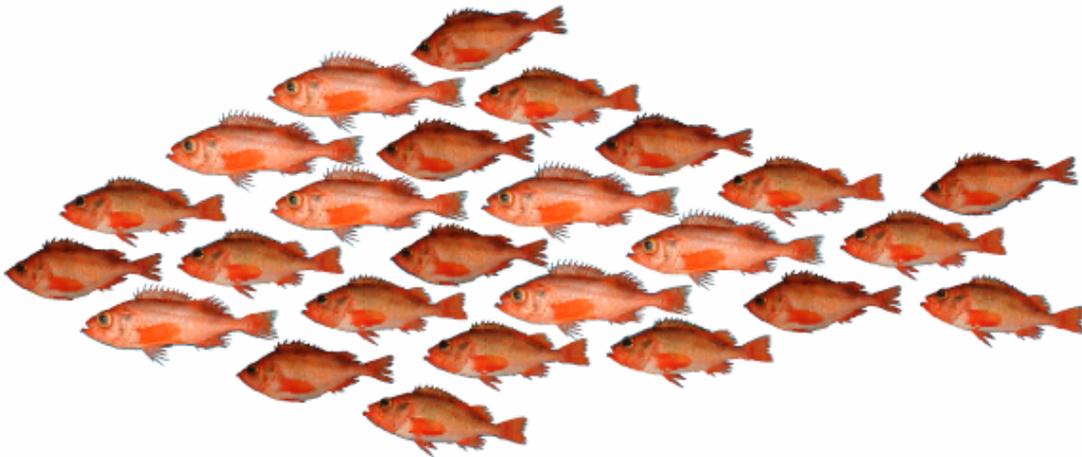




Universidade de Vigo
Departamento de Ecoloxía e Bioloxía Animal



The genus *Sebastes* Cuvier, 1829
(Pisces, Scorpaenidae) in the North Atlantic:
Species and stock discrimination using
traditional and geometric morphometrics.

Memoria de Tesis Doutoral
para optar ó título de Doutor Europeo

Dolores Garabana Barro



Instituto de Investigacións Mariñas

Juan Francisco Saborido Rey, Titulado Superior do Instituto de Investigacións Mariñas (Consejo Superior de Investigaciones Científicas)

Autoriza a presentación da memoria adxunta, titulada “The genus *Sebastes* Cuvier, 1829 (Pisces, Scorpaenidae) in the North Atlantic: Species and stock discrimination using traditional and geometric morphometrics”, realizada por D^a Dolores Garabana Barro baixo a súa dirección, para optar ó grao de Doutora Europea en Bioloxía.

E para que así conste, expídese o presente certificado en Vigo, en Abril de 2005

Juan Francisco Saborido Rey

A Rosa
Ós meus pais

Agradecementos / Acknowledgements

Xa choveu dende que saín da casa por primeira vez nun pesqueiro de Cangas cara a Terranova coa ilusión de empezar a recorrer-lo meu camiño como bióloga Mariña. Sentíame eu coma unha intrépida aventureira...hasta que á altura das Cíes tiven que saír a cuberta....Sí, o mareo duróume seis días! Foi o primeiro de moitos embarques nos que me curtín como bióloga, como mariñeira e máis como persoa. Os primeiros ós que tenho que da-lo meu agradecemento é á xente do mar, non só po-la sua axuda mentres estiven alá con eles (facíanme o traballo mais doado, eran eles os que me levantaban a moral cando a tiña polo chán, e non vos podeades imaxinar o que eso se agradece!), senon porque son a base deste traballo: Se os pesqueiros non saíran a po-la gallineta (ou cabra como eles lle chaman tamén), este traballo non tería sentido.

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1. INTRODUCTION

The Genus *Sebastes* in the North Atlantic is represented by four species, *S. marinus* (Linnaeus, 1758), *S. mentella* Travin, 1951, *S. fasciatus* Storer, 1856 and *S. viviparus* Krøyer, 1845 known under the general name of 'redfish'.

Historically, redfish species structure has been poorly understood, partially because of the strong resemblance among the species. Until the mid-20th century, only *S. marinus* and *S. viviparus* were accepted as valid species, while *S. mentella* was considered as a sub-species of *S. marinus*. In 1951 Travin described *S. mentella* as a new species, and only by the end of the 1970s *S. fasciatus* was accepted as a species, in spite of the fact that it had been described by Storer 150 years earlier. This fact has seriously conditioned the study of these species, even nowadays.

One of the consequences of the high external similarity between species is that the catches are often not split into different species, using in such cases the term 'redfish' for the catch. Another common denomination is 'beaked redfish', that is used in reference to *S. fasciatus* and *S. mentella* when they are captured together and specific identification is not made. The term 'ocean perch' is also used to refer to the whole genus.

United States and Canada, in the Northwest, Iceland and USSR in the Northeast developed redfish fisheries, as others, first in local waters and gradually moving out further and further as the closer fishing grounds became less profitable and as gear and techniques improved. The vessels of ex Soviet Union and Germany have fished redfish from the northern coast of Russia itself to certain isolated fishing grounds off the coast of Canada (Kelly and Wolf, 1959). United States, Iceland, Germany and the ex Soviet Union supported an economically important fishery since the late 1930's. It was at the end of the second world war and with the establishment of the freezing industry, when the redfish fishery started to have commercial importance. The fishery continued growing year by year, and the fishing effort reached its highest values in the sixties, when catches were about 600,000 Tm per year (Figure 1.1).

The discovery in 1981 by a Russian fleet of commercial aggregations of *S. mentella* in the open Irminger Sea, resulted in the organization of a large scale directed international fishery in the area. The catch of the ex- Soviet Union fleet was very dominant until 1989 although several East European countries (Poland, Bulgaria, GDR) took a considerable part of the total catch. The Faroe Islands joined in 1986, Iceland in 1989 and Norway in 1993. In the past, the Spanish fleet never targeted redfish. However, with the stock depletion of the traditional target species, such as cod or American plaice in the Northwest Atlantic (Figure 1.1), the Spanish fleet joined the pelagic fishery in the Irminger Sea in 1995. This fishery operates normally from March to October and from 200 to 950 meters depth, using more and more developed fishing technology. Redfish in the Irminger Sea, together with Icelandic waters support an intensive fishing effort constituting around 70% of the total catches in the North Atlantic.

Redfish are viviparous and slow growing with protracted life spans (more than 50 years). They are fairly abundant and distributed throughout the whole North Atlantic. However, many aspects of their biology and ecology are poorly studied, mainly because of the lack of a

correct species identification and a proper definition of the stock structure that prevents the correct definition of many of the population and biological parameters. In spite of the importance of these resources, basic questions regarding the integrity of the species and the delimitation of the stock units in the North Atlantic remain unresolved.

There are a number of studies where the species relationships have been studied by different disciplines such as systematics and taxonomy (Barsukov, 1968, 1972; Barsukov and Litvinenko, 1973; Litvinenko, 1974a; Moser *et al.*, 1977; Litvinenko, 1982; Barsukov *et al.*, 1984; Hureau and Litvinenko, 1984) or from morphological, biological or ecological viewpoints (Barsukov and Zakharov, 1972; Litvinenko, 1974b; Litvinenko 1980a, 1980b, 1981; Litvinenko and Tuponogov, 1981; Ni, 1981a, 1981b, 1981c, 1981d, 1982, 1984; Power and Ni, 1982; Litvinenko and Popova, 1983; Barsukov *et al.*, 1991). On the other hand, Ni, (1981e) Misra and Ni, (1983) and Kenchington (1986) were pioneers of the application of multivariate techniques to morphometric and meristic characters for redfish species identification, and Naevdal (1978), Payne and Ni (1982) and McClade *et al.* (1983) used electrophoretic methods for redfish species identification, and the results of these genetic studies led to hypotheses about the existence of hybridization among species in certain areas.

In 1992 Nedreaas *et al.* pointed out that the genetic structure of *Sebastes* populations in the Atlantic is more complicated than previously realized. Redfish from the Faroe Islands seem to be more closely related to Norwegian than to Icelandic redfish (Nedreaas and Naevdal, 1991; Reinert *et al.*, 1992). For some authors, redfish from Norway, the Faroe Islands and Iceland form distinct populations from redfish in Greenland (Nedreaas *et al.*, 1992). Morphometric studies (Reinert and Lastein, 1992) revealed the existence of three different populations for both *S. marinus* and *S. mentella* in Norway, the Faroe Islands and the Irminger Sea. A more complete morphometric study of redfish populations on both sides of the North Atlantic was carried out by Saborido-Rey (1994), who concluded that redfish from Norway, Svalbard, Flemish Cap, Grand Bank, and Saint Pierre (Canadian coast) constitute independent populations.

The identification and delimitation of the self-sustaining stock units is the prerequisite and basis for any management actions in accordance with the FAO 'Code of Conduct for Responsible Fisheries' (Food and Agriculture Organisation) and the UN Agreement on 'Straddling and Highly Migratory Fish Stocks'. However in many areas, and perhaps even in the whole North Atlantic, redfish stock structure is still under debate and controversial. This is particularly true in the Irminger Sea and adjacent waters, the areas where the redfish catches are higher today, as there is only preliminary information available about the species and population structure for *Sebastes marinus* and *S. mentella* inhabiting the shelves and continental slopes off Greenland, Iceland, the Faroe Islands and even the pelagic water column above 2000 m depth in the Irminger Sea. For larvae and juveniles, even the discrimination of species is doubtful. Preliminary information suggests the existence of various distinct gene pools but is based on limited material in terms of temporal coverage as well as the size groups investigated. The risk of overexploitation of some of the redfish stock components has lead the research community to deeper study of these features.

As a consequence, the European Union has financed the project 'Population structure, reproductive strategies and demography of redfish (Genus *Sebastes*) in the Irminger Sea and adjacent waters (ICES V, XII and XIV; NAFO 1)', a four-year research project funded within the European Commission's 5th framework programme (1998-2002), contract QLK5-CT1999-01222. This project is known by the acronym of REDFISH, and treated the main tasks from a multidisciplinary point of view. Thus, genetics, otolith shape and chemical composition, reproductive strategies, demography, meristics and morphometrics of redfish were used in the project.

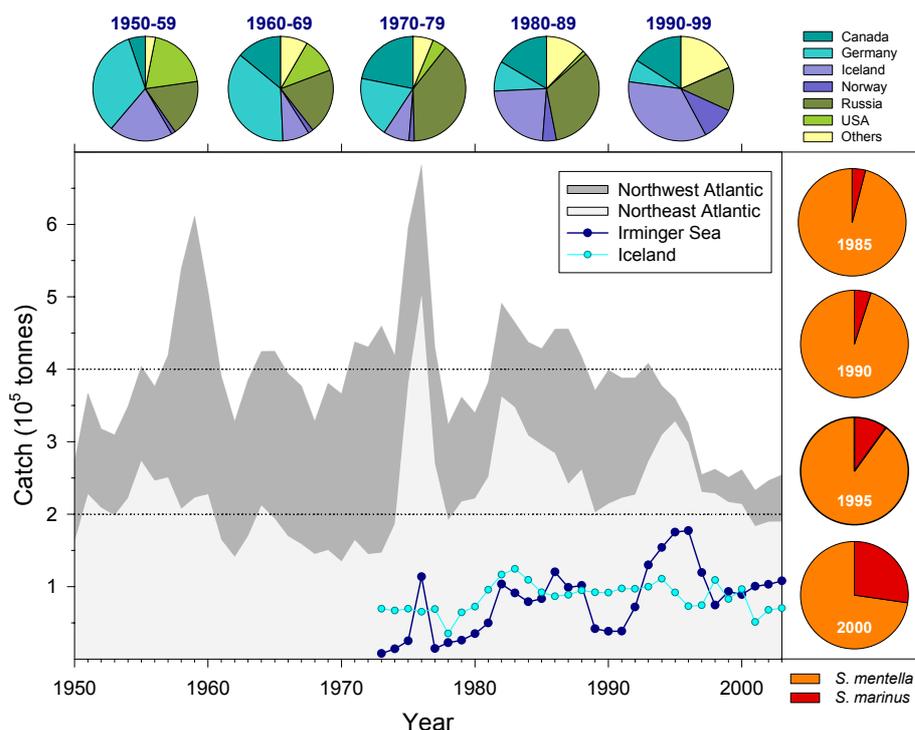


Figure 1.1. Historical redfish catches from 1950 to 2003. Central panel: landings in the two main Atlantic areas and in the Irminger Sea and Icelandic waters. Upper panel: Proportion of landings by country summarized by decade. Right panel: species composition of the catches for four selected years. Russia includes former USSR and Germany both FDR and GDR. Source: FAO, NAFO and ICES official statistics.

This PhD thesis is a part of this multidisciplinary project, centered on species and population identification, using meristic and morphometric techniques. A big part of the work was methodological, and, as a result, two protocols (Annex I and II in the present work) were developed. Two different morphometric techniques were applied, the 'traditional morphometrics' that uses interlandmark distances, and the most recently developed 'geometric morphometrics' that uses landmark coordinates as input in the analysis. While the statistical methods used in traditional morphometrics are more developed, geometric morphometrics captures better the shape differences and allow one to produce graphic displays. Both morphometric and meristic techniques were applied to the same individuals. Part of those individuals were also genetically analyzed, which allows a comparison of the results for these different techniques.

1.1. MORPHOLOGY

Sebastes are included in the Order Scorpaeniformes, Family Scorpaenidae. The order includes 25 families with 266 genera and about 1271 species, although debate continues on the systematics of the genus *Sebastes* itself (see 1.3 below). This order is defined by the presence of a suborbital stay, a posterior extension of the third infraorbital bone (counting the lacrimal), which extends across the cheek to the preoperculum and is usually firmly attached to that bone. Head and body tend to be spiny or have bony plates; pectoral fin usually rounded, membranes between lower rays often incised; caudal fin usually rounded (occasionally truncate, rarely forked).

1.1.1. Family Scorpaenidae (in the Suborder Scorpaenoidei)

The family Scorpaenidae is included in the Suborder Scorpaenoidei, which contains the world's most venomous fishes, usually brightly colored.

This family is comprised of moderately compressed to robust fishes, with large spiny heads. The mouth is moderate to large, terminal, oblique and protractile; the teeth are usually villiform (small canine teeth present in some species), and arranged in bands on the upper jaw, lower jaw, and on the vomer (sometimes on palatines); the eyes are moderate to large; very characteristic is the presence of a ridge of bone (sub-orbital stay) below the eye; the preopercular margin presents 3 to 5 spines; the opercle with 2 divergent spines or a single spine; other spines scattered on head. The members of the family present a single dorsal fin, usually notched at the posterior end of the spinous part, with 11-16 spines and 4-17 rays; The anal fin has 3 spines and 5-14 rays; the pectoral fin is broad-based, large and fan-like; the pelvic fins are thoracic in position with one spine and 3-5 rays; the caudal fin is rounded or emarginate. The body is covered with cycloid, ctenoid or rudimentary scales. The lateral line is always present, although sometimes incomplete or represented only as a scaleless groove. Fleshy skin flaps, cirri, tentacles, tabs present on head and body of many species. The gillrakers are usually short and tubercular in form.

1.1.2. Genus *Sebastes* Cuvier, 1829 (in the Subfamily Sebastinae)

The subfamily Sebastinae contains four genera, *Helicolenus*, *Sebastes* (= *Sebastodes*), *Sebasticus* and *Hozukius*, with about 128 species. The former two genera occur in all oceans, whereas the latter two occur only in the western Pacific. The live-bearing genus *Sebastes* is the largest in the family with about 110 species (almost all of them occurring in the North Pacific). Members of *Sebastes* present the maximum number of dorsal fin spines and anal soft rays, which separate this genus from the others in the family.

The head is large; The preorbital bone presents 1 or 2 spinous points over the maxilla; the sub-orbital ridge has no spine and is generally weakly marked; the supplemental preopercular spine is absent; all the 5 preopercular spines about equal in length; the upper post-temporal spine is present but the lower one is small or absent; the supracleithral spine

is also present; 2 opercular spines; other spines present include the nalsa, pre-, supra- and post-ocular and parietal. Symphysis of lower jaw more or less developed as a rounded protuberance or sharply projected. Occipital pit absent. Dorsal fin with 14-16 strong spines and 12-17 rays; anal fin with 3 spines and 6 or more rays; pectoral fin with 17-21 rays. Scales ctnoid; head, cheek and snout scaly. No flap or tab or tentacle on head and body.

1.1.3. *Sebastes* Species in the North Atlantic

The four species present in the North Atlantic are very similar in appearance and are difficult to distinguish from one another, even for experts. Only pre-adult and adult *S. viviparus* can be clearly distinguished from the other species (Figure 1.2).

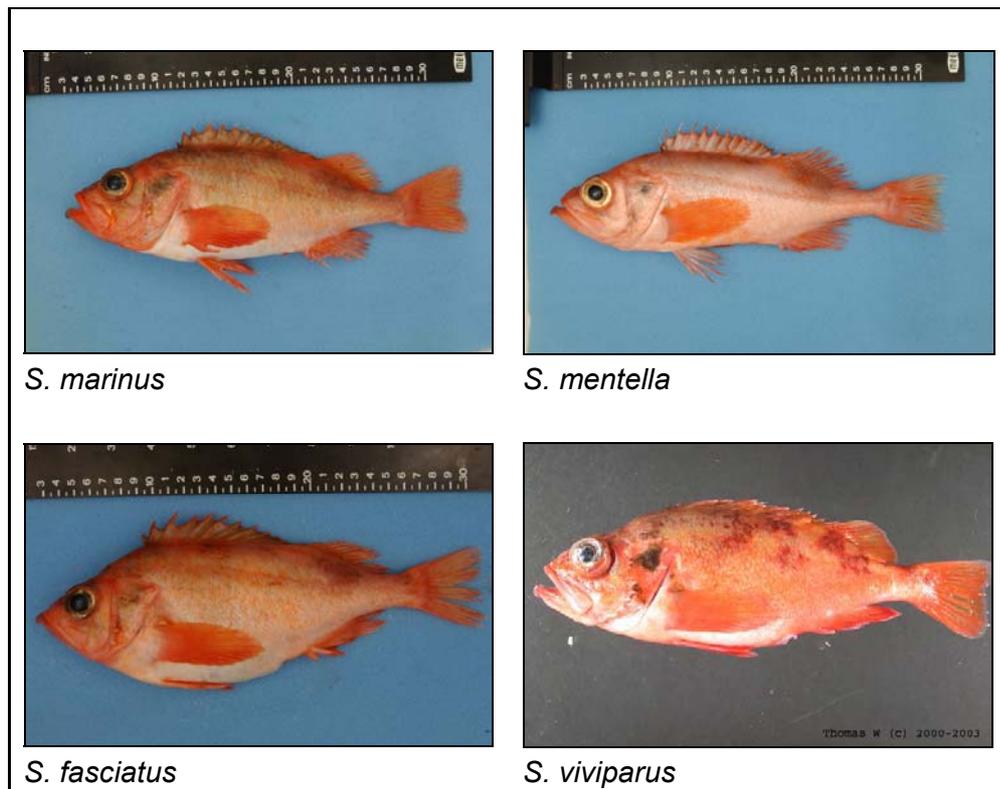


Figure 1.2. *Sebastes* species in the North Atlantic. *S. marinus*, *S. mentella* and *S. fasciatus* pictures were taken on Flemish Cap (Northwest Atlantic) by the author on those individuals that best represented the morphology of each of the species. *S. viviparus* picture was taken by Thomas W. (Institute of Marine Research of Bergen).

Several characters have been used for species discrimination, but most of them are subjective or present some exceptions. The symphyseal tubercle is well developed and sharp in *S. mentella* and *S. fasciatus* while poorly developed and blunt in *S. marinus* and *S. viviparus* (Barsukov *et al.*, 1992; Ni, 1984). There is at least one exception to that general character, and it is in some *S. marinus* from the North East Atlantic (Faroe Islands and Norway, basically), that present a well developed and sharp symphyseal tubercle, instead of the *S. marinus* 's type that is blunt.

The eye diameter is larger in *S. fasciatus*, *S. viviparus* and *S. mentella* than in *S. marinus* (Barsukov *et al.*, 1992; Power and Ni, 1985). The angle of the 3rd and 5th preopercular

spines in relation with the longitudinal axis of the body have been used traditionally to differentiate *Sebastes* species inhabiting several areas (Barsukov *et al.*, 1992). The body depth at the pectoral fin is narrower in *S. mentella*, *S. fasciatus* and *S. viviparus* than in *S. marinus* (Power and Ni, 1985).

Ni (1984) and Kenchington (1986) described a slight difference in the number of soft rays in the anal fin between *S. marinus* and *S. mentella* that present 8 or more and *S. fasciatus* and *S. viviparus* with 7 or less. However, this difference in the number of rays has not been observed in all areas (Saborido-Rey, 1994), and an important overlap in this feature among species has been recorded.

S. marinus coloration is orange-yellow or golden-yellow. *S. mentella* is bright red with soft blackish blotches near both the caudal peduncle and the operculum. *S. fasciatus* is overall orange-red with green-black blotches on the body near and below the dorsal fin and on the posterior part of the gill cover. *S. viviparus* is light red with three well-defined brown-black blotches in the dorsal area. However, color is one of the most plastic characters, and it can change in different geographic areas, with depth, and also with the food composition.

The gas bladder musculature pattern (Matsubara, 1943; Hallacher, 1974; Power and Ni, 1982; Litvinenko, 1981) has been traditionally and successfully used for species discrimination in most of the studied areas. It is a very reliable character, and species identification using the gas bladder musculature has normally yielded a high proportion of accuracy. However, the temporal stability and the suitability of this character in all areas has not been carefully studied. As shown later in this work, this character does not allow one to distinguish between the redfish species present in Greenland. However, it is the most successful of all the species identification characters, and has been used in the present study to identify species. Therefore, a detailed description of its anatomy is given in the next section.

1.1.3.1. Gas Bladder musculature

The 'gas bladder musculature' (GBM) is the common name used to define the set of muscles connecting the neurocranium with the gas bladder and with the vertebrae and ribs in the area close to the gas bladder.

The first major work that described these gasbladder muscles for a variety of scorpaenid fishes was that of Matsubara (1943) who showed that intrageneric variations in the structure of these muscles exist in Oriental Pacific Scorpaenids. Hallacher (1974) studied the GBM in the *Sebastes* genus, including principally the Pacific species, but also *S. fasciatus* and *S. viviparus* which he erroneously considered to be *S. marinus*. Litvinenko (1981) described this character in the four *Sebastes* species in the North Atlantic, confirming its specificity. Subsequently, the suitability of this character for *Sebastes* species identification in the North Atlantic has been confirmed (Ni, 1981a; Saborido-Rey, 1994).

The general pattern of the gas bladder musculature is as follows:

S. marinus muscle is thicker, and it is constituted by three or four muscular heads (Litvinenko, 1981; Power and Ni, 1982). This multi-head muscle generates the possibility of several patterns. The most common pattern consists of the presence of three muscular heads, the dorsal one directly attached to the second rib, the central going through ribs 2 and 3 and the ventral one crossing between the 3rd and 4th ribs. There are normally six tendons attached to the 5th to the 9th vertebrae, or to the corresponding ribs. (Figure 1.3).

S. mentella typically presents a single, thin and short muscle, that passes between the 2nd and 3rd ribs (Litvinenko, 1981; Ni, 1981a), and ends in a single tendon attached to the 7th vertebra. Sometimes, this tendon is divided in two, and one part is attached to the 6th vertebra. If instead of one single muscle, there are two muscular heads, one of the tendons goes between the 2nd and 3rd ribs, and the other between the 3rd and the 4th, ending attached to the 6th vertebra (Figure 1.3).

S. viviparus and *S. fasciatus* typical pattern consists of a unique muscular head crossing between the third and fourth ribs (Litvinenko, 1981), and finishing in three tendons that are attached to the 9th to 11th vertebrae. *S. fasciatus* gas bladder musculature is very similar to *S. viviparus*. Fortunately, the two species do not share distribution areas, as *S. fasciatus* is restricted to the Western North Atlantic while *S. viviparus* is found in the East (Figure 1.3).

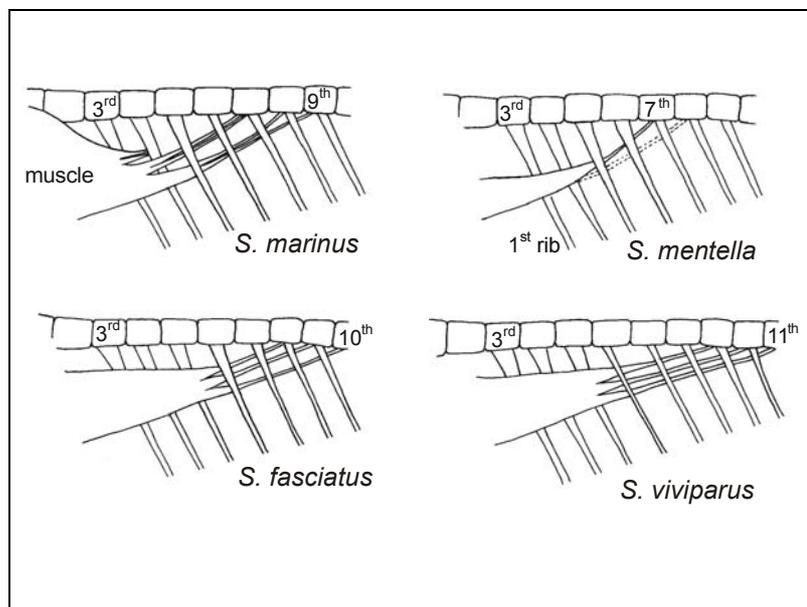


Figure 1.3. Gas bladder musculature pattern for the four *Sebastes* species in the North Atlantic. The muscle of the gas bladder can be constituted by one or more muscular heads that finish in a tendon. The tendons cross between different ribs and are attached to different vertebrae in each of the species. The numbers represent the vertebrae. Vertebrae 1 and 2 do not hold ribs.

1.2. BIOLOGY

1.2.1. Reproduction

Transitional states from oviparity to viviparity are evident in different species within the family Scorpaenidae. Viviparity is confined to the subfamily Sebastinae; gestation is luminal and the embryos usually develop to term within the egg envelop. All species of the genus *Sebastes* are viviparous, although in a primitive, unspecialized state. The embryos, which develop within egg envelopes during most of gestation, hatch several days prior to parturition (Wourms, 1991) or right after or before parturition (Saborido-Rey, 1994).

The genus *Sebastes* has historically been considered to be ovoviviparous, with all energy from embryonic development coming from the yolk present at fertilization, and with no additional nutrition provided to the embryo during gestation (Amoroso, 1960). However, studies of the maternal-fetal relationships (Wourms, 1991) indicate that ovoviviparity must be considered as an incorrect and obsolete term, and viviparity should be used instead. Thus, there are two major types of viviparity: lecithotrophic and matrotrophic viviparity. In the former, the energy for embryonic development comes exclusively from the stored yolk (lecitho) while in the latter all or part of the energy is provided directly by the mother during embryonic development. Although studies have shown that embryos of some *Sebastes* species receive some form of nutrition during later stages of gestation and are thus matrotrophic viviparous (Boehlert and Yoklavich, 1984; Boehlert et al, 1986), it is generally accepted that most species are lecithotrophic viviparous. There is no evidence of embryonic nutrition for *Sebastes* species in the North-Atlantic (Saborido-Rey, 1994).

In both types of viviparity, fertilization is internal and egg and embryo development proceeds within the female reproductive system. The evolution of these reproductive modes involved a compromise between high reproductive rates with low survival and low reproductive rates with high survival. In *Sebastes*, however, fecundity approaches that of the most highly fecund oviparous species, with individual fecundity to 2,300,000 in *S. paucispinis* (Phillips, 1964), although the Atlantic species shown lower fecundity, up to 350,000 eggs in 55 cm *S. marinus* (Saborido-Rey *et al.*, 2004a). Eggs sizes range from about 0.7 to 1.5 mm, the gestation period is approximately 1-2 months long, and larval size at birth is relatively small, ranging from 4 to 9 mm. The larvae are relatively well developed, however, and are generally born at a developmental stage with organogenesis complete, jaws developed and the ability to initiate feeding.

There is no sexual dimorphism in any of the four species in the Atlantic, except for the presence of a urogenital papilla or penis in males.

The reproductive cycle for *Sebastes* species in the North Atlantic is annual. Copulation takes place in late-fall to early-winter. There are no data about the mating behaviour of the North Atlantic species. However, an elaborate courtship and copulation behaviour has been described for *Sebastes inermis* in the Pacific by Shinomiya and Ezaki (1991). Males

performed courtship behaviour when females approached the territories. Principal motor patterns of courtship were characterized by 'lateral display' and 'rushing and turning' movements. The mating pair formed about 30 min before copulation. As the mating pair ascended 2.0 m above the bottom, the male suddenly coiled around the female's body to copulate. The spermatozoa are stored in the ovary in a dormant condition until the eggs become available. Spermatozoa can be stored in the ovary for up to 6 months, as seems to happen in *S. marinus*, in which copulation time started in October and fertilization occurs in March (Magnússon, 1955); however, spermatozooids have not yet been observed within the ovary and this aspect remains unknown. Once fertilized, egg and larval development takes around one month. The larvae are released in the period between late-winter to early-summer.

The ovarian cycle for redfish is as follows: During late summer-early autumn, the oocytes start ripening, entering the cortical alveoli stage. Macroscopically, the oocytes become clear again. Vitellogenesis lasts for several months until fertilization occurs. The ovary reaches its largest expansion and fills almost the whole abdominal cavity at that moment. Ovulation and fertilization occur at different moments depending on the species, but basically one month before parturition which occurs at different moments depending on the species and/or stocks (Magnússon, 1955; Saborido-Rey, 1994). The timing of spawning of a given species should reflect an adaptive strategy to match spawning with the optimal phase of the annual planktonic production cycle, to maximize the survival of its progeny (Cushing, 1975). However, reproductive strategies are also determined by other specific factors, such as to avoid interspecific competition in areas where more than one species lives. Thus, although parturition periods of redfish may overlap, peaks of larval extrusion can be observed in different months for the different species inhabiting the same area. Thus, on Flemish Cap *S. mentella* spawns in Feb-Mar, *S. marinus* in Apr-May and *S. fasciatus* in May-Jun (Saborido-Rey, 1994). In the Irminger Sea, although there is one main spawning area over Reijkjanes Ridge for both species inhabiting the area, *S. marinus* hatch their larvae closer to the western slopes of Iceland and much later than *S. mentella* (Saborido-Rey *et al.*, 2004a).

There are both shows interspecific and intraspecific differences in size at maturity, dependent on environmental conditions (Ni and Sandeman, 1984). Generally, *S. marinus* is larger at maturity, and *S. fasciatus* smaller (Figure 1.4), as shown on Flemish Cap (26 cm in *S. fasciatus*; 30 cm in *S. mentella* and 34 cm in *S. marinus*). Males and females showed general differences of around 10 cm, with females larger at maturity; between areas, the differences are around 2 cm (Ni and Sandeman, 1984). The size at maturity might be an indicator for species differentiation and stock discrimination, particularly for sibling redfish species and/or stocks (Ni and Sandeman, 1984).

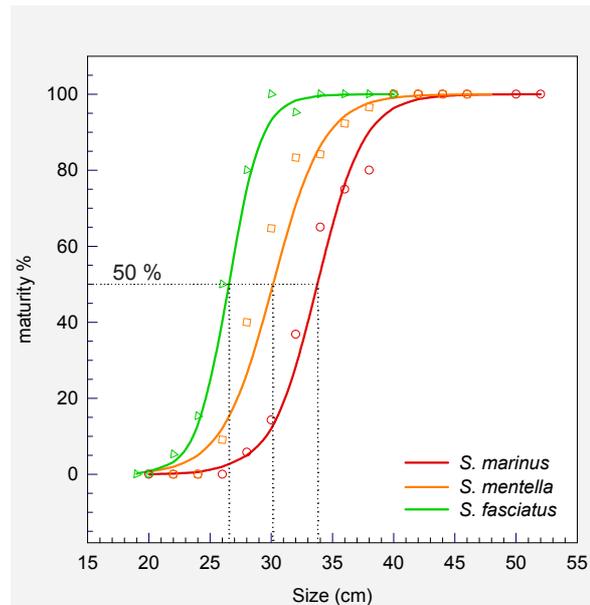


Figure 1.4. Size at maturity for *S. marinus*, *S. mentella* and *S. fasciatus* from Flemish Cap in 1994.

S. mentella in the Irminger Sea involves only mature individuals, that reach maturity at a length of 32 (males) and 33 centimetres (females) at an age of 13 and 14 years, respectively (Rikhter, 1996). According to Paulov *et al.*, 1989, the annual cycle in the Irminger Sea includes wintering (December to March), extrusion of larvae (April-May), feeding (June-August) and copulation (September-November). The extrusion of larvae takes place over Reykjanes Ridge, and the absolute fecundity of *S. mentella* females in the area varied from 4.9 to 70.4 thousands, being on average 35.8 thousands (Paulov *et al.*, 1989). A full description of the life cycle of redfish in the Irminger Sea and adjacent waters is presented in Chapter 2.

1.2.2. Age and Growth

Sebastes species grows very slowly and are long lived. Longevity of *S. aleutianus* has been estimated at around one hundred years (Archibald *et al.*, 1981). In the North Atlantic 60 year old individuals have been recorded, but following the strong fishing effort after the second world war, it is nowadays difficult to find individuals more than 30 years old.

Amongst the four species of *Sebastes* in the North Atlantic, *S. viviparus* is the smallest, reaching only 30 cm of maximum standard length; *S. mentella* and *S. fasciatus* reach 50 cm, and *S. marinus* is the largest, reaching 70 cm.

Age determination is one of the most important yet unresolved questions in research on redfish in the North Atlantic. Controversy rages around the most appropriate means of age determination (Nedreaas, 1990), and several attempts have been made to create common criteria (ICES, 1983a, 1984, 1991, 1996a). Although such criteria have not yet been established, agreement has been reached on the use exclusively of otoliths for redfish age determination. Many different methodologies have been used. For instance, Russian scientists have read scales under ordinary light (ICES, 1991); German, Danish, and Icelandic

scientists have read scales under polarized light after treatment with silver nitrate (Kosswig, 1980); North Americans have used the broken and burnt otolith technique, also used by Norwegian scientists (Nedreaas, 1990). In contrast, some Spanish scientists use otoliths, but with a slightly different technique (Saborido-Rey, 1993). Norwegians, and other Spanish scientists have used scales routinely in past years, but it is known that, after a certain age, there is little or no scale growth and, therefore, the age of older fish tends to be underestimated when scales are used (ICES, 1996). However, redfish longevity makes it difficult to interpret the growth structures, and different readers often produce different age readings, as is the case in *S. mentella* and *S. marinus* (ICES, 1996 a ; Stransky *et al.*, 2005). Thus, age validation for this commercially important species is essential to prove that interpretations of age are accurate.

Common validation techniques include direct methods such as tag/recapture or the use of known-age fish, and indirect techniques such as back-calculation, marginal increment analysis, edge progression analysis, frequency year-class progression analysis, radiometric isotope analysis and elemental analysis. Direct methods are difficult to implement in *Sebastes* owing to the low rate of survival when fish are caught. Frequency year-class progression analysis has been successfully used in North Atlantic *Sebastes*: Svalbard *S. mentella* (Nedreaas, 1990), Gulf of Maine *S. fasciatus* (then known as *S. marinus* Mayo *et al.*, 1981) and Flemish Cap *S. mentella* (Saborido-Rey *et al.*, 2004b). Radiometric studies have recently been developed for age validation in *S. mentella* and *S. marinus* from Iceland, Greenland and the Irminger Sea (Stransky *et al.*, 2004).

The growth rates of male and female redfish follow similar profiles in some areas of the North Atlantic, but females live longer (Sandeman, 1961; Surkova, 1961). However, differences in growth rate by sex were found (Figure 1.5) for the three species of redfish present on Flemish Cap (Saborido-Rey *et al.*, 2004). Differences among species have been also observed (Figure 1.5).

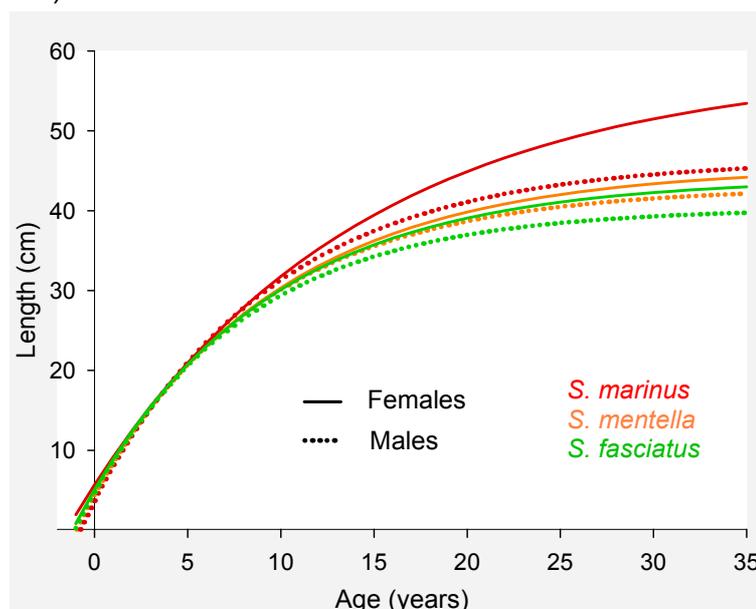


Figure 1.5. Von Bertalanffy growth curves for males and females of the three redfish species on the Flemish Cap from research vessel bottom trawl surveys carried out during summer 1990–2000 for *S. marinus* and 1991–2000 for *S. mentella* and *S. fasciatus*. Source: Saborido-Rey *et al.* (2004)

1.2.3. Other biological aspects

1.2.3.1. Food and feeding

Quantitative analysis of food and feeding in redfish is difficult due to the high percentage of everted stomach in the catches, due to the change in pressure experienced when redfish are brought from deep waters to the surface.

The first studies of redfish feeding in the Northwestern Atlantic conducted at the level of genus *Sebastes* concluded that redfish diets are very variable; planktonic invertebrates, i.e. copepods, hyperiids, euphausiids and shrimps are the main food items, and pelagic fish species are eaten to a lesser degree.

Studies on redfish larval feeding in the Gulf of Saint Lawrence show a strong link between variability in *Calanus* production and variability of the growth and survival of redfish larvae (Runge and Lafontaine, 1996). This link was also observed in the Irminger Sea (Bainbridge, 1965; Magnússon *et al.*, 1965). According to Runge and Lafontaine (1996), this may reflect the ability of redfish adults to extrude their young in potential areas of high prey abundance or, alternatively, the survival of the larvae may be related to the numbers of *Calanus* females present in the immediate area, because the females are the source of their preferred prey (Bainbridge, 1965). This latter hypothesis is supported by Anderson's (1994) results, in which he found that redfish larvae ate less and metamorphosed at larger size in those years in which *Calanus* eggs and nauplii were rarely observed in their diet.

Konchina (1983) studied *S. mentella* and *S. fasciatus* feeding characteristics in the Northwest Atlantic. She found that both species show a high degree of plasticity in food composition by season and by age; thus, prey are larger and more mobile as redfish get older. She also observed differences in prey composition by species, in young and adult individuals. Young *S. fasciatus* fed mainly on euphausiids (and with less intensity on copepods), while young *S. mentella* feeds on a wider spectrum of prey, including not only euphausiids but also on other small crustaceans, squids, hyperiids and fish, representatives of the mesopelagic complex. The adults of both species shows, like young fish, a high plasticity in their feeding, including besides the planktonic crustaceans and fish, jellyfish, *Actinaria*, *Ctenophora*, *Gastropoda*, *Bivalvia*, *Octopoda* and *Polychaetes*. *S. mentella* adults feed on fish-prey representatives of the neritic ichthyocene (capelin and sand lance) and of the mesopelagic one (*Paralepididae*, *Myctophidae*, *Stomiatidae*, etc.), as well as on young specimens of commercial fish species like cod, redfish or grenadier. *S. fasciatus* has a narrower spectrum, and members of the benthos are absent from its diet.

Differences in feeding intensity occur during the year (Janulov, 1963; Konchina, 1983); thus, young redfish feed more intensively in late spring and summer than the rest of the year, and adults show a minimum in feeding intensity during spawning time, after which the females start immediately to feed intensively, while males start later, apparently due to their sexual activity during the period of mating (Janulov, 1962), and reach the highest rates during the autumn-winter period (Atkinson, 1986).

The main trophic connections can be apparently explained by the availability of food items at the horizon of redfish hunting. Actually, in East Greenland, Magnússon and Pálsson (1988) found 0-group redfish to be an important part of the redfish diet when those 0-groups are especially abundant and thus easily available as food.

In West Greenland shrimp grounds, the most important prey items of small redfish are planktonic crustaceans (copepods, mysids, hyperiids and euphausiids, that become less important with increasing predator size, while euphausiids and shrimps become more important (Pedersen and Riget, 1991).

According to Pálsson, (1983), zooplanktonic prey (euphausiids and copepods, especially *Calanus sp.*) are the dominant prey of redfish in Icelandic waters and redfish should therefore be classified as 'pelagic' rather than 'demersal' as usually done.

Daily vertical migrations have been reported by several authors (Templeman, 1959, Janulov, 1963; Konchina, 1983). According to Konchina, 1983, *S. fasciatus* and *S. mentella* migrates to the upper layers in the evening and feed on the concentrations of numerous crustaceans species or fish of the mesopelagic complex.

In the Irminger Sea, *S. mentella* feeds mostly on zooplankton (*Calanus*, euphausiids) and squids, but shrimp and fish increase in importance with depth (Melkinov, 1998). There are, however, important differences between years regarding the frequency of the various dietary components (Magnússon and Magnússon, 1995). In areas where the commercial fleet is fishing, a high presence of offal resulting from fishing activity has been reported in the diet (Gonzalez *et al.*, 2000).

1.2.3.2. Parasites and abnormalities in the pigmentation

The species and abundance of parasites may differ between fish stocks due to biogeography, differential environmental tolerances of parasites, differences in availability of intermediate hosts, and different life history characteristics of the fish stocks themselves (Begg and Waldman, 1999).

Bakay (1988), demonstrated the isolation of *S. mentella* from the Flemish Cap and Irminger Sea areas, and a high uniformity between North-east, Central and Southern areas of the Irminger Sea, with a study of 10 parasitic species. On the other hand, larvae of anisakid nematodes and the copepod ectoparasite *Sphirion lumpi* were used to discriminate among *S. mentella* stocks in different areas of the Northwest Atlantic (Marcogliese *et al.*, 2003). *S. lumpi* is one of the most widespread redfish parasites, and occurs with varying abundance in the different areas. In addition, although its life span is limited, the cephalothorax and necrotic tissue from previous infections persist for many years (Bakay, 1988). These characteristics united to its macroscopical nature, make it widely used in studies of the population structure of its hosts. *S. lumpi* has preference for *S. mentella*, occurring over the entire area of distribution of mature *S. mentella*, i. e. from Canada to the Barents Sea, infestation being highest in the Irminger Sea and Labrador Sea areas (Bakay and Karasev, 2001). The average infestation rate in the Irminger sea, is slightly superior for females than for males, and varies by month and area (Rikhter, 1996).

Pigmented (reddish-orange and black) patches in the body are also used for *Sebastes* studies in the North Atlantic (Bakay and Karasev, 2001).

1.2.3.3. Migration

Redfish exhibit strong migrations between areas in the North Atlantic, related to oceanographic conditions, feeding and breeding places, and are perhaps more extensive in *S. mentella*, because of its pelagic behaviour and wide distribution.

Population studies on *S. marinus* and *S. fasciatus* in the Gulf of Maine-Georges Bank region have shown that there is probably a southerly migration of adults from the Baffin Island region to areas southwest of Newfoundland (Mayo *et al.*, 1980 and 1993). In the Northeast Atlantic, *S. mentella* seasonal migration patterns for spawning were described (Sorokin, 1961; Saborido-Rey and Nedreaas, 2000). Traditionally, *S. mentella* on the shelves and banks around Faroe Islands, Iceland and East Greenland are treated as one stock unit, with a common area of larval extrusion to the SW of Iceland, a drift of the pelagic fry towards the nursery areas in relatively shallow waters of East Greenland, and feeding and copulation areas on the shelves and banks around the Faroe Islands, Iceland and East Greenland. This implies extensive migrations of the mature fish (mainly females) between the feeding and the spawning areas and of the immature fish between nursery and feeding areas (ICES, 1998). In recent years, a migration of *S. mentella* juveniles was reported by Stransky (2000) from the East Greenland shelf into the central Irminger Sea. In early spring, concentrations of spawning *S. mentella* females are observed over the Reykjanes Ridge area, where the larvae are released. Part of those females are thought to migrate from the slopes of Iceland, where they return in August-September (Kothaus, 1965; Rikhter, 1996), and another part migrates from the open Irminger Sea. When the spawning has been completed, the oceanic redfish come back to the feeding areas, the feeding aggregations of these mature redfish being dependent on the dynamics and structure of the waters of the Subpolar Gyre (ICES, 2003). A detailed description of the migration pattern in this area can be found in Chapter 2. Diel migrations were observed in beaked redfish in the Northwest Atlantic (Atkinson, 1989; Gauthier and Rose, 2002), moving off the bottom at night in pursuit of their prey

1.3. SYSTEMATICS

Historically the systematics of the genus *Sebastes* was marked by strong debate over relationships among the species. There was also considerable disagreement among ichthyologists of the late 1800's concerning the proliferation of subgenera without any basis. In the early 1900's, most of the research used only two generic designations: *Sebastes* for Atlantic species and *Sebastodes* for Pacific species, disregarding the subgenera (Kendall, 2000). Matsubara (1943) joined these two genera in one, as he concluded that there was no validity in grouping the Atlantic species separately from the Pacific species, and kept the name of '*Sebastes*'. The classification of the Genus is shown in Table 1.1.

The first two genera, *Helicolenus* and *Sebastes*, occurs in all oceans, whereas *Sebasticus* and *Hozukius* occur only in the western Pacific. The live-bearing genus *Sebastes* is the

largest in the family with about 110 species, almost all of them occurring in the North Pacific (Nelson, 1994).

Table 1.1. Classification of the genus *Sebastes* (Nelson, 1994).

Classification of the genus <i>Sebastes</i> (Nelson, 1994)
Order SCORPAENIFORMES
Suborder Scorpaenoidei
Family SCORPAENIDAE
SUBFAMILY SEBASTINAE (4 genera)
Genus <i>Helicolenus</i>
Genus <i>Sebastes</i> (= <i>Sebastodes</i>)
Genus <i>Sebasticus</i>
Genus <i>Hozukius</i>

The uncertainty on this classification begins at the ordinal level, as it is not clear if *Sebastes* must be included within the Scorpaeniformes or the Perciformes. *Sebastes* and basal perciforms share many characteristics. The hallmark of the scorpaeniforms, the suborbital stay is much reduced in *Sebastes*, and except for the suborbital stay, *Sebastes* is more similar to basal perciforms than are other scorpaeniforms. In other ways, *Sebastes* is the least specialized of the scorpaeniforms: e. g. head spination is minimal, squamation is normal. It remains uncertain if this is due to convergence or a common ancestor. Nowadays *Sebastes* systematics remains in a confused state, with over 100 species, and very little obvious structure within the genus (Kendall, 2000).

1.3.1. Systematics of the genus *Sebastes* in the North Atlantic

In the North Atlantic the Genus *Sebastes* is represented by 4 species (Table 1.2), *S. marinus* (Linneo, 1758), *S. mentella* Travin, 1951, *S. fasciatus* Storer, 1856 and *S. viviparus* Krøyer, 1845. The first two species are widely distributed, while *S. fasciatus* is restricted to the East and *S. viviparus* to the West part of the North Atlantic.

Table 1.2. Systematics of the Genus *Sebastes* in the North Atlantic.

Genus <i>Sebastes</i> in the North Atlantic
Genus <i>Sebastes</i>
<i>Sebastes marinus</i>
<i>Sebastes mentella</i>
<i>Sebastes viviparus</i>
<i>Sebastes fasciatus</i>
<i>Sebastes fasciatus fasciatus</i>
<i>Sebastes fasciatus kellyi</i>

Of the four species, only *S. viviparus* is distinguished from the others by external morphology. For a long time, it was considered that there were only two species, *S. viviparus* and *S. marinus* described by Linnaeus (1758). Although *S. mentella* was described in 1951, three years later there was a new revision and two subspecies were defined, *S. marinus marinus* (Linnaeus, 1758) and *S. marinus mentella* (Andriiashev, 1954).

Ginsburg (1954) considered there was only one species, *S. marinus*, while *S. fasciatus* was considered a synonym of *S. marinus*, and *S. mentella* was not mentioned at all. *S. fasciatus* was recognized as a different species later (Scott and Scott, 1988). *S. fasciatus* is the only species subdivided in subspecies, *S. fasciatus fasciatus* and *S. fasciatus kellyi* (Litvinenko, 1974a). *S. f. fasciatus* has a more northern distribution and *S. f. kellyi* habitat is restricted to the Gulf of Maine (Hureau and Litvinenko, 1984). The common names of the *Sebastes* species in different languages can be found in Table 1.3.

Table 1.3. Redfish nomenclature in different languages.

Redfish common nomenclature in different languages				
	<i>S. marinus</i>	<i>S. mentella</i>	<i>S. fasciatus</i>	<i>S. viviparus</i>
Spanish	Gallineta nórdica	Gallineta nórdica	Gallineta nórdica	Gallineta nórdica
English	Golden redfish	Deepwater redfish	Acadian redfish	Small redfish
French	Grande Sèbaste	Sèbaste	Sèbaste américain	Petite Sèbaste
German	Goldbarsch	Schnabelbarsch		Kleiner Rotbarsch
Norwegian	Vanliguer	Snabeluer	Amerikansk uer	Lusuer
Russian	Морской окунь	Клювач	Американский окунь	Малый окунь
Icelandic	Gullkarfi	Djúpkarfi	Vínlandskarfi	Litlikarfi

1.4. DISTRIBUTION

The Genus *Sebastes* has many species and a wide distribution (Figure 1.6), inhabiting preferentially coastal waters, but also the open sea. There is a cline between bottom-dwelling and pelagic *Sebastes* species that occupy a wide range of niches (Kendall, 2000). The bathymetric distribution covers a wide depth range from shallow to deep waters, each species being associated with a different depth range. The bathymetric distribution of related species overlap in part because younger fish occur in shallower waters and associated with the adults of shallower-water forms, and also because the depth range for a given species varies with locality (Chen, 1971).

The range of *Sebastes* extends over the whole North Pacific from Japan in the west to Baja California and the Gulf of California, Mexico, in the east. However, *Sebastes* species are most abundant between 34° and 38° N. Beyond this range of latitude, the number of species falls southward and northward (Chen, 1971). The genus *Sebastes* is very common and widely distributed in the North Atlantic. It is found off the coast of Britain, along Norway, in the Barents Sea and Spitzbergen, off the Faroe Islands, Iceland, East-Greenland, West-

Greenland, and along the East coast of North America from Baffin Island South to Cape Cod (Magnússon and Magnússon, 1995). In the Southern Hemisphere, *Sebastes* is represented by two closely related species, *Sebastes oculatus* and *S. capensis*. *S. oculatus* is found from the Southern Peruvian coast to the Atlantic Argentine coast, including the Falkland Islands. *S. capensis* is found in Tristan da Cunha-Gough Islands and on the coast of South Africa. Eschmeyer (1969) was sceptical about the differences between both species, and Kong (1985) considered *S. oculatus* to be a synonym of *S. capensis*.

Most species of *Sebastes* occur in the temperate North Pacific Ocean. 69 species occurs in the East Pacific, that is, in North American Waters, and 25 species occur in the West. In the boreal North Atlantic there are four *Sebastes* species. All of these added to the species in the southern hemisphere, give an estimate of more than one hundred species in the Genus.



Figure 1.6. Genus *Sebastes* distribution

The North Pacific seems to be the center of *Sebastes* speciation. In the expansion to the southern hemisphere, the tropics are an unbridgeable boundary. However, the relationship between northern and southern species suggest that the tropics were crossed. Hubbs (1952) hypothesized that this crossing would be possible in the Pleistocene when the ocean was cooled to 8° C. Recent genetic studies (Rocha Olivares et al, 1999) in the Southern hemisphere, corroborate the existence of two different lineages of austral *Sebastes* corresponding to *S. capensis* and *S. oculatus*; it was found that *S. capensis* is not restricted to Tristan de Cunha and South Africa, but is widespread across the South Atlantic, and revealed the existence of a third evolutionary lineage with high levels of genetic divergence, particularly abundant in the south-western Atlantic, which may be recognized as a third austral species of *Sebastes*. According to Barsukov (1981), the presence of *Sebastes* species in the North Atlantic is related to a transarctic deep-water movement after the formation of the Bering Strait, at the end of the Miocene, beginning of the Pliocene. The settlement in the west of the Arctic basin ended with the penetration of one of the species into the northern

part of the Atlantic Ocean where it produced the 4 contemporary species, 3 species of north-eastern origin and 1 of north-western origin.

1.4.1. North Atlantic Distribution

In the North Atlantic, *Sebastes* occurs in boreal waters, with the Gulf Stream forming the southern boundary. In the northern area, its distribution reaches the Svalbard archipelago (80°N) in the East and The Davis Strait in the West. (Figure 1.7).

S. marinus and *S. mentella* have a very wide distribution, covering both sides of the Atlantic, Greenland, Iceland and Faroe Islands.

S. mentella occurs in colder waters, so its distribution covers the most boreal areas, but is scarce in the more southern areas. Thus, its distribution area includes Baffin Bay, David Strait, the coast off Labrador, Newfoundland and Flemish Cap. It is present on the whole Greenland coast, from Disko Bay to the South of the Greenland Sea and Reykjanes Ridge. A pelagic component inhabits the Irminger Sea, and since 1999, a displacement to both south and west has been observed, so that this pelagic component reaches NAFO convention area waters. Its distribution continues to Iceland, the Faroe Islands, the coast of Norway, the Svalbard Archipelago and the Barents Sea. However, as already mentioned, *S. mentella* is scarce in more southern areas, as south of Newfoundland (Saint Lawrence Gulf and Saint Pierre Bank), the south of Norway, the North Sea and the Irish Sea.

S. marinus and *S. mentella* distributions overlap. However, *S. marinus* prefers somewhat warmer waters than *S. mentella*. Thus, *S. marinus* is not present in the most northerly areas such as Baffin Bay and Greenland Sea. But near the coast and rarely, *S. marinus* appears in the Barents Sea, in the White Sea, and near Novaya Zemlya. Its abundance in the Svalbard Archipelago and North of Iceland is lower, and it also has a less homogeneous distribution than *S. mentella*. However, *S. marinus* distribution covers southern areas from the Gulf of St. Lawrence and Saint Pierre Bank to Gulf of Maine in the Northwest Atlantic; Rockall bank and the North Sea to the Kattegat Strait in the Northeast. Du Buit and Quèro (1989) recorded one *S. marinus* specimen in the Bay of Biscay.

S. fasciatus is only present in the western North Atlantic. Hureau and Litvinenko (1984) described 18 *S. fasciatus* specimens in Iceland and the Irminger Sea, but its presence there were occasional, and probably they were not *S. fasciatus* but a type of *S. mentella*. Its distribution does not reach the northern areas, and it is scarce in Labrador and the north of the Grand Bank of Newfoundland. However, its abundance increase towards the south, it is common on Flemish Cap, the south of the Grand Bank and the South of the Gulf of St. Lawrence, and predominate in Saint Pierre Bank, New Scottia, Gulf of Maine and in Georges Bank. There are two different forms of *S. fasciatus* in George Bank, considered different subspecies, *S. fasciatus fasciatus* and *S. fasciatus kellyi* (Barsukov *et al.*, 1984). *S. fasciatus kellyi* occupies shallower waters, while *S. fasciatus fasciatus* is in deeper waters.

S. viviparus occupies the same ecological niche as *S. fasciatus*, but in the Northeast Atlantic. Its distribution covers Irminger Sea, Iceland and the North of Norway, although that in those

areas is very scarce. However, its presence increase southerly, being very common in Faroe Islands, Rockall, in Shetland, Scotia, the North of England, Wales, Ireland and in the Norwegian coast to the Kattegat Strait.

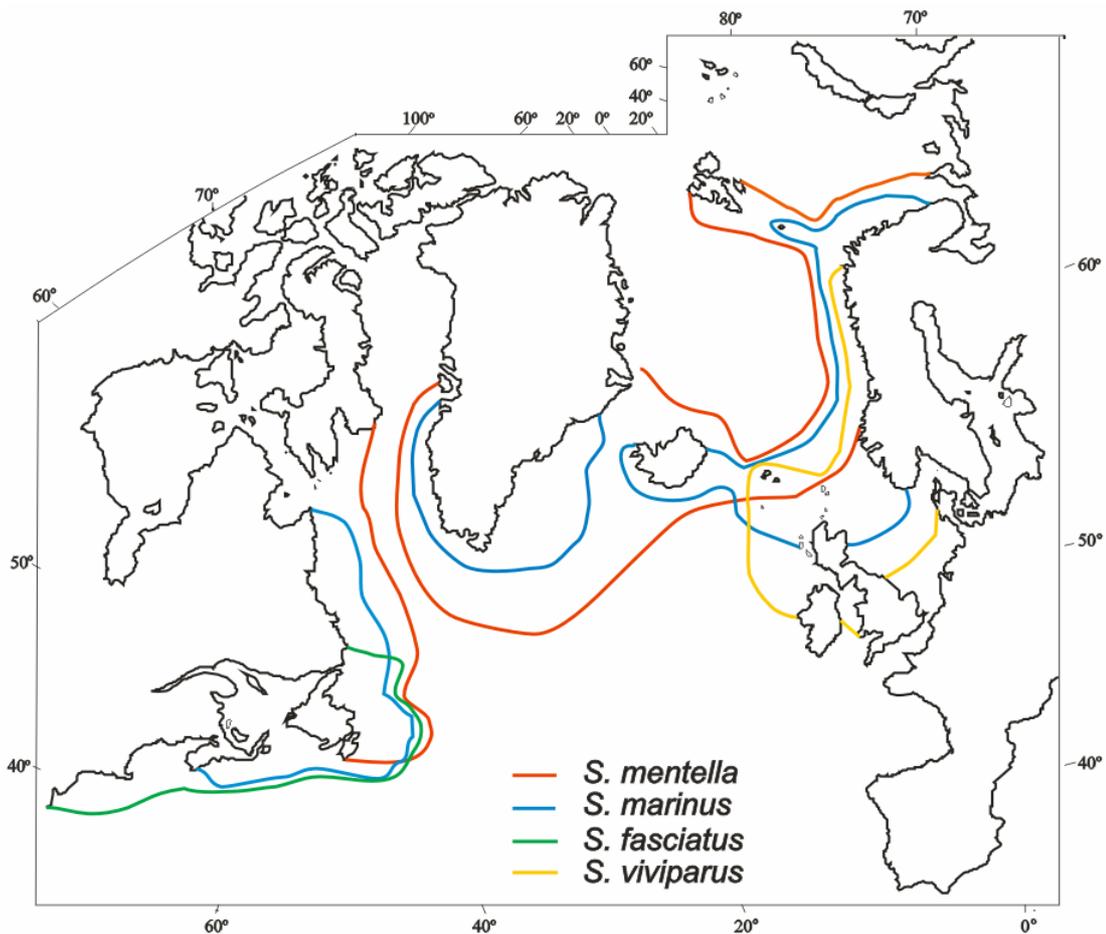


Figure 1.7. Genus *Sebastes* North Atlantic distribution.

The four species show different depth-range preferences. Thus, *S. fasciatus* and *S. viviparus* occupy shallower waters, not more than 300-350 m depth. So, its distribution area is always near the coast, with the exception of offshore banks such as Flemish Cap where those depths are also available. *S. marinus* however prefers intermediate waters, and *S. mentella* occupies deeper waters, reaching as deep as 1000 m. However the most normal depths for *S. mentella* are those beyond 400 m. *S. mentella* is the species that shows the most pelagic behaviour.

Because of the overlap of distribution areas, not only horizontally but also in depth, and the high resemblance between species, it makes sense to hypothesize about hybridization between redfish species. In fact, hybridization is a component of the discussion concerning the identification of North Atlantic *Sebastes* species (Altukhov and Nefyodov, 1968; Altukhov *et al.*, 1968; Rubec *et al.*, 1991; Johansen, 2003). Evidence for extensive introgressive hybridization between *S. mentella* and *S. fasciatus* in the Gulf of Saint Lawrence and south of Newfoundland has been described by Roques *et al.* (2001).

1.5. REDFISH POPULATIONS/STOCKS IN THE NORTH ATLANTIC

The common approaches to evaluation and management of marine finfish stocks assume discrete populations. However, the identification of redfish populations is complicated, principally due to the high external similarity between species and the occurrence of more than one species in the same area. Thus, in areas where more of one species coexist, they are normally managed as single management units. For example *S. mentella*, *S. fasciatus* and *S. marinus* in the Northwest Atlantic are assessed as one unit under the denomination of 'redfish *Sebastes spp.*', because catches are not separated into species. Table 1.4 shows the present management units used for redfish assessment in the North Atlantic, ICES (Northeastern area) and NAFO (Northwestern area), but it must be taken into account that those management units do not always correspond to redfish population units.

Table 1.4. Redfish stocks and management units in the North Atlantic.

Redfish management units in the Northeast Atlantic	
<i>S. mentella</i>	Northeast Arctic Shelves of East Greenland-Iceland-Faroe Islands Open Irminger Sea
<i>S. marinus</i>	Northeast Arctic East Greenland-Iceland-Faroe Island
'Giant' <i>S. marinus</i>	Reykjanes Ridge
<i>S. viviparus</i>	South of Iceland
Redfish management units in the Northwest Atlantic	
Davis Strait and West Greenland	NAFO subarea 0+1
Labrador and North of Newfoundland	SA2+ Div 3K
Grand Bank of Newfoundland	Div 3LN
Flemish Cap	Div 3M
Southwest (tail) of the Grand Bank	Div 3NO
St. Pierre Bank	Div 3P
Gulf of St. Lawrence	Div 4RST
Nova Scotia	Div 4VWX
Gulf of Maine-Georges Bank	Div 5

In the Northeast Atlantic, two stocks of *S. marinus* are actually defined, i. e., the Northeast Arctic stock and the East Greenland-Iceland-Faroe Island stock, and three different stocks are considered for *S. mentella*. One include the Northeast Arctic area, the second is formed by *S. mentella* on the shelves of Greenland, Iceland and the Faroe Islands, and the third stock is constituted by the *S. mentella* pelagic component in the open Irminger Sea. Because of the lack of commercial interest in *S. viviparus*, only one stock has been defined, in the south of Iceland, and the rest of the Atlantic remains without any particular division in stocks. Large redfish, named 'giant' redfish, have been found in different areas of the Reykjanes Ridge, on the continental slopes of Iceland and Greenland and the Faroe Islands. Although they are morphologically similar to *S. marinus*, some evidence (mainly genetic and size at

maturity) shows that they may constitute a different stock. However, this question remains under debate.

In the Northwest Atlantic, redfish are managed not by stocks but by nine management units in the following areas: Davis Strait and West Greenland, Labrador and Newfoundland, Grand Bank of Newfoundland, Flemish Cap, the southwest (tail) of the Grand Bank, St. Pierre, Gulf of St. Lawrence, Nova Scotia and the Gulf of Maine-Georges Bank.

1.5.1. *S. mentella* in Irminger Sea and adjacent waters

Up to three types of *S. mentella* have been described in the Irminger Sea and adjacent waters, but there is a lot of controversy about whether these types are different stocks (ICES, 1998, 1999). Historically, *S. mentella* and also *S. viviparus* and *S. marinus*, were fished on the shelves of East Greenland, Iceland and the Faroe Islands. *S. mentella* on the shelves around the Faroe Islands, Iceland and at East Greenland was considered as one stock unit. But, since 1982, when Russian vessels started the fishery in the open Irminger Sea, a new stock of *S. mentella* was defined for management purposes. This new stock in the open Irminger Sea, is mainly composed by adult *S. mentella* fished above 500m, and was named 'oceanic *S. mentella*' to distinguish it from the *S. mentella* on the shelves, and the latter were then named 'deep-sea *S. mentella*'. During the early 1990's, the pelagic fishery in the open Irminger sea moved to deeper waters beyond 500 m. At that time, some researchers considered that some of the fish caught below 500 m were different from those living above 500 m and resembling the 'deep sea *S. mentella*' on the shelves. This *S. mentella* distributed below 500 m in the open Irminger Sea was then considered as a new type and called 'pelagic deep-sea *S. mentella*' (Figure 1.8). Some researches differentiate the two *S. mentella* types in the open Irminger Sea based on variations in colour, length-weight relationship, length at first maturity and parasite infestation (ICES, 1998). In addition several criteria were used to aid in the identification of types (Magnússon, 1991):

- The general appearance is different: the oceanic type gives an impression of not being 'clean'
- The oceanic type very frequently has black and red spots on the skin, that are also observed, although rather seldom in the deep-sea type.
- Dark or grey spots are frequent in fillets of the oceanic type, but hardly seen in fillets of the deep-sea type.
- The oceanic redfish are often slightly thinner (just behind the head) than the deep-sea redfish.

Although preliminary genetic studies have given evidence of differences between these three groups of *S. mentella*, there are no significant biological or ecological differences between these groups (Saborido-Rey *et al.*, 2005). A more detailed exposition of the ecology of *S. mentella* in the Irminger Sea and adjacent waters is given in Chapter 2.

S. mentella in the Irminger Sea is a resource with economical importance for several countries. Furthermore, *S. mentella*, as most redfish species, has slow growth rates, and although the stock is considered to be inside safe biological limits, catches have decreased in recent years. Although several studies have been made to identify, delimit and discriminate redfish stocks (ICES, 1998), the basic knowledge for good assessments have not been acquired, that is, the biology, ecology and population structure remain unknown. So, in practice, sometimes the stocks have been defined attending exclusively to fish available for exploitation, without any biological basis. Thus, it was the fishery development and not biological knowledge which marked the establishment of new stocks. Although both species, *S. mentella* and *S. marinus* in the Irminger Sea and adjacent waters are the focus of the 'REDFISH project', particular emphasis has been placed on the study of the different *S. mentella* types defined in the open Irminger Sea, that is, the 'oceanic' and 'pelagic deep-sea' types, because a major controversy exists as to whether or not those types represent different stocks.

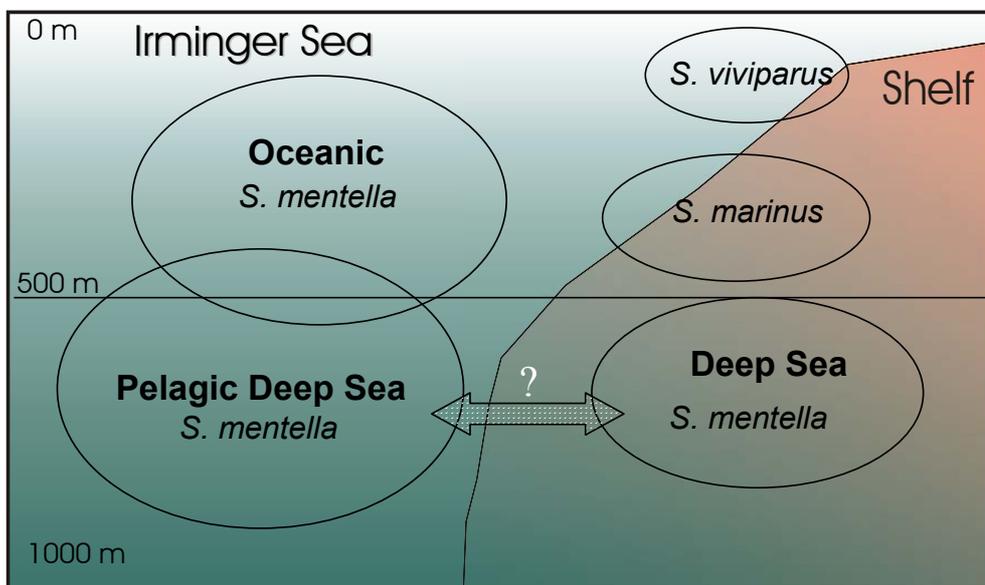


Figure 1.8. Up to three different types are considered for *Sebastes mentella* in the Irminger Sea and adjacent waters. In open waters The 'oceanic *S. mentella*' is distributed above 500m, and the 'pelagic deep-sea *S. mentella*' can be found principally below 500m. The 'pelagic deep-sea' type resembles the 'Deep-sea *S. mentella*' on the shelves, and there are authors that consider the two deep-sea types as the same stock.

1.6. STOCK IDENTIFICATION METHODS

The optimum approach to stock identification consist on the use of multiple techniques in order to confirm a particular stock structure, as the investigation of any single characteristic will not necessarily reveal stock differences, even when 'true' stock differences exist (Begg and Waldman, 1999); but combining the results obtained with several techniques can provide considerable insight into the possible stock structure of a species (Elliot *et al.*, 1995).

Traditionally, mark-recapture, catch data, life history characteristics, parasites, meristic, phenotypic variation (morphometrics) and genetics are used for stock identification. Although there is a general agreement on the need for a multidisciplinary approach, it present a number of obstacles including the varying expertise of individual scientists conducting the research, the specific stock identification issues and purposes being addressed, and the logistical costs in utilizing multiple, complementary techniques in a single study (Begg and Waldman, 1999). All these problems prevented multidisciplinary studies in the past. However, nowadays multidisciplinary studies are increasing. The EU Commission within the 5th framework program, promotes multidisciplinary approaches to different fish stocks, such as the 'HOMSIR project' (EU QLRT-PL1999-01438) that studies horse mackerel (*Trachurus trachurus*) stock structure, the 'WESTHER project' (EU QLRT-2001-01056) that deals with herring (*Clupea harengus*) or the already cited 'REDFISH' project (EU QLK5-CT1999-01222) that deals with *Sebastes* in the North Atlantic.

As mentioned above, although the best approach would be the utilization of all available techniques in the study of a stock, this is not always possible. So, a recommended protocol for integrated stock identification is the use of at least a genetic procedure and at least one phenotypic-based approach (Begg and Waldman, 1999).

Genetic differences between individuals, stocks and populations are the basis for ascertaining the degree of reproductive isolation, which is the fundamental mechanism structuring differences between these taxonomic groups (Begg and Waldman, 1999). Thus, genetics may be used to provide a direct basis for stock structuring and to interpret phenotypic-based patterns (Ihssen *et al.*, 1981; Smith, 1990).

Phenotypic variation between stocks can provide an indirect basis for stock structure, and although it does not provide direct evidence of genetic isolation between stocks, it can indicate prolonged separation of postlarval fish in different environmental regimes. Consequently, phenotypic markers may be more applicable for studying short-term, environmentally induced variation; perhaps more applicable to fisheries management, as opposed to genetic variation and endangered species management. Although in some circumstances phenotypic differences between stocks may be entirely environmentally induced, genetic components may also contribute to this variation (Begg *et al.*, 1999).

Comparing anatomical features of organisms has been the basis for taxonomic classification of organisms and differentiation among stocks. Those comparisons were traditionally carried out using differences in body measurements (morphometry) or differences in numbers of anatomical structures (meristics).

Morphometrics (from the Greek: 'morph' meaning 'shape' and 'metron' meaning 'measurement') study the form of the fish, analyzing the body shape or the shape of a relevant part of the body. Since the beginning of the past century, traditional morphometrics have been widely used for stock discrimination in different fish species. But phenotypic markers used without an understanding of their inheritance can lead to false acceptance of a stock unit (Booke, 1981). Geographic variation in morphometry has been used to discriminate local forms of fish. The historical development of stock identification methods has paralleled the advancement of morphometric techniques. The earliest analyses of morphometric variables for stock identifications were univariate comparisons, but were soon followed by bivariate analyses of relative growth to detect ontogenetic changes and geographic variation among fish stocks. As the field of multivariate morphometrics flourished, a suite of multivariate methods was applied to quantify variation in growth and form among stocks. More recent advances have been facilitated by image processing techniques, more comprehensive and precise data collection, more efficient quantification of shape, and new analytical tools (Cadrin, 2000).

Morphometrics is the study of shape variation and its covariation with other variables (Bookstein, 1991; Dryden and Mardia, 1998). From the sixties to the eighties, morphometrics was focused on the application of multivariate statistical tools to morphological variables, in order to describe shape variation within and between groups. Nowadays, this approach is referred as 'traditional morphometrics' (Marcus, 1990; Reyment, 1991) or 'multivariate morphometrics' (Blackith and Reyment, 1971). One of the difficulties of traditional morphometrics is that the geometric relationships among the variables are not preserved (a set of linear distances is usually insufficient to capture the geometry of the original object) (Adams *et al.*, 2004). To avoid this problem, alternative methods of quantifying and analyzing morphological shape were explored. Data that captured the geometry of the morphological structure was of particular interest, and methods to analyze such data were developed. This included methods for both outline and landmark data. Concurrent with these advances, David Kendall and other statisticians developed a rigorous statistical theory for shape analysis that made possible the combined use of multivariate statistical methods and methods for the direct visualization of biological forms (Adams *et al.*, 2004). This new approach is called 'geometric morphometrics'.

Furthermore, countable, morphological structures (e.g. gill rakers or fin rays) have also served as an important basis to identifying fish stocks. Other characteristics that can be set in numerically discontinuous values (such as the relative position of a spine) are considered also as meristic variables, even though they have no correspondence with the myomeres. Meristics have been used in the past both to identify species (Boetius, 1980) and to delimit populations within species (Schmidt, 1930, Templeman, 1981). Nowadays, meristic studies continue to be used as one of the tools in multidisciplinary population studies (Pepin and Carr, 1993; Tudela, 1999; Murta, 2000; Kai and Nakabo, 2002; O'Reilly and Hornt, 2004)

Several methods have been used to identify, delimit and discriminate redfish stocks, such as analysis of populational, physiological, behavioural, meristic, morphometric, biochemical and genetic parameters. (Ihssen *et al.*, 1981; ICES, 1996 b). Perhaps the most used have been morphometric (Reinert and Lastein, 1992; Saborido-Rey, 1994; and Saborido-Rey and Nedreaas, 2000), and genetic analyses (Dushchenko, 1987; Nedreaas and Naevdal, 1989, 1991; Nedreaas *et al.*, 1994; Johansen *et al.*, 2000; Johansen *et al.*, 2002).

1.7. OBJECTIVES

The main objective has been the study of the structure of the *Sebastes* species and populations in the Irminger Sea and adjacent waters by the use of morphometric and meristics tools, with special emphasis on the pelagic component of *S. mentella*.

To fulfil this main goal, several specific objectives were carried out:

Using morphometrics and meristics to differentiate between

- The four *Sebastes* species in the North Atlantic. (Species differentiation).
- Individuals of the same species but inhabiting different areas or sub-areas (Population differentiation).
- Pelagic deepsea and oceanic types described for the pelagic component of *S. mentella* inhabiting the open Irminger Sea.

Methodological

- Development of a data acquisition protocol with the aim of giving uniformity to the data taken in different laboratories.
- Development of the methodology to make comparable 3D and 2D measurements in order to introduce both types of data to the analyses.
- Compare traditional and geometric morphometric techniques.

2. REDFISH POPULATION ECOLOGY

The content of this chapter corresponds with the publication:

Saborido-Rey, F., Garabana, D., Stransky, C., Melnikov, S. and Shibanov, V., 2005. **Review of the population structure and ecology of *S. mentella* in the Irminger Sea and adjacent waters.** Rev. Fish Biol. Fish., in press.

2.1. INTRODUCTION

There are at least three species of redfish in ICES Divisions V, VI, XII and XIV, i.e. *S. marinus* (Linneo, 1758), *S. mentella* (Travin, 1951) and *S. viviparus* (Krøyer, 1845). Recently, the existence of more than one stock of *S. mentella* in the area was discussed (ICES, 2000). Historically, *S. mentella* was fished on the shelves and banks of the Faroe Islands, Iceland and East Greenland and was considered to be one stock. With the start of a new pelagic fishery in the open Irminger Sea in 1982, a new type of *S. mentella* inhabiting in this area was found, which was defined as a different stock for management purposes. In 1992, the Study Group on Redfish Stocks distinguished between these stocks as deep-sea *S. mentella* and oceanic *S. mentella* (ICES, 1992). In the early 1990s, the pelagic fishery in the open Irminger Sea moved to deeper layers beyond 500 m and some researchers considered that some of the fish caught below 500 m were different from those living above 500 m but resembling more the deep-sea *S. mentella* living on the shelves. This new type of *S. mentella* living below 500 m has been called “pelagic deep-sea *S. mentella*” to be distinguished from “shelf deep-sea *S. mentella*” (ICES, 1998) (Figure 2.1). The relationship between these three types has been subject of strong controversy, especially about whether these types are more than one population. Several hypotheses have been put forward, firstly summarized during the Study Group of Redfish Stocks (ICES, 1998) and stated in more detail (ICES, 1999):

- The **single population hypothesis** suggests that all *S. mentella* from the Faroe Islands to Greenland are a unique population, although they may be segregated according to age/size.
- The **two population hypothesis** suggests that the *S. mentella* living on the shelves (shelf deep-sea *S. mentella*) and that living in deeper pelagic waters of Irminger Sea (pelagic deep-sea *S. mentella*) constitute one population which is separated from the oceanic *S. mentella* living in upper layers of the Irminger Sea.
- The **three population hypothesis** supports the idea that each of the described types constitutes a distinct population.

Preliminary genetic studies indicate that pelagic deep-sea and oceanic *S. mentella* types in the Irminger Sea do not share a common gene pool. Nevertheless, heterogeneity among samples of these two types in the Irminger Sea could indicate sub-structuring within each group and awaits further study. Only minor differences were observed between deep-sea *S. mentella* in the Irminger Sea and on the Icelandic Continental shelf, indicating that they could share a common gene pool (Johansen *et al.*, 2000).

Apart from biochemical and genetic studies, several attempts were undertaken to investigate the population structure of redfish, especially for the highly migratory and widely distributed *S. mentella*. While Nagel *et al.* (1991a), Reinert and Lastein (1992), Saborido-Rey (1994) and Saborido-Rey and Nedreaas (2000) used meristic and morphometric characteristics of the fish body, Stransky (2001, 2002, 2003) applied otolith shape analysis to study relationships between species and populations or areas. The geographical variation of infestation by the copepod parasite *Sphyrion lumpi* was the subject of several investigations (e.g. Bakay, 1988; 2000, 2001; Magnusson, 1992; Nagel *et al.* 1991b), as well as abnormal external coloration and ectolesions (e.g. Bogovski and Bakay, 1989). Almost all studies pointed to uniformity of *S. mentella* within the Irminger Sea and close relations with the demersal occurrences on the Greenland and Iceland shelves. Regarding chemical analyses, a pilot study of Reinert *et al.* 1992 using Cs-137 as a population marker indicated a closer relationship of redfish from Faroese waters to samples from the Barents Sea than to Icelandic samples. This was partly confirmed by fatty acid analyses of Joensen and Grahl-Nielsen (2002), dividing the occurrences of *S. mentella* around the Faroe Islands into one group related with the Icelandic shelf and another group connected to the Norwegian coast. Within the Irminger Sea, however, they reported considerable heterogeneity. The analysis of trace element composition of redfish otoliths across the entire North Atlantic resulted in weak small-scale geographic separation but indicated moderate large-scale discrimination of *S. mentella* from the Northwest, central and Northeast Atlantic (Stransky *et al.* 2005a).

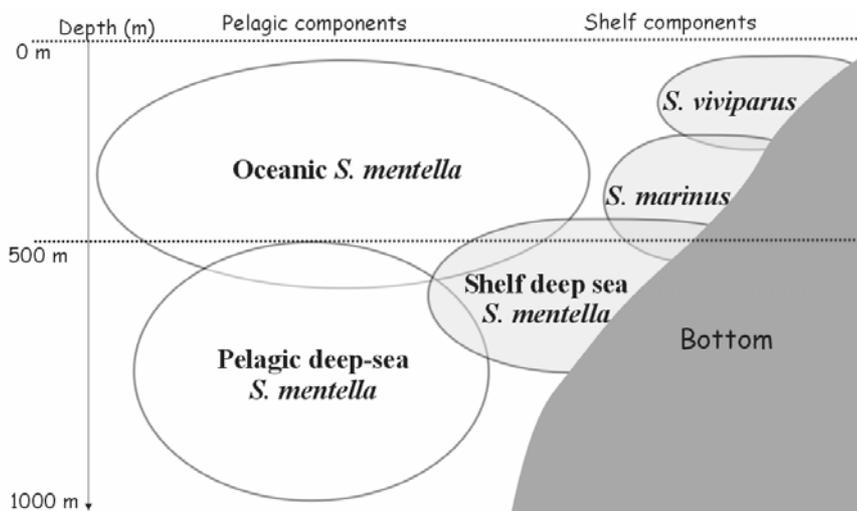


Figure 2.1. Schematic representation of the vertical distribution of redfish in the Irminger Sea and adjacent shelf areas.

The definition of the "population" term remains debatable. The number of definitions is enormous but none of them can be considered as the only thorough and correct one. However, the main criteria inherent in every population of any biological species can be chosen. Among the features described in the literature to define a population in fisheries science (see Moller, 1969; Templeman, 1979; Booke, 1981; Ihssen *et al.*, 1981; Kutkuhn, 1981; Carvalho and Hauser, 1994; Begg *et al.*, 1999; Waldman, 2005 and references therein), and acknowledging that there is not a simple and single definition of population, we consider that a fish population is characterized, beyond the genetic contiguity, by the spatial and temporal integrity of the reproductive isolation of its members, i.e. the individuals of the

population randomly mate among them but not with another congeners from other populations. A population should contain the full range of life stages, from larvae to older groups. There are more identification criteria to name that are peculiar to every specific population, but the above listed features are inherent in any fish population.

However, habitat area of the majority of widespread fish populations has a complex functionally divided structure. It manifests itself through reproductive, feeding, wintering and nursery areas, which may overlap with one another or be independent of one another. To characterize populations of such fish species the habitats of each functional division should be considered in its definition, i.e. the population is constituted by all the individuals living in each of the functional division.

When carrying out inter- and intra-population research it is important to recognize that populations like biological species are characterized primarily not by distinctions but by isolation. Such population parameters as size-age composition, growth and maturity rate, fecundity, sex ratio and other quantitative characteristics can be both distinct and similar in different populations. Differences in samples on these or other parameters alone do not always indicate that they are taken from different populations. In widespread species with complex, functionally divided habitats, differences in a number of parameters may occur at the intra-population level, particularly when observations have been made over a long period or samples under comparison have been taken in different parts of the habitat area. There can be interannual differences among parameters within any population. Some of these parameters showing variation, such as maturity ogive, size-age composition or growth rate, are used as input in many stock assessments models. Many commercial fish species showed some patterns in variation of population parameters throughout the habitat of the population. For instance, it is well known that size-age composition of fish in the nursery and reproductive areas are most distinct. Different sex ratio and proportion of immature and mature fish of the same age, dissimilarities in diet and food supply in different areas of the habitat lead to distinctions in growth and maturity rates of fish. All the above peculiarities were taken by the authors into account when considering the population structure of *S. mentella* dwelling in the Irminger Sea and adjacent waters.

In the present paper, we give an overview of the existing knowledge on the ecology of *S. mentella* inhabiting the Irminger Sea and adjacent waters (Faroe Islands, Iceland and Greenland), i.e. life cycle description, spawning behavior and different life stages distribution and drift and/or migration patterns. The population structure of the redfish is discussed with respect to recent observations on the migration of juveniles (from the East Greenland shelf into the Irminger Sea).

2.2. REDFISH SPAWNING AREAS AND TIME

Redfish is a viviparous species, and therefore copulation occurs well before spawning. This implies that the types are not necessarily disaggregated at spawning time. Spawning of redfish in the Irminger Sea takes place in a wide area southwest of Iceland and above of the

Reykjanes ridge with extension in a southwesterly direction (Figure 2.2). It has been recognized that in this wide area both *S. marinus* and *S. mentella* (all types) spawn.

For many years no one has succeeded in defining separate spawning locations for these two groups of redfish. As a result of Soviet investigations in 1962-1966, areas and time of spawning for both redfish species dwelling along the slopes of Iceland were ascertained (Zakharov, 1964, 1969). To hatch out larvae *S. mentella* migrate from the slopes of Iceland and Rosengarten Bank to the Reykjanes Ridge in the open sea. This is the same area where redfish inhabiting pelagic waters of the Irminger Sea hatch out their larvae (Magnusson, 1983; Pavlov *et al.*, 1989a; Shibanov *et al.*, 1995). During spawning *S. marinus* also migrate from the slopes of northwestern Iceland and East Greenland to the slopes of western and southwestern Iceland. Golden redfish hatch out their larvae not far from the slopes and much later than the beaked redfish. Subsequent investigations supported differences in areas and time of spawning between the above two redfish species revealed by G. P. Zakharov (Magnusson and Magnusson, 1977; Magnusson 1980; Anon., 1983; ICES, 1988).

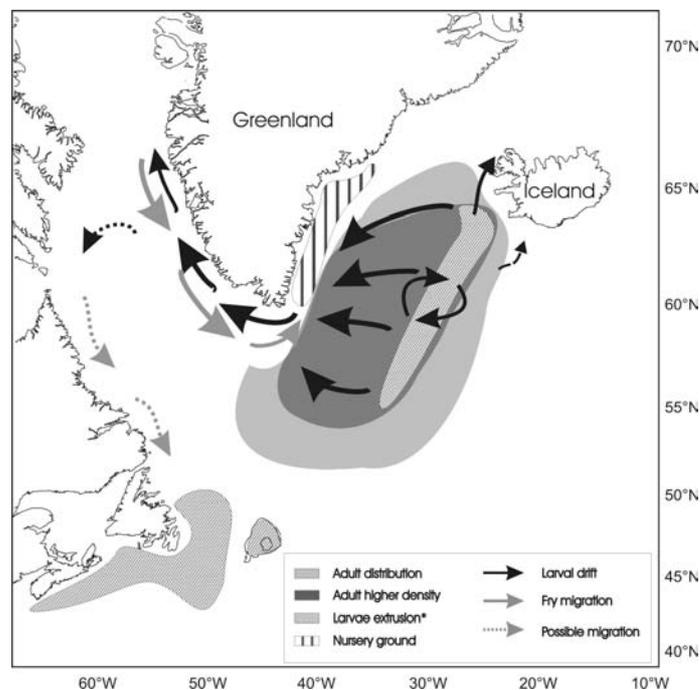


Figure 2.2. *S. mentella* larval and fry drift pattern in the Irminger Sea and adjacent waters. Larvae drift from the extrusion area in Central Irminger Sea mostly towards East Greenland. Later they drift Northwards along West Greenland Shelf. A posterior fry migration southwards towards the main Nursery area takes place. There is some larvae retention in the Central Irminger Sea. It is possible the existence of larval drift from West Greenland to Canadian waters in the Davis Strait. *Another major extrusion areas are also shown in Canada and Flemish Cap.

It has not yet been made clear which of the *S. mentella* types the extruded larvae in this area belong. It only has been hypothesized (Magnússon and Magnússon, 1995) that the larvae extruded north-eastwards are more likely shelf deep-sea *S. mentella* and that the larvae extruded southwesterly belong to the other two types (pelagic deep-sea and oceanic *S. mentella*). However, this hypothesis is only based on the proximity of each spawning area with the proposed distribution area of each type.

All types of *S. mentella* release their larvae in April to June, overlapping to a great extent in both time and space (Kotthaus, 1961; Magnússon, 1962; Magnússon *et al.*, 1965; Noskov *et al.*, 1984; Shibanov *et al.*, 1984, 1995; Pavlov *et al.*, 1989a; Magnússon and Jóhannesson, 1997). It was reported that shelf deep-sea *S. mentella* spawn later in the year from July to August (ICES, 1983). However, the Icelandic 0-group surveys (August) have not given evidence for *S. mentella* larvae around Iceland, or the presence of such larvae were scarce (Magnússon and Sveinbjörnsson, 1991; Sigurðsson *et al.*, 1997; ICES, 1998). Moreover, the size of those few larvae was over 50 mm, even bigger than those reported from the open Irminger Sea at the same time. For these reasons it is unlikely that these larvae had been released so late in the year, and were probably born in April-May. Magnússon and Magnússon (1995) support this by stating that larvae from both types are extruded at the same time. In addition, it has been reported that the main peak of larval extrusion varies year by year both in time and space in relation to oceanographic conditions (Pavlov *et al.*, 1989a; Magnússon and Magnússon, 1995; Shibanov *et al.*, 1995).

The main difference reported between the shelf deep-sea *S. mentella* and oceanic *S. mentella* is the depth at which spawning is believed to occur. While the oceanic type spawns at depths of about 200 to 500 m, the shelf deep-sea type spawns approximately at 500 m depth (ICES, 1983; Magnússon and Magnússon, 1995; Shibanov *et al.*, 1995). There are no reports, however, about the spawning depth of the pelagic deep-sea type, which is thought to live in greater depths (below 500 m) than the oceanic type. A possible migration of the pelagic deep-sea type in upward direction for spawning in shallower waters has not been reported, so it is likely that there is a continuous range in spawning depth between 200 and 500 m, representing the adults' distribution rather than spawning preferences. Thus, the spawning depth is more likely related to the size distribution and oceanographic conditions than with spawning behavior. In addition, we should not assume that released larvae will live in the same depths where they have been released. Although it has not been studied in redfish, it is likely that neutral buoyancy of redfish larvae is similar irrespective of the species/type origin and, therefore, as for most other marine fish species spawning pelagic eggs/larvae, redfish larvae will drift to shallower and more productive waters irrespective of the depth where they have been released. Thus, the distribution of redfish larvae in the Irminger Sea is more restricted than the distribution area of the mature redfish (Pavlov *et al.*, 1989b), suggesting that immediately after spawning the larvae drift to the central and eastern area of the Irminger Sea, probably driven by the cyclonic gyre (Cuny *et al.* 2002) of the Irminger current (Figure 2.3). Based on ichthyoplankton surveys the densest larvae abundance was recorded at depths of 0-50 m or 0-150 m (Noskov *et al.*, 1984; Pavlov *et al.*, 1989a; Shibanov *et al.*, 1984; Herra *et al.*, 1987; Wieland, 1991; Shibanov *et al.*, 1995). Therefore, it is very clear that spawning depth is not relevant in the ecology of the larvae since all larvae appear to follow the same drift pattern independent of their origin

No spawning activity of *S. mentella* has been reported to occur in Greenland waters. But it has been observed in the south and west of the Faroe Islands in some years, implying that there could be a local component in the area; no nursery areas, however, have been found so far (Reinert, 1990). A relationship to the North-east Atlantic areas has also been suggested (Reinert *et al.*, 1992, Reinert and Lastein, 1992). The question of a possible relationship between this stock unit and the two pelagic types in the Irminger Sea has been

raised several times (e.g. ICES, 1999; 2000). In conclusion, the existence of a single and wide spawning in the area of concern (Greenland, Irminger Sea and Iceland), with a wide depth distribution and with a single spawning peak in April-June, is very likely. Redfish larvae, irrespective of their origin (type) are then pooled and drifted towards Greenland as laid out below.

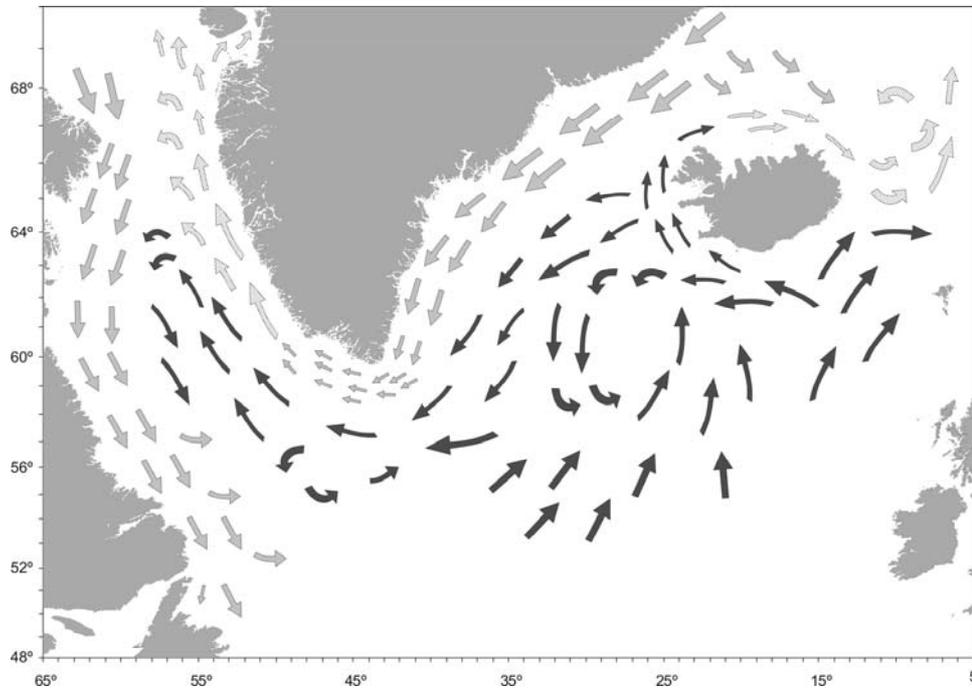


Figure 2.3. General trends of currents in the Northwest Atlantic. Figure taken from ICES, (1998). Light grey arrows represents cold waters (Labrador and Irminger currents), while dark arrows are the North Atlantic current and their branches.

2.3. LARVAE AND FRY (0-GROUP) DISTRIBUTION AND DRIFT PATTERNS

The ichthyoplankton surveys show that redfish larvae are distributed over an extensive area, although smaller than the distribution area of adult fish. During Russian larval redfish surveys in 1982-1995 it was found that distribution of just hatched larvae corresponded to distribution of the redfish spawning stock. Areas of the densest concentrations of spawning redfish and hatched larvae vary in latitude by years but their general confinement to and orientation along the Reykjanes Ridge remain unchanged (Shibanov *et al.*, 1995; Melnikov *et al.*, 2001) (Figure 2.4). The early larval distribution area is shown in Figure 2.2. Redfish larvae are widely distributed across the whole area, but patches of higher density are located in a different position year-by-year (Henderson, 1961; Pavlov *et al.*, 1989a; Magnússon and Magnússon, 1995; Magnússon and Jóhannesson, 1997).

There is a general agreement among authors about the larval drift. A general trend in the drift of larvae was indicated from the central and eastern Irminger Sea towards the slopes along the East Greenland shelf and to some extent around Cape Farewell (Anonymus, 1968; ICES,

1983, 1998; Troyanovsky, 1992; Alekseev, 1999; Melnikov *et al.*, 2001), in accordance with the general direction of currents in the area (Figure 2.3).

From Icelandic 0-group surveys, drift of redfish postlarvae from the areas of larval extrusion to areas West and North of Iceland has also been observed (Einarsson, 1960; Sveinbjörnsson, 1996; Magnússon and Jóhannesson, 1997; Sveinbjörnsson and Jónsson, 1998). However, these consist almost entirely of *S. marinus* (see e.g. Pálsson *et al.*, 1989; Pálsson *et al.*, 1997; Sigurðsson *et al.*, 1997). Only rarely are small (juvenile) *S. mentella* found around Iceland. As mentioned, it seems that *S. marinus* spawns north-easterly, for this reason it is consistent with the occurrence of *S. marinus* larvae around Iceland. Records of larvae along the Greenland shelves strongly suggest that *S. mentella* larvae initially drift to Southeast Greenland, passing Cape Farwell and moving northwards. *S. mentella* larvae and postlarvae have been observed off West Greenland (ICES, 1983, 1997).

It is unknown whether most of the larvae or only parts of them move to West Greenland. We suspect that most of the *S. mentella* larvae drift to West Greenland since there is an important nursery area for age 1 redfish as explained below.

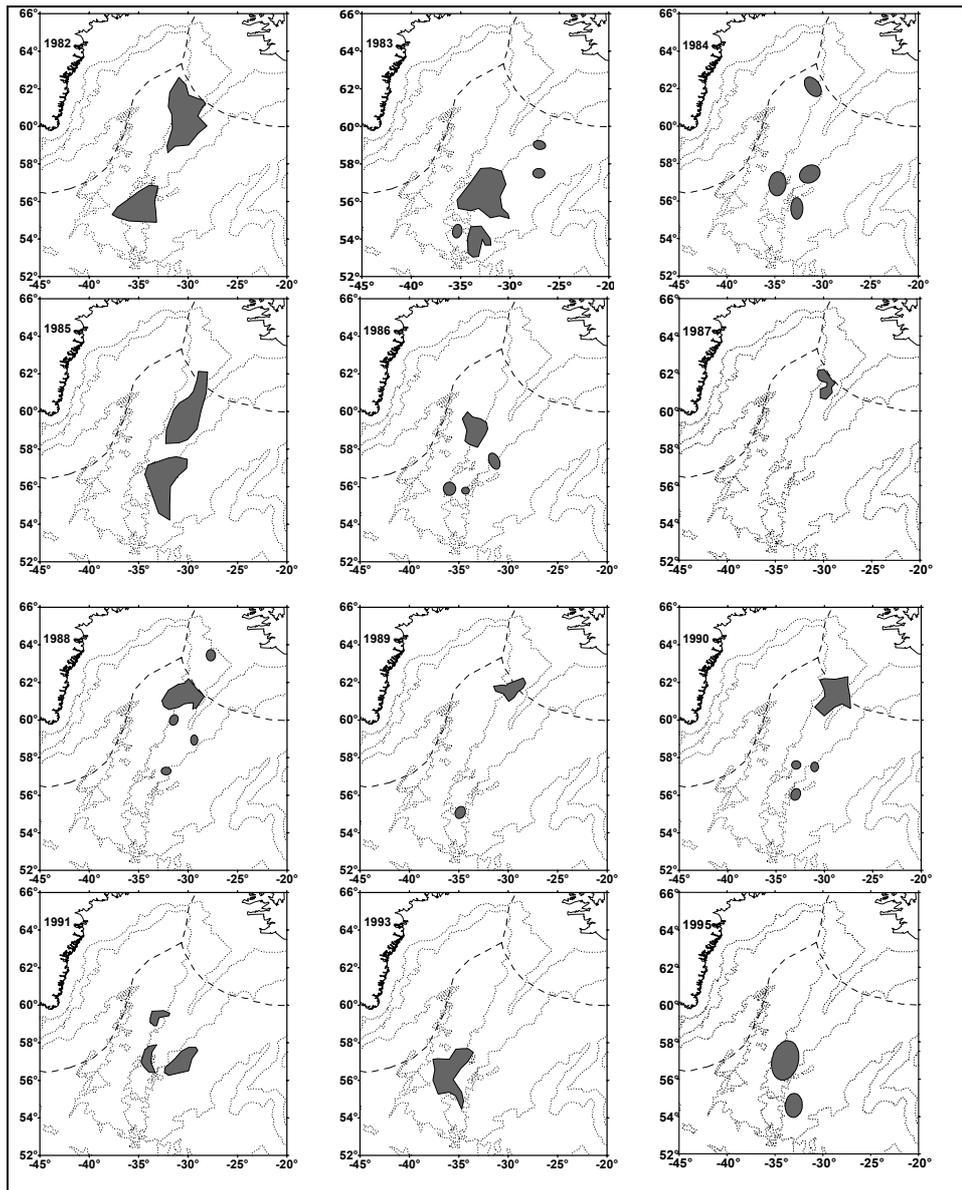


Figure 2.4. Areas of mass larvae hatching (more than 25 individuals under 1 m²) from Russian spring ichthyoplankton surveys in 1982-1995.

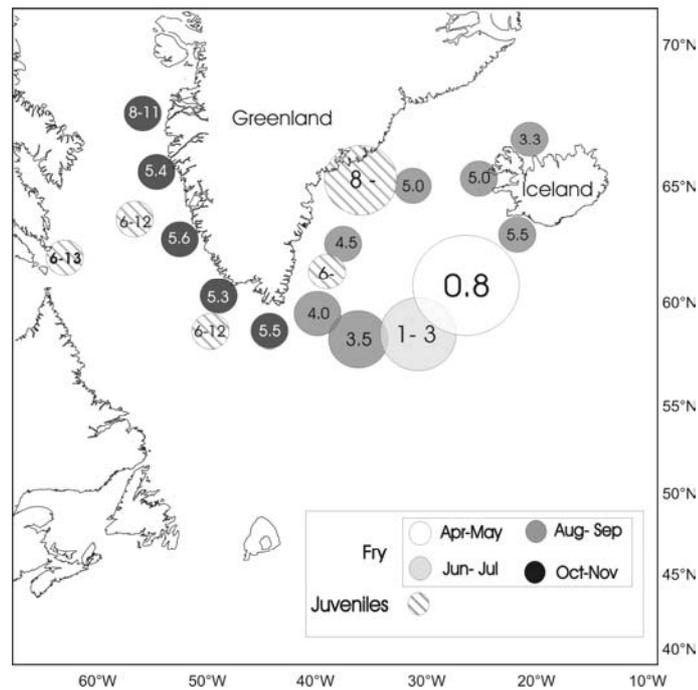


Figure 2.5. *S. mentella* fry and juveniles size. *S. mentella* fry are recorded in different positions along the year as growing. Juveniles size distributions (in mm) are shown.

The distribution area of larvae during summer extends south from Cape Farewell to 55°N (Henderson, 1961). According to several authors (Zakharov, 1966; Magnússon and Sveinbjörnsson, 1991; Wieland, 1991, 1992; Pedersen and Kanneworff, 1992), it is obvious that *S. mentella* larvae extruded in the Irminger Sea in April-June drift towards East Greenland, where high densities of 0-group redfish are found in August/September. Subsequently, most of them are carried southwards with the East Greenland current, passing Southwest Greenland in September/October and continue with a northward displacement during October and November at West Greenland (Figure 2.5). In this area, redfish is largely recruited at age 1 which could explain the occurrence of large quantities of small redfish as by-catch in the shrimp fishery in the Davis Strait (ICES, 1990; Pedersen, 1990, Pedersen and Kanneworff, 1992) or in the surveys in NAFO area 1 (Atkinson, 1987; Rätz and Stransky, 1999).

After hatching, the drifting larvae do not always reach nursery areas on the shelf of Greenland. On the evidence from some researchers (Noskov and Romanchenko, 1986; Magnússon and Sveinbjörnsson, 1991; Rikhter, 1996) in late summer/early autumn in the central Irminger Sea redfish fry of 4-6 cm long were observed. Apparently, these fry had not been drifted by the Irminger Current from the area of reproduction to the places of settling on the shelf of Greenland and retained in local circulations. In the end, the fry will be drifted and settle on the slopes of Greenland otherwise they die in autumn. The high fecundity of *S. mentella* compensates for substantial mortality of larvae and fry in the early ontogeny.

It is probable that *S. mentella* postlarvae migrate or drift from West Greenland to Canadian waters in the Davis Strait. Templeman (1961) reported small (6-13 cm) *S. mentella* off the East Coast of Canada, from 64°N to 60°N. The source of these small *S. mentella* is

supposed to be an extraordinarily strong transfer of West Greenland-Water (Templeman, 1961).

2.4. JUVENILE DISTRIBUTION, NURSERY AREAS, AND MIGRATION PATTERNS

No specific nursery grounds for *S. mentella* have been found in Icelandic and/or Faroese waters, and there is also no record of nursery grounds off the south coast of Iceland (ICES, 1983). However, major nursery grounds were found along the coast of West- and East Greenland (Figure 2.2). The main source of information about these nursery grounds is the German and Icelandic annual surveys off Greenland and Iceland respectively (see e.g. Rätz *et al.*, 2003). Unspecified small redfish (<17 cm) are distributed along both sides of Greenland. Most of the redfish larger than 17 cm are *S. mentella*, so we assume that most of the small and unidentified redfish are also *S. mentella*. In Figure 2.6, the length distributions of small redfish are shown since 1982, derived from the German surveys. It can be seen that small redfish is less abundant off West Greenland, but when occurring, they mainly and almost exclusively consist of ages 1 and 2 (6-8 cm and 11-12 cm).

In years of low abundance, these year classes are found mainly in West Greenland, but in years of higher abundance (i.e. 1985, 1993, 1995-98) the bulk is almost solely distributed off East Greenland. The size of young redfish increases northwards in East Greenland (Jørgensen, 1999; Rätz and Stransky, 1999). This indicates that first the larvae and 0-group drift from East to West Greenland, although in some years, larvae are retained in East Greenland, probably yielding a higher survivor rate and hence a good recruitment. After having spent one or two years in West Greenland a southward migration presumably takes place in this area, since the length of small *S. mentella* increases from North to South in the area of the offshore shrimp fishery (ICES, 1983). This is in accordance with the fact that no spawning of redfish has been observed in West Greenland. Thus, young redfish return to East Greenland where they finally recruit (Figure 2.6).

In recent years (1989-2000), deep-sea *S. mentella* off Greenland were basically smaller than 30 cm, although in previous years, a higher abundance of sizes over 30 cm was observed, mainly off East Greenland (Figure 2.7). This indicates that redfish over 30 cm “disappear” from the area. *S. mentella* grows in this area until near maturity.

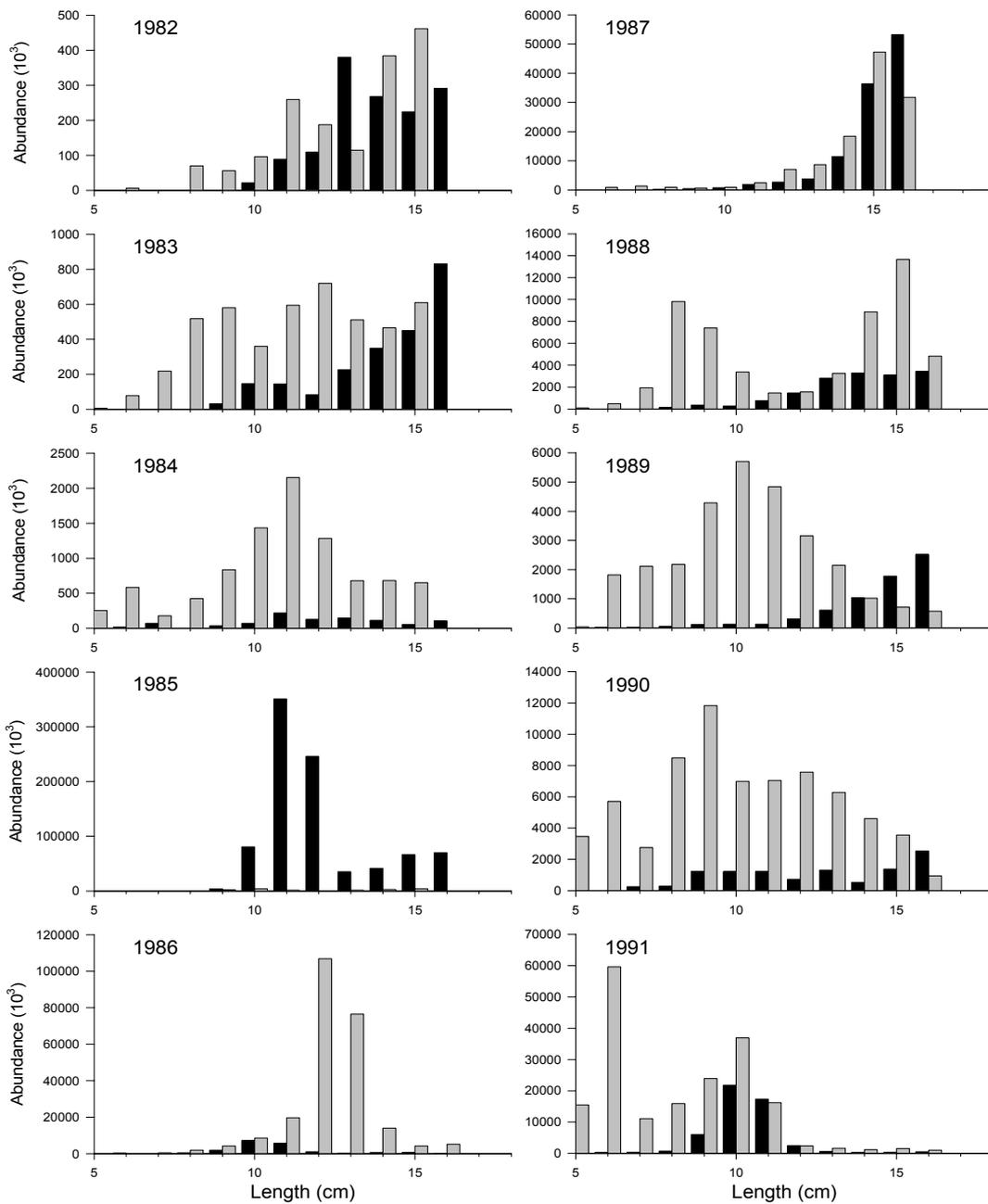


Figure 2.6. *Sebastes* spp. (<17 cm). Length frequencies for East (black) and West (grey) Greenland. 1982-1991.

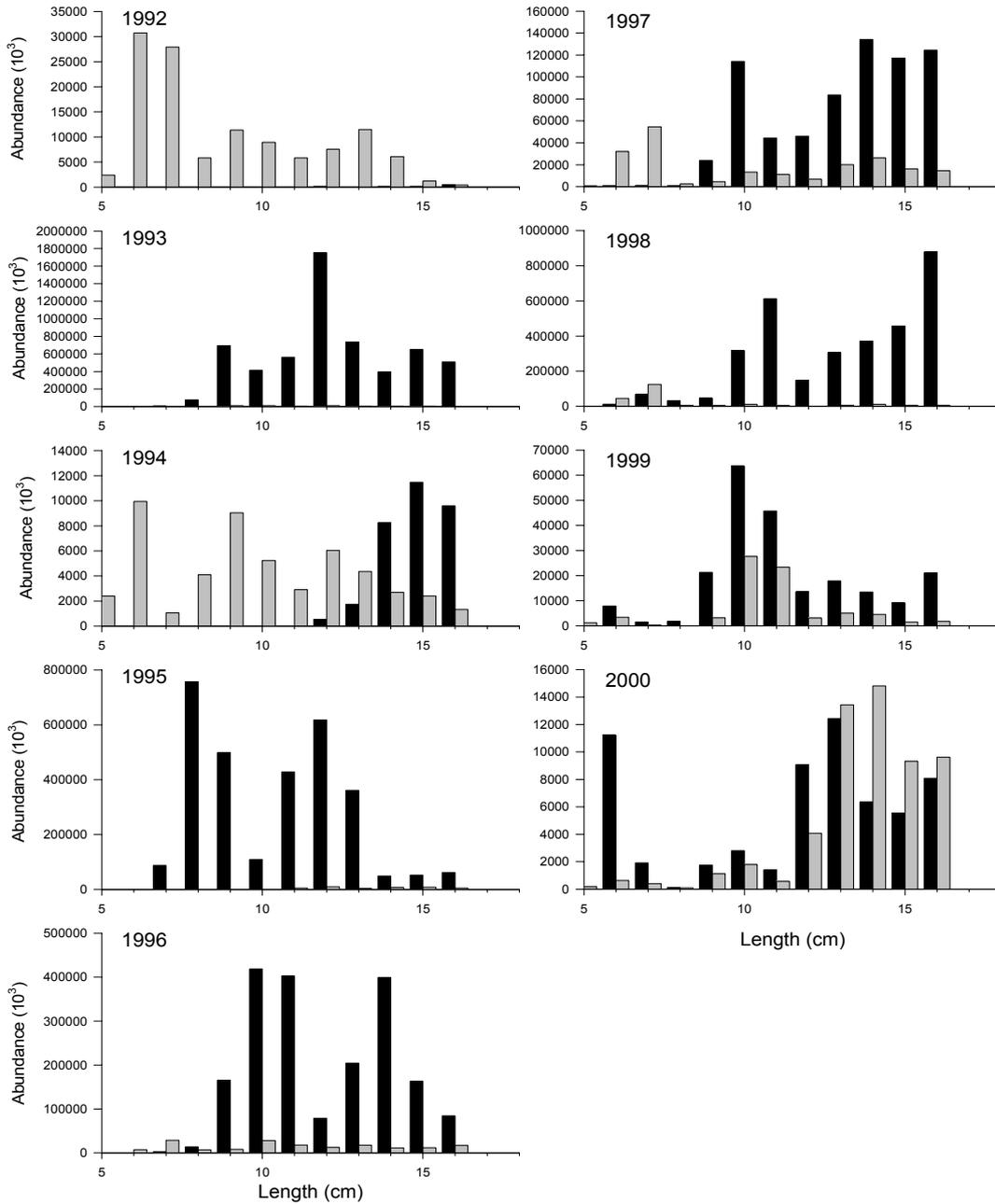


Figure 2.6 (continuation)

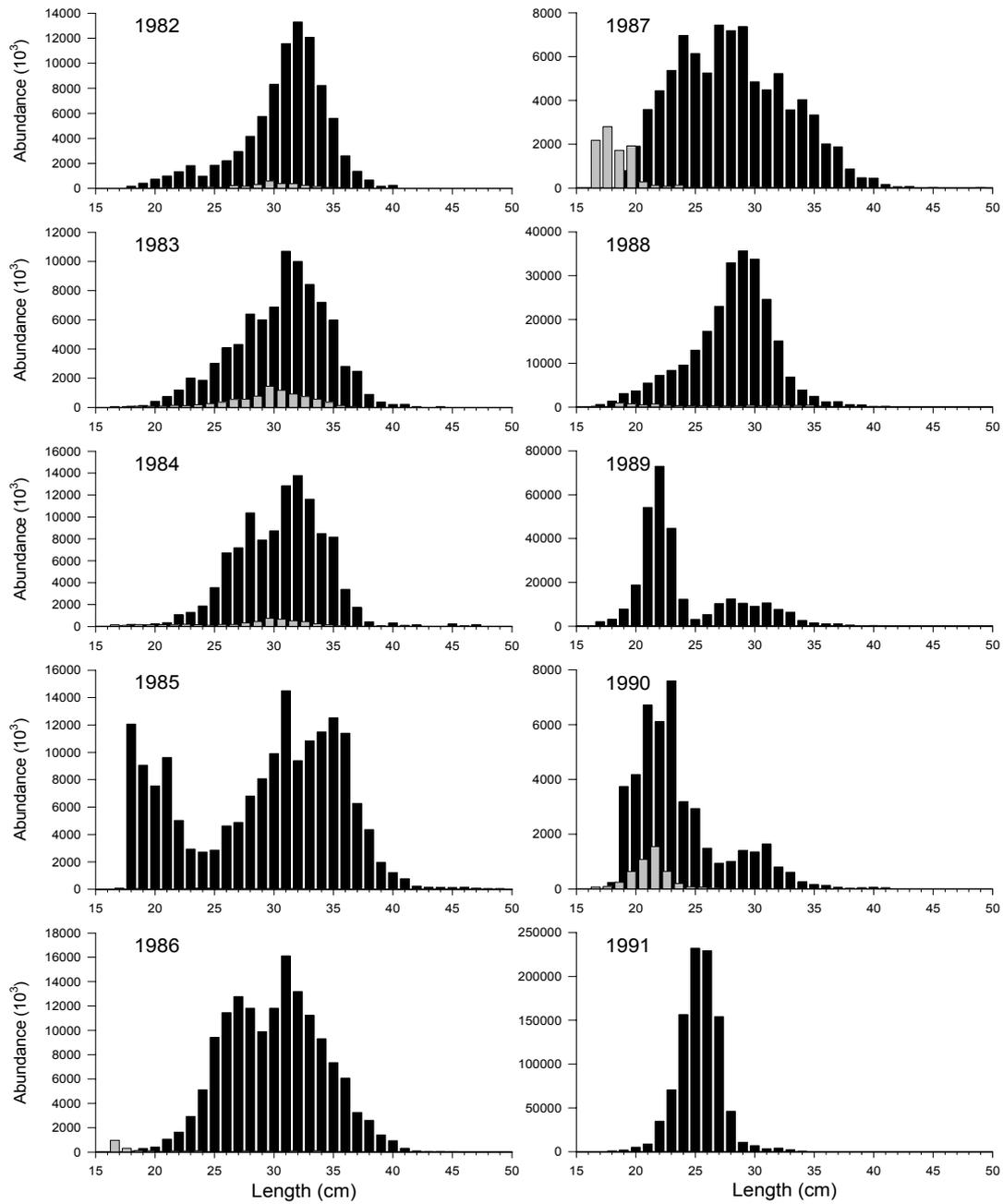


Figure 2.7. Deep-sea *S. mentella* (>=17 cm). Length frequencies for East (black) and West (white) Greenland, 1982-91

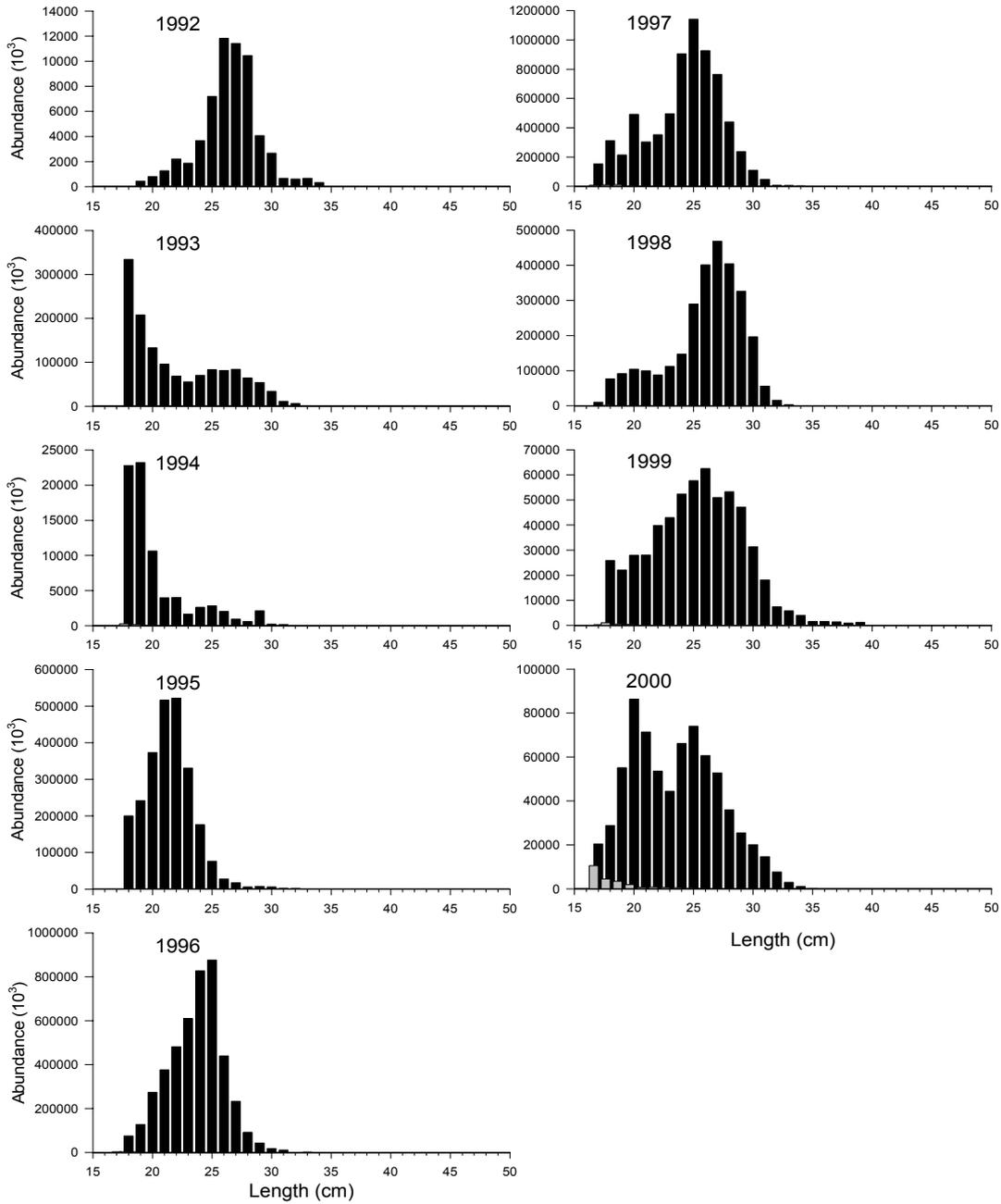


Figure 2.7 (continuation).

2.5. ADULT DISTRIBUTION

Adult *S. mentella* are found all around the studied area: Faroes, Iceland, open Irminger Sea and both East and West Greenland. However, most of the adults are found around the Faroe Islands, Iceland and in the Irminger Sea.

Both oceanic and pelagic deep-sea *S. mentella* inhabit pelagic waters of the Irminger Sea, including areas in the Icelandic and Greenland EEZ, rather close to the shelf, while the shelf deep-sea type is restricted to the shelves. The distribution areas of the above three types overlap. As is known, reproductive cycle of Atlantic redfishes includes maturation, mating, fertilization and extrusion of larvae. In males, only two stages of the sexual cycle, maturation and mating, are observed while in females all the four stages are inherent. Males are fully mature in August–November, while egg maturation occurs in January–February, showing an asynchronous maturation of male–female gonads (Sorokin, 1961; Sorokin *et al.*, 1986; Pavlov *et al.*, 1989a). This may indicate that *S. mentella* mate in August–November when mature males also migrate to the southwestern Irminger Sea in a depth range of 150 m to 400 m. In addition, *S. mentella* do not form dense concentrations in the pelagic waters of the Irminger Sea during winter (Melnikov *et al.*, 2001), suggesting an earlier copulation. Sperm would remain inactive within the ovaries until fertilization. Embryogenesis begins with ovulation–fertilization and last until March. Extrusion of larvae ensues soon afterwards, in April–May.

Change of stages of the annual cycle determines variability in spatial and vertical distribution of concentrations, timing and direction of seasonal migration of mature *S. mentella* within the population area. The general pattern of distribution and seasonal migration of *S. mentella* appear as follows. On completion of wintering, females migrate from the vast area to the central Irminger Sea for spawning. Major larval extrusion occurs in the Reykjanes Ridge area in a depth range between 250 m and 800 m (see “Redfish spawning areas and time” section). Extrusion of larvae begins in April in the southwestern Irminger Sea and ends in June in the northwestern Irminger Sea near the slopes of Iceland. After spawning, in June–August, concentrations of feeding *S. mentella* are distributed over the area more than 400 thousand mile² between 52°–64°N and 28°–54°W in a depth range of 150 m to 1100 m (Pavlov *et al.*, 1989a; Magnusson *et al.*, 1996; Shibanov *et al.*, 1996; Pedchenko *et al.*, 1996, 1997; Sigurðsson *et al.*, 1999, 2001; ICES, 2003).

Very low abundance of *S. mentella* smaller than 25 cm is found around Iceland and in the Irminger Sea (Sigurðsson, 1998; Alekseev, 1999; Stransky, 2000). And, as stated in the previous section, *S. mentella* larger than 30 cm are rare around Greenland. On the international survey on pelagic redfish in the Irminger Sea in June/July 1999, juvenile *S. mentella* of 27–29 cm were observed in the length spectrum above and below 500 m depth (Figure 2.8). However, the bulk of redfish in the Irminger Sea observed in previous years varied around 35 cm. Therefore, it can be concluded that a considerable proportion of recruits was appearing in the area during 1999. A migration of a part of the large quantities of

juvenile *S. mentella* recorded on the East Greenland shelf in 1995-1998 (Figure 2.7) into the Irminger Sea is therefore very likely. As already hypothesized by Alekseev (1999) and Stransky (2000), as redfish grow, they migrate from East Greenland eastwards (Iceland and/or Irminger Sea).

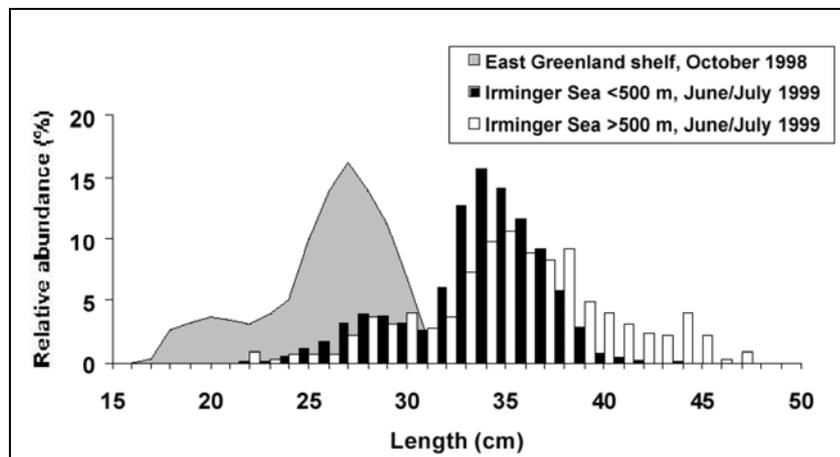


Figure 2.8. Length distribution of *S. mentella* on the East Greenland continental shelf in October 1998 and in the Irminger Sea in June/July 1999 (from Stransky, 2000).

The hypothesis of the existence of different types of *S. mentella* (Magnusson *et al.*, 1995) is supported by others based their opinion in three main criteria:

1. Length compositions
2. Length at maturation
3. Infestation of parasites

In addition, two other criteria were used: the intensity of red color on the fish body and the thickness of the neck part. However, we considered these two characters too subtle and usually connected with ecological and age specific features of the fish.

These three criteria suggested to separate out “types” of redfish have been recently examined in the Irminger Sea (Bakay, 2000; Bakay and Melnikov, 2001). These studies showed close similarity in linear and weight growth rates and maturity rates of *S. mentella*; the same peculiarities of *S. mentella* infestation with copepodite *Sphyrion lumpi* and pigment patches, and the same extent of infestation with other parasite species were revealed. These data indicated the same dwelling conditions of *S. mentella* and provided evidence for a single origin and integrity of *S. mentella* type occurring in the upper (0-500 m) and lower (500-1000 m) layers. *S. mentella* concentrations in the area of the Reykjanes Ridge deeper than 500 m are formed due to partial redistribution of young individuals from the upper layer and leaving most of the older redfish at greater depth. Similar changes in size-age composition of fish with depth are inherent in other deepwater fish species in the North Atlantic, particularly in rock grenadier (Savvatimsky, 1992a) and roughhead grenadier (Savvatimsky, 1992b). In our opinion the concentrations of *S. mentella* of older age distributing during feeding in depths greater than 500 m are a deepwater component of the single *S. mentella* population but not a separate population of *S. mentella*.

The general overview of the size range distribution of *S. mentella* is shown in Figure 2.9. The majority of oceanic *S. mentella* ranges between 30 and 40 cm in length, with a mean of 35–36 cm, rather constant over years. In Icelandic *S. mentella*, sizes ranged from 30 to 45 cm, with several modal groups present in different years (35 cm in 1995–1997, 40–42 cm in 1989–1994; Sigurðsson, 1998). Pelagic deep-sea *S. mentella*, however, is known to have a bigger size range, between 36 to 46 cm, with a mode around 42 cm (Figure 2.9).

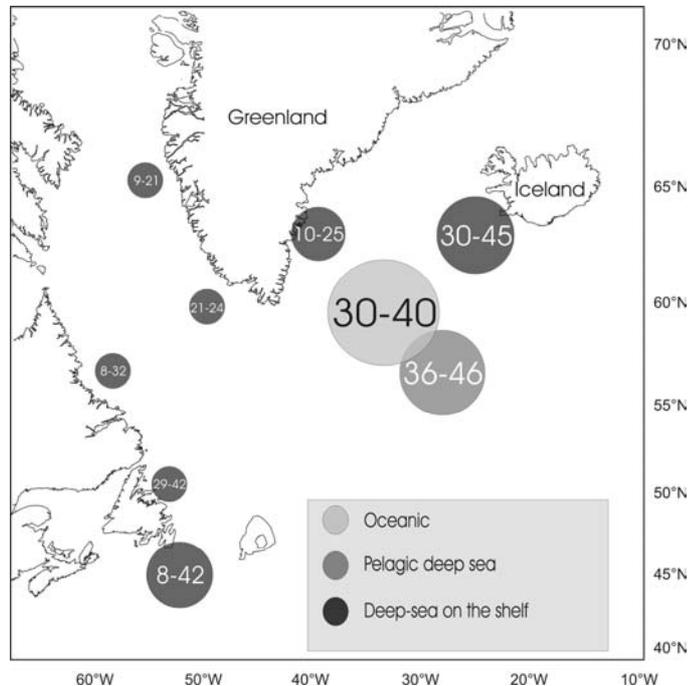


Figure 2.9. *S. mentella* adult distribution with the size range (fish total length in cm) for each area. Size is referred to the most abundant fish, not maximum and minimum values. Different redfish types are shown with different grey colours.

Analysis of spatial structure of *S. mentella* population in the pelagic Irminger Sea and adjacent waters in summer indicated the complicated structure of *S. mentella* concentrations showing high spatial and vertical variability in the size-age and sex composition and in ratio of mature and immature individuals (Melnikov and Bakay, 2002). Such variability in this part of the North Atlantic is caused by ontogenetic and geographic differences between the areas where juvenile and mature redfish dwell as well as by the fact that with growth *S. mentella* tend to change ecological conditions of their habitat. The relationship between mature and immature parts of the population is achieved through return migration of *S. mentella* reaching maturity from their habitat areas to the areas where mature fish dwell and spawn, that is from the shelf and slope of Greenland to the pelagic waters of the open sea (Pavlov *et al.*, 1989a; Melnikov *et al.*, 2001). It was found that in the upper 500-m layer the largest individuals of 35–39 cm long inhabited the central part of the feeding area. On the margins, mean length of *S. mentella* is 3–6 cm less (Figure 2.10 A). At depths below 500 m large individuals being predominantly of 40–46 cm in length occurred mostly in the northern areas of the Reykjanes Ridge. South of 55°N in the Reykjanes Ridge area, mean length of *S. mentella* was estimated at 34–37 cm (Figure 2.10 B). The study did not reveal any isolated age groups of *S. mentella* over the whole area of their concentrations. Spatial variability of size composition is not an evidence for different populations of *S. mentella* in the pelagic waters of the Irminger Sea but is caused by a number of factors. First, it is the change of life stages when with

growth *S. mentella* tend to change the ecological conditions of their habitat. Second, seasonal migrations of the redfish, when over the distribution area of the population, concentrations of *S. mentella* move actively throughout a year.

A gradient in the depth distribution in relation to the size is very well known in many marine fish species. Particularly, in redfish, it has been extensively described that fish move to deeper waters as they grow (Atkinson, 1986). Moreover, the migration to deeper waters is more related with maturation than with size (Saborido-Rey, 1994), but since maturation is correlated with size, the overall picture is that bigger fish migrate to deeper waters. At the same size, immature fish inhabit shallower waters.

Most of the oceanic and pelagic deep-sea *S. mentella* are mature fish, but it has been argued that pelagic deep-sea *S. mentella* reach maturity at a bigger size, about 38 cm, than oceanic type, around 31 cm (Magnússon and Magnússon, 1995). In both cases, the estimated size at 50% maturity is rather close to the minimum size recorded in the area for each type, so it remains unclear if such differences are a statistical artifact of the different size distribution. However, recent studies showed that *S. mentella* mature at both depths (less and greater than 500 m), displaying the same length at maturity of 37 cm in males and 38 cm in females (Bakay and Melnikov, 2001). Immature fish are more common in shelf deep-sea *S. mentella* on the Icelandic shelf, and size at 50% maturity is slightly bigger than for oceanic *S. mentella*, around 34 cm. Interestingly, if oceanic and pelagic deep-sea *S. mentella* were pooled, the resulting size at 50% maturity between *S. mentella* from the open Irminger Sea and those off Icelandic is very similar. In addition, the proportion of immature fish increases towards south and east of the Irminger Sea in the proximity of Greenland, which is consistent with the idea and data presented above of Greenland being the nursery area of *S. mentella*.

This idea is also supported by the fact that the decrease of linear sizes was observed in the direction of Greenland during the International Trawl Acoustic Survey carried out in 2001 (Sigurðsson *et al.*, 2001; Melnikov and Bakay, 2002) as well as in previous surveys (Pedchenko *et al.*, 1997). The comparative analysis of length compositions above and below 500 m, reveals that lengths of *S. mentella* were practically equal in the Southern and Eastern part of the Irminger Sea, close to Greenland. But, differences occur above Reykjanes ridge, close to Iceland, *i.e.* the main spawning area.

Ageing of *S. mentella* in this area has been conducted only sparsely. However, there is an agreement that these redfish are quite old, and older than redfish at the same size from other populations, *e.g.* Barents Sea, Flemish Cap (Saborido-Rey *et al.*, 1997; Stransky *et al.*, 2005b,c). Generally, it can be said that there could easily be 30 year classes living in the Irminger Sea at the same time (with a length range of 30 to 45 cm). During the period from 1995 to 2001 *S. mentella* was aged (Melnikov and Bakay, 2002), showing that, in general, the spatial and vertical distribution of the age composition fully reflects the dynamics of the length composition of fish. In the area of Iceland redfish at the age of 17-22 predominated in catches. Southwards in the open Irminger Sea the age of fish in concentrations decreased, and redfish at the age of 12-20 predominated. Within Greenland EEZ a further decrease in mean age is noted, and the presence of young redfish (6-10 years) is obvious (Figure 2.11).

Younger *S. mentella* are, in practice, exclusive of this area. Nevertheless, ageing of *S. mentella* from the East Greenland shelf and the Irminger Sea from samples collected during the observed migration of juveniles from the nursery grounds into the pelagial (Stransky, 2000) showed that these fish were of similar age (Stransky *et al.*, 2005b).

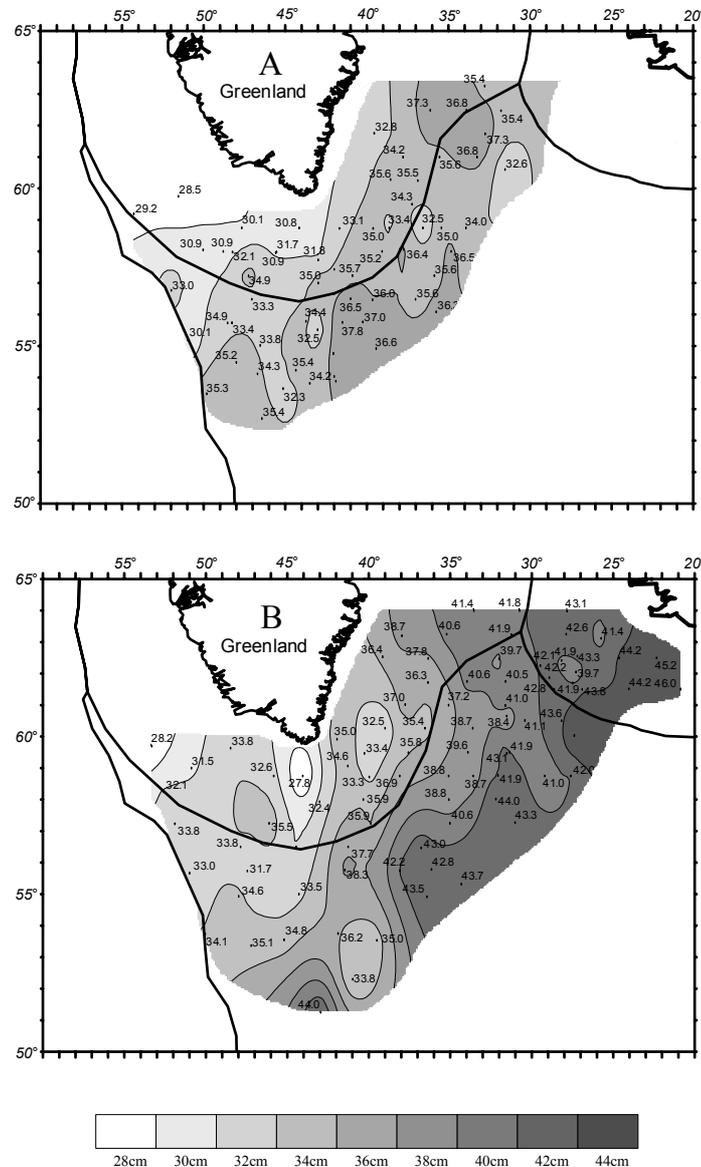


Figure 2.10. Mean length of redfish in catches taken in 0-500 m (A), 501-1000 m (B) layers by the results from international TAS in 2001.

In the Irminger Sea, a large number of year-classes occur, and between the youngest and the oldest fish, 20-30 years difference may exist. The life history of each cohort may be different due to, for example, different environmental conditions, affecting growth, maturation and parasite infestation features. Thus, during the 1980s, the occurrence of *S. mentella* larger than 30 cm was common off East Greenland (Figure 2.7) and rare in the Irminger Sea, while the opposite trend was observed in recent years. The behavior of different year classes (distribution, migration pattern, etc.) may also explain some of the recorded differences. Recent analyses of extensive time series data conducted by Bakay and Melnikov (2002) revealed the inaccuracy of the hypothesis of a considerable difference in infestation by *S.*

lumpi oceanic and pelagic deep-sea *S. mentella*, i.e. the upper and lower layers (Magnusson *et al.*, 1995). Slightly lower infestation by alive *S. lumpi* was registered at greater depths and larger fish, i.e. fish that may correspond to the pelagic deep-sea type. However, a detailed inspection showed underestimation of remains of the copepod presence, remaining on redfish body for a long time. Thus, the overall infestation rate is similar above and below 500 m and among length classes. Larger depths are known to be inappropriate for *S. lumpi* survival (Squires 1966; Pedchenko 1992). This may explain the earlier reported differences in infestation when only external presence was considered. Similarly, the pigmented patches, also used to differentiate the *S. mentella* types, seem to be more related to growth dynamics, feeding regimes and/or fish survival rate (Bakay and Melnikov, 2002).

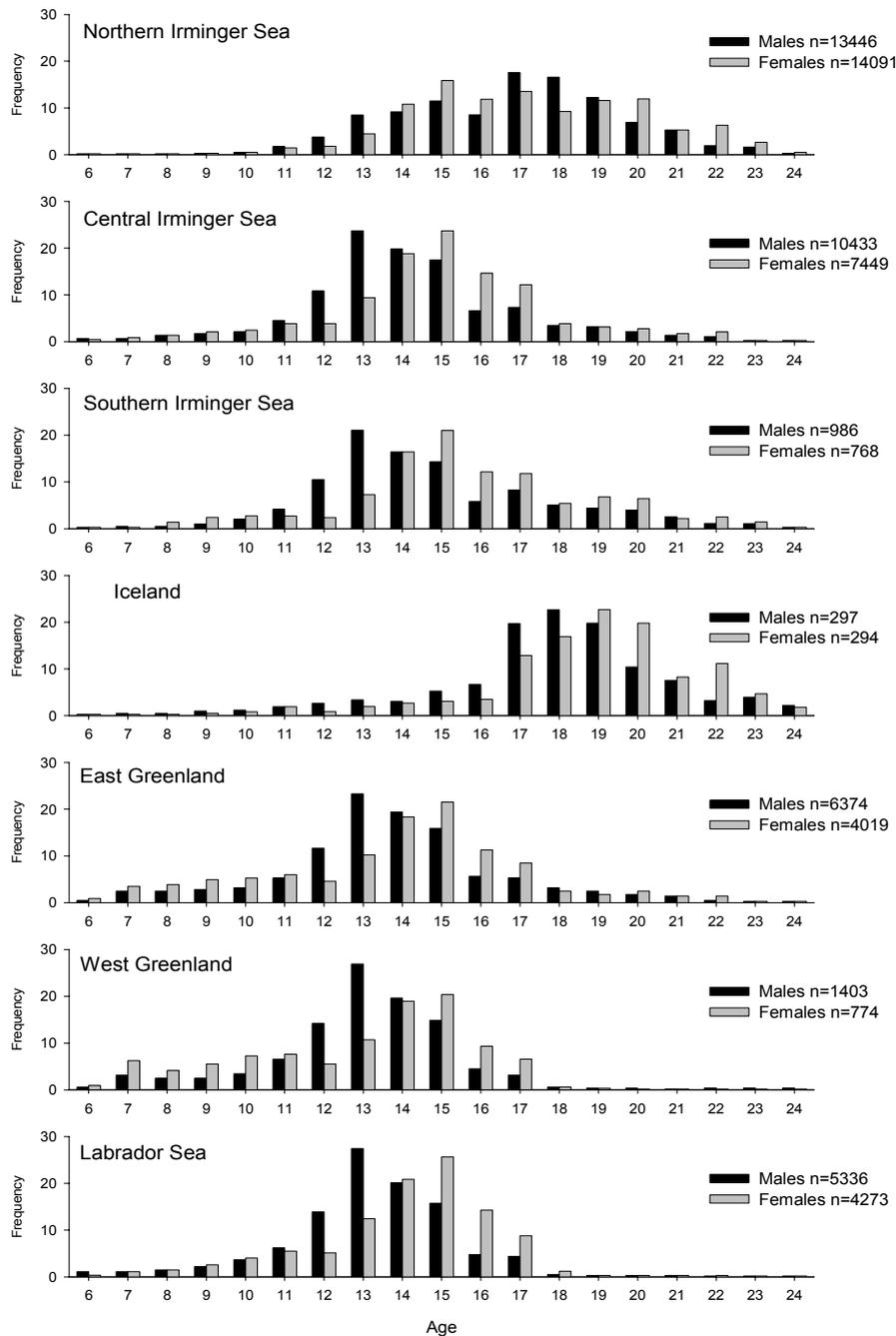


Figure 2.11. Age composition of redfish within the NEAFC and NAFO Regulatory Areas in June-August, 1995-2001

Most authors agree with the concept that *S. marinus* off Greenland and Iceland are the same population, declaring East Greenland as the main nursery area, although not the only one. Both shelf deep-sea *S. mentella* off Greenland and Iceland is believed as belonging to the same population. Pelagic deep-sea *S. mentella* resembles to shelf deep-sea *S. mentella* regarding color, morphology, infestation rate and maturity, and many authors believe that they both are the same population. The life cycles of both species, *S. marinus* and *S. mentella* are similar. No genetic differences have been found between Greenland and Icelandic *S. marinus* (Nedreaas *et al.*, 1994), but significant differences have been described between shelf deep-sea *S. mentella* and pelagic deep-sea *S. mentella* although only Icelandic *S. mentella* has been compared (Johansen *et al.*, 2000). From an ecological point of view, it is not possible to explain the existence of different populations of *S. mentella* in the Irminger Sea, while only one population of *S. mentella* and *S. marinus* is assumed to occur on the shelves.

2.6. GENETIC DIFFERENTIATION

In recent years, an increasing effort studying genetic discrimination of the redfish species and stocks has been made (Johansen *et al.*, 1996, 1997, 2000; Daniëlsdóttir and Jónsdóttir, 1999; Roques *et al.*, 1999a, 1999b, 2000, 2001, 2002; Daniëlsdóttir *et al.*, 2005; ICES, 2005 and references therein). Several techniques have been used (haemoglobin, allozymes, mtDNA, nDNA, etc.). The results of these studies do not show a clear pattern regarding stock structure of redfish, and in most of the cases they yield contradictory results. The discrimination of the *S. mentella* types is supported in some cases by the haemoglobin and allozymes analyses (Johansen *et al.*, 1996, 1997, 2000). However, the studies made in the mid-1980's in the depth range of 0 m to 500 m in the Irminger Sea pelagic waters did not reveal any genetically isolated groups of *S. mentella*. Besides, no signs of crossing with closely-related redfish species were observed (Dushchenko, 1986). In addition, allozyme analysis indicated genetic differences with age for samples collected from the Irminger Sea (Stroganov and Novikov, 2005). Regarding the molecular genetic studies conducted on microsatellites and mt-DNA, some of these studies show the presence of genetic structure in the Irminger Sea and adjacent waters (Daniëlsdóttir and Jónsdóttir, 1999; Daniëlsdóttir *et al.*, 2005; ICES, 2005) while others show lack of genetic differences in this area (Roques, 2002; ICES, 2005). In general, this studies revealed lack of genetic isolation by geographic distance, a very complex resulting structure and genetic differences among *S. mentella* types much smaller than those observed in *S. marinus* in the same area, currently considered as a single stock (ICES, 2005).

As explained above, redfish is a long-lived species with a particular life history in the Irminger Sea. Special attention has to be paid to the fact that many different cohorts, probably more than 20-30, are involved in the spawning fraction of the population. More important is the fact that larvae are pelagic and spread across a wide geographic area.

In cod, growth differences between genotypes have been shown (see Imsland and Jónsdóttir, 2003 and references therein). Thus, it is evident that there can be a link between genotypes at certain loci and important life parameters as growth. Growth can significantly

influence life history of the fish, for example affecting the survival rate. In long-lived and slow growing species such as redfish, differences in growth patterns can produce different survival rates, which can shift allelic structure in the adult population. Other studies in wild populations have indicated that some loci are affected by natural selection which appears strong enough to change allele frequencies within and between generations (Jamieson, 1975; Mork and Sundnes, 1985). In other words, to serve as reliable population markers, allele frequencies should be stable within and between generations. Loci that are detectably affected by selection are not expected to fulfill this requirement.

Hedgecock (1994a) suggests that genetic heterogeneity on microgeographic scales results from temporal variation in the genetic composition of recruits. An even more widespread observation is that very slight but significant and persistent heterogeneities of allelic frequencies have frequently been observed on microgeographic scales, embedded within the large regions over which dispersal maintains an otherwise high level of genetic similarity (Hedgecock, 1994a).

Distinct genetic subdivisions can occur in continuously distributed species, particularly those spanning biogeographic boundaries. Examples of the latter from the California Current are presented for the barnacle *Balanus glandula* and the northern anchovy *Engraulis mordax* (Hedgecock, 1994a). Microgeographic heterogeneity holds interest for biological oceanographers and fisheries scientists because it contradicts the logic of population genetics as well as commonly held notions about the structure of zooplankton and marine fish populations. This temporal variation could be a consequence of either selection on larval populations or large variance in the reproductive success of individuals, owing to chance matching of reproductive activity with windows of oceanographic conditions conducive to fertilization, larval development, retention, and recruitment. In support of the latter hypothesis, effective sizes for natural oyster populations are estimated to be only small fractions of breeding population numbers (Li and Hedgecock, 1998).

In anchovy, genetic variance may be generated by processes governing reproductive success, larval survival and recruitment to first schools. And it does permit natural selection to act among groups as well as among individuals (Hedgecock *et al.*, 1994). Moreover, differences in genetic composition (in mtDNA) among samples of larvae, produced during a single spawning season by a semi-isolated population of Pacific oysters, have been described (Li and Hedgecock, 1998) and confirm a specific prediction of the hypothesis that marine animals have large variances in reproductive success. Other examples of long-term differences have been described in haddock (Purcell *et al.*, 1996), showing an inter-decadal heterogeneity in mtDNA from Georges Bank. The temporal aspect of population genetic structure forges a strong interdisciplinary bridge to oceanographic research aimed at elucidating the temporally and spatially varying factors affecting recruitment.

Allele frequencies, when measured over time as either replicate samples, or as different year classes, often show considerable variation (Gauldie, 1984; 1991). Thus, temporal variation in allele frequencies within populations is often as great as geographical variation in allele frequencies between populations (Gauldie, 1991). The significance and analysis of temporal and geographic variation in allele frequency depend to a large extent on the underlying

assumptions of either selection or genetic drift as cause of variation (Gauldie, 1991; Hedgecock, 1994b).

In other studies on genetic structure (Hedgecock, 1994a; Stepien, 1995), most of the genetic diversity was found among individuals within samples, and only a small amount was accounted for by differences among populations. The lack of geographic structure was attributed to high levels of gene flow via larval dispersal. Larval dispersion of Irminger Sea redfish is considerable, as shown above, and much higher than for redfish in other areas such as Flemish Cap, Gulf of Saint Lawrence or Barents Sea.

In the case of Irminger Sea redfish, there are no geographic borders among the suggested genetic types, and probably neither a reproductive isolation. These facts, together with a low temporal stability because of year class variation, leave little ecological or biological support of different populations.

In a long-lived species, with a relatively high fecundity, a population can be sustained by few but abundant year-classes, which can be mature for many years. In redfish, absolute fecundity is not high but because of viviparism offspring are more likely to survive, for this reason reproductive potential may be high (Saborido-Rey, 1994). Even if there is recruitment failure over the long term, the population can be sustained if the spawning stock produces few but strong year-classes. As a result, the population can be mostly formed by a few year-classes and usually with large age differences. Species exhibiting high longevity also have high levels of mitochondrial DNA mutations, e.g. sturgeons (Brown *et al.*, 1988) while species with low longevity also have low levels of mitochondrial DNA variation, e.g. albacore tuna (Graves and Dizon 1989). Further research will be necessary to determine the age dependency of mitochondrial DNA before its role in any kind of population analysis can be determined (Gauldie 1991). The observed genetic structure may reflect cohorts (no geographic isolation) derived from a sweepstakes chance effect in which a few adult spawners successfully contribute offspring each season (Hedgecock 1994b; Geiger *et al.*, 1997). This hypothesis has been already pointed for *Sebastes* species, both in the Atlantic (Schmidt and Trautner, 2005) and in the Pacific (Matala *et al.*, 2004). Redfish genetic research should be conducted taking into account the age structure of the population. In that sense, reliable age determinations and validation will be an important task to be solved.

Genetic structure of *S. mentella* populations across the North Atlantic have been also studied using microsatellites (Roques *et al.*, 2002), concluding with the definition of three distinct population units in the North Atlantic: Eastern (Barents Sea and Norway), Western (Gulf of St. Lawrence) and Panoceanic. Among the areas within the Panoceanic unit, the Irminger Sea, Greenland and Labrador Sea were included. These results are consistent with other studies of geographical variability of *S. mentella* across the North Atlantic, such as parasite fauna (Bakay, 2001) and the ecological analysis presented here.

2.7. CONCLUSIONS

In summary, the main features drawn from this review are:

1. The different *S. mentella* types were described as the fishery developed and moved to different areas and were not based on any relevant biological feature. Criteria suggested by some researchers in the mid-1990's to separate out *S. mentella* types have no biological grounds and cannot be used for the stock management. When dealing with the management issues the term "stock" is equivalent to the "population" term in its scientific sense.
2. The ecology of *S. mentella* across the studied area supports that:
 - There is a single spawning area (East/central Irminger Sea)
 - Irrespective of time and depth of spawning, the extruded larvae drift towards the slopes of Greenland where their intermingling and settling occur. Thus, during spawning, an exchange of gene pool among *S. mentella* individuals dwelling in different habitat areas of the population takes place. The study did not reveal any isolated reproductive groups of *S. mentella*.
 - The nursery areas of *S. mentella* are located in the West and mainly in the East Greenland forming a single nursery area.
 - There are indications of a migration of *S. mentella* juveniles from the nursery into the adult distribution areas (mainly Irminger Sea, Iceland).
3. Some studies have shown genetic differences among types, but the observed genetic heterogeneity can be explained by other causes than the existence of different populations. Recent studies demonstrated, however, a prevailing pattern of genetic homogeneity.
4. Spatial and vertical variability of different biological parameters over the Irminger Sea and adjacent waters is determined by the fact of functional sub-units within the habitat area of *S. mentella* population, the change of ecological conditions during their life cycle and active seasonal migrations, but not by the existence of different types /stocks of *S. mentella*.

In conclusion and given these indications, we are of the opinion that it is more reliable the existence of a single stock of *S. mentella* in the Irminger Sea, around Iceland and off Greenland. However, more research has to be conducted to understand the mechanisms of redfish reproduction, recruitment, drift and migration. It is crucial that genetic studies should be conducted taking into account the age structure of the population. It is also essential to understand the relationship between the different components and functional units described for this species in the studied areas and to address the question of population structure from a holistic approach.

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3. MATERIAL AND METHODS

3.1. MATERIAL

Landmark coordinates and meristic data for this study were taken from 6,764 fish, following the morphologic protocol developed by the author as part of this work. The complete protocol is presented in Annex I. The main reason to develop this protocol was to coordinate data collection in the two laboratories participating in the morphological study within the Redfish project, i.e. the Institute of Marine Research in Vigo, Spain (IIM) and the Marine Research Institute in Reykjavik, Iceland (IMR). 2,776 fish were fully processed by the author at IIM while 3,988 were processed in Iceland to obtain the rough data, i. e. the landmark coordinates. In addition, measurements from 549 redfish previously used by Saborido-Rey (1994) were also included in the analyses. All of them add up to 7,313 individuals that have been used for morphometric and meristic analysis.

The collection of samples followed a general agreement among the researchers involved in the REDFISH project regarding the optimum number of samples to be collected by species, year, season and area and subarea, providing, thus, good spatial and temporal coverage. To achieve this, the Irminger Sea and adjacent waters were divided into four different areas, corresponding to four putative stocks, i.e. Faroes, Iceland, Irminger Sea and Greenland, each of them divided into different subareas (Figure 3.1).

The fish analyzed by the author were sampling in 11 surveys during 2000, 2001 and 2002, part of them obtained in close cooperation with the IEO (Spanish Institute of Oceanography) Coastal Center of Vigo, through the Observers Program. The data produced in Iceland were taken during the same years, 2000, 2001 and 2002, from samples obtained in 36 surveys (Table 3.1). The separation by species of all of them yield a total of 1,993 *S. marinus*, 79 *S. viviparus* and 4,692 *S. mentella*. Sampling was planned to cover the whole area and all the years, and to be especially extensive for *S. mentella* in the Irminger sea. *S. marinus* and *S. viviparus* individuals ordered by area, year and quarter are presented in Table 3.2, and Table 3.3 shows *S. mentella* individuals. The samples from the Faroe Islands and from Greenland were obtained only from research surveys, and thus are limited to the time where the surveys were carried out. In contrast, the samples from Iceland and the Irminger sea were not only from research surveys but also from fishing vessels, and thus are better distributed over the year. A special effort was put on Irminger Sea sampling (Table 3.4 and Table 3.4) because the study of the two types (oceanic and deep sea) described for *S. mentella* in the area was one of the main subjects of the Redfish project.

In the early 90's, earlier research by Saborido-Rey on 11 populations of *Sebastes*, used traditional morphometry. The results showed good discrimination among the populations studied (Saborido-Rey, 1994). The potential use of these data was as a reference for better understanding of the results obtained in the current study, and also as an example of the validity of the techniques. Among the 11 populations, the closest to the areas analyzed were Flemish Cap and Norway (Figure 3.1). Thus, 549 fishes, 315 from Flemish Cap and 234 from Norwegian waters, were incorporated. The four *Sebastes* species in the Atlantic are represented in those two areas of reference (Table 3.5). From those individuals, only

distances between landmarks are available, and therefore, Norway and Flemish Cap could not be included in geometric morphometric analyses, as explained below. Flemish Cap samples were taken in four of the Flemish Cap series of research cruises supported by the European Union (Vázquez, 1990, 1992, 1993 and 1994). Flemish Cap is a bank which on the basis of bottom topography and the strong associated currents, is considered somewhat isolated. Its isolated position, which makes genetic exchanges less probable, combined with wide knowledge of the genus in the area, product of the 15 years series of research cruises, made Flemish Cap a 'guide' to distinguish *Sebastes* species. Norwegian samples were taken during two research cruises, in October 1990 and March 1993. For sampling details of these specimens, see Saborido-Rey (1994).

The distribution of the samples by sex and length was well-balanced for all species. *S. fasciatus* and *S. viviparus* (Table 3.6) are smaller, and thus present a range of lengths shorter than *S. marinus* (Table 3.7) and- *S. mentella* (Table 3.8). In Greenland, the main nursery area for *S. marinus* and *S. mentella*, many individuals were smaller than 200 mm. Finally, the numbers of fish used in each of the three data analysis techniques, that is, Traditional morphometrics, Geometric morphometrics and Meristics, is presented in Table 3.9, Table 3.10 and Table 3.11 respectively. A large number of individuals from Iceland (1,192) could not be used in traditional morphometrics, but only for geometric morphometric analyses; those individuals from Norway and Flemish Cap, the reference areas, could only be used in traditional morphometrics analyses.

In summary, a high quantity of samples were taken from a wide area in the North Atlantic, and data from fish of both sides of the Atlantic were combined in the analyses. A map showing the areas sampled for *S. mentella* is presented in Figure 3.2, while Figure 3.3 shows where the other three species were sampled.

All individuals were randomly sampled and frozen onboard, with special care to keep the natural position of the fish, to avoid distortions of shape.

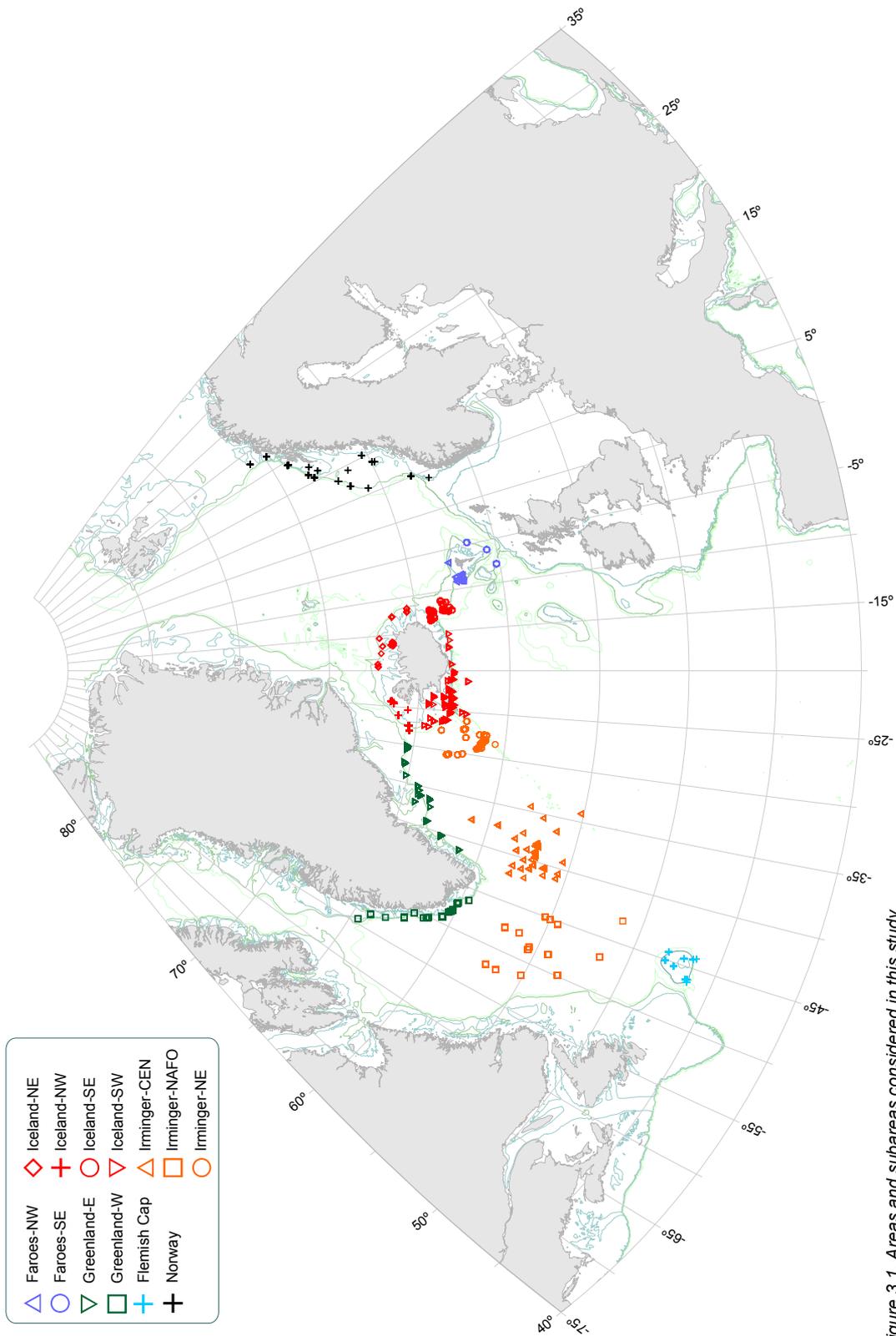


Figure 3.1. Areas and subareas considered in this study

Table 3.1. Cruises by area and year. Type of cruise (res: research; com: commercial); Gear (dem: demersal; pel: pelagic).

Area	Cruise identification	Type	Year	Depth interval	Gear
Faroes	FAER	res	2000	245-552	dem
	MH	res	2002	375-572	dem
Iceland	1277	com	2002	406-406	dem
	1351	com	2000	705-860	dem
	1369	com	2000	384-384	dem
	1376	com	2001	311-357	dem
	1880	res	2000	339-686	dem
	1902	res	2001	339-348	dem
	1972	res	2001	470-470	dem
	2165	res	2001	247-247	dem
	2170	res	2000	631-631	dem
	2182	res	2001	201-421	dem
	A12	res	2001	181-784	dem
	A42001	com	2001	109-276	dem
	A7	com	2000	183-566	dem
		com	2001	418-418	dem
	A9	com	2002	365-592	dem
	B13	com	2000	177-300	dem
		res	2001	114-415	dem
	B14	res	2002	349-349	dem
	B4	res	2000	137-137	dem
	B42000	res	2000	555-555	dem
	B42001	res	2001	308-467	dem
	B82000	res	2000	102-102	dem
	TBR1	res	2001	121-256	dem
	res	2002	552-552	dem	
TJ1	res	2000	138-438	dem	
TL1	res	2000	122-380	dem	
	res	2001	219-421	dem	
	res	2002	129-608	dem	
Greenland	WH221	res	2000	219-380	dem
	WH233	res	2001	120-770	dem
Irminger	1273	com	2001	790-790	pel
	1308	com	2001	720-768	pel
	1579	com	2001	752-752	pel
	1833	com	2000	695-695	pel
		com	2001	686-686	pel
	1868	com	2002	185-185	pel
	1902	com	2001	724-724	pel
	2170	com	2001	194-641	pel
	2203	com	2001	280-306	pel
	2350	com	2001	655-655	pel
	A4	com	2002	710-710	pel
	A8	res	2001	225-775	pel
	B82001	res	2001	225-650	pel
	IR1102	res	2002	670-805	pel
	IR2101	com	2001	725-872	pel
	IR2102	com	2002	435-440	pel
	IR3100	com	2000	727-827	pel
	IR3101	res	2001	400-415	pel
	IR3200	com	2000	368-775	pel
	IR4101	res	2001	353-428	pel
IR7100	com	2000	420-459	pel	
WH229	com	2001	159-550	pel	
Flemish Cap	FC89	res	1989	306-306	dem
	FC91	res	1991	145-324	dem
	FC92	res	1992	387-577	dem
	FC93	res	1993	327-327	dem
Norway	NOR90	res	1990	125-900	dem
	NOR93	res	1993	74-983	dem

Table 3.2. Total numbers of *S. marinus* and *S. viviparus* from Faroes, Greenland and Iceland by, area, year and quarter.

<i>S. marinus</i> and <i>S. viviparus</i>					
Year	Quarter	<i>S. marinus</i>			<i>S. viviparus</i>
		Faroes	Greenland	Iceland	Faroes
2000	1			223	
	2			131	
	3	93	156	30	
	4	19		149	
2001	1			522	
	2			59	
	4		239	161	
2002	1			71	
	3	69			79
	4			71	
Total				1993	79

Table 3.3. Total numbers of *S. mentella* from Faroes, Greenland Iceland and Irminger Sea, by area, year and quarter.

<i>S. mentella</i>						
Year	Quarter	<i>S. mentella</i>				Total
		Faroes	Greenland	Iceland	Irminger	
2000	1			175		175
	2			11	267	278
	3	97	101		251	449
	4		77	271	92	440
2001	1			133		133
	2			33	591	624
	3				756	756
	4		873	182	83	1138
2002	1			163		163
	2				241	241
	3	131			36	167
	4			101	27	128
Total		228	1051	1069	2344	4692

Table 3.4. Irminger Sea *S. mentella* by sub-area, phenotype (deepsea and oceanic) and year.

Irminger Sea					
Sub-area	year	<i>S. mentella</i>			Total
		Deep-sea	Oceanic	Undef	
Irminger-CEN	2000	27	83		110
	2001	292	368	67	727
	2002	11	51	1	63
Irminger-NAFO	2000	6	86		92
	2001	135	124		259
Irminger-NE	2000	219	187	2	408
	2001	401	28	15	444
	2002	177	56	8	241
Total		1268	983	93	2344

Table 3.5. Numbers of individuals by species in the areas of reference, Flemish Cap and Norway.

Flemish Cap and Norway					
Area	<i>S. marinus</i>	<i>S. mentella</i>	<i>S. viviparus</i>	<i>S. fasciatus</i>	Total
Flemish Cap	102	111		102	315
Norway	64	75	95		234
Total	166	186	95	102	549

Table 3.6. Total numbers of *S. fasciatus* and *S. viviparus* by area, sex and length.

<i>S. fasciatus</i> and <i>S. viviparus</i>					
Sex	Length (mm)	<i>S. fasciatus</i>		<i>S. viviparus</i>	
		Flemish	Faroes	Norway	
Males					
	<100				15
	100-150		1		12
	150-200		32	16	8
	200-250		18	8	3
	Total		51	24	38
Females					
	<100				8
	100-150				10
	150-200		20	13	20
	200-250		7	39	8
	250-300		2	3	1
	300-350		1		
	Total		30	55	47
Unknown					
	<100				7
	100-150				1
	150-200		11		1
	200-250		8		1
	300-350		1		
	350-400		1		
	Total		21		10
Grand total			102		174

Table 3.7. Total numbers of *S. marinus*, by area, sex and length.

<i>S. marinus</i>							
Sex	Length (mm)	Faroes	Flemish	Greenland	Iceland	Norway	Total
Males							
	<100			4			4
	100-150		2	15	4		21
	150-200		4	23	12		39
	200-250	1	8	31	66	4	110
	250-300	16	9	46	223	8	302
	300-350	21	6	32	199	15	273
	350-400	26		36	103	7	172
	400-450	15		6	18		39
	450-500	2		1	3	1	7
	500-550				1		1
	550-600				1		1
	Total	81	29	194	630	35	969
Females							
	<100			2	2		4
	100-150			15	5		20
	150-200		2	20	8		30
	200-250	3	6	31	78	2	120
	250-300	8	1	53	174	6	242
	300-350	15	3	35	238	7	298
	350-400	21		38	130	8	197
	400-450	33		5	30		68
	450-500	17		1	6		24
	500-550	2			2		4
	650-700				1		1
	700-750				1		1
	Total	99	12	200	675	23	1009
Unknown							
	<100				3		3
	100-150		4				4
	150-200		25		1		26
	200-250	1	20		13	2	36
	250-300		4		31		35
	300-350		7		36	3	46
	350-400		1	1	24		26
	400-450				4	1	5
	Total	1	61	1	112	6	181
Grand total		181	102	395	1417	64	2159

Table 3.8. Total numbers of *S. mentella* by area, sex and length.

<i>S. mentella</i>								
Sex	Length (mm)	Faroes	Flemish	Greenland	Iceland	Irminger	Norway	Total
Males								
	<100			30	2			32
	100-150			231	2			233
	150-200		27	202	9	13		251
	200-250		10	91	63	131	2	297
	250-300	12	14	19	135	540	16	736
	300-350	37	2	5	182	409	16	651
	350-400	42			132	160	1	335
	400-450	17			12			29
	450-500	1				1		2
	600-650					1		1
	Total	109	53	578	537	1255	35	2567
Females								
	<100			18	1			19
	100-150			173	1			174
	150-200		19	164	11	8		202
	200-250		12	79	58	117		266
	250-300	14	11	31	137	296	7	496
	300-350	15	7	2	140	438	25	627
	350-400	63			119	197	4	383
	400-450	27			22	3		52
	500-550					1		1
	800-850				1			1
	Total	119	49	485	490	1060	36	2221
Unknown								
	100-150			1	2			3
	150-200			4	2	1		7
	200-250		8	1	14	1		24
	250-300		1		17	3	2	23
	300-350				6	9	2	17
	350-400					14		14
	400-450				1			1
	700-750					1		1
	Total		9	6	42	29	4	90
Grand total		228	111	1051	1069	2344	75	4878

Table 3.9. Numbers of fish by subarea, species and phenotypes used for traditional morphometric analysis.

Traditional morphometric analysis								
Subareas	<i>S. marinus</i>	<i>S. mentella</i>				<i>S. viviparus</i>	<i>S. fasciatus</i>	Total
	demersal	demersal	deep-sea	oceanic	undef	demersal	demersal	
Faroese-NW	110	172				60		342
Faroese-SE	66	53				19		138
Greenland-E	322	539						861
Greenland-W	65	483						548
Iceland-NE	47	50						97
Iceland-SE	371	467						838
Iceland-SW	254	276						530
Irminger-CEN			315	456	65			836
Irminger-NAFO			135	197				332
Irminger-NE			638	247	16			901
Flemish Cap	102	111					102	315
Norway	61	75				95		231
Total	1398	412	1088	900	81	174	102	5969

Table 3.10. Total numbers of fish by area, subarea, species and phenotypes used for geometric morphometric analysis.

Geometric morphometrics							
Subarea	<i>S. marinus</i>		<i>S. mentella</i>			<i>S. viviparus</i>	Total
	demersal	demersal	deep-sea	oceanic	undef	demersal	
Faroese-NW	113	172				60	345
Faroese-SE	66	53				19	138
Greenland-E	323	539					862
Greenland-W	65	485					550
Iceland-NE	121	51					172
Iceland-NW	136						136
Iceland-SE	375	474					849
Iceland-SW	762	513					1275
Irminger-CEN			321	493	65		879
Irminger-NAFO			135	197			332
Irminger-NE			758	268	22		1048
Total	1961	2287	1214	958	87	79	6586

Table 3.11. Numbers of individuals in the meristic analysis by subarea, phenotype and species.

Meristics						
Subareas	<i>S. marinus</i>	<i>S. mentella</i>				Total
	demersal	demersal	deep-sea	oceanic	undef	
Faroese-NW	148	141				289
Faroese-SE	61	59				120
Greenland-E	219	151				370
Greenland-W	46	63				109
Iceland-NE	122	54				176
Iceland-NW	136					136
Iceland-SE	382	484				866
Iceland-SW	772	528				1300
Irminger-CEN			330	502	68	900
Irminger-NAFO			141	210		351
Irminger-NE			797	271	25	1093
Total	1886	653	1268	983	920	5710

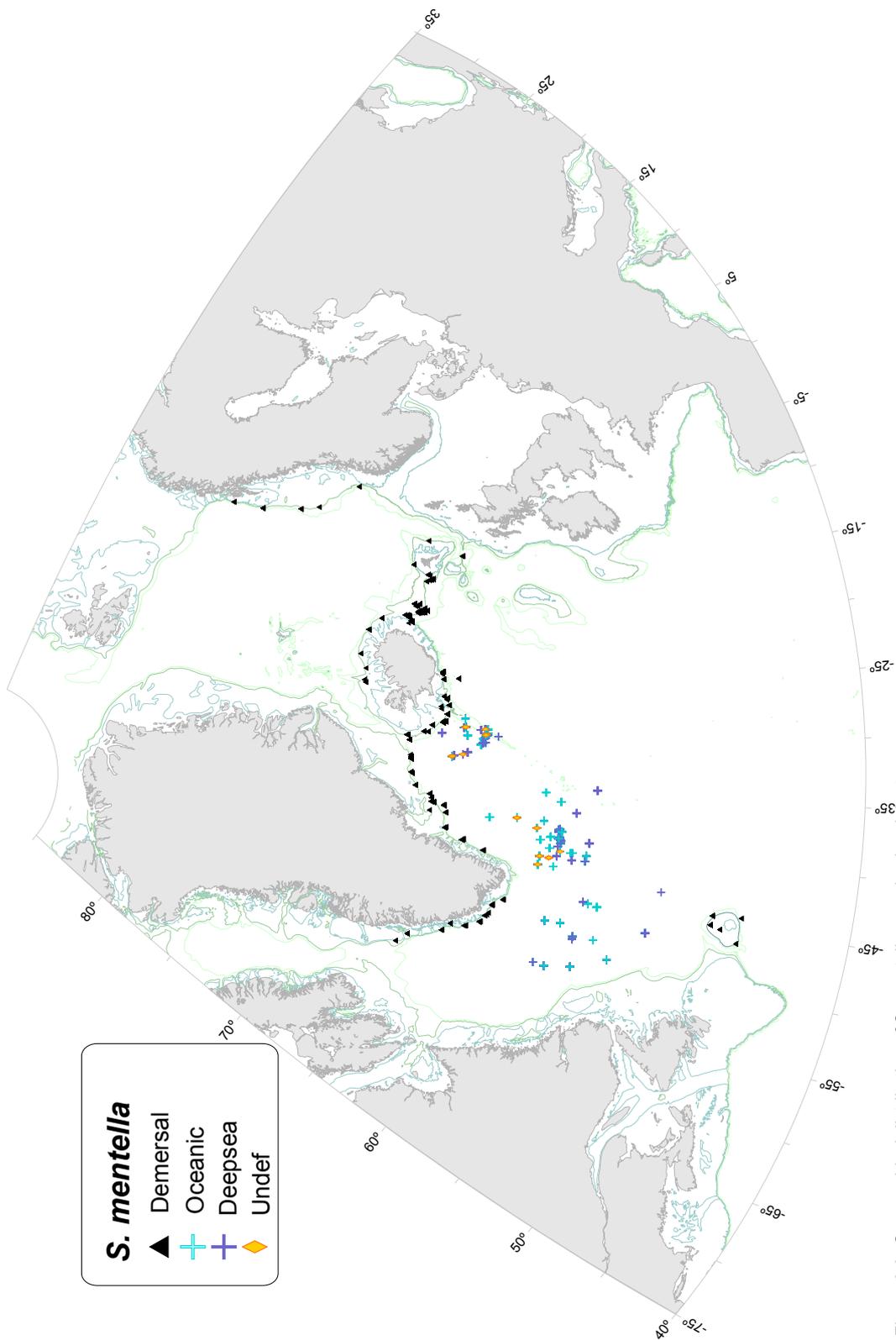


Figure 3.2. Geographical distribution of *S. mentella* samples by phenotype.

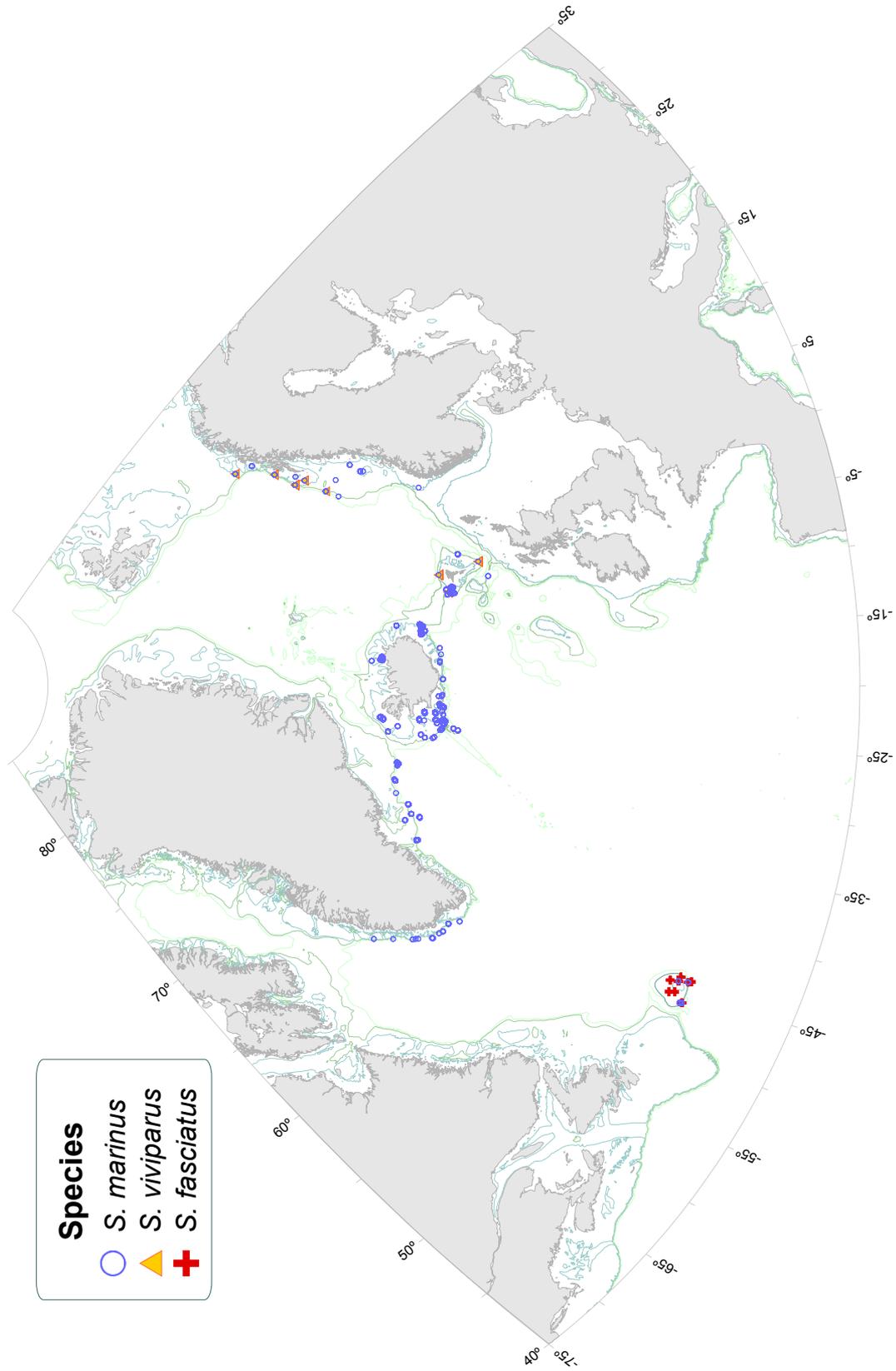


Figure 3.3. Geographical distribution of *S. marinus*, *S. fasciatus* and *S. viviparus* samples.

3.2. METHODS

This section is divided in two parts, the methods used in data acquisition (3.2.1), and the methods used in data analyses (3.2.2).

The data acquisition methods are presented in the same order as they were collected, starting from the frozen sample, and explaining how the samples were processed in the laboratory. A detailed explanation of the whole process is found in Annex I; the following is a summary, highlighting the most relevant features.

3.2.1. Data Acquisition

Since one of the main goals of the Redfish project was to compare results with different stock identification techniques, genetic samples were taken from a selected number of fish. Gill filaments, muscle and liver samples were taken from fish while they were still frozen, to avoid damage to the genetic material. These samples were taken from the right side of the fish, as the side used for morphometry was the left. Special care was taken to avoid modification of the fish shape.

Total length, preanal length and total weight of each fish were recorded prior to all analyses.

3.2.1.1. Acquisition of meristic data

During defrosting, meristic data were recorded, as they are taken from external features that can be measured before the fish is completely defrosted.

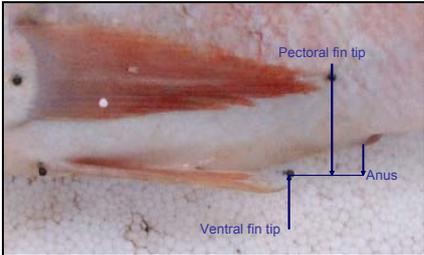
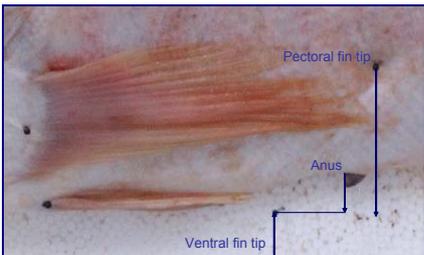
11 meristic variables were recorded (Table 3.12); A detailed guide on how the meristic variables were taken is given in Annex I. However, meristic variables other than ray-counting are explained in the following paragraphs.

Table 3.12. Meristic variables, with acronyms and description.

Meristic variables	
Acronym	Description
PPA	Pectoral fin position in relation to pelvic fin and anus.
RDF1	No. of first dorsal fin spines.
RDF2	No. of second dorsal fin soft rays.
RAF	No. of anal fin rays.
RPF	No. of pectoral fin rays.
RVF	No. of ventral fin rays.
A3S	Third preopercular spine angle.
A5S	Fifth preopercular spine angle.
GHO	No. of horizontal segment gill rakers.
GVO	No. of vertical segment gill rakers.
GTO	Total gill rakers.

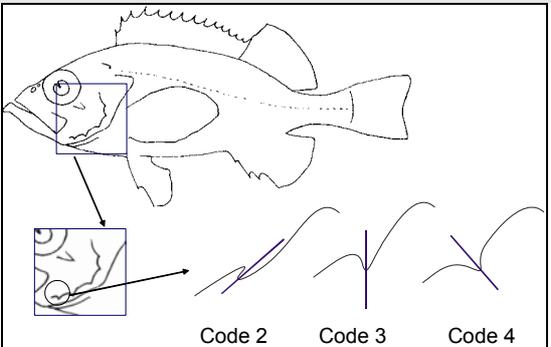
PPA: This is not a typical meristic variable, as it refers to the position of the pectoral fin tip in relation to the pelvic fin and anus. It is coded as explained in Table 3.13. The main reason for using this variable is that it has been used successfully in previous research on redfish (Saborido-Rey, 1994 and references therein).

Table 3.13. Description of the PPA meristic variable

PPA		
Code	Description	Examples
1	Pectoral fin tip does not reach ventral fin end	
2	Pectoral fin lies between ventral fin tip and 1/2 the distance between the end of the pelvic fin and the anterior part of the anus.	
3	Pectoral fin lies beyond 1/2 the distance between the end of the pelvic fin and the anterior part of the anus.	
4	It lies over the anus	
5	Goes beyond the posterior part of the anus	

A3S and A5S: These meristic characters refer to the angle of the third (A3S) and fifth (A5S) preopercular spines. They are coded as explained in Table 3.14.

Table 3.14. Codification of the A3S and A5S meristic variables

A3S and A5S codes		
Code	Description	Examples
1	Spine pointed forward	
2	Spine pointed down-forward	
3	Spine pointed downward	
4	Spine pointed down-backward	
5	Spine pointed backward	

GHO, GVO and GTO: These are the numbers of gill rakers in the horizontal (GHO) and vertical (GVO) gill branches, whilst GTO is the combination of both, i.e., the total number of gill rakers (Figure 3.4).

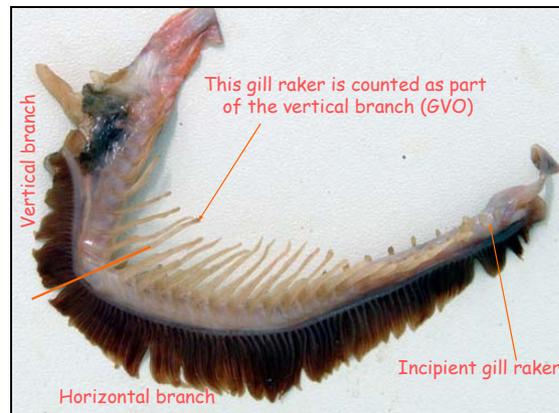


Figure 3.4. Gill rakers

RVF was excluded from the analysis because it gave the same value (5 rays) in all individuals. Gill raker measurements (GHO and GVO and hence GTO) were not taken in the samples collected at IMR in Iceland. Thus there are no data for this variable in the specimens collected around Iceland, and for Faroes, Greenland and Irminger Sea areas, data are only available in some individuals (those collected by IIM).

3.2.1.2. Acquisition of morphometric data

Once the genetic samples were removed, and the meristic data recorded, the next step was to obtain the morphometric data, that is, the coordinates (x,y) of the landmarks. The landmark coordinates were captured from digitized picture of the fish.

Taking the photo

The fish was placed on a polystyrene base, left side up, in such a way that all landmarks were visible in the photo. The perfect localization of the landmarks in the photo was guaranteed by marking each landmark with a black-headed entomological pin. A graduated rule was placed on the base where the fish lay, and subsequently used as a reference in the image calibration. A label with the fish information was added. Once the fish was prepared (Figure 3.5), the next step was to take the photo. Later, the landmark coordinates were recorded with the aid of image analysis software.

The principal advantage of digital images is the possibility to interchange them to control possible differences between laboratories. Image collections also permit checks for possible errors, and new landmarks can be added if necessary. This was impossible to do with the traditional methodology, which consisted of taking measurements directly from the fish with the aid of calipers, since fish were normally discarded after measurement. But digitalization also creates some problems, principally due to the loss of the third dimension.

However, during the present work, the principal problem to solve was derived from the introduction of data from the reference areas, Norway and Flemish Cap. These data had been taken directly from the fish with calipers, that is, taken in a 3 dimensional space, and

the question was how to make comparable the measurements taken from our digital images (2D) with those 3D measurements. Both problems are discussed below, and a more detailed explanation can be consulted in Annex II.



Figure 3.5. Details of one of the digital photos taken, where the fish and the 19 landmarks used for the morphometric analyses are displayed.

The digital cameras used to obtain the images were a NIKON D1X at the IIM and a Canon Power Shot G1 at the IMR. The cameras were attached to a column with a

sliding camera head. A focal length of 35 mm was used to avoid lens aberrations, i. e., pincushion and barrel distortions. The equivalence between the photos used in both laboratories was checked. In the following sections, both camera aberrations and differences due to the use of different material in each of the laboratories are further described.

The photo was captured and displayed in the moment on a connected PC. Thus, the picture was checked on the PC screen, to be sure that all the pins, the rule and the identification label were visible and readable. Thus, the shot could be repeated until the image was correct. The images were stored in the PC, and security copies were made.

The landmark coordinates were acquired from the digital images with the aid of image analysis software (AnalySIS, ® Soft Imaging System, GmbH at IIM and SigmaScan Pro5 in IMR).

Data were placed in an Excel spreadsheet, with a matrix format, the landmarks (variables) in columns and each fish (cases) in rows. For geometric morphometrics, the input variables for the set of analyses were the landmark coordinates, but traditional morphometrics uses the distances between landmarks as input. Therefore, interlandmark distances were calculated with a simple application of the Pythagorean Theorem and used as variables in traditional morphometrics. Subsequently, other variables used in the analyses as area, sex, species or phenotype were added to this matrix.

Landmarks and distances between landmarks

A total of 19 landmarks (Figure 3.6) were fixed on the fish. Description of the technique to accurately mark the points with pins is available in Annex I. The points were chosen in order to delimit a truss network, marking the broad features of the fish body. Other landmarks reflecting anatomical features traditionally used for redfish species identification, like fin lengths, eye diameter, snout length, etc. were added.

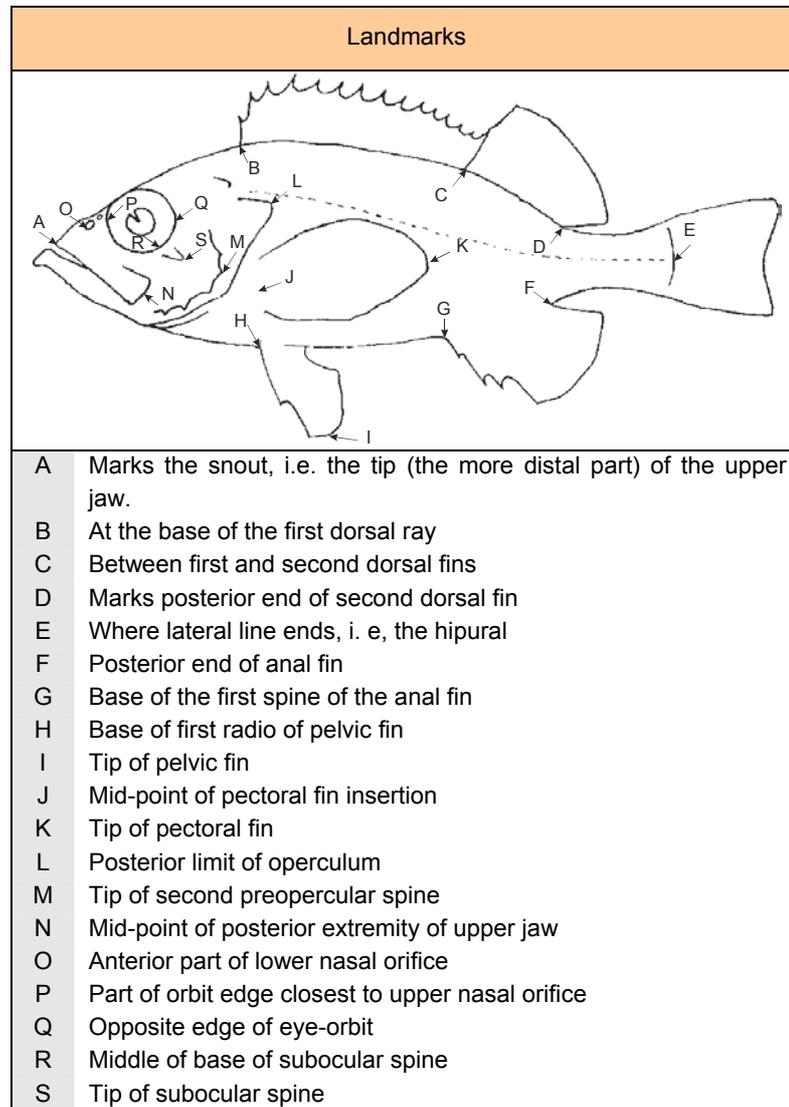


Figure 3.6. Location of the 19 landmarks on the fish.

Figure 3.7 shows the interlandmark distances used in traditional morphometrics. They are divided into those distances that conform with the truss network, those that mark head features, and fin lengths, and the two distances that, in the end, were not considered in the analyses, as explained below (section “The detection of outliers”).

mandible is mobile, it was stated in the morphologic protocol (Annex I) that the mouth of the fish had to be closed when fixing this point.

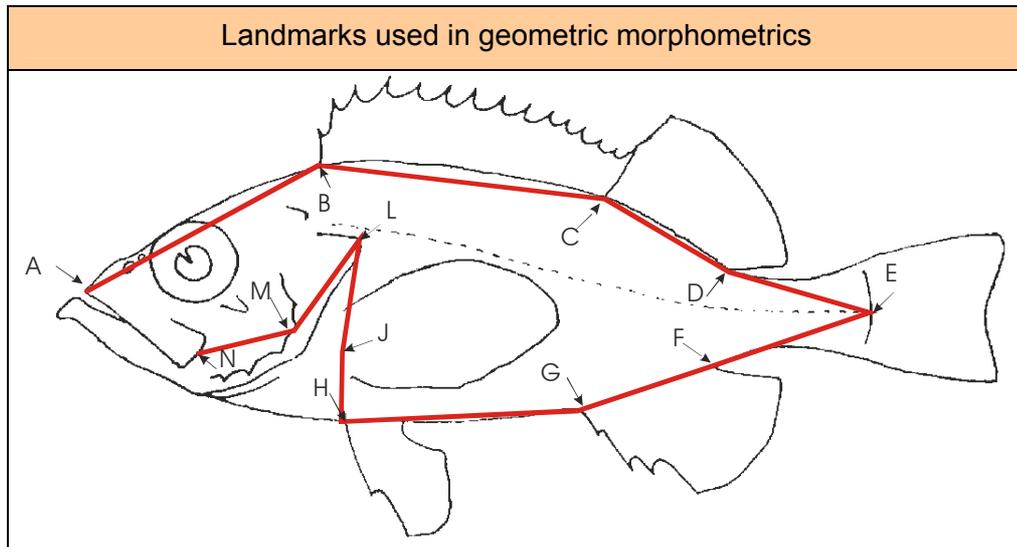


Figure 3.8. Landmarks used as variables in geometric morphometric analysis.

Problems associated with the use of digital images

Measuring distances or coordinates from images presents various problems: first, there are problems inherent to the camera and its appropriate use, and second, an image is bidimensional (2D) and cannot be used for metric measurements of the tridimensional (3D) world.

Cameras

The problems related to the camera are basically the distortions produced by the optics, the focal length effect or incorrect camera orientation. Optical distortion is due to the inherent aberration in bad quality camera lenses and only avoided with the use of a good quality camera. The focal length effects produce 'barrel distortion' and 'pincushion distortion' (Figure 3.9).

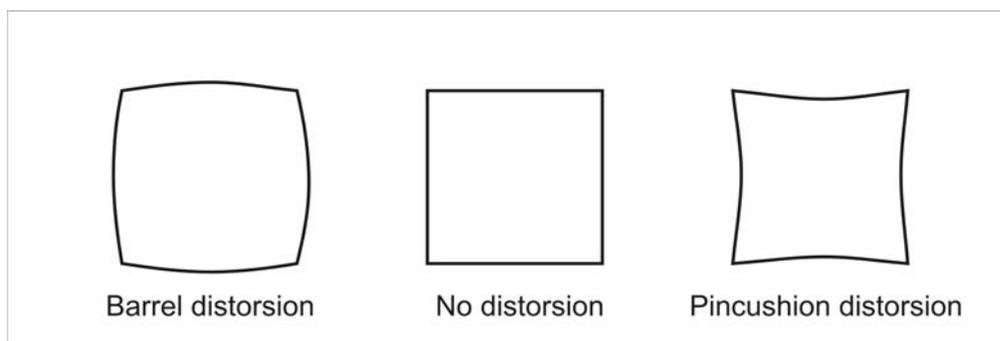


Figure 3.9. different distortions produced by the focal length.

Barrel distortion is a lens effect that causes images to appear 'spherical' at their centre. Some digital cameras suffer from barrel distortion at small focal lengths (wider angles: <35mm in traditional photography). Pincushion distortion is a lens effect which causes images to be pinched at their centre. Pincushion distortion is associated with zoom lenses or when adding telephoto adapters and only occurs at the telephoto end of a zoom lens with

long focal distance. It is most noticeable when there is a very straight edge near the side of the image frame. We can easily avoid barrel and pincushion distortion by using an intermediate focal length, that is, 50 mm in traditional photography, the same focal distance as the human eye. The CCD sensor in a digital camera is much smaller than a 35 mm negative. So, the focal length in a digital camera equivalent to the 50 mm in traditional photography, is calculated multiplying the CCD size by the "focal length multiplier", that is a value mentioned in the camera manual.

The camera used by the author at IIM was a Nikon D1X. The distortion was analyzed and it was concluded that no distortion existed using a focal length of 35 mm (equivalent to the 50 mm in traditional photography). A complete report of this distortion study can be found in Annex II. Similarly, the appropriate focal length was employed in the camera PowerShot G1 used at the IMR in Iceland.

The third and last source of distortion is the orientation of the camera. The camera had to be placed in an horizontal plane, parallel to the base were the fish lay, because if the camera is slanting, the image will show distortion.

2D images vs 3D objects

Distances taken on images (2D) are not, initially, comparable to real (3D) distances for several reasons related to the properties of perspective projection, scaling and foreshortening:

1. The distance from the focal plane to an object is inversely proportional to its size in the image, i.e. the objects closest to the camera look bigger in the image.
2. When a line or surface is parallel to the image plane, the effect of perspective projection is scaling.
3. When a line or surface is not parallel to the image plane, the term foreshortening is used to describe the projective distortion (i.e., the dimension parallel to the optical axis is compressed relative to the frontal dimension).

Most of the distances measured on the fish are lines not parallel but oblique to the focal (image) plane, i.e. the CCD. So, their projections on the image plane suffer 'foreshortening' distortion. This means that oblique distances measured directly on the fish with a caliper are always longer than the same distances measured on an image.

There are landmarks on the fish that are closer to the camera than others. The projection on the image will be inversely proportional to the distance to the camera. Thus, closer features will be magnified, and as the distance increase, the image size decreases.

Differences in small fish are smaller than in large fish, as the former are flatter. There can be differences even among distances taken on the same fish, as the head region is thicker than the tail, as is the case in redfish, and hence head landmarks are closer to the focal plane.

A method was developed to overcome, as far as possible, these problems, creating a calibration protocol, explained in the next section.

Calibration

Distances in an image are always measured in number of pixels. To convert pixels to metric units, such as millimetres, it is necessary to have a reference in the image of a known distance. Usually this is solved by introducing a ruler in the image to make the calibration. However, the position of the ruler is critical to estimate distances between landmarks, because the fish is not flat. If the calibration ruler is placed on the base, a distance in a higher position, such as the pectoral fin, will appear larger than its real value. On the contrary, if the ruler is in the higher position, distances that are close to the base, such as anal fin, will appear to be smaller. The calibration would not be a problem if all measurements were taken from images, because they would be comparable. But the introduction in the analysis of distances measured with calipers made it necessary to find a way to make new distances taken from the images as real as possible. A detailed report of the research conducted to choose the best calibration is presented in Annex II. However, the two methods selected are briefly described below.

Calibration with a ruler

As a first approach, five *S. mentella* were measured with calipers, and these real distances were then compared with the same distances taken from the image. For testing the best position for the ruler, it was placed at two different heights, i.e., on the base where the fish lies and at 3.5 cm height.

The results showed that some of the measurements were best calibrated with the ruler on the base, but others with the ruler in the higher position, the error always being too high for the measurements wrongly calibrated. Therefore, there was no optimum height to place the calibration ruler .

Calibration with D2D

One of the hypotheses developed in this work was the suitability of calibrating the image with a real distance taken from the fish itself, a distance in an intermediate position in the fish width, that might be valid for most of the distances. Initially, the D2D distance was chosen (Figure 3.7) as it is at an intermediate height and is large enough to be considered quite representative.

To evaluate the suitability of this distance for calibration, 107 *S. mentella* were studied, 17 distances were measured, both directly on the fish with calipers (3D measurements) and on the image (2D measurements). The images were calibrated with both, the ruler placed on the base where the fish lies and also with the D2D distance.

Using D2D for calibration, the accuracy of many of the measurements improved considerably, reducing to a minimum and acceptable level the differences between real distances and their projections on the images. Nevertheless some of the distances were still more accurate using the ruler on the base for calibration. Only three distances showed an error higher than 3% independently of the calibration method. In these cases we think that the source of the error comes from the fact that these distances are too small, thus slight errors in measuring (both with calipers and on the image) cause important relative errors.

From this study, it was concluded that AH, H2D, LPO, LMO and DO should be calibrated with the ruler placed on the base, whilst LD, LV, VA, 2DA, DV, LP, LA, LAV, LC, LM and LMS should be calibrated using D2D. However, LMO always showed an error higher than the 5%, and this was one of the major reasons for excluding it from all the analyses.

Comparing morphometric data from different partners

The data used in this study were taken in two different laboratories. Although the same protocol was followed, the digital cameras and the image analysis software used to acquire the landmark coordinates were different. So, possible differences between the softwares and the cameras were checked.

Software comparison

Looking for possible differences between softwares, the variables (distances between landmarks) in 25 individuals were compared. The same digital pictures were used to record the landmark coordinates, using both softwares. Later, the distances between landmarks were calculated and compared. Figure 3.10 shows raw differences in mm for each of the measurements. Looking at the ANOVA test results (Table 3.15) we conclude that there were no significant differences in measurements calculated with both softwares.

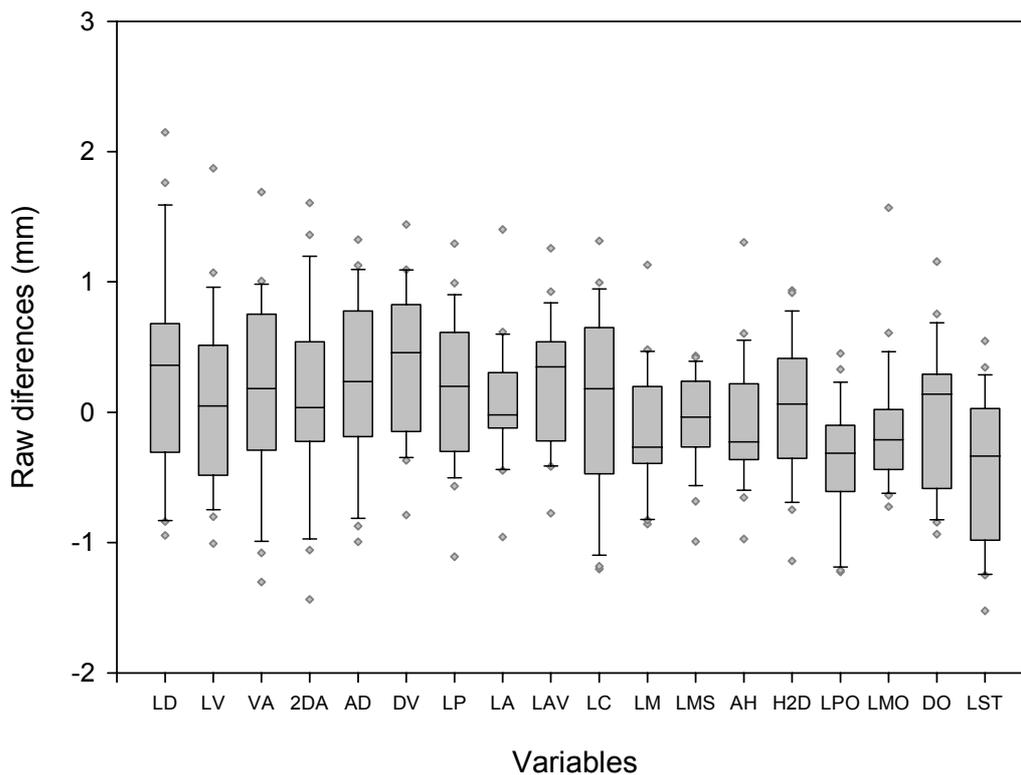


Figure 3.10. Software comparison. Raw differences (in mm), between the distances calculated with each of the softwares.

Camera comparison

Possible differences between the images taken with the Canon PowerShot G1 used in Iceland and the Nikon D1X used by the author were tested by taking photographs of the same 30 fishes with the two cameras. Subsequently, distances between landmarks were calculated in the 60 photos, and compared by pairs. Table 3.15 shows the resultant ANOVA from which it can be concluded that there are no significant differences between cameras. Because this analysis was performed just to compare the cameras, it was not necessary to compare all the variables. Thus, LAV and DO were not used because of the time consumed using the related landmarks. This comparison was performed in Reykjavik once it was decided to exclude LMS and LMO from the analyses (see below section 0), therefore these two variables were not used for the camera comparison

Table 3.15. ANOVA results of the comparison of measurements taken with both softwares and cameras.

	ANOVA			
	Software		Camera	
	F	p	F	p
LD	0.02	0.9015	0.09	0.7614
LV	0.00	0.9717	0.07	0.7853
VA	0.01	0.9346	0.02	0.8992
2DA	0.00	0.9503	0.00	0.9989
AD	0.01	0.9316	0.00	0.9751
DV	0.02	0.8786	0.03	0.8685
LP	0.03	0.8673	0.03	0.8621
LA	0.01	0.9367	0.00	0.9812
LAV	0.00	0.9616		
LC	0.00	0.9739	0.13	0.7162
LM	0.02	0.8817	0.45	0.5045
LMS	0.00	0.9619		
AH	0.00	0.9631	0.21	0.6444
H2D	0.00	0.9899	0.33	0.5662
LPO	0.05	0.8237	0.17	0.6779
LMO	0.06	0.8015		
DO	0.00	0.9715		
LST	0.01	0.9422	0.01	0.9129

3.2.1.3. Acquisition of data on parasite and skin pigmentation

Data on infestation by *Sphirion lumpi* and on pigmented patches in redfish were collected parallel to morphological studies, following the methodology described in Bakay and Karasev (2001). Live *Sphirion lumpi*, or remains of its presence such as ulcers or cysts were checked and counted from the left side of the fish, specifying the situation, i. e. the filet area (dorsal and tail), the ventral area, the head and the anus regions. Pigmented patches of grayish-black or reddish-orange colour that sometimes occur on redfish skin were also recorded. Both parasites and pigmented patches, were used to create predefined groups for the discriminant analysis in an attempt to discriminate between oceanic and deep-sea phenotypes in *S. mentella* from the Irminger Sea.

3.2.1.4. Acquisition of gas bladder musculature data

As explained in the introduction, the gas bladder musculature pattern (Figure 3.11) is a widely used character to distinguish between *Sebastes* species in the North Atlantic.

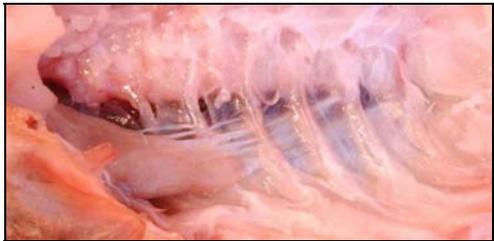
Gass bladder musculature pattern	
	<i>S. mentella</i> Typically a single, thin and short muscle. The tendon passes between the 2nd and 3rd ribs and is attached to the 5th rib, i. e., the 7th vertebrae.
	<i>S. marinus</i> : Normally the muscle is constituted by three or four muscle heads with several tendons each. The first two tendons (in a dorsal position) are directly attached to the 2nd and 3rd ribs. The following tendons cross between the second and third ribs and are attached to the 5th rib, and the principal tendon crosses between the third and fourth ribs and is attached to the sixth rib.
	<i>S. viviparus</i> The typical pattern is a unique muscular head crossing between the third and fourth ribs. This muscular head is attached to vertebrae 9 to 11 by three tendons. (The gas bladder musculature of <i>S. fasciatus</i> is similar to this one).

Figure 3.11. Gas bladder musculature of *S. marinus*, *S. mentella* and *S. viviparus* from Faroe Islands

The 2,780 fish processed by the author were dissected, and records made of the numbers of muscular heads, the numbers of tendons, the numbers of the ribs that those tendons crossed between, and the ribs where the tendons were attached. Digital photos of the gas bladder musculature of 100 *S. marinus*, 100 *S. mentella* and 80 *S. viviparus* from Faroe Islands were taken and stored.

3.2.2. Data Analyses

Two different groups of analyses, Meristic and Morphometric, were performed to study the redfish species and population structure in this work. Meristic traits differ from morphometric traits in a fundamental way: whereas counts (meristic) usually become stable in number during growth after a threshold body size has been attained, mensural traits (morphometric) change continuously with size and age. Thus, morphometric data are continuous and must be corrected for size differences among specimens whilst meristic data are discontinuous values that, in theory, remains constant through fish ontogeny once the juvenile stage is attained.

The statistical treatment was also different: morphometric data were analyzed using multivariate statistical techniques that study continuous variables, while for meristic data, nonparametric statistical techniques are the only appropriate tool.

Two different morphometric approaches were, in addition, used, Traditional and Geometric morphometrics. Even though geometric methods are those usually followed these days, these techniques are not exempt from difficulties in their application, especially due to the still poorly developed statistical procedures behind the techniques. Therefore, Traditional morphometrics are still widely used, and there is still a extensive range of applications where traditional morphometrics can give satisfactory results. From previous studies (Misra and Ni, 1983; Power and Ni, 1985; Kenchington, 1986; Saborido-Rey, 1994), traditional morphometry have been proved to be a good tool for redfish species and population discriminations in the North Atlantic. Hence, the first approach within this study was to carry out Traditional morphometric analyses. Later, the same analyses were carried out with Geometric morphometric techniques, that reinforce traditional morphometry and also complement it with graphical displays of the comparisons between species or populations. Meristic analyses were performed only on *S. mentella* and *S. marinus* from the central area, since this was considered the main goal of the study.

3.2.2.1. Data analysis in Traditional morphometrics

Traditional morphometrics consists in applying multivariate statistical analysis to sets of morphological variables. Usually, linear distance measurements are used, but sometimes counts, ratios, and angles may be included. With these approaches, covariation in the morphological measurements is quantified, and patterns of variation within and among samples can be assessed. Statistical analysis typically includes principal components analysis, factor analysis, canonical variables analysis (CVA), and discriminant function analysis. Many studies have investigated allometry, or changes in shape with change in size (Jolicoeur, 1963). Because linear distance measurements are usually highly correlated with size (Bookstein *et al.*, 1985), much effort has been spent developing methods for size correction, so that size-free shape variables can be extracted and patterns of shape variation elucidated. (e.g., Sundberg, 1989; Jungers *et al.*, 1995, Adams *et al.*, 2004).

The detection of outliers

One of the main purposes of initial data analysis is data screening to look for possible anomalous values and, if possible, correct them. Thus, outliers were checked for all individuals.

For this purpose, a regression of all variables against standard length was performed for each species in each of the studied areas. Data have been screened area by area for a better detection of outliers. Outliers were identified in the residuals of this regression, with the aid of graphical methods such as the distribution of raw and standard residuals, and the distribution of Mahalanobis and Cook's distances. Statistical 6.0 software was used for these analyses.

All standard residuals with unexpected values were checked and errors were corrected when possible; on the other hand, if the rogue values were genuine, then it was considered whether or not they should be retained.

The detection of outliers in the whole dataset yield a total of 186 outliers detected and hence removed from the analyses.

Variables excluded from the analyses

Although, a precise protocol was developed (see Annexes), it was necessary to detect if there was any kind of systematic error on measuring the landmarks, especially because there was two laboratories involved. To achieve this it was selected 100 specimens measured in each laboratory of *S. mentella* from Central Irminger Sea collected in 2001 and from the same size range (240-330 mm). An ANOVA was then performed for each variable with laboratory as factor. No significant differences occurred between laboratories except for LMS, as shown in Table 3.16. This difference was further investigated; regressions of each variable with standard length were plotted and it was observed that systematically LMS was larger at all sizes in one of the laboratories. Figure 3.12 show the regression against standard length of LMS and to LC as a representation of the rest of the variables, in order to compare the differences. Digital images were revised and it was realized that pin R was placed in different position in both laboratories. This variable was, therefore, excluded from the analyses.

On the other hand, as stated in the Technical protocol (Annex II), LMO showed always a high error when compared with the measurements taken with the calliper, independently of the calibration procedure. High error occurred also when the same specimens were measured by two different persons and results compared (section 7.3.3. in Annex II). The landmark O, which defines LMO, is in one of the narinas of the fish, and it is easily deformed when the pin is driving into the hole. This fact introduced uncertainty in the position of the landmark. In addition, it was discovered at latter stage that this pin was systematically placed in different position in the fishes measured in this study and those available from Flemish Cap and Norway, measured earlier (Saborido-Rey, 1994). The uncertainty of the position of the landmark and the inherent inaccuracy when marking the landmark O, were the causes for the exclusion of this variable from the analyses.

Table 3.16. Results of the ANOVA between variable measurements taken in both laboratories (IMR-Iceland and IIM-Spain).

ANOVA		
	F	p
LD	0.00	0.981
LV	0.22	0.642
VA	6.71	0.010
2DA	3.19	0.076
AD	6.28	0.013
DV	0.18	0.668
LP	3.85	0.051
LA	0.09	0.769
LAV	5.38	0.021
LC	0.43	0.514
LM	0.87	0.353
LMS	0.46	0.500
AH	184.32	0.000
H2D	2.26	0.135
LPO	3.63	0.058
LMO	0.29	0.588
DO	2.42	0.121

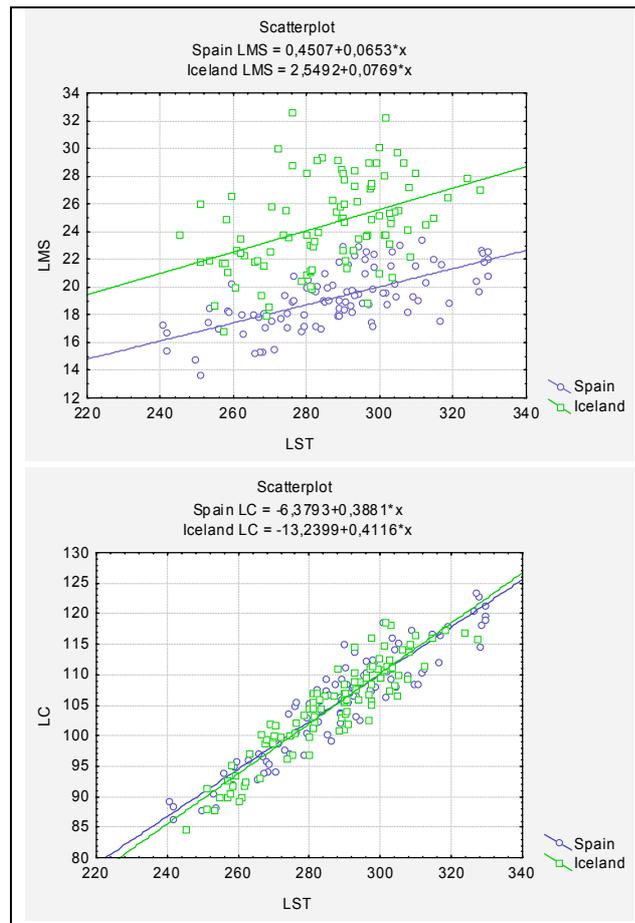


Figure 3.12. Scatterplot showing LMS and LC regressions against standard length (LST) for each laboratory (IMR-Iceland and IIM-Spain).

Normality

Discriminant analysis assumes that the underlying structure of the data for each group is multivariate normal. This permits the precise computation of tests of significance and probabilities of group membership. When this assumption is violated, the computed probabilities are not exact and will not be optimal in the sense of minimizing the number of misclassifications, even though they may still be quite useful if interpreted with caution (Lachenbruch, 1975). There is no objective way to fully evaluate the assumption of multivariate normality. The best reassurance is a sufficiently large sample, although even an infinitely large sample will not normalize an inherently non normal distribution.

Univariate normality of the 16 variables (Figure 3.7) has been computed; normal, half-normal and detrended normal probability plots of the residuals of the regression of each variable against the standard length were analyzed for each group. Normal probability plots of samples against corresponding percentages of a standard normal variable. If the data are from a normal distribution, the pooled values will lie on a straight line.

However, whereas multivariate normality implies univariate normality, univariate normality does not imply multivariate normality. Hence, these diagnostics are of limited use only. However, it is usually assumed that univariate normality is a good step toward multivariate normality (McGarigal *et al.*, 2000).

In the present study, the data present univariate normality and all groups are large enough to consider the multivariate analyses robust enough to be unaffected by departure from multivariate normality.

Size and shape

One of the main problems in traditional morphometrics is that linear distance measurements are usually highly correlated with size (Bookstein *et al.*, 1985). Thus, a size correction among specimens must be carried out prior to data analyses. To do that, the 'size-free' part of the variance must be obtained, i.e. the shape variation. There are different methods to separate the effects of size differences from variation in body shape, i.e., ratios, regression related, and multivariate methods (Strauss and Bond, 1990) .

Size and shape: Ratios

A conventional technique for assessing shape differences is to use ratios (proportions or percentages) of measurements as characters. Ratios are assumed to remove the effects of body size by dividing out a 'size variable' such as standard length. The ratio of head length to standard length, for example, is often assumed to reflect relative head size independent of body size.

Size and shape: Regression-related methods

Size effects can be removed from a morphometric data set in several ways that rely on some type of regression analysis (Thorpe, 1976; Bookstein *et al.*, 1985). Regressing a size-dependent character against an 'independent' size character such as standard length has been suggested (Atchley *et al.*, 1976; Atchley, 1978; Gould, 1966). By definition, residuals are orthogonal to the regression line, in this case size variation, i.e., are independent of size.

Three types of slopes or coefficients may be estimated for data in which a grouping structure is inherent: pooled groups, common within-groups and individual within-groups. Each distance variable is replaced by its residual after the regression. To properly use residuals, the assumption of homogeneity between group slopes must be tested. If violated, the common within-groups slope must be used to estimate residual or allometric variables (Reist, 1983; Thorpe, 1975, 1976) to obtain the so called “adjusted residuals”.

The residuals obtained by any of these regressions, i.e. common residuals or adjusted residuals, are then used as input for subsequent multivariate analyses.

Size and shape: Multivariate methods

These methods are based on the concept that size is not equal to any single length, but has a multivariate nature. Many alternative methods have been proposed for multivariate size correction. Each method yields a slightly different result. It appears that the most effective and valid procedure is multiple group principal components analysis and a related method, Burnaby’s size-adjusted discriminant analysis (Burnaby, 1966; Rohlf and Bookstein, 1987; Klingenberg, 1996).

Burnaby’s method corresponds to the projection of the data onto a space orthogonal to a size vector. The method removes all variation parallel to a specified vector and thus reduces the dimensionality of the variation (Rohlf, 1990). If the first principal component axis is used as a size vector, the major source of variation in the sample is being removed.

All the case studies presented here were performed using three methods: residuals, adjusted residuals and Burnaby’s method, and the results were compared. The results from each of the methods in each case study, although slightly different, never lead to a different conclusion. In view of this, and the fact that the presentation of the results using the three different methods would be too large, due to the high number of analyses, only the results of analyses using Burnaby’s method have been displayed.

Once the size effect has been removed, multivariate analyses were performed over pre-defined groups, searching for differences in shape between groups.

Multivariate Statistical analyses

Two types of multivariate analysis were performed: Discriminant analysis and Cluster analysis:

Discriminant analysis

The goal of Discriminant analysis (DA) is to discriminate among prespecified groups of individuals based on a sample of observations from each group (i. e. discriminating variables). Discriminant Analysis allows study of the differences between two or more groups of individuals with respect to several variables simultaneously.

The foundations of DA were based on the study of differences between various types of Iris plants by Fisher (1936) who sought the linear function that would best separate samples of two types of Iris plants. Fisher argued that the coefficients in this function should be given by the values maximizing the ratio of squared group mean difference to within-group variance.

There are two main objectives of DA, discrimination and classification. Discrimination tries to obtain the optimal separation of groups, based on certain linear combinations of the discriminating variables (discriminant function), while classification predicts the group membership for an individual based on its measured values on the discriminant variables. Specifically, DA produces linear combinations (i. e. canonical functions, discriminant functions or canonical roots) of the two or more discriminating variables that will 'best' discriminate among the a priori defined groups. Each canonical root is a linear combination of the original variables, where each variable is weighted according to its ability to discriminate among groups:

$$D_i = \beta_1 X_{i1} + \beta_2 X_{i2} + \dots + \beta_n X_{in} + \beta$$

where D_i is the canonical score of each individual, X are the values of the n original independent variables of each i individual and β are the discriminant coefficients.

By averaging the canonical scores for all entities within a particular group, we arrive at the group mean canonical score, i.e. the centroid.

The best linear combination of variables is achieved by the statistical decision rule of maximizing the among-group variance relative to the within-group variance; that is, maximizing the ratio of among group to within-group variance in canonical scores (McGarigal *et al.*, 2000). This is especially relevant for the subsequent interpretation of the output of the discriminant analysis, since this analysis searches for the differences, maximizing them. In other words, potential similarities are not considered.

Classification is the process by which a decision is made that a specific entity belongs to or most closely resembles one particular group. Mahalanobis distance (Mahalanobis, 1963) is used to measure the distance from each entity to each of the group centroids. Later each entity is classified with the group to which it is closest. That group is the one in which the typical profile on the variables most closely resembles the profile of this entity. Because Mahalanobis distance has the same properties as the Chi-square statistic we can convert the distance between each entity and each group centroid into a probability that an entity belongs to each group (Klecka, 1980).

Results of the classification are presented as the classification matrix (or confusion matrix). In this matrix the numbers of individuals classified correctly or incorrectly into each group are displayed. As a direct measure of predictive accuracy, the correct classification rate (i.e. the percentage of samples classified correctly) is the most intuitive measure of discrimination. However, caution is necessary in interpreting the magnitude of the correct classification rate, as it has to be interpreted in relation to the expected percentage of correct classifications when group assignments have been randomly made. In any dataset, it is always expected that a certain number or percentage of individuals will be classified correctly. For example, if we have two groups of equal size and 50% of prior probabilities, we can expect to get 50% of the classifications right by pure random assignment. However, since the discriminant

analysis maximizes the differences, random classification always results in higher values, roughly 70%.

Furthermore, the expected probability of classification into any group by chance is proportional to the group size. Therefore, as the relative size of any single group becomes predominant, the correct classification rate based on chance alone tends to increase toward unity. In extreme situations, greatly different group sizes may lead to a very high correct classification rate, but the improvement over random classification may be slight. An example of this can be found at the end of this section.

There are several techniques to assess the accuracy of classification rates compared with those expected by random assignment. From them, Cohen's Kappa (K) statistic (Cohen, 1960) has been selected. It is defined as:

$$K = \frac{N_c - \sum_{i=1}^G EF_i}{N - \sum_{i=1}^G EF_i}$$

where N_c is the number of individuals correctly classified, N the total number of individuals in the analysis, G is the number of groups and EF_i is the expected frequency for the number of individuals correctly classified in each group i that would have been expected by chance:

$$EF_i = \frac{p_i * q_i}{N}$$

where p_i is the number of individuals in the i th group (sum of each row in the classification matrix), and q_i is the number of individuals classified into the i th group (sum of each column in the classification matrix).

Kappa ranges from 0 to 1, where 0 indicates a random classification and 1 a perfect assignment. To evaluate K it has to be considered that:

- if K is less than 0.7, classification is unreliable
- if K is greater than 0.7, the K value should be compared with the total correct classification; if K is much lower than the correct classification rate it suggests that the classification power is due to chance.

The Wilks' lambda statistic is another procedure often used to evaluate the discrimination power. Wilks' λ , is a likelihood ratio statistic for testing the hypothesis that group means are equal; Lambda approaches zero if the groups are well separated, and one if there is no discrimination. However, there is no rule of thumb about the critical value of Wilks' λ , i.e. a threshold indicating if the discrimination power is good enough.

Thus, the confusion matrix should be interpreted together with Cohen's Kappa and Wilks' λ values.

Although not presented, many other outputs from the discriminant analyses were computed. Some of them are particularly interesting to evaluate the analysis, such as those derived from the canonical analysis (particularly the canonical correlation). Especially important is the computation of the Distances between groups through the Mahalanobis distance. However, a cluster analysis was occasionally performed based on this distance (see the cluster analysis section below).

Mahalanobis distance (D^2) was the measure used to compute the resemblance (or classification) matrix. The Mahalanobis distance between two entities (j and k) based on P variables is defined as:

$$D^2 = (x_j - x_k)' \Sigma^{-1} (x_j - x_k)$$

Where Σ is the pooled within-groups variance-covariance matrix, x_j is the vector of scores for the i th entity, and x_k is the vector of scores for the j th entity.

Cluster analyses

Cluster analyses (First used by Tryon, 1939) refers to a large family of classification algorithms, that attempts to organize observed data into meaningful groups. Amongst these techniques, the most appropriate for this data are called Polythetic agglomerative hierarchical clustering (PAHC). These are included in the group of 'Hierarchical procedures', that combine similar entities into classes or groups and arrange these groups into a hierarchy, thereby revealing interesting relationships among the entities classified. PAHC techniques use the information contained in all variables. First, each entity is assigned as an individual cluster. Subsequently, PAHC agglomerates these clusters in a hierarchy of larger and larger clusters until finally a single cluster contains all entities (McGarigal *et al.*, 2000). Polythetic agglomerative HC involves two major steps. Briefly, the first step is to compute a resemblance matrix from the original data matrix. The second step is to agglomerate or fuse entities successively to build up a hierarchy of increasingly large clusters. The entire agglomeration sequence is summarized in agglomeration tables, which are usually portrayed as dendrograms (tree-like plots) that are ideal to interpret relationships.

The Hierarchical Tree Plot (dendrogram) should be interpreted as follows:

On the left of the plot, each object is in a class by itself and they are successively aggregated in larger clusters of increasingly dissimilar elements. Finally, in the last step, all objects are joined together. The horizontal axis denotes the linkage distance. Thus, for each node in the graph (where a new cluster is formed) we can read off the criterion distance at which the respective elements were linked together into a new single cluster. When the data contain a clear "structure" in terms of clusters of objects that are similar to each other, then this structure will often be reflected in the hierarchical tree as distinct branches. As the result of a successful analysis with the joining method, one is able to detect clusters (branches) and interpret those branches.

In this study, the cluster analysis was not performed from the raw data, but from the Mahalanobis distances matrix resulting from the stepwise discriminant analysis. Among the different amalgamation rules available, the Complete linkage (furthest neighbor) were

selected. In this method, the distances between clusters are determined by the greatest distance between any two objects in the different clusters. This method usually performs quite well in cases when objects actually form naturally distinct 'clumps'.

An example explaining statistical artifacts due to the unequal size of groups.

In this section the influence of group size on the discriminant analysis results and how those results should be interpreted are described. For this purpose, the 276 *S. mentella* from SW Iceland have been divided randomly into two identical groups. It was assumed that no morphometric differences exist between individuals, since all of them come from the same area and belong to the same species. A set of three discriminant analyses were carried out, the first one with all specimens divided into two identical groups (A and B) of 138 individuals each; a second one where group A is reduced to only 100 individuals and group B continues with 138; and finally, a third analysis was performed with group A reduced to only 30, and B continues with the same 138 individuals.

In the first analysis, 60.5 % of the specimens were correctly classified, 62.3 in group A and 58.7 in group B. These figures are higher than the 50% expected, showing that random classification is not 50% (Table 3.17 -A).

In the second analysis, the unequal group size is reflected in a higher classification rate for the dominant group. Thus, there is an increase in the percent of correct classifications in group B that increases from 58 to 83%, and a decrease of the percent of correct classifications in group A, that declines from 62% to a 26%. Note that most of the group A samples were classified into group B (74%) (Table 3.17 -B).

Finally, when the group sizes were strongly unbalanced, 30 and 138 individuals respectively for group A and B, the classification matrix shows that 100% of the specimens are classified as group B, and the total classification rate reaches as much as 82.1%. But obviously no differences exist among groups, since groups overlaps completely (Table 3.17 -C).

For a good interpretation of the classification matrix, it is highly recommended to observe Wilks' lambda and Cohen's Kappa values. In the three analysis of this example, both statistics reflect the absence of classification, as they got very poor values. (Remember that Wilk's lambda ranges between 0 and 1, 0 indicating total discrimination and 1 no discrimination, and Cohen's kappa ranges between 0 and 1, 1 indicating perfect discrimination and 0 no discrimination). It is very significant that in the third analysis, Kappa value is 0 in spite of the fact that the correct classification rate is 82%.

Table 3.17. Statistical artifacts in unequal sized groups

Statistical artifacts in unequal sized groups				
A. Equal size groups (138 each)	Wilks' Lambda: 0.95744 Cohen's Kappa= 0.21			
		Percent	A	B
	A	62.3	86	52
	B	58.6	57	81
	Total	60.5	143	133
B. Group A with only 100 samples	Wilks' Lambda: .97811 Cohen's Kappa= 0.10			
		Percent	A	B
	A	26.0	26	74
	B	83.3	23	115
	Total	59.2	49	189
C. Group A with only 30 samples	Wilks' Lambda: .94996 Cohen's Kappa= 0			
		Percent	A	B
	A	0.0	0	30
	B	100.0	0	138
	Total	82.1	0	168

The analyses performed

There are several ways to perform a discriminant analysis, but probably the most widely used and the one selected for this study is the forward stepwise discriminant analysis (SDA). This procedure selects only the variables that best discriminate. The variables are selected based on some previous criteria in order to enter in the model. One of the most used criteria is Wilks' lambda statistic. Thus, at each step, a variable is selected if it minimizes the overall Wilks' Lambda statistic. Before being considered for entry in the model, a variable should meet certain conditions specified a priori. One of the characteristics of the model is that a variable that has previously entered may lose its discrimination power and be removed from the model. A variable in the model that does not reach certain conditions is removed. These conditions are usually a tolerance test and a partial F-test.

Tolerance represents the percentage of variance in a variable not accounted for by the variables already entered. A variable with a small tolerance is likely to cause computational inaccuracies in the eigenanalysis because of the rapid accumulation of rounding errors, and may cause the matrix to be singular. Moreover, a variable with a small tolerance is highly redundant with the variables already entered, and thus, has little unique information to contribute. Hence, at each step of the procedure, each potential variable-to-enter must pass some minimum tolerance level.

The partial F-test consist on both and F-to-enter and F-to-remove. The F-to-enter tests the significance of the added discrimination introduced by the variable being considered after taking into account the discrimination achieved by the other variables already entered (Klecka, 1980). If this F is smaller than a specified significance level, the variable is not introduced in the analysis. The F-to-remove tests the significance of the decrease in

discrimination if that variable should be removed from the list of variables already selected. A variable may lose its unique discriminatory power because of correlations with other variables subsequently entered into the model.

The whole set of analyses carried out in this study was performed with the same criterion, i.e. F-to-enter was always set at 1, F to remove was always set at 0.99, and Tolerance was 0.01.

For each of the analyses, several outputs are presented: The classification matrix, the Wilks' lambda and the Cohen's Kappa are displayed together in the same table, as it is important to take into account all together for a good interpretation of the discrimination, and the scatterplots of the canonical scores are also displayed.

The classification matrix contains information about the percent of correctly classified cases in each group and the number of cases correctly and incorrectly classified. The computations for the classification of the cases were based on a priori classification probabilities proportional to group sizes. Wilks' Lambda and Cohen's Kappa were always displayed with the classification matrix. The Wilk's lambda presented is that for the overall model.

In most of the analyses performed, the difference in number of individuals per group was very large, which may lead to a potential bias in the classification procedures. It has long been acknowledged that mixture models perform best when each source stock contributes equally to the mixture (Mulligan *et al.*, 1988). In order to avoid this potential error, extra analyses were computed with the same number of individuals per group. To build the new groups, the individuals were randomly chosen. For this purpose, a random number was assigned to each of the cases in the analysis, employing Microsoft® Excel functions. Then, the cases were ordered by the random number, and the first n cases selected. The number of individuals taken to create the new groups, i.e., n , was constrained by the number of individuals available in the smaller group. On the other hand, if a group included more than one area (or subarea), the same numbers of individuals were taken for each of these areas or subareas, in order to avoid over- or under-representation of one particular area. For example, if 100 *S. mentella* were to be taken from Greenland, 50 were taken from East and 50 from West Greenland. These extra analyses gave a better overview of the separation between the groups, but part of the information was lost, because only some of the available individuals were used.

The scatterplot of the canonical scores for each sample are, when possible, presented for the first two discriminant functions or canonical roots, and occasionally for the first three canonical roots, in a 3D plot. This plot is not available if only one canonical function is extracted (i.e. when the discriminant analysis is performed between only two groups). In this case, the scatterplot is substituted by the histogram of the canonical scores by group, where the frequency of each group along the canonical root values is depicted. These plots show graphically the separation of the groups and how each discriminant function contributes to this discrimination.

In some of the case studies, a cluster analysis was performed and the Hierarchical Tree Plot presented.

Statistica 6.0 for Windows software was used to perform all the analyses described up to now.

3.2.2.2. Data analysis in Geometric morphometrics

Geometric morphometrics consist on a set of new techniques that were developed for morphometric studies, inspired by D'Arcy Thompson's theory of form-change. D'Arcy Thompson (1917) consider the transformations between forms making homologous points correspond, and the deformations from one form to another were illustrated in deformation grids.

With this new techniques, what is compared are the shapes themselves, instead of shape measurements. Thus, the initial variables are the two dimensional coordinates of the landmarks placed on the specimens being compared. Each specimen is characterized by a landmark configuration. But direct analysis of those landmark coordinates is not appropriate, because variations of position, orientation and scale of the specimens are present. Thus, the first step is to eliminate this non-shape variation, transforming the coordinates into shape variables that can be compared statistically; the differences between configurations can be displayed graphically.

The so called 'superimposition methods' eliminate the non-shape variation from configurations of landmarks. One of the most popular superimposition method is GPA, Generalized Procrustes Analysis (Rohlf, 1990). In this method the centroid of each configuration is translated to the origin, and configurations are scaled to a common unit size by dividing by the centroid size (Bookstein, 1986). The centroid size is the sum of squared distances between all landmarks and the specimen's centroids. Finally the configurations are rotated to minimize the interlandmark distances (Rohlf and Slice, 1990). The process is iterated to compute the mean shape or consensus of the sample of specimens.

As a result of all the preceding manipulations, the landmark configurations lie in Kendall's shape space (Kendall, 1984). Its metrics are called Procrustes metrics as it is formed basically by the Procrustes distance. This space is non-Euclidean. Its geometry, for the simplest case of a plane object described by three landmarks, can be visualized as a hypersphere surface. The shapes are points of this surface (Goodall, 1991). To avoid non-linearity effects, Kendall's space is approximated by a tangent space that has Euclidean geometry. The shapes can be now defined as projections of the initial points onto this hyperplane, and their dissimilarities can be evaluated as distances in the tangent space, which are Euclidean distances.

Some differences remain among specimens after their alignment, indicated by dispersion of landmarks around reference configuration. These correspond by definition to differences of landmark configurations in the tangent space, which is to dissimilarity of the shapes. This dispersion around each of the landmarks is expressed by the so called 'procrustes residuals'

which are considered as an special case of shape variables, the 'Procrustes coordinates' (Rohlf, 1999). Their sum gives overall Procrustes distance among the specimens.

In this study, landmark configurations of all fish in each case study were superimposed, i.e., scaled, translated and rotated, using the generalized procrustes analysis (GPA), in order to minimize the sum of squared distances between homologous landmarks. The consensus of the whole individuals, i. e. the mean of the landmarks configurations of the whole individuals were calculated with the aid of *Tpsrewl* version 1.35.

Comparisons of shapes

There are two alternative methods to compare shapes in geometric morphometrics. One is based on differences in coordinates of corresponding landmarks between objects (procrustes distance matrix), and the other is to use a thin-plate spline to map the deformation of shape from one object to another.

The second method was the one used in this study, and what follows is a brief introduction to this thin-plate spline method. Thin-plate spline analysis is based on analogy of a 2D morphological object to a thin homogenous deformable metallic plate (Bookstein, 1991). According to its methodology, one specimen is fit to another and the numerical estimate of the degree of such deformation is called the bending energy.

The thin-plate spline algorithm uses the consensus configuration to compute a number of orthogonal shape components called principal warps (Bookstein, 1990). All individual landmark configurations are then projected onto the principal warps; the parameters of the function relative to each individual (partial warp scores) represents the new set of variables, and can be used for statistical comparisons of variation in shape within and between populations.

The matrix containing the partial warp scores is the weight matrix. The columns are called partial warps and represent nonlinear components of deformation. The last two columns of the weight matrix represent the uniform component, which represents the non-localized component of the deformation, i. e. the result of stretching and shearing.

Shape changes can be visualized as splines (deformation grids), and provide a fast and intuitive way to visualize those shape changes. But these images are deduced from approximate algorithms and should not be taken too literally: they are visual 'metaphors' of shape transformations rather than exact mappings of them (Bookstein, 1996).

Graphical methods based on thin-plate spline analysis illustrate partial warps in two ways. One of them is the 'transformation grid', and the other is a set of vectors. The 'transformation grid' is initially orthogonal, and the bigger the difference among shapes, the bigger is the deformation of the grid. Vectors give for each landmark the visualization of the corresponding bending energy and the direction of the change.

The weight matrix for each of the analyses was calculated by the aid of *Tpsrewl* version 1.35. They have been used as input to the subsequent stepwise discriminant analysis.

The stepwise discriminant analysis and the cluster analysis were performed following the same procedure as in traditional morphometrics, as previously described. The only difference was the input data, that for traditional morphometrics was the size-free distances between landmarks resulting from Burnaby's method, and for geometric morphometrics was the weight matrix (with the uniform component included) of each of the samples.

If the number of individuals by group was uncompensated, an extra analysis was performed, taking randomly for each group the same number of individuals. The classification matrix of this new analysis is presented.

Outliers

All landmarks coordinates were closely inspected over the digitized images from which they had been collected, with the help of the software *tpsdig* version 1.40 (Copyright © 2004, F. James Rohlf, Ecology and Evolution, SUNY at Stony Brook). The *Tpsdig* program displays landmark coordinates as points over the digitized images, allowing the user to change the points if necessary. Thus, all errors made when marking the points over the digital images were checked, and corrected when necessary.

In a second step, outliers were checked with the aid of the relative warps software, *tpsrewl* version 1.35 (Copyright © 2003, F. James Rohlf, Ecology and Evolution, SUNY at Stony Brook). The program allows one to visualize the vectors on landmarks of the superimposed specimens (Figure 3.13) and to identify each vector departing from the cloud of landmark coordinates.

Two *S. mentella* and two *S. marinus* that showed vectors larger than normal (outliers) were found, and the individuals were completely removed from the analysis.

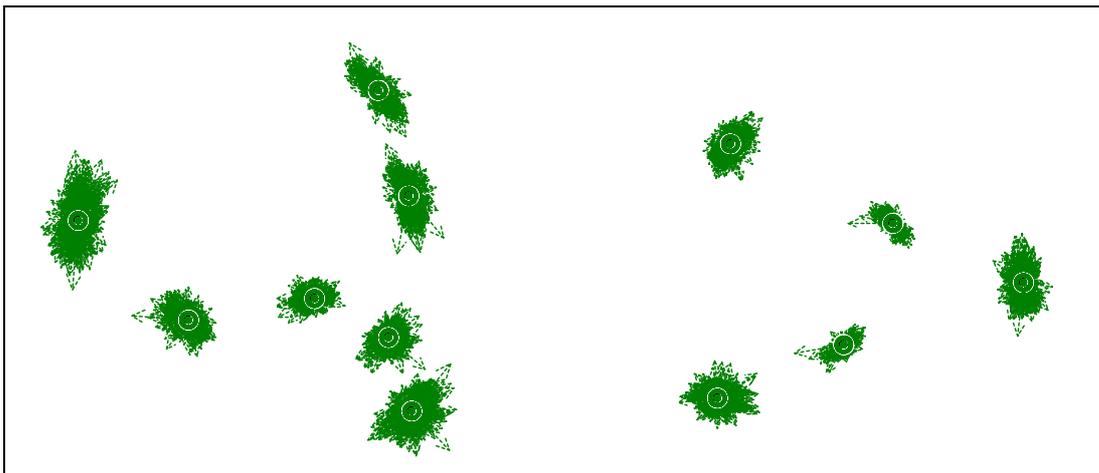


Figure 3.13. Plot showing the vectors on landmarks of the individuals superimposed in *S. marinus*.

Graphical displays

Another difference between Traditional and Geometric morphometrics is that the latter offers graphical displays that facilitate the interpretation of shape variation between pairs of groups. In the present study, two different types of graphics were used.

The first type displays the raw data superimposed and the consensus (mean of the data); both plots were depicted by the aid of *TwoGroup6 IMP* software, developed by David Sheets (Department of Physics, Canisius Collage, Buffalo, NY 14208; sheets@canisius.edu;

The second type of graphics consist on a grid that presents a deformation equivalent to the difference between two groups, i.e. the difference between the consensus of all the individuals in each group. Thus, what deforms the grid is the bending energy necessary to change the shape from one consensus (used as reference) to match the shape of the other consensus (used as target). The deformation grid plots are complemented with the correspondent plots of 'vectors on landmarks' that shows the bending energy in each of the landmarks, in the form of a vector. As the differences in shape of the different species and populations of *Sebastes* are very small, the vectors have been magnified by 5.0, for a better visualization of their directions, which allows better differentiation of those landmarks that contribute most to the deformation. This second type of graphical displays was computed using the *Morpheus* software (Slice, Dennis E. 1998. *Morpheus et al.*: software for morphometric research. Revision 01-30-98. Department of Ecology and Evolution, State University of New York, Stony Brook, New York).

It should be taken into account that the available software analyzes only pairs of groups. Thus, when more than two groups were involved in a particular study, the graphs show the relationships by pairs.

3.2.2.3. Data analysis in Meristics

General considerations

Meristic characters are countable morphological features of fishes (Waldman, 2005a). Meristics are controlled by both genetic and environmental factors, in unknown proportions (Barlow, 1961). The number of parts formed in developing fish can be greatly influenced by the environment. This environmental dependence must be taken into account not only in the design of the analysis but also in interpretation of the results.

In the meristic analyses neither Flemish Cap nor Norway have been included. It was preferred to concentrate meristic analyses on the main area (Faroes, Iceland, Greenland and Irminger Sea) and on the differences between *S. marinus* and *S. mentella*. *S. viviparus* was not included in the analysis because samples for this species were restricted to the Faroes area, and a comparative analysis among areas was not possible.

Nonparametric statistics

Meristic characters are discrete, so, nonparametric techniques are appropriate for their analysis. To study the differences among groups, Kruskal-Wallis H-test is one nonparametric alternative to the between groups one way analysis of variance, and it has been used in this study.

The Kruskal-Wallis H-test assumes that the variable under consideration is continuous and that it was measured on at least an ordinal (rank order) scale. The H-test assesses the hypothesis that the different samples in the comparison were drawn from the same distribution or from distributions with the same median. Thus, the interpretation of the Kruskal-Wallis test is basically identical to that of the parametric one-way ANOVA, except that it is based on ranks rather than means. Post-hoc comparisons of mean ranks for all pair of groups (Siegel and Castellan, 1988) have been computed. z values for each two groups were calculated as:

$$z_{u,v} = \left| \bar{R}_u - \bar{R}_v \right| / \left[N(N+1) / 12(1/n_u + 1/n_v) \right]^{1/2}$$

Where \bar{R} denotes the average ranks for the two groups and n_u and n_v are the number of observations in the two groups (u and v).

Statistica 6.1 StatSoft, software was used to perform the meristic analysis.

4. RESULTS

To analyze the structure of *Sebastes* species and populations in the target area, three different approaches were followed:

- 1 TRADITIONAL MORPHOMETRICS
- 2 GEOMETRIC MORPHOMETRICS
- 3 MERISTICS

In each of these approaches different methodologies have been used, and so the type of results and the manner of presenting them are also different. Therefore, in order to make the reading of this chapter clearer, results are presented in three different sections, 4.1 to 4.3, one per approach. However, within each section the same text and heading structures have been maintained, which basically correspond with the four different types of analyses conducted to deal with the specific objectives:

1. Morphological analyses performed to investigate the differences between species
 - 1.1. The first step was to compare the four species considering all the studied areas as a whole.
 - 1.2. Then the analyses were carried out within each of the selected areas: Flemish Cap, Norway, Faroe Islands, Iceland and Greenland
2. Morphological analyses conducted to ascertain the stock structure of each species:
 - 2.1. *S. marinus*
 - 2.2. *S. mentella*
3. A joint analysis with the four species and the six areas together was then performed.
4. And finally, the phenotypes deep-sea and oceanic *S. mentella* in the Irminger Sea were compared.

4.1. TRADITIONAL MORPHOMETRICS

One of the main problems in traditional morphometrics is that linear distance measurements are usually highly correlated with size and must be corrected for size differences among specimens. At present, Burnaby's method is considered by most authors as the most valid procedure for multivariate size correction (see Material and Methods, chapter 3). After size correction, the correlation between the variables and the fish body standard length decreased considerably.

Burnaby's method has been used for size correction, and the resultant matrix of size-corrected data has been used as input in the subsequent stepwise discriminant analyses (SDA) between groups. The classification matrix, the Wilks' lambda and the scatterplot of canonical scores for each of the analyses have been selected among the different outputs of the SDA because they allow the goodness of the discrimination to be ascertained.

The classification matrix must be interpreted in two ways: first, by observing the proportion of fish that are correctly classified in each group and second, the proportion of fish from a given

group that are classified into another, i.e. the so called confusion matrix. Wilks' lambda is a statistic that denotes the statistical significance of the discriminatory power of the model. Its value ranges from 1 (no discriminatory power) to 0 (perfect discriminatory power). Canonical analysis produces $n-1$ canonical roots, where n is the number of groups considered in the analysis. Thus, when more than two groups were analyzed, the scatterplot of canonical scores of the first two or three canonical roots are presented. When only two groups were analyzed, a histogram of the canonical scores for the single canonical root is presented instead.

In some of the analyses, the number of individuals in each of the groups in the discriminant analysis was very different. This decompensation creates problems in the classification (see Material and Methods, Chapter 3). So, extra analyses were performed, but with an equal number of individuals by group. The individuals for these new groups were chosen randomly, and the number of individuals by group is specified in each of the analyses, because they vary as a function of the maximal number of individuals available in all groups. Although part of the information is lost because only some of the available individuals are used, the classification rates are more in accordance with the confusion that existed between groups.

Individuals included in the analysis

6,764 individuals have been sampled within the REDFISH project, which added to those from Norway and Flemish Cap, totals 7,313 individuals (Table 4.1). In 1,185 fish measured in Iceland, the D2D distance was not taken, which is critical to calibrate the image (see Material and Methods, chapter 3). These fish were, therefore, excluded from the traditional morphometric analyses. Those 1,185 fish nevertheless were used in the geometric morphometric analyses.

Table 4.1. Summary information of individuals involved in traditional morphometrics analyses, by area, species and phenotype. The excluded individuals are divided into those unsuitable for calibration because they have no D2D measurements, those with some of the data missing, and those that presented some outlier.

Area	Total individuals			Used for traditional morphometrics			
	species	Phenotype	Total	Without D2D	Data missing	Outliers	Total
Irminger	<i>S. mentella</i>	deep-sea	1268	132	8	40	1088
		oceanic	983	60		23	900
		undef	93	8	2	2	81
Iceland	<i>S. marinus</i>	demersal	1417	727	1	17	672
	<i>S. mentella</i>	demersal	1069	253	3	20	793
Greenland	<i>S. marinus</i>	demersal	395	1	1	6	387
	<i>S. mentella</i>	demersal	1051	2	1	26	1022
Faroe Islands	<i>S. marinus</i>	demersal	181	2	1	2	176
	<i>S. mentella</i>	demersal	228		1	2	225
	<i>S. viviparus</i>	demersal	79				79
Norway	<i>S. marinus</i>	demersal	64		3		61
	<i>S. mentella</i>	demersal	75				75
	<i>S. viviparus</i>	demersal	95			1	94
Flemish Cap	<i>S. marinus</i>	demersal	102				102
	<i>S. mentella</i>	demersal	102				102
	<i>S. fasciatus</i>	demersal	111				111
Total			7313	1185	21	139	5968

A total of 21 individuals presented missing data in some of the morphometric variables, and they were excluded from the analysis too. The screening of the whole dataset detected 139 outliers that were removed for any kind of analyses.

Therefore, 5,423 fishes were available for traditional morphometric analyses from those collected within the REDFISH project, that added to the fish from Flemish Cap and Norway sums 5,968 fishes that were finally used for traditional morphometric analyses (Table 4.1).

4.1.1. Discrimination between species

4.1.1.1. Discrimination between species in the whole area

The first step was to analyze the differences between the four species inhabiting the North Atlantic in the whole studied area, without consideration of possible population structure. Thus the data from the Irminger Sea, Iceland, Greenland, the Faroe Islands, Flemish Cap and Norway were pooled for each species. Species assignment was accomplished using the external appearance of the fish, i.e. through visual inspection of fish morphology, except for the individuals of Flemish Cap and Norway that were identified using gas bladder musculature.

The classification matrix in numbers, the percentage of total correct classification, Wilks' lambda and Cohen's Kappa for the discriminant analysis by species, of all individuals in all areas, are shown in Table 4.2. *S. fasciatus* showed the lowest percent of correct classification (56.9%), while *S. marinus* (78.8%) and *S. viviparus* (79.9%) classify correctly in around the 80% of individuals. The main reason for these low classification rates is the confusion with *S. mentella*, as 24.5% of the *S. fasciatus*, 19.5% of *S. marinus* and 17.8% of *S. viviparus* classified as *S. mentella*. The confusion between individuals of *S. fasciatus* and *S. marinus* (15.7%) is also remarkable, but the confusion within the other groups was less important. On the other hand, *S. mentella* was the species that showed the highest percent of correct classification with 95.5%. The discrimination within species can be graphically observed in the plot of the canonical scores for each case presented for the two canonical roots in Figure 4.1. The total correct classification for the whole analysis was of 90.5 %, which is considered as a good discrimination, and the relatively low value of Wilks' lambda (0.29) corroborates this discriminatory power. However, the partial results for each of the species showed relatively low discrimination (lower than 85%) in *S. marinus*, *S. fasciatus* and *S. viviparus*. Although *S. mentella* showed a high proportion of correct classifications, there are a relatively high number of *S. marinus*, *S. fasciatus* and *S. viviparus* individuals that classified as *S. mentella*, which implies that the discrimination between species is not as good as the total percent of correct classification indicates. *S. mentella* scored a high proportion of correct classifications due to the fact that most of the specimens analyzed (71%) belong to this species, which, as explained in the discussion section, constrain most of the individuals to be classified with this group. Thus, to have a good view of the separation between species, all confusion rates have to be taken into account together.

RESULTS

Table 4.2. Classification matrix in numbers, percentage of total correct classification and Wilks' lambda and Cohen's Kappa resulting to the discriminant analysis of all cases in the whole area, evaluating discrimination among the four species, i. e. *S. marinus*, *S. mentella*, *S. fasciatus* and *S. viviparus*. The percentage of misclassification is shown at the bottom.

Wilks' Lambda= 0.292897 Cohen's Kappa= 0.77046					
	Correct %	<i>S. mentella</i>	<i>S. marinus</i>	<i>S. fasciatus</i>	<i>S. viviparus</i>
<i>S. mentella</i>	95.5	4102	162	14	17
<i>S. marinus</i>	78.8	272	1102	13	11
<i>S. fasciatus</i>	56.9	25	16	58	3
<i>S. viviparus</i>	79.9	31	3	1	139
Total	90.5	4430	1283	86	170
Percentage of misclassification (%)					
<i>S. mentella</i>			3.8	0.3	0.4
<i>S. marinus</i>		19.5		0.9	0.8
<i>S. fasciatus</i>		24.5	15.7		2.9
<i>S. viviparus</i>		17.8	1.7	0.6	

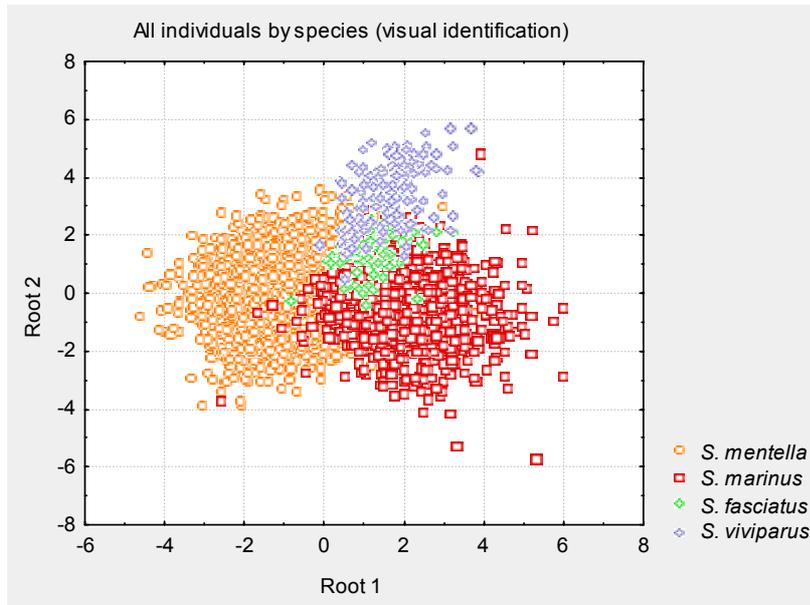


Figure 4.1. Plot of canonical scores for each case for the first and second canonical roots.

4.1.1.2. Discrimination between species in each of the selected areas

FLEMISH CAP AND NORWAY

These areas are out of the core area of study, that is Irminger Sea and adjacent waters. However, previous research on 11 North Atlantic populations of *Sebastes* had been conducted, showing good discrimination among the populations studied (Saborido-Rey, 1994). Among the 11 populations, the closest to the core area were Flemish Cap and Norway. The potential use of these data is as reference for a better understanding of the results, as well an example of the validity of the techniques used here.

Flemish Cap and Norway represent the extremes of the target area, i. e. The Irminger Sea and adjacent waters. *Sebastes* species are well identified in both areas, as was demonstrated by previous traditional morphometric studies carried out by Saborido-Rey (1994).

In both areas, assignment of each individual to its respective species was performed on board, and later the species assignment was corrected by looking at the gas-bladder musculature pattern used in the analyses. The variables were taken directly from the fish with calipers, measuring distances between landmarks. It is important to remark that the gas-bladder musculature anatomy was used for species identification in both areas, Flemish Cap and Norway, and made the species assignment much more accurate, which is often a very difficult task using external features.

The results presented in the next two sections for Flemish Cap and Norway are based on the same data used by Saborido-Rey (1994), but the method to remove the size effect is different from that used in the 1994 analysis. Here, Burnaby's method has been used as in the rest of the analyses.

FLEMISH CAP

The classification matrix in numbers, the percentage of total correct classification, Cohen's Kappa and Wilks' lambda are shown in Table 4.3.

The overall discrimination reaches a value of 93.7 %, accompanied by a very low lambda value (0.08). The highest classification rate corresponds to *S. mentella* with 99% discrimination, almost total, whilst *S. marinus* and *S. fasciatus* show reciprocal, but low, confusion. But the classification rate for both species is nevertheless high. As a result, it is considered that the species in Flemish Cap are clearly different, as shown also in Figure 4.2 representing the canonical scores for each case.

Table 4.3. Classification matrix, percentage of total correct classification and Wilks' lambda and Cohen's Kappa resulting to the discriminant analysis of *Sebastes* species in Flemish Cap. The percentage of misclassification is shown at the bottom.

Wilks' Lambda= 0.08292 Cohen's Kappa= 0.90467				
	Correct %	<i>S. mentella</i>	<i>S. marinus</i>	<i>S. fasciatus</i>
<i>S. mentella</i>	99.1	110	1	0
<i>S. marinus</i>	90.2	0	92	10
<i>S. fasciatus</i>	91.2	2	7	93
Total	93.7	112	100	103
Percentage of misclassification (%)				
<i>S. mentella</i>			0.9	0.0
<i>S. marinus</i>		0.0		9.8
<i>S. fasciatus</i>		2.0	6.9	

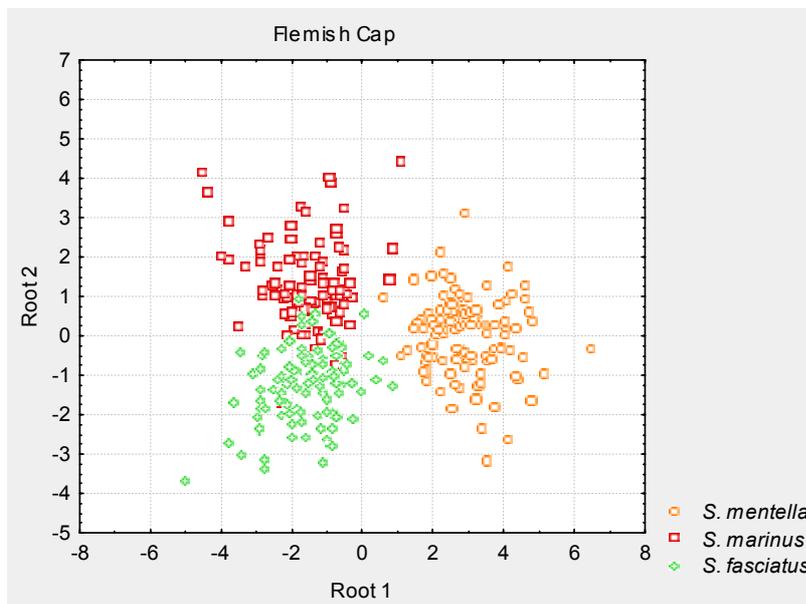


Figure 4.2. Plot of canonical scores for each case for the first and second canonical roots of the analysis performed with the three species inhabiting Flemish Cap.

NORWAY

The classification matrix in numbers, the percentage of total correct classification, Cohen's Kappa and Wilks' lambda are shown in Table 4.4.

The overall discrimination and the very low lambda value (0.02) show almost total discrimination, reaching a value of 98.7 %. Only two specimens of *S. marinus* are classified as *S. viviparus* while 100% of *S. mentella* and *S. viviparus* are classified into their respective species. The good discrimination for these species in Norway is also clear from inspection of the plot of canonical scores (Figure 4.3).

In summary, the species in Flemish Cap and Norway are morphometrically different; showing that the technique used (morphometry based on Burnaby's method) is accurate for species distinction. These results also allow us to use these two areas as reference for posterior comparisons and ecological interpretation.

Table 4.4. Classification matrix, percentage of total correct classification and Wilks' lambda and Cohen's Kappa resulting to the discriminant analysis of *Sebastes* species in Norway. The percentage of misclassification is shown in the bottom.

Wilks' Lambda= 0.02863 Cohen's Kappa= 0.98008				
	Percent	<i>S. mentella</i>	<i>S. marinus</i>	<i>S. viviparus</i>
<i>S. mentella</i>	100.0	75	0	0
<i>S. marinus</i>	95.1	1	58	2
<i>S. viviparus</i>	100.0	0	0	94
Total	98.7	76	58	96
Percentage of misclassification (%)				
<i>S. mentella</i>			0.0	0.0
<i>S. marinus</i>		1.6		3.3
<i>S. viviparus</i>		0.0	0.0	

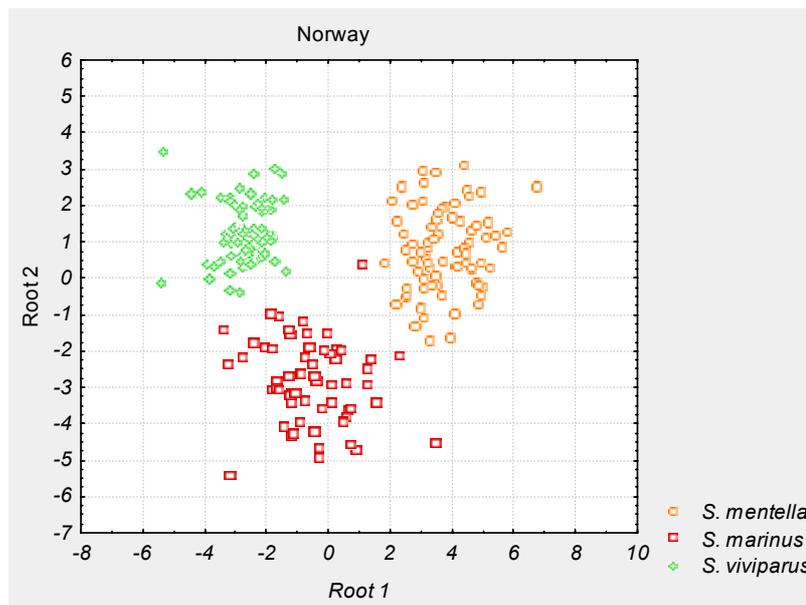


Figure 4.3. Plot of canonical scores for each case for the first and second canonical roots of the analysis performed with the three species inhabiting Norway.

FAROE ISLANDS

The samples available from Faroe Islands waters were collected from two different research cruises, FAER2000 and MH2002, in September and October 2000 and 2002, respectively. The samples from FAER2000 were collected and measured in Iceland. All samples from this survey were classified into species using the external morphology, because gas bladder musculature anatomy (GBM) is not used by Icelandic researchers to identify species on a routine basis. On the other hand, samples from MH2002 were collected by Norwegian researchers but measured in Bergen (Norway) by the author, who separated the individuals into species using GBM. Digital photos of the gas bladder muscle of each individual were taken and stored, making possible the revision of the species assignments when necessary. At the same time, all fish from MH2002 have been genetically analyzed by Norwegian researchers. In consequence, three different types of species assignment are available for Faroe Island samples, i. e., one based on external morphology of the fish, another based on GBM, and the third based on genotype.

It is important to note that the samples from FAER2000 cruise were taken exclusively from the Northwest area of Faroe Island waters, the “Faroes Plateau”, while MH2002 samples were taken from this area and also from the South East of Faroe Islands. The SE of Faroe Islands is called the “Faroes Bank”, and is an area well-known to Norwegian researchers, who have conducted several cruises in the area. They have found in the past a special type of individual in which visual identification disagrees with subsequent species identification using genetic methods. Some of the samples taken by Norwegian researchers during the MH2002 cruise were selected from these special types because they were considered to be interesting for this study.

After the redfish were visually split into species, GBM inspection revealed that there was a large number of (38 of 200) individuals with the external appearance of *S. mentella* but with the typical GBM of *S. marinus*. Also 9 out 200 *S. marinus* showed the GBM *mentella* type. However, contrasting GBM and genetic species assignment, only 2.5% (7 specimens) of mismatch was found, showing good agreement between both methods.

In order to check how these differences in species assignment were reflected in the analysis of fish morphometry, several analyses were run:

- Step 1: Using all individuals, of both cruises, the species being identified by the external morphology, i.e. the appearance of the fish, in all individuals.
- Step 2: Using GBM to assign individuals to species. Therefore only data from MH2002 was used.
- Step 3: Using only FAER2000 fishes, that although only the external morphology was available to classify individuals to species, they were collected on the Faroes Plateau, where theoretically no difficult classifications of individuals are expected.

Step 1: Species classified by external morphology.

For this analysis a total of 480 individuals was employed, belonging to both cruises, FAER2000 and MH2002.

As shown in Table 4.5, the 79 individuals of *S. viviparus* were classified as this species and not confused with others. This good classification for *S. viviparus* is the reason for the low lambda value (0.12). However the correct classification rate for *S. marinus* and *S. mentella* was relatively low, below 85%. From 176 *S. marinus*, 30 (17%) were classified as *S. mentella*, while from 225 *S. mentella*, 32 individuals (14.2%) were classified as *S. marinus*. The good separation of *S. viviparus* but the overlap in the classification of *S. marinus* and *S. mentella* is also visible in the plot of canonical scores (Figure 4.4)

Step 2: Species classified by gas bladder musculature.

Only 278 fish were available for this analysis, those collected during the MH2002 survey. The results of this analysis show an important increase in the discrimination power (Wilks' lambda = 0.02), with a total correct classification of 97.1 % (Table 4.6). Again, *S. viviparus* were classified as this species and not confused with others. However the correct classification rate for *S. marinus* and *S. mentella* increased notably to 94.8 and 97.1 % respectively. Only 4.1% of *S. marinus* and 2.9% of *S. mentella* were misclassified. Thus, the rate of correct

classification for *S. marinus* has increased from 82% to 94,8% and for *S. mentella* from 84.9% to 97% when using the GBM. The good separation of the species is also observed in Figure 4.5 that shows the scatterplot of the canonical scores of each case for the two first canonical roots. The results of this analysis corroborate the conclusion that visual inspection yields a poor species classification in this area, and it is also concluded that GBM species identifications are in full agreement with morphometric methods to identify redfish species.

Step 3: FAER 2000 - Species classified by the external morphology.

As stated above, the samples from FAER2000 were collected in the Northwest, i. e., on Faroes Bank, where no special difficulties in species identification were expected. Table 4.7 shows the classification matrix of the discriminant analysis, Cohen's Kappa, Wilk's lambda, and the percentage of misclassification. 89.1 % correct classification is achieved and lambda value is 0.41, which although higher than in the step 2 analysis (GBM), must be considered as an indicator of a good discrimination between species. *S. viviparus* was not sampled in this survey, and thus, the histogram of frequencies of the canonical scores for each species (Figure 4.6) is displayed instead of the scatterplot of canonical scores.

Final step: Final analysis

Most of the 'problematic' fish were sampled in the SE, but in those fish GBM is available. On the other hand, fish sampled in FAER-2000 show quite good classification although only external appearance was available for species identification. Thus, the final analysis was performed using the species assignment of GBM when available (i. e., in MH2002 samples), and the external morphology where not (FAER2000).

The classification matrix, the percentage of total correct classification, Cohen's Kappa and Wilks' lambda are shown in Table 4.8. As expected, the percent of correct classification has decreased compared with Step 2 analysis, but the total correct classification proportion is high (92.5%). Lambda value (0.08) is perhaps lower than expected for this percent of correct classification, due to the good classification rate attained by *S. viviparus* (100%). Only 2 specimens of *S. marinus* and 1 of *S. mentella* are classified as *S. viviparus*. The 92.2% of *S. marinus* and the 89.8% of *S. mentella* were classified into their respective species.

In consequence it was concluded that the whole dataset of Faroes samples can be used in the subsequent analyses, with the species assignment made by the GBM when available and the external morphology for the rest of the cases.

RESULTS

Table 4.5. Classification matrix, percentage of total correct classification, Wilks' lambda and Cohen's Kappa resulting from the discriminant analysis of *Sebastes* species in Faroe Islands using external features (visual inspection) of the individuals for species assignment.

Wilks' Lambda= 0.12615 Cohen's Kappa= 0.781887				
	Percent	<i>S. mentella</i>	<i>S. marinus</i>	<i>S. viviparus</i>
<i>S. mentella</i>	84.9	191	32	2
<i>S. marinus</i>	82.4	30	145	1
<i>S. viviparus</i>	100.0	0	0	79
Total	86.5	221	177	82
Percentage of misclassification (%)				
<i>S. mentella</i>			14.2	0.9
<i>S. marinus</i>		17.0		0.6
<i>S. viviparus</i>		0.0	0.0	

Table 4.6. Classification matrix, percentage of total correct classification, Wilks' lambda and Cohen's Kappa resulting from the discriminant analysis of *Sebastes* species in Faroe Islands using the gas bladder musculature for species assignment. The percentage of misclassification is shown in the bottom.

Wilks' Lambda= 0.02847 Cohen's Kappa= 0.956599				
	Percent	<i>S. mentella</i>	<i>S. marinus</i>	<i>S. viviparus</i>
<i>S. mentella</i>	97.1	99	3	0
<i>S. marinus</i>	94.8	4	92	1
<i>S. viviparus</i>	100.0	0	0	79
Total	97.1	103	95	80
Percentage of misclassification (%)				
<i>S. mentella</i>			2.9	0.0
<i>S. marinus</i>		4.1		1.0
<i>S. viviparus</i>		0.0	0.0	

Table 4.7. Classification matrix, percentage of total correct classification and Wilks' lambda and Cohen's Kappa resulting from the discriminant analysis of *Sebastes* species in Faroe islands, using the samples from FAER2000 cruise (external morphology for species assignment). The percentage of misclassification is shown at the bottom.

Wilks' Lambda= 0.41494 Cohen's Kappa= 0.780889			
	Percent	<i>S. mentella</i>	<i>S. marinus</i>
<i>S. mentella</i>	86.3	82	13
<i>S. marinus</i>	91.6	9	98
Total	89.1	91	111
Percentage of misclassification (%)			
<i>S. mentella</i>			13.7
<i>S. marinus</i>		8.4	

Table 4.8 Classification matrix, percentage of total correct classification and Wilks' lambda and Cohen's Kappa resulting from the discriminant analysis of *Sebastes* species in Faroe Islands using external morphology for FAER samples and GBM criteria for those fish where it is available, that is, for MH2002 samples. The percentage of misclassification is shown at the bottom.

Wilks' Lambda= 0.08628 Cohen's Kappa= 0.880056				
	Percent	<i>S. mentella</i>	<i>S. marinus</i>	<i>S. viviparus</i>
<i>S. mentella</i>	89.8	177	19	1
<i>S. marinus</i>	92.2	14	188	2
<i>S. viviparus</i>	100.0	0	0	79
Total	92.5	191	207	82
Percentage of misclassification (%)				
<i>S. mentella</i>			9.6	0.5
<i>S. marinus</i>		6.9		1.0
<i>S. viviparus</i>		0.0	0.0	

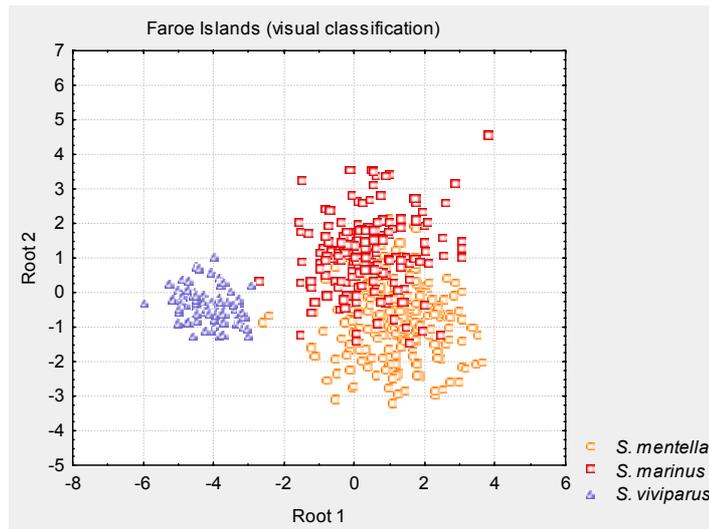


Figure 4.4. Plot of canonical scores for each case for the first and second canonical roots of the analysis performed with the three species inhabiting Faroe Islands waters using external morphology as criterion to classify species.

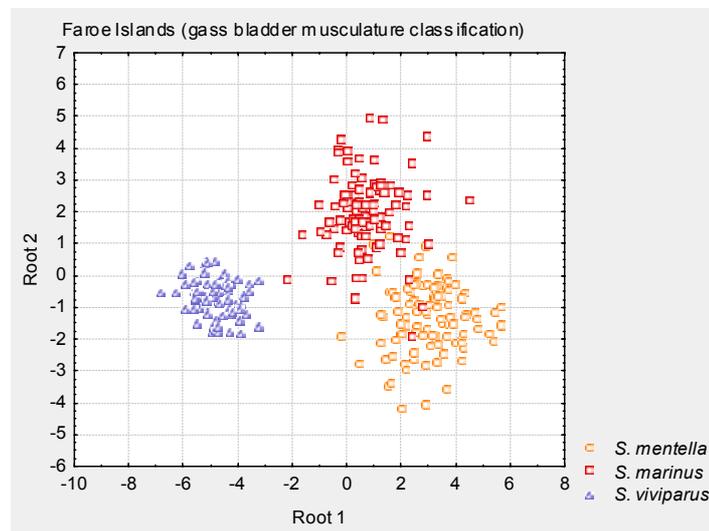


Figure 4.5. Plot of canonical scores for each case for the first and second canonical roots of the analysis performed with the three species inhabiting Faroe Islands, using the gas bladder musculature as criterion to classify species.

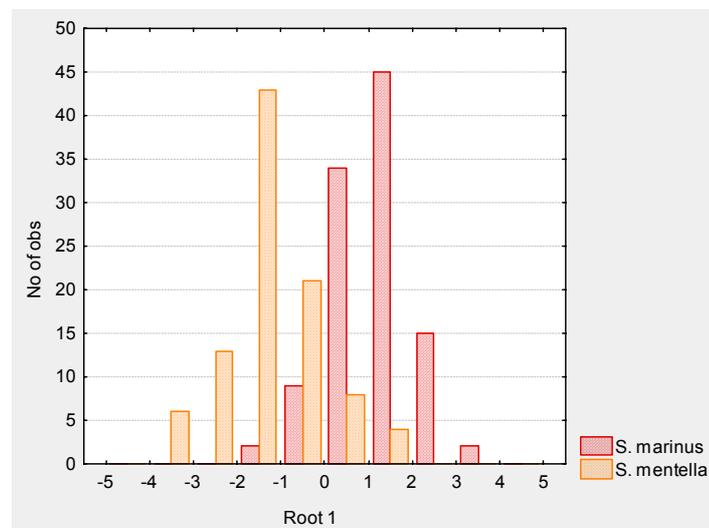


Figure 4.6. Histogram of canonical scores for *S. marinus* and *S. mentella* from Faroes Plateau, showing the discrimination when the individuals of this area are classified into species by external appearance.

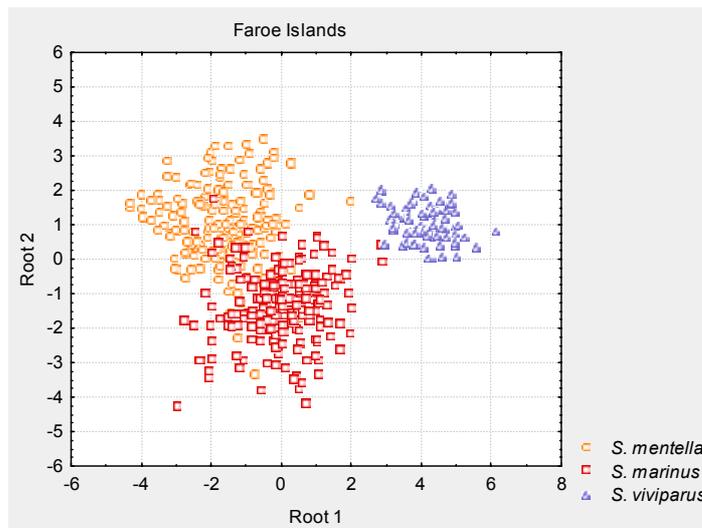


Figure 4.7. Plot of canonical scores for each case for the first and second canonical roots of the analysis performed with the three species inhabiting Faroe Islands waters using both external morphology and the GBM when available for species identification.

ICELAND

This area extends over the entire Icelandic coast. For statistical and analytical purposes, it was divided into three sub-areas, based on geographical situation. These subareas are: Iceland-NE, Iceland-SE and Iceland-SW (Table 4.1).

The samples, species classification and measurements were taken by Icelandic researchers. The individuals were classified into species only following the external appearance of the fish. However, most of the samples come from research surveys where the species are carefully identified. In addition, the classification into species made onboard was revised in the laboratory, when the picture was taken. Most of the individuals (1368) were collected in the South of Iceland (SE and SW), while only 97 individuals from the North are used in traditional morphometrics.

The classification matrix in numbers, the percentage of total correct classification, Cohen's Kappa and Wilks' lambda for the discriminant analysis by species in Iceland are shown in Table 4.9. The separation between species was good, as the overall discrimination reached a value of 91.1%, with an associated Wilks' lambda value of 0.38. This classification rate is lower than the one reached in Norway, but it is only slightly below the Flemish Cap's classification rate. The species are clearly distinct morphometrically as can also be observed in the histogram of the canonical scores (Figure 4.8).

Table 4.9. Classification matrix, percentage of total correct classification, Wilks' lambda and Cohen's Kappa resulting from the discriminant analysis of *Sebastes* species in Iceland.

Wilks' Lambda= 0.38771 Cohen's Kappa= 0.821792			
	Percent	<i>S. mentella</i>	<i>S. marinus</i>
<i>S. mentella</i>	90.3	716	77
<i>S. marinus</i>	92.1	53	619
Total	91.1	769	696
Percentage of misclassification (%)			
<i>S. mentella</i>			9.7
<i>S. marinus</i>		7.9	

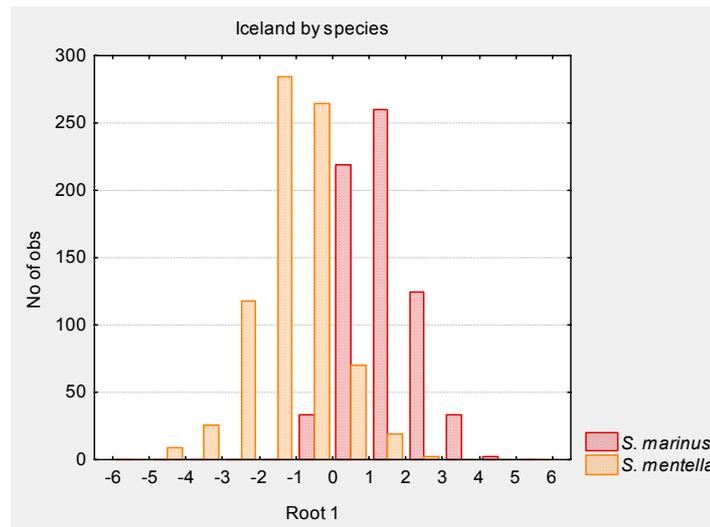


Figure 4.8. Frequencies of canonical scores values for the canonical root resulting of the discriminant analysis performed with the two species inhabiting Iceland.

Greenland

The samples available from this area were taken during two research surveys, 'WH221 cruise' in September-October of 2000 and 'WH233 cruise' in October-November of 2001. The main difference between sampling conducted in Greenland compared with other areas was the high amount of small fish collected in Greenland, especially of *S. mentella*. Greenland, and especially the eastern part, is known as the principal redfish nursery area, and as the samples were collected in research surveys that extend over the whole area and with small mesh size of the trawl gear, a high quantity of juveniles are included in the samples. The smaller the size of the fish, the more difficult species identification becomes and thus, more errors in species assignment may occur in small fish. To avoid this potential problem, some analyses were conducted excluding fish smaller than 200 mm. This threshold was established after observing the size distribution of each species in each area. Fish smaller than 200 mm are called from now onwards the "juveniles" (Table 4.10).

Both Greenland surveys (WH221 and WH233) are part of the long German historical series conducted in autumn around Greenland. The experience in species identification of German researches is high, but still they have reported serious difficulties on distinguishing *S. mentella* and *S. marinus* in Greenland, due to the high resemblance between them, especially in small fish.

RESULTS

As with the Faroe Island analyses, the samples from Greenland were treated in two different manners: samples from WH221 were processed in Iceland, and species identification was made only through external morphology. The author, however, measured the WH233 samples, and hence in addition to the external morphology, the gas bladder musculature was also inspected. Thus, two types of species assignment are available for 1,085 of the 1,409 total fish.

Genetic analyses were conducted in 531 individuals (Table 4.11 and Table 4.12). These analyses were performed in Norway and Germany. But only in 452 of these 531 individuals the gas bladder musculature (GBM) information was also available. The availability of these different species assignments allows several analyses to confirm the suitability of the different methods. The coincidence between the genetic species assignments and the GBM was high with fish analyzed in Germany (Table 4.12), and also in *S. mentella* analyzed in Norway (Table 4.11), but the coincidence was poor for *S. marinus* analyzed in Norway (Table 4.11).

In summary, the morphometric comparison of the species inhabiting Greenland was conducted in three initial steps, each of them using different criteria for species assignment.

Table 4.10 Samples around Greenland by species, cruise and subarea (W: West and E: East). Species identifications are based on external morphology.

Samples in Greenland					
Cruise	Species	Sub-area	No. juveniles	No. adults	Total
WH221	<i>S. marinus</i>	Greenland-E	1	152	153
	<i>S. mentella</i>	Greenland-E	50	50	100
		Greenland-W	63	8	71
WH233	<i>S. marinus</i>	Greenland-E	70	99	169
		Greenland-W	8	57	65
	<i>S. mentella</i>	Greenland-E	315	124	439
		Greenland-W	376	36	412

Table 4.11. Individuals genetically analyzed in Norway. Classification into species has been made by genotype. Juveniles: fish smaller than 20 cm. The mismatch are the number of specimens with different species assignments for genetic or GBM method.

Genetics					
Cruise	Species	Sub-area	Total	Juveniles	Mismatch
WH233	<i>S. marinus</i>	Greenland-E	141	23	42
		Greenland-W	47	5	10
	<i>S. mentella</i>	Greenland-E	98	7	2
		Greenland-W	45	23	3

Table 4.12. Individuals genetically analyzed in Germany. Classification into species has been made by genotype. Juveniles: fish smaller than 20 cm. The mismatch are the number of specimens with different species assignment using genetic and GBM method.

Genetics in Germany					
Cruise	Species	Sub-area	Total	Juveniles	mismatch
WH221	<i>S. marinus</i>	Greenland-E	78	0	No musc
	<i>S. mentella</i>	Greenland-E	1	0	No musc
WH233	<i>S. marinus</i>	Greenland-E	43	2	1
		Greenland-W	28	2	2
	<i>S. mentella</i>	Greenland-E	50	4	1

Step 1. Species classified by external morphology

In a first approach, all fish from both cruises were introduced in the analysis, with the species identifications made on board (by its external appearance).

The classification matrix, the percentage of total correct classification, Cohen's Kappa and Wilks' lambda are shown in Table 4.13. Although the total correct classification was relatively high 90.1% and Wilks' lambda shows a medium value (0.48), the high proportion of *S. marinus* that was classified as *S. mentella* (23.3%) yield a poor discrimination between the species. The high percent of correct classification for *S. mentella* group is spurious because of the higher number of *S. mentella* (1,022) compared with 393 *S. marinus*. The number of individuals by species in the WH221 survey is more evenly distributed, 153 *S. marinus* and 171 *S. mentella*. So, another discriminant analysis was performed on the WH221 samples. The results (Table 4.14) showed the same tendency of *S. mentella* group to a higher classification, but much weaker, 84.8%, which is already considered a poor discrimination.

Step 2. Species classified by gas bladder musculature

As demonstrated in the Faroe Islands' analyses, the use of the gas bladder musculature to identify the species considerably improved the discrimination power. Therefore, the discrimination using GBM instead of external morphology was investigated, using only WH233, i. e. the samples with the GBM identification available. However, the results of the discriminant analysis still showed a low classification rate and a medium lambda value (0.5) (Table 4.15): 22.7% of *S. marinus* were classified as *S. mentella*. As with the previous analyses, the high values of *S. mentella* classification, and the total correct classification, are affected by the unbalanced high number of *S. mentella*.

Due to the high number of small fish involved in this analysis, it was decided to run a new one, but only with fish larger than 200 mm and when GBM information was available (WH233). The classification matrix, the percentage of total correct classifications, Cohen's Kappa and Wilks' lambda for this new analysis are shown in Table 4.16. Although, in general, the results yielded a better discrimination than in previous analyses, the proportion correctly classified is low for both species: 86.8% for *S. marinus* and 83.5% for *S. mentella*, and lambda value (0.49) shows a relatively poor discriminant power.

These results indicate that there are low morphometric differences between species in Greenland. However, the possibility was considered that GBM is not a suitable character for species identification in this area. In order to test this hypothesis, a third analysis was performed, using the individuals for which genotypes were available.

Table 4.13. Classification matrix, percentage of total correct classifications and Wilks' lambda and Cohen's Kappa resulting from the discriminant analysis of *Sebastes* species in Greenland using external morphology of the individuals for species classifications. The percentage of misclassifications is shown at the bottom.

Wilks' Lambda= 0.48788 Cohen's Kappa= 0.742355			
	Percent	<i>S. mentella</i>	<i>S. marinus</i>
<i>S. mentella</i>	95.1	972	50
<i>S. marinus</i>	76.7	90	297
Total	90.1	1062	347
Percentage of misclassification (%)			
<i>S. mentella</i>			4.9
<i>S. marinus</i>		23.3	

RESULTS

Table 4.14. Classification matrix, percentage of total correct classification and Wilks' lambda and Cohen's Kappa resulting from the discriminant analysis of *Sebastes* species in Greenland using external morphology for the WH221 samples. The percentage of misclassifications is shown at the bottom.

Wilks' Lambda= 0.53608 Cohen's Kappa= 0.627458			
	Percent	<i>S. mentella</i>	<i>S. marinus</i>
<i>S. mentella</i>	84.8	145	26
<i>S. marinus</i>	77.8	34	119
Total	81.5	179	145
Percentage of misclassification (%)			
<i>S. mentella</i>			15.2
<i>S. marinus</i>		22.2	

Table 4.15. Classification matrix, percentage of total correct classification and Wilks' lambda and Cohen's Kappa resulting from the discriminant analysis of *Sebastes* species in Greenland using gas bladder musculature examination for species assignment. The percentage of misclassifications is shown at the bottom.

Wilks' Lambda= 0.50142 Cohen's Kappa= 0.736426			
	Percent	<i>S. mentella</i>	<i>S. marinus</i>
<i>S. mentella</i>	95.5	842	40
<i>S. marinus</i>	77.3	46	157
Total	92.1	888	197
Percentage of misclassification (%)			
<i>S. mentella</i>			4.5
<i>S. marinus</i>		22.7	

Table 4.16 Classification matrix, percentage of total correct classification and Wilks' lambda and Cohen's Kappa resulting from the discriminant analysis of *Sebastes* species in Greenland using the gas bladder musculature examination for species assignment, and excluding the juveniles. The percentage of misclassifications is shown at the bottom.

Wilks' Lambda= 0.49714 Cohen's Kappa= 0.702603			
	Percent	<i>S. mentella</i>	<i>S. marinus</i>
<i>S. mentella</i>	83.5	137	27
<i>S. marinus</i>	86.8	20	132
Total	85.1	157	159
Percentage of misclassification (%)			
<i>S. mentella</i>			16.5
<i>S. marinus</i>		13.2	

Step 3. Species classified by genotype.

The genetic analyses revealed the presence of three major genotypes: two were clearly *S. marinus* and *S. mentella* respectively, and the third one could not be assigned to one or another species, the so called *Sebastes* type. The fish used in the following analysis were those for which genetic results offered no doubts about the species assignment. Therefore, those specimens in which the genotype was not clearly *S. marinus* or *S. mentella* were not included in the current analyses. Samples from WH221 survey were analyzed in Germany, and for those fish the GBM pattern was not available. However, there was a 100% coincidence in species assignment between the external morphology and the genetic analyses. WH233 individuals were analyzed in both Germany and Norway, yielding basically the same genotypes, except for some fish that had been identified as *S. marinus* in Norway and as *Sebastes* type in Germany. Because of these differences, two morphometric analyses were performed, one with the species assignment made in Germany and a second with that made in Norway.

The classification matrix in numbers, the percentage of total correct classification, Cohen's Kappa and Wilks' lambda resultant of the discriminant analysis between *S. mentella* and *S. marinus* in Greenland, attending to the genotype ascription made in Germany are shown in Table 4.17. Species discrimination is very good, up to 98%, and both species discriminate correctly, in 98.7% *S. marinus* and in 95.8% *S. mentella*. Only 4 out 200 specimens are classified in different species than their a priori assignments. The lambda value (0.29) corroborates this good classification.

The results of the discrimination analysis using Norwegian genetic species ascription showed a similar trend. The classification matrix in numbers, the percentage of total correct classification, Cohen's Kappa and Wilks' lambda for this analysis are shown in Table 4.18. The lambda value of 0.36 reflects a good discrimination, and in fact, both species showed a proportion correctly classified close to the 94%, which is also the total correct classification. Only 20 out 331 individuals were classified as different species.

Table 4.17. Classification matrix, percentage of total correct classification and Wilks' lambda and Cohen's Kappa resulting from the discriminant analysis of *Sebastes* species in Greenland using genetic species identification from German research.

Wilks' Lambda= 0.29964 Cohen's Kappa= 0.944911			
	Percent	<i>S. mentella</i>	<i>S. marinus</i>
<i>S. mentella</i>	95.8	46	2
<i>S. marinus</i>	98.7	2	147
Total	98.0	48	149
Percentage of misclassification (%)			
<i>S. mentella</i>			4.2
<i>S. marinus</i>		1.3	

Table 4.18. Classification matrix, percentage of total correct classification and Wilks' lambda and Cohen's Kappa resulting from the discriminant analysis of *Sebastes* species in Greenland using genetic species identification from Norwegian researches.

Wilks' Lambda= 0.36320 Cohen's Kappa= 0.877084			
	Percent	<i>S. mentella</i>	<i>S. marinus</i>
<i>S. mentella</i>	93.7	134	9
<i>S. marinus</i>	94.1	11	177
Total	94.0	145	186
Percentage of misclassification (%)			
<i>S. mentella</i>			6.3
<i>S. marinus</i>		5.9	

Final step: final analysis.

The morphometric analyses conducted with the species assigned well with the external musculature, well with the GBM, yielded a poor discrimination. However, when genetic ascription was used, a very good morphometric discrimination occurred. It is interesting to note that the species ascription following the external morphology and the genetic results for the survey WH221 were in full agreement. In this survey 75% of the sampled fishes were above 180 mm, while in WH233, 65% of the fishes analyzed were below 180 mm, i.e. the number of small fish in the WH233 survey is considerably higher. Another difference between surveys was the area sampled, while WH221 collected more of the samples from East Greenland, the proportion of fish collected from West Greenland in WH233 was 44%. It must also be noted that in West Greenland the size of the fish is generally smaller. Figure 4.9 shows the standard length distribution of *S. marinus* from WH233, split into two groups: blue represents the lack of coincidence between species ascription based on GBM and

genetics; green represents those individuals where both species designations yield the same results. In all *S. marinus* with standard length larger than 280 mm, both techniques yield the same species, whilst for all individuals with standard length smaller than 150 mm they never match. The intermediate sizes (from 150 to 280mm) show equal probability to belong to one or another group.

GBM was not available for WH221 samples, but only available for WH233 samples. As shown in Table 4.11, the quantity of mismatch between genetic and GBM species assignment is low for *S. mentella*, but noticeable for *S. marinus*.

It was, therefore, observed that small size may be the main reason for species identification failure, not only when this identification is made using external morphology of the fish, but also using GBM anatomy.

Due to the impossibility of classifying all Greenland sampled individuals accurately into species, the morphometric analysis for species discrimination in Greenland was performed using only those individuals for which genetic species classification was available, i.e. 411 specimens from WH221 and WH233, analyzed in German and Norwegian laboratories.

The classification matrix in numbers, the percentage of total correct classification, Cohen's Kappa and Wilks' lambda of this new analysis are shown in

Table 4.19. As expected, the results showed a good discrimination between species, 94.4% of total classification (Wilks' lambda = 0.38), with a partial discrimination of 93.1% for *S. mentella* and 95.1% for *S. marinus*. It must be concluded that both are clearly morphometrically distinct species, as can also be observed in the plot of the canonical scores (Figure 4.10).

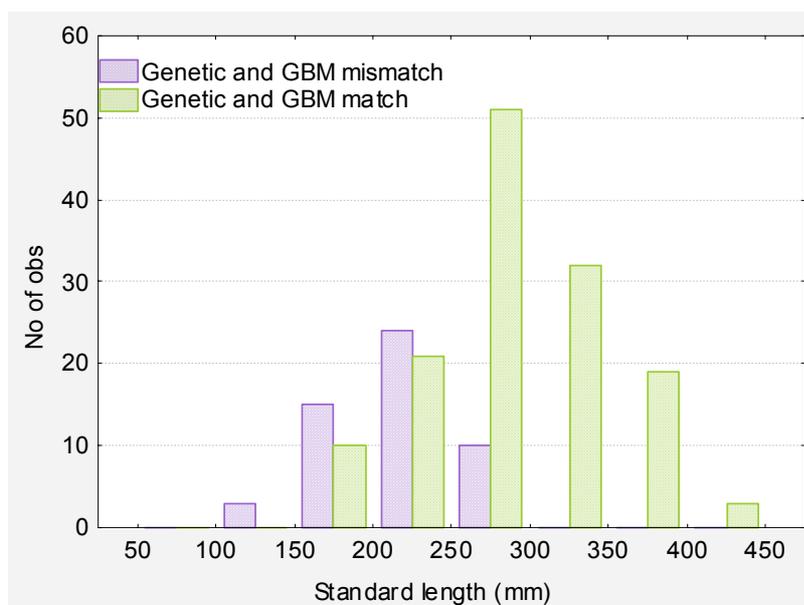


Figure 4.9. Frequency of match and mismatch of species assignment by genetic and GBM by size range in *S. marinus* from WH233.

Table 4.19. Species classification in Greenland area using WH233 and WH221 genetic species identification. The percentage of misclassification is shown at the bottom.

Wilks' Lambda= 0.38847 Cohen's Kappa= 0.877655			
	Percent	<i>S. mentella</i>	<i>S. marinus</i>
<i>S. mentella</i>	93.1	134	10
<i>S. marinus</i>	95.1	13	254
Total	94.4	147	264
Percentage of misclassification (%)			
<i>S. mentella</i>			6.9
<i>S. marinus</i>		4.9	

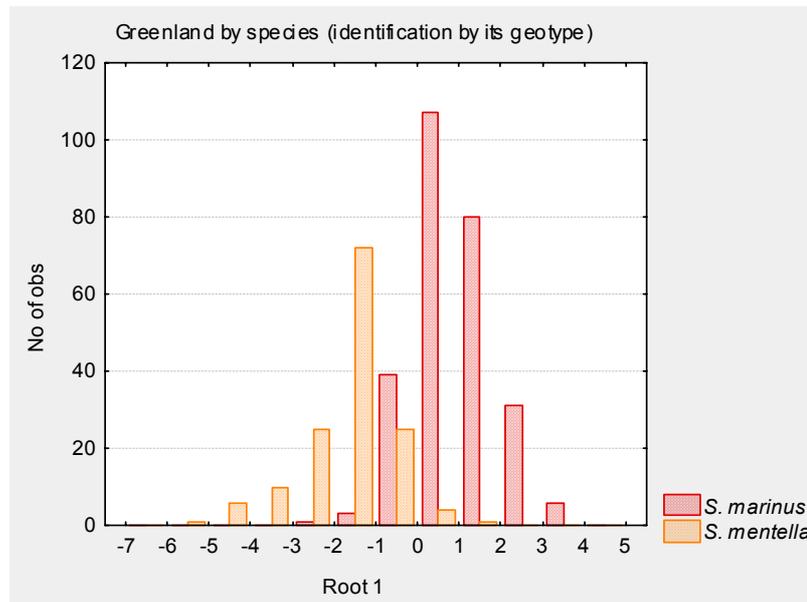


Figure 4.10. Frequencies of canonical score values for the canonical root resulting from the discriminant analysis performed with the two species inhabiting Greenland identified by genotype.

4.1.1.3. Final species analysis in the whole area

Due to the difficulties found in Faroe Islands and Greenland for species assignation using external morphology, it was decided, for subsequent analyses, to use only the individuals where a reliable species assignation was available, and thus, the GBM was used in Norway, Flemish Cap, Faroe Islands and the Irminger Sea samples analyzed by the author; genetic assignation was used in Greenland; and finally, in Iceland and the Irminger Sea samples analyzed by Icelandic researchers, the external morphology was used, as it has been proved they were reliable (Table 4.20).

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Table 4.20. Individuals with a reliable species assignment. This species assignment has been used in the final analysis studying the discrimination between the four redfish species in the whole area.

Individuals used in the final morphometric analyses								
Species	Area	Standard body length					Total	
		<200	200-250	250-300	300-350	350-400		>400
<i>S. marinus</i>	Faroes		5	23	50	57	69	204
	Iceland	30	118	196	179	122	27	672
	Greenland	28	45	65	54	66	9	267
	Flemish Cap	37	34	14	16	1		102
	Norway		8	12	24	15	2	61
<i>S. mentella</i>	Faroes			27	38	92	40	197
	Iceland	25	88	226	255	172	27	793
	Greenland	30	75	32	7			144
	Irminger	22	245	792	742	265	3	2069
	Flemish Cap	46	30	26	9			111
	Norway		2	25	43	5		75
<i>S. fasciatus</i>	Flemish Cap	64	33	2	2	1		102
<i>S. viviparus</i>	Faroes	29	47	3				79
	Norway	81	12	1				94
Total		392	742	1444	1419	796	177	4970

The classification matrix in numbers, the percentage of total correct classification, Wilks' lambda and Cohen's Kappa for this new analysis with a more accurate species classification are shown in Table 4.21. The overall correct classification increased to 92.9 % (compared to 90.5% from the initial analysis) and Wilks' lambda dropped to 0.23 (compared to 0.29 in the first analysis). The proportion correctly classified increased for *S. marinus*, *S. fasciatus* and *S. viviparus* (see Table 4.2 for comparisons). However, *S. fasciatus* still showed a low correct classification, 60.8%, beyond the admissible limits of good classification. It is interesting to note that *S. fasciatus* was absolutely well classified in Flemish Cap (Table 4.3). It is surprising, therefore, that this species yielded so low a discrimination when the areas are pooled, and it is remarkable that the main confusion is with *S. marinus*. The group with the largest sample size is, by far, *S. mentella*, and it would be expected that in case of poor discrimination the higher confusion rate would be with this group. However, this is not the case for *S. fasciatus*, neither for *S. viviparus* that also showed more confusion with *S. marinus* (8.1%) than with *S. mentella* (1.2%).

The same analysis was carried out but with a more balanced design, i.e. the same number of individuals in each of the species; 100 individuals were randomly selected for each species. In this new analysis, part of the information was lost, as most of the sampled individuals were not used, but on the other hand the classifications may not be biased. The classification matrix, the percentage of total correct classification, Wilks' lambda and Cohen's Kappa are shown in Table 4.22 while the scatterplot of canonical scores is shown in Figure 4.12. Now *S. mentella* and *S. viviparus* showed a high discrimination, 92 and 100% respectively of correct classification, and a low confusion rate with other species. *S. fasciatus* showed 93% correctly classified, a value much higher than in the previous analysis. On the contrary, *S. marinus* classification diminished to 80%, due principally to confusion with *S. fasciatus* (12%). Therefore, *S. fasciatus* and *S. marinus* still showed an important confusion rate.

Considering that both species classified correctly in the analyses for each area separately, the low classification rate when pooling all areas can be due to an area effect, i.e. *S. fasciatus* is morphometrically different to *S. marinus* in Flemish Cap, but may be similar to *S. marinus* in other areas. This issue is discussed below, but an environmental effect should not be discarded.

Thus, it is necessary to study first the differences and similarities between areas within each species to ascertain how the relationship between the potential populations.

Table 4.21. Classification matrix, percentage of total correct classification and Wilks' lambda and Cohen's Kappa resulting from the discriminant analysis of *Sebastes* species in the whole area with new species assignation in Greenland area, based on genotype. The percentage of misclassification is shown at the bottom.

Wilks' Lambda= 0.238237 Cohen's Kappa= 0.849162					
	Correct %	<i>S. mentella</i>	<i>S. marinus</i>	<i>S. fasciatus</i>	<i>S. viviparus</i>
<i>S. mentella</i>	95.3	3230	142	6	11
<i>S. marinus</i>	89.7	102	1171	19	14
<i>S. fasciatus</i>	60.8	7	30	62	3
<i>S. viviparus</i>	90.2	2	14	1	156
Total	92.9	3341	1357	88	184
Percentage of misclassification (%)					
<i>S. mentella</i>			4.2	0.2	0.3
<i>S. marinus</i>		7.8		1.5	1.1
<i>S. fasciatus</i>		6.9	29.4		2.9
<i>S. viviparus</i>		1.2	8.1	0.6	

Table 4.22 Classification matrix in numbers, percentage of total correct classification and Wilks' lambda and Cohen's Kappa resulting from the discriminant analysis of all cases in the whole area evaluating discrimination among the four species, i. e. *S. marinus*, *S. mentella*, *S. fasciatus* and *S. viviparus*, taking 100 random individuals of each species. The percentage of misclassification is shown at the bottom.

Wilks' Lambda= 0.060119 Cohen's Kappa= 0.883333					
	Correct %	<i>S. mentella</i>	<i>S. marinus</i>	<i>S. fasciatus</i>	<i>S. viviparus</i>
<i>S. mentella</i>	92.0	92	7	1	0
<i>S. marinus</i>	80.0	5	80	12	3
<i>S. fasciatus</i>	93.0	1	4	93	2
<i>S. viviparus</i>	100.0	0	0	0	100
Total	91.3	98	91	106	105
Percentage of misclassification (%)					
<i>S. mentella</i>			7.0	1.0	0.0
<i>S. marinus</i>		5.0		12.0	3.0
<i>S. fasciatus</i>		1.0	4.0		2.0
<i>S. viviparus</i>		0.0	0.0	0.0	

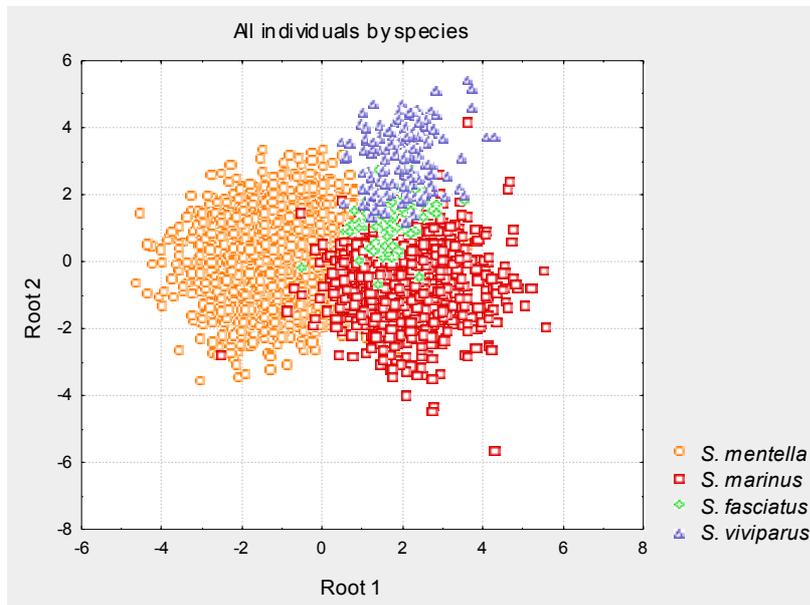


Figure 4.11. Plot of canonical scores for each case for the first and second canonical roots resulting from the discriminant analysis performed with the four species inhabiting the whole analyzed area.'

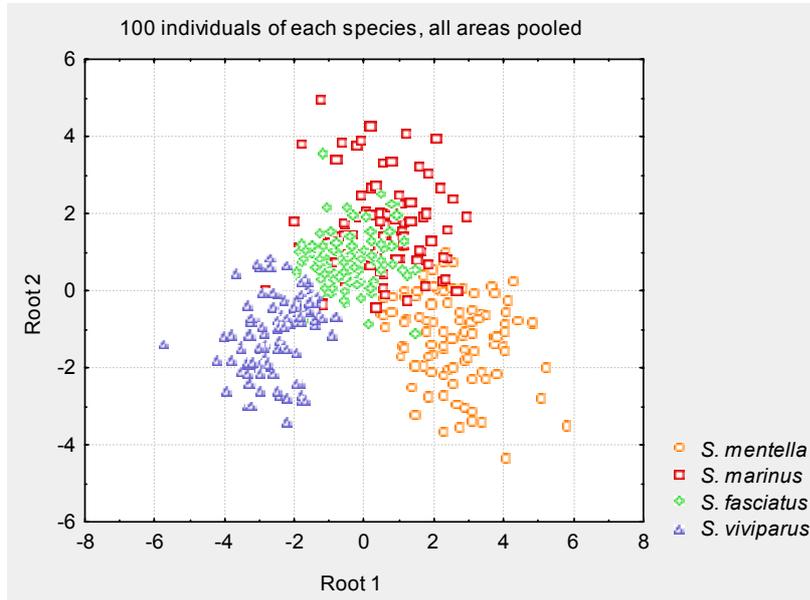


Figure 4.12 Plot of canonical scores for each case for the first and second canonical roots.

4.1.2. Discrimination between areas and subareas

In this section, the individuals of each species in the different areas and subareas are morphometrically compared. The areas correspond to the shelves of Faroes, Greenland, Iceland, and Norway, the Flemish Cap bank and the pelagic fishery in the open Irminger Sea. The subareas have been defined as follows:

Faroe Islands.- Samples have been divided into two subareas: Northwest and southeast based on the geographical positions where the samples were taken. This division does not correspond to different fishing banks or potential stocks, they are just geographical divisions, but the NW is exclusively within the Faroes Plateau and is very close, maybe related, to Southeast Iceland, while the SE includes the so called Faroes Bank. Most of the samples were taken in the Northwest (Faroes-NW).

Iceland.- It was initially divided into four quadrants, Northeast (NE), Northwest (NW), Southeast (SE) and Southwest (SW). For traditional morphometric analysis, however, no samples from the Northwest were available. As in the Faroe Islands, these divisions are purely geographical, and no biological meaning lies behind them. However, redfish is not evenly distributed, and hence nor are the samples (see maps in Chapter 3, Figure 3.2 and 3.3); thus most of the SW Iceland samples are in the vicinity of the Irminger Sea, while the southeast samples are very close to the Faroes.

Greenland.- It has been divided into East and West Greenland using Cape Farewell as reference.

These three major areas together, the Faroes, Iceland and Greenland are called the “central area” in this study.

Irminger Sea, where only *S. mentella* occurs, has been divided into three areas; the Northeast, over Reykjanes Ridge and close to SW Iceland waters (Irminger-NE); the central Irminger Sea (Irminger-CEN) south of Greenland, and, finally, Irminger-NAFO corresponds to pelagic *S. mentella* sampled in the NAFO-1F area, over the Labrador Basin.

Flemish Cap and Norway are very distinct geographical areas from the central area and the Irminger Sea, and are considered as reference areas.

Table 4.23 summarizes the areas and subareas described above. It is important to recall that Greenland samples used in all the following analyses are only those with available genetic species assignment.

Table 4.23. Areas and Sub-areas. The central area is constituted by Irminger Sea and adjacent waters, that is, Irminger Sea, Faroe Islands, Iceland and Greenland.

	Central area				Border areas	
Areas	Faroe Islands	Iceland	Greenland	Irminger	Flemish Cap	Norway
Subareas	Faroes-NW	Iceland-NE	Greenland-E	Irminger-NE		
	Faroes-SE	Iceland-SE	Greenland-W	Irminger-CEN		
		Iceland-SW		Irminger-NAFO		

4.1.2.1. *S. marinus*

To study the potential populations of *S. marinus*, the relationships between areas were first analyzed, and later the relationships between the subareas described above. *S. marinus* samples are available from Norway, Faroes, Iceland, Greenland and Flemish Cap.

The total number of *S. marinus* samples available by area, subarea and size range is shown in Table 4.24.

Table 4.24. *S. marinus* samples in the different areas and subareas by standard body length.

<i>S. marinus</i>								
Area	Sub-area	Standard body length						Total
		<200	200-250	250-300	300-350	350-400	>400	
Faroes	NW		5	23	48	35	32	143
	SE				2	22	37	61
Iceland	NE	9	25	10	3			47
	SE	9	62	115	101	66	18	371
	SW	12	31	71	75	56	9	254
Greenland	E	23	31	47	46	64	9	220
	W	5	14	18	8	2		47
Flemish Cap		37	34	14	16	1		102
Norway			8	12	24	15	2	61
Total		95	210	310	323	261	107	1306

Differences by area (*S. marinus*)

Five different potential populations were used in this analysis: Norway, the Faroe Islands, Iceland, Greenland and Flemish Cap. A total of 1,306 specimens was used, 61, 204, 672, 267 and 102 in each area respectively (Table 4.24).

The classification matrix in numbers, the percentage of total correct classification, Cohen's Kappa and Wilks' lambda are shown in Table 4.25. The total correct classification was low, 69.9%, which indicates a very poor discrimination. Although Wilks' λ value was also low, indicating a good discrimination for some of the areas, Cohen's Kappa value is lower than 0.7, indicating that the classification is not reliable. Greenland and the Faroe Islands showed a very low percent of correct classification (43.7 and 44.6 % respectively), most of the fish of these two areas being classified as Icelandic. Although the specimens from Iceland were mostly correctly classified (86.5%), the confusion rate with Greenland and Faroes was very high, suggesting a lack of structuring for *S. marinus* in the central area. From the classification matrix a gradient can be observed in the confusion rate, i.e. the proportion of fish that are classified into a different group is proportional to the geographical distance between areas. Thus, Faroe Islands showed a higher confusion with Iceland than with Greenland; Greenland showed most of the confusion with Iceland, and Iceland, that is between Greenland and the Faroes, showed more or less the same level of confusion with both areas. Discrimination was 93.4% for Norway but only 67.6% for Flemish Cap. Flemish Cap is far away from the central area, and a higher percent of correct classification was expected.

However, as stated before, the results might be biased by the high number of individuals in Iceland. Thus, a new analysis was performed by randomly choosing 100 individuals from each of the areas. The results of this analysis can be seen in Table 4.26 and Figure 4.14. The correct percent of classification in Iceland diminishes to more logical values (62%) considering the confusion with the neighboring areas observed in the previous analysis. However, now, as expected, Flemish Cap showed a higher percent of correct classification. Thus, in this new analysis, the two reference areas Flemish Cap and Norway, showed a high percent of correct classification (89% and 93.4% respectively), validating not only the technique but also the logic of the results obtained here: Norway and Flemish Cap are different populations, while Faroe Islands, Iceland and Greenland are the same, although a geographic pattern exists in these central areas. This pattern can be observed also in the plot of the canonical scores shown in Figure 4.14. In this scatterplot, it can be observed that Flemish Cap and Norway cluster apart from the Central area, although Flemish Cap showed a closer relationship with the central area than *S. marinus* from Norway, that appears very different from all the other areas, including the Faroes.

Table 4.25. Classification matrix, percentage of total correct classification and Wilks' lambda and Cohen's Kappa resulting from the discriminant analysis of *S. marinus* by area

Wilks' Lambda= 0.204187 Cohen's Kappa= 0.516377						
	Correct %	Iceland	Greenland	Faroe Islands	Flemish Cap	Norway
Iceland	86.5	581	45	33	13	0
Greenland	43.1	133	115	7	12	0
Faroe Islands	44.6	91	17	91	5	0
Flemish Cap	67.6	15	15	3	69	0
Norway	93.4	1	1	0	2	57
Total	69.9	821	193	134	101	57
Percentage of misclassification (%)						
Iceland			6.7	4.9	1.9	0.0
Greenland		49.8		2.6	4.5	0.0
Faroe Islands		44.6	8.3		2.5	0.0
Flemish Cap		14.7	14.7	2.9		0.0
Norway		1.6	1.6	0.0	3.3	

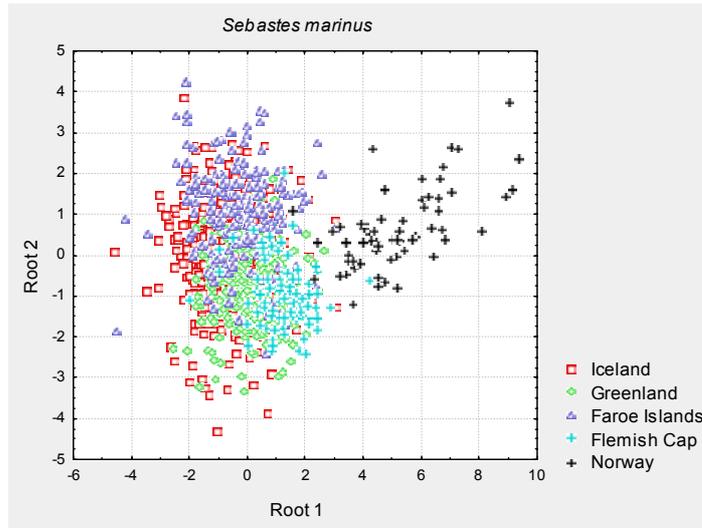


Figure 4.13. Plot of canonical scores for each case for the first and second canonical roots resulting from the discriminant analysis performed with *S. marinus* by area.

Table 4.26. Classification matrix, wilks' Lambda and Cohen's Kappa resulting from the discriminant analysis of *S. marinus* by area, taking 100 individuals in each of the areas.

Wilks' Lambda= 0.096461 Cohen's Kappa= 0.641902						
	Correct %	Iceland	Greenland	Faroe Islands	Flemish Cap	Norway
Iceland	62.0	62	15	11	12	0
Greenland	59.0	18	59	10	13	0
Faroe Islands	63.0	16	13	63	8	0
Flemish Cap	89.0	5	3	3	89	0
Norway	93.4	0	0	0	4	57
Total	71.6	101	90	87	126	57
Percentage of missclassification (%)						
Iceland			15.0	11.0	12.0	0.0
Greenland		18.0		10.0	13.0	0.0
Faroe Islands		16.0	13.0		8.0	0.0
Flemish Cap		5.0	3.0	3.0		0.0
Norway		0.0	0.0	0.0	6.6	

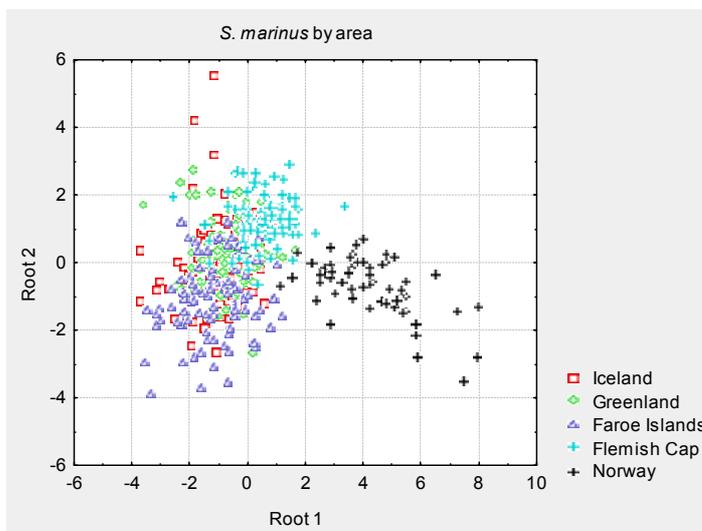


Figure 4.14. Plot of canonical scores for each case for the first and second canonical roots, resulting from the discriminant analysis of *S. marinus* by area, taking 100 individuals for each of the areas.

Differences by Subarea (*S. marinus*)

The subareas considered were those reflected in Table 4.23. To analyze the morphometric relationships among subareas can provide us with important information about the population structure, not only within a given area, but also about the whole population structure. In this sense it has been considered more interesting to perform a single analysis with all the subareas as groups to obtain a general picture of the relationships among them. This is especially true for this case because a lack of discrimination has been observed for the central area.

The classification matrix in numbers, the percentage of total correct classification, Cohen's Kappa and Wilks' lambda are shown in Table 4.27. As before, an extra analysis was performed with a more balanced design on the group size, to avoid the possible bias produced by the difference in numbers of individuals by group. The results are displayed in Table 4.28 and Figure 4.15. Only Flemish Cap and Norway showed a good discrimination, 88 and 93.4 % respectively. These two good classification rates produce a very low Wilks' λ (0.06). As expected from previous analyses, all the other subareas presented a very low classification rate. However, it is very interesting to analyze the confusion matrix which yielded a geographical pattern, also observed in the plot of canonical scores (Figure 4.15) and in the tree diagram of the cluster analysis (Figure 4.16):

There were no individuals from other areas classified into Norway and this area clearly clustered separately. The confusion between the subareas included in the central area is evident. However, Greenland subareas misclassified basically with Iceland subareas. The confusion of Iceland subareas was with Greenland and Faroes in similar proportion. While Faroes subareas confused essentially with South Iceland. Flemish Cap classification is not especially high (88%). Both subareas of Greenland showed a relatively high proportion of confusion with Flemish Cap, showing a connection between these areas. This is reflected very well in the cluster analysis (Figure 4.16)

In summary *S. marinus* showed a good discrimination in Norway, a low classification rate for the "central area", suggesting a lack of structuring for this species in this area (Greenland, Iceland and the Faroe Islands), and it is not clear if Flemish Cap is a separate population because some relation exist between *S. marinus* in this area and in the central area, specially with Greenland. Further analyses would be necessary, perhaps with samples of areas on the coast of Canada. A clear geographical pattern exists connected with the morphometric similarities between subareas.

RESULTS

Table 4.27. Classification matrix, percentage of total correct classification and Wilks' lambda and Cohen's Kappa resulting from the discriminant analysis of *S. marinus* by subarea.

Wilks' Lambda= 0.126929 Cohen's Kappa= 0.457907										
	Correct %	Iceland			Greenland		Faroe Islands		Flemish	Norway
		NE	SE	SW	E	W	NW	SE		
Iceland-NE	10.6	5	30	1	10	0	0	0	1	0
Iceland-SE	66.3	8	246	41	42	0	22	1	10	1
Iceland-SW	56.7	0	73	144	6	1	18	8	4	0
Greenland-E	51.4	2	70	12	113	0	11	0	12	0
Greenland-W	0.0	0	14	6	18	0	5	0	4	0
Faroese-NW	36.4	1	29	33	12	0	52	11	5	0
Faroese-SE	62.3	0	3	14	1	0	5	38	0	0
Flemish Cap	75.5	0	4	7	10	1	3	0	77	0
Norway	93.4	0	0	1	0	0	0	0	3	57
Total	56.0	16	469	259	212	2	116	58	116	58
Percentage of misclassification										
Iceland-NE			63.8	2.1	21.3	0.0	0.0	0.0	2.1	0.0
Iceland-SE		2.2		11.1	11.3	0.0	5.9	0.3	2.7	0.3
Iceland-SW		0.0	28.7		2.4	0.4	7.1	3.1	1.6	0.0
Greenland-E		0.9	31.8	5.5		0.0	5.0	0.0	5.5	0.0
Greenland-W		0.0	29.8	12.8	38.3		10.6	0.0	8.5	0.0
Faroese-NW		0.7	20.3	23.1	8.4	0.0		7.7	3.5	0.0
Faroese-SE		0.0	4.9	23.0	1.6	0.0	8.2		0.0	0.0
Flemish Cap		0.0	3.9	6.9	9.8	1.0	2.9	0.0		0.0
Norway		0.0	0.0	1.6	0.0	0.0	0.0	0.0	4.9	

Table 4.28 Classification matrix, percentage of total correct classification, Wilks' lambda and Cohen's Kappa resulting from the discriminant analysis of *S. marinus* by subarea with a balanced design.

Wilks' Lambda= 0.069881 Cohen's Kappa= 0.525122										
Subarea	Correct %	Iceland			Greenland		Faroe Islands		Flemish	Norway
		NE	SE	SW	E	W	NW	SE		
Iceland-NE	40,4	19	15	2	7	0	0	0	4	0
Iceland-SE	50,0	8	50	11	15	0	11	1	4	0
Iceland-SW	60,0	1	17	60	1	0	11	6	4	0
Greenland-E	53,0	6	13	6	53	1	9	1	11	0
Greenland-W	2,1	1	5	7	17	1	8	0	8	0
Faroese-NW	49,0	1	11	15	7	0	49	12	5	0
Faroese-SE	68,9	0	1	11	1	0	4	42	2	0
Flemish Cap	88,0	0	4	3	2	1	1	1	88	0
Norway	93,4	0	0	1	0	0	0	0	3	57
Total	58,5	36	116	116	103	3	93	63	129	57
Percentage of misclassification										
Iceland-NE			31,9	4,3	14,9	0,0	0,0	0,0	8,5	0,0
Iceland-SE		8,0		11,0	15,0	0,0	11,0	1,0	4,0	0,0
Iceland-SW		1,0	17,0		1,0	0,0	11,0	6,0	4,0	0,0
Greenland-E		6,0	13,0	6,0		1,0	9,0	1,0	11,0	0,0
Greenland-W		2,1	10,6	14,9	36,2		17,0	0,0	17,0	0,0
Faroese-NW		1,0	11,0	15,0	7,0	0,0		12,0	5,0	0,0
Faroese-SE		0,0	1,6	18,0	1,6	0,0	6,6		3,3	0,0
Flemish Cap		0,0	4,0	3,0	2,0	1,0	1,0	1,0		0,0
Norway		0,0	0,0	1,6	0,0	0,0	0,0	0,0	4,9	

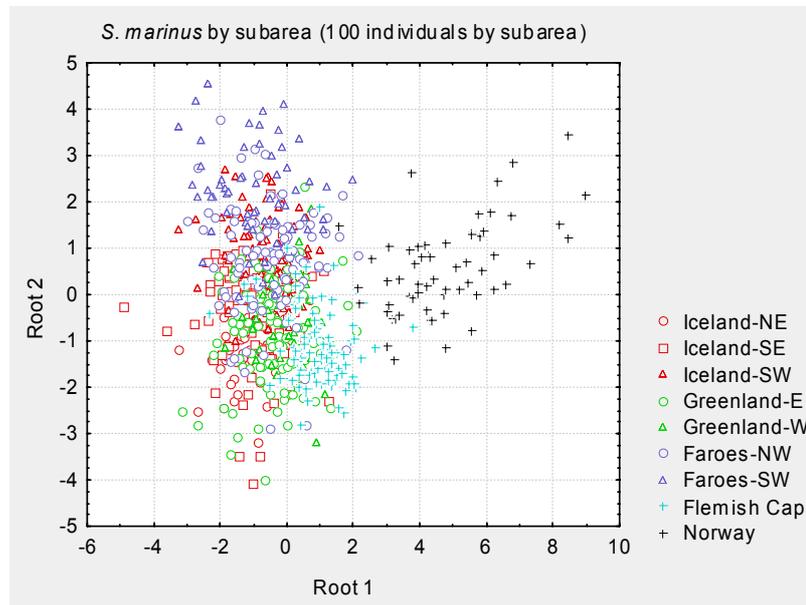


Figure 4.15. Plot of canonical scores for each case for the first and second canonical roots resulting from the discriminant analysis performed with *S. marinus* by Sub area, with a balanced design.

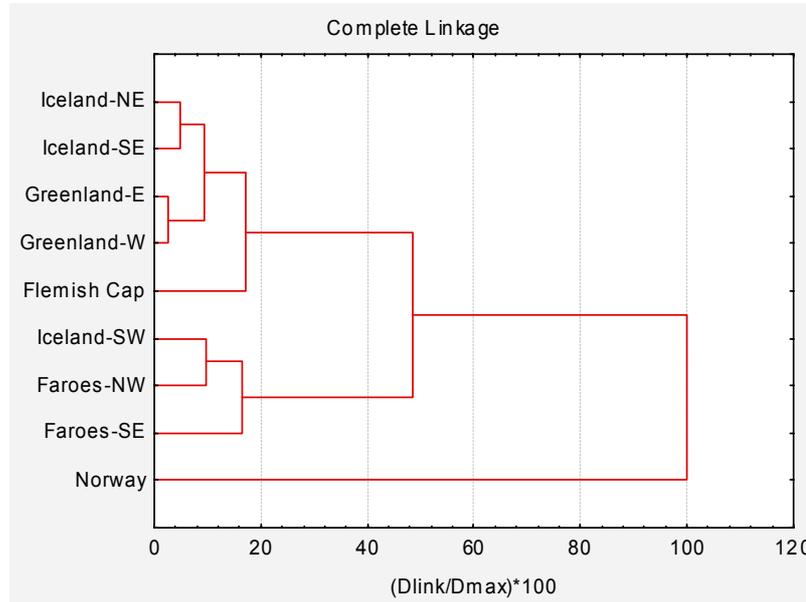


Figure 4.16 Tree diagram of a Cluster analysis based in Mahalanobis distances resulting from the discriminant analysis performed with *S. marinus* by Subarea with a balanced design.

4.1.2.2. *S. mentella*

The areas analyzed for *S. mentella* were the same as for *S. marinus*, i. e., Iceland, Greenland, the Faroe islands, Norway and Flemish Cap, and additionally, the Irminger Sea where only *S. mentella* is present.

The open Irminger Sea is the major fishing ground for *S. mentella* in the whole Atlantic, and its abundance is very high, being, thus, the main area of study of the redfish project. The vast distribution of *S. mentella* in this area extends to the surroundings of Iceland and Greenland, to the south of parallel 54°N, and towards the West reach the vicinity of the Labrador Sea.

The total number of *S. mentella* samples available by area, subarea and size range is shown in Table 4.29. A total of 3,389 *S. mentella* were analyzed. The individuals were assigned to species following the external morphology of the fish in Iceland and Irminger Sea, using the GBM pattern in Flemish Cap, Norway and the Faroe Islands and the genotype in Greenland.

Table 4.29. *S. mentella* samples in the different areas and subareas by standard body length.

<i>S. mentella</i>								
Area	Sub-area	Standard body length						Total
		<200	200-250	250-300	300-350	350-400	>400	
Faroes	NW			26	36	59	18	139
	SE			1	2	33	22	58
Iceland	NE	13	34	3				50
	SE	11	32	138	163	102	21	467
	SW	1	22	85	92	70	6	276
Greenland	E	7	59	26	7			99
	W	23	16	6				45
Irminger	NE		22	298	345	233	3	901
	CEN	10	150	380	268	28		836
	NAFO	12	73	114	129	4		332
Flemish Cap		46	30	26	9			111
Norway			2	25	43	5		75
Total		123	440	1128	1094	534	70	3389

Differences by area (*S. mentella*)

The number of specimens per area was: 75 in Norway, 197 in Faroe Island, 793 in Iceland, 2,069 in the Irminger Sea and 111 in Flemish Cap. Obviously, there was a strong bias in sample size towards the Irminger Sea and to a lesser extent towards Iceland. The former area represented 61% of the total of individuals analyzed, while Iceland accounted for 24%. Each of the remaining areas represented less than 6% of the individuals analyzed. Therefore, a second analysis was performed with a more balanced number of individuals per area, i.e., 75 random individuals in each of the areas. In this analysis, the bias due to the imbalance of the number of individuals was avoided, but part of the information was lost because many of the samples did not enter the analysis. The individuals were selected randomly within each subarea, to reach 75 fishes in each of the areas (Table 4.30). In this manner it was avoided that, by chance, the selected fishes were from a particular subarea, introducing undesirable noise in the analysis. It was decided to select only 75 individuals by

area because this is the maximum number of *S. mentella* individuals available in one of the areas, Norway (Table 4.1).

The classification matrix in numbers, the percentage of total correct classification, Cohen's Kappa and Wilks' lambda resulting from the discriminant analysis with all individuals are shown in Table 4.31. The correct classification value is low, 81.5%, and so it is considered that there is no discrimination between groups. Although Wilks' λ value was low (0.23), Cohen's Kappa value (0.65) indicated the unreliability of the classification. Irminger Sea individuals classified correctly in high proportion (95.5%), but most of the individuals from other areas classified into Irminger Sea or Iceland (Table 4.31) which is very likely a consequence of the unbalanced number of samples in Irminger and Iceland. This high confusion is also visible in the plot of canonical scores (Figure 4.17). This is a typical artifact in discriminant analyses, and to test if the results are spurious, a second analysis was performed with the same number of fish in all areas.

Table 4.30 Number of individuals by area and subarea randomly taken to introduce in the discriminant analysis of *S. mentella* by areas with a balanced number of individuals in each of the groups.

<i>S. mentella</i> by area and subarea		
	By subarea	By area
Irminger-NE	25	
Irminger-CEN	25	
Irminger-NAFO	25	75
Iceland-NE	25	
Iceland-SE	25	
Iceland-SW	25	75
Greenland-E	38	
Greenland-W	37	75
Faroese-NW	38	
Faroese-SE	37	75
Flemish Cap	75	75
Norway	75	75
Total		450

The analysis with 75 individuals per area was performed three times with different random groups to investigate if different selections produced different results. The three analyses yielded similar results, and the interpretation of the relationships between groups was always the same. The classification matrix in numbers, the percentage of total correct classification, Cohen's Kappa and Wilks' lambda resulting from this second discriminant analysis are shown in Table 4.32 and the plots of the canonical scores in Figure 4.18. First, the total correct classification dropped to 65.3%. In all the areas, the percent of correct classification was below 75%, indicating poor discrimination and a high confusion rate. Thus, Iceland confused basically with Greenland (20%) and with the Faroe Islands (21.3%), and, to lesser degrees, with Irminger (4%) and Norway (4%). Greenland confuses mainly with Iceland (16%), and to a much lesser extent with the other areas. The Faroe Islands individuals, which classified correctly only in 60%, were assigned to Iceland (17.3%) and Greenland (14.7). In other words, these three areas, the Faroe Islands, Iceland and Greenland, cluster very much together, as can be observed in Figure 4.18.

RESULTS

The Irminger Sea showed 73% correctly classified, and the misclassified individuals occurred in all the other areas. Flemish Cap, with 73% correctly classified, misclassified mostly with Norway and Greenland. Norway, however, showed a bigger confusion with Flemish Cap (18.7%) and 9.3% with Irminger, the confusion with other areas being low (2.7% in Greenland) or none.

The first canonical root (Figure 4.18) split the areas in two groups; to the right, Irminger, Flemish Cap and Norway have positive values and to the left, Faroes, Greenland and Iceland have negative values. Faroes, Greenland and Iceland are neighbouring areas, and the similarity of *S. mentella* inhabiting the three areas was expected. However, a reason other than geographical proximity should explain the relation between Irminger, Flemish Cap and Norway and so, complementary analyses were performed to go into this topic in more depth.

Table 4.31. Classification matrix in numbers, percentage of total correct classification and Wilks' lambda and Cohen's Kappa resulting from the discriminant analysis of *S. mentella* by area.

Wilks' Lambda= 0.233906 Cohen's Kappa= 0.650385							
	Correct %	Irminger	Iceland	Greenland	Faroes	Flemish	Norway
Irminger	95.5	1976	54	9	4	15	11
Iceland	78.7	105	624	16	45	2	1
Greenland	14.6	57	65	21	1	0	0
Faroe Islands	39.1	21	93	4	77	2	0
Flemish Cap	32.4	70	3	1	0	36	1
Norway	37.3	42	2	0	0	3	28
Total	81.5	2271	841	51	127	58	41
Percentage of misclassification (%)							
Irminger			2.6	0.4	0.2	0.7	0.5
Iceland		13.2		2.0	5.7	0.3	0.1
Greenland		39.6	45.1		0.7	0.0	0.0
Faroe Islands		10.7	47.2	2.0		1.0	0.0
Flemish Cap		63.1	2.7	0.9	0.0		0.9
Norway		56.0	2.7	0.0	0.0	4.0	

Table 4.32 Classification matrix in numbers, percentage of total correct classification and Wilks' lambda and Cohen's Kappa resulting from the discriminant analysis of *S. mentella* by area but with the same number of individuals (75) per area.

Wilks' Lambda= 0.13447 Cohen's Kappa= 0.584							
	Correct %	Irminger	Iceland	Greenland	Faroes	Flemish	Norway
Irminger	73.33	55	3	5	1	7	4
Iceland	50.67	3	38	15	16	0	3
Greenland	65.33	5	12	49	2	4	3
Faroe Islands	60.00	5	13	11	45	1	0
Flemish Cap	73.33	3	3	7	0	55	7
Norway	69.33	7	0	2	0	14	52
Total	65.33	78	69	89	64	81	69
Percentage of misclassification (%)							
Irminger			4.0	6.7	1.3	9.3	5.3
Iceland		4.0		20.0	21.3	0.0	4.0
Greenland		6.7	16.0		2.7	5.3	4.0
Faroe Islands		6.7	17.3	14.7		1.3	0.0
Flemish Cap		4.0	4.0	9.3	0.0		9.3
Norway		9.3	0.0	2.7	0.0	18.7	

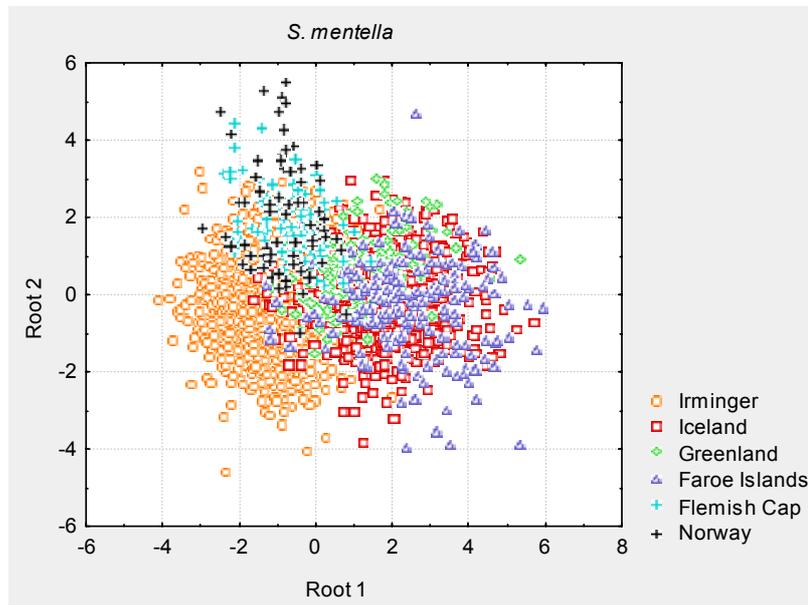


Figure 4.17. Plot of canonical scores for each case for the first and second canonical roots resulting from the discriminant analysis performed with *S. mentella* by area.

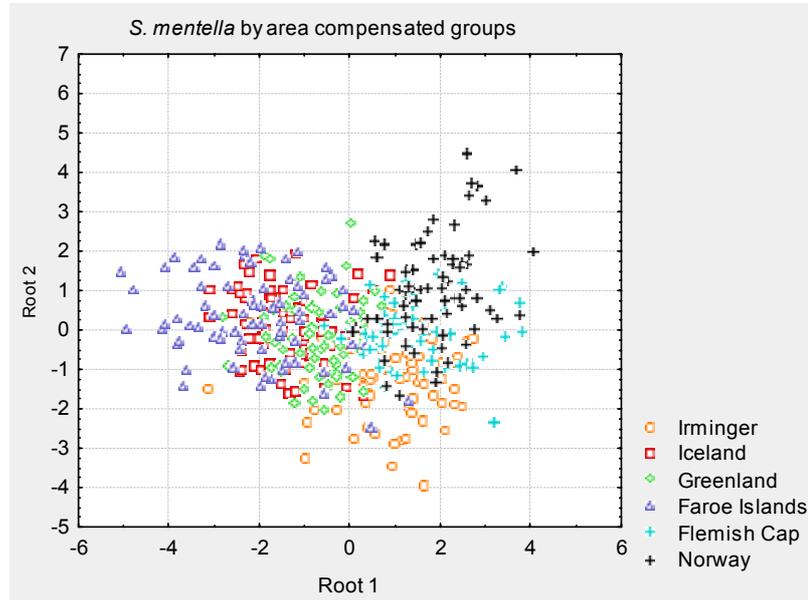


Figure 4.18. Plot of canonical scores for each case for the first and second canonical roots resulting from the discriminant analysis performed with *S. mentella* by area, but with the same number of individuals per area.

Differences by Subarea (*S. mentella*)

The subareas considered are those reflected in Table 4.23. As in the discrimination analysis by subarea performed with *S. marinus*, a single analysis including all the subareas was performed instead of several analyses, one per area. The total numbers of *S. mentella* samples available by area, subarea and size range are shown in Table 4.29.

The classification matrix in numbers, the percentage of total correct classification, Wilks' lambda and Cohen's Kappa are shown in Table 4.33. The result of this analysis gives a more detailed view of the species structure in the area, although again the unbalanced number of individuals per area and subarea make it difficult to interpret the results. The discrimination power was extremely low, 55.7%, but Wilks' λ was very low, suggesting some structure in the subareas. As in the previous analysis, this was the result of the existence of two groups, the three subareas of Irminger, Flemish Cap and Norway on one hand, and the seven subareas of the Faroes, Iceland and Greenland on the other, as shown in the scatterplot of the canonical scores (Figure 4.19).

To avoid spurious results due to the difference in number of individuals by group, a new analysis was performed with the same number of individuals (50) in each of the subareas. As explained before, part of the information may be lost in this analysis because not all the available individuals are used, but the classification is more reliable. The classification results of this new analysis can be consulted in Table 4.34 and the plot of canonical scores in Figure 4.20. The percent of total classification was similar to the previous analysis, 55.3%, but the principal difference is that there are no large confusions with those subareas that were over-represented in the previous analysis, and on the other hand, the subareas that were under-represented in the previous analysis now show a higher percent of correct classifications. In this analysis with an equal number of individuals by group, the confusion is principally produced between subareas belonging to the same area. This can be seen in the percentage of misclassification in Table 4.34, where the highest confusion was produced between subareas within the same area (framed in a box). However, in some cases there was also confusion between other subareas belonging to different areas. Thus, the confusion between SE Iceland and NW Faroes areas is remarkable, 20% and 18% respectively, as is the confusion that Norway showed with the Central Irminger Sea (12%) and Flemish Cap (14%). The subareas to the East of Iceland showed around 8% of confusion with both subareas in Greenland. The plot of canonical scores reveals the presence of these two groups separated by the first canonical root, as in previous analyses of *S. mentella* (Figure 4.20). Thus, Irminger, Norway and Flemish Cap present negative values and Iceland, Greenland and Faroe Islands positive values. The relationships between subareas are reflected also in the tree diagram of the cluster analyses (Figure 4.21). The North West of the Faroe Islands clustered with the two subareas from South of Iceland, and then with Greenland (both, East and West). However, NE Iceland and SE of Faroes seems to be the most different sub-areas in this group. On the other hand, the three areas of Irminger cluster together, and this group clusters with Flemish Cap and Norway.

In summary, the results are in accordance with those obtained in the analysis of *S. mentella* by areas, but give more detail of the relationships among subareas. There is no clear stock structure for *S. mentella*, as the percent of correct classification is always lower than the acceptable minimum (85%). Although differences between groups are not high enough to yield different groups (populations), the grouping of the areas in the same two different groups is repeated in all the analyses of *S. mentella* by area and subareas, and somewhat similar also to the *S. marinus* structure.

Table 4.33. Classification matrix, percentage of total correct classification and Wilks' lambda and Cohen's Kappa resulting from the discriminant analysis of *S. mentella* by subarea.

Wilks' Lambda= 0.138588 Cohen's Kappa= 0.452996													
	Correct %	Irminger			Iceland			Greenland		Faroes		Flem	Nor
		NE	CEN	NAFO	NE	SE	SW	E	W	NW	SE		
Irm-NE	66.8	602	205	33	1	23	11	4	0	5	1	8	8
Irm-CEN	65.4	200	547	49	0	8	10	6	2	0	0	8	6
Irm-NAFO	19.9	96	161	66	0	0	3	0	0	0	0	2	4
Ice-NE	66.0	0	1	0	33	9	0	4	3	0	0	0	0
Ice-SE	72.4	24	17	1	6	338	48	7	0	15	7	2	2
Ice-SW	35.1	38	14	1	4	94	97	7	0	16	4	1	0
Gre-E	35.4	6	21	0	2	26	5	35	3	1	0	0	0
Gre-W	6.7	3	18	0	1	9	1	8	3	0	0	2	0
Far-NW	27.3	2	14	0	0	61	13	4	0	38	6	1	0
Far-SE	58.6	1	1	1	1	12	3	0	0	5	34	0	0
Flemish	56.8	6	37	0	0	0	3	1	0	0	0	63	1
Norway	44.0	9	27	1	0	0	2	0	0	0	0	3	33
Total	55.7	987	1063	152	48	580	196	76	11	80	52	90	54
Percentage of misclassification													
Irm-NE			22.8	3.7	0.1	2.6	1.2	0.4	0.0	0.6	0.1	0.9	0.9
Irm-CEN			23.9	5.9	0.0	1.0	1.2	0.7	0.2	0.0	0.0	1.0	0.7
Irm-NAFO			28.9	48.5	0.0	0.0	0.9	0.0	0.0	0.0	0.0	0.6	1.2
Ice-NE			0.0	2.0	0.0		18.0	0.0	8.0	6.0	0.0	0.0	0.0
Ice-SE			5.1	3.6	0.2	1.3		10.3	1.5	0.0	3.2	1.5	0.4
Ice-SW			13.8	5.1	0.4	1.4	34.1		2.5	0.0	5.8	1.4	0.4
Gre-E			6.1	21.2	0.0	2.0	26.3	5.1		3.0	1.0	0.0	0.0
Gre-W			6.7	40.0	0.0	2.2	20.0	2.2	17.8		0.0	0.0	4.4
Far-NW			1.4	10.1	0.0	0.0	43.9	9.4	2.9	0.0		4.3	0.7
Far-SE			1.7	1.7	1.7	1.7	20.7	5.2	0.0	0.0	8.6		0.0
Flemish			5.4	33.3	0.0	0.0	0.0	2.7	0.9	0.0	0.0	0.0	0.9
Norway			12.0	36.0	1.3	0.0	0.0	2.7	0.0	0.0	0.0	0.0	4.0

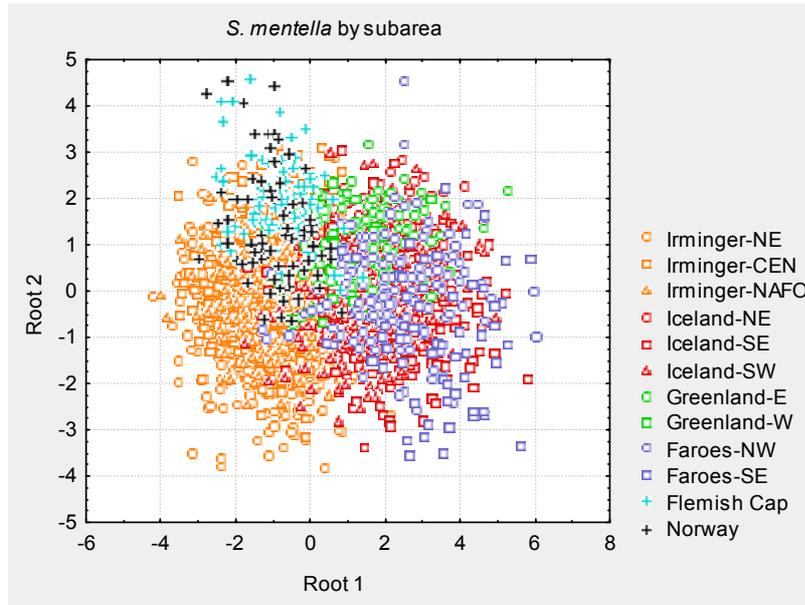


Figure 4.19. Plot of canonical scores for each case for the first and second canonical roots resulting from the discriminant analysis performed with *S. mentella* by subarea.

Table 4.34. Classification matrix, percentage of total correct classification, Wilks' lambda and Cohen's Kappa resulting from the discriminant analysis of *S. mentella* by subarea with the same number of individuals (50) by subarea.

Wilks' Lambda = 0.48353 Cohen's Kappa= 0.512288													
	Correct %	Irminger			Iceland			Greenland		Faroes		Flem	Nor
		NE	CEN	NAFO	NE	SE	SW	E	W	NW	SE		
Irm-NE	64.0	32	4	5	0	1	2	0	1	2	0	1	2
Irm-CEN	40.0	10	20	9	0	0	2	0	2	1	0	3	3
Irm-NAFO	42.0	3	18	21	0	0	2	0	1	0	0	3	2
Ice-NE	76.0	0	0	0	38	2	0	4	4	2	0	0	0
Ice-SE	42.0	2	0	1	0	21	11	1	4	10	0	0	0
Ice-SW	44.0	2	1	1	1	9	22	4	1	4	2	2	1
Gre-E	54.0	1	2	0	2	1	2	27	12	2	0	1	0
Gre-W	40.0	2	1	0	2	1	2	12	18	3	0	3	1
Far-NW	48.0	0	2	1	0	9	5	1	0	24	6	1	1
Far-SE	70.0	0	0	1	3	2	2	2	1	4	35	0	0
Flemish	78.0	0	1	0	0	0	2	0	3	1	0	39	4
Norway	64.0	0	6	4	0	0	0	0	1	0	0	7	32
Total	55.3	52	55	43	46	46	52	51	48	53	43	60	46
Percentage of misclassification													
Irm-NE			8.0	10.0	0.0	2.0	4.0	0.0	2.0	4.0	0.0	2.0	4.0
Irm-CEN		20.0		18.0	0.0	0.0	4.0	0.0	4.0	2.0	0.0	6.0	6.0
Irm-NAFO		6.0	36.0		0.0	0.0	4.0	0.0	2.0	0.0	0.0	6.0	4.0
Ice-NE		0.0	0.0	0.0		4.0	0.0	8.0	8.0	4.0	0.0	0.0	0.0
Ice-SE		4.0	0.0	2.0	0.0		22.0	2.0	8.0	20.0	0.0	0.0	0.0
Ice-SW		4.0	2.0	2.0	2.0	18.0		8.0	2.0	8.0	4.0	4.0	2.0
Gre-E		2.0	4.0	0.0	4.0	2.0	4.0		24.0	4.0	0.0	2.0	0.0
Gre-W		4.4	2.2	0.0	4.4	2.2	4.4	26.7		6.7	0.0	6.7	2.2
Far-NW		0.0	4.0	2.0	0.0	18.0	10.0	2.0	0.0		12.0	2.0	2.0
Far-SE		0.0	0.0	2.0	6.0	4.0	4.0	4.0	2.0	8.0		0.0	0.0
Flemish		0.0	2.0	0.0	0.0	0.0	4.0	0.0	6.0	2.0	0.0		8.0
Norway		0.0	12.0	8.0	0.0	0.0	0.0	0.0	2.0	0.0	0.0	14.0	

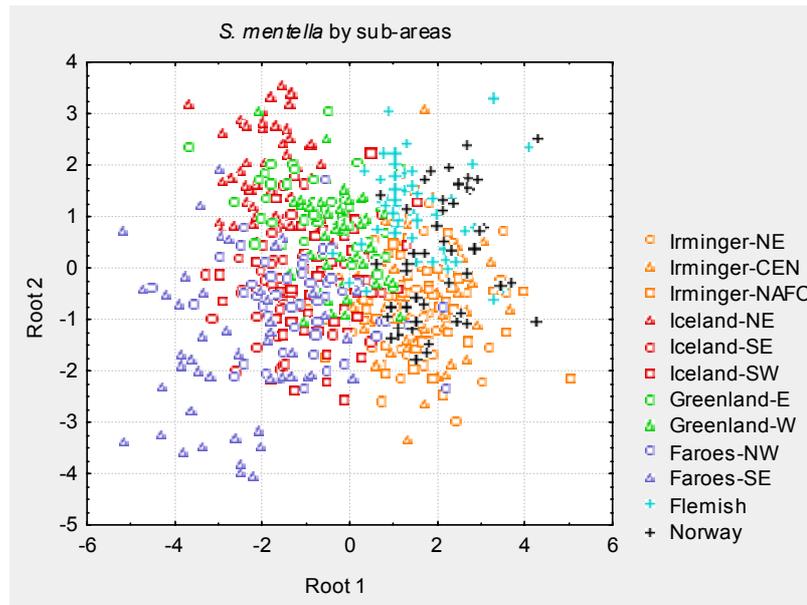


Figure 4.20 Plot of canonical scores for each case for the first and second canonical roots resulting from the discriminant analysis performed with *S. mentella* by subarea with the same number of individuals in each subarea.

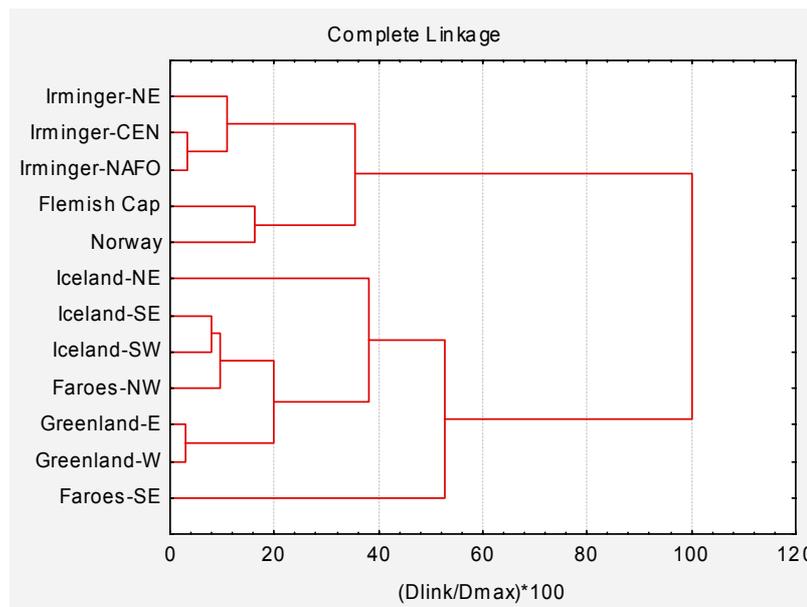


Figure 4.21 Tree diagram of a Complete Linkage Cluster Analysis based on Mahalanobis distances resulting from the discriminant analysis performed with *S. mentella* by Subarea with the same number of individuals in each subarea.

4.1.3. Species-area groups discrimination

To conclude the study of the relationships among species and areas, it was decided to pool the whole dataset and perform a single analysis with a group definition based on species and area assignments. The total number of fish considered in this analysis increased to 4,970, distributed as shown in Table 4.35.

The classification matrix in numbers, the percentage of total correct classification and Wilks' lambda are shown in Table 4.36. First, the total correct classification was low (73.7%), as a consequence of the high confusion among most of the groups. However, Wilks' λ is very low,

0.053 reflecting structure in the data. This structure is clearly understood from the classification matrix, but difficult to observe in the canonical scores plot (Figure 4.22) due to the high number of cases. From the classification matrix (Table 4.36) it can be concluded that most times the confusion occurs between individuals of the same species inhabiting different areas (framed in boxes).

The analysis with a balanced design in sample size was performed with 61 individuals by group. The classification matrix is shown in Table 4.37. The percent of total correct classification was lower (68.6%), due to the lower correct classification of Irminger. Similarly, most of the confusion rate was within species, as expected. It is remarkable that *S. marinus* and *S. fasciatus* in Flemish Cap showed interspecific confusion (9.8% each). Note, however, that although in low proportion, some *S. marinus* were classified as Icelandic *S. mentella*. And, in parallel, some *S. mentella* were classified as Icelandic *S. marinus*. The best correct classification was scored by *S. viviparus* in the Faroe Islands and Norway, and by *S. marinus* in Norway. In the plot of canonical scores the first root divided *S. mentella* from other species (Figure 4.23). In fact the AD variable is the one that shows a larger correlation with the first discriminant function, and is the same variable that has the larger correlation with the discriminant function in the discriminant analysis by species. Second and third roots allowed the separation of *S. marinus*, *S. fasciatus* and *S. viviparus* as shown in the 3D plot of the centroids resulting from the discriminant analysis (Figure 4.25). This figure illustrates clearly the separation among species and the geographical relationship among areas within each species. The second root separates the central area, i. e., Greenland, Iceland, the Faroe Islands, and Irminger from the reference areas, i. e., Norway and Flemish Cap, regardless of the species.

The results of the cluster analysis (Figure 4.24) shown that the most different groups were *S. marinus* from Norway and the two areas of *S. viviparus*, that formed separate clusters. All *S. mentella* clustered together, although *S. mentella* from the coastal regions in the central area cluster before. But Irminger Sea *S. mentella* clustered with *S. mentella* in Norway and Flemish Cap, although at a little distance from the central area *S. mentella* cluster. All *S. marinus* also joined in a separate cluster, that also included *S. fasciatus*. The results of the cluster analysis are in accordance with previous analyses. *S. marinus* in Norway appeared as a very different group when studying *S. marinus* by areas, and here clustered apart. The closeness between Iceland, Greenland and the Faroe Islands is reflected in the intraspecific similarities of the individuals inhabiting those areas, in both *S. mentella* and *S. marinus*. And *S. mentella* from Irminger, Flemish Cap and Norway that clustered together, are also related in the analysis of *S. mentella* by area. What is different in this analysis is the fact that *S. marinus* and *S. fasciatus* from Flemish Cap clustered together at low values of linkage distance, while in the analysis by species conducted in Flemish Cap both were revealed as very distinct morphometrically.

Combining the cluster analysis with the scatterplots of the centroids of each species-area group, if we focus in greater detail on *S. mentella*, it can be observed that Greenland, Iceland and the Faroe Islands, i. e. *S. mentella* from the coastal regions in the central area, cluster together and with a short linkage distance. Although Irminger Sea *S. mentella* clustered with

S. mentella in Norway and Flemish Cap, in the scatterplot it can be observed that the second root separates Irminger from Norway and Flemish Cap.

Table 4.35. Number of fish considered in the species-area analysis.

Species	Area	Number	Code
<i>S. mentella</i>	Irminger Sea	2069	T-IRM
	Iceland	793	T-ICE
	Greenland	144	T-GR
	Faroes	197	T-FR
	Flemish Cap	111	T-FC
	Norway	75	T-NO
<i>S. marinus</i>	Iceland	672	M-ICE
	Greenland	267	M-GR
	Faroes	204	M-FR
	Flemish Cap	102	M-FC
	Norway	61	M-NO
<i>S. fasciatus</i>	Flemish Cap	102	F-FC
<i>S. viviparus</i>	Faroes	79	V-FR
	Norway	94	V-NO

Table 4.36. Classification matrix, percentage of total correct classification and Wilks' lambda and Cohen's Kappa resulting from the discriminant analysis of all species-area groups. Codes are described in Table 4.35.

Wilks' Lambda=0.053704 Cohen's Kappa= 0.652143															
	Correct %	T						M				F		V	
		IR	ICE	GR	FR	FC	NO	ICE	GR	FR	FC	NO	FC	FR	NO
T-IRM	95.2	1969	78	5	5	3	5	2	0	0	0	0	1	0	1
T-ICE	65.7	130	521	5	36	3	0	65	11	3	8	0	2	5	4
T-GR	9.0	21	95	13	1	0	0	4	4	0	4	0	2	0	0
T-FR	39.1	29	70	1	77	0	1	12	2	3	1	0	1	0	0
T-FC	34.2	60	13	0	0	38	0	0	0	0	0	0	0	0	0
T-NO	24.0	48	0	0	0	3	18	0	0	0	0	6	0	0	0
M-ICE	70.1	4	67	0	1	0	0	471	70	46	6	0	0	0	7
M-GR	44.6	0	22	1	0	0	0	104	119	8	6	0	6	0	1
M-FR	40.2	1	19	0	2	2	0	65	22	82	2	0	5	4	0
M-FC	60.8	1	1	1	0	1	0	10	7	2	62	0	11	0	6
M-NO	93.4	0	0	0	0	0	0	1	1	0	2	57	0	0	0
F-FC	75.5	1	2	0	1	0	0	4	4	0	9	1	77	1	2
V-FR	96.2	0	0	0	0	0	0	0	0	0	0	0	1	76	2
V-NO	90.4	0	0	0	0	0	0	0	0	0	1	2	2	4	85
Total	73.7	2264	888	26	123	50	24	738	240	144	101	66	108	90	108
Percentage of misclassification (%)															
T-IRM			3.8	0.2	0.2	0.1	0.2	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
T-ICE		16.4		0.6	4.5	0.4	0.0	8.2	1.4	0.4	1.0	0.0	0.3	0.6	0.5
T-GR		14.6	66.0		0.7	0.0	0.0	2.8	2.8	0.0	2.8	0.0	1.4	0.0	0.0
T-FR		14.7	35.5	0.5		0.0	0.5	6.1	1.0	1.5	0.5	0.0	0.5	0.0	0.0
T-FC		54.1	11.7	0.0	0.0		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
T-NO		64.0	0.0	0.0	0.0	4.0		0.0	0.0	0.0	0.0	8.0	0.0	0.0	0.0
M-ICE		0.6	10.0	0.0	0.1	0.0	0.0		10.4	6.8	0.9	0.0	0.0	0.0	1.0
M-GR		0.0	8.2	0.4	0.0	0.0	0.0	39.0		3.0	2.2	0.0	2.2	0.0	0.4
M-FR		0.5	9.3	0.0	1.0	1.0	0.0	31.9	10.8		1.0	0.0	2.5	2.0	0.0
M-FC		1.0	1.0	1.0	0.0	1.0	0.0	9.8	6.9	2.0		0.0	10.8	0.0	5.9
M-NO		0.0	0.0	0.0	0.0	0.0	0.0	1.6	1.6	0.0	3.3		0.0	0.0	0.0
F-FC		1.0	2.0	0.0	1.0	0.0	0.0	3.9	3.9	0.0	8.8	1.0		1.0	2.0
V-FR		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.3		2.5
V-NO		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.1	2.1	2.1	4.3	

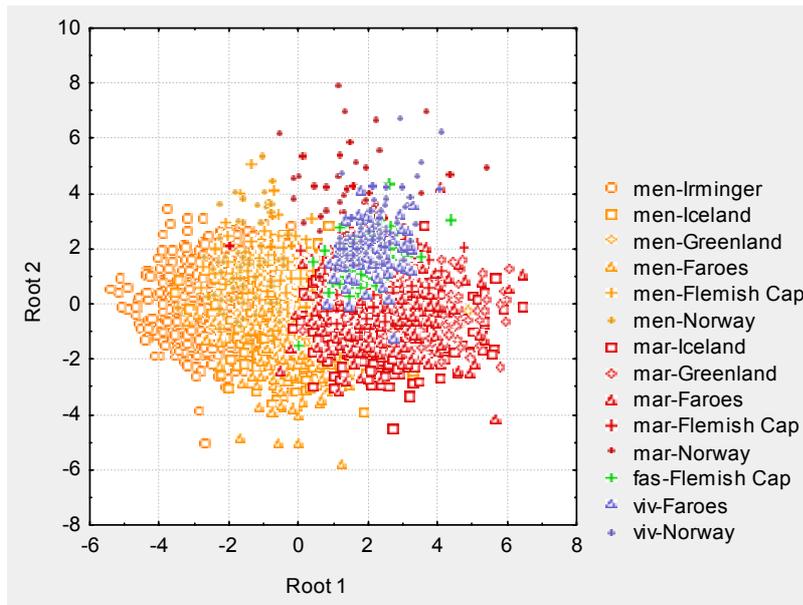


Figure 4.22. Plot of canonical scores for each case for the first and second canonical roots resulting from the discriminant analysis performed with the four species and all areas studied.

Table 4.37 Classification matrix, percentage of total correct classification and Wilks' lambda and Cohen's Kappa resulting from the discriminant analysis of all species-area groups, with the same number of individuals by group. Codes are described in Table 4.35

Wilks' Lambda=0.010333																
Cohen's Kappa=0.662043																
	Correct %	IR	ICE	T				M				F		V		
				GR	FR	FC	NO	ICE	GR	FR	FC	NO	FC	FR	NO	
T-IRM	75.4	46	1	3	1	5	5	0	0	0	0	0	0	0	0	0
T-ICE	47.5	5	29	9	9	2	0	3	1	1	1	0	0	0	0	1
T-GR	62.3	3	8	38	3	2	0	1	2	0	4	0	0	0	0	0
T-FR	63.9	5	9	3	39	1	1	1	0	1	0	0	1	0	0	0
T-FC	80.3	3	0	5	0	49	4	0	0	0	0	0	0	0	0	0
T-NO	57.4	12	1	1	2	6	35	0	0	0	0	4	0	0	0	0
M-ICE	50.8	0	1	2	0	0	0	31	8	15	3	0	0	0	0	1
M-GR	55.7	0	0	5	0	0	0	5	34	11	4	0	2	0	0	0
M-FR	47.5	1	6	4	0	1	0	6	6	29	3	0	4	1	0	0
M-FC	67.2	0	0	3	0	3	0	2	0	2	41	0	6	0	4	4
M-NO	90.2	0	0	0	0	4	0	0	0	0	1	55	0	0	1	0
F-FC	77.0	0	0	1	1	1	0	0	2	1	6	0	47	0	2	2
V-FR	95.1	0	0	0	0	0	0	0	0	0	0	0	0	58	3	3
V-NO	90.2	0	0	0	0	0	0	0	0	0	1	0	1	4	55	55
Total	68.6	75	55	74	55	74	45	49	53	60	64	59	61	63	67	67
Percentage of misclassification (%)																
T-IRM		1.6	4.9	1.6	8.2	8.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
T-ICE		8.2	14.8	14.8	3.3	0.0	4.9	1.6	1.6	1.6	0.0	0.0	0.0	0.0	1.6	1.6
T-GR		4.9	13.1	4.9	3.3	0.0	1.6	3.3	0.0	6.6	0.0	0.0	0.0	0.0	0.0	0.0
T-FR		8.2	14.8	4.9	1.6	1.6	1.6	0.0	1.6	0.0	0.0	0.0	1.6	0.0	0.0	0.0
T-FC		4.9	0.0	8.2	0.0	6.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
T-NO		19.7	1.6	1.6	3.3	9.8	0.0	0.0	0.0	0.0	6.6	0.0	0.0	0.0	0.0	0.0
M-ICE		0.0	1.6	3.3	0.0	0.0	0.0	13.1	24.6	4.9	0.0	0.0	0.0	0.0	0.0	1.6
M-GR		0.0	0.0	8.2	0.0	0.0	0.0	8.2	18.0	6.6	0.0	3.3	0.0	0.0	0.0	0.0
M-FR		1.6	9.8	6.6	0.0	1.6	0.0	9.8	9.8	4.9	0.0	6.6	1.6	0.0	0.0	0.0
M-FC		0.0	0.0	4.9	0.0	4.9	0.0	3.3	0.0	3.3	0.0	9.8	0.0	6.6	0.0	6.6
M-NO		0.0	0.0	0.0	0.0	6.6	0.0	0.0	0.0	0.0	1.6	0.0	0.0	0.0	1.6	1.6
F-FC		0.0	0.0	1.6	1.6	1.6	0.0	0.0	3.3	1.6	9.8	0.0	0.0	0.0	3.3	3.3
V-FR		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	58	3	3
V-NO		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.6	0.0	1.6	4	55	55

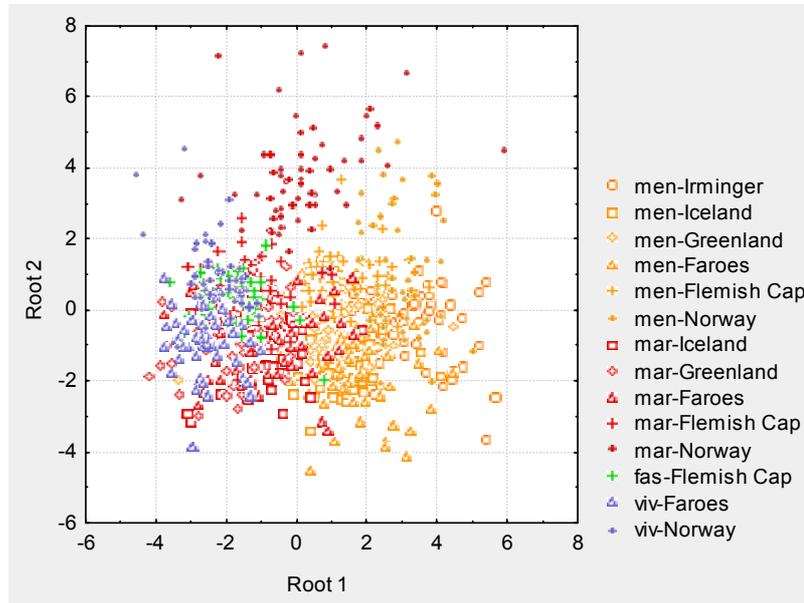


Figure 4.23 Plot of canonical scores for each case for the first and second canonical roots resulting from the discriminant analysis performed with the four species and all areas studied divided in groups with equal number of individuals.

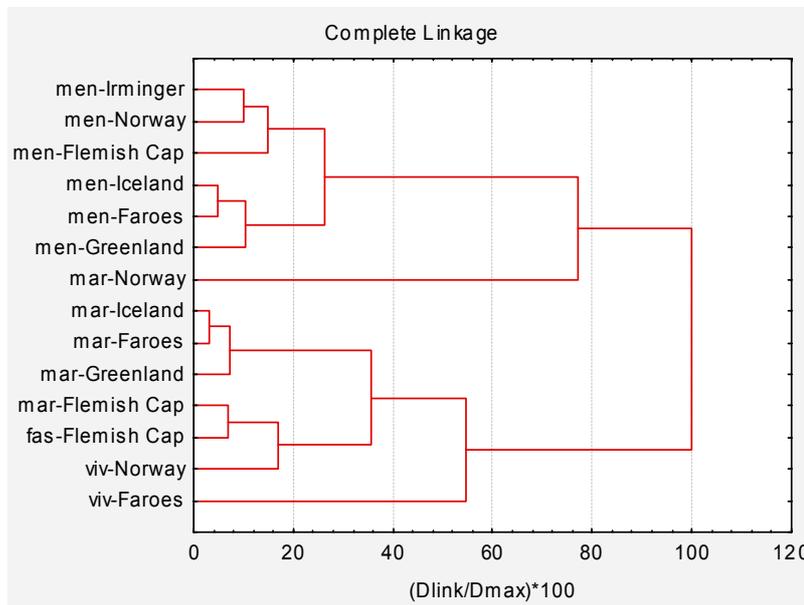


Figure 4.24 Tree diagram of the Cluster analysis based in Mahalanobis distances resulting from the discriminant analysis performed with all species-area groups.

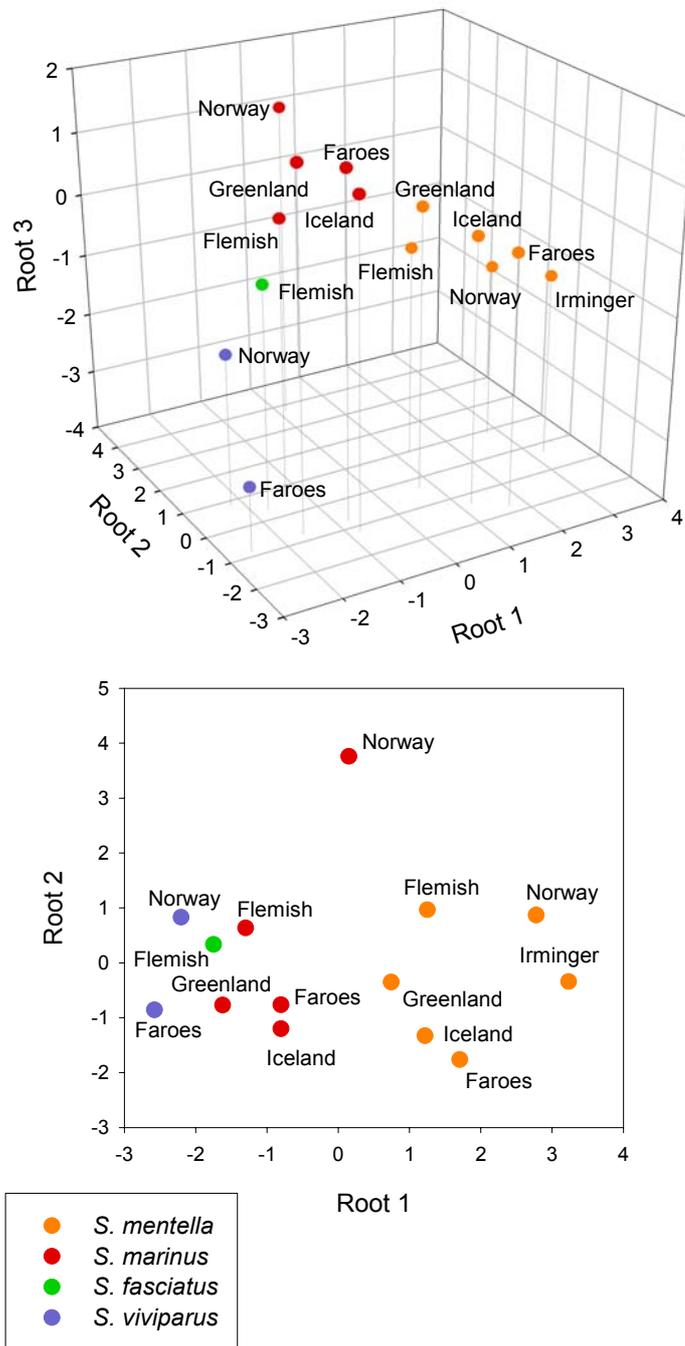


Figure 4.25. Scatterplots showing the species-area group centroids in three dimensions. The projection of the centroids on the Root 1 and Root 2 plane are also displayed.

4.1.4. Discrimination between *S. mentella* phenotypes in the Irminger Sea

One of the most relevant problems, and also one of the main goals of the REDFISH project, deals with the number of stocks inhabiting the Irminger Sea. Two types of *S. mentella* have been defined in this area, the so called oceanic and deep-sea *S. mentella*. Some researchers are of the opinion that these are separate stocks, while others believe they form part of the same population.

Both types live in the open Irminger Sea, but on many occasions these pelagic fish are close to the Icelandic and Greenlandic shelves. Within this project, a total of 2,069 fishes were identified as pelagic, i.e. the fish usually inhabiting the Irminger Sea and taken by pelagic hauls. Although all of these fish were assigned as Irminger Sea in the previous analyses, some were taken on the Icelandic shelf, in the proximity of the typical demersal *S. mentella*. From these pelagic fish, 1,988 individuals were identified as oceanic or deep-sea phenotype, while in 81 cases it was not possible to identify the phenotype. The most important feature regarding the morphometric analysis involved here is how these fish were classified a priori between the two types. The definitions of the characters that allow identifying both types were developed by Icelandic researchers for several years. Iceland has therefore the skill for the identification. An identification key was prepared by Icelandic researchers at the beginning of the project, and it was used by the author to coach observers collecting the fish onboard, but also for use in the laboratory to identify the type before being measured.

The identification key is based on several morphological characters as described in material and methods chapter. The characters used are very subtle and in many instances, subjective, such as the color. However, some of them allowed a construction of a more objective identification guide, although some characters, such as the position of the spine and parasitism may be unrealistic. Those *S. mentella* with the 5th preopercular spine looking downwards, with *Sphirion lumpi* parasitization (alive or as cyst), and with abnormal skin pigmentation, were considered as oceanic, ; fish that did not show these features were considered as deep-sea type.

After a fish was measured, the gas bladder musculature was inspected and its pattern recorded. An overview of the different patterns allowed identifying two possible, although very weak patterns:

- A) The first tendon passed between two ribs (normally the 2nd and 3rd, and less often the 1st and 2nd) attaching to some of the posterior vertebrae.
- B) The first tendon attached directly to a rib without passing between two ribs.

These two morphotypes were not related with the classical oceanic and deep sea types. In fact in both A and B morphotypes, approximately half of the fish were oceanic and the other half deep-sea.

Four different stepwise discriminant analyses were carried out bearing in mind the particularities mentioned above. All of them were performed with the same number of individuals in each of the groups to avoid bias in the classification.

1. A first analysis with all the pelagic *S. mentella*.
2. A second one using only those fish that were phenotyped into oceanic and deepsea types by Icelandic researches, as they have experience identifying these types.
3. In the third analysis, all fish were introduced, but divided into oceanic and deepsea using the meristic and parasitism characters mentioned above.
4. And finally, the fourth analysis was done with those fish studied in Spain, divided into two groups, A and B using the GBM new pattern.

The first analysis was performed with 1,800 fishes, 900 deep-sea, and 900 oceanic. The classification matrix in numbers, the percentage of total correct classification and Wilks' lambda are shown in Table 4.38. The total correct classification was low (63.44%), and a consequence of a random distribution (Kappa equal to 0.27). Deep-sea and oceanic classification showed also extremely low values, 62 and 64.9% respectively. Wilks' λ was very high (0.88) showing clearly that no morphometric discrimination between types existed. The lack of differences can also be observed from the plot of canonical scores for the single canonical root (Figure 4.26).

The second analysis was made with the fish collected from the Irminger Sea measured in Iceland. 468 individuals were included in the analysis, 234 in each group. The results were similar to the previous analysis. The classification matrix in numbers, the percentage of total correct classification and Wilks' lambda are shown in

Table 4.39. All the statistics showed the lack of discrimination, a high Wilks' λ (0.74), a low percent of correct classification (71.15%), and a low value of Kappa (0.42). The overlap between both types is considerable as shown also in the histogram of the canonical scores (Figure 4.27).

1,344 *S. mentella* from the Irminger Sea were measured by the author, and hence data on meristic, parasitism and skin abnormalities were available. In this analysis to balance the group size, only 466 individuals were included in each of the groups. The results of the discrimination analysis yielded, again, a complete lack of discrimination, as shown in

Table 4.40. The total proportion of correct classification is very low, 63.52 %, and Wilks' λ is also high, 0.90, and therefore no morphometric differences existed between the two groups. The histogram of the canonical score frequencies are presented in Figure 4.28 showing a great overlap between the two types.

In every fish measured in Spain, the GBM was recorded. This fourth analysis, was performed with 1,000 fish, 500 in each of the groups. The GBM patterns were called A and B types, as explained above. No discrimination was found with this pattern either, as shown in

Table 4.41. Wilks' λ got the highest value among the four analyses, 0.96, demonstrating the absolute lack of discrimination. Also 57% of total correct classification confirm this point. This overlap is also obvious from the histogram of canonical scores (Figure 4.29).

Table 4.38. Classification matrix, percentage of total correct classification and Wilks' lambda and Cohen's Kappa resulting from the discriminant analysis of *S. mentella* in the Irminger Sea by phenotype.

Wilks' Lambda= 0.88635 Cohen's Kappa= 0.268889			
	Percent	Oceanic	Deepsea
Oceanic	64.89	584	316
Deepsea	62.00	342	558
Total	63.44	926	874

Table 4.39. Classification matrix, percentage of total correct classification, Wilks' lambda and Cohen's Kappa resulting from the discriminant analysis of *S. mentella* in the Irminger Sea by phenotype using only fish phenotyped by Icelandic researchers.

Wilks' Lambda= 0.74952 Cohen's Kappa= 0.423067			
	Percent	Oceanic	Deepsea
Oceanic	69.66	163	71
Deepsea	72.65	64	170
Total	71.15	227	241

Table 4.40. Classification matrix, percentage of total correct classification, Wilks' lambda and Cohen's Kappa resulting from the discriminant analysis of *S. mentella* in the Irminger Sea by phenotype using selected anatomical characters.

Wilks' Lambda= 0.90967 Cohen's Kappa= 0.270386			
	Percent	Oceanic	Deepsea
Oceanic	62.66	292	174
Deepsea	64.38	166	300
Total	63.52	458	474

Table 4.41. Classification matrix, percentage of total correct classification, Wilks' lambda and Cohen's Kappa resulting from the discriminant analysis of *S. mentella* in the Irminger Sea by phenotype using GBM.

Wilks' Lambda= 0.96935 Cohen's Kappa= 0.142			
	Percent	Type A	Type B
Type A	58.4	292	208
Type B	55.8	221	279
Total	57.1	513	487

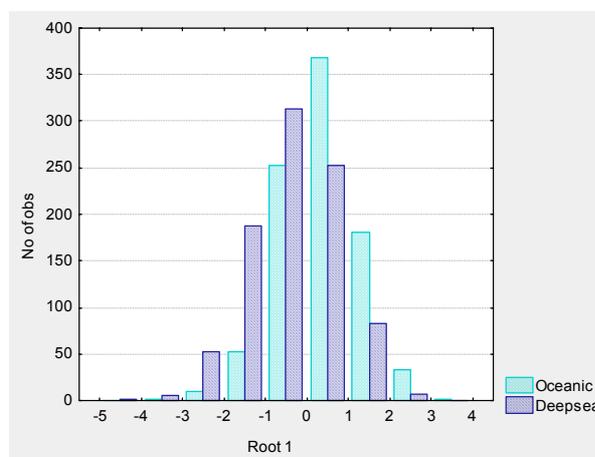


Figure 4.26. Plot of canonical scores for each case for the first and second canonical roots resulting from the discriminant analysis performed with *S. mentella* in the Irminger Sea by phenotype.

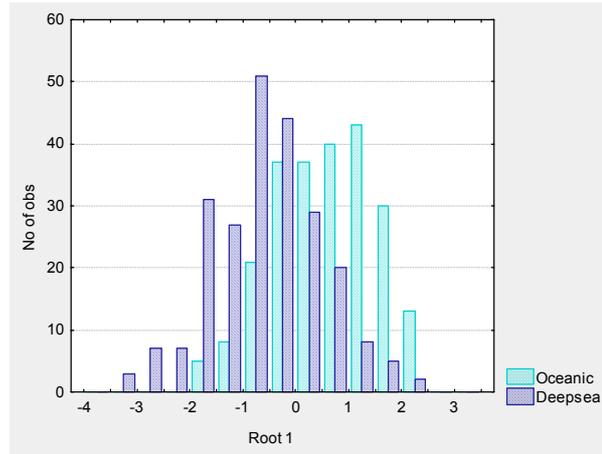


Figure 4.27. Plot of canonical scores for each case for the first and second canonical roots resulting from the discriminant analysis performed with *S. mentella* in the Irminger Sea by phenotype using only fish phenotyped by Icelandic researchers.

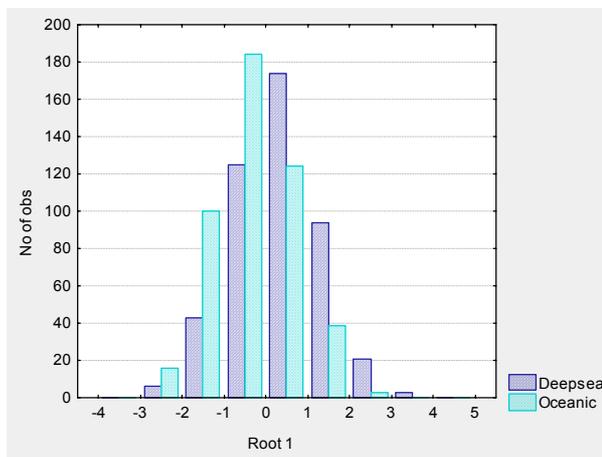


Figure 4.28. Plot of canonical scores for each case for the first and second canonical roots resulting from the discriminant analysis performed with *S. mentella* in the Irminger Sea by phenotype using selected anatomical characters.

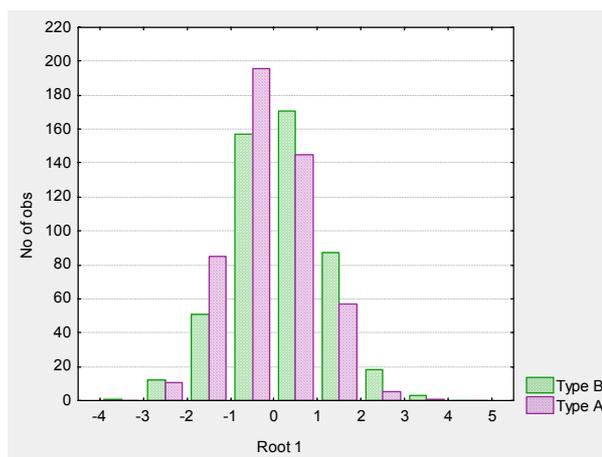


Figure 4.29. Plot of canonical scores for each case for the first and second canonical roots resulting from the discriminant analysis performed with *S. mentella* in the Irminger Sea by phenotype using GBM new pattern.

4.1.5. Further considerations of genetics and morphometrics

In Germany, 652 fishes (398 *S. marinus* and 254 *S. mentella*) were analyzed using microsatellites. For the same fish morphometric data also existed. The individuals were collected in the Faroes, Iceland, Greenland and the Irminger Sea (Table 4.42). The genetic analyses revealed the presence of three different genotypes: typical *S. marinus*, typical *S. mentella* and a third genotype with a no clear species assignment that was called “*Sebastes*”. Only 1 individual that had been classified as *S. marinus* by its external appearance, presenting a typical *S. mentella* genotype, and 6 *S. mentella* showed typical *S. marinus* genotype (Table 4.42). So, genotypes are in accordance with the external morphology in most cases. However, an important fraction of fish showed the *Sebastes* genotype, i. e., 101 *S. marinus* and 57 *S. mentella*. Except in *S. mentella* from the Irminger Sea, and in *S. marinus* from the Faroe Island, the number of individuals with the *Sebastes* genotype was relatively high; thus, in Faroes *S. mentella*, only 12.5% of the fish had a *S. mentella* genotype.

To study the existence of morphometric differences among different genotypes, a new set of analyses based on these data was performed.

4.1.5.1. Differences between species

The first analysis compared the typical genotypes. Thus, the inputs for this analysis were 302 fish with *S. marinus* genotype and 185 with *S. mentella* genotype.

The classification matrix in numbers, the percentage of total correct classification, Cohen’s Kappa and Wilks’ lambda are shown in Table 4.43. The discrimination power was almost complete, with a total correctly classified of 99.34% and a low Wilks’ λ , 0.15; 100% of the *S. marinus* were correctly classified and only 3 out 185 *S. mentella* were classified as *S. marinus*. The plot of the canonical scores shows this good discrimination between the groups (Figure 4.30). In conclusion, if we separated the samples into species using their genotypes, the resultant groups are morphometrically different.

4.1.5.2. Differences between areas

A second analysis was carried out to compare in both species the differences between areas. Five different groups were defined with the combination of the species defined by genotype and the different areas.

Table 4.44 shows the classification matrix in numbers, the percentage of total correct classification and Wilks’ lambda. Although the total correct classification was not especially high (89.9%), Wilks’ λ was very low (0.02), due to the good correct classification of most of the groups except *S. marinus* in Iceland. When confusion occurred, it was between individuals of the same species but in other areas. In fact, only one *S. marinus* was classified as *S. mentella* and one *S. mentella* as *S. marinus*. Thus, two groups were clearly identified in the scatterplot of the canonical scores in relation with root 1 (Figure 4.31). *S. mentella* individuals gave positive scores and *S. marinus* negative scores. The UPGMA cluster

analysis also showed the formation of these two groups related with the species (Figure 4.32).

More interesting were the relationships of the individuals of the same species inhabiting different areas. *S. marinus* in Greenland and Iceland overlapped, showing a clear confusion rate (Figure 4.31); *S. marinus* in Iceland showed only 81.3% classification and most of the misclassified fish were from Greenland. However, low confusion existed between these two areas (Greenland and Iceland) and Faroes (Table 4.44), meaning that, morphometrically, the typical *S. marinus* genotype in Faroes is a very distinct group, as observed also in Figure 4.31 (regarding root 2). In fact, from the tree diagram, *S. marinus* from the Faroe Islands clustered at higher values than the two areas of *S. mentella* (Figure 4.32). *S. mentella* showed also a good discrimination between areas.

These results are very relevant for the general interpretation of all the results. It is interesting to compare them with the analyses conducted in the Faroe Islands and Greenland. Especially interesting is the case of the Faroe Islands, where the total correspondence between the GBM pattern and the haemoglobin-genotype was demonstrated for species asignment.

The results of the analysis of *S. marinus* by area (Table 4.25) had shown a big overlap between the Faroe Islands and Iceland. In that analysis, the Faroe Islands sample was constituted by fish randomly taken on two different cruises. However, in this last analysis, the overlap between those areas disappears. Why did the two analyses yield different results? One hypothesis is that in the last analysis, the fish could be selected by their genotype, contrary to the former analysis where the fish were randomly taken. And if fish are selected, the sample is not representative of the whole population. So, fish have to be randomly sampled, and randomly compared. Differences between areas resulting from analysis of samples that were not randomly taken must be interpreted with caution. This issue will be discussed later.

4.1.5.3. The “*Sebastes*” genotype

To evaluate the potential of this hypothesis a third discriminant analysis was carried out but incorporating the unspecific genotype “*Sebastes*”. The results are presented in Table 4.45. This time the correct classification rate for *S. marinus* in the Faroes, Iceland and Greenland dropped to the level of no discrimination, always below 85%. *S. mentella* was still well classified in the two areas. Regarding the genotype *Sebastes*, most of the fish were classified in their respective groups, but in very low proportions. “*Sebastes*” from Greenland were, in a high proportion, classified into Greenland *S. marinus* (Table 4.45). “*Sebastes*” in Iceland were misclassified with “*Sebastes*” in Greenland, and with all the areas of *S. marinus*. Also “*Sebastes*” in the Faroes showed confusion with *S. marinus*, especially Iceland. In the scatterplot of canonical scores (Figure 4.33) the relationship of the genotype “*Sebastes*” (represented by crosses) and *S. marinus* (represented by squares) is clear. Also in the Cluster analysis (Figure 4.34) the genotype “*Sebastes*” clustered with *S. marinus* in their respective areas.

The genotype “*Sebastes*” probably corresponds to another genotype of *S. marinus* .

Unfortunately, only one genotype of *S. mentella* was sampled, which prevented a test of the hypothesis that the morphometric differences are related to genotypes, but not with species and populations. Thus, when the samples are selected by genotype, morphometric differences occurred, but when they are taken randomly, the differences among areas disappear. This is the only explanation found for the fact that important morphometric differences existed in *S. mentella* genotype between Greenland and the Irminger Sea, but low differences when considering the random samples taken for morphometric purposes. Errors in species assignment may occur, but it is difficult to have errors regarding the geographical origin of samples.

The lack of morphometric differences among areas (when all samples are considered) doesn't mean that the different areas have the same population, simply that they are morphometrically similar. However, and this is especially relevant, the absolute morphometric discrimination between areas when a particular genotype is considered, fully validate the use of morphometric tools to discriminate species and populations.

Table 4.42. Number of fishes by area and species assigned by genetic methods (performed in Germany) in comparisons with the external morphology.

No. of samples genetically analyzed in Germany						
By external morphology	By genotype	Faroes	Greenland	Iceland	Irminger	Total
<i>S. marinus</i>	<i>S. marinus</i>	54	146	96		296
	<i>S. mentella</i>		1			1
	<i>Sebastes</i>	2	49	50		101
<i>S. mentella</i>	<i>S. marinus</i>	3	3			6
	<i>S. mentella</i>	5	47		137	191
	<i>Sebastes</i>	32	27			57
Total		96	273	146	137	652

Table 4.43. Classification matrix in numbers, percentage of total correct classification and Wilks' lambda and Cohen's Kappa resulting from the discriminant analysis conducted with *S. marinus* and *S. mentella* genetically analyzed in Germany.

Wilks' Lambda: 0.15312 K= 0.9869			
	Correct %	<i>S. marinus</i>	<i>S. mentella</i>
<i>S. marinus</i>	100.00	302	0
<i>S. mentella</i>	98.38	3	182
Total	99.38	305	182

Table 4.44. Classification matrix in numbers, percentage of total correct classification and Wilks' lambda and Cohen's Kappa resulting from the discriminant analysis conducted between areas and genotypes (*S. marinus* and *S. mentella*) genetically analyzed in Germany.

Wilks' Lambda: 0.024284 K=0.4701						
	Correct %	<i>S. marinus</i>			<i>S. mentella</i>	
		Faroes	Greenland	Iceland	Greenland	Irminger
<i>S. marinus</i> -Faroes	91.2	52	0	4	1	0
<i>S. marinus</i> -Greenland	91.3	2	136	11	0	0
<i>S. marinus</i> -Iceland	81.2	2	16	78	0	0
<i>S. mentella</i> -Greenland	91.7	0	1	0	44	3
<i>S. mentella</i> -Irminger	93.4	0	0	0	9	128
Total	89.9	56	153	93	54	131

Table 4.45. Classification matrix in numbers, percentage of total correct classification and Wilks' lambda and Cohen's Kappa resulting from the discriminant analysis conducted between areas and the three genotypes (*S. marinus*, *S. mentella* and *Sebastes*). 'Far'=Faroe Islands; 'Gre'=Greenland 'Ice'=Iceland; 'Irm'=Irminger.

Wilks' Lambda: 0.02037 Cohen's Kappa= 0.3136									
	Percent	<i>S. marinus</i>			<i>S. mentella</i>		<i>Sebastes</i>		
		Far	Gre	Ice	Gre	Irm	Far	Gre	Ice
<i>S. marinus</i> -Far	82.46	47	1	4	0	0	1	1	3
<i>S. marinus</i> -Gre	83.22	3	124	9	0	0	1	7	5
<i>S. marinus</i> -Ice	78.13	1	17	75	0	0	0	1	2
<i>S. mentella</i> -Gre	89.58	0	0	0	43	3	0	1	1
<i>S. mentella</i> -Irm	92.70	0	0	0	10	127	0	0	0
<i>Sebastes</i> - Far	76.47	3	0	1	0	0	26	3	1
<i>Sebastes</i> -Gre	67.11	1	18	1	1	0	1	51	3
<i>Sebastes</i> -Ice	58.00	2	4	5	0	0	0	10	29
Total	80.68	57	164	95	54	130	29	74	44

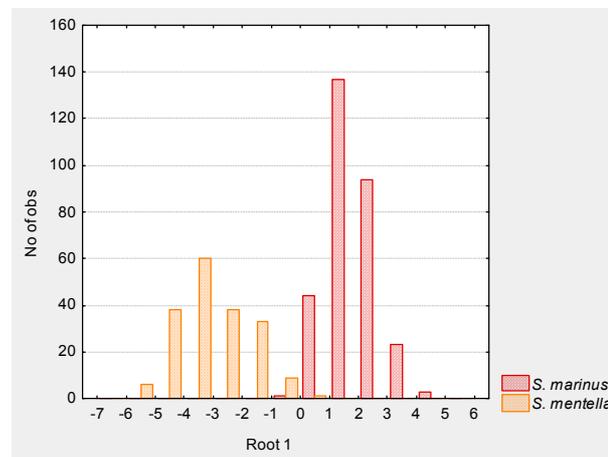


Figure 4.30. Frequencies of canonical scores for the canonical root resulting from the discriminant analysis performed with *S. marinus* and *S. mentella* genetically analyzed in Germany.

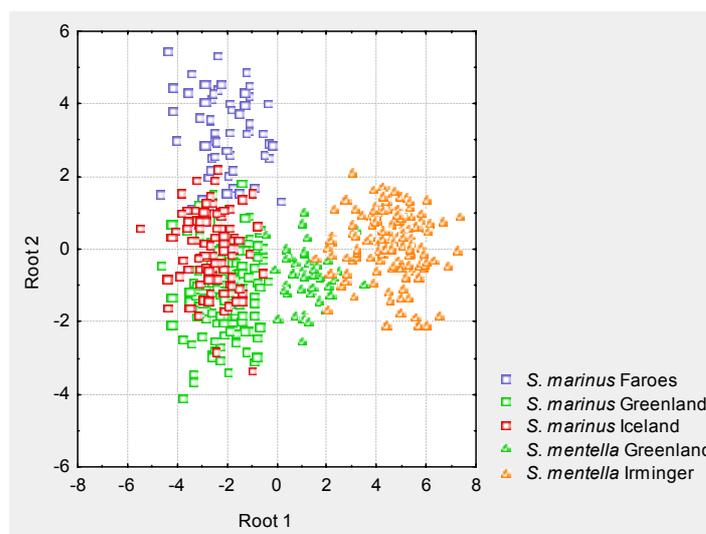


Figure 4.31. Plot of canonical scores for each case for the first and second canonical roots resulting from the discriminant analysis performed with areas and genotypes (*S. marinus* and *S. mentella*).

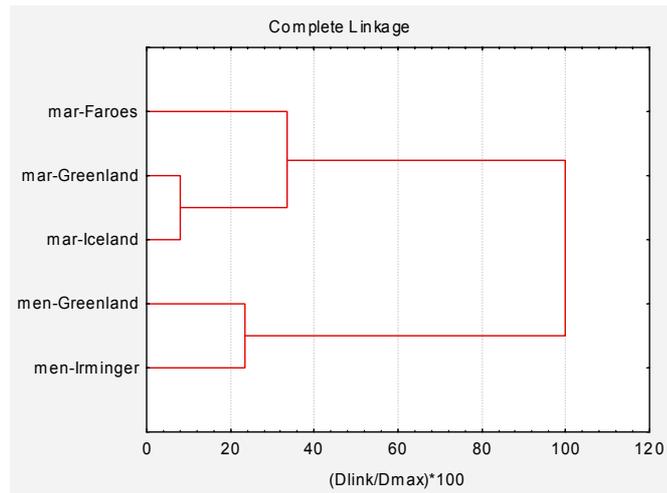


Figure 4.32. Tree diagram of the Cluster analysis based on Mahalanobis distances resulting from the discriminant analysis performed with areas and genotypes (*S. marinus* and *S. mentella*).

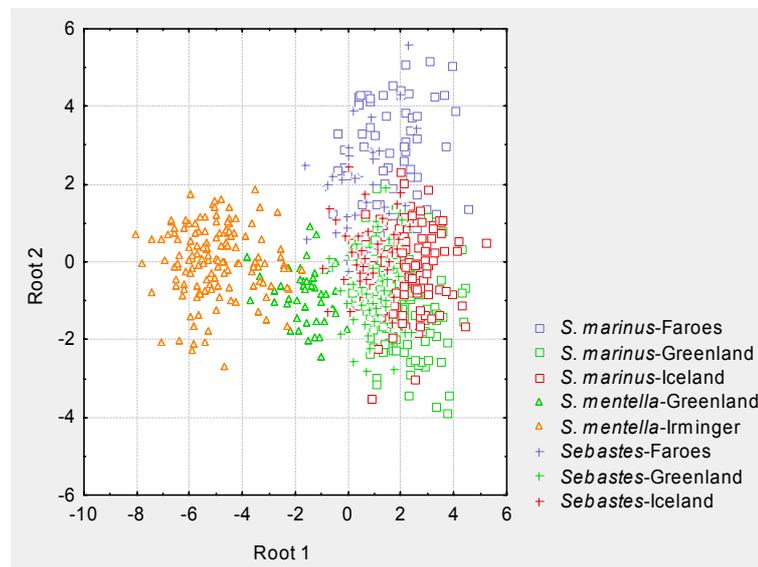


Figure 4.33. Plot of canonical scores for each case for the first and second canonical roots resulting from the discriminant analysis conducted between areas and the three genotypes (*S. marinus*, *S. mentella* and *Sebastes*).

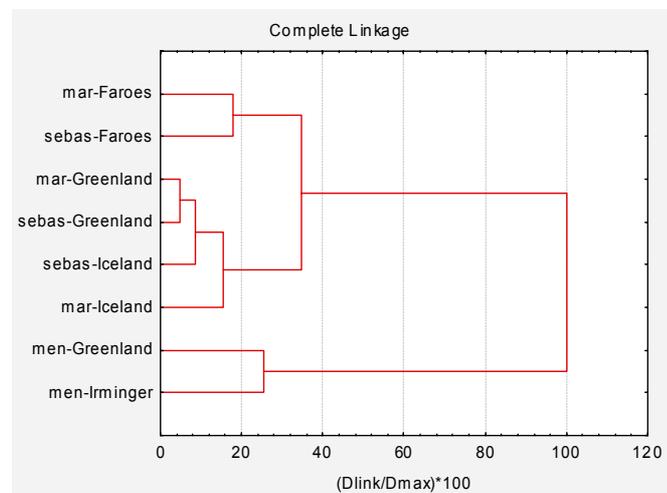


Figure 4.34. Tree diagram of the Cluster analysis based on Mahalanobis distances resulting from the discriminant analysis conducted between areas and the three genotypes (*S. marinus*, *S. mentella* and *Sebastes*).

4.2. GEOMETRIC MORPHOMETRICS

Two main reasons led to the use of geometric morphometrics; first, it more efficiently removes the size dependence of the morphometric variables, and hence a more precise morphometric analyses can be carried out for observing potential differences between the species and areas considered. Secondly, one of the outputs of geometric morphometric analysis is a set of graphical displays that offer a good intuitive interpretation of shape variation, which can help to ascertain where are the differences among the groups studied.

However, geometric morphometrics is less developed statistically, and interpretation of the results is sometimes confusing and difficult, in particular of the statistics associated purely with the geometric analyses. Some are so recently developed that their interpretation is still poorly understood. One of the major alternatives is to produce a new matrix, representing in some way the original variation, but where the size dependency has been removed; this is the so-called weight matrix (explained in Methods section), and to perform a stepwise discriminant analysis using this new matrix as input. Results of these multivariate analyses are well known and understood. In the weight matrix the partial warps and the uniform component are included (See Methods section for a detailed explanation of these topics).

The analyses performed within this section follow the same general structure used in the traditional morphometrics section, i.e. the following were compared:

1. The differences among species.

1.1 First, in the whole area.

1.2 Then, in each of the areas separately.

2. The differences between different areas and subareas within each species:

2.1 in *S. marinus*

2.2 and in *S. mentella*

3. The differences between the four species and the six areas together to study better the relationships between the geographical trends.

4. and finally, the differences between the phenotypes, oceanic and deep-sea, in the Irminger Sea.

Individuals included in the analysis

Only individuals collected during the REDFISH project have been used for geometric morphometrics, because there were no landmark coordinates available for those individuals from Norway and Flemish Cap, but only distances between landmarks taken with a caliper, as they were measured for traditional morphometric analysis exclusively. On the other hand, due to the failures in species identification discovered during the traditional morphometric analysis, only those individuals with a reliable species identification have been used in geometric morphometrics. Thus, in Greenland only those individuals with a genetic based identification have been used.

The raw number of individuals available for geometric morphometric analyses was 6,764. But from those, 998 were excluded from the analysis because in traditional morphometric analysis, it has been discovered that they did not have reliable species identifications.

So, for the geometric morphometric analysis, only 5,766 have been used, but all of them have reliable species identifications. However, of those, 178 were missing values for one of various landmark coordinates, and have been also removed from the analysis (Table 4.46). The remainder, 5,588 individuals, were screened looking for outliers on the plots of the superimposed landmarks of all specimens split by species (see Methods section). Only four outliers were found, two *S. mentella*, one from Iceland and the other from the Irminger Sea, and two *S. marinus*, both from Iceland.

Thus, a total of 5,584 individuals was used in geometric morphometric analysis, 483 from the the Faroe Islands, 414 from Greenland, 2,429 from Iceland, and 2,258 from the Irminger Sea. A summary of individuals by area, species and phenotype is shown in Table 4.46.

Table 4.46. Summary of the total individuals used for geometric morphometrics by area, species and phenotype. Data missing individuals and outliers are also shown. The final individuals used for geometric morphometrics are displayed in the last column.

Area	Species	Phenotype	Sampled	Data missing	Outliers	Total
Faroe Islands	mar	demersal	209	2		207
	men	demersal	200	3		197
	viv	demersal	79			79
Greenland	mar	demersal	280	12		268
	men	demersal	168	22		146
Iceland	mar	demersal	1417	23	2	1392
	men	demersal	1069	31	1	1037
Irminger	men	deepsea	1268	54		1214
		oceanic	983	25	1	957
		undef	93	6		87
Total			5766	178	4	5584

Shape analysis using geometric morphometrics

Following the conclusions obtained with the analyses performed using traditional morphometric methods, the assignation of each individual to species was performed using the genotypes in Greenland, the gas bladder musculature in the Faroes and, for Iceland, the external morphology of the fish.

4.2.1. Discrimination between species

4.2.1.1. Discrimination between species In the whole area

The first step was to analyze the differences between the three species inhabiting the Faroes, Iceland, Greenland and the Irminger Sea, considering all the areas as a whole. All the landmark coordinates for those areas were pooled and a weight matrix common to all individuals was performed.

The classification matrix in numbers, the percent of total correct classification, Wilks' lambda and Cohen's Kappa are shown in Table 4.47, and also the percentage of misclassification, that is the percentage of individuals that classified in other groups.

The overall correct classification was around the 95%, and Wilks' lambda value was low (0.22). Cohen's Kappa value is higher than 7, and in agreement with the total of the percent correctly classified. *S. mentella* showed 95.8% correct classification, although there are some confusions with *S. marinus* as 151 *S. mentella* classified as *S. marinus*. This represent only 4.1% of the total *S. mentella*, but we have to take into account that most *S. mentella* come from the Irminger Sea, where only *S. mentella* occurs, so the percent of misclassification can be larger than 4.1% in other areas. Only one *S. mentella* classified as *S. viviparus*.

S. marinus showed 95.3% correctly classified, presenting the highest confusion with *S. mentella* (4.4%); 83 *S. marinus* were classified as *S. mentella*. The lower confusion was, again, with *S. viviparus* as only 4 *S. marinus* (0.21%) were classified as *S. viviparus*.

The percent correctly classified for *S. viviparus* was also high, as 94.9% of them classified as this species. Only 3 *S. viviparus* (3.8%) classified as *S. marinus* and one individual (1.2%) as *S. mentella*.

The good discrimination between the three species is also observed from the plot of canonical scores for the two canonical axes (Figure 4.35). The first root discriminates between *S. mentella* and the other two species, and the second root allowed discrimination between *S. marinus* and *S. viviparus*.

The percents correctly classified are high, but looking at the classification matrix of the analysis performed with a balanced number of individuals (Table 4.48), the group that had the minor number of individuals in the previous analysis, i. e., *S. viviparus*, improves its classification, reaching 100% correctly classified, and the total percent correctly classified is even higher than in the previous analysis (96.6%).

Figure 4.36 shows graphically the superimposed raw data for pair of species and their corresponding means (consensus), and Figure 4.37 shows the comparisons by pairs of the consensus as vectors on landmarks and the deformation grids. From the comparisons, the following conclusions were made:

A. Differences between *S. mentella* and *S. marinus*:

- a) In *S. mentella*, the anterior insertion of the first dorsal fin (landmark B) and the situation of the opercula distal point (landmark L) are placed more posterior.
- b) In *S. marinus*, the tail (landmarks C to G) is thicker than in *S. mentella*, which is more evident around the anal area (landmark G).
- c) The ventral and anal fins (landmarks H and G respectively) are more posterior in *S. marinus* than in *S. mentella*.

B. Differences between *S. marinus* and *S. viviparus*.

The main difference in shape is that *S. viviparus* is wider in a dorsoventral direction than *S. marinus*, as can be observed in the more exterior position in most of the landmarks (especially in B, C, G and N). In other words, *S. viviparus* shows a more humpbacked shape and a thicker tail.

C. Differences between *S. mentella* and *S. viviparus*.

The main difference between both species is, again, the dorsoventral height, that is larger in *S. viviparus* than in *S. mentella* as landmarks B, C, F and G are in a more interior position in *S. mentella* than in *S. viviparus*.

Thus, there is a general trend in which *S. mentella* is slimmer while *S. viviparus* is more robust, and *S. marinus* is intermediate.

It is known that *S. fasciatus* has a dorsal humpback shape and a thicker tail, i.e. a similar shape to *S. viviparus*. This is in accordance with previous analyses (Saborido-Rey, 1994) where both species, although morphometrically different, were more closely related than the other two.

Table 4.47. Classification matrix in numbers, percentage of total correct classification, Wilks' Lambda and Cohen's Kappa resulting from the discriminant analysis of all cases in the whole area, evaluating discrimination among the three species, i. e. *S. marinus*, *S. mentella*, and *S. viviparus*

Wilks' Lambda: 0.22786 Cohen's Kappa= 0.9069				
	Percent	<i>S. mentella</i>	<i>S. marinus</i>	<i>S. viviparus</i>
<i>S. mentella</i>	95.8	3486	151	1
<i>S. marinus</i>	95.3	83	1780	4
<i>S. viviparus</i>	94.9	1	3	75
Total	95.6	3570	1934	80
Percentage of misclassification				
<i>S. mentella</i>			4.15	0.03
<i>S. marinus</i>		4.45		0.21
<i>S. viviparus</i>		1.27	3.80	

Table 4.48. Classification matrix in numbers, percentage of total correct classification, Wilks' Lambda and Cohen's Kappa resulting from the discriminant analysis of all cases in the whole area, evaluating discrimination among the three species, i. e. *S. marinus*, *S. mentella*, and *S. viviparus*. Each of the species are represented by the same number of individuals.

Wilks' Lambda= 0.07161 Cohen's Kappa= 0.949793				
	Percent	<i>S. mentella</i>	<i>S. marinus</i>	<i>S. viviparus</i>
<i>S. mentella</i>	96.25	77	3	0
<i>S. marinus</i>	93.75	3	75	2
<i>S. viviparus</i>	100.00	0	0	79
Total	96.65	80	78	81
Percentage of misclassification				
<i>S. mentella</i>			3.8	0.0
<i>S. marinus</i>		3.8		2.5
<i>S. viviparus</i>		0.0	0.0	

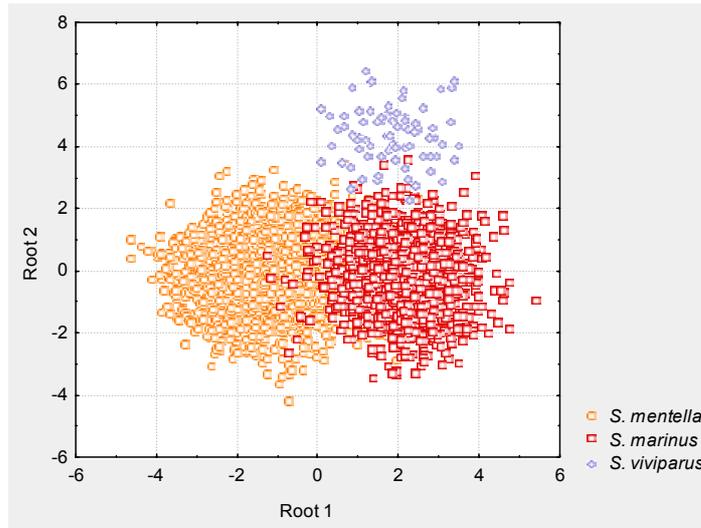


Figure 4.35. Plot of canonical scores for each case for the first and second canonical roots.

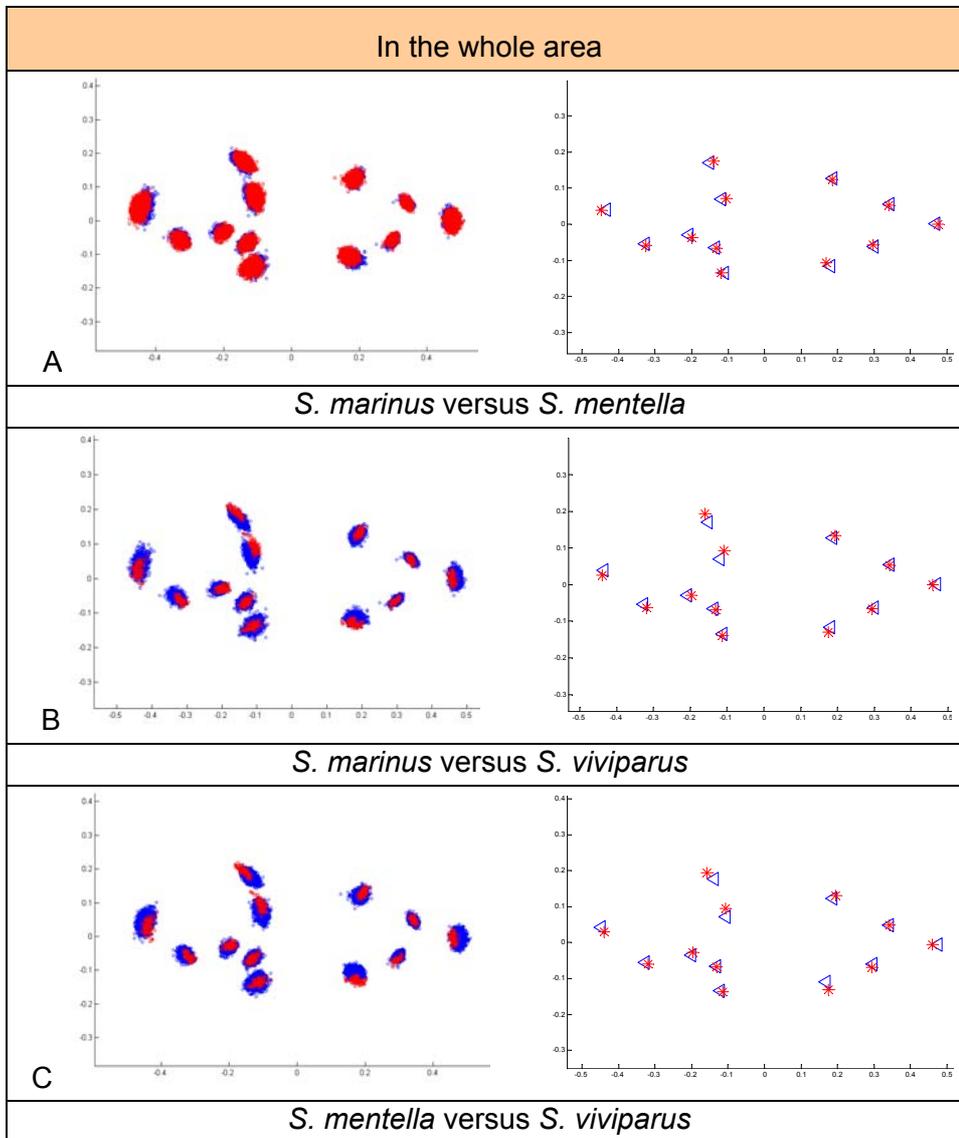


Figure 4.36. Graphical displays of the landmark configurations. Raw data are superimposed on the left, whilst the right plots the consensus of the landmark configurations. The species compared are A. *S. marinus* versus *S. mentella*. B. *S. marinus* versus *S. viviparus* and C. *S. mentella* versus *S. viviparus*.

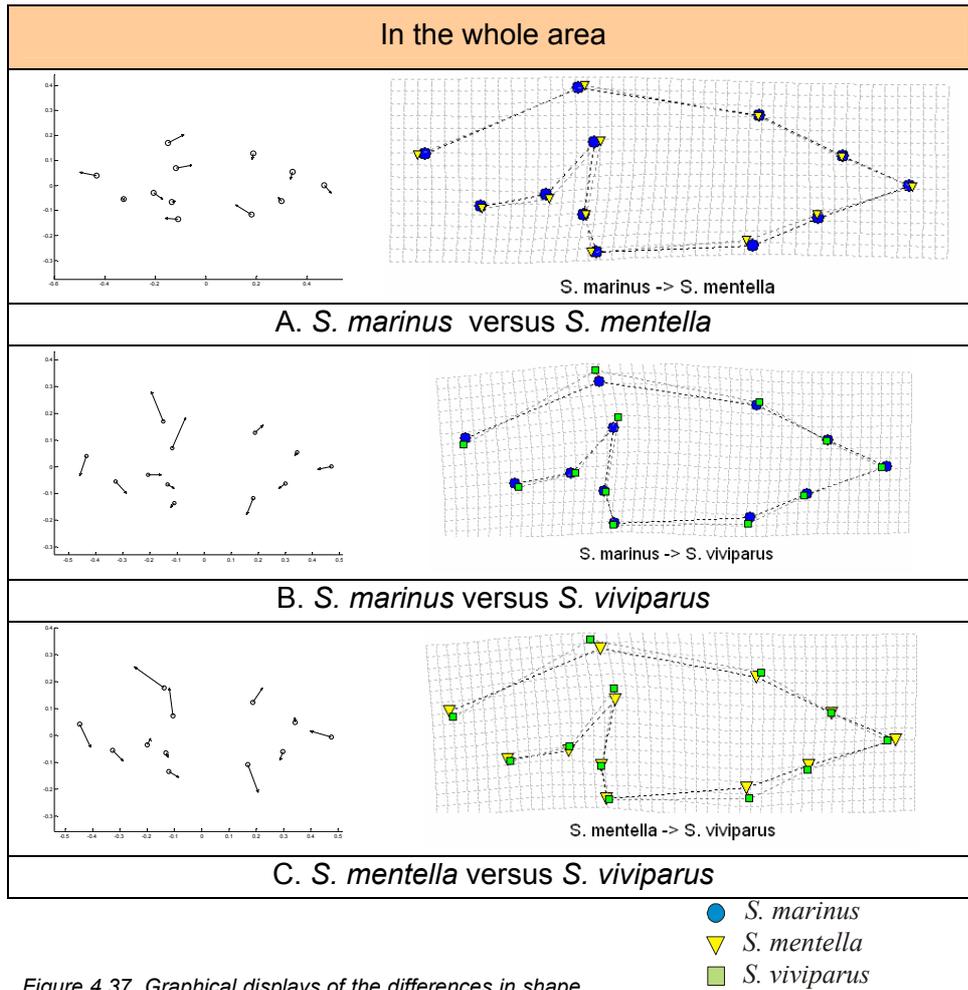
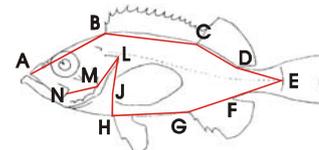


Figure 4.37. Graphical displays of the differences in shape between species in the whole area. Plots on the left represent vectors on landmarks (exaggeration factor = 5), and plots in the right represent the deformation grid. A. *S. marinus* against *S. mentella*. B. *S. marinus* versus *S. viviparus* and. C. *S. mentella* versus *S. viviparus*.



4.2.1.2. Discrimination between species In each of the areas separately

FAROES

Species were classified following the gas bladder musculature criteria in those samples from MH2002 and the external features in those fish coming from the FAER2000 cruise.

The classification matrix in numbers, the percentage of total correct classification and Wilks' lambda are shown in Table 4.49. The *Sebastes* species in Faroes waters showed a good discrimination, as the total percent correctly classified was 93.8, Wilks' lambda dropped to 0.08, and Cohen's Kappa showed a high value.

S. viviparus reached 98.7% correctly classified, and only one specimen was classified as *S. marinus*. *S. mentella* and *S. marinus* showed a similar classification rate, 92.9 and 92.7 respectively. The number of *S. mentella* that classified as *S. marinus* (14 individuals, (7.11%)

was quite similar to that of *S. marinus* classified as *S. mentella* (15 individuals, 7.25%). But none of the specimens of these two species were classified as *S. viviparus*. This result is observed also in the scatterplot of the canonical scores (Figure 4.38), where the first canonical root discriminates *S. viviparus* from the other two species, and the second canonical root, separate, subsequently, *S. marinus* and *S. mentella*.

The analysis with the same number of individuals by group, improves the classification of all species, and reaches 100% for *S. viviparus* (Table 4.50).

Figure 4.39 shows graphically the superimposed raw data for pair of species and their corresponding means (consensus), and Figure 4.40 shows the comparisons by pairs of the consensus as vectors on landmarks and the deformation grids. These graphs show that the highest difference was between *S. viviparus* consensus and the other two species, which is in agreement with the discriminant analysis. The smallest difference was between *S. marinus* and *S. mentella*, as they are closer in shape.

Regarding the landmarks marking the differences between species (Figure 4.40)), it can be observed that *S. marinus* and *S. mentella* shapes are more similar (upper panel), while *S. viviparus* is much thicker (medium and lower panels) than the other two species. Differences between *S. viviparus* and the other two species are principally concentrated in landmarks B and G. The differences between species in Faroes waters are almost identical than those observed when all the areas were analyzed as a whole, and the same trends can be identified (comparing Figure 4.37 and Figure 4.40).

This is, indeed, a very interesting result, and very relevant for later discussions, as the species here showed the same differences as in the whole area, indicating, as a first approach, that the shape of each species in the Faroes are similar to the rest of the areas for each respective species.

Table 4.49. Classification matrix, percentage of total correct classification , Wilks' Lambda and Cohen's Kappa resulting from the discriminant analysis of *Sebastes* species in the Faroes.

Wilks' Lambda= 0.08017 Cohen's Kappa= 0.9003				
	Percent	<i>S. viviparus</i>	<i>S. mentella</i>	<i>S. marinus</i>
<i>S. viviparus</i>	98.7	78	0	1
<i>S. mentella</i>	92.9	0	183	14
<i>S. marinus</i>	92.7	0	15	192
Total	93.8	78	198	207

Table 4.50. Classification matrix, percentage of total correct classification , Wilks' Lambda and Cohen's Kappa resulting from the discriminant analysis of *Sebastes* species in Faroes. Each of the species is represented by the same number of individuals.

Wilks' Lambda= 0.04250 Cohen's Kappa= 0.968619				
	Percent	<i>S. viviparus</i>	<i>S. mentella</i>	<i>S. marinus</i>
<i>S. viviparus</i>	100.0	79	0	0
<i>S. mentella</i>	97.5	0	78	2
<i>S. marinus</i>	96.3	0	3	77
Total	97.9	79	81	79

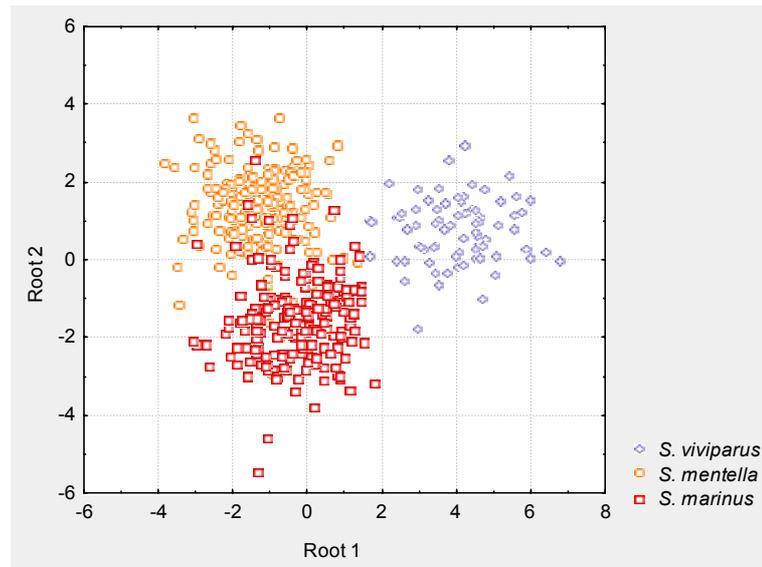


Figure 4.38. Plot of canonical scores for each case for the first and second canonical roots resulting from the discriminant analysis performed with the three species inhabiting the Faroes area.

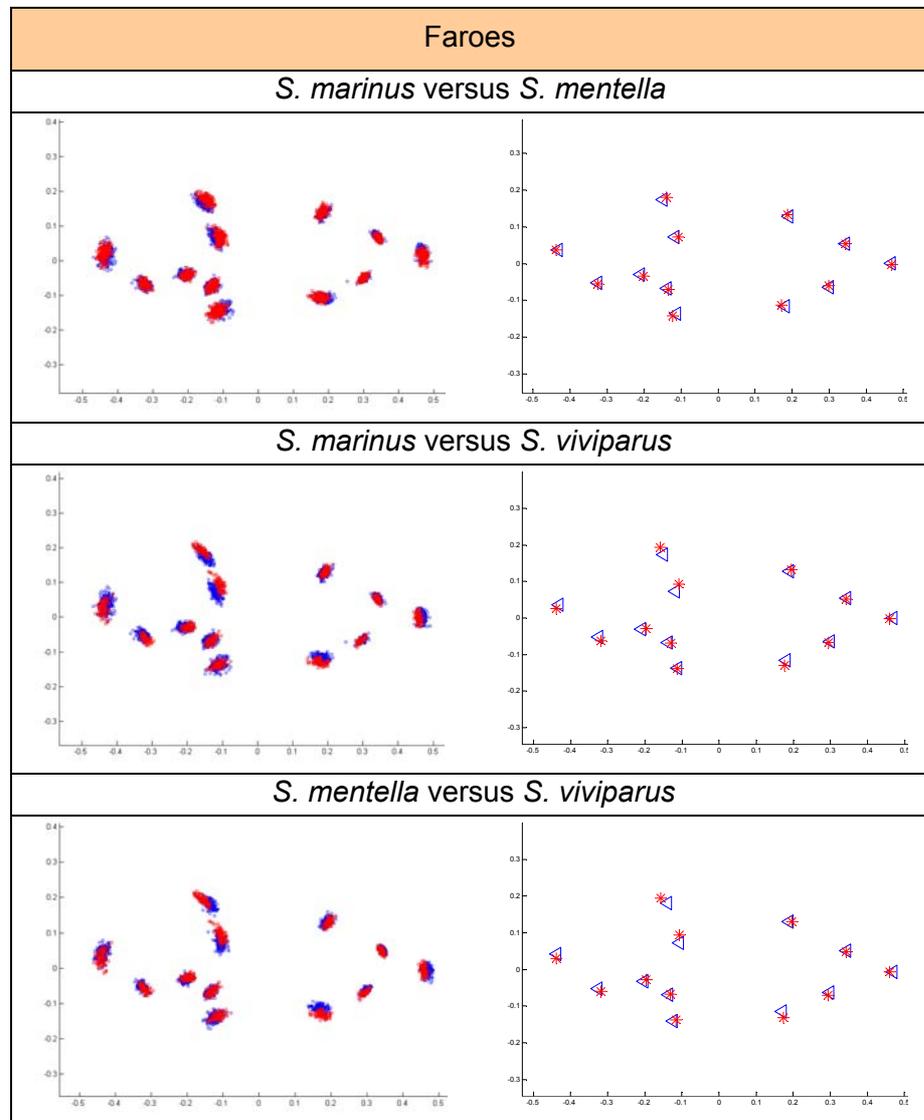


Figure 4.39. Graphical displays of the landmark configurations. Raw data are superimposed on the left. The plot of the consensus (means of the landmark configurations) are on the right.

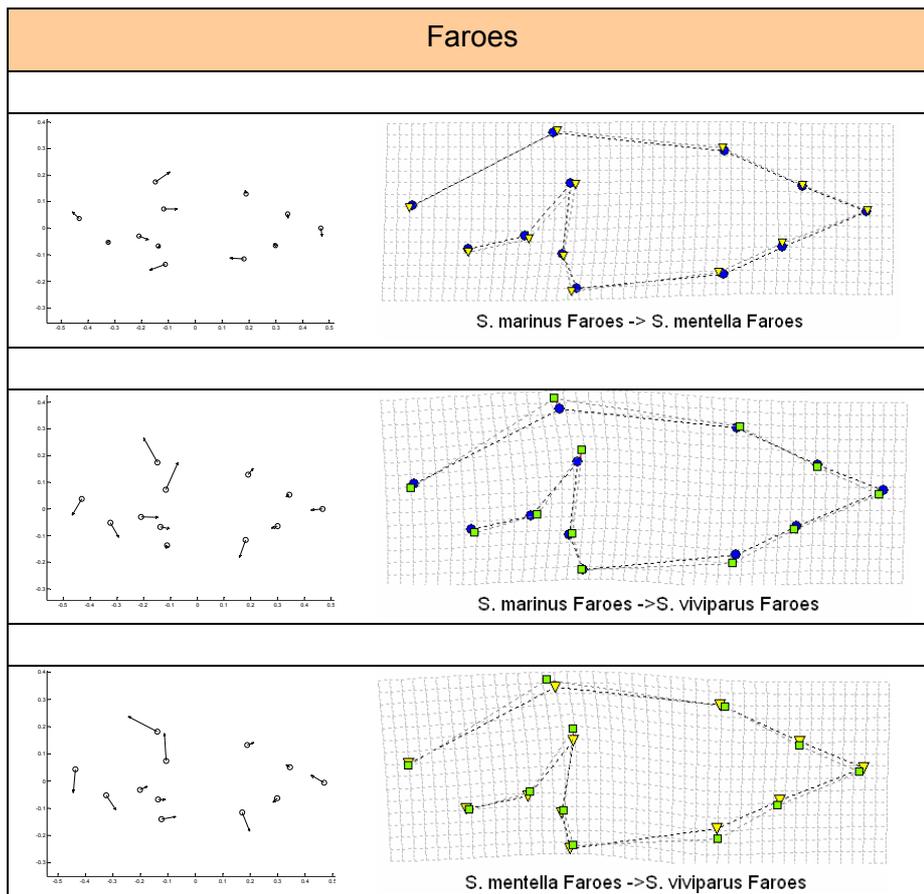
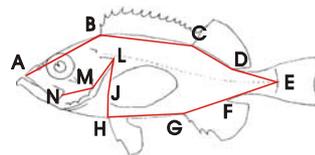


Figure 4.40. Graphical displays of the differences in shape between species in the Faroes. Plots on the left represent vectors on landmarks (exaggeration factor = 5), and plots on the right represent the deformation grid. A. *S. marinus* against *S. mentella*. B. *S. marinus* versus *S. viviparus* and. C. *S. mentella* versus *S. viviparus*.

- *S. marinus*
- ▼ *S. mentella*
- *S. viviparus*



ICELAND

All the fish analyzed in Iceland were identified by their external morphology, and therefore neither the gas bladder musculature, nor the genetic results have been used. The external morphology has been proved as efficient in species discrimination in Iceland in the same analysis (species discrimination in Iceland) performed with traditional morphometric methods. The classification matrix in numbers, the percent of total correct classification, Wilks' lambda and Cohen's Kappa are shown in Table 4.51. The discrimination between *S. marinus* and *S. mentella* in Iceland was good and at the same level as in the other areas studied. The total percent correctly classified was 93.5%. Wilks' lambda reached a relatively low value (0.32) confirming, in addition to the high value of Cohen's Kappa (0.86), a good discrimination. Some confusion existed, nevertheless, mainly in *S. mentella*, which classified correctly in 91.2%, while 8.7%, i. e. 91 individuals, classified as *S. marinus*. On the other hand, *S.*

marinus correct classification is slightly higher (95.2%) and only 4.8% of the individuals classified as *S. mentella*. The histogram of the frequencies of canonical scores shows also the good separation between species in Iceland (Figure 4.41).

The results of the analysis with the same number of individuals in each group were basically the same, probably because the imbalance in number of individuals in the previous analysis was already very small. Thus, it is observed that *S. marinus*, the group with a higher number of individuals in the previous analysis, now shows a slightly lower percent correctly classified (93.9 instead of 95.2%) and that in contrast, *S. mentella* increases its percent correctly classified from 91.2 to 92.6%.

The Figure 4.42 shows graphically the superimposed raw data for pairs of species and their corresponding means (consensus), and Figure 4.43 shows the comparisons by pairs of the consensus as vectors on landmarks and the deformation grids. Differences between species in Iceland are, once more, concentrated in the same regions as in the previous analyses, i.e. the anterior insertion of the first dorsal fin (landmark B), the situation of the distal point of the opercula, the preoperculum and the pectoral fin (landmarks L, M and J respectively) are placed farther back in *S. mentella*; The ventral and anal fins (landmarks H and G respectively) are farther back in *S. marinus*; and in *S. marinus* the tail (landmarks C to G) is thicker than in *S. mentella*, more evidently around the anal area (landmark G).

Table 4.51. Classification matrix, percentage of total correct classification, Wilks' Lambda and Cohen's Kappa resulting from the discriminant analysis between *S. marinus* and *S. mentella* in Iceland.

Wilks' Lambda= 0.32078 Cohen's Kappa= 0.8667			
	Percent	<i>S. mentella</i>	<i>S. marinus</i>
<i>S. mentella</i>	91.2	946	91
<i>S. marinus</i>	95.2	67	1325
Total	93.5	1013	1416

Table 4.52. Classification matrix, percentage of total correct classification, Wilks' Lambda and Cohen's Kappa resulting from the discriminant analysis between *S. marinus* and *S. mentella* in Iceland. All species are represented by the same number of individuals.

Wilks' Lambda= 0.32214 Cohen's Kappa= 0.864995			
	Percent	<i>S. mentella</i>	<i>S. marinus</i>
<i>S. mentella</i>	92.6	960	77
<i>S. marinus</i>	93.9	63	974
Total	93.2	1023	1051

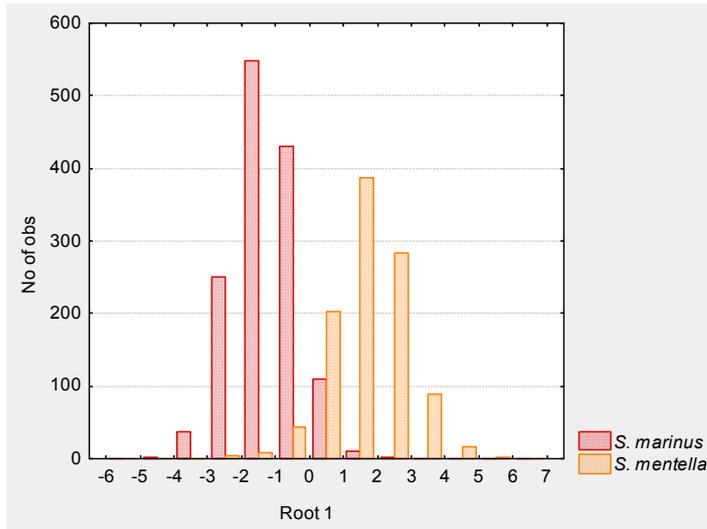


Figure 4.41. Histogram of frequencies of the canonical scores for *S. marinus* and *S. mentella* in Iceland.

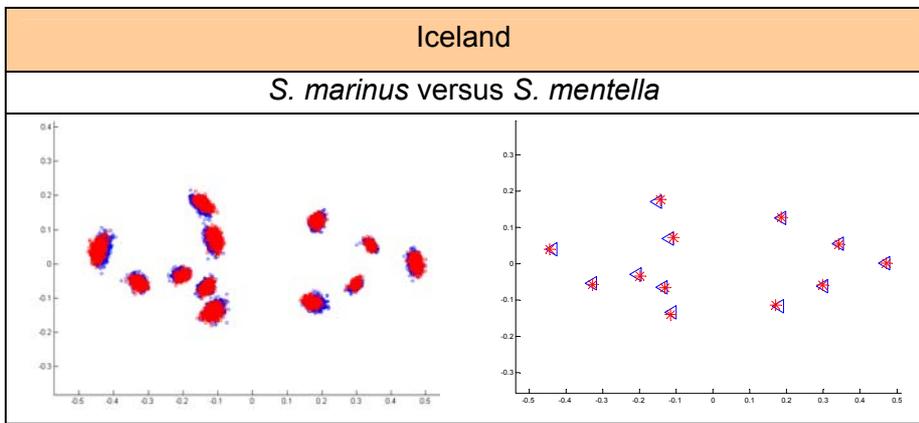


Figure 4.42. Graphical displays of the landmark configurations. Raw data are superimposed on the left, whilst the plot of the consensus (means of the landmark configurations) are on the right. The species compared are *S. marinus* (in blue) versus *S. mentella* (in red) in Iceland.

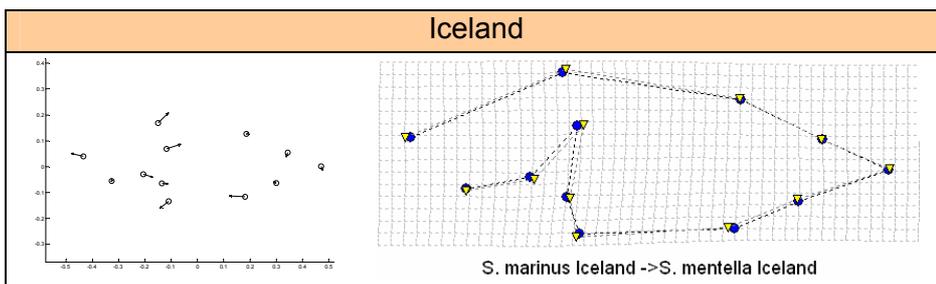
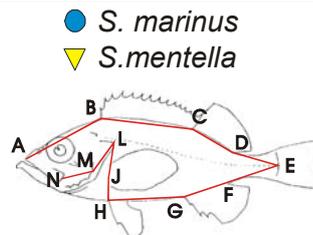


Figure 4.43. Graphical displays of the differences in shape between species in Iceland. The plot on the left represent vectors on landmarks (exaggeration factor=5), and the plot on the right represent the deformation grid.



GREENLAND

Only those individuals assigned to species by genotype were used in this analysis, because external features and gas bladder musculature were not effective for assignment to species in this area, probably due to misclassification of the individuals while sampling (see Greenland section in the traditional morphometric analyses).

The classification matrix in numbers, the percentage of total correct classification and Wilks' lambda resulting from the discriminant analysis of *S. mentella* and *S. marinus* in Greenland are shown in Table 4.53. The total percent correctly classified was very good (95.4%), and Wilks' lambda was low (0.29). The classification rate of *S. mentella* was 91.8% and only 12 individuals were classified as *S. marinus*, while only 2.6% of the *S. marinus* was assigned to *S. mentella*. Cohen's Kappa value is high, and confirms that the discrimination between *S. marinus* and *S. mentella* is very good, indicating that both species are morphometrically distinct in this area too. The separation of the two species is also evident from the histogram of the canonical scores (Figure 4.44.).

The same analysis but with the same number of *S. mentella* and *S. marinus* individuals, although that diminishes slightly the total percent correctly classified, yield more compensated values of classification for the two species (Table 4.54).

Figure 4.45 shows graphically the superimposed raw data for pairs of species and their corresponding means (consensus), and Figure 4.46 shows the comparisons by pairs of the consensus as vectors on landmarks and the deformation grids. The deformation grids for *S. marinus* and *S. mentella* consensus shown that *S. marinus* is slightly deeper than *S. mentella*, as can be observed from the positions of landmarks C to H, i.e. those corresponding to the tail and the ventral area. The relative position of the rest of the landmarks and their deformations are very similar to the differences observed in the previous two analyses, but perhaps smaller, as observed from the lengths of the vectors (left panel of Figure 4.46). These differences affected especially the anterior insertion of the first dorsal fin (landmark B), and the situation of the opercula distal point (landmark L) are placed further back in *S. mentella*; and the ventral and anal fins (landmarks H and G respectively) are further back in *S. marinus*.

Table 4.53. Classification matrix, percentage of total correct classification, Wilks' Lambda and Cohen's Kappa resulting from the discriminant analysis of *Sebastes* species in Greenland.

Wilks' Lambda= 0.29872 Cohen's Kappa= 0.8987			
	Percent	<i>S. mentella</i>	<i>S. marinus</i>
<i>S. mentella</i>	91.8	134	12
<i>S. marinus</i>	97.4	7	261
Total	95.4	141	273

Table 4.54 Classification matrix, percentage of total correct classification, Wilks' Lambda and Cohen's Kappa resulting from the discriminant analysis of *Sebastes* species in Greenland. All species represented by the same number of individuals.

Wilks' Lambda= 0.30736 Cohen's Kappa= 0.883562			
	Percent	<i>S. mentella</i>	<i>S. marinus</i>
<i>S. mentella</i>	94.5	138	8
<i>S. marinus</i>	93.8	9	137
Total	94.2	147	145

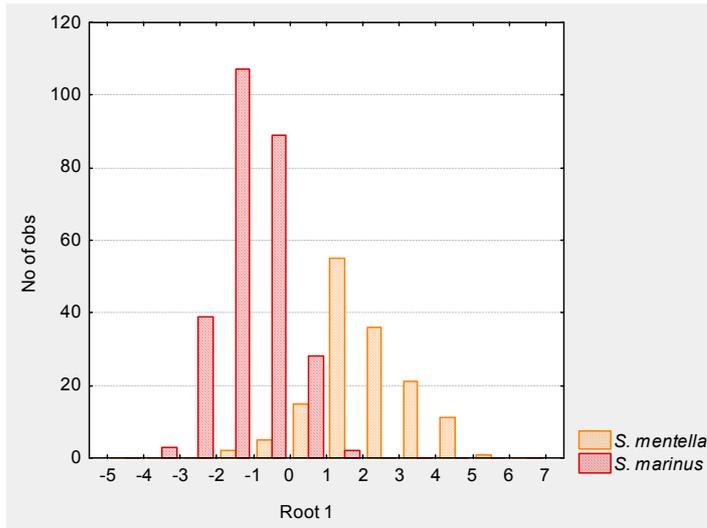


Figure 4.44. Histogram of frequencies of canonical scores for *S. marinus* and *S. mentella* in Greenland.

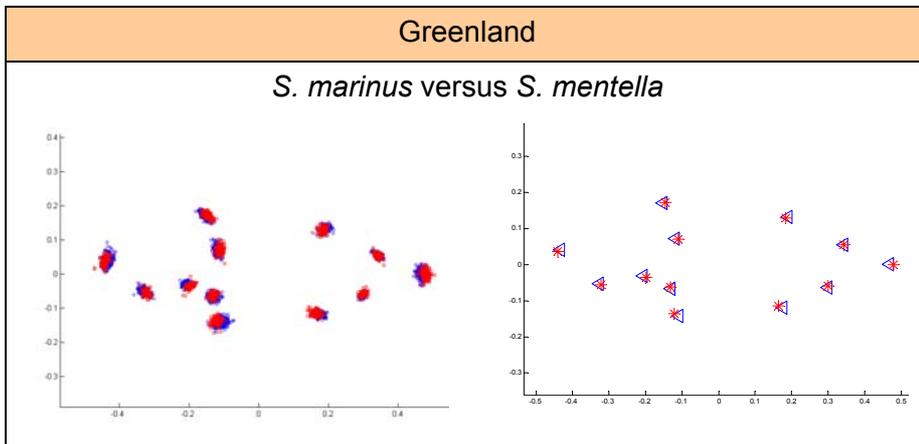
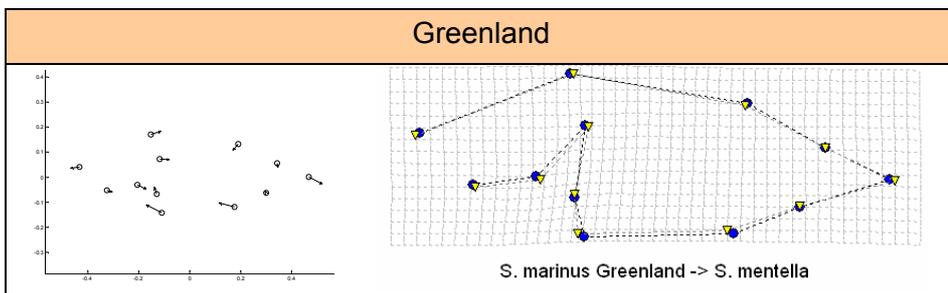
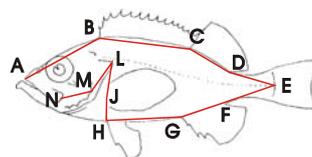


Figure 4.45. Graphical displays of the landmark configurations. Superimposed raw data are on the left, whilst the plot of the consensus (means of the landmark configurations) are on the right. The species compared are *S. marinus* (in blue) versus *S. mentella* (in red) in Greenland.



● *S. marinus*
▼ *S.mentella*

Figure 4.46. Graphical displays of the differences in shape between species in Greenland. The plot on the left represent vectors on landmarks (exaggeration factor=5), and the plot on the right represents the deformation grid.



4.2.2. Discrimination between areas and subareas

4.2.2.1. *S. marinus*

Discrimination between areas (*S. marinus*)

The classification matrix in numbers, the percentage correctly classified, Wilks' lambda and Cohen's Kappa are shown in Table 4.55. The overall percent correctly classified is low, 80.3%, indicating a very poor discrimination, confirmed by the high lambda value (0.6) and low K (0.43). In spite of the low discrimination among areas of *S. marinus*, Iceland shown a percent correctly classified higher than expected (94%); but this result is biased by the unequal quantity of individuals sampled in this area. Thus, the 35 *S. marinus* misclassified in the Faroes group represent only 2.5% of the total Icelandic *S. marinus*, and the 45 individuals misclassified in the Greenland group represent only 3.5% of the *S. marinus* from Iceland. However, the majority of *S. marinus* from Faroes (132) and from Greenland (136) were misclassified with Icelandic *S. marinus* (63.7% and 50% respectively), while a lower rate of confusion was observed between Greenland and Faroes (no more than 12%). In other words, the confusion matrix indicates the lack of structure of *S. marinus* in the areas studied. The lack of stock structure is also observed from the plot of the canonical scores for *S. marinus* by areas shown in Figure 4.47. In the analysis performed with the same number of individuals by group, the percent correctly classified in the areas is more in accordance with the confusion observed between them. Thus, all the areas presented values lower than the 80%. The result of this stepwise discriminant analysis based on the geometric morphometry (Weight matrix) showed the same trend as that performed with traditional morphometry, that is the lack of structure for *S. marinus* in the central area.

In the comparisons by pairs of *S. marinus* in the different areas (Figure 4.48), it is most evident that the high rate of classification observed for *S. marinus* in Iceland was spurious. This is because in this graph, the means of all fish from each area are displayed, and the effect of the number of individuals is removed. Thus, it can be seen in Figure 4.49 that there were no differences in the consensus between the different areas, neither between Iceland and the others (the two upper panels in both figures). Very small differences can be observed, however, between the Faroes and Greenland, especially in landmarks G to J. It is advisable to compare the graphs presented in this case study, with those resulting from the species comparisons to observe the lack of differences.

Table 4.55. Classification matrix, percentage of total correct classification, Wilks' Lambda and Cohen's Kappa resulting from the discriminant analysis of *S. marinus* by area.

Wilks' Lambda= 0.60985 Cohen's Kappa= 0.4369				
	Percent	Iceland	Faroes	Greenland
Iceland	94.0	1308	35	49
Faroes	30.4	132	63	12
Greenland	47.8	136	4	128
Total	80.3	1576	102	189
Percentage of misclassification				
Iceland			2.5	3.5
Faroes		63.8		5.8
Greenland		50.7	1.5	

RESULTS

Table 4.56 Classification matrix, percentage of total correct classification, Wilks' Lambda and Cohen's Kappa resulting from the discriminant analysis of *S. marinus* by area. The same number of individuals has been taken in each of the areas.

Wilks' Lambda= 0.31565 Cohen's Kappa= 0.63				
	Percent	Iceland	Greenland	Faroes
Iceland	72.0	72	15	13
Greenland	76.0	13	76	11
Faroes	78.0	10	12	78
Total	75.3	95	103	102
Percentage of misclassification				
Iceland			15.0	13.0
Greenland		13.0		11.0
Faroes		10.0	12.0	

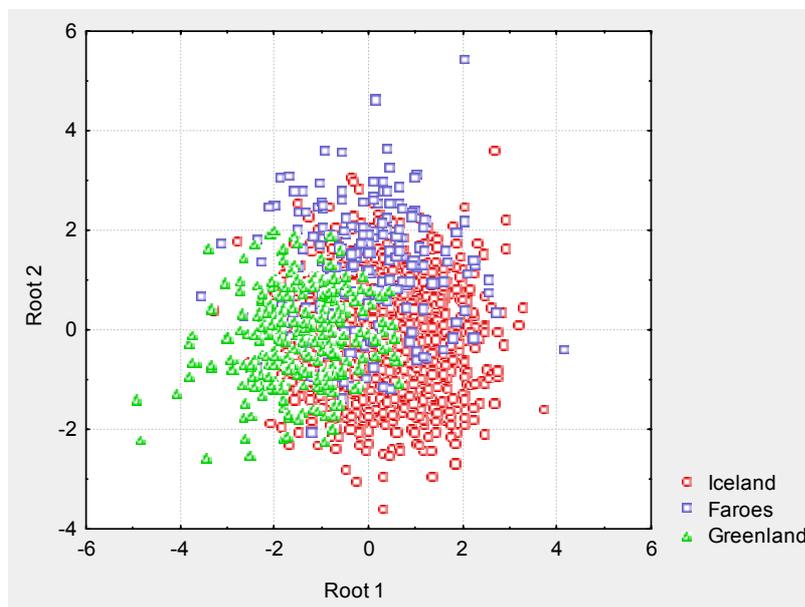


Figure 4.47. Plot of canonical scores for each case for the first and second canonical roots resulting from the discriminant analysis performed with *S. marinus* by area.

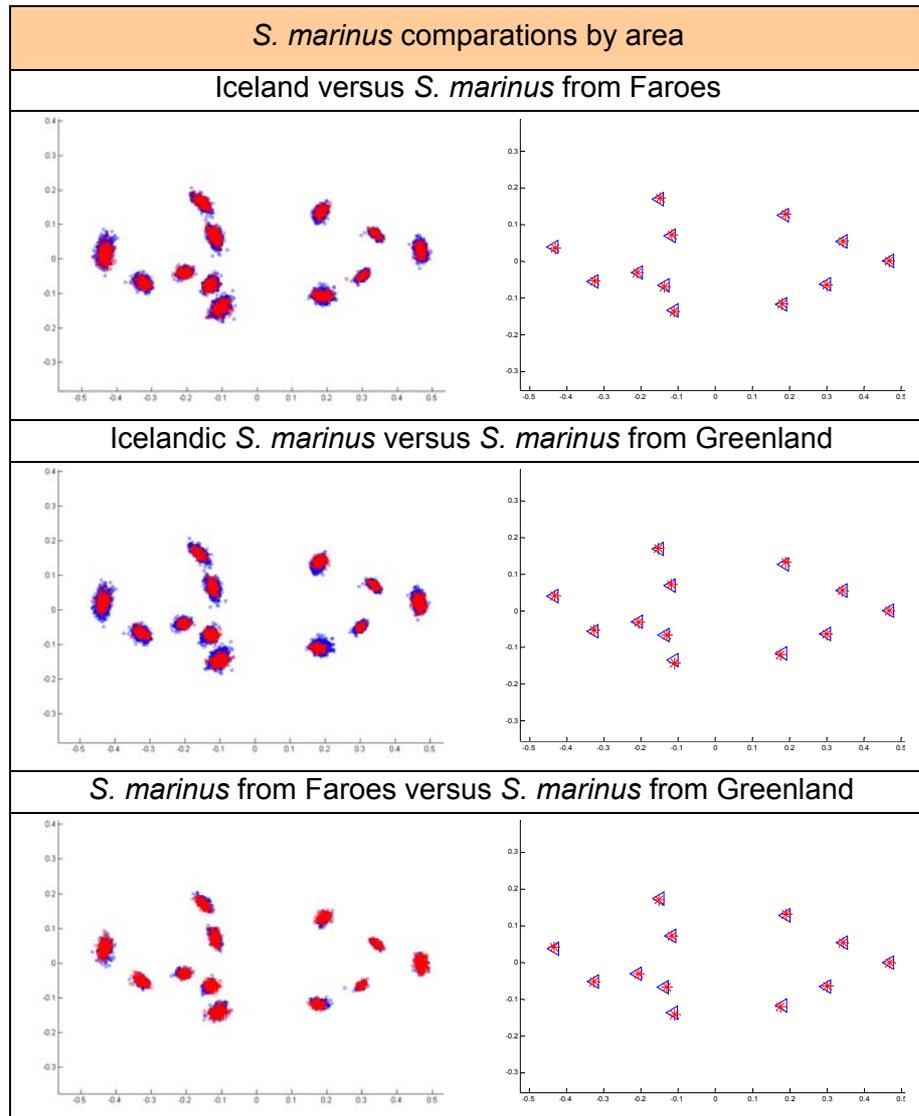


Figure 4.48. Graphical displays of the landmark configurations of *S. marinus* in the different areas. Superimposed raw data are on the left, whilst the plot of the consensus (means of the landmark configurations) are on the right.

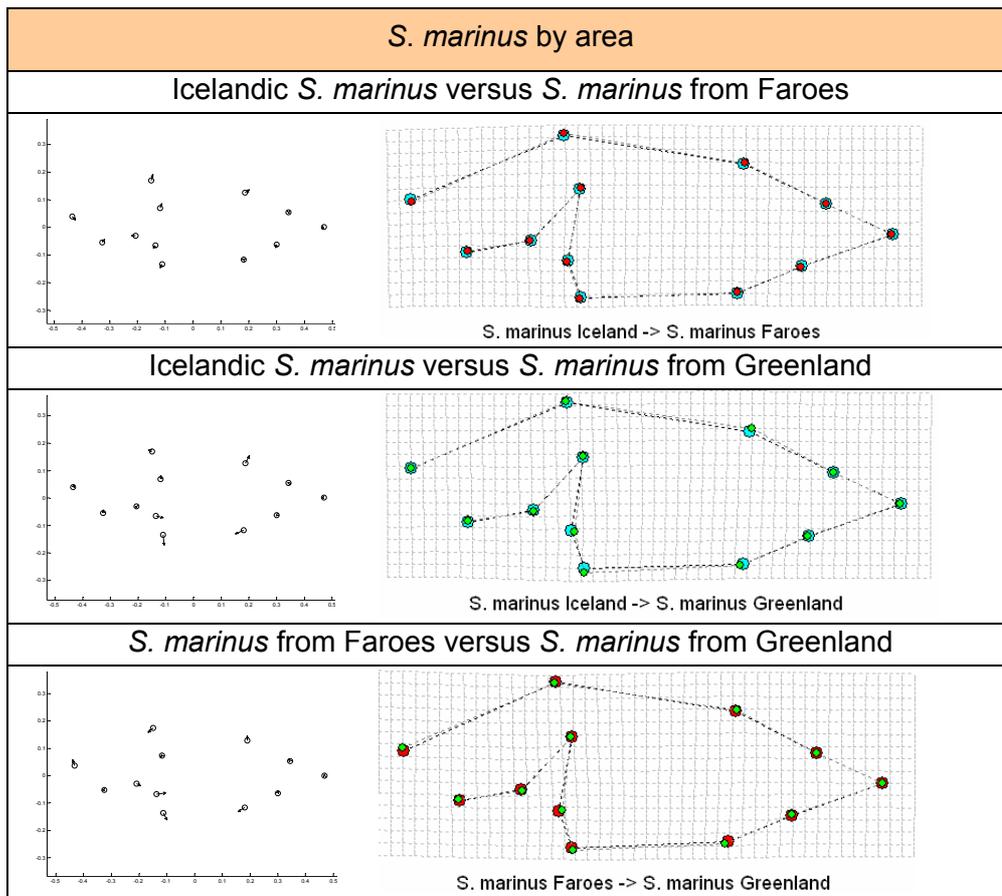
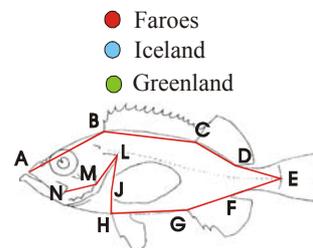


Figure 4.49. Graphical displays of the *S. marinus* differences in shape among the different areas. Plots on the left represent vectors on landmarks (exaggeration is 5), and plots on the right represent the deformation grid. A. Iceland versus Faroes. B. S. Iceland versus Greenland and. C. Faroes versus Greenland.



Discrimination between subareas (*S. marinus*)

Eight different subareas were compared. Two from Greenland, two from the Faroes and four from Iceland. In the geometric analysis, fish were included from NW Iceland which were not available for the traditional morphometric analysis.

The classification matrix in numbers, the percentage correctly classified, Wilks' lambda and Cohen's Kappa are shown in Table 4.57. This table gives a more detailed view of the species structure in the area. As expected from the previous analysis, all the subareas presented a very low correct classification rate, that can be also observed in the plot of canonical scores (Figure 4.50) where a clear pattern cannot be identified. Wilks' lambda and K values confirm this poor classification.

The analysis performed with the same number of individuals by group showed a different percent correctly classified for all the subareas (Table 4.58). Thus, now the southeast of the Faroes showed the highest, although low, classification rate (85.4%). In fact, SE of the Faroe Islands only showed confusion with the Northwest of the Faroe Islands (8.3 and 20.8 % respectively). However, NW Faroe Islands also showed important confusion with both subareas of West Iceland. This pattern is also reflected in the tree plot of the cluster analysis (Figure 4.51).

West Greenland, however, show a big increment in the percent correctly classified compared with the anterior analysis, but the correct classification rate is still too low (63.8%).

In summary *S. marinus* showed a low classification rate for Greenland, Iceland and the Faroes, suggesting a lack of structuring for this species in this area. However, there was a geographic structure as shown in Figure 4.51 where it can be observed that the two subareas in the West of Iceland were more related to NW Faroes than to the East of Iceland subareas. Greenland and East Iceland appeared as separate clusters.

Table 4.57. Classification matrix, percentage correctly classified, Wilks' Lambda and Cohen's Kappa resulting from the discriminant analysis of *S. marinus* in the different subareas.

Wilks' Lambda= 0.30343 Cohen's Kappa= 0.4113									
	Percent	Iceland				Faroes		Greenland	
		SW	NW	SE	NE	NW	SE	E	W
Iceland-SW	81.47	620	13	72	5	10	9	28	4
Iceland-NW	15.56	80	21	18	9	1	0	6	0
Iceland-SE	60.00	84	3	225	16	7	3	36	1
Iceland-NE	19.83	30	9	41	24	2	1	13	1
Faroes-NW	15.75	71	2	17	4	23	12	14	3
Faroes-SE	55.74	18	0	3	0	4	34	2	0
Greenland-E	56.11	33	1	53	0	4	1	124	5
Greenland-W	4.26	16	0	9	1	1	0	18	2
Total	57.47	952	49	438	59	52	60	241	16
Percentage of misclassification									
Iceland-SW			1.7	9.5	0.7	1.3	1.2	3.7	0.5
Iceland-NW		59.3		13.3	6.7	0.7	0.0	4.4	0.0
Iceland-SE		22.4	0.8		4.3	1.9	0.8	9.6	0.3
Iceland-NE		24.8	7.4	33.9		1.7	0.8	10.7	0.8
Faroes-NW		48.6	1.4	11.6	2.7		8.2	9.6	2.1
Faroes-SE		29.5	0.0	4.9	0.0	6.6		3.3	0.0
Greenland-E		14.9	0.5	24.0	0.0	1.8	0.5		2.3
Greenland-W		34.0	0.0	19.1	2.1	2.1	0.0	38.3	

RESULTS

Table 4.58. Classification matrix, percentage correctly classified , Wilks' Lambda and Cohen's Kappa resulting from the discriminant analysis of *S. marinus* by subarea. The different groups are composed by the same number of individuals.

Wilks' Lambda= 0.30343 Cohen's Kappa= 0.4113										
	Percent	Iceland				Faroes		Greenland		
		SW	NW	SE	NE	NW	SE	E	W	
Iceland-SW	50.0	24	9	2	1	5	2	0	5	
Iceland-NW	41.7	7	20	5	7	6	0	2	1	
Iceland-SE	54.2	2	4	26	5	2	2	5	2	
Iceland-NE	52.1	2	9	8	25	2	0	1	1	
Faroes-NW	33.3	3	5	1	2	16	10	5	6	
Faroes-SE	85.4	1	0	1	0	4	41	1	0	
Greenland-E	56.3	2	0	3	0	5	1	27	10	
Greenland-W	63.8	5	0	3	2	2	0	5	30	
Total	54.6	46	47	49	42	42	56	46	55	
Percentage of misclassification										
Iceland-SW			18.8	4.2	2.1	10.4	4.2	0.0	10.4	
Iceland-NW		14.6		10.4	14.6	12.5	0.0	4.2	2.1	
Iceland-SE		4.2	8.3		10.4	4.2	4.2	10.4	4.2	
Iceland-NE		4.2	18.8	16.7		4.2	0.0	2.1	2.1	
Faroes-NW		6.3	10.4	2.1	4.2		20.8	10.4	12.5	
Faroes-SE		2.1	0.0	2.1	0.0	8.3		2.1	0.0	
Greenland-E		4.2	0.0	6.3	0.0	10.4	2.1		20.8	
Greenland-W		10.6	0.0	6.4	4.3	4.3	0.0	10.6		

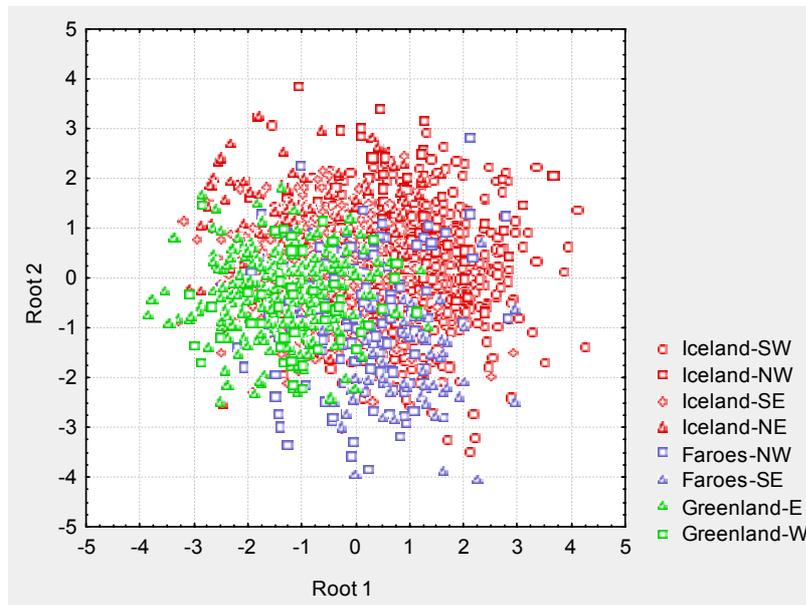


Figure 4.50. Plot of canonical scores for each case for the first and second canonical roots resulting from the discriminant analysis performed with *S. marinus* by sub-area.

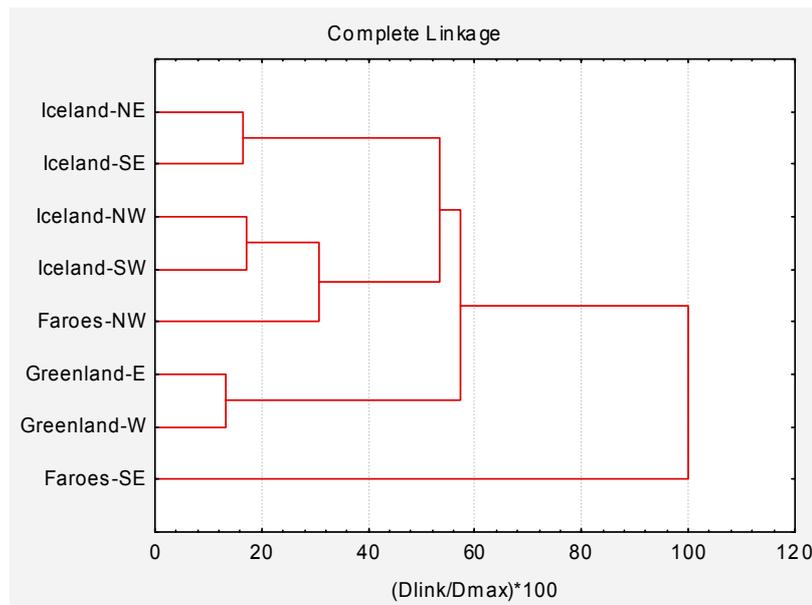


Figure 4.51. Tree diagram showing hierarchical differences in shape among *S. marinus* from the different subareas.

4.2.2.2. *S. mentella*

Discrimination between areas (*S. mentella*)

A total of 3,760 *S. mentella* were analyzed. The species assignments were done by the external morphology in Iceland and the Irminger Sea, using the GBM in the Faroes, and the genotype in Greenland. The numbers of specimens per area were: 146 in Greenland, 197 in the Faroes, 1037 in Iceland and 2258 in the the Irminger Sea. Obviously, there was a bias towards Iceland and mainly towards the Irminger Sea. The classification matrix in numbers, the percentage correctly classified, Wilks' lambda and Cohen's Kappa are shown in Table 4.59. The total correctly classified was relatively high, 84%, supported also by the low Wilks' lambda value (0.3). But this can be caused by the high percent correctly classified in the Irminger Sea. Regarding the Faroes, Iceland and Greenland, all of them showed very low correctly classified rates as a result of important confusion between them. The percentage of fish from a particular area that was classified into another showed a clear trend, with confusion larger among closer areas, i. e. The Faroes classified mainly as Iceland (44.7%) and then as Irminger Sea (14.7%). Greenland classified in high proportion as Iceland (45.2%) and Irminger Sea (23.3%). Iceland confused with Irminger Sea 16.9% of cases. Irminger Sea was classified correctly in a very high proportion, 95%, but again the large number of fish sampled in this area could be the cause of the increment of the percent classified in an spurious way. However, the amount of fish from other areas that are classified into Irminger was important, especially from Greenland with 23.3%. This tendency is also observed in the scatterplot of canonical scores (Table 4.52) where Iceland, the Faroes and Greenland clustered together, but Irminger Sea, although with an important overlap, grouped apart.

The classification matrix of the analysis with the same number of individuals by area, showed that Irminger sea continued being a different group (Table 4.60) with a 91% of correct classification. Although the percent of correct classification in Greenland and Faroes were

higher now (83 and 81% respectively) they were still too low to be considered as different groups. The percent of individuals from other areas that classifies into Irminger sea diminishes considerably, and contrary, there are an increment of the percent of individuals from Iceland that classifies in Faroe Islands and in Greenland.

Figure shows graphically the superimposed raw data for pair of species and their corresponding means (consensus), and Figure 4.54 shows the comparisons by pairs of the consensus as vectors on landmarks and the deformation grids. It can be observed in the deformation grids between the different areas that Irminger Sea *S. mentella* shape is always more fusiform (slim) than *S. mentella* in the other areas. The differences affected especially the dorsal and anal fin (landmarks C and H). In the areas different from Irminger, there are no appreciable differences, except very small ones between Greenland and the Faroes, the more distant areas.

Table 4.59. Classification matrix, percentage of correctly classified , Wilks' Lambda and Cohen's Kappa resulting from the discriminant analysis of *S. mentella* in the different areas.

Wilks' Lambda= 0.30965 Cohen's Kappa=0.6967					
	Percent	Irminger	Iceland	Faroes	Greenland
Irminger	95.1	2148	99	6	5
Iceland	77.5	175	804	38	20
Faroes	40.6	29	88	80	0
Greenland	31.5	34	66	0	46
Total	84.6	2386	1057	124	71
Percentage of misclassification					
Irminger			4.4	0.3	0.2
Iceland		16.9		3.7	1.9
Faroes		14.7	44.7		0.0
Greenland		23.3	45.2	0.0	

Table 4.60. Classification matrix, percentage correctly classified , Wilks' Lambda and Cohen's Kappa resulting from the discriminant analysis of *S. mentella* in the different areas. The same number of individuals has been randomly taken in each of the areas.

Wilks' Lambda= 0.14261 Cohen's Kappa= 0.743959					
	Percent	Irminger	Iceland	Greenland	Faroes
Irminger	91.1	92	2	5	2
Iceland	69.0	7	69	12	12
Greenland	83.0	4	12	83	1
Faroes	80.0	4	12	4	80
Total	80.8	107	95	104	95
Percentage of misclassification					
Irminger			2.0	5.0	2.0
Iceland		7.0		12.0	12.0
Faroes		4.0	12.0		1.0
Greenland		4.0	12.0	4.0	

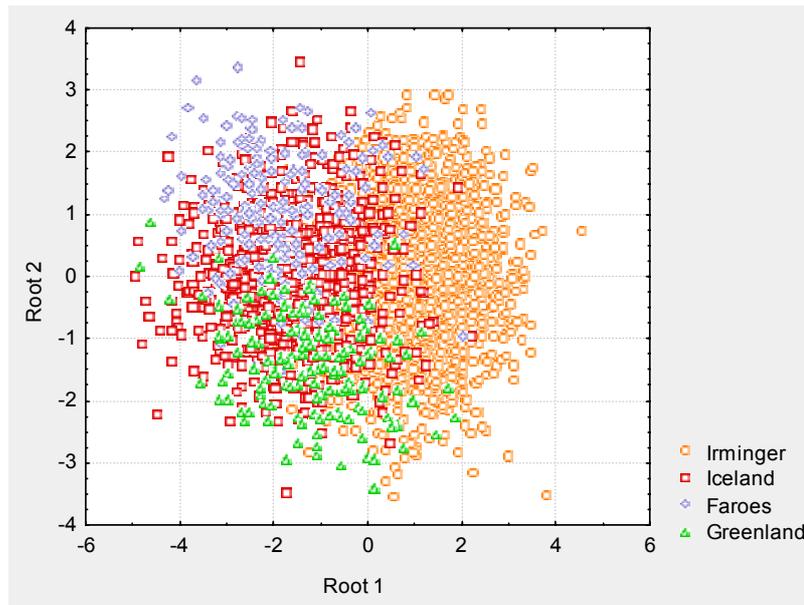


Figure 4.52. Plot of canonical scores for each case for the first and second canonical roots resulting from the discriminant analysis performed with *S. mentella* by area.

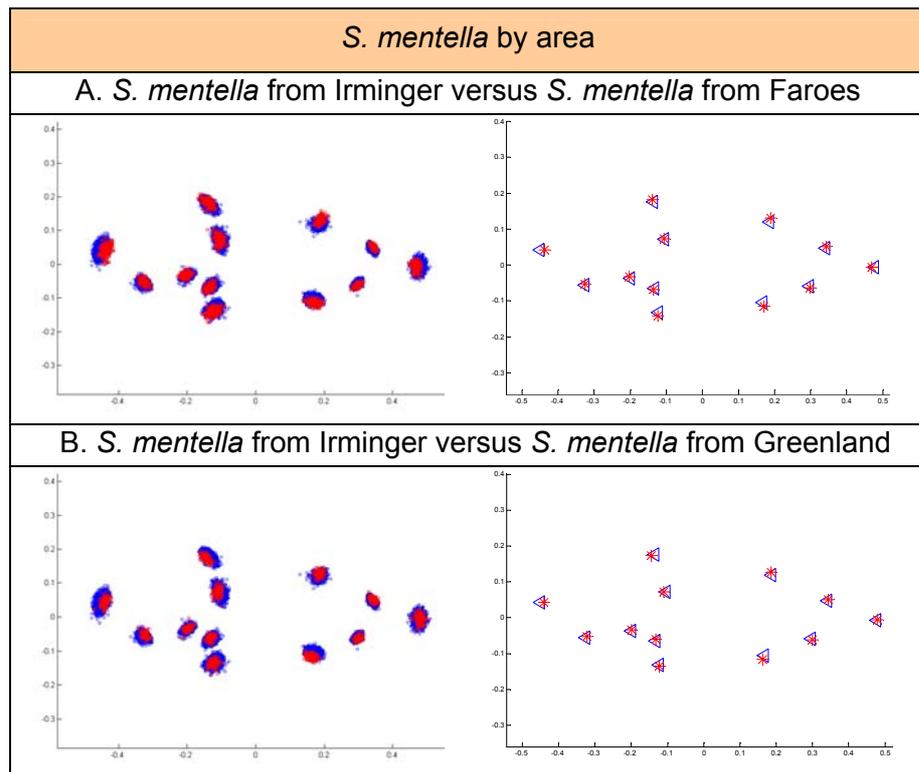


Figure 4.53. Graphical displays of the landmark configurations of *S. mentella* in the different areas by pairs. Superimposed raw data are on the left, whilst the plot of the consensus (means of the landmark configurations) are on the right.

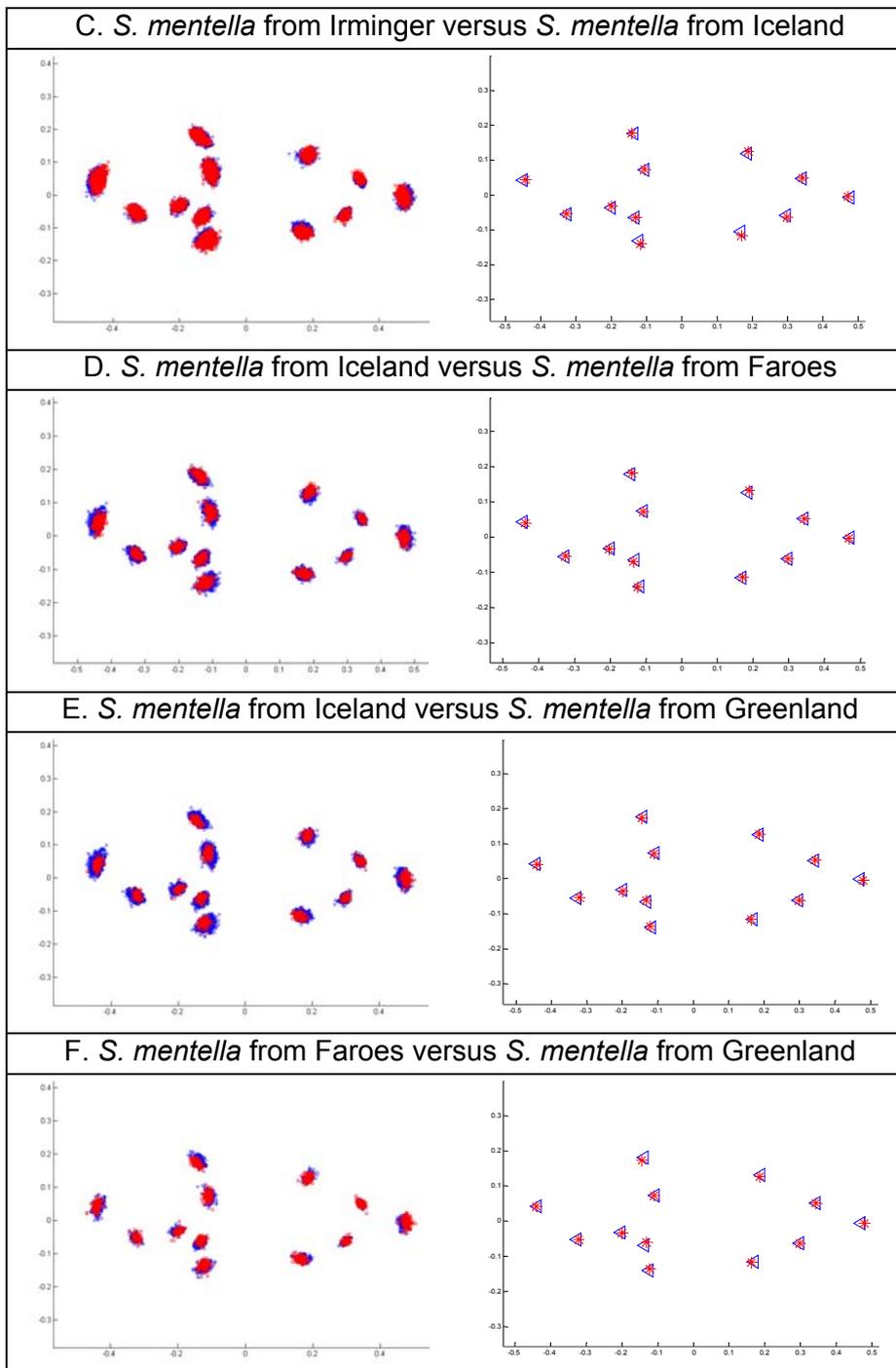
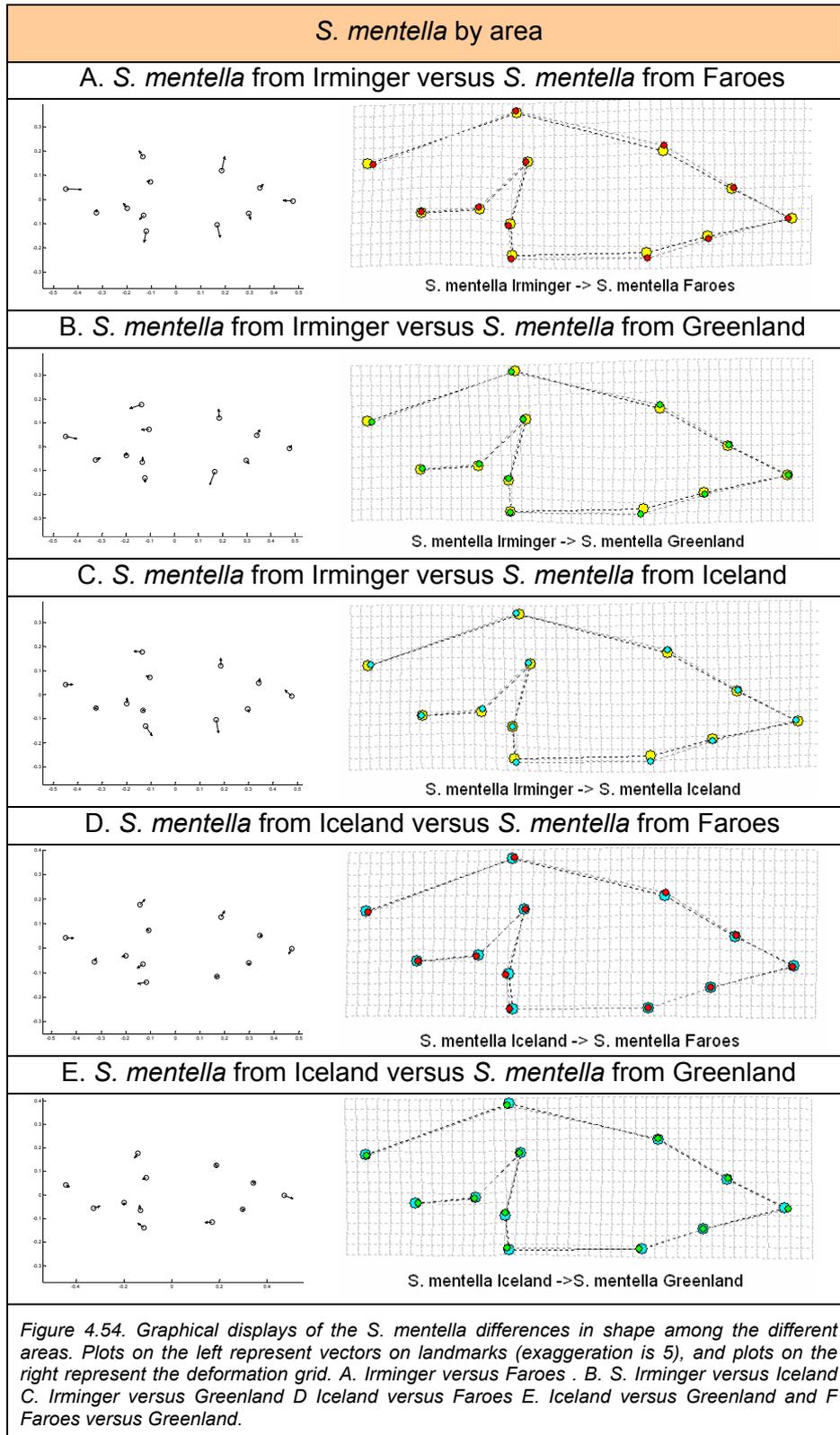


Figure 4.53 (continuation).



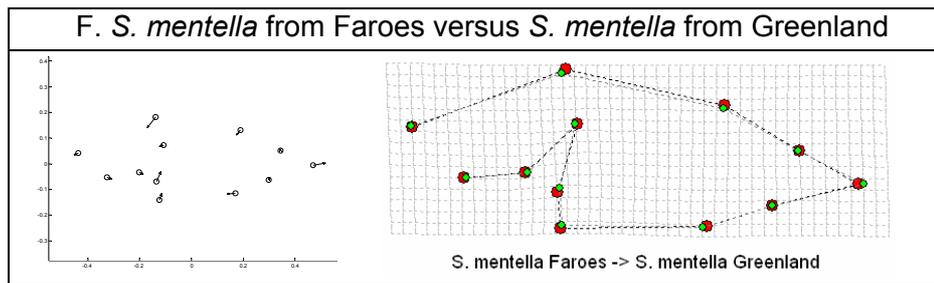
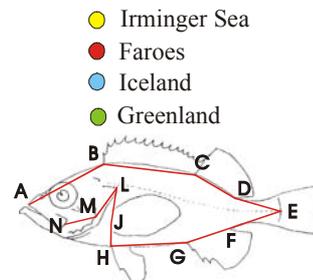


Figure 4.54. (continuation)



Discrimination between subareas (*S. mentella*)

Ten different subareas were compared. Two from Greenland, two from the Faroes, three from Iceland and three from the Irminger Sea. They are exactly the same areas as in the study conducted using traditional morphometry (Table 4.23).

The classification matrix in numbers, the percentage correctly classified, Wilks' lambda and Cohen's Kappa are shown in Table 4.61. This table gives a more detailed view of the species structure in the area. The discrimination power was extremely low, 54.2%, and also Cohen's Kappa (0.42) but Wilks' λ is very low (0.19) suggesting some structure in the subareas.

Between the Faroes and Iceland, there was a high quantity of confusion, especially between NW Faroes and both sub-areas in the south of Iceland, especially with SE Iceland (33.8%) but also with Central Irminger Sea (17%).

Similarly, East and West Greenland showed a great degree of confusion with SE Iceland (34 and 29% respectively). In the confusion among Greenland and Irminger subareas it is interesting to note that East Greenland confused principally with Central Irminger Sea (10%), the closest area while West Greenland did with Irminger NAFO (12,8%) and Central Irminger (10.6%). The confusion of SW Iceland individuals was mainly with SE Iceland (27%) and the NE Irminger Sea (17.2%). Surprisingly, few misclassifications occurred with the Faroes and Greenland, although, as explained above, a high proportion of fish from the Faroes and Greenland were classified into Iceland. Most of the misclassification of SE Iceland was Iceland SW. Irminger Sea individuals classified only in other Irminger sub-areas, showing that there were no differences between subareas. Irminger Sea classified apart as shown in the scatterplot of the canonical scores (Figure 4.55).

The analysis performed with a balanced number of individuals by subarea, yields a more equilibrated percentage of misclassification between the subareas belonging to the same area (in boxes in the Figure 4.62), but the general picture remains as in the previous

analysis. The percents of individuals from other areas that classify in Irminger and Iceland subareas diminish, and the percent of individuals from Iceland that classifies in Greenland and Faroe Islands subareas increases. There are also an increment of individuals from Irminger subareas that misclassified in Greenland and Faroes subareas. The subareas that presented a larger percent correctly classified are NE Iceland and SE Faroes, which is in accordance with the cluster analysis (Figure 4.56). Greenland subareas cluster in one group, while South of Iceland and NW Faroes cluster together. The three Irminger Sea subareas form a cluster apart from Greenland, Faroes and Iceland, but clustered with Iceland and Greenland before NE Iceland and SE Faroes.

In summary we have not observed a clear stock structure for *S. mentella*. Samples off the coasts of Greenland, the Faroes Islands and Iceland do not indicate differences between areas. There was no difference between subareas within the Irminger Sea.

Table 4.61. Classification matrix, percentage correctly classified , Wilks' Lambda and Cohen's Kappa resulting from the discriminant analysis of *S. mentella* by subarea.

Wilks' Lambda = 0.19205 Cohen's Kappa = 0.4266											
	Percent	Irminger			Iceland			Greenland		Faroes	
		NE	CEN	NAFO	NE	SE	SW	E	W	NW	SE
Irminger-NE	68.3	715	230	38	2	15	37	3	0	7	0
Irminger-CEN	55.2	275	485	65	0	8	35	2	5	4	0
Irminger-NAFO	29.2	85	138	97	0	2	8	1	0	1	0
Iceland-NE	52.9	0	0	0	27	19	3	2	0	0	0
Iceland-SE	63.5	13	13	0	5	301	102	15	1	16	8
Iceland-SW	45.3	88	25	0	2	138	232	7	0	18	2
Greenland-E	29.3	6	10	2	2	34	9	29	5	2	0
Greenland-W	29.8	1	5	6	0	14	2	5	14	0	0
Faroes-NW	25.2	0	24	0	3	47	24	0	0	35	6
Faroes-SE	63.8	0	2	0	0	12	3	0	0	4	37
Total	54.2	1183	932	208	41	590	455	64	25	87	53
Percentage of misclassification											
Irminger-NE			22.0	3.6	0.2	1.4	3.5	0.3	0	0.7	0
Irminger-CEN			31.3	7.4	0	0.9	4.0	0.2	0.6	0.5	0
Irminger-NAFO			25.6	41.6	0	0.6	2.4	0.3	0	0.3	0
Iceland-NE			0	0	0	37.3	5.9	3.9	0	0	0
Iceland-SE			2.7	2.7	0	1.1	21.5	3.2	0.2	3.4	1.7
Iceland-SW			17.2	4.9	0	0.4	27.0	1.4	0	3.5	0.4
Greenland-E			6.1	10.1	2.0	2.0	34.3	9.1	5.1	2	0
Greenland-W			2.1	10.6	12.8	0	29.8	4.3	10.6	0	0
Faroes-NW			0	17.3	0	2.2	33.8	17.3	0	0	4.3
Faroes-SE			0	3.5	0	0.0	20.7	5.2	0	0	6.90

RESULTS

Table 4.62. Classification matrix, percentage correctly classified , Wilks' Lambda and Cohen's Kappa resulting from the discriminant analysis of *S. mentella* by subarea. The same number of individuals has been taken in each of the subareas.

Wilks' Lambda = 0.06026 Cohen's Kappa = 0.535646											
	Percent	Irminger			Iceland			Greenland		Faroes	
		NE	CEN	NAFO	NE	SE	SW	E	W	NW	SE
Irminger-NE	57.4	27	9	6	0	2	1	0	0	2	0
Irminger-CEN	53.2	8	25	7	0	0	1	2	3	1	0
Irminger-NAFO	53.2	5	13	25	0	1	1	0	2	0	0
Iceland-NE	80.9	0	0	0	38	6	0	3	0	0	0
Iceland-SE	53.2	0	1	0	3	25	7	1	3	6	1
Iceland-SW	23.4	4	0	3	4	6	11	5	2	9	3
Greenland-E	54.3	1	0	0	2	1	4	25	12	0	1
Greenland-W	74.5	0	1	2	0	3	2	4	35	0	0
Faroes-NW	48.9	2	1	1	4	3	3	2	3	23	5
Faroes-SE	83.0	0	0	0	0	1	0	1	0	6	39
Total	58.2	47	50	44	51	48	30	43	60	47	49
Percentage of misclassification											
Irminger-NE			19.1	12.8	0.0	4.3	2.1	0.0	0.0	4.3	0.0
Irminger-CEN		17.0		14.9	0.0	0.0	2.1	4.3	6.4	2.1	0.0
Irminger-NAFO		10.6	27.7		0.0	2.1	2.1	0.0	4.3	0.0	0.0
Iceland-NE		0.0	0.0	0.0		12.8	0.0	6.4	0.0	0.0	0.0
Iceland-SE		0.0	2.1	0.0	6.4		14.9	2.1	6.4	12.8	2.1
Iceland-SW		8.5	0.0	6.4	8.5	12.8		10.6	4.3	19.1	6.4
Greenland-E		2.2	0.0	0.0	4.3	2.2	8.7		26.1	0.0	2.2
Greenland-W		0.0	2.1	4.3	0.0	6.4	4.3	8.5		0.0	0.0
Faroes-NW		4.3	2.1	2.1	8.5	6.4	6.4	4.3	6.4		10.6
Faroes-SE		0.0	0.0	0.0	0.0	2.1	0.0	2.1	0.0	12.8	

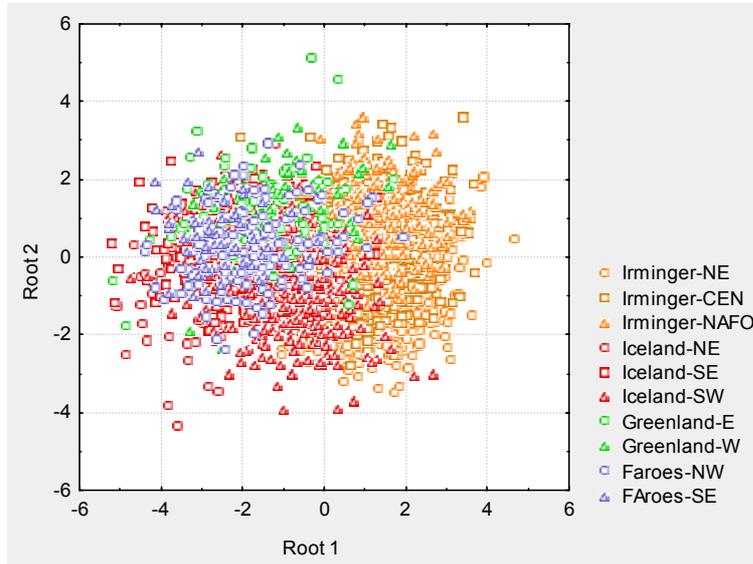


Figure 4.55. Plot of canonical scores for each case for the first and second canonical roots resulting from the discriminant analysis performed with *S. mentella* by sub-area.

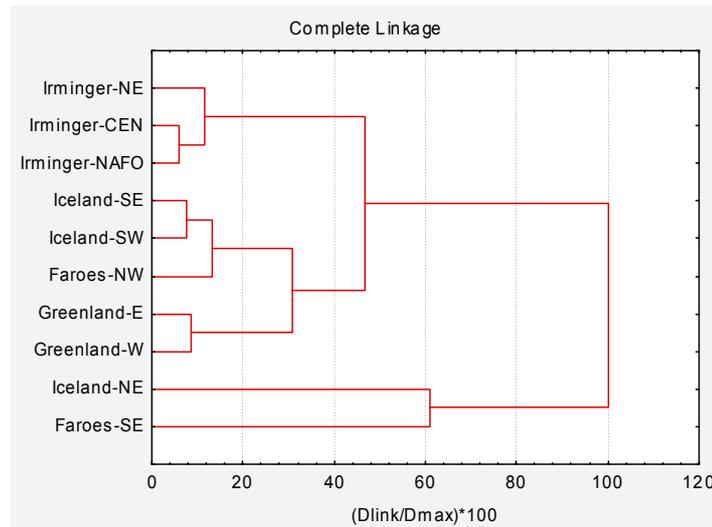


Figure 4.56. Dendrogram showing differences in shape among *S. mentella* from the different subareas.

4.2.3. Discrimination between species-area groups

The whole dataset has been pooled to perform a single analysis with a group definition based on species and area assignments. The total number of fish considered in this analysis was 5,584 distributed as shown in Table 4.63.

The classification matrix in numbers, the percentage correctly classified, Wilks' lambda and Cohen's Kappa are shown in Table 4.64. As expected, the figures showed a logical view of the relationships between species and areas. First, the total correct classification is low (79.7%) as a consequence of the high confusion among most of the groups. Cohen's Kappa is in the limit (0.7). However, Wilks' λ is very low, 0.08 reflecting structure in the data. This structure is clearly understood from the classification matrix, and can be observed in the canonical scores plot (Figure 4.57).

S. viviparus classified as a single group with a high percent correctly classified (97.4%). Few individuals from other groups were classified as *S. viviparus*. So, *S. viviparus* is a different group. *S. marinus* and *S. mentella* individuals confuse mainly with individuals of the same species but from other areas. Note, however, that although in low proportion, some *S. marinus* were classified as *S. mentella*, especially in Iceland; few *S. mentella* were classified as *S. marinus* either in Iceland or Greenland. In spite of the high classification rate of the Irminger Sea, 20 % of *S. mentella* from Iceland, another 20% of *S. mentella* from Greenland and 15 % of Faroes *S. mentella* classified in Irminger.

The analysis performed with the same number of individuals by group (100 in each except *S. viviparus* with 79) yielded, as expected, a reduction in the percent of misclassification in those areas that had a small number of individuals, i. e., *S. mentella* and *S. marinus* from Greenland and Faroes.

Figure 4.58 shows the cluster analysis. There were three main clusters, one for each species. Irminger sea clustered apart of the Central area within the *S. mentella* cluster.

Table 4.63. Number of fish in the species-area analysis.

Species	Area	Number
<i>S. marinus</i>	Faroes	207
	Greenland	268
	Iceland	1,392
<i>S. mentella</i>	Faroes	197
	Greenland	146
	Iceland	1,037
	Irminger	2,258
<i>S. viviparus</i>	Faroes	79
		Total 5, 584

Table 4.64. Classification matrix, percentage correctly classified , Wilks' Lambda and Cohen's Kappa resulting from the discriminant analysis of the species-area groups. 'Irm' : Irminger; 'Ice'=Iceland; 'Gre'= Greenland ; 'Far' = Faroes; 'men'=S. mentella; 'mar'= S. marinus and 'viv'= S. viviparus.

Wilks' Lambda: 0 .08300 Cohen's Kappa= 0.7175									
		<i>S. mentella</i>				<i>S. marinus</i>			<i>S. viviparus</i>
	Percent	Irm	Ice	Far	Gre	Ice	Far	Gre	Far
men-Irm	94.55	2135	97	6	13	6	1	0	0
men-Ice	67.40	185	699	31	21	83	2	15	1
men-Far	38.57	29	76	76	0	15	1	0	0
men-Gre	33.56	27	63	0	49	2	0	5	0
mar-Ice	89.36	5	65	1	1	1244	15	57	4
mar-Far	24.15	1	13	4	0	127	50	12	0
mar-Gre	45.14	0	16	0	3	125	2	121	1
viv-Far	97.46	0	0	1	0	0	1	0	77
Total	79.70	2382	1029	119	87	1602	72	210	83
Percentage of misclassification									
men-Irm			4.3	0.3	0.6	0.3	0.0	0.0	0.0
men-Ice			17.8		3.0	2.0	8.0	0.2	1.4
men-Far			14.7	38.6		0.0	7.6	0.5	0.0
men-Gre			18.5	43.2	0.0		1.4	0.0	3.4
mar-Ice			0.4	4.7	0.1	0.1		1.1	4.1
mar-Far			0.5	6.3	1.9	0.0	61.4		5.8
mar-Gre			0.0	6.0	0.0	1.1	46.6	0.7	
viv-Far			0.0	0.0	1.3	0.0	0.0	1.3	0.0

Table 4.65. Classification matrix, percentage correctly classified, Wilks' Lambda and Cohen's Kappa resulting from the discriminant analysis of the species-area groups. The same number of individuals has been taken for each of the groups. 'Irm': Irminger; 'Ice'=Iceland; 'Gre'= Greenland; 'Far' = Faroes; 'men'=S. mentella; 'mar'= S. marinus and 'viv'= S. viviparus.

Wilks' Lambda= 0.02344									
Cohen's Kappa= 0.708194									
	Percent	S. mentella				S. marinus			S. viviparus
		Irm	Ice	Gre	Far	Ice	Gre	Far	Far
men-Irm	91.1	92	2	5	2	0	0	0	0
men-Ice	48.0	9	48	12	13	8	7	3	0
men-Gre	81.0	5	10	81	1	1	2	0	0
men-Far	77.0	4	11	4	77	2	0	2	0
mar-Ice	64.0	0	8	1	0	64	13	14	0
mar-Gre	68.0	0	8	3	0	11	68	9	1
mar-Far	74.0	0	2	0	3	9	12	74	0
viv-Far	97.5	0	0	0	1	0	0	1	77
Total	74.5	110	89	106	97	95	102	103	78
Percentage of misclassification									
men-Irm			2.0	5.0	2.0	0.0	0.0	0.0	0.0
men-Ice			9.0		12.0	13.0	8.0	7.0	3.0
men-Gre			5.0	10.0		1.0	1.0	2.0	0.0
men-Far			4.0	11.0	4.0		2.0	0.0	2.0
mar-Ice			0.0	8.0	1.0	0.0		13.0	14.0
mar-Gre			0.0	8.0	3.0	0.0	11.0		9.0
mar-Far			0.0	2.0	0.0	3.0	9.0	12.0	
viv-Far			0.0	0.0	0.0	1.3	0.0	0.0	1.3

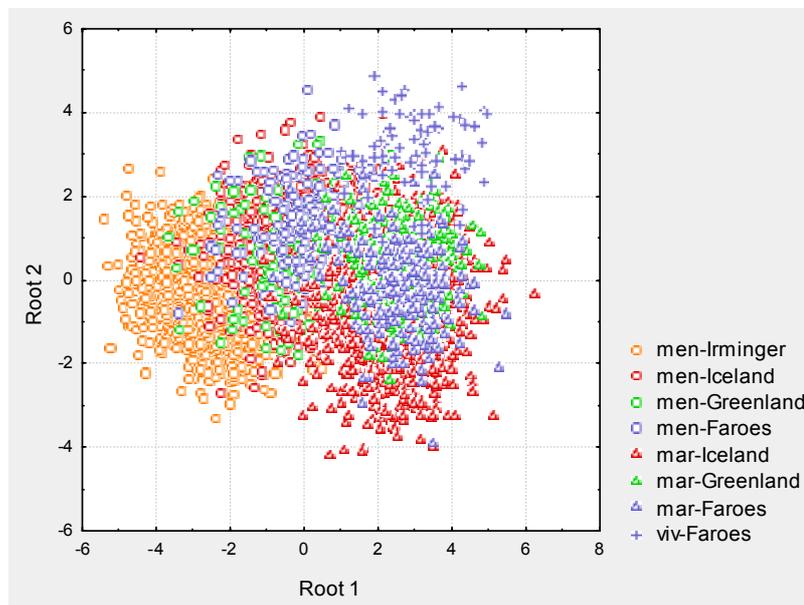


Figure 4.57. Plot of canonical scores for each case for the first and second canonical roots resulting of the discriminant analysis performed with the eight species-area groups.

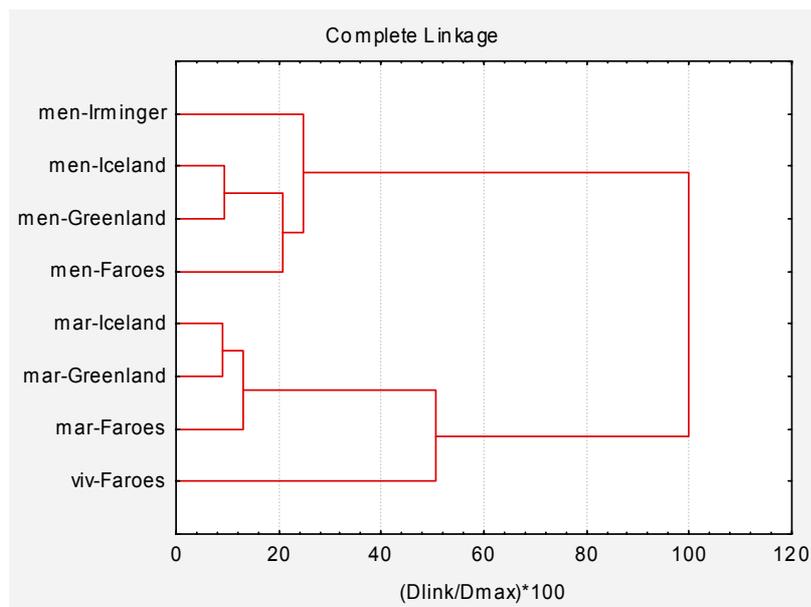


Figure 4.58. Tree diagram of the Cluster analysis based on Mahalanobis distances resulting from the discriminant analysis performed on the species-area groups.

4.2.4. Discrimination between *S. mentella* phenotypes in the Irminger Sea

One of the main goals of the REDFISH project concerns the number of stocks in the Irminger Sea. To investigate Irminger *S. mentella*, four different analyses were performed, as explained in the traditional morphometric section 4.1.4.

1. A first analysis included all *S. mentella* in the Irminger Sea.
2. A second one included only those fish that were divided into oceanic and deepsea types by Icelandic researches, as they have the experience to recognise these types.
3. In the third analysis all fish were introduced, but divided into oceanic and deepsea using meristic and parasitism data.
4. And finally, a fourth analysis was done with those fish with the GBM data available, divided into two groups, A and B using the new pattern of GBM as explained in section 4.1.4.

1. The first analysis was performed with 1,914 fishes, 957 deep-sea and 957 oceanic. The classification matrix in numbers, the percentage correctly classified and Wilks' lambda are shown in Table 4.66. The total correctly classified was very low (66.4%). The classification rate of both deep-sea and oceanic, was also very low, 63.5 and 69.3% respectively, and the classification was done randomly (K value much lower than 0.7). Wilks' λ was very high (0.85), indicating no discrimination between the types. The lack of differences can also be observed in the plot of canonical scores (Figure 4.59).

2. 582 fish phenotyped in Iceland were used in this analysis, 291 labeled as oceanic and 291 labeled as deepsea. The results were similar to those in the previous analysis. The classification matrix in numbers, the percentage correctly classified and Wilks' lambda are shown in Table 4.67. All the statistics showed the lack of discrimination, a high Wilks' λ (0.64),

a low correct classification (77.7%), a low Kappa value (0.55) and an important confusion rate, i.e. 24.1% of the deep-sea fish were classified as oceanic. The overlap between both types is considerable as shown also in Figure 4.60.

3. 932 *S. mentella* have been used for this analysis using the selected anatomical features, resulting, again, in a complete lack of discrimination. The classification matrix in numbers, the percentage correctly classified and Wilks' lambda are shown in Table 4.68. The total correctly classified is very low, 61.2%, and Wilks' λ present a high value, 0.91, and therefore no morphometric differences exist between the groups. The histogram of frequencies of the canonical scores are presented in Figure 4.61 showing the great overlap between the two types.

4. The last analysis was performed with 1,012 fishes. The GBM patterns were called A and B as explained in the same analysis performed with traditional methods. The classification matrix in numbers, the percentage correctly classified and Wilks' lambda (Table 4.69) showed no discrimination between groups. Wilks' λ got the highest value among the four analyses, 0.98, demonstrating the absolute lack of discrimination. Also 55.7% correctly classified confirm this point. In fact, the overlap of both groups are almost total, since half of the individuals of each group were classified as belonging to the other group, i.e. morphometrically they are almost identical. This overlap is also obvious in the histogram of frequencies of the canonical scores (Figure 4.62).

Comparisons of the consensus of the phenotypes yielded the same lack of differences between phenotypes in all analyses, as can be seen in the plots of the raw data and the consensus (Figure 4.63), and the plots of vector on landmarks and deformation grids (Figure 4.64) where the four analyses consensus comparisons are displayed.

Table 4.66. Classification matrix, percentage correctly classified, Wilks' Lambda and Cohen's Kappa resulting from the discriminant analysis of *S. mentella* in the Irminger Sea by phenotype.

Wilks' Lambda= 0.85862 Cohen's Kappa= 0.328109			
	Percent	oceanic	Deepsea
Oceanic	69.3	663	294
Deepsea	63.5	349	608
Total	66.4	1012	902
Percentage of misclassification			
Oceanic			30.7
Deepsea		36.5	

Table 4.67. Classification matrix, percentage correctly classified, Wilks' Lambda and Cohen's Kappa resulting from the discriminant analysis of *S. mentella* in the Irminger Sea by phenotype using only partner 3 data.

Wilks' Lambda= 0.64835 Cohen's Kappa= 0.553265			
	Percent	oceanic	Deepsea
Oceanic	79.4	231	60
Deepsea	75.9	70	221
Total	77.7	301	281
Percentage of misclassification			
Oceanic			20.6
Deepsea		24.1	

RESULTS

Table 4.68. Classification matrix, percentage correctly classified , Wilks' Lambda and Cohen's Kappa resulting from the discriminant analysis of *S. mentella* in the Irminger Sea by phenotype using selected anatomical characters.

Wilks' Lambda= 0.90672 Cohen's Kappa= 0.223176			
	Percent	oceanic	Deepsea
Oceanic	60.7	283	183
Deepsea	61.6	179	287
Total	61.2	462	470
Percentage of misclassification			
Oceanic			39.3
Deepsea		38.4	

Table 4.69 Classification matrix, percentage correctly classified , Wilks' Lambda and Cohen's Kappa resulting from the discriminant analysis of *S. mentella* in the Irminger Sea by phenotype using GBM.

Wilks' Lambda= 0.97813 Cohen's Kappa= 0.114625			
	Percent	Type A	Type B
Type A	55.1	279	227
Type B	56.3	221	285
Total	55.7	500	512
Percentage of misclassification			
Type A			44.9
Type B		43.7	

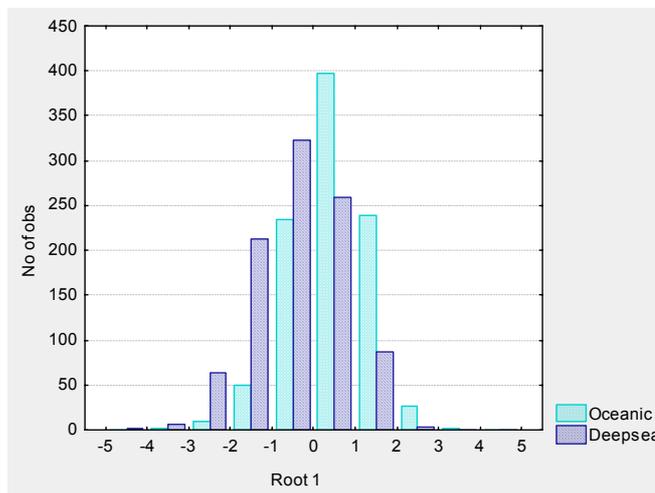


Figure 4.59. Plot of canonical scores for each case for the first and second canonical roots resulting from the discriminant analysis performed with *S. mentella* in the Irminger Sea by phenotype.

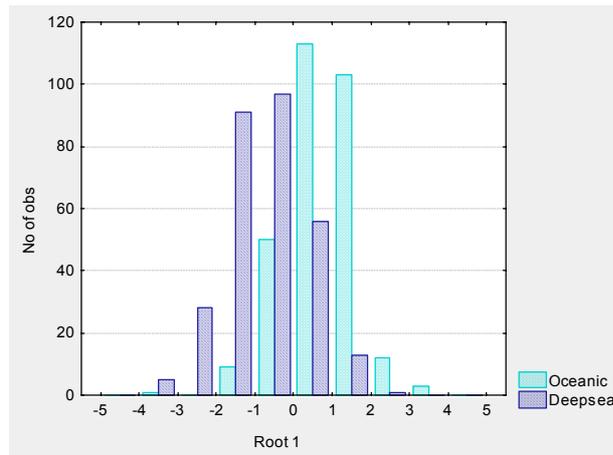


Figure 4.60. Plot of canonical scores for each case for the first and second canonical roots resulting from the discriminant analysis performed with *S. mentella* in the Irminger Sea by phenotype using only data from Iceland.

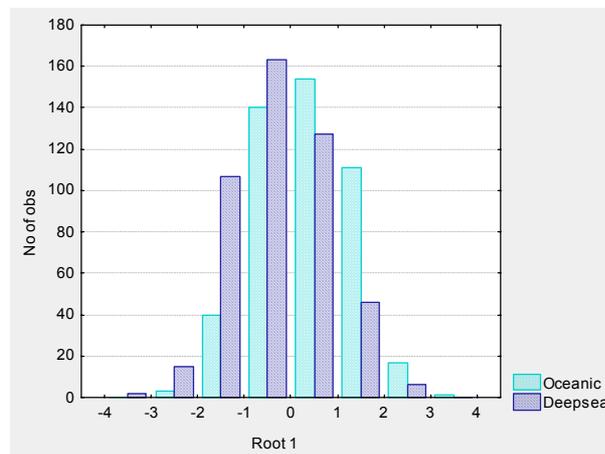


Figure 4.61. Histogram of frequencies of canonical scores for *S. mentella* in the Irminger Sea by phenotype using selected anatomical characters.

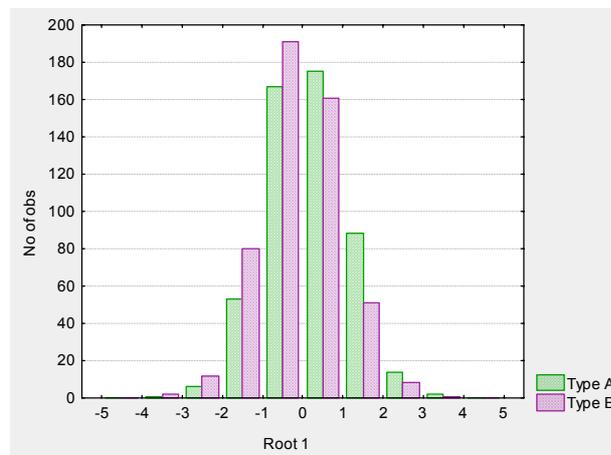


Figure 4.62. Histogram of frequencies of canonical scores for *S. mentella* in the Irminger Sea by phenotype using the gas bladder musculature.

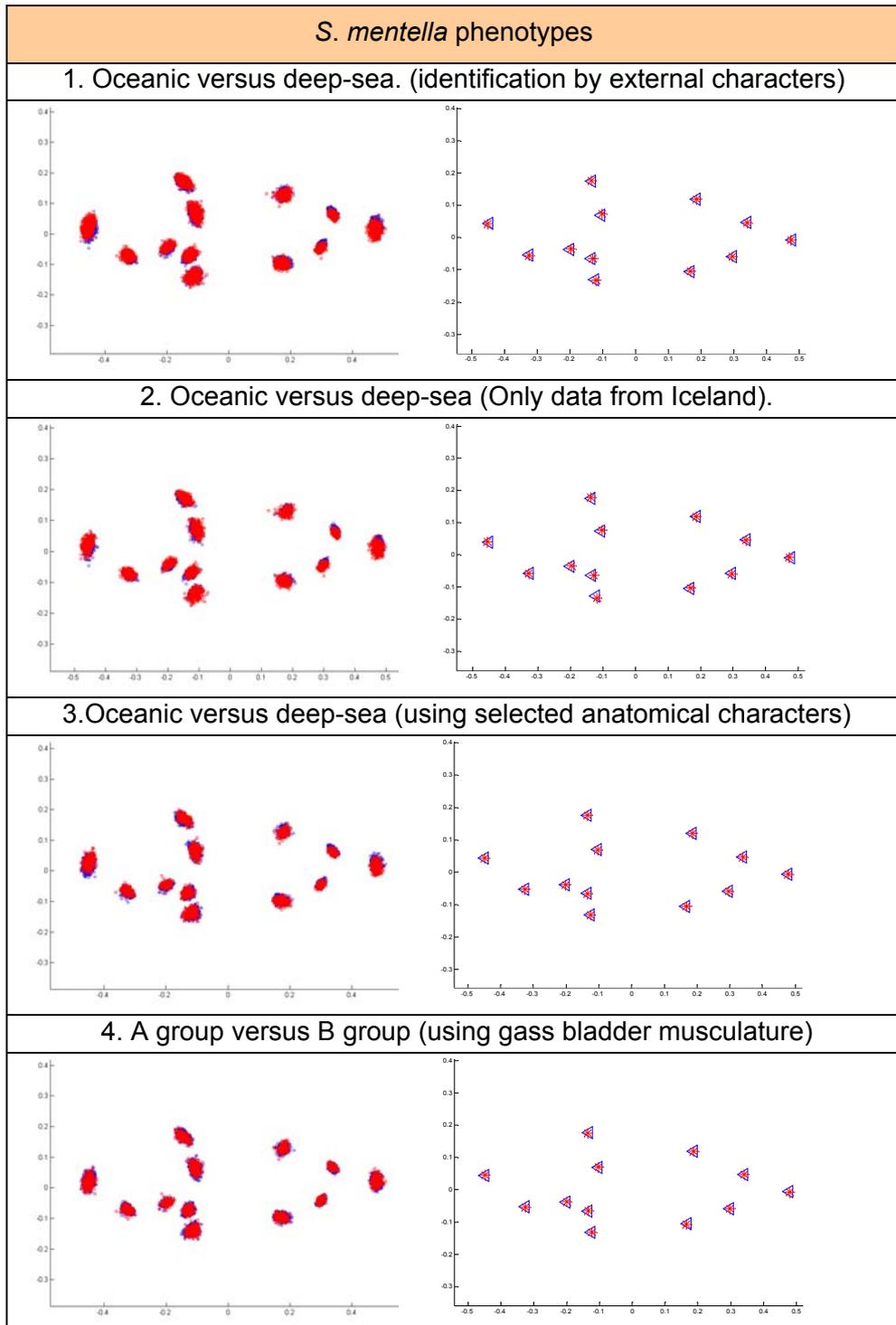
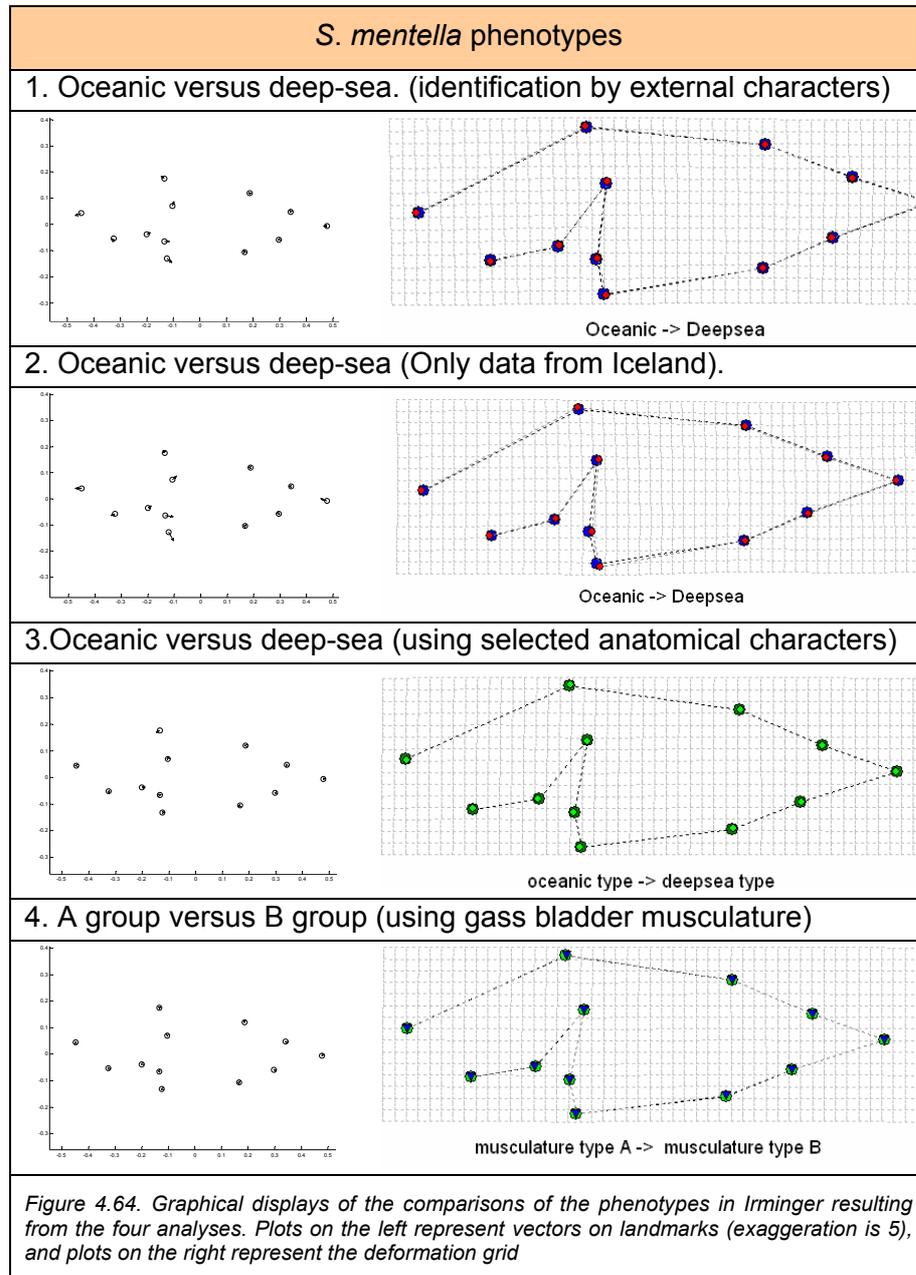


Figure 4.63. Graphical displays of the landmark configurations of *S. mentella* phenotypes resulting from the different analyses. Superimposed raw data are on the left, whilst the plots of the consensus (means of the landmark configurations) are on the right.



4.3. MERISTICS

Meristics are controlled by both genetic and environmental factors, in unknown proportions (Barlow, 1961). The number of parts formed in developing fish can be greatly influenced by the environment. This environmental dependence must be taken into account not only in the design of the analysis but also in interpretation of the results. This topic will be discussed later.

Another important feature of the meristic analysis is the fact that, because these variables are discrete, parametric analysis is not possible. Two classical nonparametric methods were therefore selected, the Kruskal-Wallis H-test and the multiple comparisons z' values. The use of nonparametric statistics prevents another interesting approach, multivariate analysis. All of this, together with the less developed formulation for nonparametric analysis produces a much weaker type of analysis than the morphometric, especially the traditional morphometry analysis.

Furthermore, the potential dependence of the meristic with the size of the fish is difficult, or impossible, to remove, as was done with the morphometric data. All these considerations are well developed in the methods section and should be kept in mind when interpreting the results.

Individuals included in the analysis

In the meristic analyses neither Flemish Cap nor Norway have been included. The relationships between populations are clearer from the morphometric analyses and it was preferred to concentrate on the main problem dealt with in this project, i.e. the differences between *S. marinus* and *S. mentella* in the core area: the Faroes, Iceland, Greenland and the Irminger Sea.

4.3.1. Discrimination between species

Because of the simplicity of the meristic analyses, compared with the morphometric analyses, the results are not presented in different sections, but all together, which also facilitates the comparisons.

The nonparametric analysis of each meristic variable to compare *S. marinus* and *S. mentella*, in the whole area and in each of the areas separately are presented in Table 4.70. The results showed that all variables had significant values when the whole area was considered, but also in Iceland. In the Faroes and Greenland, most of the variables also showed significant values, only RPF in the Faroes and RDF1 and RPF in Greenland showed non significant z and H values. Because the analysis involved two groups (species), z values and H tests are highly correlated. Figure 4.66 shows the histograms of the frequencies for each meristic variable. In spite of the statistical differences, it is difficult to observe a visible pattern between some of the variables, as the rays of the dorsal fins (RDF1 and RDF2); although they were the variables with lower H and z values, were still significant. Only the angle of the

5th preopercular spine (A5S) and, possibly, the number of rays in the anal fin (RAF) showed a different normal distribution.

Table 4.70. z values and Kruskal-Wallis H-test (in bold) for *S. marinus* versus *S. mentella* in the whole area and by area.* Significant at $p < 0.05$

		Meristic variables									
		PPA	RDF1	RDF2	RAF	RPF	A3S	A5S	GHO	GVO	GTO
Whole											
area		29.21*	2.54*	5.21*	30.17*	16.87*	20.74*	35.81*	10.89*	12.14*	14.18*
	H	968.82*	33.62*	33.41*	1068.37*	414.72*	726.82*	1628.46*	136.01*	192.70*	215.06*
Faroes											
		5.65*	1.11	2.84*	4.46*	1.87	6.92*	9.96*	7.02*	4.14*	6.88*
	H	35.99*	6.69*	10.37*	24.17*	9.34	79.55*	115.31*	53.77*	21.43*	49.59*
Iceland											
		18.11*	1.41	5.63*	12.13*	12.20*	17.94*	28.86*			
	H	366.30*	13.40*	41.39*	260.79*	177.10*	492.29*	1088.60*			
Greenland											
		9.12*	0.26	3.45*	8.22*	0.02	3.44*	8.89*	6.80*	6.15*	8.23*
	H	94.23*	0.64	14.24*	85.05*	0.00	20.31*	115.55*	51.81*	48.37*	71.81*

4.3.2. Discrimination between areas and subareas

Environmental conditions are probably different in each of the areas of study and, due to the different currents; even the subareas can show environmental differences. It is assumed that meristics are fixed at early stages of ontogeny, therefore it is the environment at the place of birth or where the early stages live which affects the meristic. Thus, it is pertinent to study the difference of each species by both areas and subareas, looking also for potential geographical differences within species, as was done with the morphometric data.

4.3.2.1. *S. marinus*

The first analyses involved only *S. marinus*. Two analyses were performed; one to study possible differences by area and the other differences by subarea. Both of them are presented in Table 4.71.

In the multigroup Kruskal-Wallis test by areas, all the variables, except RDF1, presented significant differences among groups (Table 4.71). Z values, which allowed pairwise comparisons, showed that larger differences occurred between Iceland and Greenland. Between Iceland and the Faroes, only two variables showed differences, the number of rays in the anal and pectoral fins, RAF and RPF respectively, but both presented relatively high z values. Between Greenland and the Faroes, up to 4 variables displayed differences; z was relatively low in all of them, but still significant.

By subareas, Kruskal-Wallis test showed the same pattern as the previous analysis, as expected: All, except the number of rays in the first dorsal fin (RDF1) showed significant values, the highest corresponding to RAF and RPF (Table 4.71). The interpretation of the results among subareas is a bit more complicated, due to the high number of comparisons involved. In general, it can be said that:

- PPA showed low differences and RDF1 showed no variability at all among subareas.
- The number of rays in the anal fin (RAF) showed no differences among the Icelandic subareas. The highest differences occurred among East Iceland subareas and Greenland.
- The number of rays in the pectoral fin (RPF), showed no differences within and between Greenland and Faroes subareas. All the differences occurred between some of the Icelandic subareas and some other.
- The angle of the third preopercular spine (A3S) showed the highest differences basically between Iceland and Greenland.
- The angle of the fifth preopercular spine (A5S) showed differences between Greenland and the Faroes and NE Iceland.

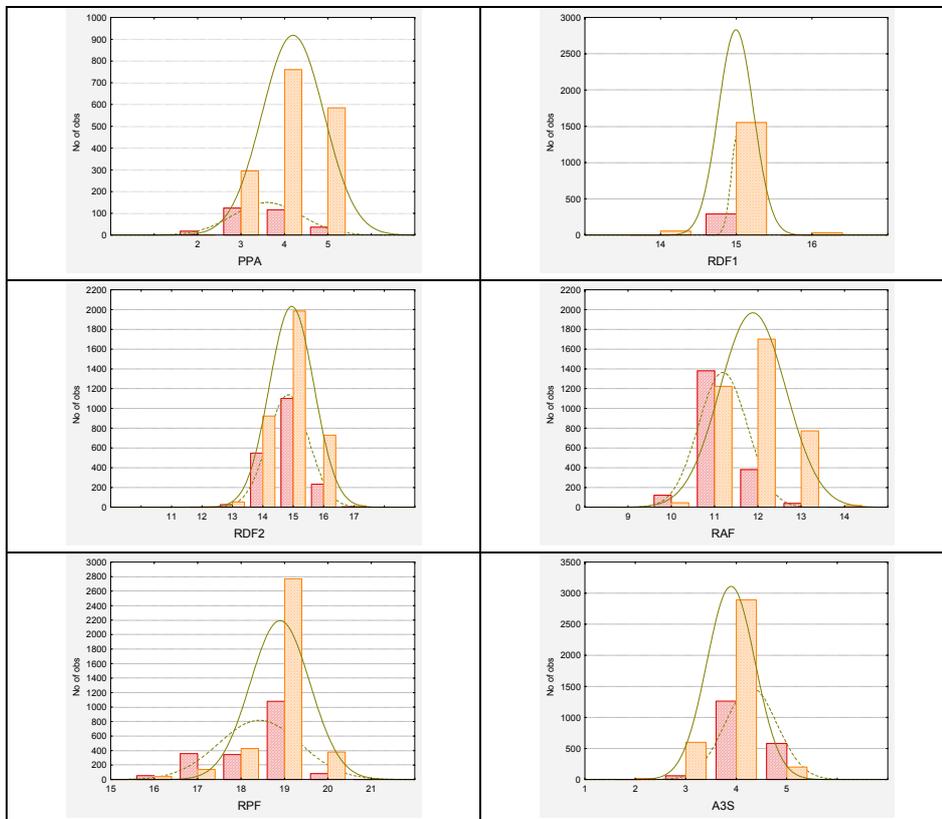


Figure 4.65. Histograms of the frequencies of the meristic variables for *S. mentella* (in orange) and *S. marinus* (in red) in the whole area. Curves correspond with the estimated normal distribution

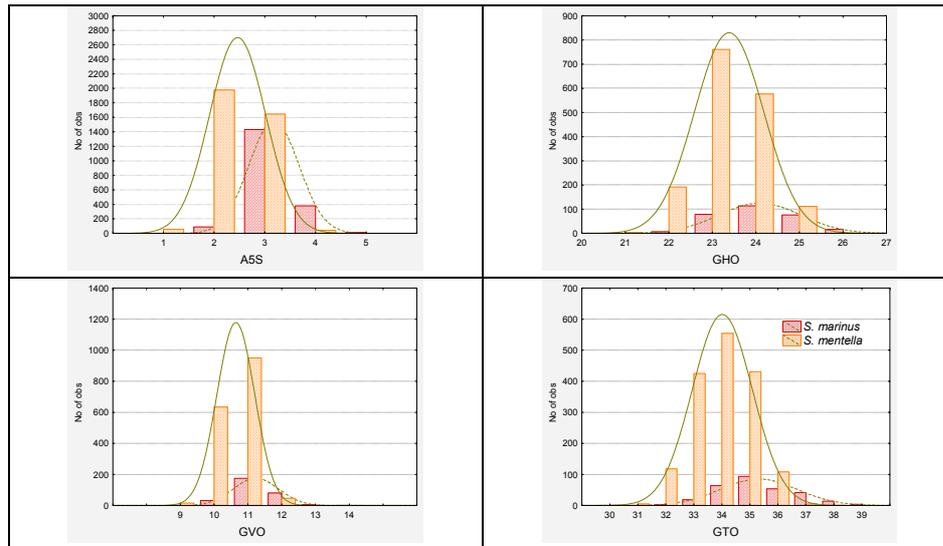


Figure 4.65. (continuation)

4.3.2.2. *S. mentella*

Both analyses, by area and by subarea, are presented in Table 4.72. In the analysis by areas, the multigroup Kruskal-Wallis test showed that for all the variables, except RDF1, there were significant differences among groups, which is very much the same as with *S. marinus*. The highest values were obtained with RAF, as well, followed by RDF2. The Z values, used to compare area by area, showed that, excluding RDF1:

- Irminger Sea and Iceland showed the highest differences, in all the variables.
- Except between these two areas, the angle of the spines was not different.
- The second highest differences were obtained between Iceland and Greenland.
- And the lowest between the Irminger Sea and two areas: Greenland and the Faroes.

By subareas, Kruskal-Wallis test showed the same pattern as the previous analysis: All, except, the number of rays in the first dorsal fin (RDF1) showed significant values, the highest corresponding to RAF and RDF2 (Table 4.72). The interpretation of the results among subareas is very complicated, since it involved 45 pairwise comparisons. In general, it can be said that:

- The number of rays in the first dorsal (RDF1) fin showed no variability among subareas.
- The highest variability was observed in two variables: the number of rays in the anal and second dorsal fin (RAF and RDF2, respectively).
- Basically these two variables showed differences between the subareas from the Irminger Sea; between each of the subareas of the Irminger Sea and those from Iceland; between Iceland and Greenland; and Iceland and the Faroes.
- The rest of the significant differences did not follow a defined pattern.
- Few differences existed regarding the preopercular spines
- And, in comparison with *S. marinus*, the number of rays of pectoral fin (RPF) was not so relevant.

Table 4.71 . z values and Kruskal-Wallis H-test (in bold) for *S. marinus* studied by areas and subareas. * Significant at $p < 0.05$

Meristic variables for <i>S. marinus</i>							
	PPA	RDF1	RDF2	RAF	RPF	A3S	A5S
Areas							
Iceland-Greenland	2.85*	0.28	10.19*	17.38*	13.92*	7.58*	6.50*
Iceland-Faroes	0.01	0.09	2.20	10.57*	10.66*	1.13	1.57
Greenland-Faroes	1.99	0.27	5.30*	3.42*	0.92	4.39*	5.86*
H	9.28*	1.03	132.66*	587.75*	331.08*	84.45*	85.53*
Sub-areas							
Iceland-SW-Iceland-NW	6.17*	0.07	0.14	0.16	5.25*	1.23	1.99
Iceland-SW-Iceland-NE	1.91	0.21	3.20*	1.05	0.08	0.02	1.06
Iceland-SW-Iceland-SE	4.03*	0.32	1.91	1.83	16.76*	5.93*	4.01*
Iceland-NW-Iceland-NE	3.05	0.11	2.39	0.94	3.97*	0.93	0.63
Iceland-NW-Iceland-SE	3.23*	0.13	1.07	1.00	15.42*	4.87*	4.38
Iceland-NE-Iceland-SE	0.59	0.00	1.84	2.08	10.06*	3.54*	3.40*
Iceland-SW-Greenland-E	0.33	0.27	8.54*	14.06*	15.18*	7.64*	4.66*
Iceland-SW-Greenland-W	0.70	0.27	7.84*	11.31*	8.32*	4.48*	3.66*
Iceland-NW-Greenland-E	5.67*	0.25	5.66*	9.40*	14.91*	6.27*	1.43
Iceland-NW-Greenland-W	4.20*	0.20	6.79*	9.83*	10.31*	4.63*	2.07
Iceland-NE-Greenland-E	1.92	0.37	2.76	10.13*	9.87*	4.96*	2.08
Iceland-NE-Greenland-W	1.75	0.11	4.87*	10.39*	7.13*	3.84*	2.51
Iceland-SE-Greenland-E	3.46*	0.49	6.10*	11.07*	0.43	2.20	7.26*
Iceland-SE-Greenland-W	2.42	0.13	6.76*	10.16*	0.85	1.79	5.27*
Iceland-SW-Faroes-NW	0.07	0.07	0.85	6.42*	12.15*	2.30	1.38
Iceland-SW-Faroes-SE	3.49*	0.26	7.13*	10.28*	6.51*	0.70	1.78
Iceland-NW-Faroes-NW	4.79*	0.00	0.75	4.73*	13.29*	2.70	2.60
Iceland-NW-Faroes-SE	0.77	0.18	6.07*	8.78*	8.78*	1.34	2.74
Iceland-NE-Faroes-NW	1.49	0.11	3.17*	5.55*	8.85*	1.66	1.86
Iceland-NE-Faroes-SE	1.71	0.09	4.06*	9.37*	5.47*	0.58	2.17
Iceland-SE-Faroes-NW	2.55	0.14	2.02	4.75*	0.34	1.72	1.33
Iceland-SE-Faroes-SE	1.51	0.10	6.00*	9.07*	1.39	2.04	0.12
Greenland-E-Greenland-W	0.50	0.39	3.31*	3.95*	0.59	0.55	1.21
Greenland-E-Faroes-NW	0.29	0.26	6.68*	4.22*	0.01	3.34*	4.46*
Greenland-E-Faroes-SE	3.43*	0.38	2.35	2.50	1.59	3.23*	4.03*
Greenland-W-Faroes-NW	0.66	0.20	7.42*	6.44*	0.57	2.68	4.02*
Greenland-W-Faroes-SE	3.00	0.02	0.88	1.27	1.68	2.90	4.03*
Faroes-NW-Faroes-SE	3.00	0.18	6.73*	5.20*	1.48	0.75	0.74
H	70.52*	4.26	219.64*	664.97*	793.66*	148.79*	137.27*

Table 4.72 . z values and Kruskal-Wallis H-test (in bold) for *S. mentella* studied by areas and subareas. * Significant at $p < 0.05$.

Meristic variables for <i>S. mentella</i>							
	PPA	RDF1	RDF2	RAF	RPF	A3S	A5S
Areas							
Irminger-Iceland	5.19*	0.61	16.84*	25.61*	6.59*	3.44*	5.60*
Irminger-Greenland	1.15	0.48	6.76*	4.63*	1.37	2.34	1.97
Irminger-Faroes	5.73*	0.31	0.29	3.50*	2.43	1.99	0.96
Iceland-Greenland	3.42*	0.19	14.03*	15.89*	4.25*	0.70	0.62
Iceland-Faroes	2.98*	0.59	8.40*	8.97*	5.47*	0.24	1.78
Greenland-Faroes	4.91*	0.59	4.97*	6.00*	0.67	0.38	0.83
H	67.49*	3.01	456.92*	841.15*	100.69*	32.78*	42.88*
Subareas							
Irminger-NE-Irminger-CEN	1.63	0.32	5.56*	6.25*	2.28	2.98	3.76*
Irminger-NE-Irminger-NAFO	6.02*	1.08	9.98*	11.41*	0.15	2.84	1.11
Irminger-CEN-Irminger-NAFO	7.04*	0.82	5.73*	6.64*	1.48	4.93*	1.62
Irminger-NE-Iceland-SW	0.39	0.86	10.06*	14.17*	12.14*	5.08*	2.29
Irminger-NE-Iceland-NE	4.57*	0.90	1.57	6.78*	1.99	1.54	1.98
Irminger-NE-Iceland-SE	4.89*	0.38	6.30*	12.66*	0.80	0.87	3.44*
Irminger-CEN-Iceland-SW	0.95	0.57	14.33*	18.82*	9.87*	7.39*	5.30*
Irminger-CEN-Iceland-NE	4.03*	0.79	3.36*	8.73*	2.71	0.60	0.77
Irminger-CEN-Iceland-SE	3.44*	0.11	10.58*	17.26*	2.60	1.55	6.34*
Irminger-NAFO-Iceland-SW	5.65*	0.29	16.70*	21.07*	9.22*	1.41	2.76
Irminger-NAFO-Iceland-NE	6.87*	0.41	5.67*	11.19*	1.96	2.63	1.43
Irminger-NAFO-Iceland-SE	9.08*	0.65	13.68*	19.85*	0.76	3.17	3.67*
Irminger-NE-Greenland-E	1.05	0.96	7.68*	5.46*	1.26	1.80	1.22
Irminger-NE-Greenland-W	1.08	0.20	4.86*	4.88*	0.33	0.91	0.01
Irminger-CEN-Greenland-E	1.77	0.80	5.09*	2.59	2.27	3.13	2.91
Irminger-CEN-Greenland-W	1.61	0.31	3.01	2.80	0.42	1.89	1.25
Irminger-NAFO-Greenland-E	2.44	0.27	1.37	1.47	1.24	0.03	1.75
Irminger-NAFO-Greenland-W	1.54	0.65	0.40	0.22	0.24	0.35	0.48
Irminger-NE-Faroes-NW	5.13*	0.55	1.13	3.36*	0.65	1.01	1.30
Irminger-NE-Faroes-SE	1.80	0.71	3.59*	4.26*	2.46	1.44	1.96
Irminger-CEN-Faroes-NW	4.26*	0.71	1.68	6.43*	1.77	2.49	0.59
Irminger-CEN-Faroes-SE	1.24	0.60	1.69	2.14	3.21	2.44	3.21
Irminger-NAFO-Faroes-NW	8.32*	1.16	5.15*	10.04*	0.68	0.84	0.48
Irminger-NAFO-Faroes-SE	4.34*	0.21	0.96	0.93	2.40	0.13	2.35
Iceland-SW-Iceland-NE	4.32*	0.56	2.20	1.43	6.42*	3.34*	2.78
Iceland-SW-Iceland-SE	3.90*	0.40	3.03	1.00	10.93*	5.05*	1.06
Iceland-NE-Iceland-SE	2.60	0.73	0.86	1.86	1.63	1.18	3.23
Iceland-SW-Greenland-E	1.20	0.47	12.50*	12.44*	7.39*	0.90	0.01
Iceland-SW-Greenland-W	1.20	0.52	8.54*	10.12*	4.27*	1.05	0.86
Iceland-NE-Greenland-E	4.49*	0.19	5.91*	8.96*	0.93	2.36	2.40
Iceland-NE-Greenland-W	4.13*	0.80	4.64*	8.47*	1.69	1.79	1.46
Iceland-SE-Greenland-E	3.55*	0.71	10.58*	11.76*	0.77	2.16	0.65
Iceland-SE-Greenland-W	2.95	0.34	7.16*	9.63*	0.63	1.22	1.33
Iceland-SW-Faroes-NW	4.62*	1.01	6.74*	4.78*	7.37*	1.91	2.52
Iceland-SW-Faroes-SE	1.60	0.36	7.42*	9.64*	7.09*	0.58	1.01
Iceland-NE-Faroes-NW	1.14	1.10	2.00	4.08*	1.37	1.90	1.01
Iceland-NE-Faroes-SE	2.13	0.17	3.71*	8.05*	0.26	2.16	2.86
Iceland-SE-Faroes-NW	2.01	0.73	4.67*	4.08*	0.15	1.44	3.19
Iceland-SE-Faroes-SE	0.20	0.54	5.99*	9.14*	2.06	1.74	0.52
Greenland-E-Greenland-W	0.27	0.75	0.55	0.77	1.03	0.33	0.74
Greenland-E-Faroes-NW	4.46*	1.14	5.23*	6.65*	0.52	0.69	1.88
Greenland-E-Faroes-SE	2.14	0.00	1.76	0.17	1.27	0.09	0.87
Greenland-W-Faroes-NW	3.85*	0.14	3.59*	6.13*	0.65	0.22	0.75
Greenland-W-Faroes-SE	2.08	0.66	1.02	0.53	2.00	0.36	1.40
Faroes-NW-Faroes-SE	1.41	0.93	2.44	5.61*	1.74	0.66	2.44
H	164.40*	16.32	606.30*	1041.19*	345.41*	141.97*	83.71*

4.3.3. Discrimination between *S. mentella* phenotypes in Irminger

One of the most relevant problems, and also one of the main goals of this project, concerns the number of stocks in the Irminger Sea. Two types of *S. mentella* have been defined in the Irminger Sea, the so called oceanic and deep-sea *S. mentella*. Some researchers are of the opinion that these are separate stocks, while others believe they form part of the same population.

Both types live in the open Irminger Sea, but on many occasions these pelagic fish are close to the Icelandic and Greenlandic shelves. Within this project, a total of 2,069 fish were identified as pelagic, i.e. the fish usually inhabiting the Irminger Sea and taken by pelagic hauls. Although all of these fish were assigned as Irminger Sea in the previous analyses, some were taken on the Icelandic shelf, in the proximity of the typical demersal *S. mentella*. Four different nonparametric analyses were carried out taking into consideration the same particulars described in previous sections to study the morphometric differences between phenotypes:

1. A first analysis with all *S. mentella* in the Irminger Sea.
2. A second one using only those fish that were phenotyped into oceanic and deepsea types by Icelandic researchers, as they have the experience identifying these types.
3. In the third analysis all fish were introduced, but divided into oceanic and deepsea using meristic and parasitism data.
4. And finally, a fourth analysis was done with those fish with the GBM data available, divided into two groups, A and B using the new pattern of GBM.
- 5.

The four nonparametric analyses of each meristic variable to compare deep-sea and oceanic *S. mentella* are presented in

Table 4.73. For the first, third and fourth analyses, the results revealed that the same three variables showed significant differences between phenotypes: the number of rays in the anal and pectoral fins (RAF and RPF), and the 5th preopercular spine (A5S). However, when using the Icelandic phenotypes (second analysis), anal counts (RAF) was not significant, but instead, PPA, RDF2 and A3S were significant in addition to RPF and A3S. However, in comparison with the analyses between areas of *S. mentella*, the values of Kruskal-Wallis and z statistics were low.

The high values obtained by A5S should not be considered, since this variable is part of the key to identify the two types, and it is thus logical to find such differences, especially in the third analysis where this character was used and hence the very high value. Note, however, that in the fourth analysis (using GBM) the value was very low, although significant.

Figure 4.66 show the histograms of the frequencies for each meristic variable. In spite of the statistical differences, it is difficult to observe a visible pattern between types.

Table 4.73. z values and Kruskal-Wallis H-test (in bold) for comparing deep-sea and oceanic *S. mentella* in the Irminger Sea.* Significant at $p < 0.05$

Meristic variables										
	PPA	RDF1	RDF2	RAF	RPF	A3S	A5S	GHO	GVO	GTO
Phenotype	0.87	0.96	1.50	4.41*	2.38*	1.75	7.91*	0.15	1.33	0.80
H	0.90	3.77	2.72	23.05*	10.36*	5.85	81.24*	0.02	2.39	0.68
Icelandic data	2.82*	0.67	2.30*	1.04	3.89*	2.80*	5.20*			
H	9.83*	1.17	6.60*	1.36	23.04*	17.75*	34.98*			
Anatomical	0.65	0.09	2.03	0.27*	1.51*	1.58	16.65*	0.11	0.29	0.01
H	0.50	0.05	5.31	0.09*	4.90*	4.36	360.9*	0.01	0.11	0.99
GBM	0.44	0.09	0.76	2.02*	2.26*	0.50	0.19*	0.82	0.13	0.56
H	0.23	0.05	0.74	5.42*	11.04*	0.43	2.19*	0.79	0.02	0.56

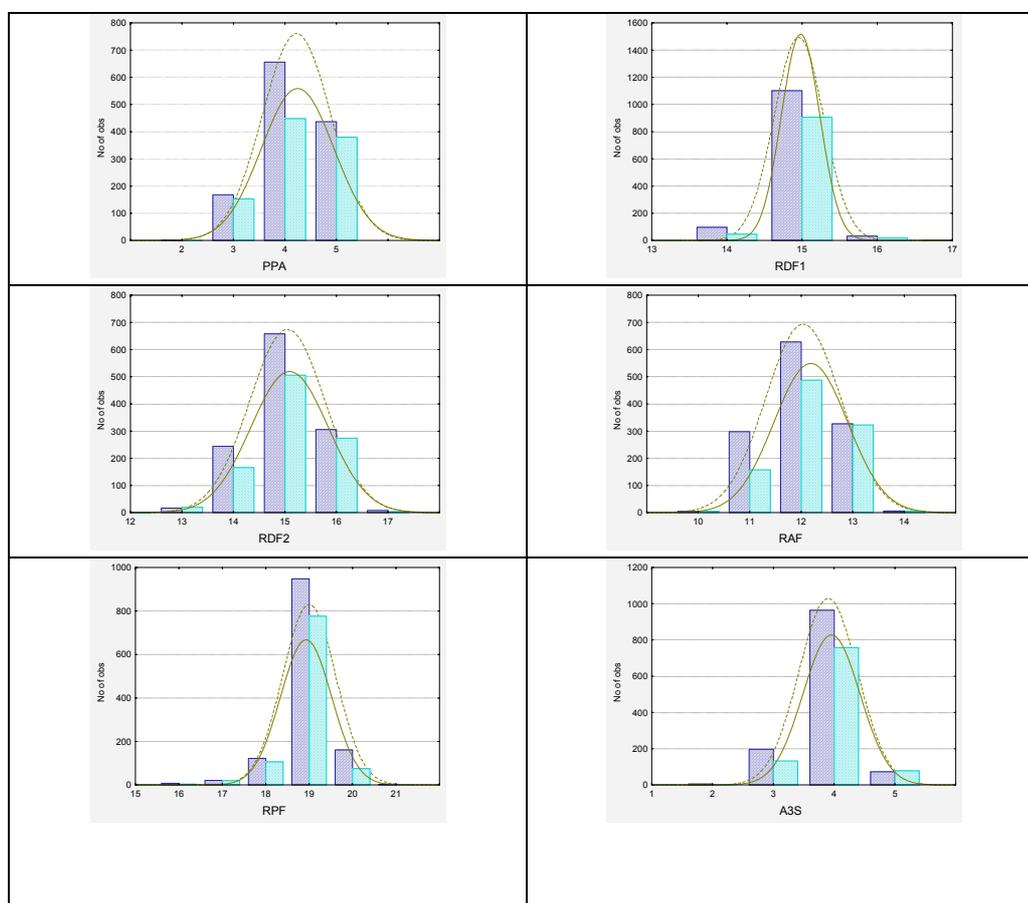


Figure 4.66. Histograms of the frequencies of the meristic variables for *S. mentella* in the Irminger Sea by phenotype. In dark blue deep-sea; in light blue oceanic. Data correspond to the first analysis done with the whole dataset and original phenotype assignment

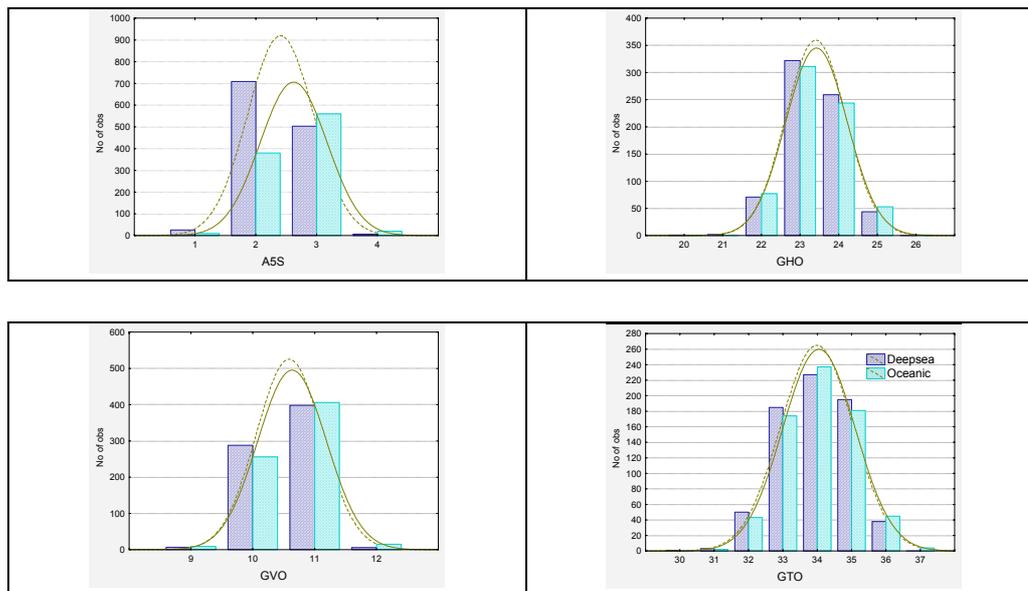


Figure 4.66. (continuation).

4.3.4. Meristics and size

A test was performed to determine if the observed differences were due to differences in fish body size of the samples among the areas. Thus, it was necessary to verify the independence of meristic variables in relation to size. Spearman Correlation was used to analyze size dependence for each of the meristic variables within each species and by area and sub-area.

Table 4.74 shows the Spearman correlation coefficients. The variables that most often showed significant correlation were, precisely, those that showed significant differences in the Kruskal-Wallis and z statistic analyses. The amount of significant correlations was high, although the correlations were in general low, but significant.

More in detail, PPA is correlated with size in all the areas, except for *S. mentella* in Greenland. In some degree, this was expected, since PPA is a dummy variable defined by the relative distance between the pectoral tip and the anus, which initially can be affected by size.

In contrast, RDF1 was independent from the size except for *S. marinus* in Greenland. RDF1 is perhaps the only variable that is not correlated with size, but this is not surprising as this variable represents the count of the dorsal fin spines, and spines are less labile than other meristic characters such as fin-rays. RDF1 did not show differences in the previous analyses, except between species. In contrast with the spines, the rays of the fins i.e. RDF2 and RAF, and to a lesser extent RPF, showed the highest correlations with size.

A3S and A5S are not exactly meristic variables, as they represent respectively the relative position of the 3rd and 5th opercula spines (see material and methods). Both variables showed a significant correlation with size for Faroes *S. marinus*, Irminger *S. mentella* and Greenland *S. mentella* in the case of A3S. Gill rakers (GHO and GVO) are not correlated with the size, except for *S. marinus* and *S. mentella* from the Faroes. All areas showed significant correlations with size in one or another variable.

It was hypothesized that these significant correlations were not related to size variation as the expression of ontogeny, but with the presence in the dataset of different year classes. Each cohort probably underwent very different environmental conditions, especially those with many years between them. This issue will be discussed later.

The presence of significant correlation of the meristics with size is a problem with which it is difficult to deal. Because the nonparametric statistics are less developed in their mathematical formulation compared with the parametric, the approach performed with the morphometric data was not possible. The only manner that was found as a possibility to overcome this problem was to select a short range of sizes and perform the meristic analyses again with the hope that the number of year classes involved in such a range was low enough. The selected range could not be too small because of the lack of enough specimens. The size interval chosen was 250-350 mm, since this interval has enough individuals in all the areas (Table 4.75). We must take special care with the low number of individuals for *S. marinus* and *S. mentella* from SE Faroes, and *S. mentella* from NE Iceland and West Greenland, when interpreting the results. The following step was to analyze if the correlation with size persisted in that interval of sizes. Results of the Pearson's correlation analysis are shown in Table 4.76. The significant correlations still persisted, but in less occasions and with lower values. PPA and especially RAF still showed important correlations. It is important to consider also that in the Irminger Sea, 5 out of 9 variables still showed significant correlations. Faroes *S. mentella* and Greenland *S. marinus* were also seriously affected by this phenomenon. It will make it difficult to interpret the results of any meristic analyses with the selected size range. However, the significant correlations were lower than previously (where the full size interval goes from 106 to 803 mm). It was decided to continue with the new meristic analyses performed with the selected size range.

Table 4.74. Spearman correlation coefficient for each of the meristic variables in relation to the standard length, by species and area. * Significant at $p < 0.05$

Spearman correlation coefficient									
	PPA	RDF1	RDF2	RAF	RPF	A3S	A5S	GHO	GVO
<i>S. marinus</i>									
Faroes	-0,569*	0,098	0,326*	0,167*	-0,069	-0,058	-0,036	-0,395*	-0,286*
Iceland	-0,146*	-0,012	0,139*	0,066*	-0,060*	0,116*	-0,085*		
Greenland	-0,295*	-0,111*	-0,021	-0,364*	0,076	0,202*	0,138*	-0,078	-0,071
<i>S. mentella</i>									
Faroes	-0,297*	0,009	0,2347*	0,268*	0,154*	-0,168*	-0,051	-0,253*	-0,245*
Iceland	-0,110*	0,056	-0,019	0,044	-0,015	-0,182*	-0,074*		
Greenland	-0,066	0,064	0,014	-0,155*	0,096	-0,094	-0,256*	0,055	-0,069
Irminger	-0,188*	0,021	-0,082*	-0,115*	0,081*	-0,024	-0,020	-0,028	0,001

RESULTS

Table 4.75. Number of individuals in the interval between 250 and 350 mm used for meristic analysis by area and subarea.

Area	Subarea	Species		Total
		<i>S. marinus</i>	<i>S. mentella</i>	
Faroes	Faroes-NW	71	62	133
	Faroes-SE	2	3	5
Greenland	Greenland-E	108	42	150
	Greenland-W	27	7	34
Iceland	Iceland-NE	61	4	65
	Iceland-NW	94		94
	Iceland-SE	222	310	532
	Iceland-SW	524	303	827
Irminger	Irminger-CEN		700	700
	Irminger-NAFO		259	259
	Irminger-NE		736	736
Total I		1109	2426	3535

Table 4.76. Spearman correlation for meristic variables with size for those individuals in the size interval of 250-350mm.* Significant at $p < 0.05$

	Spearman correlation coefficient								
	PPA	RDF1	RDF2	RAF	RPF	A3S	A5S	GHO	GVO
<i>S. marinus</i>									
Faroes	-0.3045*	0.0559	0.1701	0.2995*	-0.1726	0.1236	0.1295	-0.1496	-0.1240
Iceland	-0.0535	0.0545	0.0610	0.0407	-0.0134	0.0579	-0.0677*		
Greenland	-0.1992*	-0.1090	0.0924	-0.3241*	0.2016*	-0.0121	-0.0322	-0.1534	-0.2150*
<i>S. mentella</i>									
Faroes	0.0059	-0.2153	0.3404*	0.3722*	-0.0315	-0.0215	-0.0880	0.5218*	0.2211
Iceland	0.0167	-0.0072	0.0229	0.0142	0.0251	-0.1038*	-0.0184		
Greenland	0.0052	-0.0174	-0.0280	-0.2254	0.0115	0.1288	-0.2507	0.0568	-0.2201
Irminger	-0.2059*	0.0493*	0.0552*	0.0562*	0.0511*	-0.0169	-0.0191	0.0353	0.0545

Species discrimination with selected size range

The analyses carried out to compare species yielded important differences in all the meristic variables when the whole area was considered (Table 4.77). Especially high values were obtained in A5S, RAF, PPA and RPF. Significant differences occurred in both the Kruskal-Wallis and z values.

In each of the areas, and in general, the differences were clear. All variables showed variation between species in Iceland. In the Faroes and Greenland, three variables in each did not show variation: RDF1, RAF and RPF in the Faroes and RDF1, RDF2 and RPF in Greenland.

Although significant, the values of H and z were much lower than in the analysis of the full size range (Table 4.70).

Discrimination between area and subarea

S. marinus showed significant H values in all, except RDF1, variables (Table 4.78). By far, the highest values were obtained in those variables that consist of a count of fin soft rays (RDF2, RAF and RPF), which are more labile (environmentally affected) than spines. There

were trends to a lower variance between Faroes and Iceland, and Faroes and Greenland. On the contrary, the larger variance is between Iceland and Greenland.

The differences between subareas reflected very much this general trend. In general, it can be said that:

- a) Most of the differences are concentrated in RDF2, RAF and RPF
- b) Most of the differences occurred basically when some of the subareas from Greenland were involved
- c) No differences between Greenland subareas were detected.
- d) Small differences existed between the subareas of Iceland except in the comparisons of RPF of SE Iceland with the rest of the subareas.
- e) Few significant differences were observed between the subareas of the Faroes and Iceland, and those significant occurred in variables that still showed correlation with size.
- f) No differences between subareas in the Faroes were observed.

Similarly, in *S. mentella*, significant differences among areas were observed (Table 4.79) in the soft rays (RDF2, RAF, and RPF) and in the preopercular spines (A3S, A5S). No significant differences occurred in PPA and RDF1. Highest z values appeared between Irminger and Iceland (RDF2 and RAF) and at lower level between Iceland and Greenland.

The differences between subareas logically reflected very much this trend. In general, it can be said that:

- a) Most of the differences were concentrated in the variables of soft rays, RDF1, RAF and RPF.
- b) At the same time, most of the significant differences occurred between the subareas of the Irminger Sea and the subareas of Iceland, but also between the three subareas of the Irminger Sea itself.
- c) No significant differences were detected between the subareas of the Faroes, nor between East and west Greenland.
- d) Few significant differences were observed in the rest of the comparisons, and there were no clear patterns in these differences.

In the Irminger Sea most of the variables were still correlated with size, and this fact maybe affects the observed results. Especially, because differences were found among the subareas of the Irminger Sea, which is unexpected considering the lack of differences in the morphometric analyses. This perhaps reflects a structure of the size. Figure 4.67 shows the box and whisker plot for the size distribution in each of the Subareas within the Irminger Sea. Significant differences in size were observed, as also reflected from the ANOVA performed ($F=14.95$, $p<0.001$).

In spite of the relatively short range of sizes selected, the ANOVA performed to compare the size of *S. mentella* among areas was significant ($F=13.42$, $p<0.001$) as shown in Figure 4.68. The differences in size distribution between areas and even subareas prevented a correct comparison of the potential differences in the meristic variables. Size effects should be removed from the variables before any kind of conclusion can be made.

RESULTS

Table 4.77. z values and Kruskal-Wallis H-test (in bold) for *S. marinus* versus *S. mentella* in the whole area and by area with individuals between 250 and 350 mm. * Significant at $p < 0.05$

Meristic variables										
	PPA	RDF1	RDF2	RAF	RPF	A3S	A5S	GHO	GVO	GTO
whole area	23.04*	2.41*	5.82*	25.77*	16.76*	16.64*	27.17*	6.83*	7.74*	8.68*
H	607.40*	30.38*	41.98*	778.31*	404.95*	471.41*	953.56*	53.71*	79.96*	81.12*
Faroes	2.99*	0.64	3.14*	1.05	0.29	3.53*	5.47*	3.97*	2.43*	3.69*
H	10.82	2.05	13.49*	1.41	0.29	24.65*	35.65*	17.06*	6.76*	13.99*
Iceland	15.38*	1.84	3.18*	9.85*	12.18*	13.90*	23.13*			
H	263.59*	22.74*	13.29*	177.57*	174.83*	291.99*	713.42*			
Greenland	4.95*	0.14	1.34	4.22*	0.95	3.85*	6.16*	4.08*	4.18*	5.29*
H	28.30*	0.29	2.14	24.17*	2.47	24.85*	60.18*	18.44*	23.58*	29.91*

Table 4.78. z values and Kruskal-Wallis H-test (in bold) for *S. marinus* studied by areas and subareas with individuals between 250 and 350 mm. * Significant at $p < 0.05$

Meristic variables								
Areas	PPA	RDF1	RDF2	RAF	RPF	A3S	A5S	
Iceland-Greenland	0.76	0.41	8.24*	12.73*	10.78*	4.78*	4.17*	
Iceland-Faroes	3.86*	0.30	1.66	5.00*	7.77*	1.23	0.83	
Greenland-Faroes	2.80*	0.01	6.58*	3.78*	0.21	1.97	3.32*	
H	16.70*	2.69	94.10*	314.16*	189.04*	33.49*	37.45*	
Sub-areas								
Iceland-SW-Iceland-NW	5.48*	0.06	0.05	0.09	3.52*	0.29	1.86	
Iceland-SW-Iceland-NE	0.26	0.16	3.15*	0.12	1.79	0.21	1.46	
Iceland-SW-Iceland-SE	4.81*	0.31	1.28	1.27	13.74*	5.26*	2.65	
Iceland-SW-Greenland-E	0.61	0.35	6.57*	10.43*	11.26*	4.99*	3.19*	
Iceland-SW-Greenland-W	1.08	0.03	6.65*	8.17*	6.55*	3.12	2.49	
Iceland-SW-Faroes-NW	2.44	0.24	1.28	4.77*	9.29*	2.08	0.83	
Iceland-SW-Faroes-SE	0.53	0.01	0.50	2.51	0.79	0.20	0.78	
Iceland-NW-Iceland-NE	3.88*	0.17	2.62	0.04	0.93	0.37	0.06	
Iceland-NW-Iceland-SE	1.86	0.25	0.88	0.75	12.17*	3.70*	3.42*	
Iceland-NW-Greenland-E	3.98*	0.21	4.90*	7.66*	11.18*	3.94*	0.87	
Iceland-NW-Greenland-W	1.86	0.00	6.03*	7.34*	7.73*	2.97	1.30	
Iceland-NW-Faroes-NW	5.88*	0.15	0.99	3.77*	9.98*	1.88	2.00	
Iceland-NW-Faroes-SE	1.40	0.00	0.50	2.47	1.33	0.16	1.06	
Iceland-NE-Iceland-SE	2.85	0.02	2.23	0.59	9.31*	2.68	2.83	
Iceland-NE-Greenland-E	0.61	0.36	1.59	6.69*	8.86*	3.03	0.82	
Iceland-NE-Greenland-W	1.07	0.12	3.83*	6.90*	6.64*	2.52	1.27	
Iceland-NE-Faroes-NW	1.53	0.30	3.37*	3.36*	8.12*	1.32	1.74	
Iceland-NE-Faroes-SE	0.47	0.04	0.10	2.45	1.11	0.24	1.04	
Iceland-SE-Greenland-E	2.83	0.53	4.99*	8.44*	0.50	0.82	4.70*	
Iceland-SE-Greenland-W	0.86	0.15	5.93*	7.40*	0.92	0.94	3.46*	
Iceland-SE-Faroes-NW	5.10*	0.40	1.94	3.67*	0.51	1.17	0.79	
Iceland-SE-Faroes-SE	1.08	0.04	0.35	2.36	0.77	0.80	0.47	
Greenland-E-Greenland-W	0.71	0.14	2.96	2.50	0.61	0.45	0.76	
Greenland-E-Faroes-NW	2.46	0.04	5.56*	3.14*	0.08	1.68	2.88	
Greenland-E-Faroes-SE	0.62	0.04	0.45	0.98	0.85	0.93	1.23	
Greenland-W-Faroes-NW	2.32	0.11	6.52*	4.46*	0.52	1.55	2.64	
Greenland-W-Faroes-SE	0.81	0.00	1.31	0.22	1.00	1.04	1.42	
Faroes-NW-Faroes-SE	0.09	0.03	0.72	1.63	0.86	0.57	0.62	
H	68.52*	4.31	119.63*	333.04*	478.44*	77.42*	71.38*	

Table 4.79. *z* values and Kruskal-Wallis *H*-test (in bold) for *S. mentella* studied by areas and subareas with individuals between 250 and 350 mm. * Significant at $p < 0.05$.

Meristic variables							
Areas	PPA	RDF1	RDF2	RAF	RPF	A3S	A5S
Irminger-Iceland	2.08	0.06	12.71*	20.13*	3.33*	2.29	6.43*
Irminger-Greenland	0.34	0.52	3.67*	2.03	1.87	3.13*	2.65*
Irminger-Faroes	0.21	0.40	1.36	5.25*	0.48	0.62	0.51
Iceland-Greenland	0.29	0.53	7.40*	8.01*	2.83*	2.38	0.66
Iceland-Faroes	0.55	0.37	3.29*	2.20	1.67	0.23	1.85
Greenland-Faroes	0.13	0.67	3.76*	4.97*	1.16	2.06	1.76
H	5.26	1.91	225.81*	497.06*	28.24*	27.72*	59.93*
Subareas							
Irminger-NE-Irminger-CEN	3.15	0.22	2.77	3.10	1.48	2.93	3.76*
Irminger-NE-Irminger-NAFO	2.92	1.39	8.37*	9.28*	0.42	2.24	0.63
Irminger-CEN-Irminger-NAFO	5.19*	1.22	6.29*	6.97*	1.49	4.36*	2.12
Irminger-NE-Iceland-SW	0.14	0.49	7.75*	11.65*	8.11*	2.79	2.89
Irminger-NE-Iceland-NE	1.44	1.01	0.41	3.01	0.11	0.28	1.79
Irminger-NE-Iceland-SE	3.93*	0.07	5.37*	11.05*	2.54	0.71	3.61*
Irminger-CEN-Iceland-SW	2.55	0.31	9.82*	13.94*	6.92*	5.02*	5.75*
Irminger-CEN-Iceland-NE	1.15	0.99	0.71	3.33*	0.24	0.01	1.39
Irminger-CEN-Iceland-SE	1.46	0.24	7.48*	13.38*	3.66*	1.56	6.49*
Irminger-NAFO-Iceland-SW	2.37	0.79	13.42*	17.32*	6.90*	0.36	2.87
Irminger-NAFO-Iceland-NE	1.79	0.81	1.61	4.32*	0.05	0.56	1.69
Irminger-NAFO-Iceland-SE	5.67*	1.24	11.52*	16.87*	1.68	2.49	3.46*
Irminger-NE-Greenland-E	0.02	0.86	4.49*	2.91	2.00	2.77	1.61
Irminger-NE-Greenland-W	1.49	0.37	1.27	1.11	0.40	0.79	1.45
Irminger-CEN-Greenland-E	0.99	0.79	3.63*	1.95	2.46	3.67*	2.78
Irminger-CEN-Greenland-W	1.05	0.40	0.89	0.67	0.19	1.20	1.97
Irminger-NAFO-Greenland-E	1.17	0.26	0.91	0.98	1.75	1.74	1.80
Irminger-NAFO-Greenland-W	2.03	0.63	0.32	0.66	0.47	0.36	1.55
Irminger-NE-Faroes-NW	0.38	0.28	0.18	4.09*	0.06	0.17	0.60
Irminger-NE-Faroes-SE	0.58	0.18	0.18	0.87	0.94	0.65	1.89
Irminger-CEN-Faroes-NW	0.88	0.37	1.29	5.32*	0.65	1.34	0.91
Irminger-CEN-Faroes-SE	0.29	0.16	0.08	0.58	1.08	0.92	2.24
Irminger-NAFO-Faroes-NW	1.85	0.97	4.45*	8.57*	0.15	0.99	0.24
Irminger-NAFO-Faroes-SE	0.94	0.00	0.87	0.29	0.89	0.37	1.96
Iceland-SW-Iceland-NE	1.45	0.94	0.65	1.40	1.06	0.61	2.18
Iceland-SW-Iceland-SE	3.40*	0.47	2.05	0.64	8.98*	2.96	0.58
Iceland-NE-Iceland-SE	0.97	1.02	0.32	1.51	0.19	0.20	2.27
Iceland-SW-Greenland-E	0.04	0.65	7.37*	7.36*	5.09*	1.58	0.43
Iceland-SW-Greenland-W	1.50	0.45	2.66	3.19	1.06	0.28	0.92
Iceland-NE-Greenland-E	1.39	0.68	1.85	3.80*	0.47	1.06	2.22
Iceland-NE-Greenland-W	0.38	1.03	1.10	3.07	0.31	0.67	2.31
Iceland-SE-Greenland-E	1.53	0.86	6.43*	7.08*	0.97	2.96	0.16
Iceland-SE-Greenland-W	0.78	0.36	2.22	3.06	0.85	0.91	0.79
Iceland-SW-Faroes-NW	0.43	0.51	3.65*	1.86	4.04*	1.22	2.00
Iceland-SW-Faroes-SE	0.59	0.12	1.09	2.25	1.90	0.32	1.54
Iceland-NE-Faroes-NW	1.32	1.05	0.36	1.87	0.09	0.31	1.58
Iceland-NE-Faroes-SE	0.61	0.53	0.40	2.63	0.59	0.66	2.61
Iceland-SE-Faroes-NW	1.56	0.24	2.46	1.50	1.18	0.51	2.34
Iceland-SE-Faroes-SE	0.11	0.18	0.81	2.16	0.65	0.73	1.46
Greenland-E-Greenland-W	1.38	0.70	0.69	0.19	1.19	0.42	0.66
Greenland-E-Faroes-NW	0.25	0.88	3.77*	4.95*	1.59	2.15	1.69
Greenland-E-Faroes-SE	0.56	0.07	1.11	0.01	0.34	0.16	1.37
Greenland-W-Faroes-NW	1.29	0.26	1.27	2.41	0.40	0.70	1.58
Greenland-W-Faroes-SE	0.34	0.35	0.55	0.12	1.01	0.11	0.79
Faroes-NW-Faroes-SE	0.48	0.24	0.21	1.76	0.91	0.60	1.99
H	58.25*	17.81*	317.84*	603.33*	177.77*	87.61*	91.85*

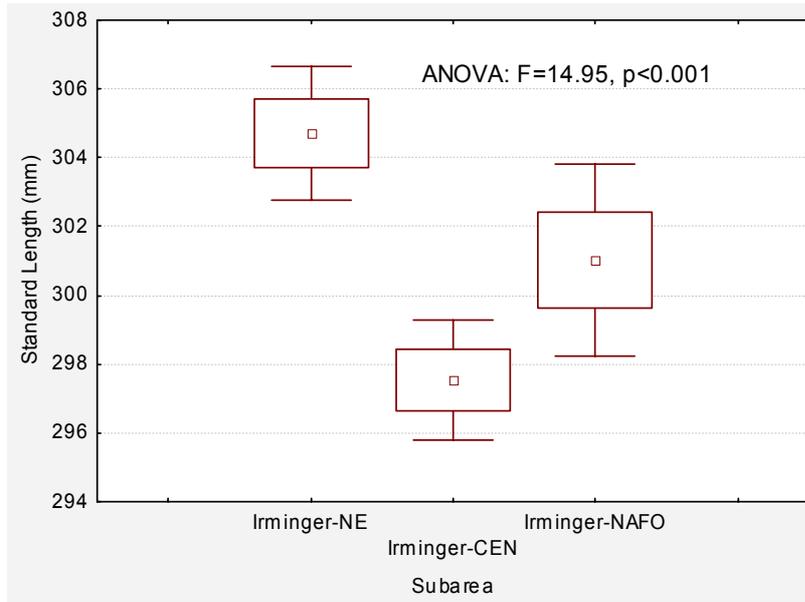


Figure 4.67. Box and whisker graph of the Standard length between 250 and 350 mm in the three subareas of the Irminger Sea. Square-Mean; Box- Standard error; Whisker-1.96 Standard error. Results of the ANOVA presented in the plot.

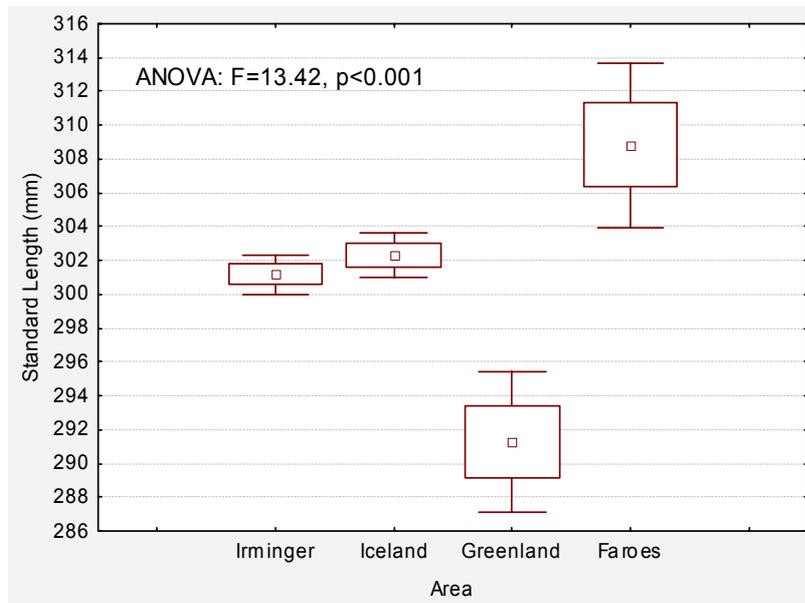


Figure 4.68. Box and whisker graph of the Standard length between 250 and 350 mm in the four areas of *S. mentella*. Square-Mean; Box- Standard error; Whisker-1.96 Standard error. Results of the ANOVA presented in the plot.

5. DISCUSSION

Fisheries management and stock identification

Serious declines in fish stocks during the last decades and the collapse of important fisheries, such as North Sea herring or the Northern Cod off Newfoundland, have driven different fisheries organizations to revise the principles that they apply to fisheries management. Several fisheries have collapsed before, but they were not under a precise control and their collapse had been attributed to the immaturity of fisheries science, or to the failure to implement the recommended management measures. But the collapse of the Northern cod and the North Sea Herring, that were considered to be well managed and under control, provided evidence that uncertainties in fishery science are greater than presupposed (Hilborn *et al.*, 2001). Changes in fisheries systems are difficult to control and not yet well understood, subject to changes in the environment and human values, and are only slowly reversible. So, in order to reduce the risk of irreversible damage, the adoption and implementation of a precautionary approach was requested in a number of international instruments of importance to fisheries, such as the FAO International Code of Conduct for Responsible Fisheries (FAO, 1995) and the United Nations Agreement on Straddling and Highly Migratory Fish Stocks (United Nations, 1995). Thus, modern fisheries management is moving towards the precautionary approach (FAO, 1996) in order to ensure a sustainable utilization of our marine resources. The precautionary approach has been widely adopted by a number of fishery institutions and between them, the International Council for the Exploration of the Sea (ICES) is progressing fast. A fundamental requirement of the precautionary approach is to consider the full impact of management actions, including identification of the stock complexity (Begg *et al.*, 1999).

Fisheries management is based on stock assessment, that describes the condition of the stock and also predicts how the stock is going to respond to a particular management system. The identification and delimitation of stock units is the prerequisite and basis for any management actions. These stock units should be based on the biological reality of the species. However, operational considerations such as historical consistency, political boundaries or some aspects of the scale of fisheries statistical data, have been taken into account in the establishment of management units, resulting in a mismatch between the management units and the biological reality of species. Common stock assessment techniques and management strategies assume discrete populations, while it has long been recognized that the boundaries of management areas are confounded by migration, mixing, political and administrative considerations, and do not always match biological population structure (Stephenson, 1999, 2002). In many cases management is not of single, simple populations, but rather of complex populations containing components which are susceptible to overfishing and erosion, even under management measures thought to be appropriate to the overall management unit (Stephenson, 1999). On several occasions, the biological structure of the stock is not well understood. There is uncertainty in several species regarding the degree of discreteness of spawning components, the value of specific genes and genetic variations, the number of sub-populations necessary for ensuring stock viability in all conditions, and how fishing affects genetic resources (Stephenson, 2002). Disregard of stock structure and ineffective fisheries management can result in dramatic changes in the biological attributes and productivity rates of a species, as well as the genetic diversity of a species (Begg *et al.*, 1999 and references therein). To obtain an optimal yield, each stock

must be managed separately, as failure to recognize the stock structure of an exploited species can lead to overfishing and depletion of less productive stocks (Begg *et al.*, 1999). Although stock structures and their complexity are components of 'biodiversity', and are fundamental aspects to take into account in fisheries management in accordance with the precautionary approach, nowadays few assessments implement stock identification requirements, resulting in the prevalence of a level of uncertainty in stock assessment models concerning the actual stock structure (National Research Council, 1998). Given this uncertainty, and under the 'precautionary approach', sub-units of a population should be treated as discrete and conserved (Stephenson, 1999).

So, it is obvious that the delimitation of the stock units and an understanding of their structure are essential for an appropriate management regulations design (Ricker, 1981).

Stock concept

The definition of the term "stock" remains debatable. There is no a single and simple definition of this concept, as it can be dealt from different perspectives. Initially, in fisheries science, the term stock was considered from an operational point of view, a 'stock unit' being any grouping of a fish species that was available for exploitation in a given area (Milton and Shaklee, 1987). But the term began to drift from the practical towards more theoretical definitions more related to the biology of the species, and recognizing subdivisions occurring below the species level. So, beyond its definitions related to fisheries, stocks may be considered to be units below the species level that are occur naturally, and are of interest to managers and scientists (Waldman, 2005b).

The stock definition even varies depending on the discipline or tools used for its definition. Thus, for example, 'phenotypic stocks' are those separated by their morphometric variation, and if the discrimination is a function of different genotypes, they would be called 'genetic stocks' (Booke, 1981). Among the several definitions that have been proposed for the stock concept, Ihssen *et al.* (1981), defined the fish stock as an intraspecific group of randomly mating individuals with temporal or spatial integrity. Saila and Jones (1983) proposed to define stock units as 'characteristic populations or sets of populations within subareas of the geographic range of a species'. Commonly, a stock is considered to correspond to a population, at least partly reproductively isolated from other populations, and genetically different from them as a result of adaptation to its local environment (Swain *et al.*, 2005). In this sense, the terms 'population' and 'stock' are often used rather interchangeably (Begg and Waldman, 1999), as we do in this study.

Life history parameters as such abundance, yield, age composition, growth, age at maturity, fecundity, recruitment and mortality, describe the dynamic properties of a population (Ihssen *et al.*, 1981). The differences in those parameters between populations have long been used as a basis for the identification of stocks (Begg *et al.*, 1999). However, the observed temporal variations in the life history parameters within stocks, in comparison with the spatial differences between stocks, raise questions about the long-term stability of these parameters, and their suitability as indicators of stock structure (Ihssen *et al.*, 1981, Begg *et al.*, 1999 and references therein). Many of these parameters, if not all, are very sensitive to environmental shifts, including exploitation patterns, which may change even in the short

term, and dramatically change life history parameters. Life history parameters should be the first data examined in any stock identification study, as these data are often routinely collected for assessment and management purposes, and used to describe stock boundaries that may assist in directing future studies to refine stock structures using more advanced approaches such as genetics, morphometrics or elemental analysis (Begg, 2005). Although life history parameters can be used for stock identification, they become less efficient with the increase of stock complexity, and other techniques have to be used for stock identification: mark-recapture, catch data, parasites, otolith microchemistry, meristics, morphometrics, scale and otolith analyses and genetics (protein variation, mitochondrial DNA and nuclear DNA).

A large proportion of fisheries occur on complex-stocks, including different populations or even different species. In order to identify the different components of these stocks, a holistic approach involving a broad spectrum of techniques, appears to be particularly pertinent. Begg and Waldman (1999) suggested the use of at least a genetic procedure and at least one phenotypic-based approach.

Furthermore, the use of several techniques may confirm a particular stock structure first detected by a single procedure used in isolation (Begg and Waldman, 1999). Although all the techniques used for stock identification contribute to the knowledge of the differences between groups (species, populations or subpopulations), the results obtained have to be integrated, and the conclusions must be coherent with the general population structure and ecology of the species, inferred from knowledge of spawning areas, larval drift, nursery areas and size/age composition of the population in the different areas.

The redfish problem

Genus *Sebastes* comprises the second major group of species of economic importance around the world. Most *Sebastes* species live in the Pacific, where *Sebastes* comprise the core of the US Pacific coast demersal fishery (Parker *et al.*, 2000). Only four species lives in the North Atlantic, *S. marinus*, *S. mentella*, *S. fasciatus* and *S. viviparus*, with *S. mentella* the one that represents the largest biomass, principally due to the high abundance of the pelagic component in the open Irminger Sea, that in 1995 was estimated to be 2,5 million tonnes, decreasing to approximately 1,1 million tonnes in 2003.

Despite their commercial importance, little biological research has been conducted on redfish in the North Atlantic. The very high external resemblances between species, together with a continuous distribution throughout the North Atlantic, and the absence of clear boundaries, have prevented a proper assessment and management of these resources (ICES, 2000). Thus, in the Northwest Atlantic, the three species living there are managed together as a result of the impossibility of splitting the catches into species. The defined management units are, in most of the cases, the consequence of statistical or political divisions, but seldom based on biological information. The special biological features of redfish contributes to this complex situation, among them, reproductive ecology, growth patterns, and habitat selection. Redfish is a viviparous species, with a relative long embryonic development of around one month (Magnússon, 1955; Saborido-Rey, 1994); therefore egg fertilization and parturition are not coupled. In addition, it seems that copulation occurs long before egg fertilization, even as

much as 6 months earlier (Magnússon, 1955), although this is still a poorly known aspect of its biology. This means that during egg fertilization and parturition time, males and females do not necessarily share the same habitat. Moreover, in some areas, a migration pattern has been identified related with spawning, where males and females are disaggregated (Sorokin, 1961; Saborido-Rey and Nedreaas, 2000). Spawning areas may overlap spatially among species, although often separately in time (Saborido-Rey, 1994; Saborido-Rey *et al.*, 2005). On the other hand, redfish are known to be a very long-lived species (Archibald *et al.*, 1981) with a very slow growth rate (Sandeman, 1961; Surkova, 1961); this has made the issue of accurate age determination particularly difficult to resolve, and also produces important differences in growth rate among cohorts when density dependent growth occur (Saborido-Rey *et al.*, 2004b). Both spawning pattern and growth rates, have produced difficulties in some of the stock identification analyses performed (Saborido-Rey and Nedreaas, 2000; Saborido-Rey *et al.*, 2005), and may explain some of the differences encountered among stocks, as discussed later.

Although redfish live in connection with the bottom, i.e. they may be considered demersal species, they also show very important pelagic behaviour, especially *S. mentella* and to a lesser extent, probably also *S. marinus*. Thus, traditionally, and in most of the areas, redfish is fished by bottom trawl, although a mixed pelagic-bottom trawl fishery has been common also in many areas, especially where fleets from certain countries operate, such as Russia, for example. However, more recently, and basically in the Irminger Sea, redfish is targetted exclusively by pelagic trawling. This is indeed a very interesting and important subject, because this behaviour may affect the interpretation of some of the conclusions about redfish stock structure, and therefore will be a recurrent topic in this section. It is remarkable that *S. mentella* is basically demersal in Greenland, the Faroe Islands and Iceland, but exclusively pelagic in the adjacent waters of the Irminger Sea, the only area in the North Atlantic where redfish show this behaviour, and which is probably due to the depths in this area, that extend beyond 1100-1200 m over the Reykjanes Ridge, 2400-3000m in the Irminger Sea Basin, and reach 4000 m in the Labrador Sea Basin.

Only preliminary information was available about the species and population structure for *Sebastes marinus* and *S. mentella* inhabiting the shelves and continental slopes off Greenland, Iceland, the Faroe Islands and the pelagic waters of the deep Irminger Sea. In this area, annual exploitation rates exceeded 200,000 tons. This fishery is relatively new and has developed very rapidly, basically since 1991, with up to 15 countries participating in some years, Spain amongst them, although Iceland, Germany and Russia have in recent years been the major participants (Sigurdsson *et al.*, 2003). The exploited area extends without interruption through the Exclusive Economic Zones (EEZ) of the Faroe Islands, Iceland, Greenland and in the international waters of the Irminger Sea. Like most straddling resources, *S. mentella* in the Irminger Sea is the subject of important controversy and debate regarding its stock structure. Preliminary studies suggests the existence of various distinct gene pools, but the ecology and reproductive behaviour of the species in the area indicates the opposite (Chapter 2). The preliminary information has created a serious controversy about the population structure among redfish scientists, discussed in many ICES Working groups, and the main reason for the development of the EU REDFISH project.

Up to three types of *S. mentella* have been described in the Irminger Sea and adjacent waters, 'deep sea' *S. mentella* living on the shelves, and both 'oceanic' and 'pelagic deep-sea' *S. mentella* in the open Irminger Sea. However, there is a lot of controversy as to whether these types are one, two or three different stocks (ICES, 2000). *S. mentella* in the Irminger Sea is a resource with great economic importance for several countries. Furthermore, *S. mentella*, like most redfish species, has slow growth rates, and although the stock is considered to be inside safe biological limits, catches have decreased in recent years, increasing concern about the status of the stock; this has not favoured healthy discussion among scientists.

The existence of a single stock of *S. mentella* in the Irminger Sea, around Iceland and off Greenland is supported by the ecology of *S. mentella* in the area (Saborido-Rey *et al.*, 2005); the existence of a single spawning area in the East Central Irminger Sea from where the extruded larvae drift towards the single nursery area located on the slopes of Greenland, and the migration of *S. mentella* juveniles from the nursery into the adult distribution areas (mainly the Irminger Sea and Iceland) provide no evidence for the existence of more than one stock in the area. Furthermore, the different *S. mentella* stocks were described as the fishery developed, and were not based on any relevant biological feature.

However, some researches differentiate two *S. mentella* types in the open Irminger Sea based on variations in colour, length-weight relationship, length at first maturity and parasite infestation (ICES, 1998), and preliminary genetic studies have given evidence of differences between the different types of *S. mentella* (Johansen *et al.*, 2000). But the observed genetic heterogeneity can be explained by other causes than the existence of different stocks, and recent studies demonstrated a prevailing pattern of genetic homogeneity (Saborido-Rey *et al.*, 2005 and references therein). Spatial and vertical variability of different biological parameters over the Irminger Sea and adjacent waters is determined by the existence of functional sub-units within the habitat area of the *S. mentella* population, the change of ecological conditions during their life cycle and active seasonal migrations, but not by the existence of different types/stocks of *S. mentella* (Saborido *et al.*, 2005).

Although several studies have been made to identify, delimit and discriminate redfish stocks (ICES, 1998), the basic knowledge for a good assessment has not been reached, that is, the biology, ecology and population structure remain unknown, as is common in widespread highly migratory species. In practice, the stocks have been defined attending exclusively to fish availability for exploitation, without any biological base.

Due to the importance of the management of the redfish resource in this area, ICES established a study group on redfish stocks in 1992, with the aim to identify, discuss and coordinate present and future redfish research. In addition, the European Union financed in January 2000 a research project (QLRT-CT-1999-01222, Acronym: 'REDFISH'), composed of researchers from Germany, Iceland, Norway and Spain, which was expected to clarify the population structure of the redfish in the area. A variety of methods have been used in the REDFISH project to enlighten redfish population structure:

- Morphologic analysis including morphometrics and meristics.
- Otolith structures (shape) and elemental composition.
- Genetic analyses using selected molecular markers (such as isozymes and other proteins, DNA based methods such as microsatellites, amplified fragment length polymorphisms (AFLP), nuclear DNA and mitochondrial DNA genes).
- Reproductive cycles by species, stock and sex (spawning grounds and season, maturity stages).
- Female fecundity by species and stock.

Morphometrics and meristics: pro and cons.

Taxonomic classification and differentiation among stocks has been based on differences in fish morphology. Comparisons were traditionally carried out using differences in body measurements (morphometrics) or differences in numbers of anatomical structures (meristics). Morphometrics and meristics are clearly appropriate to distinguish redfish populations and stocks. A combination of both techniques have been satisfactorily used for north Atlantic redfish identification of species (Ni, 1981e, Misra and Ni, 1983 and Kenchington, 1986; Saborido-Rey, 1994; Valentin *et al.*, 2002) and populations (Reinert and Lastein, 1992, Saborido-Rey, 1994; Saborido-Rey and Nedreaas, 2000).

Genetic markers are usually assumed to be neutral or nearly neutral to selection, while morphometric and meristic characters are modulated by selection, reflecting local adaptation. The main advantage in using morphological traits in studies of population structure is that these traits are often related to fitness and respond to selection, and thus, may reveal genetic differentiation not evident in neutral genetic traits, while their main disadvantage results from phenotypic plasticity, the ability of a genotype to produce different phenotypes across an environmental gradient (Swain *et al.*, 2005).

Morphometric characters may be labile to environmental influences throughout life, making it possible to separate groups of fish that, although sharing the same genotype, have followed divergent paths in different environmental regimes. This phenotypic plasticity usually produces gradual changes in fish shape parallel to environmental gradients, and if the sampling does not cover the whole range, spurious differences can be identified when discrete samples are collected along the cline (Bowering, 1988). In widespread pelagic migratory populations such as *S. mentella*, it is very likely that the sampling does not cover the whole area of distribution of the species, and so, special care has to be exercised when interpreting the results. In this study, this aspect has been considered and mostly avoided with a very extensive sampling scheme.

Begg *et al.* (1999) considered that phenotypic markers may be more applicable for studying short-term, environmentally induced variation; perhaps more applicable to fisheries management. Thus, the importance of delineating groups of fish characterized by phenotypic differences that may be entirely environmentally induced, is being increasingly emphasized (Swain *et al.*, 2005 and references therein). For example, Cadrin and Friedland (1999) argue that intraspecific groups with persistent phenotypic differences in life history traits need to be

recognized in stock assessment and fisheries management, even if these differences do not reflect genetic differentiation.

Normally, multidisciplinary approaches include genetic and morphometric techniques as they are considered basic techniques for stock identification (Begg and Waldman, 1999). It remains important that traditional identifiers (morphometrics and meristics) are congruent with genetic results, and in the case that population structure analyzed genetically does not coincide well with the ecology of the species, comparisons with morphometric and meristic results are essential (Hammer and Zimmermann, 2005).

Traditionally, meristics is used in alpha taxonomy, and also to look for differences among individuals of the same species in order to delimit different stocks (Schmidt, 1930, Templeman, 1981). Nowadays meristics continues to be one of the tools used in the multidisciplinary approaches for stock identification (Pepin and Carr, 1993; Tudela, 1999; Murta, 2000; Kai and Nakabo, 2002; O'Reilly and Hornt, 2004).

The results of this and previous studies show a high degree of overlap of the meristic counts among *Sebastes* species in the North Atlantic. Reinert and Lastein (1992) found that five of the meristic counts in *S. mentella* and *S. marinus* were exactly the same for all the specimens examined, and that other five counts had the same mean and total range. Thus, most of the meristic variables are useless for *Sebastes* species discrimination, However, their use in combination with other techniques can be advantageous, as in the study of *S. mentella*, *S. fasciatus* and its putative hybrids in the Gulf of Saint Lawrence, where Valentin *et al.* (2002) combined the number of soft rays of the anal fin and the gas bladder musculature pattern with genetic techniques.

One of the most outstanding peculiarities of meristic characters is their dependence on the environment (i. e., temperature, salinity, oxygen, pH, food availability and growth rate) (Waldman, 2005a and references therein). However, contrary to morphometric characters that may be labile throughout life, meristic traits are fixed early in ontogeny and remain stable throughout life, thus reflecting environmental effects over the relatively brief period of larval development and metamorphosis. Because of this, significant statistical differences can occur within a stock among year classes of geographic subgroups subjected to varying environmental conditions. (Begg and Waldman, 1999). Those characteristics are very relevant and they must be taken into account when interpreting the results.

The meristic analyses developed in this study indicate the existence of differences between *S. mentella* and *S. marinus*. But significant intraspecific differences by area and subarea were also obtained for both species in Irminger, Greenland, Iceland, and the Faroe Islands, and among the two different *S. mentella* types, 'oceanic' and 'deep-sea', in the Irminger Sea. This results are in disagreement with those obtained from morphometry, which yielded a lack of structure for both species in the core area of study, a homogeneity in accordance with the biology of the species in the area. Furthermore, the histograms of the meristic variables do not allow such differences to be tracked. This leads to the suspicion that some other reasons were behind such differences other than pure differences among species or stocks.

The study carried out to elucidate the causes of this meristic heterogeneity in the different areas revealed, surprisingly, the possible existence of size dependence of the meristic

counts, since results showed significant Pearson correlation of these variables with standard length. However, the significant correlations did not show a clear pattern, or even a logical one, because in some instances they were positive while in others they were negative. If size dependence exists, the correlations should always be positive or negative, although the latter is improbable, since it would indicate that as fish grows, it loses rays or gill rakers. Furthermore, for the same meristic variable, the correlation was positive in some areas, and negative in others. The variables that showed significant correlation with size were, precisely, those that showed significant differences in the Kruskal-Wallis and z statistic analyses. It was very surprising to find meristic variables dependent on size because, as stated before, meristic characters are supposed to be fixed early in ontogeny, and remain unchanged regardless of subsequent environmental changes. The individuals analyzed were exclusively adults, and it is difficult to believe that as fish grow new rays, for example, are formed. So, this variation in meristic characters between different sized individuals should not be due to the size that one individual has at that moment, but to the fact that the different sized individuals were born at different periods, under different environmental conditions.

In our opinion, the observed differences in meristics are the consequence of the presence in the stock of different years classes with very different ages. Because meristic characters reflect environmental effects over the relatively brief time of larval development, significant statistical differences can occur within a stock among year classes or geographical subgroups subjected to varying environmental conditions (Begg and Waldman, 1999). As mentioned, *Sebastes* are long-lived species and the adult population in the Irminger Sea and adjacent waters can be constituted by a large number of year classes, and between the youngest and the oldest, up to 30 years of difference may exist (ICES, 2004) It is obvious that in such a wide period of time, environmental conditions may have changed, yielding meristic differences between year-classes. Another point is that several year-classes can be found in the same size-interval, due to the different life history of each year-class.

In this study, the individuals could not be separated by yearclass, because the individual ages were not available. However, when a narrow interval of sizes was selected, the correlation with size decreased considerably. Although different yearclasses can share the same size, a decrease in the number of yearclasses when restricting the size interval is very likely. It is hypothesized that the meristic differences observed in the analyses are very likely to be due to differences between cohorts that underwent different environmental conditions in early ontogeny. The impossibility of separating individuals by age prevents confirmation of this hypothesis. For this reason, meristic results were not taken into account in the interpretation of the species and population structure. Probably some of the meristic variables used are affected by growth, such as PPA, but most of them were not.

However, these results are interesting in the sense that they revealed potential differences among year classes, rather than between stocks. Because meristics are believed to be invariant in the life of a single fish, these results indicate that caution has to be used when interpreting observed differences in what is believed invariant, such as the genotypes. But also caution is needed when analyzing fish from many different year classes, especially if the age differences between them are large enough to imply exposure to very different environmental conditions.

Size and shape in morphometry

One of the main problems in traditional morphometrics is that linear distance measurements are usually highly correlated with size (Bookstein *et al.*, 1985). The elimination of size influence from the data is basic in traditional multivariate morphometrical analysis, because the majority of the variation of measurements taken from samples constituted by different sized fish, would obviously be due to the size.

Ontogeny produces two kinds of morphometric variance: isometric size variation due to growth, and allometric shape variance arising from developmental changes in form. Isometric size variation is related exclusively to size of the fish; the larger the fish, the larger the measured distances. It is therefore a simple scale problem, and easily removed from the data. Allometric variation, however, is more related to developmental change, and not necessarily every distance measured has the same allometric variation. Hence, the removal of allometric variation is more complicated. Only a small part of the covariance expresses functional regulation, morphological integration, shape differences between subgroups at constant size, or other specifically biological processes or constraints (Bookstein, 1997).

Stock identification is generally affected by size differences, but can be improved by removing the allometric patterns associated with development or maturation. If differences among stocks are exclusively based on isometric growth, stock identification can be a function of size at age (Cote *et al.*, 1980; Hedgecock *et al.*, 1989; Schweigert, 1987; Tully and Hillis, 1995). However, differences in size distributions are often temporary or artificial for several reasons: stock-specific recruitment events (Fabrizio, 1985, 1987; Waldman and Fabrizio, 1994), geographic patterns in fishing mortality (Cadrin, 1995; Tully and Hillis, 1995), or loss of scale information in historical images (Bookstein, 1991). Loy (1996) concluded that intraspecific size variation often represents morphometric noise, and is a nongeographic source of variation (Cadrin, 2000).

To avoid this morphometric noise, the 'size-free' part of the variance, i.e. the shape variation, must be obtained. There are different methods to separate the effects of size from variation in body shape, i.e., ratios, regression related, and multivariate methods (Strauss and Bond, 1990). In this study three methods, residuals, adjusted residuals and the multivariate Burnaby's method have been applied to our data. The discrimination of the species and populations were computed from the data transformed using each of the three methods, and the discrimination obtained with each of the methods compared to test which of the methods was the most suitable for eliminate the size effect.

Ratios have not been used because, despite their apparently simplicity, there are many ecological and statistical problems associated with their use as 'dimensionless' shape descriptors (Atchley *et al.*, 1976; Albrecht 1978; Atchley and Anderson, 1978; Bookstein *et al.*, 1985). For example, ratios generally have larger sampling errors than the original measurements, and they also have non-normal frequency distributions that violate the assumptions of standard statistical tests. The use of ratios can also introduce spurious character correlations that were not present in the original data, and can introduce nonlinearities into previously linear relationships. Most important, however, ratios do not

adequately compensate for differences in body size except in special circumstances (Strauss, 1985), as for example the isometry, but never the allometry. Thus, consider the equation of simple allometry (Gould, 1966):

$$Y=aX^b$$

Where the line passes through the origin, a and b are constants, X is a size expression, as for example standard length and Y a morphometric character. Using ratios:

$$\frac{Y}{X} = aX^{(b-1)}$$

where the ratio is still affected by X , except in the special case of $b=1$, isometry.

As regards regression related methods, two of them have been used in this study, residuals and adjusted residuals against standard length. The main reason for choosing regression related methods has been that by definition, residuals are orthogonal to the regression line, in this case size variation, i.e., are independent of size. Furthermore, in previous analyses carried out by Saborido-Rey (1994), it had been proved that using residuals, *Sebastes* populations discriminate well. In addition, the results were similar to using multivariate methods. However, for some authors, these regression approaches are generally ineffective for removing size variance from data because they only remove the effect of the standard length distance, which is not necessarily a comprehensive measure of general size (Humphries *et al.*, 1981; Klingerberg, 1996). Thus, a multivariate method to eliminate the size influence, the Burnaby's method, was also performed, because it deals with size in a multivariate approach. After size correction using Burnaby's method, the correlation between the variables decreased considerably, principally the correlations between the variables and standard length. However, this method also makes an important assumption: that the first eigenvector on a Principal component analysis corresponds to a size vector, which may not be true. Although logically, most of the size effect is probably contained in the first eigenvector, other sources of variation that are not size-specific can also be present in the first eigenvector. On the other hand, part of the size-related variation may remain in the other eigenvectors, and thus, be introduced in the discriminant analyses. However, the results from each of the methods in each case study, although slightly different, never lead to a different conclusion. Taking into account that both ratios and residuals implicitly assume isometry between the independent and dependent variables (Strauss and Bookstein, 1982; Bookstein *et al.*, 1985), and that multivariate analysis of ratios or residuals may be statistically invalid (Atchley *et al.*, 1976, Atchley and Anderson, 1978; Misra and Ni, 1983; Trippel and Hubbert, 1990), Burnaby's method was chosen as the preferred method to be presented here, and from which the conclusions were drawn.

Geometric versus traditional morphometrics

One of the drawbacks of traditional morphometrics is that the geometric relationships among the variables are not preserved (a set of linear distances is usually insufficient to capture the geometry of the original object) (Adams *et al.*, 2004). Thus, the same set of measurements can be obtained from two different shapes. For example maximum height and width can be identical from oval- and teardrop-shaped objects. Another problem of the traditional methods consists in the high correlation between linear measurements and size, that although in less intensity, persist even after using the best method for the elimination of the size-effect (Bookstein *et al.*, 1985). Additionally, geometric morphometrics automatically avoid the size

effect when the specimen is scaled. Variation in size is removed from the data by standardizing for centroid size, and shape changes alone are included in the analysis (Klingenberg, 1996).

While in traditional morphometrics some of the variables are distances defined by non-homologous points (for example maximal eye diameter, or body width), these variables cannot be used in geometric morphometrics, and thus variables traditionally used for species identification can not be included when using geometric morphometric techniques. With traditional morphometrics, it is not possible to perform graphical displays because the geometry of the shape is lost. On the other hand, geometric morphometrics conserves the geometry, and thus graphical displays of the shape changes are available, and used as a good intuitive interpretation of shape variation, which can help one to see where the differences among the studied groups are located. A disadvantage of geometric morphometrics, is that variables lose their biological significance. In traditional morphometrics the variables, although modified in order to avoid the size effect, do not lose their identity; thus, the analytical results indicate which of the variables contributes the most to the discrimination. In contrast, variables used in geometric morphometrics, the partial warps, have no biological meaning.

Although geometric morphometrics has been used for *S. mentella* and *S. fasciatus* differentiation in the Northwest Atlantic (Valentin *et al.*, 2002), traditional morphometry has been more widely used for species and stock identification, and its utility in discrimination between the four *Sebastes* species and populations in the North Atlantic had been verified in previous studies (Saborido-Rey, 1994; Saborido-Rey and Nedreaas, 2000). Thus, the traditional approach was designed to be used as the main technique, and the same analyses repeated subsequently using geometric morphometrics. In doing so, it was possible to compare the results that each technique yields in the same analysis, the results being interpreted with the aid of different outputs, such as the geometric morphometric graphics. In this study, geometric morphometrics always yielded the same results as traditional morphometrics, although the differences between groups, when they existed, were more sharply marked by the geometric methods.

Methodological improvements

In this study, the most recent advances in technology has been employed in order to improve the traditional methods of data acquisition. Thus, landmark coordinates have been taken with the aid of image analysis software over digital images of the fish, which made the process quick and straightforward. In order to avoid inaccuracies when capturing the landmark coordinates on the photos, landmark points were marked with pins driven into the fish. A detailed protocol was performed with graphical indications of the exact point where the landmark is allocated and with warnings to avoid mistakes in specially difficult points. This protocol was a guarantee of the repeatability of the points by different persons, and it was necessary because two laboratories of different countries were involved in the data acquisition.

The use of digital photos optimized the work, as outliers, once evaluated, were corrected directly on the photos, while failures in measurements taken with traditional methods were

not correctable, because normally the individuals were discarded after taking the measurements, and if they had been preserved, the shape would probably have changed after a second period of frozen storage.

The use of digital photos also allowed the interchange of material between laboratories. This allows revision of the pin positions, i.e. the exact point of the landmarks, revealing possible differences or inaccuracies. In fact, in this analysis, the variable describing the suborbital ridge was finally not considered in the analyses because it was significantly different in the two laboratories. The origin of those differences was confirmed in the photos, where it was observed that one of the pins had been systematically driven into a different position in one of the laboratories. Moreover, collections of digital photos can be stored, for future use, with the advantage that new landmarks can be added if required.

The coordination of data measurement in two different laboratories in different countries has being beneficial, because the number of samples was very high, improving the sampling scheme, not only covering a wide area but also different years and seasons. This good coverage allowed the very comprehensive analysis as conducted here, but also allows future analyses focused with different perspectives, such as the study of seasonal variation, similar to that conducted in northern Norway (Saborido-Rey and Nedreaas, 2000). However, coordination between the two laboratories in data acquisition, the development of protocols and verification of the uniformity of the data, caused a great deal of extra work. Although the protocol was designed with the maximum detail, differences among laboratories existed in one of the variables, as mentioned above. Furthermore, the use of different digital cameras and software in each laboratory, implied the need to test possible dissimilarities among labs. Thus, an extra effort was made to compare data from the two laboratories. Overall, however, results indicated that it is highly recommended to follow a similar approach as taken here in future morphometric studies, especially if several laboratories are involved.

In this study, measurements taken previously with traditional methods, i.e. calipers, were used. More precisely, the data from Flemish Cap and Norway used by Saborido-Rey (1994) to study *Sebastes* populations across the North Atlantic were incorporated. The importance of using these two areas was already decided in the design of the REDFISH project. Since the main goal of the project was to study the population structure in the Irminger Sea and adjacent waters, sampling was designed and scheduled for these areas. Flemish Cap and Norway were the two closest areas where data was available, and were considered important referents to understand the population structure in the Irminger Sea. Moreover, a close relationships between these two reference areas and the Irminger Sea and adjacent waters had been shown (Reinert and Lastein, 1992; Roques *et al.*, 2002). Therefore the use of Flemish Cap and Norway data added value to the analyses presented here.

However, in order to make the measurements taken on pictures (two dimensional) comparable with those measurements taken directly on the fish with calipers (three dimensional), a special double calibration was devised. This calibration uses a measurement taken directly on the fish with calipers to calibrate some of the variables, and a ruler placed on the base where the fish lay to calibrate the rest of the variables. This was shown to be a very good solution, since errors were minimized. It was, indeed, a novelty within the

morphometric field. Using the length of the first dorsal ray, D2D, the calibration improved notably for most of the variables. The main reason for the usefulness of this distance probably lies in the fact that it is long enough and is placed at mid distance of the fish height, being thus very representative of most of the distances. Probably, some other variables would yield similar results, but D2D was one of the easiest to measure, and landmarks defining it were clearly identifiable. In another species, it is recommended to study the best suitable variable as was done here, and presented in Annex II.

Another alternative method to compare the two types of measurements would have been to convert the interlandmark distances from previous studies to cartesian coordinates (Carpenter *et al.*, 1996). However, this conversion is restricted to those variables that are part of the truss network, i.e. to those landmarks that share at least three distances. Some of the variables used in the previous analyses would thus have been discarded. Among them, some that play an important role in discrimination, such as for example the eye diameter in species discrimination. Taking this approach therefore would have reduced the reliability of the analyses, and hence the method used here was preferred.

The sampling schedule was elaborated in order to cover the widest area possible, the four seasons in the year and different years. The ultimate reason of this sampling schedule was to study in the future putative migrations of the species between areas, i.e. some kind of seasonal pattern as shown in northern Norway (Saborido-Rey and Nedreaas, 2000). However, as stated above, phenotypic plasticity usually produces gradual changes in fish shape parallel to environmental gradients, and if the samples do not cover the whole range, spurious differences can be obtained between individuals that, although belonging to the same population, are located at the extremes of the gradient. This is especially true in a species as widely distributed as redfish, and particularly in *S. mentella* which is not restricted to the shelves, but also present throughout almost the entire Irminger Sea. In fact, results presented here showed a morphometric gradient of similarities, the closest areas being more related than the distant ones. For this reason, a special effort was placed on sampling *S. mentella* in the entire area, but especially in the Irminger Sea, because the pelagic component of *S. mentella* in this area was the prior objective. As a consequence, and unavoidably, an unequal sampling effort was obtained for each area, yielding important differences in numbers of individuals available from each of them. Those differences in number of samples also depended on the existence of commercial fishery activity and the possibility to obtain samples from research cruises in the different areas. Thus, in most of the analyses performed, the differences in numbers of individuals per group were very large, and this fact might produce a bias in the classification procedures because the models perform better if all groups have the same number of individuals (Mulligan *et al.*, 1988; Fabrizio, 2005). Furthermore, for the classification of the cases, the statistical program calculates the 'a priori classification probabilities'. The value of those probabilities is proportional to the number of individuals in each of the groups, and the probabilities are used in the classification to assign the uncertain individuals to one or another group. Thus, if the analysis is performed between groups with very different numbers of individuals, the doubtful individuals would be assigned to the larger group. The close similarities between *Sebastes* species emphasize this effect. Thus, overclassification was observed in the larger groups, while the smaller were underestimated. To what extent these over- and misclassifications are

anomalies due to artefacts of the analyses is unknown. However, when performing the same analysis with a balanced number of individuals, the results changed, and this bias disappeared from the larger groups. However, in this balanced analysis the numbers of individuals were fewer, and thus, part of the information was lost. However, to minimize this decrease of information, the individuals were randomly taken, and, special care was put on the equal representation of all the sub-areas. The results of both analyses were displayed, as the results of both analyses were taken into account when interpreting the relations between the groups.

Discrimination of *Sebastes* species

Although the main goal of the project was to clarify the population structure of each of the *Sebastes* species in the Irminger Sea and adjacent waters, the basic concept of the relationships among the species were not clear yet. The high resemblance of the four species not only creates difficulties to separate the species in the commercial catches. Most of the research surveys conducted in the Northwest Atlantic do not separate *S. mentella* and *S. fasciatus*, which are grouped together as beaked redfish. *S. marinus* is, however, easier to identify. On the contrary, it is well known that in Norway, and in Faroes waters, *S. marinus* and *S. mentella* are more similar than in the Northwest, and have created some difficulties in separating the catches, both at commercial and at scientific levels. Although the integrity of each species is not questionable, several hypotheses have been developed about the potential for hybridization among the species (Altukhov and Nefyodov, 1968; Altukhov *et al.*, 1968; Rubec *et al.*, 1991; Roques *et al.*, 2001; Johansen, 2003).

The impossibility of differentiating *Sebastes* species by their external appearance, prevents accurate knowledge of basic biological parameters such as maximum age, natural mortality rates, fecundity and age at maturity for each of the species separately. These parameters are the basis for stock assessment. However, although a perfect separation of species might be performed on research cruises, and a good assessment could then be implemented for each of them separately, the mixed-species nature of redfish fisheries prevents the separation of species onboard commercial fishing vessels, and consequently, to know the landings by species. Species of *Sebastes* in the Pacific, despite their diversity, share attributes that make them extremely vulnerable to fishing pressure. In fact they are classified in the very low productivity category following the AFS productivity criteria (Musick 1999a; 1999b). The impossibility of sustaining high fishing mortality is due to their reproductive strategies which limit them to relatively low intrinsic rates of increase (Adams, 1980). Perhaps the same warnings should have been applied for *Sebastes* in the North Atlantic.

To ascertain the stock structure, it is necessary to understand first the morphometric relationships among species. At least 22 different definitions of species concept have been described (Mayden, 1997) depending on the discipline, giving an idea of the high controversy around this concept. Between all, the most extended in the last 50 years has been The Biological Species Concept (BSC) (Mayr, 1942), based on the idea of isolating reproduction, considering a species as a population (or a group of populations) within which there is interbreeding (or there would be interbreeding if they were not geographically separated), but which does not interbreed with other populations. Other more recent definitions of the species concept follow a more practical point of view. Thus, Mallet (1995) defines species as

recognizable 'morphological and genotypic clusters'. This definition corresponds to taxonomic practice of most workers (Turner, 1999). Mallet's concept has been considered as a return to the darwinian species concept, as Darwin (1859) maintained that species were simply well-marked varieties and that there was no discontinuity between individual variation and variation at the level of species or higher taxa. The BSC has been questioned since evidence for transfer of genes between closely related species has been found. Many organisms hybridize in nature, fishes probably more than most other animals. Although early works tended to emphasize the high frequency of sterility or inviability of hybrids (Hubbs, 1955), it is indisputable in fish that some hybrids are able to interbreed successfully with one or both parental forms, leading to introgression (Turner, 1999). It is interesting to note that hybrids are not necessarily intermediate forms and may exhibit novel morphologies outside the range of the parental species (Crapon de Caprona and Fritzsh, 1984; McElroy and Kornfield, 1993).

Taxonomists define different species (i. e., introduce the individuals in the Linnean hierarchy) in terms of morphology, based around a specimen considered the 'type'. Species identification traditionally uses the description of the individual, using taxonomic characters based on the phenotype. But this view of the species as a distinct biological entity that can be described and differentiated from others using morphological characters, is questionable, because the biological reality of a species may not always be amenable to a rigid definition (Carvalho and Hausen, 1999). This seems to be the case for redfish. The morphology of the four *Sebastes* species in the North Atlantic is very similar, and most of the taxonomic characters normally used to identify species overlap. Furthermore, some morphological characters that allow species distinctions in determined areas are useless in others, making it impossible to use a 'type specimen' for a species in the whole area. For example, in most of the Atlantic, *S. mentella* has big eyes and a distinctly long symphyisial tubercle, but in Norway and in the south of the Faroe Islands, these characters can be also found in some *S. marinus* individuals.

In the morphometric analyses conducted to compare the *Sebastes* species present in each of the areas separately, it was concluded that species are clearly distinguished by their morphometry. However, a prerequisite to the discriminant analyses is to classify properly the individuals into species; because the discriminant analysis looks for the maximal differences between predefined groups, errors in species assignation of the specimens may lead to wrong interpretation of the results. It is obvious that the unmixed nature of those predefined groups is essential. So, one of the main constraints during this study was the identification of species *a priori*. In Flemish Cap and Norway, the species were identified using the gas bladder musculature and the analyses yielded a very good discrimination of species. In the other areas, i.e. Iceland, the Faroe Islands and Greenland, the species assignation was based on the external morphology given by the researcher measuring the distances. In this case the results were uneven.

The results of the morphometric analysis in Iceland yielded good discrimination between *S. marinus* and *S. mentella*, despite the fact that the samples had been separated into species using the visual inspection of the external features. The discrimination was not as high as in the reference areas (Norway and Flemish Cap), but in those areas the species had been

separated using the GBM pattern. The reason for the incorrect classification of some individuals in Iceland could be that they had been incorrectly classified into species. But another possibility, although less likely, is perhaps the that different redfish species are more similar in Iceland than in the reference areas. In any case, the separation of redfish species in Iceland by their external features yielded good discrimination, similar to that obtained in Flemish Cap.

In the Faroe Islands, when the species were separated only by external morphology, the analysis yielded a total lack of discrimination. *S. viviparus* was clearly different from *S. mentella* and *S. marinus*. But between these last species, discrimination was poor. The samples had been taken from two different locations, the NW or 'Faroes plateau' and the SW or 'Faroes Bank'. As in Iceland, the samples taken on Faroes Plateau yielded good discrimination when the individuals were classified into species using external morphology. The problems increased when analyzing the samples from the Faroes Bank, because the external features were not useful for species identification, as shown by the lack of discrimination. The problem of species identification in this area was already a known feature. Previous investigations in redfish genetics from this area have warned scientists about the existence of individuals that although externally like *S. mentella*, present *S. marinus* genotypes (Torild Johansen, personal communication). Because of this previous knowledge, the samples in this area were not randomly collected. Instead, those individuals especially difficult to identify *into species* were consciously selected. The main purpose of this selection was to conduct a deeper genetic analyses on this feature. However, this is not the ideal sampling scheme for stock structure studies, and its consequences in the interpretation of the results are discussed below. The lack of morphometric discrimination in the first approach was exclusively due to the wrong assignation into species when using external features. This was demonstrated with the complete morphometric discrimination attained in the discriminant analysis performed using the gas bladder musculature to identify the species. So, the external features can be deceitful in Faroes Bank, but the GBM is a good tool for species identification in this area. Moreover, the species assignation using GBM was in almost total concordance with the genetic analyses, reinforcing the GBM pattern as a good tool for species identification. It is interesting to note that the difficulties in species identification using external morphology affected basically the samples collected in the southwest of the Faroe Islands. Therefore, once the problems about species identification were clarified and the samples were reorganized in groups constituted by individuals correctly classified into species, the discriminant analysis showed that, morphometrically, redfish species are clearly distinct in the Faroe Islands. However, it is a question for debate in the future whether the external features still should be used to identify the species, at least in the research surveys conducted here. To what extent errors in species identification affect the resource management remains unknown, but should be studied.

The situation in Greenland is even more complicated, this being the area that presented the biggest difficulties in interpreting the results. Although only two species, *S. mentella* and *S. marinus*, were recorded, the problems of species identification resulted in a complex scenario. The possibility that *S. fasciatus* or *S. viviparus* are present in Greenland is remote. Hureau and Litvenko (1984) described 18 *S. fasciatus* specimens in Iceland and the Irminger Sea, but either its presence there was occasional, or alternatively they were not *S. fasciatus*

but a type of *S. mentella* or *S. marinus*, because *S. fasciatus* is scarce in such northern latitudes. On the other hand, Johansen (2003) stated that neither *S. viviparus* nor *S. fasciatus* were found in Greenland. Thus, the possibility of the presence of another species different from *S. marinus* or *S. mentella* in Greenland was discarded.

East Greenland is the main nursery area for *S. mentella* and *S. Marinus*, and a large quantity of juveniles are present in this area. It is very difficult to separate these juveniles into species, and thus, they were removed from the analyses, on the assumption that most of the errors identifying the species comes from these specimens. However, problems in species identification persist in the adults, and thus, unfortunately, only those individuals for which a typical *S. mentella* or *S. marinus* genotype was available were included in the final analyses. Thus, the quality of the analyses decreased. First, because the low number of genotyped fish reduced the sample size of this area compared with others, and second, because there is the possibility that these genotypes represents two typical morphotypes, and hence, the morphometric variation was reduced, affecting the analysis in an unknown manner.

Neither the use of external features nor the gas bladder musculature were able to discriminate among species. The GBM pattern that allowed us to distinguish between species in Flemish Cap, Norway and the Faroe Islands was ineffective in Greenland. Thus, *S. marinus* and *S. mentella* from Greenland could not be accurately separated either by the inspection of external features, or by the GBM pattern. Part of the sampled individuals were juveniles (fish smaller than 18 cm standard length) from both species, and since the smaller the fish the more difficult species identification becomes, initially, it was hypothesized that the small size of the fish was the cause of the high proportion of misclassification. But even removing the juveniles from the analyses, the classification did not improve. Several approaches were taken to investigate the lack of discrimination using the GBM. The GBM pattern in Greenland was different from that in the other areas studied, and the principal characteristic was the presence of a high polymorphism, that hampers species asignation. Until now, the GBM pattern showed some variation within species, but always with a common within species pattern that allowed the different species to be clearly distinguished. Surprisingly, this was not the case for Greenland. Attempts were made to find a pattern in GBM that permitted discrimination of species, even if the new pattern were different from that in other areas, i.e. from that traditionally described. All attempts failed, and the discrimination was always poor, even using only adults. Thus, at this point, both the external morphology and the GBM pattern had proved ineffective to accurately separate the individuals into species, and were rejected as criteria. As an alternative, 267 *S. marinus* and 144 *S. mentella* genetically identified in Germany (Cathrin Schmidt, Federal Research Centre for Fisheries, Hamburg) and Norway (Torild Johanssen, University of Bergen) were used to compare their morphometry. Thus, groups were constructed separating species by their genotypes, and these were used as input in a new discriminant analysis, resulting in a very clear discrimination between species; the conclusion, therefore, was that both species are morphometrically (and genetically) different, but very difficult to distinguish by traditional means. Moreover, genetics and morphometry show full agreement in species identification, as the morphometric discrimination was excellent.

A new attempt was performed to find a GBM pattern for species identification using exclusively fish in which species had been identified genetically, but also morphometrically. However, even in these specimens, it was impossible to find a clear GBM pattern among species. Maybe redfish in Greenland do not show strong differences in this anatomical feature, but the existence of some imprecision when recording the GBM pattern for these fish cannot be rejected. The GBM inspection was performed in 1,085 individuals from the WH233 research cruise, a large part of them (769) being juveniles, i. e., with standard length smaller than 18 cm, and within those juveniles, 456 had a standard length smaller than 15 cm. These individuals had previously been frozen, and despite the fact that all individuals were in good condition, the tendons of the gas bladder musculature in such small fish are thinner and more easily broken than normally.

For those individuals genetically analyzed in Germany, it was observed that misclassification when using the GBM occurred mainly in smaller *S. marinus*. This means that, at those sizes, there were individuals with *S. mentella* genotype but with *S. marinus* GBM pattern. One explanation for this misclassification was hypothesized: as the number of muscle heads in which the tendons originate was not considered, nor recorded, perhaps *S. mentella* at this sizes possess many tendons but originating from only one or two muscle heads. If this is the case, this pattern is different from the typical pattern for *S. mentella* in other areas, and also different from *S. marinus* typical pattern. However, as only the number of tendons had been recorded, the individuals would have been wrongly assigned to *S. marinus*. The opposite situation, however, is not possible, and it may explain why few misclassifications occurred in *S. mentella*. In the Faroe Islands, GBM and genetic species assignment matched perfectly, but the sample in this area was composed of specimens larger than 300 mm. In large individuals, the tendons are thicker, easy to handle, and distinguishable from other tendons present that do not belong to the GBM.

It has to be pointed out that only the fish that showed a typical 'marinus' or 'mentella' genotype were included in the final morphometric analyses in Greenland. However, there were other specimens in the population with genotypes different from the typical ones. When introducing those individuals with a non-typical genotype into the analysis, they classified clearly in the *S. marinus* group. The conclusion for the Greenland area is that morphometric differences exist between the individuals that present the typical 'mentella' or 'marinus' genotype, i. e., those well defined genetically, but this affirmation cannot be extended to the whole population. The genotype was available for only a small part of the individuals sampled in Greenland, although the quantity was enough to conduct new morphometrical analyses. Nevertheless, the individuals for which both genotypes and morphometric variables were available were very likely not representative of the population. In other words, these individuals correspond to what can be called "pure" specimens, or pure morphotypes, if preferred. This seems to be confirmed with the morphometric analysis conducted with the 487 clear genotypes from different areas (The Faroe Islands, Iceland, Irminger Sea and Greenland) which resulted in an astonishing discrimination rate of 99.3%. It is not known, at this time, whether the atypical genotypes represent other morphotypes of each species, basically, *S. marinus*, or whether they are the consequence of hybridization. Therefore to what extent the presence of intraspecific genetic variability in Greenland is the consequence of hybridization between *S. mentella* and *S. marinus* remains debatable. As mentioned, two

different genetic analyses have been performed on the same *S. mentella* and *S. marinus* individuals from Greenland, one analyzing haemoglobin performed in Norway, and the other using microsatellites and performed in Germany.

In the haemoglobin analyses performed in Norway, Hb-1* variation was found in *S. marinus*. To test how this variation in the genotype is reflected in the morphometry of the individuals, a morphometric analysis was performed, introducing as many groups as existing genotypes i. e., *S. mentella*- Hb-1*35/35, *S. marinus* Hb-1*100/100, *S. marinus* Hb-1*70/100, *S. marinus* Hb-1*70/70, *S. marinus* Hb-1*45/100, *S. marinus* Hb-1*45/45 and Giants Hb-1*40/40 and Hb-1*40/70. The results of the morphometric analysis performed between these different Hb patterns showed that 89.5 % of *S. mentella* classified as *S. mentella* and 90.5% of *S. marinus* classified as *S. marinus* or another of the *S. marinus* genotypes. Thus, all the individuals with *S. marinus* genotypes have the same morphometry, but the number of specimens with deviating Hb-1* genotypes is too low to draw solid conclusions.

On the other hand, microsatellite analysis performed in Germany on *S. mentella* and *S. marinus* from Greenland, yielded three different genotypes; the typical *S. mentella*, the typical *S. marinus* and a third one different from the others. The morphometric analysis of those individuals in the present study, showed that individuals with this third genotype had a *S. marinus*-like shape.

The possibility of hybridisation has been a main subject when discussing the North Atlantic *Sebastes* species identification (Johansen, 2003 and references therein). Evidence for extensive introgressive hybridization between *S. mentella* and *S. fasciatus* in the Gulf of Saint Lawrence and south of Newfoundland has been described by Roques *et al.* (2001). Following Johansen (2003), introgression in some areas of the Northwest Atlantic may involve not only *S. fasciatus* and *S. mentella* but also *S. marinus*. In East Greenland, the complicated haemoglobin system in *S. marinus* had been observed already by Nedreaas and Naevdal (1991). Those authors found that *S. marinus* haemoglobin may show intermediate patterns between common *S. marinus* and *S. mentella*, although the morphology was clearly *S. marinus*. Later investigations pointed to extensive hybridization with *S. mentella* as the possible explanation of those uncommon haemoglobin patterns of *S. marinus* at Greenland (Nedreaas *et al.*, 1994). The morphological study of *S. mentella*, *S. fasciatus* and its hybrids in the Gulf of St. Lawrence, stated that the hybrids were not intermediate between the parental species, but a parental phenotype, *S. mentella*-like, was dominant in the hybrid group (Valentin *et al.*, 2002). But hybrids are not necessarily intermediate forms, so molecular identification of hybrids is much more convincing than that based on inferences from morphology alone (Turner, 1999 and references therein). Hybridization in Greenland has not been proved, nor rejected (Johansen, 2003). In this study, the morphological 'marinus-type' for all those individuals that present atypical genotypes were tested for both microsatellite and haemoglobin results. The fact that hybrids can have a shape different from the intermediate between the parental ones, joined to the fact that this is the case for *S. mentella* and *S. fasciatus* hybrids in the Gulf of Saint Lawrence, leaves an open window on the existence of hybridization between *S. mentella* and *S. marinus* in Greenland.

In conclusion, the species in Greenland waters are phenotypically distinct; the problem is whether they can be distinguished from the external appearance or the GBM. Problems in species assignment are very similar in Greenland and in the Faroe Islands, but in the Faroe Islands the larger size of the sampled fish made the gas bladder musculature suitable for species identification. *S. marinus* and *S. mentella* are very similar in both areas, so special care must be taken with species identification in these areas.

Summarizing, the species were clearly morphometrically distinct in each of the studied areas. However, it must be taken into account that in Greenland it was very difficult to identify the species, and only those individuals with a clear genotype *S. mentella* or *S. marinus* were used in the final analyses, although it is not clear if these samples are representative of the whole population. So, in Greenland, we only demonstrated that it is possible to distinguish morphologically those individuals that present a clear genotype *S. mentella* or *S. marinus*. But in the rest of the areas, and despite of the high similarity between the different species of *Sebastes*, it was possible to define morphological groups that correspond to the different species. Thus, the overall conclusion was that the four *Sebastes* species inhabiting the North Atlantic are morphometrically distinct. The differentiation of redfish species using morphometry had been previously proved in the study performed by Saborido-Rey (1994), but now it was confirmed for areas not analyzed before: the Faroe Islands, Iceland, and Greenland. Although a previous morphometric analysis had been performed by Reinert and Lastein (1992) including individuals from the Faroe Islands, Greenland, Iceland and Irminger, it did not deal with the species but with population differentiation. In addition, that study had important methodological limitations that reduced its usefulness.

Traditional morphometric techniques allow us to know which of the variables contributes the most to the discrimination between groups. However, as in all forward stepwise discriminant analysis, if any of the variables are removed, the order, and hence the importance of the variables entering the stepwise analysis can change. Between the variables, those that form part of the truss network, i.e. the outline of the fish, better capture the shape of the whole fish. But the other variables were included because they are helpful in *Sebastes* species discrimination as shown in previous analyses (Misra and Ni, 1983; Power and Ni, 1985; Kenchington, 1986; Saborido-Rey, 1994).

In the discriminant analysis between species involving all species in all areas, AD, LV and DO were the variables that separate most between the four species (see figure 3.7 for the variables' acronyms). The most important variables for the discrimination of *S. mentella* and *S. marinus* in Iceland and Greenland, were two included in the truss network, AD and LV, and also the ventral fin length (LAV). It is remarkable that in both areas the first three variables entering the stepwise analyses were the same. In the Faroe Islands, the separation of *S. mentella*, *S. marinus*, and *S. viviparus* was marked by AD, H2D and LV, while in Norway it was marked by AD, LP and AH. The only area where *S. fasciatus* was present was Flemish Cap, and AD, LP and LM were the most important variables in the discrimination of this species, *S. mentella* and *S. marinus*.

There is a coincident point in all the analyses, independent of the number of species entering in the discrimination or the areas where the analyses were performed; the variable that contributed most to species differentiation was always, and with a higher contribution, AD, i.

e., the transversal distance from the beginning of the dorsal fin to the beginning of the anal fin. And this was also the most important discriminant variable in the study performed by Saborido-Rey (1994) when analyzing differences between redfish species in different areas of the North Atlantic, such as Flemish Cap and Norway (which data were used in the present study) but also in three other areas not present in our analysis, i. e., Grand Bank and Saint Pierre in the Northwest and Svalbard in the Northeast Atlantic.

Graphical displays from the geometric morphometric analyses were only available for Irminger Sea, Greenland, Iceland and the Faroe Islands. Thus, although data from *S. fasciatus* were not available for geometric morphometrics, the shape differences between *S. marinus*, *S. mentella* and *S. viviparus* are reflected in them. Thus, *S. mentella* and *S. marinus* share a more similar shape and are separated from *S. viviparus*, that clearly has a deeper body. This relation between these three species was observed when all the areas were studied together, and also when the relation between species was studied in each of the areas separately. Perhaps differences in shape are influenced by the habits of the different species, as body shape in fishes is generally thought to reflect adaptation to their ecological niches (Swain, 2005); *S. viviparus* is more bottom related than the other species, and *S. marinus* and particularly *S. mentella* are more pelagic. A deep body is associated with superior swimming burst performance whereas a fusiform shape is superior for sustained swimming (Swain and Foote, 1999). This is only a theory, but supported by differences between species, as a gradient from the shallower body shape of *S. mentella* and the deeper body shape of *S. viviparus* through the intermediate shape of *S. marinus* is in accordance with the more pelagic habits of *S. mentella* and the more bottom-related habits of *S. viviparus* through the intermediate habits of *S. marinus*. Furthermore, Valentin *et al.* (2002) found that *S. mentella* exhibits a more fusiform body shape than *S. fasciatus* in the Gulf of St. Lawrence, where *S. fasciatus* occupy the same ecological niche of *S. viviparus* in the East Atlantic. In this sense, AD is more likely the variable that better captures the information on this body dimension.

When all areas were pooled, and a general analysis to compare species was performed, unexpected results were obtained. The results shown that *S. viviparus*, *S. mentella* and *S. fasciatus* were clearly morphometrically distinct, but poor discrimination of *S. marinus* occurred. The question is, if the species (including *S. marinus*) were clearly different when analyzing each of the areas separately, why did *S. marinus* show confusion with the other species when all areas were combined *S. marinus* showed confusion basically with *S. fasciatus* and the overlap affected almost exclusively *S. marinus* from the Flemish Cap, i.e. the confusion is between *S. marinus* and *S. fasciatus* from Flemish Cap. However, those species are clearly different in the analyses performed including only Flemish Cap individuals.

A possible explanation is that discriminant analysis maximizes the differences between the predefined groups. If only individuals from Flemish Cap are present, the differences between *S. fasciatus* and *S. marinus* are obvious and the species are well distinguishable. However, when *S. marinus* from other areas were introduced into the analysis, *S. marinus* from Flemish Cap became more similar to *S. fasciatus*, indicating that the differences between *S. marinus* from Flemish Cap and *S. marinus* from the other areas are bigger than the

differences that show with *S. fasciatus*, a different species but from the same area. The reason for this issue can be derived from the fact that the environment has molded *S. marinus* and *S. fasciatus* from Flemish Cap in the same direction. It can be hypothesized that since the data from Flemish Cap were obtained in a different way (the measurements were taken with calipers, and not on a digital photo), it has produced such an effect. Although it was proved with one hundred fish that with double calibration the differences taken with a caliper were not significantly different from those taken on the photos, perhaps a minor difference in the way the measurements were taken in Flemish Cap could yield this similarity between *S. marinus* and *S. fasciatus* from Flemish Cap. However, if variations when taking the measurements were the cause, *S. fasciatus* confusion would be produced also with *S. marinus* from Norway, as individuals from this area were also measured with calipers, and with the same criteria as those from Flemish Cap, but this is not the case. In addition, as shown in the stock structure analyses, Flemish Cap did not differ from other areas, in spite of the different methodology used. This hypothesis must, therefore, be rejected. However, this enhances the importance of the homogeneity in the data acquisition; as discriminant analysis is able to find slight differences, it is patently obvious that a clear protocol is essential to avoid differences in data acquisition, and that if data from different sources has to be compared, a deep study must be performed in order to find all the kinds of difference that can yield spurious discrimination between groups.

The answer to the problem presented by *S. marinus* and *S. fasciatus* from Flemish Cap could thus be the existence of several stocks of *S. marinus*, the morphometric differences between stocks being higher than between different species sharing the same area. But then, why does it affect only *S. marinus* and *S. fasciatus* in Flemish Cap? The cluster analysis performed in the species-area analysis gave a wider view of the relationships not only between individuals of the same species living in different areas, but between the different species separated by areas. In this analysis the relation between *S. marinus* and *S. fasciatus* from Flemish Cap is evident and confirmed, as *S. marinus* cluster first with *S. fasciatus* previous to clustering with other *S. marinus* individuals from other areas, except Norway, as *S. marinus* from this area seems to be very different. Unfortunately only one area of *S. fasciatus* was available and few conclusions can be derived from this analysis. Furthermore, there are no *S. fasciatus* data available for the geometric morphometric analysis. *S. fasciatus* and *S. marinus* were clearly different in the morphometric analyses conducted by Saborido-Rey (1994) in Flemish Cap, Grand Bank and Saint Pierre Bank, both when analyzed in each area separately, and when analyzed together. However, in that study, the areas were also clearly different when analyzed within each species, but when pooled, the populations become more similar, significantly reducing the discrimination. Differences between individuals of the same species living in different areas, that is, the possibility of the existence of different populations, was also studied.

Population structure

Overall, the analysis of morphometric variation in *S. marinus* defined at least two geographically distinct populations; one of them includes Norway, and the other includes the core area (i. e., Greenland, Iceland and the Faroe Islands), Flemish Cap being a questionable population. *S. viviparus* samples were morphometrically differentiable in the two areas from which samples were available, that is, the Faroe Islands and Norway. The results of the discriminant analyses for *S. mentella* defined a single population for all the areas, including Flemish Cap and Norway, although these two may constitute different populations.

The ecology of *S. marinus* in the main area of this study (Iceland, Greenland and the Faroe Islands) supports the existence of one population in the area, i.e. there is one main spawning area located in the West and Southwest of Iceland (Magnússon and Magnússon, 1977; Magnússon, 1980; ICES, 1983b), and one main nursery area located in the East of Greenland; no spawning has been cited in Greenland waters, and in the Faroe Islands spawning has been observed only in some years, implying that there could be a local component in the area, although no nursery areas have been found (Reinert, 1990). Flemish Cap is known to be a spawning area for the three *Sebastes* species which live there (Saborido-Rey, 1994). Although there are few studies dealing with the isolation of fish species in Flemish Cap, it is an isolated bank separated from the Grand Bank by more than 1000 m depth, the Flemish pass. The water masses over the Cap have a typical anticyclonic gyre movement (Ross 1981; Kudlo *et al.* 1984) that would favour the retention of water. In cod, however, there are studies suggesting the lack of isolation of Flemish Cap (De Cárdenas, 1995). Spawning activity of *S. marinus* has been observed on Flemish Cap, and the juveniles are also present, indicating the presence of a local component. On the other hand, it is thought that *S. marinus* is not strongly pelagic, and so, can only be found near the coast, Flemish Cap being the major exception. In the surveys conducted on Flemish Cap since 1988 (Saborido-Rey and Vázquez, 2003), *S. marinus* biomass seems to have been very stable, except for three particular years when the biomass increased notably, in 1994, 1997 and 2000. These increases are not explained by the population dynamics, nor by recruitment. Moreover, they are produced by the occurrence of a few hauls with unexpectedly large and old *S. marinus*. This may be the product of shifts in availability, either because of the pelagic behaviour of this species, or due to local migration. The distribution of *S. marinus* is continuous along the east coast of Canada, Greenland, Iceland and the Faroe Islands, but in this study the sampling does not cover all the area of distribution; there is a gap on the coast of Canada. However, a gradient of similarity is observed, as West Greenland showed the maximum confusion with Flemish Cap, while the Faroe Islands (the most distant area) the minimum. Further studies should be made including sampling from the coast of Canada, in order to study the gradient of similarity in shape for *S. marinus* in the whole area.

Differences between *S. marinus* from Norway and the other areas were located principally in variables related to the head length, LC, LD, and LPO, resulting in 93,2% correctly classified *S. marinus* from Norway. There are no geometric data from Norway and Flemish Cap, and thus, those areas were not included in the geometric analyses. The graphical displays shown subtle differences between *S. marinus* from Iceland, Greenland and the Faroe Islands, corroborating the existence of a single population for *S. marinus* in these three areas.

Previous analysis performed by Saborido-Rey (1994) on *S. marinus* in the North Atlantic showed a discrimination higher than the 96% in all the areas, which included Flemish Cap and Norway, and in addition Saint Pierre bank on the coast of Canada, not analyzed in this study. His results do not contradict those presented here, since in both studies Norway had a different population. The relationships of Flemish Cap with the Irminger Sea and adjacent waters were not analyzed in his study. Combining both studies, the conclusion is that Norway and Saint Pierre are different populations, Iceland, the Faroe Islands and Greenland another one, and the status of Flemish Cap must be carefully analyzed. The discrimination rate of Flemish Cap was always slightly below 90% (89 and 88% in the two analyses conducted) and the confusion rate did not follow a clear pattern since few specimens were classified in almost all the other areas, except Norway. These values are at the limit of what is considered good discrimination, although there is no rule of thumb at about this in discriminant analysis. Many authors would consider this level as a good discrimination, especially when dealing with population discrimination. There are several methods to evaluate the power of a discrimination function, but they usually involve only two groups, and become more complex when more than two groups exist. Kappa values indicated that the discrimination obtained was not due to chance but probably due to the high correct classification values of Norway and Flemish Cap. More complex statistical analyses have to be conducted to elucidate the status of Flemish Cap, in particular crossvalidation. However, combining the biological knowledge on *S. marinus* and Flemish Cap, it can be easily hypothesized that Flemish Cap fish belong to a different population.

In the analysis performed by Saborido-Rey (1994), body width was the most important variable in the discrimination between *S. marinus* populations. However, this variable has not been included in the present study. Reinert and Lastein (1992) in their morphometric study divided *S. marinus* of the North-east Atlantic into three different populations i. e., Iceland, the Faroe Islands and Norway, *S. marinus* from the Faroe Islands being more related to *S. marinus* in Norway than to *S. marinus* in Iceland. However, in that study, the size influence was not properly removed, and thus, the results are not reliable. *S. marinus* meristic variation from West Greenland to the Grand Bank-Gulf of Saint Lawrence was studied by Ni (1984), who concluded that *S. marinus* from Flemish Cap may be distinct from *S. marinus* in the adjacent areas.

Studies of the Cs-137 content in *S. marinus* (Reinert *et al.*, 1992) indicated that *S. marinus* from the Faroe Islands and Norway are more closely related than this species in Norway and Iceland or in the Faroe Islands and Iceland. These results are not confirmed by our morphometric study, that for *S. marinus* yielded a different population in Norway, and another common one for Iceland, Greenland and the Faroe Islands.

S. viviparus is the species with the less commercial importance, and few studies have been made to clarify its population structure. Nedreaas *et al.* (1994) studied this species in Iceland, and concluded that it is composed of genetically different subunits, that do not seem to be connected to specific geographical areas but related to sex-dependent migrations.

In this study, only two *S. viviparus* samples were available, one from Norway and other from the Faroes Plateau, insufficient to perform population studies. The sample from the Faroe

Islands discriminated perfectly from the one taken in Norway, and in the species-area analysis in which all species in all areas were represented, *S. viviparus* from the Faroe Islands proved very different from the other groups. In contrary, the sample from Norway clustered with *S. marinus* and *S. fasciatus* from Flemish Cap.

Although the reference areas, i. e., Norway and Flemish Cap, could not be included in the geometric morphometric analysis, graphic displays from the species differentiation in the main area reflected the fact that *S. viviparus* (from the Faroe Islands) shape is very different from the shape of *S. marinus* (from several areas but not from Flemish Cap). So, taking into account the differences between *S. marinus* and *S. viviparus* shape, it is reasonable to question what is the cause of the similarities between *S. viviparus* from Norway, and *S. marinus* and *S. fasciatus* from the Flemish Cap. Morphometrical affinity between *S. fasciatus* and *S. viviparus* was observed by Saborido-Rey (1994) in the discriminant analysis of eleven redfish populations in the North Atlantic, where *S. fasciatus* from three different areas were included. *S. fasciatus* and *S. viviparus* occupied the same ecological niche on both sides of the Atlantic, and, if the shape of the fish is a function of the habits of the fish as stated before, the shape in both species should be similar. Thus, it is reasonable to think that it is *S. marinus* from the Flemish Cap group that has a shape in some way different from the shape of this species, but after clustering with *S. fasciatus* and with *S. viviparus* from Norway, the three groups clustered with the other *S. marinus* populations.

S. mentella is the most widely distributed of the four *Sebastes* species in the North Atlantic. Its distribution reaches from the Northeast Arctic area (the Barents Sea and the Norwegian coast) through the central area represented by the Irminger Sea and the adjacent areas, i. e., the Faroe Islands, Iceland, Greenland East and West, to the Canadian coasts and the Flemish Cap in the West.

The analysis performed with traditional morphometric techniques in this study yields a lack of structure for *S. mentella* in all the areas studied, including Flemish Cap and Norway. It has to be taken into account that in the present study the western part of the Atlantic was only represented by Flemish Cap, and the East only by samples from the Norwegian coasts. No samples from the coast of Canada or from the Barents Sea were used. The most surprising result was to observe that Flemish Cap and Norway did not discriminate as different groups. Moreover, when interpreting the classification matrix, it is clear that these two areas confuse basically among them and to a lesser extent with the Irminger Sea. Both areas clustered apart and looked different to the others. In previous analysis (Saborido-Rey, 1994) both were revealed as different stocks. In such studies, differences occurred when *S. mentella* was analyzed separately, but when pooled with other species, Norway and Flemish Cap increased their respective confusion. It is not known to what extent consideration of the central areas (the Faroe Islands, Iceland, Greenland and Irminger Sea) affects the current analyses in a similar manner, but more analyses have to be conducted to clarify the status of these two areas. In the analyses conducted with all species and areas, there was a clear separation of species by the first canonical root, while the second root separated Flemish Cap and Norway from the rest of the areas for all the species. Although this is the expected structure, the relationships among the putative stocks yielded a very complex scenario. Similarly, and following the same reasoning, Irminger Sea confused more with Flemish Cap

and Norway than with other areas. Thus, initially two possible alternatives may arise, either Irminger Sea is a different stock, as Flemish Cap and Norway, or all the areas conform to a single stock. More alternatives will be discussed later.

The variable that discriminated the most between *S. mentella* in the different areas was 2DA, that measures body depth at the anus. This variable was the first entering the stepwise analysis, and with it, Wilk's lambda drops to 0.34. The second variable in importance for the discrimination was DO (eye diameter).

Geometric and traditional morphometrics yielded slightly different results in the morphological study performed with *S. mentella*. However, the results were not contradictory, and seem to differ from two main causes, the different resolving power of both techniques, and the fact that Norway and Flemish Cap data were not available for geometric morphometrics, as explained next. In the geometric morphometric analysis, Irminger Sea *S. mentella* appears as a different group from the one constituted by Faroe Island, Greenland and Iceland. Geometric morphometrics capture better the shape and differences not detected by the traditional approach can emerge with the geometric techniques. But using traditional morphometrics this difference was also detected: the first canonical root separated two groups, Irminger-Norway-Flemish and Faroes-Greenland-Iceland. However, perhaps Irminger Sea similarities with Norway and Flemish Cap decreased the percent of correct classification to a level less than the minimum to be considered as a different group. The geometric morphometric graphs shows that the difference between *S. mentella* in Irminger and in the other areas is principally related to two landmarks that made the shape of *S. mentella* in Irminger more hydrodynamic (i. e., thinner in the tail region). On the other hand, in traditional morphometrics, the variable that measures the body depth at anus (2DA) appears as the one that contributes most to differentiate between the two groups. However, it is not known if the body depth is larger or smaller for *S. mentella* in Flemish Cap and Norway, since these two areas were not analyzed with geometric morphometry. However, it can be hypothesized that this variable would be similar to *S. mentella* in Irminger, as Irminger, Norway and Flemish Cap are very close in the traditional approach (they cluster together and are separated from the rest of the areas by the first canonical root).

Irminger Sea *S. mentella* are more pelagic, with important feeding and reproductive migrations carried out each year. This pelagic character can mark the difference in body shape with the more sedentary *S. mentella* from the surrounding coastal areas. This different shape does not necessarily imply genetic differences between *S. mentella* from Irminger and the other areas, because morphometrical characters have a labile condition throughout life (Wainwright *et al.*, 1991). In other words, the adaptive phenotypic differences between groups of fish may reflect phenotypic plasticity instead of indicating genetic differentiation between the groups (Swain and Foote, 1999). Thus, once the *S. mentella* enter in the Irminger Sea and adopt pelagic behaviour, the fish form a stable group that does not migrate strongly to the shelves and hence gradually change shape. This is only a theory, and further studies are necessary to test it. However, the differences between species support this theory, as stated before in this discussion, a gradient from the shallower body shape of *S. mentella* and the deeper body shape of *S. viviparus* through the intermediate shape of *S. marinus* is in accordance with the more pelagical habits of *S. mentella* and the more bottom-

related habits of *S. viviparus* through the intermediate habits of *S. marinus*. Furthermore, Valentin *et al.* (2002) found that *S. mentella* exhibits a more fusiform body shape than *S. fasciatus* in the Gulf of St. Lawrence, where *S. fasciatus* occupy the same ecological niche of *S. viviparus* in the East Atlantic.

The analysis of microsatellite variation performed by Roques *et al.* (2002) defined three geographically distinct population units in the North Atlantic: Eastern (Barents Sea and Norway), Panoceanic and Western (Gulf of Saint Lawrence and Laurentian channel). However, the third population is restricted to a particular area, the Gulf of Saint Lawrence and the Laurentian Channel, where evidence of introgressive hybridization between *S. mentella* and *S. fasciatus* was found (Roques *et al.*, 2001); but in another area in the West Atlantic, the Grand Bank, it was part of the Panoceanic population. Thus, excluding this particular area, *S. mentella* is genetically one population from the Grand Bank to the Faroe Islands. Although *S. mentella* in the Northeast Arctic has traditionally being considered as a single stock, and this independence is supported by microsatellite variation (Roques *et al.* 2002), genetic studies of haemoglobin and enzymes shown great uniformity within each of the *Sebastes* species in the Northeast Atlantic, from Norway to East Greenland (Nedreaas and Naevdal, 1991).

In the study of Saborido-Rey (1994), *S. mentella* from the East (Svalbard and Norway) and the West (Flemish Cap and the Grand Bank) were introduced in a discriminant analysis, yielding four different populations. The confusion between populations occurred principally among the geographically closer areas, i. e., among Flemish Cap and Grand Bank and among Svalbard and Norway.

Rikhter (1996) studied the structure of *S. mentella* in the Irminger Sea in relation to larval drift, and concluded that two different *S. mentella* populations live in the Irminger Sea, one on the coasts of Greenland and Iceland and another pelagic, and that although some of the individuals of the pelagic stock sometimes penetrate onto the eastern Greenland shelf, they never returned to the open sea. However his theory was opposed to the existence of a movement of individuals from the Greenland slopes into the open Irminger Sea, demonstrated by Stransky (2000). Furthermore, no juveniles were founded in the open Irminger Sea, but only adult individuals.

Joensen and Grahl-Nielsen (2004) studied the population structure of *S. mentella* using the fatty acid profile in the heart tissue. The author found similarities between one sample from the Faroes Plateau and another from the Norwegian Sea; similarities were also found between one sample from the Faroes Bank, another from southern Iceland, and a third sample from the deep Irminger Sea; a sample taken in the eastern part of the Icelandic Plateau clustered apart, and finally the sample taken in the oceanic layer of the Irminger Sea was also independent from the others. So, a high complexity on *S. mentella* structure in this area is apparent from this study. However, the results lack congruence, and the similarities seems to be aleatory, as there are no gradients of similarity or dissimilarity. Perhaps further analysis based on more continuous sampling is desirable to give consistency to the relations found with the study of fatty acid profiles.

Several studies taking into account different aspects of the biology of *S. mentella* have been carried out by different authors, trying to elucidate the structure of *S. mentella* in the Irminger Sea and adjacent waters (Pavlov *et al.*, 1989; Alekseev, 1999; Saborido-Rey *et al.*, 2001; Melnikov and Bakay, 2002) or trying to find biological significance for the genetic differences found in the pelagic component of *S. mentella* in the Irminger Sea (Melnikov, 1998; Bakay and Melnikov, 2002). Several biological aspects were revised, such as length distributions and maturation curves in different areas around Iceland, Greenland and on the coast of Canada from the north of the Labrador Peninsula to Flemish Cap, analyzing the various stages of the life cycle and the seasonal cycles of *S. mentella*; several characters considered to discriminate between oceanic and pelagic deep-sea *S. mentella* in the Irminger Sea, such as infestation with the copepod *Sphyrion lumpi* or the presence of pigmented patches on the skin have also been extensively studied. All these studies conclude that *S. mentella* is represented by a single population subdivided into reproductive and vegetative regions, and enclosed in the common subarctic cyclonic circulation system, and it is remarkable that no differences were found between oceanic and pelagic deep-sea types in the Irminger Sea.

Genetic results obtained in the Redfish project seems to confirm the relation between redfish in Flemish Cap and Norway, despite of the fact that those areas are located on opposite sides of the North Atlantic. Although the existence of shallow submarine plateaux which extend from Greenland to Scotland (and passing through the Faroe Islands and Iceland) present a major obstacle at depths greater than about 400m, and a complete barrier at depths greater than about 850 m, there is a fairly free passage of surface water between the Norwegian and Greenland Seas and the North Atlantic, with water flowing into these seas mainly between Scotland and Iceland, and out mainly between Iceland and Greenland (Brown *et al.*, 1991). *S. mentella* is known to perform extensive migrations, and advantage can be taken from the surface currents that enters the Norwegian Sea between Iceland and Scotland, after crossing the most southerly part of the Irminger Sea. Morphometrically, the maximal confusion occurred between Norway, Flemish Cap and the Central and NAFO-Irminger Sea subareas, but the reason for the similarity between *S. mentella* from these areas is unknown. A possibility is the existence of a migration of adult fish between these areas, that is, in accordance with the water movements in the north Atlantic. However, perhaps a migration of adult fish between these far areas is not very likely. Another explanation for these similarities could be the existence of ecological convergence in the absence of competition. This ecological convergence can also explain the similarities found in *S. marinus* from Norway and Flemish Cap, as this species is not as pelagic as *S. mentella*, and thus, migrations between both areas are very unlikely.

Excluding the reference areas, and taking into account only the core area, that is, the Irminger Sea, Greenland, Iceland and the Faroe Islands, a gradient of similarity proportional to the geographic distance has been observed for both *S. mentella* and *S. marinus* populations. Thus, individuals from closer areas are morphometrically more similar, and this similarity diminishes with the increase of geographic distance. Phenotypic plasticity has been defined as 'the ability of a single genotype to produce more than one alternative form of morphology in response to environmental conditions' (West-Eberhard, 1989). This phenotypic variation modulated by environmental conditions is typically continuous (Swain

and Foote, 1999), and can indicate the prolonged separation of postlarval fish in different environmental regimes (Campana *et al.*, 1995).

The existence of a main extrusion region for the whole area where the larvae are distributed, and from where they drift to the East Greenland slopes where the nursery area is located; the movements of those juveniles towards West-Greenland (with the sporadic passage of some of them to the Canadian coasts if the currents are favorable and from there to southern areas), and back to East Greenland and their redistribution to the Icelandic shelves, and from there to the Faroe Islands shelves, gives sense to the homogeneity of *S. mentella* and *S. marinus* in the whole area. However, redfish from different areas are not the result of a single annual spawning event concentrated in one particular area, but local, although minor, spawning areas exist. This means that fish living, for example, around the Faroe Islands come partially from spawning grounds close to the Faroes, and partially from the major spawning grounds, i.e. south and southwest of Iceland in the case of *S. marinus*, and in the Irminger Sea for *S. mentella*. On the other hand, although fish growing in Greenland are able to appear in the Faroe Islands, and the opposite, the normal situation is that migration takes place between closer intermediate areas. If the most different fishes from Greenland were compared with the most different fishes from the Faroe Islands, morphometric differences would probably occur. But what actually occurs is that fish from neighboring areas are mixed, and what is most important, the new recruitment spreads from the main spawning area, where adults of all the surrounded areas migrate to release larvae and are all mixed, producing the lack of significant differences among areas and leading us to consider single populations in both species. The pelagic behaviour of *S. mentella* in the Irminger Sea could produce a change in shape that, although slight, is enough to detect by geometric morphometrics, and separate Irminger Sea *S. mentella* in an independent group.

S. mentella phenotypes in the Irminger Sea

With regard to the proposed phenotypes for *S. mentella* in the Irminger Sea, *oceanic* and *pelagic deep-sea*, they are, without doubt, morphometrically identical. The absolute lack of discrimination between the two types leads to the conclusion that both belong to the same population, at least morphometrically. Genetic data of the individuals analyzed for the putative *S. mentella* phenotypes in the Irminger Sea were not available, and therefore, it was not possible to check if morphometrical differences exist in those individuals that present genetic differences, as it was done in Faroes and Greenland.

Fish separated into phenotypes by Icelandic experts were analyzed, and morphometric differences were not found between the types, neither with traditional nor with geometric morphometrics that analyze better the shape of the individuals, and so, enhance the differences. In addition, some of the characters used to identify the two types were recorded in the laboratory by the author; the presence of parasites (*Sphirion lumpi*), the position of the third preopercular spine, and the presence of pigmented patches on the skin were used to divide the *S. mentella* from Irminger into the two types. However, no differences were found.

The three different stocks of *S. mentella* in the Irminger Sea and adjacent waters have been described from the management point of view; initially, *S. mentella* was only fished on the coast, and only one stock was considered. With the discovery of a pelagic component, the

fishery moved to the open Irminger Sea, and at that moment, a second stock was defined (oceanic *S. mentella*). With the movement of the Irminger Sea fishery to deeper waters, the third one was described (pelagial deep-sea *S. mentella*). Obviously, these are 'Fishery stocks' as described by Smith *et al.* (1990), that is, groups of fish exploited in a specific area by a certain method, that is, the fish caught in a certain management unit. The term 'fishery stock' is used without clear correspondence to biological stock (Hammer and Zimmermann, 2005).

However, genetic indications of the existence of different 'types' of *S. mentella* in Irminger were described (Johansen *et al.*, 2000). It is not clear whether those types are members of different stocks (Hammer and Zimmermann, 2005), and they completely lack morphometrical differences, even using geometric morphometrics that has a larger power of discrimination between groups.

In recent years, an increasing effort to study genetic discrimination of the redfish species and stocks has been made (Johansen *et al.*, 1996, 1997, 2000; Daniélsdottir and Jónsdottir, 1999; Roques *et al.*, 1999a, 1999b, 2001, 2002; Daniélsdottir *et al.*, 2005; ICES, 2005 and references therein). Several techniques have been used (haemoglobin, allozymes, mtDNA, nDNA, etc.). The results of these studies do not show a clear pattern regarding stock structure of redfish, and in most cases they yield contradictory results. The discrimination of the *S. mentella* types is supported in some cases by the haemoglobin and allozymes analyses (Johansen *et al.*, 1996, 1997, 2000). However, studies made in the mid-1980's in the depth range of 0 m to 500 m in the Irminger Sea pelagic waters did not reveal any genetically isolated groups of *S. mentella*. Besides, no signs of crossing with closely-related redfish species were observed (Dushchenko, 1986). In addition, allozyme analysis indicated genetic differences with age for samples collected from the Irminger Sea (Stroganov and Novikov, 2005). Regarding the molecular genetic studies conducted on microsatellites and mt-DNA, some of these studies show the presence of genetic structure in the Irminger Sea and adjacent waters (Daniélsdottir and Jónsdottir, 1999; Daniélsdottir *et al.*, 2005; ICES, 2005) while others show lack of genetic differences in this area (Roques *et al.*, 2002; ICES, 2005). In general, these studies revealed lack of genetic isolation by geographic distance, a very complex resulting structure, and genetic differences among *S. mentella* types much smaller than those observed in *S. marinus* in the same area, currently considered as a single stock (ICES, 2005).

The existence of two stocks in the Irminger Sea was first hypothesized by Icelandic researchers (Magnússon, 1977, 1983, 1990; Magnússon and Magnússon, 1995), and the skill to distinguish the putative stocks was developed exclusively in Iceland. It can therefore be assumed that only fish identified by Icelandic researchers are suitable to study morphometric differences. This was tested in this study, and an analysis was made to discriminate between the two types using only those individuals separated into types by Icelandic research, but giving the same result: a complete lack of morphometric differences. Moreover, because differences between stocks are based on some qualitative characters, and to overcome possible errors of the phenotypes classification, samples from the Irminger Sea were reclassified into phenotypes based in part of those characters, i. e., the presence

of parasites (*Sphirion lumpi*), the position of the third preopercular spine, and the presence of pigmented patches on the skin. However, as stated before, no differences were found. On the other hand, the ecology of *S. mentella* in the area does not support the existence of two different stocks. There are contradictory results, and if we assume as correct some of the genetic results, the question arising is, what is the explanation for the existence of different genetic types in the Irminger Sea? Variation exists at many levels below that of species, occurring among subspecies, stocks, substocks, year classes, family lineages, individuals and even intraindividually (i. e., heteroplasmy, bilateral asymmetry), but much of this variation is tangential to the purposes of stock discrimination (Waldman, 1999).

For rockfish in the Pacific it was stated that a dominant feature of reproduction is a pattern of infrequent and irregular years with successful recruitment during periods with favorable environmental conditions, and many years with poor recruitment (Leaman and Beamish 1984; Botsford *et al.*, 1994; Ralston and Howard, 1995). However, an entire year class may not experience favorable environmental conditions because of variation in the timing of larval release. Larson *et al.*, (1998) found that recruits of *S. jordani* exhibited reduced genetic variability compared to the adult population, suggesting that surviving young of the year are the products of reproduction by only a small fraction of the adult population. Reproductive success appears then to be restricted to narrow spatial and temporal windows when conditions are favorable for larval survival. (Parker *et al.*, 2000). In the Irminger Sea, redfish release their larvae in a wide area over the Reikjanes Ridge. The currents bring the larvae to the nursery area in Greenland. So total or partial failure of recruitment would not being uncommon. In addition, redfish is a long-lived species, and many different cohorts, probably more than 20-30, are involved in the spawning fraction of the population. In a long-lived species, with relatively high fecundity, a population can be sustained by few but abundant year-classes, which can be mature for many years. So, even if there is recruitment failure during long periods, the population can be sustained if the spawning stock produces a few strong year-classes. As a result, the population can mostly be maintained by a few year-classes, and usually with large age differences. To find genetic differences is therefore not surprising. This hypothesis is supported by genetic differences found between redfish cohorts by Schmidt and Trautner (2005).

Comparing genetics and morphometrics. The importance of the sampling procedure.

One of the most interesting analyses performed was that comparing genetic and morphometric results in the same individuals. The comparisons showed a very good concordance between both methodologies, as the fish separated into species by genotype also shown differences in body shape. This has been done in samples from the Faroe Islands and Greenland, using genetic data from Cathrin Smith (Institute of Marine Research of Hamburg, Germany) and Torild Johansen (University of Bergen, Norway). Unfortunately, no individuals from Iceland or the Irminger Sea in which both morphometric and genetic analyses had been performed were available.

The aim of sampling must always be the selection of a sample representative of the population. It is important to obtain a good coverage of the area and the target species. However, sometimes the sample is far from representative of the population, and the results obtained from this data can not be extrapolated to the whole population. One exercise illustrating this problem was carried out with those individuals for which genetic data were available. The aim of the exercise was to point out the inadequacies of selecting the individuals instead of making a random sampling. Thus, *S. marinus* showed a complete overlap between the the Faroe Islands and Iceland when all fish were taken randomly from the surveys and fisheries in both areas. In that analysis, the species were assigned by both GBM and haemoglobin, with a total correspondence between these two methods. However, when fish that clearly showed a *S. marinus* genotype were selected, morphometric differences occurred between Iceland and the Faroe Islands. It is not possible to make mistakes about the origin of the samples, those collected in Iceland were really collected there and the same is true for the Faroe Islands. Therefore, the lack of differences when comparing the whole dataset (random samples) means, without doubt, that the fish in both areas belong to the same stock, and that the differences occurred because the fish were selected for “pure” genotypes. This may mean that when a fish is selected to analyze its genotype, it may drive the results, and hence the interpretation and conclusions reached. So, fish have to be sampled randomly, and randomly compared.

Similarly, if morphometric comparisons are made between the individuals at the extremes of a shape gradient, that is, between completely different morphotypes, differences can be found. But the populations are constituted not only by the ‘pure morphotype’ fish but also by the whole range of shapes between them. If the morphometric analyses were performed on the all (random sampled) kinds of individuals, such differences in body shape would not be found; on the other hand, when morphometric analyses were conducted on selected fish, differences occurred. This implies, again, that selection of the samples may lead to wrong interpretation of the results.

So, the morphometric results follow a coherent pattern that is very much in agreement with the ecological theory of population structure developed in Saborido-Rey *et al.* (2005). This leads also to consideration of the importance of random sampling, because differences may appear if fish at the extremes of morphotypes are selected, but those differences would not be representative of the reality.

This is, nevertheless, a theory that potentially explains the morphometric pattern encountered, but further morphometric analysis had to be performed.

The sampling of the present study was also conceived to study fish movements between and within areas in the same or in different years. This kind of study can be performed in Iceland, Greenland and the Irminger Sea with the available data. The long period of acquisition of the samples, the problems to solve in the data acquisition and coordination of the two laboratories, together with the long data screening and the large quantity of analyses following two different morphometric techniques (traditional and geometric morphometrics) and meristic, have so far prevented those studies from being performed, but a future perspective lies in this direction.

Summarizing, the morphometric analysis indicated that, without doubt, both *S. marinus* and *S. mentella* on the shelves of Greenland, Iceland and the Faroe Islands constitute single populations respectively. The differences between *S. mentella* on the shelves and *S. mentella* in the open Irminger Sea were very slight, and only geometric morphometrics detected these differences at a level that may lead us to consider the Irminger Sea as a separate population from the adjacent areas. What would be revealed if Norway and Flemish Cap could be introduced into the geometric morphometric analyses is unknown. However, whether *S. mentella* in the Irminger Sea classifies as a different group or not, some differences exist, as they were also detected by the traditional morphometric analysis. What is relevant is the fact that the reason for these differences between the demersal and pelagic *S. mentella* may be derived only from the pelagic behaviour that made the body shallower, more hydrodynamic. If, following the ecological hypothesis, *S. mentella* in the whole area release their larvae in the same time period and in the overlapped areas, and if there is only one main nursery area where the juveniles recruit until they became adults, the question arises, do the fact of that some of these fish remain on the shelves or enter the pelagic Irminger Sea make them different populations?

Another question is the relation of *S. mentella* and *S. marinus* from this central area and the reference areas, i. e., Norway and Flemish Cap. In those areas, all fish stages are represented, as spawning has been observed. *S. marinus* in Norway is a different population, and probably also *S. marinus* in Flemish Cap, although the lack of samples from the Canadian Coast diminishes the potential of the analyses performed; thus, no final conclusions can be derived for the case of Flemish Cap. On the other hand, the similarities found for *S. mentella* in Norway and Flemish Cap could reflect exclusively that *S. mentella* in those areas have undergone a convergent shape evolution, since it is unreasonable to believe they constitute the same population. It is hypothesized here that they are different populations. However, to ascertain the morphometric relationships between all these areas, it would be interesting to have more samples from other areas in the West and East Atlantic and perform both traditional and geometric morphometric with all samples.

Implications for redfish management

ICES advice for redfish species in the Irminger Sea and adjacent areas considers one stock of *S. marinus* and two of *S. mentella* i. e., a demersal unit on the continental shelf of Iceland, Greenland and the Faroe Islands, and the pelagic unit in the Irminger Sea and adjacent areas.

Most of the studies dealing with population structure are concerned with the problem derived from managing multiple stocks as if they were a single unit. However, there is also considerable risk in the opposite situation, i. e., managing one single stock as if there were different units. The greatest risk is a biased perspective of stock productivity (S. Cadrin, pers. comm.).

The latter would be the situation if ICES continues to assess *S. mentella* as two stocks, or even three, if it really were one single stock, as the results of this morphometric study suggest. Pelagic *S. mentella* in Irminger is the bulk of the adult part of the stock. The juveniles, however, are principally distributed in East Greenland. Both parts of the same stock are managed as if they were different stocks. *S. mentella* reproduction is very likely to follow a pattern of irregular years with successful recruitment during periods with favorable environmental conditions and many years with poor recruitment. The number of adults does not affect recruitment in a direct proportion, as several factors take part. The adults release larvae in a wide area over the Reykjanes Ridge, and the larvae drift with the currents to Greenland slopes. How many of those larvae reach the Greenlandic coasts would depend on the area that the adults occupied when releasing the larvae. Furthermore, the time interval could be diminished if the number of the breeding fish decrease, diminishing also the probabilities that some of the larvae were released in an optimum environmental window.

On the other hand, part of the adult population stays on the shelves, and part migrates from the nursery to the open sea, as was demonstrated. It is not known if the contribution of these young adults to the open Irminger Sea is density-dependent or not, since this migration was observed in years with the presence of very strong recruitment. Nevertheless, the settlement on the shelves of adult fish in some years and in the open Irminger Sea in other years, may lead to the false idea that both putative stocks are very productive. However, both depend of the same spawning stock, i.e. Irminger Sea, which may be overfished when relying on false productivity estimates. The risk is even higher if one of the areas is normally underexploited, as in the case of Greenland, but exploited later in the belief that it is a self sustained stock. The rates of exploitation of the newly fished area may be perceived as sustainable when they are not, since recruitment comes from a different area, already heavily exploited. The most precautionary assessment is that managing single biological stocks, the opposite, in one or another direction, would be a hazardous option.

The management of the two areas, the shelves and the open Irminger Sea, should be made as a single stock until more research demonstrates the existence of separate stocks. The consideration of a single stock is based on strong evidence showing that Greenland is the nursery area of the Irminger Sea; there are no differences within Greenland, and this area constitutes a single stock with the Faroe Islands and Iceland. Although a complex structure may exist, the strong relationships among the components prevents a separate management which may cause the overexploitation of the stock.

6. CONCLUSIONS

6.1. CONCLUSIONS DRAWN FROM THE METHODOLOGY IMPLEMENTATION

6.1.1. Data acquisition protocol

A precise protocol for data acquisition has been developed. It has been shown as an important advance and a critical tool in this type of research. It is highly recommendable to implement this type of protocols in similar studies, principally if more of one laboratory is involved.

- Landmarks

Landmarks placed on soft tissues may become easily deformed introducing uncontrolled error in the analyses. They should not be used as landmarks. Instead landmarks should be placed in easily recognizable positions and analogous from fish to fish.

Short distances, i. e. distances from landmarks placed too close, showed a high coefficient of variance as consequence of the intrinsic measurement error. It has been illustrated when distances were measured by different persons but also in repeat measurements performed by the same person. Although the error should be random, it may introduce important noise in multivariant analysis if the precision is not high enough.

- Digital camera

Therefore, the use of low resolution digital cameras diminishes the precision and accuracy in the measurements. One pixel (the minimum unit in image analysis) in a small distance creates relative large errors.

It is recommended the use of a lens without aberration and placed the camera lenses parallel to the fish.

It is recommended to use a focal distance equivalent to 50 mm in traditional photography to avoid optic distortions.

- Calibration

Fish body width produces that part of the measurements lay in tilted planes, but they become flat in the photos. Differences in body width among fish with different size yield biased distances in images. In order to compare 2D and 3D measurements, the utility of a double calibration, being one of the calibrations based in a 3D measurement made in each of the individuals, was demonstrated. Thus, using the most appropriate of the calibration distances for each of the measurements, the accuracy between 3D and 2D measurements reaches a relative error less than 3%. This error is similar to the error produced when the fish is measured by two different persons.

6.1.2. Data analyses

- The meristic approach

The different *Sebastes* species and populations showed significant differences in meristics. However, these differences were biologically groundless.

Meristic variables showed significant correlation with fish body size. The correlations did not follow a particular trend and ontogeny was discarded as origin of this variation. Instead, differences among cohorts has been hypothethized as the factor producing such correlations.

Thus, special caution must be taken when studying meristic variation in long lived fish species, as differences can exist within different cohorts of the same population, as they could have been born under very different environmental conditions.

- Multivariate discriminant analysis for morphometric differentiation

Discriminant analysis is an useful tool in morphometric studies, as shown also for redfish. However, it requires a proper definition of *a priori* groups. The identification of redfish species is, thus, of major concern. If no accurate methods are available for the species identification, it has to be taken into account when interpreting the results.

It was demonstrated that the gas bladder musculature pattern is a useful tool for species identification in all the studied areas, except in Greenland. This method was in total accordance with genotypes.

The use of the gass bladder musculature for species identification is recommended in the Faroe Islands, especially in the Faroes Bank, as the external appearance is specially deceitful in this area.

It is highly recommended to compare groups with the same number of individuals in order to avoid the overestimation of the bigger groups, and in consequence the underestimation of the smaller groups. The high morphometric similarity between redfishes enhance this problem.

- Sampling strategies

Samples has to be taken randomly. If the individuals are selected, spurious results will be obtained.

In species widely distributed, as redfish, the sampling should cover all the phenotypic gradient range, to avoid spurious differences that can be attained when comparing discrete areas of the gradient.

- Comparing traditional and geometric morphometric techniques.

Geometric morphometrics seems to be more powerful than traditional techniques to detect differences between redfish. However, both techniques yielded the same results, although

slight differences in traditional morphometric methods become bigger when using geometric morphometrics.

Geometric morphometrics graphical displays provided a visual and intuitive overview of the differences between groups. It was complemented with traditional morphometry, as with this technique, the variables that contributed the most to the discrimination were specified.

6.2. CONCLUSIONS DRAWN FROM THE MORPHOMETRIC STUDY

6.2.1. Conclusions derived from the species differentiation

In morphometric analysis special care must be taken when samples from different areas are included to study species differences. Due to the high similarity among redfish species, the environmental influence on the shape cause individuals of different species but living in the same area to be more related than individuals of the same species living in different areas.

The four *Sebastes* species in the North Atlantic are morphometrically distinguishable. The variable that most contributes to the discrimination is the oblique distance that joins the first ray of the dorsal fin with the first ray of the anal fin.

In Greenland, no morphometric differences were found when species were identified by the external appearance or the gas bladder musculature. However, morphometrical differences were found between *S. mentella* and *S. marinus* when species were identified by its genotype. However, given the limited number of genotyped fish, it is very likely that the analyses was not representative of the whole population.

There is a gradient from the deep body shape of *S. viviparus* to the shallower body shape of *S. mentella* with an intermediate form for *S. marinus*. Probably, *S. fasciatus* has a similar body shape to *S. viviparus*.

6.2.2. Conclusions derived from the population differentiation

Different redfish populations can be distinguished with the aid of morphometric tools. This is the first time that samples from Irminger Sea, the Faroe Islands, Greenland and Iceland are analyzed.

Individuals of the same species present a differences-similarities gradient in agreement with the geographical distance within the distribution area.

Three different populations of *S. marinus* have been identified: one in Norway, another in Flemish Cap and the third one comprised by the Faroe Islands, Iceland and Greenland.

The Central Atlantic population, the Faroe Islands, Iceland and Greenland, has a closed relation with Flemish Cap than with Norway. However, more samples covering the whole distribution area are necessary in order to draw a firm conclusion.

S. mentella from the Faroe Islands, Iceland, Greenland, and the Irminger Sea conforms a single population. However, the former three areas showed closed relationship between them than with Irminger Sea.

S. mentella in Irminger Sea present a more fusiform shape that *S. mentella* in the other more coastal areas, what is in accordance with its more pelagical behaviour.

No morphometric differences were found among the two *S. mentella* phenotypes described in the Irminger Sea. The pelagic component of *S. mentella* living in the Irminger Sea is morphometrically a single unit.

The similarities between *S. mentella* from Flemish Cap and Norway are probably due to a convergent evolution.

- Morphometrics versus genetics

A high agreement between results of the morphometric and the genetic approaches has been obtained when comparing the different redfish species in the areas where both kind of data were available, i. e., the Faroe Islands and Greenland.

No morphometric differences were found between the two *S. mentella* phenotypes in the Irminger Sea, i. e., pelagic deep-sea and oceanic *S. mentella*, although preliminary genetic studies evidence differences between them. This lack of morphometric differences was found also in individuals separated by phenotype by Icelandic experts. However, unfortunately, there were no individuals in which both, genetic and morphometric data, were available to test in the same individuals if those genetic differences corresponds also to morphometric differences.

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MORPHOLOGIC PROTOCOL

8.1. MORPHOMETRICS

8.1.1. Introducción

The main goal of this protocol is to develop and explain a methodology that helps the measurement of morphometric and meristic variables. This protocol refers to redfish species, although most of the explanations are general statements and can be easily used for other species.

Traditionally, morphometric measurements were taken with a caliper measuring the distances between defined landmarks (defined and easily recognized points on the fish body, which permit take the same measurement from fish to fish). This methodology has several limitations; first, only few distances can be taken to optimize manpower, because it is a great time consuming; second, fish is usually discarded after, so repetition of measurements (in case of detecting errors), or measuring new variables is not possible. And third, if several people participate in taking measurements (as different countries), intercalibration become a difficult task.

To overcome these, and other limitations, and because of the improved technology on digital photography and image analysis, it was decided to use fish digitized image to measuring distances between landmarks. However a detailed protocol should be developed and described on how to proceed, since several laboratories are involved on this task. Thus, the aim of this protocol is to describe the methodology to prepare fish for taking a digital photo where different landmarks must be easily recognizable. Later, digitized images of each fish will be used to take morphometric measurements directly on the image aided by an image analysis software package.

This methodology will provide a complete database of images to exchange among partners for future comparison of results. On the other hand, to record digitally each fish will permit the possibility of taking new measurements at a later state if it is necessary or revise those already taken.

However, fish is a three-dimensional object, while a picture represents a 2-D object. This means that fish width (i.e. fish height when laying down ready for the picture) will drastically affect to the distances between landmarks when the landmarks are not in the same plane. This fact has two major effects: first, as biggest the fish, smallest the distance between landmarks taken from the picture; and second, distances from the picture are not real. The former effect will unavoidably affect the morphometric analysis, so it must be considered and solved. The later is only important if the measurements digitally taken are to be compared with measurements taken with caliper (as it is the case in this project). Both questions were studied and solved as explained in the Annex. Briefly, it can be said that these problems were solved taking two calibration distances, one from a ruler placed in the base, and other taking a real distance in the fish with a caliper. It was decided to use de so-called D2D (distance between landmarks B-C, see section 8.1.3.5).

8.1.2. Material

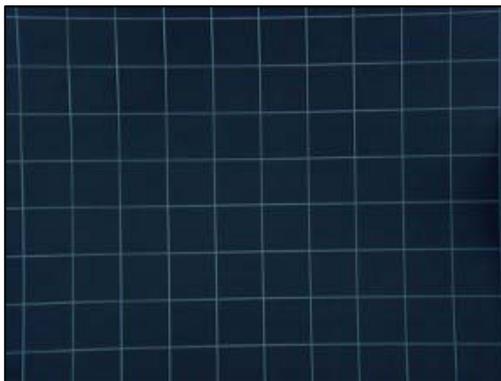
In order to take good quality images, we should have the follow set of photographic material (Figure 8.1).



- ✓ Digital camera
- ✓ Baseboard and column with sliding camera head.
- ✓ Lighting assemblies with tungsten lamps.
- ✓ A graduate rule without a reflective surface (at least 20 cm long). Flat and thin.
- ✓ Polyspan plate/panel
- ✓ A digital caliper
- ✓ Entomological pins (N° 4). The color of the head must be well distinguishable from the fish body.
- ✓ Waterproof identification labels.
- ✓ Pencil or waterproof pen.

Figure 8.1. Photographic material.

The **digital camera** needs a very good lens that avoids aberrations like the one shown in Figure 8.2. This is a very important issue because we are taking measurements from the pictures and these measurements have to be as much real as possible. Before buy or use a



camera, aberration has to be checked. It is highly recommendable to use a camera with exchangeable lenses. Use standard focal distance (between 28 and 35 mm in digital imaging). Aberration and how to deal with it are explained in Annex II.

A **lighting system** can be used if necessary. If so, consider that lights must be placed in such position that shadows are minimized and reflection over the fish avoided.

Figure 8.2. Aberration produced by bad quality lenses.

A **graduate ruler** is used to calibrate each picture. Therefore this is a critical question. The rule has to appear in each image, otherwise it is not useful. It's essential that the ruler lines be clearly distinguishable. Lines on the ruler have to be always well defined and be exact. It is also recommend to use stainless steel or plastic ruler to avoid rusting; it must be flat and rigid, but as thin as possible. A digital caliper will be used to take D2D distance, for a later calibration. (See Introduction and Annex II).

It will be used **entomological pins** to mark the fish landmarks, some of them need to be driven into the base where the fish lies, and therefore we need a soft base, preferably a white polyspan plate. The entomological pins have to be black headed to be easily recognized in the image analysis software. If the pins are golden headed (usually), they should be painted.

Labels have to be waterproof and easily readable in the final picture. The fish identification will be writing on it, in the way we describe later in this text. If the samples were taking by an observer, and has another label, it's very advisable that this original label appear in the picture too, for posterior consultation if necessary.

8.1.3. Methods

8.1.3.1. Data to be recorded

Total length, preanal length, fork length and total weight must be recorded from the whole fish, before any kind of analyses.

8.1.3.2. Combining genetic and morphometric analyses in the same fish

The first question is how combine morphometric and genetic analyses. In order to compare results, it's fundamental to study the same fishes both genetically and morphologically. Genetic samples must be taken from the frozen fish before morphometry, and that's why these samples must be taken without destroy the fish shape. As for morphometric analysis we are going to use a photo taken from the left side of the fish, genetic samples, i.e. gill filaments, muscle and liver, can be removed from the right side of the fish avoiding the fish shape be altered.

8.1.3.3. How to place the fish in position



Figure 8.3. Dealing with evaginated stomach.

For morphometry the fish must be completely (or almost) thawed because we have to drive the pins into the flesh.

Sometimes the fish need to be washed (mucus in excess, dirty, etc), but it must be dried before taking the picture, to avoid reflections.

If the stomach is evaginated, return it into the abdominal cavity (to put the fish in a vertical position as in Figure 8.3, may be useful).

It is very important that the fish looks as much natural as possible, therefore discard fish with broken parts or deformed due to a bad treatment while frozen or whatever.

Place the fish on a white base (polispan is very highly advised), the head in the left and the tail in the right, in order to take measurements from the left part of the body.

The fish must be in a horizontal position and all the pins visible in the photo. If the fish rounds, a little wedge helps to maintain the position.

In that sense, if the fish has been completely thawed it adapts easily to the horizontal position, except perhaps big *Sebastes marinus*.

8.1.3.4. Landmarks

The goal is to take measurements (distances) between points. These points have to be, obviously, the same in each of the fish analyzed. Therefore they must be located in places easily recognized fish after fish. Those points are called landmarks. The distances will be measured aided with an image analysis system, so we are going to take a picture of each fish with a digital camera.

Figure 8.4 shows the 19 landmarks considered essential. They will be marked with the entomological pins.

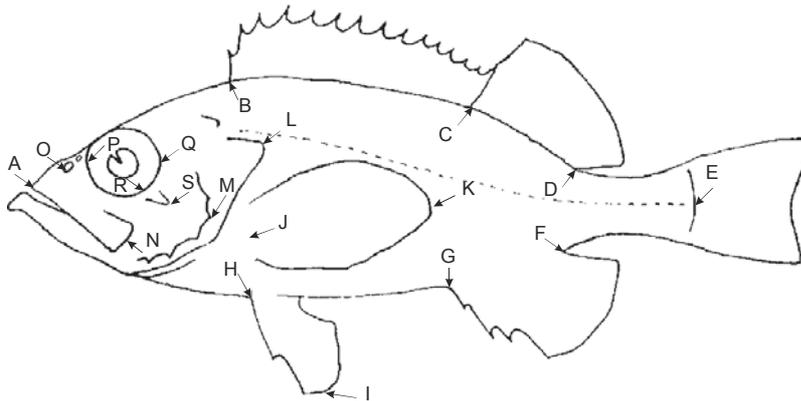


Figure 8.4. Landmarks considered in this study.

To avoid forget put the pin in some of the landmarks, it's advisable to drive into the top of the polystyrene plate exactly the 19 pins. (Figure 8.5). Besides, it makes work faster.



Figure 8.5. Fish on the polystyrene plate with the pins.

It is very important that the pins were placed in the same position in all the fishes analyzed. It is the reason for the following full description on how to place the pins in the fish.

Point A

It marks the snout, i.e. the tip (the more distal part) of the upper jaw. The pin must be introduced under the lip, the pin head must be touching the lip, without deforming it. But attention, this point is not in the center of the upper jaw, but on the left side, in the more distal point of the left side as shown in the pictures

**Point B**

In the base of the first dorsal ray. To know exactly where the base of the fin is, first pull ahead the first spine, this action will create a fold, a corner, showing the exact point where the pin has to be (left picture). The pin is driven from up to down into the flesh, a little bit above the midline of the fish avoiding the bone in the base of the spine and also the vertebra (right hand picture), thus the head of the pin matches exactly over the base of the fin and it is visible from the top (where the camera will be).



Point C

Between the last dorsal spine and the first soft ray of the dorsal fin, i.e. between the first and the second dorsal fins.

As in the point B, the pin is driven from up to down into the flesh, in the same direction as the rays

Point D

Placed in the base of the most posterior ray of the second dorsal fin.

As in point B and C, pin is driven from up to down into the flesh.

Note that at the end of the second dorsal fin, there are a membrane joining the last radio with the body. The reference point is close to the base of the radio, ignoring the membrane.

**Point F**

Marks the end of the anal fin. Note that there is a little membrane at the end of the fin, but the reference point is in the base of the last radio (Figure not shown).

Point G

In the base of the first radio (spine) of the anal fin.

To know where the insertion point of the spine with the body is, proceed as with point B, i.e. pull ahead the first radio, this action will create a fold, a corner, showing the exact point where the pin has to be (left picture)

Point H

In the base of the first radio of the pelvic fin.

To know where the insertion point of the spine with the body is, proceed as with point B and F, i.e. pull ahead the first radio, this action will create a fold, a corner, showing the exact point where the pin has to be (right picture). Then insert the pin completely in the fish flesh upwards.

**Points P, Q, R, S:**

The first three points are in the margin of the ocular orbit. The eye often covers this margin. When this occurs, just cut the ocular membrane (left picture) and fast the ocular globe with a pin (right picture), it will help to keep visible the insertion zones. Another option is to remove the eye.

**Point P**

Right in the orbit edge, in the closest point to the upper nasal orifice.

**Point Q**

The opposite point in the eye-orbit.



Point R

Midpoint of the base of the subocular spine.



Point S

Tip of the subocular spine. If you can not distinguish it well, move this part of the head.



Points L, M, N:

Keep the mouth of the fish closed when driving the pins into these three points.

Point L

Is the most posterior point of the operculum.



Point M

It marks the tip of the second preopercular spine.



Point N

Marks the midpoint of the jaw tip.

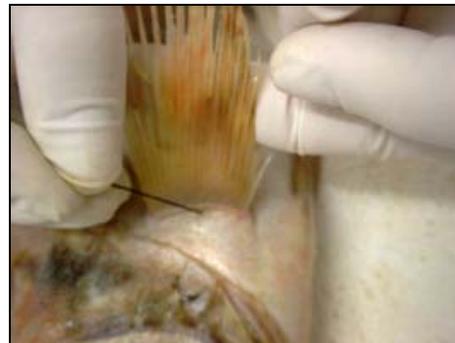


Point O

Marks the anterior part of the lower nasal orifice. Be careful; don't change the shape of the orifice.

**Point J**

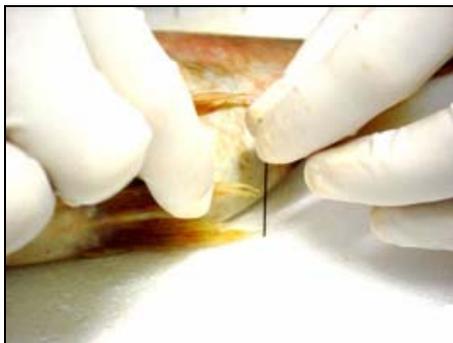
In the midpoint of the pectoral fin insertion. Lift up the fin until 90 degrees to have the correct vision of the insertion point.

**Point K**

In the tip of the pectoral fin (figure not shown). The pin is driven in the abdominal region. Be careful don't sink too much the pin in the abdomen, because it create a shadow around the pin head that will create difficulties to recognize the point later on.

Point I

Extend the pelvic fin, close to the fish body. Drive the pin into the polystyrene base, keeping the head at the same height than the pin previously fasted in the point G.

**Point E**

Hipural point. It is the point where the lateral line ends, in the tail of the fish (Figure not shown).

Once you have fasten all the pins, open the mouth of the fish and fix it with other pin (preferably other color than black), in order to see clearly the beak and the 'point A' pin in the picture.



8.1.3.5. Calibration

At this moment it is very important to take the D2D measurement (distance between points B and C) with a caliper, preferably a digital one. D2D distance will be used to calibrate and calculate some of the distances.

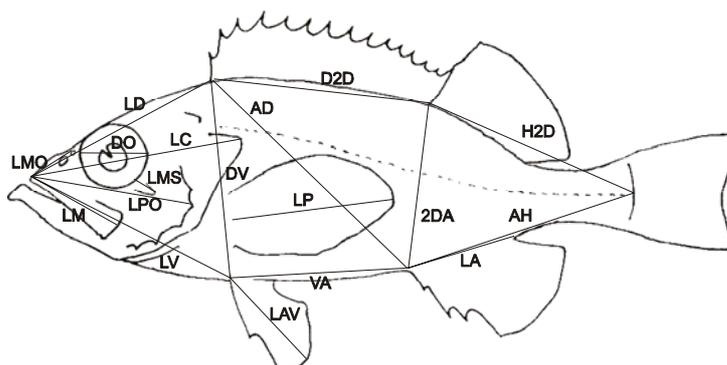


Figure 8.6. Fish ready to take pictures

8.1.3.6. Digitizing

Once all the pins are placed (Figure 8.6), be sure they are clearly visible from above, in the view the camera is going to take. For this, remove flesh, scales or other particles covering pin heads.

Place the labels on the base. As explained in section 8.1.2, label should have the required information to identify the fish. We recommend including the original label too, this is the label that was written when collecting the fish.

A ruler with the characteristics explained in the paragraph 8.1.2, must be placed on the base. If a lighting system is used, take into account that lights must be placed in such position that shadows are minimized. At the same time, reflection over the fish has to be avoided. Once these two premises have been achieved, the system doesn't need to be changed anymore. As have been told above, D2D distance is measured with a caliper. If a digital caliper is used, once the distance has been measured it can be carefully placed with the fish to take the picture. Thus, D2D distance will be recorded.

There are some questions about the camera position. In order to be sure that the lens is parallel to the baseboard, once you turn the camera on in the holder, slide it down the column placing the lens cap on the baseboard in such a way that the whole lens cap is in contact with the baseboard. Then, you must slide the camera up to the top of the column carefully, without change the camera angle. The importance to maintain this parallel position is explained in Annex II.

Camera should be at a height where focusing is possible, field depth is long and aberration doesn't exist. It is recommended to use a focal distance equivalent to 50 mm in traditional photography. Avoid place the camera too far using tele lens. It's also advising to place the camera in such height that further adjustments are not required when fish of different sizes are recorded.

Remember that the pins are in different horizontal planes, so field depth must be adjusted to ensure all the pins are focused. To achieve this, consider not only the distance fish-camera, but also narrow the diaphragm aperture to increase field depth.

Initially picture should be as much similar as possible to the original (regarding color, for example). However several settings of a digital camera can be changed to obtain a more contrasted picture (as the white balance or exposure time, for example). This fact allows to enhance pins position. It is recommended to take several pictures of the same fish, a first one with normal settings (a picture similar to the original) and others more contrasted. Taking several pictures is quick and inexpensive, but it will considerable help the later work with image analysis.

The images should be stored in TIFF raw format (uncompressed, avoiding reduces quality). Most of the digital cameras allow to save the image together with camera settings. This is a

very important feature and it should be done if possible. However, usually to store camera settings with the image is only possible in a special image format (for example, Nikon save images as NEF format). Other cameras allow saving settings in ASCII. Anyway, the goal is to have a Tiff image (the useful one) and camera settings together with the image in another format.

8.1.3.7. Making measurements

Once the pictures have been digitized and with the aid of image analysis software, the X, Y coordinates of each pin should be recorded and stored in a spreadsheet. Pin coordinates must be recorded in the same order as defined (from A to S) to avoid misreading.

In addition, the coordinates of two points in the ruler must be recorded. These two points are the ends of a given distance in the ruler. Be aware that the bigger this distance, the smaller the error in the calibration, so we recommend take 15 cm or larger. Remember that this distance will be used to calibrate the distances between some of the landmarks.

The two points in the ruler must be in equivalent position, see Figure 8.7 for explanation.

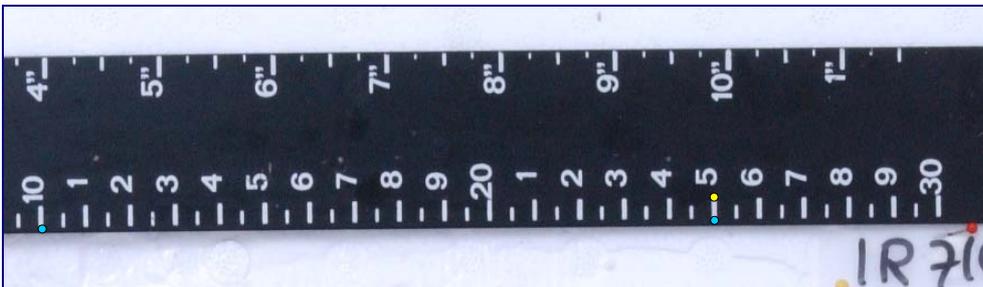


Figure 8.7. Taken the distances on the ruler for calibration.

If the left blue point is first selected, the second point must be in the same relative position, i.e. the right blue point. Avoid, therefore to select the yellow point. Consider that the distance between the two blue points is 15 cm, but between the left blue point and the yellow is longer, so it is not valid for calibration.

Next step is to calculate the distances between landmarks. Using Pythagoras' theorem is easy to calculate distances in pixels between landmark coordinates. To translate distance in pixels to distance in metric units (mm, for example) is necessary to calibrate the image. For this purpose we will use two calibration systems:

The distance (in pixels) between the two points in the ruler correspond to a known distance (15 cm, for example) and will be used to calculate real distances for the variables: AH, H2D, LPO, LMO and DO.

The distance between B and C correspond to D2D (which real distance was measured with a caliper) and it will be used as calibration for the rest of the variables i.e., LD, LV, VA, 2DA, DV, LP, LA, LAV, LC, LM and LMS.

8.2. MERISTICS

8.2.1. Introduction

Meristic variables are those that are counted. Thus they are discrete as opposed to continuous variables are those described above (morphometric). The predictive capacity for stock discriminations is much lower than the morphometric ones, and because of its nature, they must be statistically separately analyzed.

Fish have several easily recognized meristic variables: spines, rays, gill rakers and bones (especially vertebrae). Normally, the number of these structures is measured. But often, in morphological studies, some variables are “invented” and codes defined, as for example the position of a given structure which can be above or below a reference point and its position coded as 1 or 2, respectively. Similarly angles can be coded.

In this project 11 meristic variables are to be used; 8 are number of spines, rays or gill rakers, 2 are spine angles or positions and one more refers to relative position of the pectoral fin.

The measurement of meristic variables is independent of morphometry, so it is not necessarily to be done in connection with morphometric measurements.

8.2.2. Material and methods

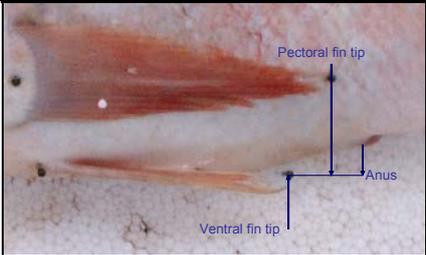
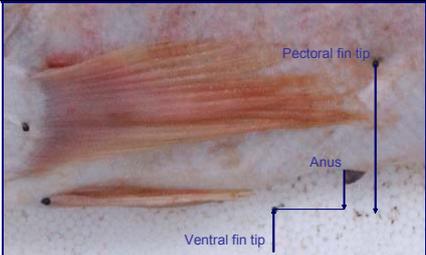
Next table list the variables used in this analysis, with the variable Acronym and their descriptions.

Meristic variables	
PPA	Pectoral fin position relating to the pelvic fin and the anus.
RDF1	N° first dorsal fin spines.
RDF2	N ° second dorsal fin soft rays.
RAF	N° anal fin rays.
RPF	N° pectoral fin rays.
RVF	N° ventral fin rays.
A3S	Third preopercular spine angle.
A5S	Fifth preopercular spine angle.
GHO	N° horizontal segment gill rakers.
GVO	N° vertical segment gill rakers.
GTO	Total gill rakers.

Description on how to measure each of these variables

PPA

It refers to the position of the pectoral fin tip in relation to the pelvic fin and the anus. It is coded as follows:

Code	Description	Examples
1	Pectoral fin tip don't reaches the ventral fin end	
2	It lies between ventral fin tip and the 1/2 distance between the end of the pelvic fin and the anterior part of the anus.	
3	Between 1/2 distance between the end of the pelvic fin and the anterior part of the anus and the anterior part of the anus	
4	Anus	
5	Goes beyond the posterior part of the anus	

RDF1

It is referred to the first dorsal fin, which have only spines.

RDF2

It is referred to the second dorsal fin, which is formed exclusively by soft rays.

RAF

Rays of anal fin, both, spines and soft rays together. Attention should be paid to the base of the rays, i.e. the insertion point of the rays with the body; because the anal rays are usually bifurcated at the end, and therefore the double counting of the same ray must be avoided.

RPF

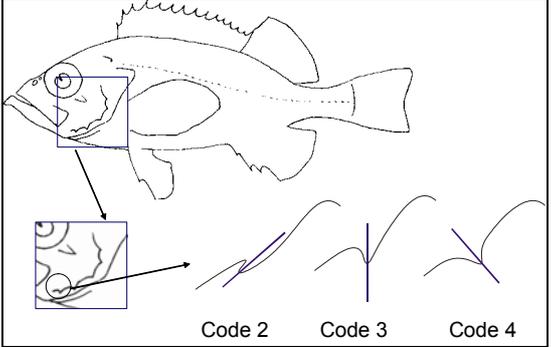
Rays of pectoral fin. Note that the outer rays are very small and they can be easily not recorded.

RVF

Rays of ventral fin, both, spines and soft rays together.

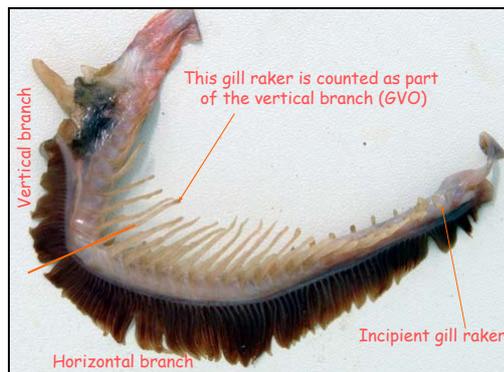
A3S and A5S

Those meristic characters made reference to the position of the third (A3S) and fifth (A5S) preopercular spines.

Cod e	Description	Examples
1	Spine pointed in a forward direction	
2	Spine pointed in a down-forwards direction	
3	Spine pointed downwards	
4	Spine pointed down-backwards	
5	Spine pointed backwards	

GHO GVO GTO

Remove the first gill arch (the outermost) of the left side of the fish. Be careful that none of the gill-raker is damaged. The incipient gill rakers are counted as well. The gill-raker that lies just where the gill arch divides in two branches, is included in GVO.



8.3. SUMMARY

Summary

- 1 Take total, preanal and fork length, total weight. If necessary, take samples from the right side of the fish (for genetic purposes, for example)
- 2 Place the fish on a base, with the left side up
- 3 Put the pins in landmarks “A” to “S” as explained in “landmarks” section
- 4 Place a ruler on the base where the fish lies
- 5 Measure D2D with a caliper, preferably a digital one, and note it down
- 6 Is advisable to extend the original fish identification label on the base, and another with the new identification if necessary
- 7 Take the picture with a digital camera. But some pictures with different camera settings can be also taken
- 8 Save the images both, as Tiff raw images and with source format, i.e. with a format where the camera settings are stored
- 9 Take meristic characters
- 10 Take eviscerate weight and remove otoliths
- 11 Create a backup of the images
- 12 Translate landmark and rule point positions to x-y coordinates, and store them in a spreadsheet
- 13 Calculate the distances between landmarks with the two calibration systems

Steps 9 can be done immediately after step 1, or several days after finishing the morphometric procedure. Step 10 must be done always after step 8, but it can be delayed as step 9.

TECHNICAL PROTOCOL

9.1. INTRODUCTION

Digital pictures of each of the individuals were taken for an easy exchange of material between laboratories. Digital pictures databases let to take more measurements in the future, or to test for possible errors made when measuring. But besides the advantages, using images present also some problems:

To take measurements from a digital picture has two major constrains: First, the image thus taken has to keep the proportion between different parts of the fish, independently of the size of the fish, and secondly, images must represent, as much as possible, the real distances between landmarks in the fish. In other words, fish is a three-dimensional object, while a picture represents a 2-D object. This means that fish width (i.e. fish height when laying down ready for the picture) will drastically affect to the distances between landmarks when the landmarks are not in the same plane. This fact has two major effects: first, as biggest the fish, smallest the distance between landmarks taken from the picture; and second, distances from the picture are not real. The former effect will unavoidably affect the morphometric analysis, so it must be considered and solved. The later is only important if the measurements digitally taken are to be compared with measurements taken with caliper (as it is the case in this project). Both questions were studied and solved as explained later in this Annex.

In addition cameras are a complex of lenses. Quality of lenses will affect to quality of picture and hence to the morphometry. In particular, optic distortions are a serious problems and it occurs in bad quality lenses, but also when using short or long focal length. This problem is also addressed in this Annex.

These two problems are described in the next two sections from two different points of view, the optics distortion and the fish geometry.

9.2. OPTICS DISTORTION

Optics distorsion is due to aberration of the camera lenses, due to the bad quality of the lenses mounted in the camera but even in good quality lenses distorsion is caused in the extremis values of the focal length. Most of the new digital cameras, are equipped with non-exchangeable (fixed) and bad quality lenses. Those cameras show strong aberrations as those shown in Figure 9.1:



Figure 9.1. Aberrations caused using a short focal length (picture in the right) and using a long focal length (picture in the left)

To avoid problems related to the bad quality of the cameras, it's highly recommended to use digital cameras with exchangeable normal lenses, that are of better quality.

But even good quality cameras produce distortions related to the focal length:

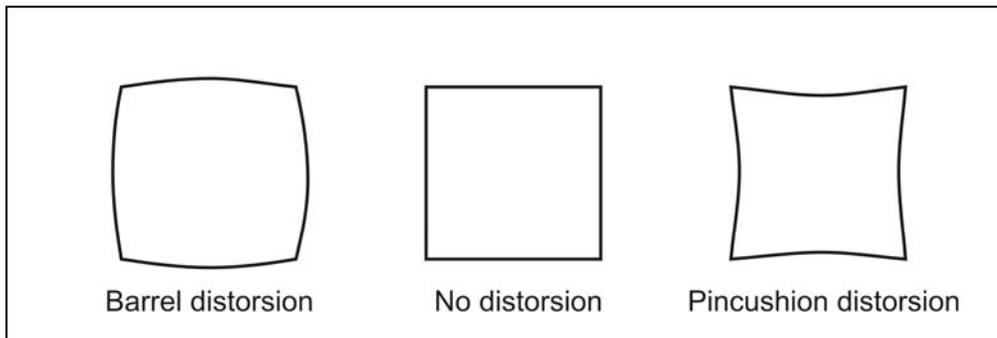


Figure 9.2. Distorsions related to the focal length.

Barrel distortion is a lens effect which causes images to see 'spherised' at their centre (Figure 9.2). The barrel distortion occurs at short focal lengths (wider angles: <35mm in traditional photography).

Pincushion distortion is a lens effect which causes images to be pinched at their centre (Figure 9.2). Pincushion distortion is associated with zoom lenses or when adding telephoto adapters and only occurs at the telephoto end of a zoom lens with long focal distance. It is most noticeable when you have a very straight edge near the side of the image frame.

We can easily avoid barrel and pincushion distortion taken an intermediate focal length, that is, 50mm in the traditional photography, the same focal distance than human eye.

Because the CCD sensor in a digital camera is much smaller than a 35mm negative, the lenses can be made smaller (because of this they have to be of a much higher quality). To get the true focal length you need to multiply this small size by a value called the "focal length multiplier". For Nikon D1X, that is the camera used by partner 2 within REDFISH project, 35mm focal length is equivalent to 50mm in traditional photography. That's why in

the test of the camera we used 35mm of focal length to avoid barrel and pincushion aberrations.

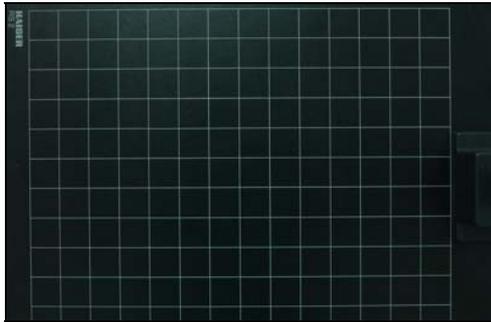


Figure 9.3. Picture taken with a 35mm focal length

To test in NIKON D1X distortion, a picture was taken of a grid at 35mm focal length (Figure 9.3) (but remember this is equivalent to a 50 mm lens in traditional photography).

The distortions in the lens for this focal length were tested measuring the width of each square in the grid using image analysis software.

As can be seen in Figure 9.4, width increases in each row from left to right. It probably is due to the fact that the grid and the camera were not in parallel. It's not the expected result if there were barrel or pincushion distortion.

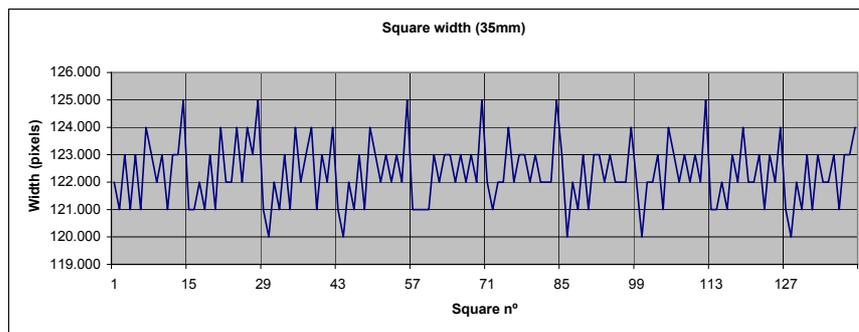


Figure 9.4. X axis represents the squares in the picture; every 14 squares (marked with a vertical line) represent a row in the grid. Y axis is the width of each of the squares (in pixels).

In the next example, similar analysis was done but with a picture taken with a 28mm focal length



Figure 9.5. Photo made with the Nikon D1X at 28mm focal length.



Figure 9.5), so it is expected a barrel distortion.

The distance from the camera to the grid is the same as in the previous

example. Note that if camera is moved closer to the grid the barrel distortion increases.

Figure 9.6 shows that central squares in each row present a longer width than the squares in the periphery. This is a clear result of barrel distortion. Pincushion distortion should be the opposite, i.e., the central squares with a smaller width than those in the periphery.

It is therefore recommended the use of a lens without aberration and placed the camera (lens) parallel to the fish

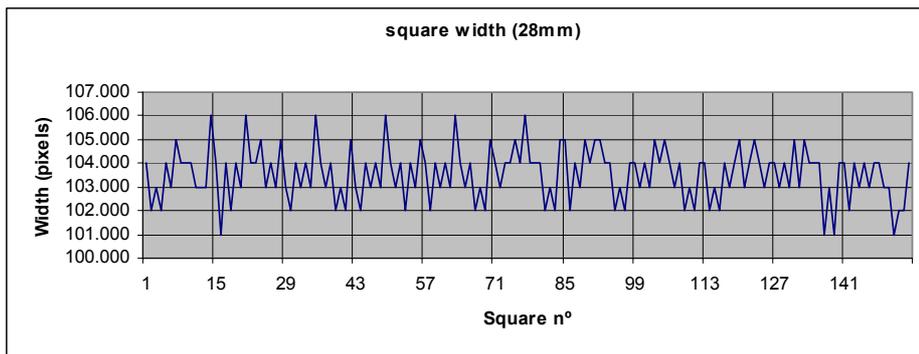


Figure 9.6. X axis represent de squares in the picture; every 14 squares (marked with a vertical line) represent a row in the grid. Y axis is the width of each square (in pixels).

9.3. FISH GEOMETRY

Landmarks in the fish are not in the same horizontal plane, i.e. fish is a three-dimensional object, while the picture is, however, a 2D image. Thus, distances taken from a picture are always smaller than the real distances, unless the landmarks were in an horizontal plane (Figure 9.7).

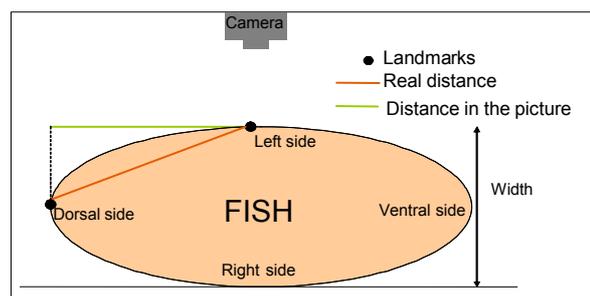


Figure 9.7. Difference between real distances and the distance capted by the camera.

Size of the fish, or more precisely, width of the fish, i.e. fish height when laying down ready for the picture will drastically affect to the distances between landmarks, i.e. the bigger the fish, the smaller the relative distance between landmarks taken from the picture. The difference between real distance and image distance is lower in small fish than in big fish (Figure 9.8).

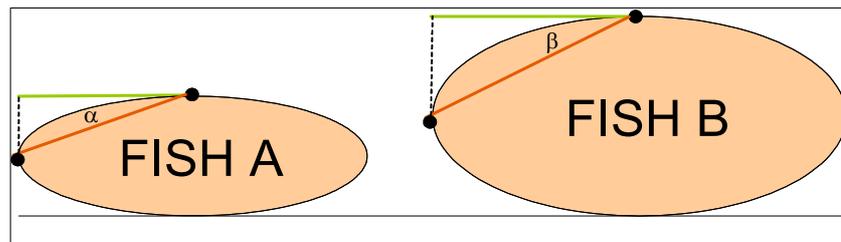


Figure 9.8. The difference between green (image) and red (real) lines is smaller in Fish A than in Fish B, i.e. angle α is smaller than β angle

Calibration consist in turn the pixels into metric units, and it is necessary to have a reference in the image of a known distance to make the conversion. Usually a ruler is include in the photo, and so the fish can be calibrated in relation to a known distance in the rule. However, because of the width of the fish, the position of the calibration ruler is critical to estimate distances between landmarks. Thus, If we put the calibration ruler on the base, distances in a higher position, as pectoral fin, will be magnified from its real value. And contrary, if we put the ruler in the higher position, distances that are close to the base, as anal fin, will be diminished.

It is important to obtain from the images distances equal or the most equal to the real ones, because real distances taken with a calliper are going to be compared with the distances taken from the pictures. So, the goal is to found the proper callibration to made the distances taken from the image as closer to the real ones as possible.

The length of the redfish included in this analysis vary from 10 to 75 cm, and in this size-range there are important differences in the width. So fish width is another important question to have into account to obtain an optimun calibration method.

9.3.1. Calibration with a ruler

Investigating possible solutions to the problems explained in the previous section, in a first approach we measured five *S. mentella* with a caliper, and we contrast this measurements with image analysis measurements. Testing the best position for the ruler, we placed it in two different positions, i.e., on the base where the fish lies and at 3,5 cm height.

Relative error between caliper and image analysis measurements for all variables (i.e. the 17 measurements shown in Figure 9.9), are shown in figures from 10 to 26. Bars represent the relative error of image analysis measurements respect to the caliper measurements; the light green ones the error if we made the calibration with the ruler 3.5 cm height, and dark green columns represent the relative error if the ruler is placed on the base.

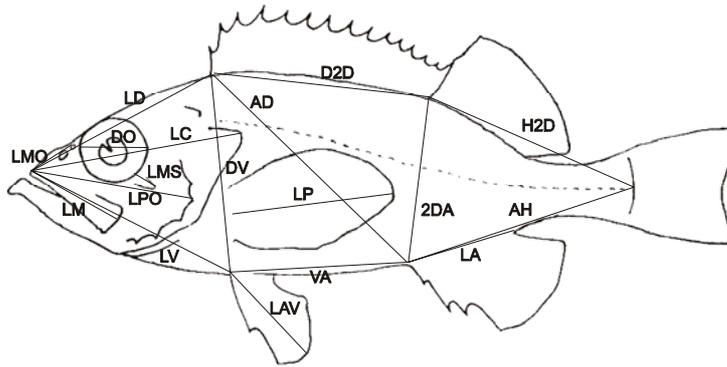


Figure 9.9. Interlandmark distances

For a better understanding we split the measurements in three groups, those delimiting the fish shape; the measurements of the fins and those measurements located in the fish head.

9.3.1.1. Shape measurements

First, we have measurements that are close to the base, i.e., 2DA (Figure 9.10), H2D (Figure 9.11) and AH (Figure 9.12). The error is bigger, as expected, when we calibrate with the ruler in a high position (light green bars).

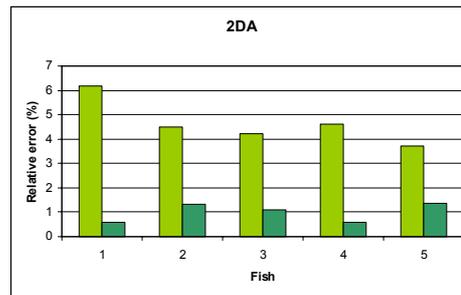


Figure 9.10

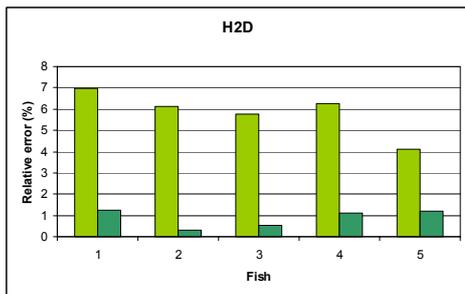


Figure 9.11

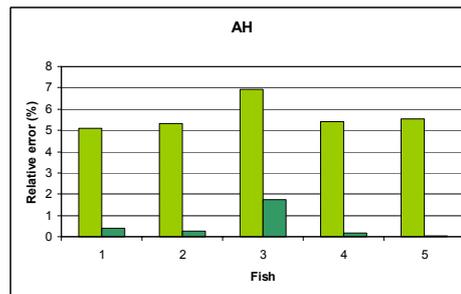


Figure 9.12

In those features that are in a higher position, i.e., LD (Figure 9.13), LV (Figure 9.14) and DV (Figure 9.15), the error is bigger when we put the ruler in the base (dark green bars)

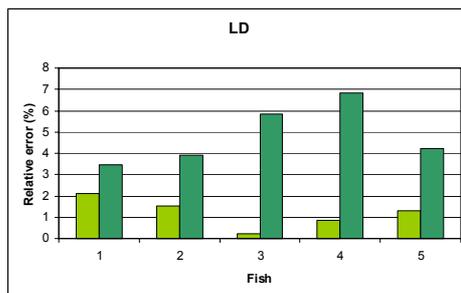


Figure 9.13

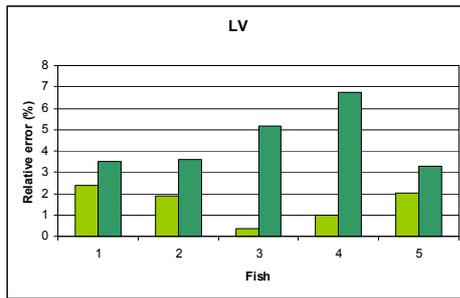


Figure 9.14

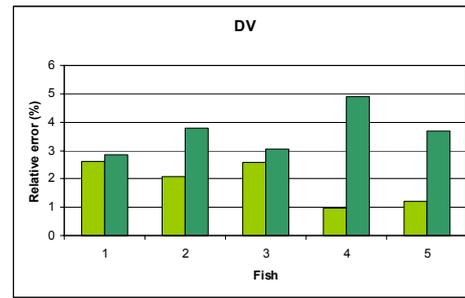


Figure 9.15

VA goes from a high landmark in the ventral fin to the anal fin that is close to the base. This inclination introduces another error factor, which must be added to the error produced by the relative position to the camera (Figure 9.16).

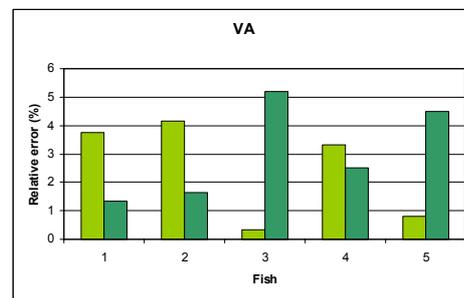


Figure 9.16

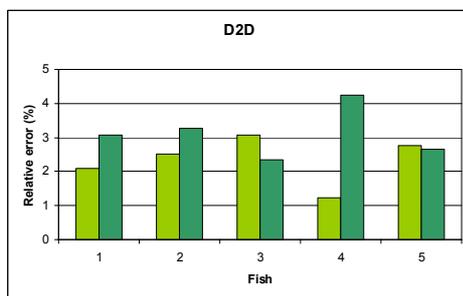


Figure 9.17

Finally, in D2D graph, both relative errors are small (most of them about 3% or less) and don't show variation from one calibration to the other (Figure 9.17). D2D are in an intermediate position between high and low features.

All this "shape" measurements have a not very high error (never bigger than 7% of the real measurement).

9.3.1.2. Fin measurements

LP and LAV are located in a high position. So, the calibration made with the ruler in a high position makes that in most of fishes error decrease to less than 2% (in light green in Figure 9.18 and Figure 9.19).

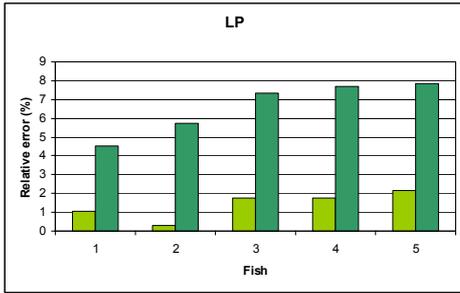


Figure 9.18

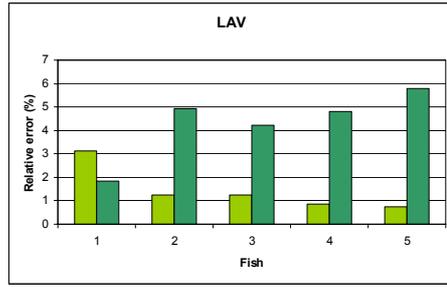


Figure 9.19

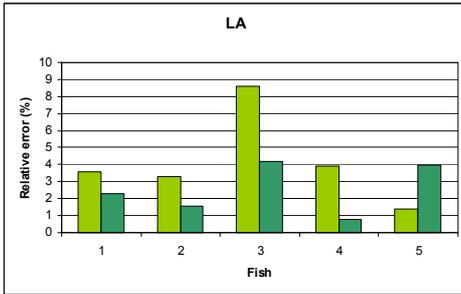


Figure 9.20

Contrary, LA is closer to the base, and error increases if we use the ruler in a high position as calibration (Figure 9.20). But it's not true in all the fishes, and there is not a clear bias. The problem is again that this measurement has the inclination factor that increases the % error.

9.3.1.3. Head measurements

LC presents an inclination, because it runs from a landmark in the tip of the lip to the highest part of the fish, that is, in the end of the opercle. So there is not a clear relation between high and low calibration (Figure 9.21).

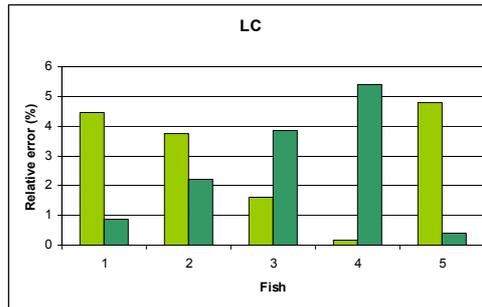


Figure 9.21

LMS, LPO, LMS, LMO and DO are small distances and are influenced by different degrees of inclination. So, the relative error increases very much in these measurements (Figure 9.22, Figure 9.23, Figure 9.24, Figure 9.25 and

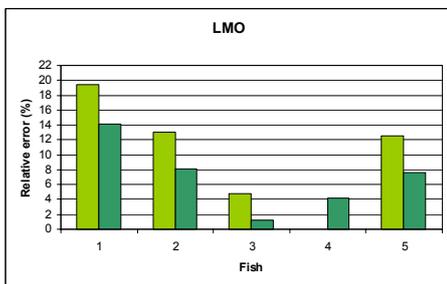


Figure 9.26)

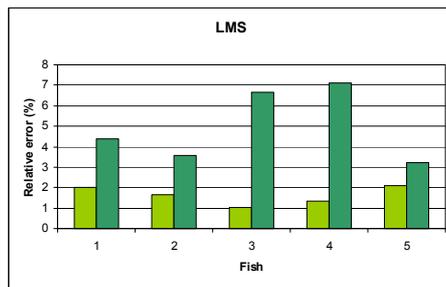


Figure 9.22

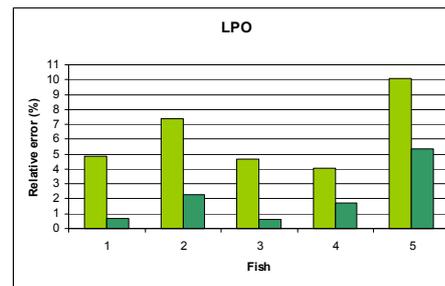


Figure 9.23

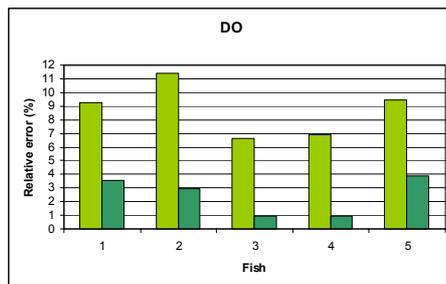


Figure 9.24

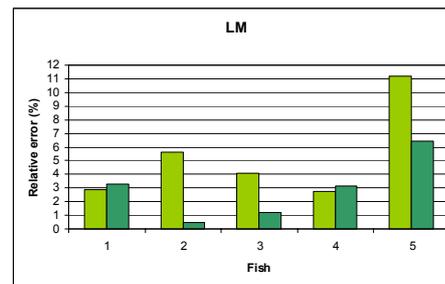


Figure 9.25

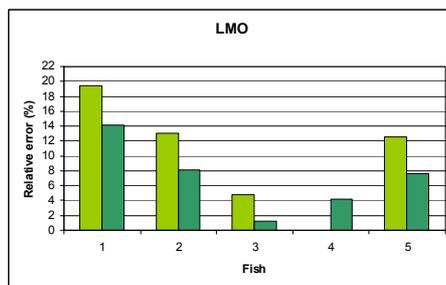


Figure 9.26

The results with this five fishes were a first approach and allow us to conclude that, as expected, although some of the measurements improved with the ruler in a higher position, other became worst so there is not an optimum place where to put the calibration rule. So, there is always a certain error in some of the distances.

9.3.2. Calibration with D2D

A possible solution would be calibrate with a real distance taking from the fish. This distance should have an intermediate length and should be large enough to minimize the error. Between the distances tested, the best option was the D2D distance (Figure 9.9), that is located in an intermediate height and is large enough.

So, the next step was to use the D2D for calibration. Thus, a double calibration was made in 107 *S. mentella*, using both, the D2D and also a ruler placed on the base where the fish lies, in order to compare results.

Figure 9.27 shows the relative error between measurements made in the images and measurements taken with a caliper, using the ruler as calibration.

As expected, those variables that are close to the base, (i.e. AH and H2D) showed the lower error. Contrary, those variables that are in a higher position, have a larger error (i.e. LP). Notice also that LMO present a bigger error than LP, despite of LMO is closer to the base than LP, that is one of the measurements placed in a highest plane.

The explanation to this LMO high relative error is that LMO is a small distance, and small differences in absolute values result in high relative errors, as shown in Figure 9.28.

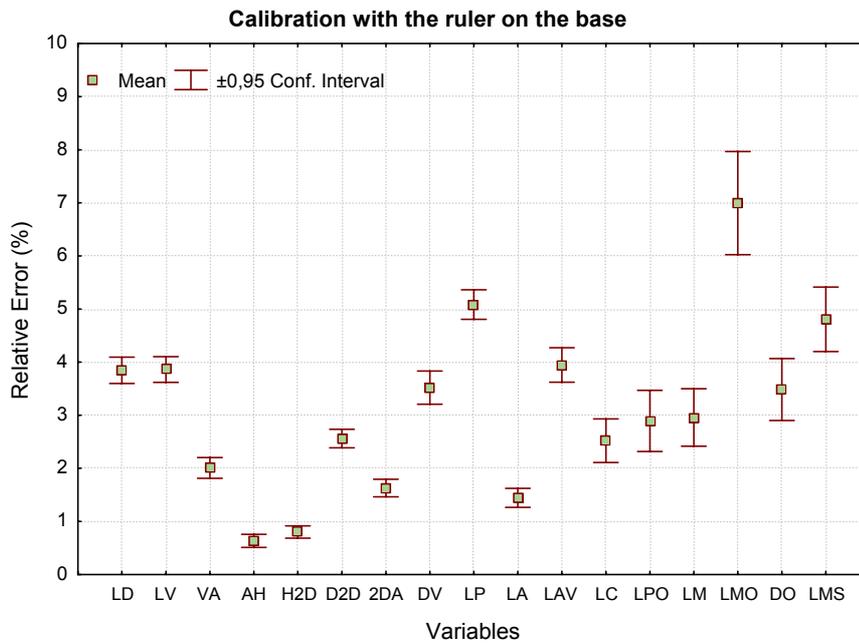


Figure 9.27. Relative error (%) between Image analysis measurements and measurements taken by hand with a caliper, using a ruler placed in the base for calibration.

Figure 9.29 shows the relative error between measurements made with image analysis and measurements taken with a caliper, calculating the image analysis measurements with D2D as calibration.

Using D2D for calibration, the relative error is lower than 3% in most of the variables. Only LPO, LM, LMO, DO and LMS have a higher error. AH, H2D and LP show an error between 2 and 3%, and the other fish shape and fin variables shows an error lower than 2%. LC is around 2%, but the other head variables have a large relative error. However, as explained before for LMO, head measurements are small distances, and a small variation produces a big relative error.

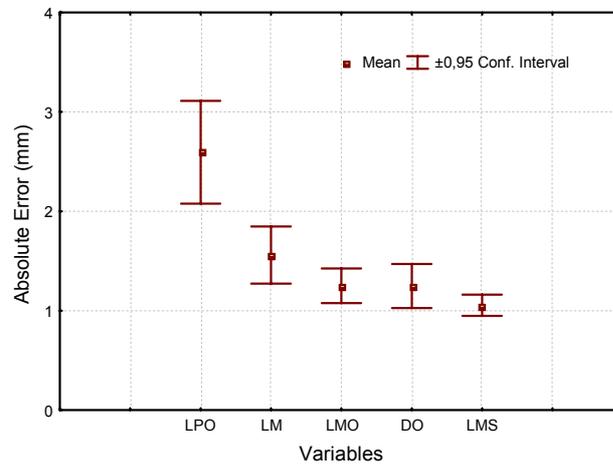


Figure 9.28. Absolute error in several distances show the low LMO absolute error (less than 0.5 mm) between real measurements and the measurements taken in the image.

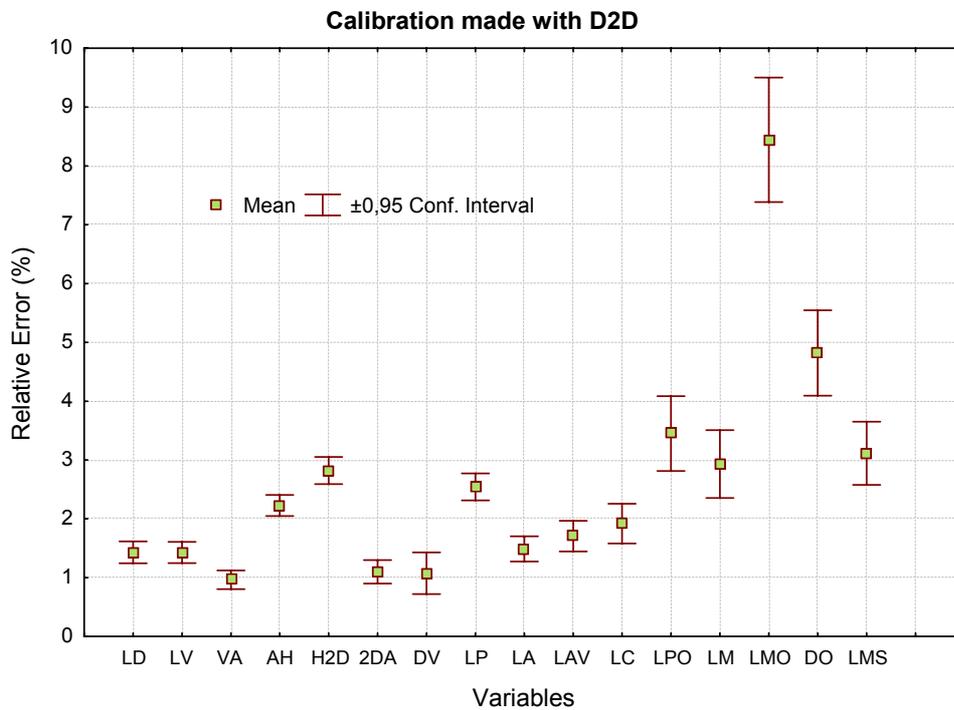


Figure 9.29. Relative error (%) between image analysis measurements and measurements taken by hand with a caliper, using the D2D for calibration.

Summarizing, we improve considerably the accuracy of many of the measurements using D2D for calibration. Differences between real and image distances are thus reduced to a minimum and acceptable level. Nevertheless, some of the distances are still more accurate using the ruler in the base to calibrate the image. Only three distances show error higher than 3% independently of the calibration method. In these cases we think that the source of the error comes from the fact that these distances are too small, thus a slight error measuring (both with caliper or in the image) causes an important relative error.

In conclusion, some measurements will be calibrated with the ruler, and other with the D2D distance. Thus, AH, H2D, LPO, LMO and DO will be calibrated with the ruler and LD, LV, VA, 2DA, DV, LP, LA, LAV, LC, LM and LMS will be calibrated using the D2D distance.

9.3.3. Source of errors in the methodology

As even using the D2D for calibration there are differences between real measurements and image measurements, the importance of that error was compared with the error produced using a caliper. For quantified the error produced using a caliper, we measured the 17 measurements in two fish several times and by two different persons. To be more precise, the first person repeated the measurements 5 times in each of the two fish and the second person made it 3 times.

First, the differences in measuring with the same caliper between both persons were tested. In two fish. Results of that comparisons are shown in Table 9.1. ANOVA results show that there are no significant differences between measurements made by both persons in most of the variables (See table below). In our opinion, the significant differences are due to the low number of fish measured. Besides, one of the persons has not experience in measure fish.

Then it was studied the relative error within each person separately and for both persons combined.

Observer 1 measured 5 times each fish, and observer 2 did it 3 times. Even though that observers were told to be very careful making measurements, we found differences. The relative errors (Figure 9.30) and the coefficient of variation (Figure 9.31) are shown. The measurements located in the fish head showed the highest relative errors. Those are, however the smaller distances, and a little absolute error implies a high relative error.

If we compare this relative errors with those that were produced when testing the accuracy of each of the persons, most of them don't differ greatly. In addition, those are also similar to those errors produced between real and image measures. One pixel in a small distance creates relative big errors, and it should be considered than in image analysis one pixel is the minimum unit (there is not half pixel). It can also explain the errors found in LMO and LMS.

Table 9.1. Results of the ANOVA looking for significative differences between the caliper measurements made in two fish by two different persons

ANOVA					
Fish 1	F		Fish 2	F	
LD	0,005	ns	LD	16,884	**
LV	15,599	**	LV	39,646	**
VA	3,396	ns	VA	0,011	ns
AH	0,006	ns	AH	36,875	**
H2D	0,165	ns	H2D	0,307	ns
D2D	0,175	ns	D2D	2,188	ns
2DA	11,508	*	2DA	0,700	ns
AD	0,172	ns	AD	4,883	ns
DV	0,008	ns	DV	3,106	ns
LP	7,964	*	LP	1,899	ns
LA	1,882	ns	LA	62,809	**
LAV	0,984	ns	LAV	1,324	ns
LC	6,377	*	LC	4,582	ns
LPO	0,001	ns	LPO	15,944	**
LM	6,531	*	LM	2,985	ns
LMO	1,577	ns	LMO	17,200	**
DO	2,203	ns	DO	0,006	ns
LMS	6,586	*	LMS	3,399	ns
LB	2,941	ns	LB	0,733	ns

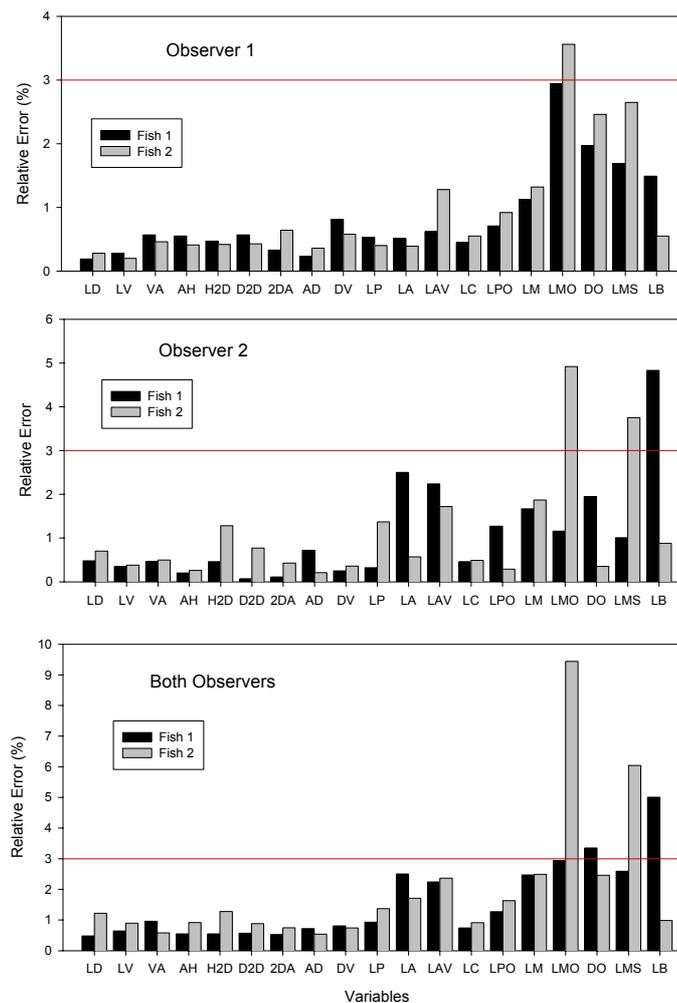


Figure 9.30. Relative Error between the different measurements made by two persons in the same fishes.

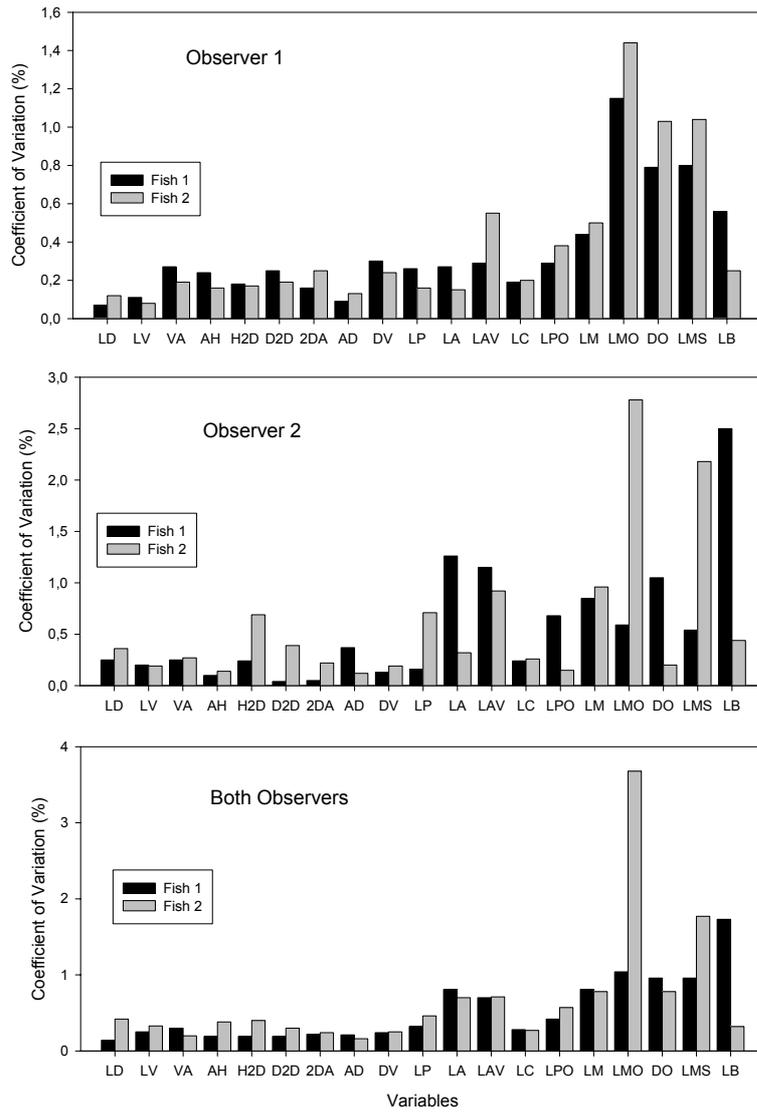


Figure 9.31. Coefficient of Variation between different measurements made by two observers in the same fishes.

