were not significantly different, with the exception of anal fin ray counts and although slightly overlapping, the pectoral fin ray count had a mode of 19 in *S. norvegicus*-B while it typically ranged from 17-18 in *S. norvegicus*-A (). However, with the introduction of Greenland specimens, the test implied several significant differences between the two types. This may be caused by a size difference, which has been found previously in meristic analysis of redfish (Garabana 2005). Although focusing on Greenland and Iceland specimens, Garabana (2005) also found that the number of fin rays in the first dorsal fin was consistent across *S. norvegicus*, *S. mentella* and *S. viviparus*. The number of gill rakers was found to correlate with length, indicating that meristic features are likely associated with size, as observed by Garabana (2005). Standardization of values and reduction of potential size dependency in meristic data is prevented by the non-parametric nature of the data, and the finding of meristic feature variations among the specimens in the present study are therefore relevant for the included size range but may vary further outside the represented sizes.

Meristic features are known to be restricted to a certain degree by underlying genetics but are greatly affected by ontogenetic environment, although the relative contributions of these mechanisms in the development of meristic features are uncertain (McDowall 2008). Generally, features that are developed last seem to be affected by environmental conditions to the greatest extent (Barlow 1961). Fish larva have been shown to exhibit phenotypic plasticity in meristic features induced by temperature gradients in controlled experiments (Lindsey 1954, Sfakianakis et al. 2011). The phenomena of meristic variation along a latitudinal gradient has been described as Jordan's rule (Jordan 1891), an ecogeographical rule proposed to explain the observed positive correlation between increasing latitude and meristic counts, initially detected in the numbers of vertebrae. This latitudinal gradient in meristic counts is believed to be associated with the accompanying temperature gradient, but the successful application of this rule to expected changes in anatomy varies between species (McDowall 2008). As the ontogenetic environment may vary spatially and temporally between populations of the same species, the intraspecific range in counts may consequently vary, potentially reducing the diagnostic value of meristics for species with extensive distributions across the latitudinal

gradient. However, meristic features may provide good discrimination between populations or stocks on a smaller spatial scale (Ozerov et al. 2016), and be useful for distinguishing closely related species when applied in combination with morphometrics. For discrimination between the two proposed cryptic species, meristic features should be interpreted with caution.

4.3 Growth pattern

The growth patterns varied substantially between *S. norvegicus*-A, *S. norvegicus*-B and *S. mentella* for the data available in this study. Average length-at-age and growth pattern for *S. norvegicus*-A were overlapping with *S. mentella*, while *S. norvegicus*-B exhibited a range of length trajectories resulting in a comparably large spread in data (Figure 9). The observed variation in growth of *S. norvegicus* has been suggested as conforming to two main distinct growth patterns: slow growth subsiding after maturity, and rapid growth where the fish continues to grow presumably throughout its life (Monferrer and Planque 2019). Separation of *S. norvegicus*-A and *S. norvegicus*-B in the present study did not resolve the proposed existence of two growth patterns, as specimens of *S. norvegicus*-A exclusively displayed slow growth which overlapped with slow growing specimens of *S. norvegicus*-B. Monferrer and Planque (2019) could not eliminate genetic variation as a leading cause of growth pattern divergence but showed that larger *S. norvegicus* specimens were generally associated with deeper, colder waters. This is in agreement with Bergmann's rule, which is an ecogeographical rule describing the observation of increasing body size for some ectotherms with increasing latitude (Angilletta and Dunham 2003). As the genetic method used in the present thesis did not allow identification of *S. norvegicus* giants, the attribution of a faster growth rate to a genetic component cannot yet be discarded.

4.4 Species complex

The *Sebastes* species are notoriously similar regarding external morphology (Frable et al. 2015) (Frable et al. 2015, Hyde et al. 2008, Gharret et al. 2008), which may delay the detection of ongoing speciation within the genus. As discussed in the introduction, *Sebastes* life history provides great potential for speciation events either partially in sympatry or for short periods of reproductive isolation allowing for allopatric speciation (Hyde and Vetter 2007). Genetic analysis conducted on the redfish of the North Atlantic indicate that the genetic variation may be more extensive than the four currently recognized species represent (Johansen et al. 2000a, Johansen et al. 2000b, Danıelsdóttir et al. 2008, Cadrin et al. 2010, Saha et al. 2016, Saha et al. 2017). This was supported by the genetic analysis performed for this thesis, detecting the proposed species complex formed by *S. norvegicus*-A and *S. norvegicus*-B in Norwegian waters.

It can be challenging to set the criteria for recognizing and establishing boundaries between cryptic species. Typically, the traditional definition of a biological species is based on the reproductive isolation of a group of organisms, with hybrid offspring incapable of reproducing. However, this narrow definition does not necessarily cover the range of genetic, ecological, behavioral, physiological, morphological, and evolutionary traits of separately evolving metapopulations (De Queiroz 2007). Saha et al. (2017) investigated the extent of gene flow between the three proposed cryptic species, and compared it with both the gene flow between the established species *S. fasciatus* and *S. viviparus* used for establishing a reference value between geographically separated species as well as the three ecomorphs of *S. mentella* (Saha et al. 2016). Their analysis showed that the reproductive isolation between the proposed cryptic species was not complete, but comparable of that between *S. viviparus* and *S. fasciatus*, showing lower levels of gene flow than the exchange between the three *S. mentella* ecomorphs (Schmidt 2005). The observed continuation of gene flow between groups supports a theory of recent speciation of redfish (Hyde and Vetter 2007), where evidence of hybridization and introgression indicates that reproductive isolation between the established species is incomplete. Gene flow between closely related populations undergoing genetic divergence does not necessarily prevent speciation (Bolnick and Fitzpatrick 2007, Nosil 2008, Potkamp and Fransen 2019).

Previously, these proposed cryptic species were only known to be present in the Greenland and Iceland area with indications of S. norvegicus-A in Norwegian waters (Saha et al. 2017). Among the samples in this study, *Sebastes norvegicus*-B was considerably more abundant than *S. norvegicus*-A both in Norwegian waters and in the Greenland and Iceland waters. Schmidt (2005) found through the use of mitochondrial DNA sequence analysis and microsatellites that there were possibly two distinct groupings of *S. norvegicus* in their data; one common type, which was found across the Atlantic Ocean from Norway to the Flemish Cap, and one less abundant type that became apparent in highly intraspecific genetic divergence on the continental slope of Greenland, Flemish Cap and Iceland. In the present study, the uncommon type described by Schmidt (2005) and Saha et al. (2017) is likely equivalent to the *S. norvegicus*-A detected in this study thereby expanding its known range to include the Norwegian Sea and the Barents Sea shelf, while *S. norvegicus*-B likely represents the common type. In addition to *S. norvegicus*-A and *S. norvegicus*-B, Saha et al. (2017) described the third *giant* type as an additional cryptic species in the *S. norvegicus* complex in Greenland waters, which could not be identified using the SNP markers in this study. The potential prevalence of the giant type of *S. norvegicus* in Norwegian waters remains undetermined and requires further investigation.

The high classification accuracy for *S. mentella* and *S. viviparus* could be expected considering their long history of genetic divergence (Hyde and Vetter 2007), and surprisingly, the morphometric classification of the proposed cryptic species were similarly high in the two methods applied. Cryptic speciation and the development of reproductive isolation may be masked by a less rapid evolution in external morphology and ecology facilitated by homogenous environmental conditions that favor the retention of the current biological expression in redfish (Colborn et al. 2001). This may cause the differentiation in morphological expression of genotype to be delayed despite low levels of gene flow for maintaining morphological similarities. However, considering the great potential for reproductive barriers (Hyde and Vetter 2007) between *S. norvegicus*-A and *S. norvegicus*-B and distinct morphological features revealed in the present study, it would be reasonable to assume that that these two types may have had the opportunity to diverge for a prolonged time, achieving the observed morphological distinct traits and low levels of gene flow comparable to that of established species (Saha et al. 2017).

Geographical overlap in the distribution of *S. norvegicus*-A and *S. norvegicus*-B in Norwegian waters along the Eggakanten and on the Barents shelf (Figure 10) and the presence of both types in single hauls provide evidence that the two types exist in sympatry. This is concurrent with the findings of Saha et al. (2017), who detected low but significant rates of geneflow among the proposed cryptic species comparable to the geneflow between *S. mentella* and *S. viviparus*, and far lower than between three recognized ecomorphs of *S. mentella* in Greenland waters (Saha et al. 2016). Several whole specimens of female *S. norvegicus*-A examined in the morphological part of the study were found to carry eggs. Collected in the Lofoten area (N68°25, E11°20) in March, close in space and time to the known larval release (Figure 10) of *S. norvegicus* along the coast of Norway (Drevetnyak et al. 2011). In regards to the potential of larval release as mechanism for reproductive barrier to sustain a genetic divergence between closely related species, adjusting the timing or area of larval release can prevent the mixing of larvae between populations through altered dispersal (Bernardi 2013). As redfish eggs are not fertilized at the moment of copulation but rather by delayed self-fertilization (Raitt and Hall 1967), this potential observation of reproductive overlap does not necessarily provide the enough information about the reproductive habits of S. norvegicus-A to make an inference about reproductive barriers or gene flow. The majority of *S. norvegicus*-A specimens were collected in spring, with only one specimen collected in autumn located in the northern part of the Norwegian Sea. Previous morphological studies have suggested that seasonal migrations cause possibly separated populations to aggregate in certain areas (Saborido-Rey and Nedreaas 2000), and it is therefore unclear whether the sampled specimens in the morphological and archived material represent separate populations of *S. norvegicus*-A, and where they spend the rest of the year. The migration patterns, preferred habitat and distribution of *S. norvegicus*-A throughout the year remains to be explored further, but this study reveals that reproducing females of *S. norvegicus*-A may overlap spatially and temporally with *S. norvegicus*-B for the release of larvae in spring along the Norwegian coast.

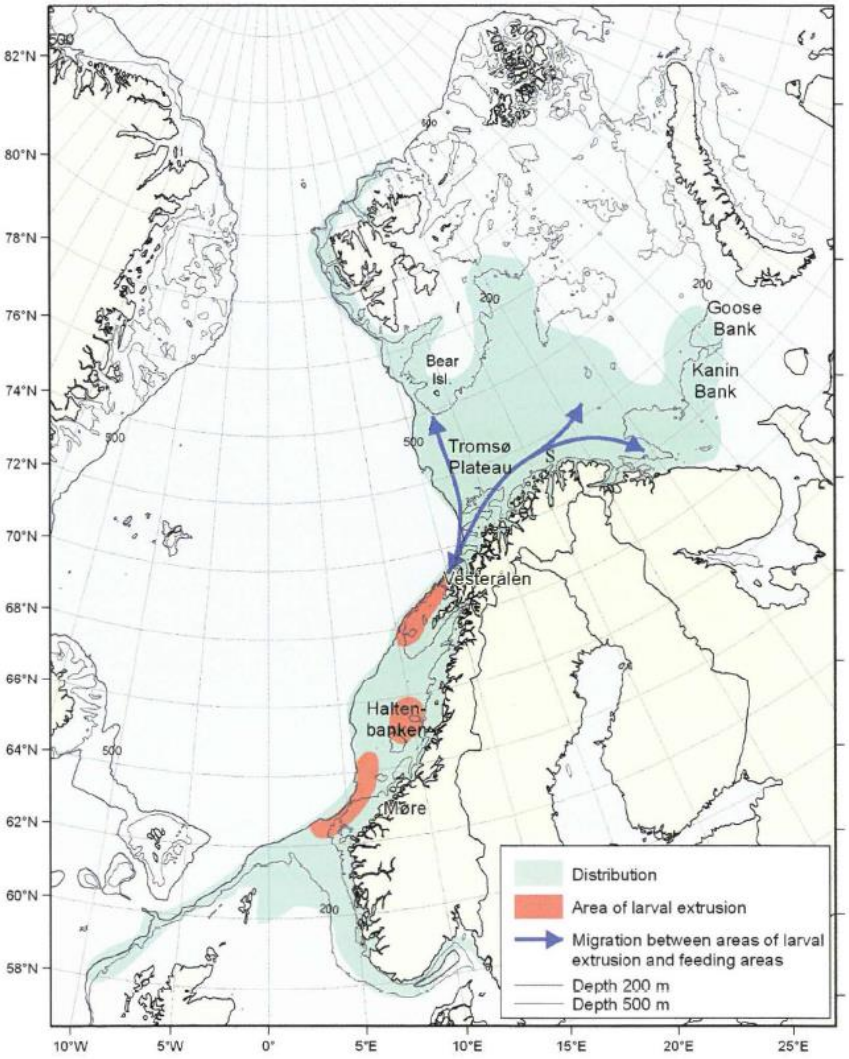


Figure 10. Distribution of S. norvegicus *in Norwegian waters, showing known larval extrusion area (Vesterålen, Lofoten area) and migration routes. From: Drevetnyak et al. (2011)*

4.5 Practical application of morphological characters

Considering the somewhat volatile nature of meristic features which may make them difficult to implement as identifiable characters (Kendall 2000), the morphometric characters obtained in the present study showed the strongest potential for good species discrimination without further studies. The three morphometric characters best separating the proposed cryptic species *S. norvegicus*-A and *S. norvegicus*-B based on ratio-transformed data were eye orbit diameter (DO), beak length (LB), and the distance from the snout to the preopercular spine (LPO). A combination of morphometric characters, such as eye orbit size and beak, may be required for highly accurate classification at sea as suggested by Power and Ni (1985) for discriminating between *S. mentella* and *S. norvegicus*. For morphological identification of *Sebastes* spp. in Norwegian waters, the currently available data indicate that specimens of *S. norvegicus*-A show similar growth pattern to *S. mentella* and that they do not reach the same maximum length as *S. norvegicus*-B. As a consequence, specimens larger than ~50 cm may be easier to identify quickly as *S. norvegicus*-B. Future investigations of meristic features and combination with extended knowledge on habitats and environmental influence may provide better results for discriminating between the proposed cryptic species. Regardless, the morphometric variables presented in the present study have shown potential for practical application.

4.6 Limitations of the study

Due to the low frequency of *Sebastes norvegicus*-A in Norwegian waters, the morphological analysis was constrained to 14 specimens sampled in a single haul. Although this does not necessarily mean that the fish belonged to the same population due to unknown migration patterns of *S. norvegicus*-A in Norwegian waters (Saborido-Rey and Nedreaas 2000), collecting and examining additional whole specimens from a greater geographical range will likely contribute to expanding our knowledge on morphological variation among the cryptic specimens and valuable characters for classification. This is especially interesting considering the phenotypic plasticity of redfish to the environmental gradients (West-Eberhard 1989) they are likely subjected to along their distribution in Norwegian waters potentially creating morphological gradients (Garabana 2005). Furthermore, the current knowledge of

morphometric and meristic features in *S. norvegicus*-A is limited to the age range and length range available in the dataset, which, considering the potential development of shape over time, means that the morphological characters found to be important in this study cannot necessarily be directly applied to classification of younger individuals and juvenile fish which is important in monitoring recruitment and population fluctuations.

4.6.1 Methodological limitations

Despite the frequent use of morphological tools in species classification in marine scientific literature, there are limitations to the methods developed for shape identification and separation between groupings. Size dependency between variables caused by allometric growth is a central issue in morphological studies (Rohlf and Bookstein 1987, Bookstein 1989, Klingenberg 1996, Baur and Leuenberger 2011, Klingenberg 2016). The strong influence of size on morphometric measurements can mask variations in shape reflecting differences in biological processes, functional regulation, or other morphological expressions within and between species which may be interesting in the context of morphometric species delineation (Bookstein 1989). Multiple approaches to size correction have been developed to assess the problem of size dependency in morphometric studies. Calculating the ratio between a linear measurement and a size measurement is a widely used method for its simplicity (Power and Ni 1985, Albrecht et al. 1993, Doyle et al. 2018). Other regression-related methods such as residuals, adjusted residuals, shearing or allometric vectors have been suggested to more efficiently correct for size correlations than ratios in multivariate morphometric analyses (Burnaby 1966, Humphries et al. 1981, McCoy et al. 2006, Baur and Leuenberger 2011, Klingenberg 2016). Although ratio transformation was used in this study, this method is less than ideal, and further options to apply appropriate transformation to the data should be exhausted. In addition, the characters used in morphometric analysis are often adaptive traits. According to Kendall (2000), adaptive traits such as eye size, beak length and fin musculature may reflect the of a fish rather than ancestry, making them suboptimal traits for classification compared to features that are not subjected to selection. Consequently, the traits observed in specimens may be shaped by external factors such as depth rather than genetics.

Limitations of the multivariate analysis methods are also relevant, especially regarding the robustness of linear discriminant analysis (LDA) discussed earlier, as well as the limitations of using data with missing values. In the present study, the robustness of LDA was addressed using the non-parametric method of classification provided by Random Forest, confirming the general trends displayed in the LDA results. Other multivariate methods for analysis of traditional morphometrics such as linear regression analysis can be useful. Historically, stepwise discriminant methods have been applied to morphometric data for classification and determination of variable importance, but this method can be sensitive to which variables are present in the data set and the removal of a variable may cause the analysis outcome to change (Garabana 2005). An additional limitation of the present study is the lack of a pilot study for adjustment of the morphometric and meristic sampling protocol to streamline the sampling and achieve the most consistent results across different samplings, as well as performing repeated measurements for the estimation of a measurement error (Claude 2008). However, all measurements and counts in the morphological part of the study were performed by the author, eliminating potential measurement inconsistencies between multiple observers. Increasing the sample sizes of both the morphological and the growth pattern sections of the data could strengthen the results and allow for additional patterns to be examined.

In the present study, the whole fish used in morphological analysis went through a process of freezing and defrosting before measurements were taken, which may alter the weight and length properties of the fish. As the fish were processed in separate sessions, the fish used in the study may have experienced inconsistent durations of thawing in freshwater. Freezing whole fish has been observed to cause a length reduction of which the proportion depends on whether the fish is frozen in water, the duration of freezing, and the biological properties of the fish such as fat and muscle content (Sayers Jr 1987, McQueen et al. 2019). Thawing fish in freshwater can cause water to spontaneously absorb into the tissue of the fish through osmosis, thereby artificially inflating the weight of the fish after defrosting (Halliday and Roscoe, 1969). This effect can vary with duration of submersion in freshwater after the tissue is defrosted, and smaller fish may consequently absorb more water relative to larger specimens. Combined, the freezing and subsequent thawing of fish create some uncertainty regarding the accuracy of weight and length measurements compared to the weight and length of the fish at the time of capture which is important to keep in mind when comparing measurements of frozen fish to those of fresh fish.

4.7 Future research

Future studies can further address the unresolved knowledge gaps regarding the genetic structure of the *Sebastes* genus in the North Atlantic, and the morphological, biological, physiological, and behavioral aspects of redfish life history intertwined with it. Building on the present study, morphological characters for species identification should be further examined and assembled for classification at sea. Furthermore, reviewing supplementary morphological characters historically used in taxonomic identification of redfish can be of great value in simplifying the classification process. For example, color and color patterns have been described in the context of differentiating *S. mentella* ecotypes (Artamonova et al. 2013). In West Atlantic redfish fishery, gas bladder musculature is used to separate the species *S. mentella* and *S. fasciatus* which could be investigated among *S. norvegicus*-A and *S. norvegicus*-B, although time consuming (Ni 1981, Garabana 2005). As mentioned, the genetic method applied in this study did not allow for the identification of the giant type *Sebastes norvegicus* (Johansen et al. 2000b, Pampoulie and Daníelsdóttir 2008, Saha et al. 2017), and additional genetic analysis combined with the use of morphological tools on specimens in Norwegian waters may aid in resolving both the question of species composition as well as possible mechanisms behind growth pattern variations observed in *S. norvegicus* specimens (Monferrer and Planque 2019). Correctly identifying and consequently gaining more information about *S. norvegicus*-A and *S. norvegicus*-B has great potential in ensuring sustainable management of redfish and evaluating the impact of fishery on the genetic composition in Norwegian waters.

4.8 Conclusions

Genetic methods are powerful tools for investigating species structure and identifying potential emerging species among seemingly morphologically similar species. The findings of this study revealed that the proposed cryptic species complex of *S. norvegicus* is prevalent in Norwegian waters, with the detection of the *S. norvegicus*-A and *S. norvegicus*-B previously described by Saha et al. (2017) and Schmidt (2005) from Icelandic and Greenland waters. The combined approach using genetic and morphological tools allowed for the separation of four groups of redfish in the Norwegian waters. High classification accuracy of *S. norvegicus*-A and *S. norvegicus*-B at levels comparable to the classification accuracy of the recognized species *S. mentella* and *S. viviparus* in light of the high degree of reproductive isolation found by Saha et al. (2017) encourages this thesis to strengthen the proposition that *S. norvegicus*-A and *S. norvegicus*-B are separate species composing a cryptic species complex in the context of the research questions this thesis aims to elaborate on. Furthermore, the morphological analysis reveals that the cryptic species are distinguishable by external morphology. Finally, the thesis was not able to resolve the variation in growth patterns observed in *S. norvegicus* with application of Single Nucleotide Polymorphisms, although the results imply that the variations in growth patterns between *S. norvegicus*-A and *S. norvegicus*-B are not of the same magnitude. However, this distinct genetic and morphological variation should be considered in management plans regarding redfish stocks to identify and conserve potentially vulnerable populations.

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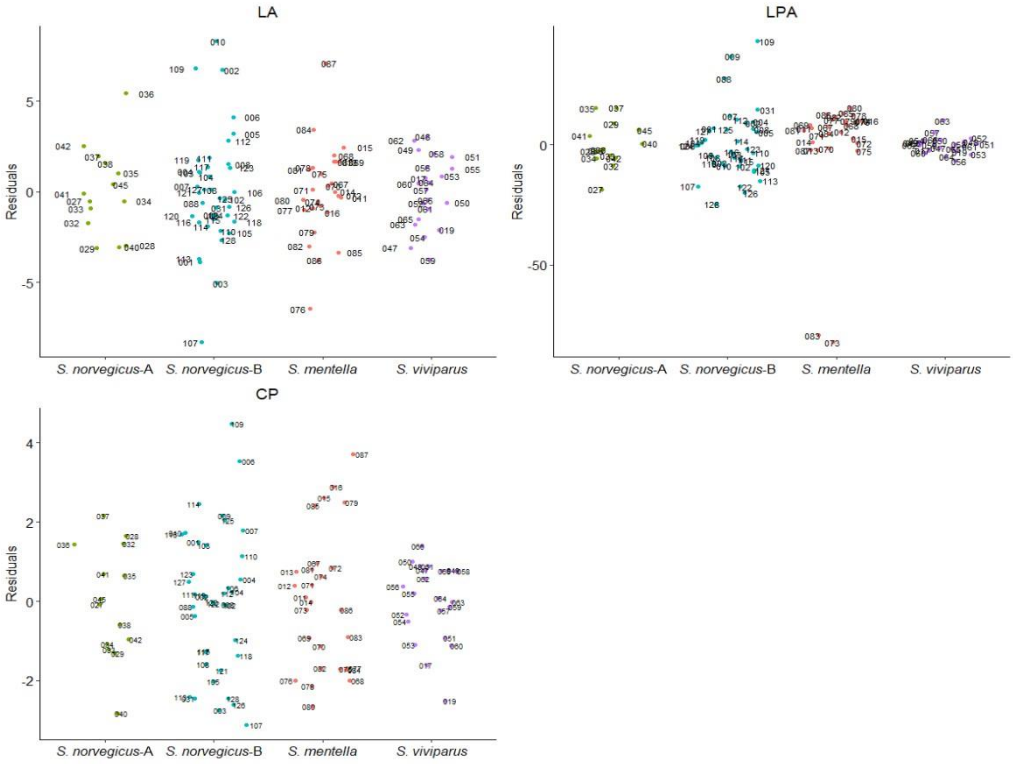
Appendix 1

Species	Unknown length	a	b	Known length	Length range (cm)	Sex of fish
S. norvegicus	SL	0.224	0.865	TL	10.2 – 50	Unsexed
S. mentella	SL	0.000	0.805	TL	-	Unsexed

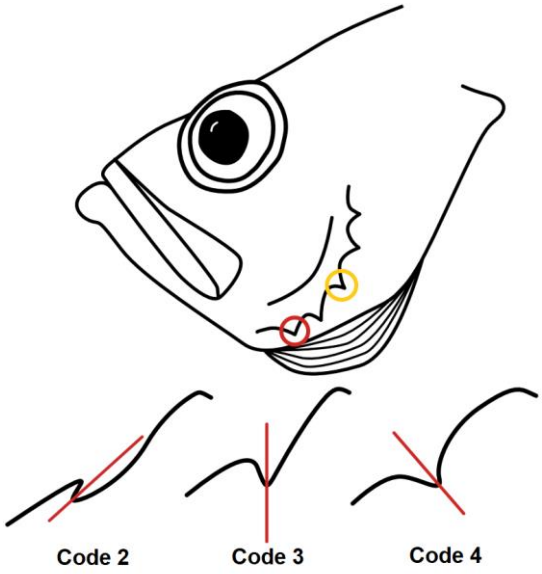
Appendix 1. Table of conversion units for length estimation of S. norvegicus *and* S. mentella*. Source: (Binohlan et al. 2011)*

Appendix 2



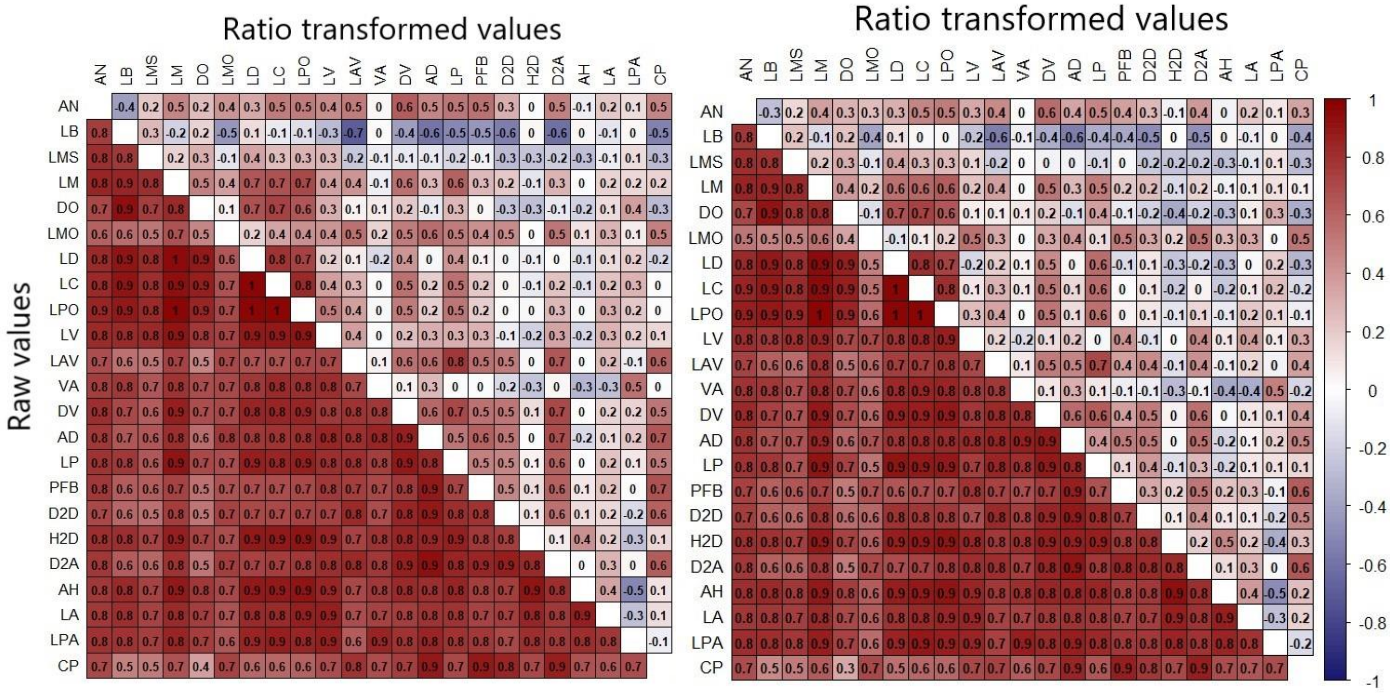
Appendix 2. A selection of residual plots for all morphometric variables for identification of potential outliers. Numbers represent individual fish ID.

Appendix 3



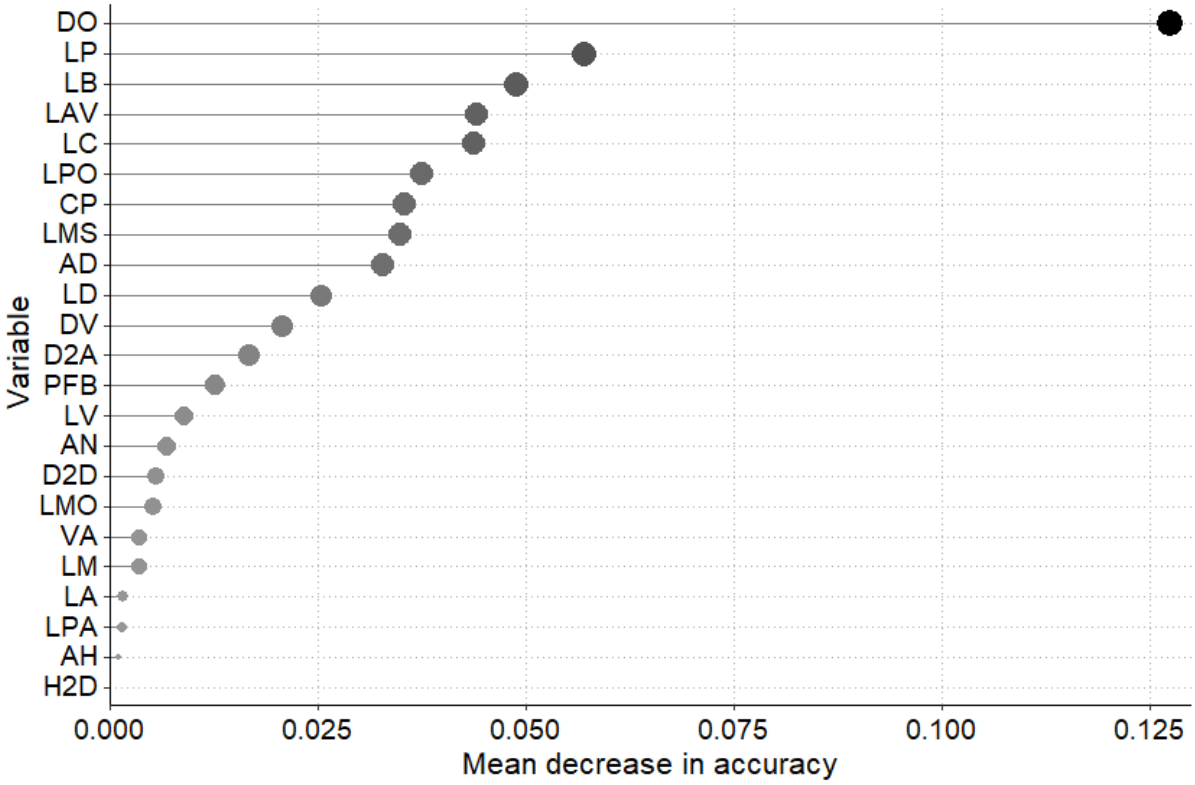
Appendix 3. Codes used to determine the angle of the third preopercular spine (PS3) and the fifth preopercular spine (PS5) in the meristic analysis. Yellow circle: PS3. Red circle: PS5. Code 2: Spine pointed down-forward. Code 3: Spine pointed downwards. Code 4: Spine pointed down-backward. Figure adapted from Garabana (2005).

Appendix 4



Appendix 4. Correlation plots showing Spearman's correlation for raw values and ratio transformed values. Left: Raw and ratio transformed values for specimens collected in Norwegian waters (n = 99). Right: Raw and ratio transformed values for species collected in both Norwegian and East Greenland waters (n = 129).

Appendix 5



Appendix 5. Mean decrease in accuracy indicating relative variable importance ranked by Random Forest for all samples in the total morphometric dataset (Norwegian + East Greenland samples). Higher values indicate greater variable importance. Top ten linear distance variables: Eye orbit diameter (DO), pectoral fin length (LP), beak length (LB), pelvic fin length (LAV), length from snout to edge of operculum (LC), length from snout to preopercular spine (LPO), caudal peduncle height (CP), length from snout to upper nostril (LMS), length from first dorsal fin to anal fin (AD), length from second dorsal fin to anal fin (D2A).

Appendix 6

Actual class

Norwegian waters

Predicted class		*S. norvegicus*-A	*S. norvegicus*-B	*S. mentella*	*S. viviparus*
	S. norvegicus-A	12	0	1	1
	S. norvegicus-B	0	38	0	0
	S. mentella	0	0	24	1
	S. viviparus	1	0	0	21
	Class error	0.14	0.0	0.04	0.05

Norwegian and East Greenland waters

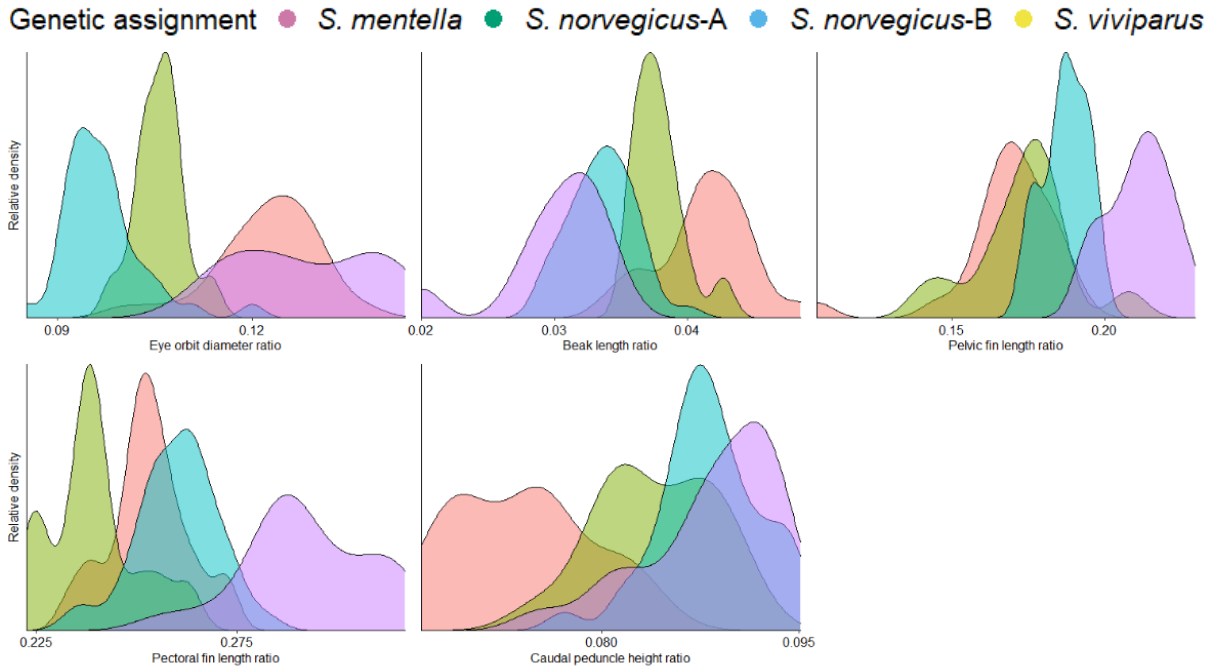
Predicted class					
	S. norvegicus-A	32	2	1	1
	S. norvegicus-B	4	41	1	0
	S. mentella	0	1	24	0
	S. viviparus	0	1	0	21
	Class error	0.11	0.11	0.04	0.05

Appendix 6. Classification matrix produced by Random Forest from morphometric data on Sebastes *spp. Estimated out-of-bag error rates were 4.04% and 8.54% for dataset with specimens from Norwegian waters (top, n = 99) and total dataset including East Greenland specimens (bottom, n = 30).*

Appendix 7

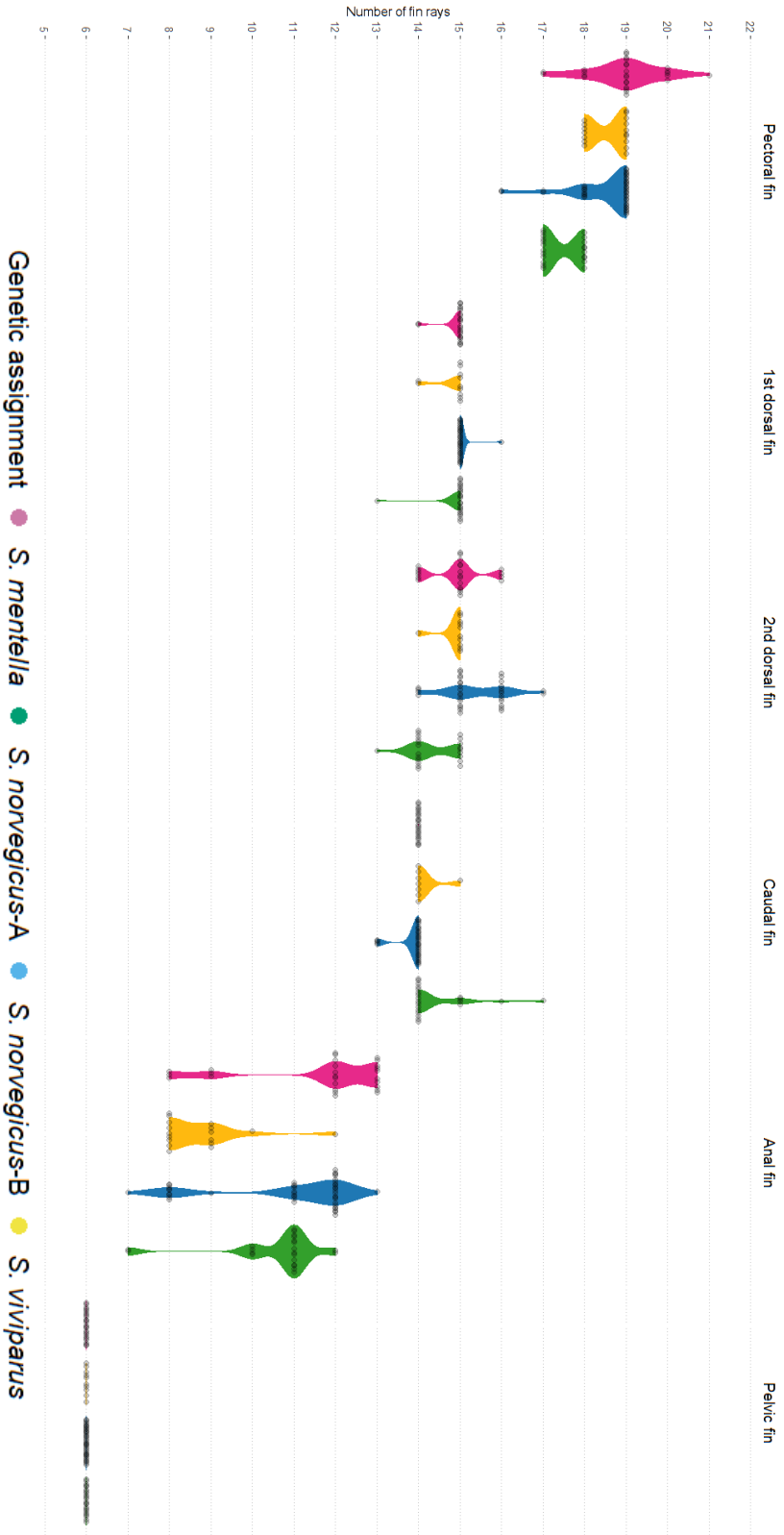
The classification of *Sebastes* spp. varied slightly between Norwegian waters and East Greenland/Icelandic waters. In Norwegian waters, the majority of *S. norvegicus*-A were classified as *S. mentella*, while many were classified as *S. norvegicus*. Among the fish collected in Icelandic and East Greenland waters, nearly all individuals were initially classified as *S. mentella*. This variation between areas could be caused by differences in classification protocols between areas or individual vessels (ICES 2019), or that the redfish are more difficult to classify in the Greenland area due to morphological similarities as found by Garabana (2005). It is important to note that the samples from East Greenland and Icelandic waters were collected by a single commercial vessel, and the differences may therefore also be attributed variations in the experience of samplers performing the morphological classification at sea. Within the Norwegian samples, the classification accuracy was slightly higher in data from the commercial vessels than data from the research vessels. This difference in classification accuracy may similarly be attributed to differences in classification procedures between vessels or variations in the experience of samplers performing the morphological classification at sea. This difference may also reflect the greater sampling area covered by research vessels and possibly a spatial gradient in morphology as described by Garabana (2005).

Appendix 8



Appendix 8. Relative frequencies of morphometric measurement ratios indicating the relative length of morphometric measurements between groups. The top five variables of highest importance ranked by Random Forest are displayed: eye orbit diameter, beak length, pelvic fin length, pectoral fin length and caudal peduncle height.

Appendix 9



Appendix 9. Meristic features for the Norwegian and East Greenland specimens.