FiskDir.Skr.Ser.HavUnders., 16: 359—368.

DIFFERENTIATION BETWEEN «MARINUS» AND «MENTELLA» TYPES OF REDFISH BY ELECTROPHORESIS OF HAEMOGLOBINS

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ABSTRACT

NÆVDAL, G. 1978. Differentiation between «marinus» and «mentella» types of redfish by electorphoresis of haemoglobins. *FiskDir.Skr.Ser.HavUnders., 16* : 359–368.

Haemoglobins of 225 specimens of redfish from the Barents Sea and 357 specimens from Icelandic waters were analysed by electrophoresis to search for genetic differences between the morphologian *marinus* and *mentella* types. Two main haemoglobin patterns were commonly found, one characteristic for redfish of the *mentella* type and one characteristic for the *marinus* type. Specimens of *Sebastes viviparus* showed the *marinus* haemoglobin pattern. Morphological «intermediates» showed the *marinus* pattern in the Barents Sea while some specimens, which showed the *mentella* hemoglobin patterns, but could not be separated morphologically from the *marinus* type, were recorded in the Icelandic-Faeroe Ridge area. Four specimens showed a haemoglobin pattern which indicated hybridization between individuals with different haemoglobin pattern.

The results indicate that *Sebastes mentella* Travin is a species distinct form *Sebastes marinus* (L), possibly with occasional interbreeding. However, for final conclusion about the species of redfish in the North Atlantic samples have to be collected from the total range of the redfish's distribution.

INTRODUCTION

LUNDBECK (1940) claims that besides the common redfish, *Sebastes marinus* (L), a deep sea type excists in the Bear Island area and on the Iceland-Faeroe Ridge. TRAVIN (1951) described the deep sea redfish from the Bear Island area and the Barents Sea as a new species, *Sebastes mentella* Travin. This species prefers deeper water and somewhat higher temperature than *S. marinus* although they also occur sympatrically. *S. mentella* is characterized by a beak on its lower jaw, possesses a greater eye diameter, grows slower and reaches a smaller total length than *S. marinus*. The colour of *S. mentella* is bright red and of *S. marinus* usually orange-red.

ANDRIIACHEV (1954) and TEMPLEMAN (1959) restricted *Sebastes mentella* to the status of a subspecies, *S. marinus mentella,* because the differences between *marinus* and *mentella* were not clear enough to justify them as two distinct species. TEMPLEMAN (1959) and others have found that *S. mentella* is not restricted to the Northeast Atlantic, but is common also in the Northwest Atlantic and occurred here in most localities in greater quantities than *S. marinus*.

By meristic, morphometric or morphologic methods clearcut separation of redfishes into *marinus* and *mentella* groups has been difficult (KOTTHAUS 1961, TEMPLEMAN 1959, KELLY, BAKER and CLARKE 1961). A great proportion of the specimens possessed the characteristics of both groups in a varying manner. However, genetical differences between the two groups were suggested by TEMPLEMAN and SANDEMAN (1959) who found great differences between *marinus* and *mentella* types in relative occurrence of caudal melanophores of pre extrusion larvae.

YANULOV (1962 a) found significant differences between the two types in infestation rate of various parasites in parts of the Northwest Atlantic.

By immuno-diffusion techniques, photron-reflectometric measurements and two separate methods of chromatography O'ROURKE (1961) found evidence for biochemical specificity of the two forms to such a degree that they could be considered as two distinct species. Similarly SCHAEFFER (1961) found that the two types differed significantly in content of certain free amino acids and total nitrogen content in muscle tissue.

In a series of papers (ALTUKHOV *et al.* 1968, ALTUKHOV and NEFYODOV 1968, ALTUKHOV, NEFYODOV and PAYUSOVA 1968, NEFYODOV 1969) dealing with the redfish problem, were described differences in muscle termostability and also differences in frequencies of some polymorphic serum proteins chartateristics (haptoglobins, albumins, and β-globulins) between samples of *marinus* and *mentella* types from the waters between Iceland and Greenland, showing that the analysed samples were not drawn from one homogenous population.

In the present paper differences in electrophoretic mobility of haemoglobins of the two types of redfish are described, and the results are discussed in relation to their significance on the systematic and the management of the redfish.

MATERIALS AND METHODS

Redfish for blood sampling was caught by bottom trawl. Blood was obtained by a syringe from the heart region, or the fish was cut open and blood collected into small glass tubes. After centrifugation the serum was pipettet off and the cells lysed by adding destilled water.

The haemoglobins were analysed by the agar gel electrophoresis at pH 7.2 describe by SICK (1965). Selected specimens were also analysed by the combined starch and agar gel electrophoresis at pH 9.0 described by MØLLER (1966). The haemoglobins were always analysed within 24 hours, usually

Table 1. Distributions of haemoglobin patterns in redfish from the Barents Sea and Icelandic waters.

Sample no.	Locality	Date of sampling	Depth m	Mentalla-pattern	Marinus-pattern	Others	Sum
1	71°oo'N, 20° 00'E	7 November 1970	200	—	30	—	30
2	75°40'N, 16°50'E	10 » »	350	40	—	—	40
3	76°30'N, 13°50'E	12 » »	200	6	24	—	30
4	76°58'N, 12°30'E	12 » »	400	20	—	—	20
5	73°00'N, 33°45'E	17 » »	218	2	18	—	20
6	71°00'N, 34°42'E	19 » »	200	6	19	—	25
7	71°00'N, 29°06'E	19 » »	218	5	55	—	60
8	71°20'N, 26°40'E	20 » »	150	—	100	—	100
	Total, Barents Sea			79	246	—	325
9	64°20'N, 12°36'W	19 August 1971	236	9	27	2	38
10	63°20'N, 25°08'W	13 » »	300	—	158	2	160
11	65°38'N, 26°47'W	16 » »	280	3	156	—	159
	Total, Icelandic waters			12	341	4	357

within 12 hours from sampling. Parts of the material were reanalysed after one or two days. Some haemoglobin specimens were also frozen and later reanalysed at the Institute of Marine Research in Bergen. The haemoglobins were stained by Amidoblack 10 B.

All samples from the Barents Sea were collected and analysed during a cruise by R.V. «G. O. Sars» in November 1970 and the samples from Icelandic waters likewise in August 1971. Fishing localities, depth, date of sampling and number of specimens in each sample are listed in Table 1. Fishing localities are also shown in Fig. 1 and 2.

All redfish specimen were separated morphologically into *Sebastes viviparus* [backward directed anterior preopercular spine (ANDRIIASHEV 1954)], *mentella* type, *marinus* type and intermediates [according to the description by TRAVIN (1951)], and separation by morphology was compared to the results from the haemoglobin analyses. Total length, usually also sex, were recorded for the fishes of which blood was collected.

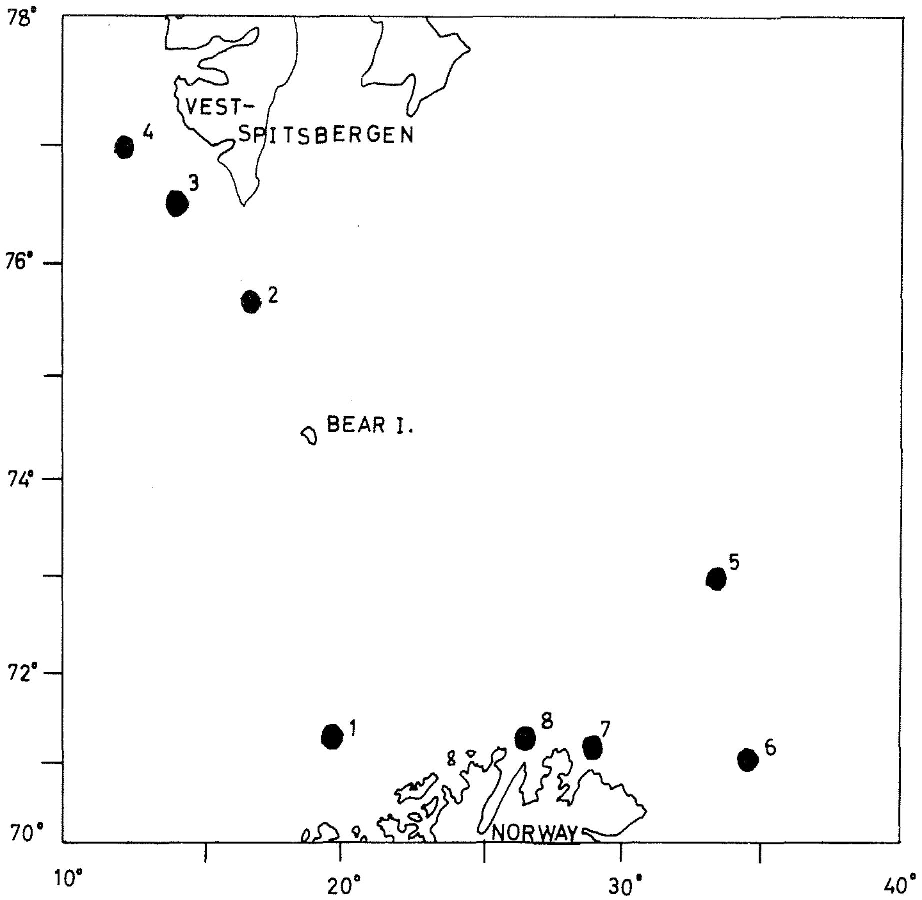

Fig. 1. Sampling localities of redfish in the Barents Sea.

RESULTS

HAEMOGLOBIN TYPES OF REDFISH FROM THE BARENTS SEA

Among the samples from the Barents Sea were found two main haemoglobin patterns (Fig. 3). The first pattern occurred in specimens which according to TRAVIN (1951) were of the *mentella* type. In agar gel at pH 7.2 this pattern showed a strong, but rather diffuse anodic moving component, and one weak component stayed near the origin. By combined starch and agar gel electrophoresis at pH 9.0 the strong component showed high anodic mobility. Two weak components also showed anodic mobility while one very weak component moved slightly towards the cathode. This pattern was tentatively called the *mentella* pattern.

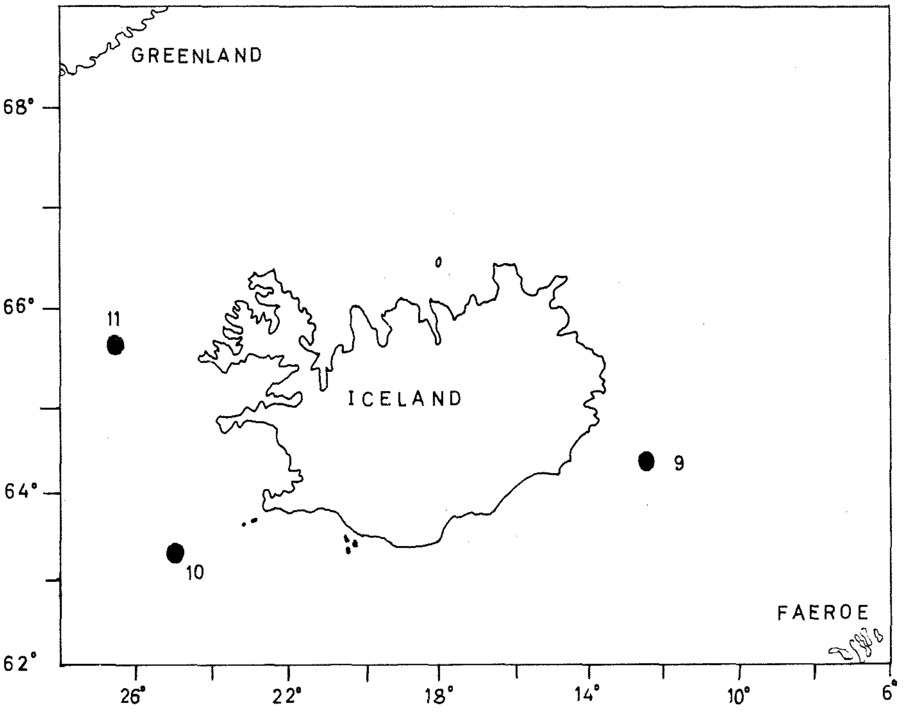

Fig. 2. Sampling localities of redfish in Icelandic waters.

The second pattern was found in specimens morphologically determined to be of the *marinus* type and consequently was called the *marinus* pattern. At pH 7.2 all components moved towards the cathode. Two weak components showed the highest mobility, and one strong component moved only slowly.

Individual variations were indicated in strength and occurrence of the two weak components, but this variation was not clear enough to form the basis of clearcut classification of the specimens. At pH 9.0 the strong component of the *marinus* pattern moved slower towards the anode than the strong component of the *mentella* pattern. Also in the *marinus* pattern was seen two weak components, one with intermediate anodic mobility and one with slight cathodic mobility.

One *Sebastes viviparus* from the Barents Sea (sample 1) showed the same haemoglobin patterns as individuals of the *marinus* type. Some specimens possessed morphological characteristics both of *marinus* and *mentella* types. This was especially evident at one locality on the Norwegian coast (sample 8) where the greater part of the specimens were recorded as such «intermediates» (SMESTAD, unpublished). However, with no exception all these specimens showed the *marinus* haemoglobin pattern.

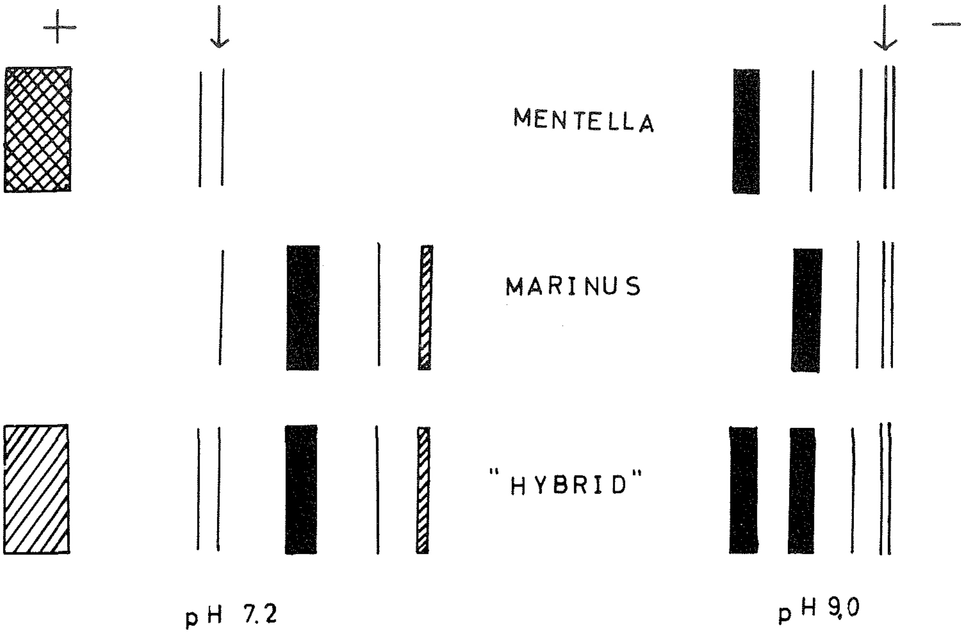

Fig. 3. Outline of haemoglobin patterns in redfish obtained by gel electrophoresis. Filled in bars: Strong bands. Hatched bars: Moderately strong bands. Single lines. Faint bands. Arrow indicate the points of application.

The analysed specimens varied in length from about 15 to 40 cm, and both haemoglobin patterns were observed among the smaller as well as among the greater specimens. Consequently, no indications of ontogenetic variation in haemoglobin patterns were found in redfish.

The haemoglobin pattern could be recognized also after freezing and thawing of the hemolysate, but the clearest patterns were obtained with fresh material. No *post mortem* variation, except that the total patterns became diffuse, could be observed after proponged storage in the refrigerator.

The distribution of the two different patterns in the samples from the Barents Sea are shown in Table 1 (sample 1–8). The *mentella* pattern was found most frequently in the area between Bear Island and Spitsbergen and were rare in the eastern parts of the Barents Sea and near the Norwegian coast. Both were found together in the same trawl haul, but *mentella* occurred more frequently in deeper water. This is in accordance with the general appearence of the *mentella* type of redfish for instance from the description of TRAVIN (1951).

Similar *mentella* and *marinus* haemoglobin patterns occurred among redfish samples from Icelandic waters as in the samples from the Barents Sea. In some specimens a modified *marinus* pattern occurred. This pattern showed an extra component which in agar gel at pH 7.2 moved slightly towards the anode and in combined starch and agar gel electrophoresis at pH 9.0 moved towards the anode with a mobility greater than the common strong component of the *mentella* pattern.

Totally 88 specimens of sample 10 and 19 specimens of sample 11, which were supposed to be *S. viviparus* from their backward directed preopercular spines, showed one of the two *marinus* patterns.

Few *mentella* patterns were found in Icelandic waters. West of northern Iceland (sample 11) were found three specimens out of 159 which showed the *mentella* pattern, and the fishes which possessed these patterns, were easily distinguished morphologically. West of Reykjanes (sample 10) were found no *mentella* patterns, and on Iceland-Faeroe Ridge (sample 9) were found 9 *mentella* patterns out of totally 38 specimens. In this sample all but one of the smaller fishes (9–13 cm) showed the *mentella* pattern, and also three fishes about 40 cm in length showed this pattern. However, in contrast to all other samples, morphological differentiation of the fishes showing the different haemoglobin types was nearly impossible, even for the greater specimens.

Four specimens, two in each of sample 9 and 10, showed a haemoglobin pattern with both the component of *mentella* and *marinus* pattern (Fig. 3). Morphologically these four specimens could not be distinguished from the *marinus* type, but because the pattern indicates hybridization between individuals with different haemoglobin types, this pattern tentatively was called the «hybrid» pattern. The fishes which showed this pattern ranged in length from 36 til 45 cm.

DISCUSSION

The compositions by amino acids of the proteins polypeptide chains and consequently the electrophoretic mobility of the proteins are controlled by genetic factors (MANWELL and BAKER 1970). The electrophoretic patterns of fish haemoglobins usually are species specific, illustrated by among others TSUYUKI *et al.* (1968) who described species specific electrophoretic patterns of haemoglobins of 28 species Pacific Ocean Scorpaenidae. Intraspecific variations of haemoglobin patterns, controlled by codominant alleles, have been described for several species (see de LIGNY 1969 for references). In other species, for example salmon (KOCH, BERGSTRØM and EVANS 1966) and herring (WILKINS and ILES 1966), intraspecific variations have been observed to be connected with ontogeny. Also variations due to changes in haemoglobin components after prolonged storage of samples have been described for several species, but these variations usually are found in the minor haemoglobin components (SICK 1965, MØLLER and NÆVDAL 1969, TSUYUKI *et al.* 1968, NÆVDAL 1968).

Neither ontogenetic cariation nor *post mortem* changes can account for the observed patterns of redfish haemoglobins because the two common patterns were observed in fishes ranging from less than 10 cm to more than 40 cm in length, all specimens were given the same treatment, and all were analysed within 24 hours from sampling. Control specimens did not show any major change in patterns even after two or three days in refrigerator or after freezing and thawing.

Segregation of two codominant alleles within one species could produce three patterns like the main patterns observed (the *mentella* and *marinus* patterns as homozygotes and the «hybrid» pattern as heterozygote). However, according to the Hardy-Weinberg law the hypothetical heterozygote (here the «hybrid» pattern) should then be expected to occur much more frequently than observed. It therefore seems unlikely that haemoglobin variation within one species can account for these patterns, but the modified *marinus* pattern in redfish from Icelandic waters may be normal intraspecific variation, probably genetically controlled.

However, the two common patterns may be explained by assuming that the *mentella* and *marinus* types of redfish really belong to different species, each with their own haemoglobin pattern. The «hybrid» pattern may be due to occasional hybridization between individuals of the two species. The good agreement, except in sample 9, between the results from the analyses of haemoglobins and morphological differenciation strongly support the theory of two species. Four hybrids out of more than 650 individuals do not show that the two types are conspecific because hybridization between related species is not uncommon among fishes. According to a second theory the «hybrid» pattern may represent an intraspecific variant of the *marinus* type. This theory is supported by the fact that the four specimens with the «hybrid» haemoglobin pattern could not be distinguished morphologically from the *marinus* type.

Although the *mentella* and *marinus* type of redfish seem to represent different species with only occasional interbreeding in the Barents Sea and Icelandic waters, the possibility still excist that the two types may be connected through intermediate populations in other areas. A definite conclusion about the species of redfish in the North Atlantic can therefore not be drawn until samples have been collected from the total geographic range of the redfish. However, the *marinus* and *mentella* types of redfish surely represent different *gene pools* with a minimum exchange of genetic material, and they therefore should be treated as separate units in management of the Northeast Atlantic fisheries. In addition, both the *mentella* and the *marinus* types may be composed of smaller units (populations, stock units) as claimed by SINDERMANN (1961) and YANULOV (1962 a, b) and others in their studies of parasites and meristic characteristics.

The fact that *Sebastes viviparus* showed the *marinus* haemoglobin pattern has no effect upon this conclusion. *Sebastes viviparus* doubtless is a true species (ANDRIIASHEV 1954, TROUT 1961), and two related species may have similar haemoglobin structure while others differ widely.

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Received 15 November 1976
Printed 20 January 1978