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DIFFERENTIATION BETWEEN "MARINUS" AND "MENTELLA" TYPES OF REDFISH BY ELECTROPHORESIS OF HEMOGLOBINS

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INTRODUCTION

Lundbeck (1940) claimen that besidws the common redfish, Sebastes marinus (L.), there excist a deep sea type 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 prefére 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, and this species possess a greater eye diameter, grows slower, and reach a smaller total length than S. marinus. The colour of mentella is bright red and of 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. A.o. Templeman (1959) found that mentella was not restricted to the Northeast Atlantic, but was common also in the Northwest Atlantic, and in most localities here occurred in greater quantities than marinus.

By meristic, morphometric or morpholgic metods clear-cut 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 specimems posessed 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-diffuison 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. Similary 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 characteristics (haptoglobins, albumins, - and - 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 hemoglobins 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. Besides hemoglobins, total serum and mucle proteins, serum and muscle esterase, lactate dehydrogenase and aspartate aminotransferase were analysed by electrophoresis.
The result of these analyses, however, were not very conclusive, and are omitted in the present report.

MATERIAL 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 pipetted off and the cells lysed by adding destilled water.

The hemoglobins were analysed by the agar gel electrophoresis at pH 7.2 described 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 hemoglobins were always analysed within 24 hours, and usually within 12 hours from sampling, and part of the material were reanalysed after one or two days, Some hemoglobin specimens were also frozen and later reanalysed at the Institute of Marine Research in Bergen. The hemoglobins were stained by Amidoblack 10 B.

All samples from the Barent 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, and fishing localities are also shown in Figs. 1 and 2.

All redfish specimen were separated morphologically into Sebasted viviparus (backward directed anterior pre-opercular spine (Andriiashev 1954)), mentella - type, marinus - type and intermediates (according to the describtion by Travin(1951); and separation by morphology was compared to the results from the hemoglobin analyses. Total length, usually also sex and age, were recorded for the fishes of which blood was collected.

RESULTS

HEMOGLOBIN TYPES OF REDFISH FROM THE BARENT SEA

Among the samples from the Barent Sea were found two main hemoglobin patterens, 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.

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 clear-cut 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 Barent Sea (sample 1) showed the same hemoglobin patterns as invividuals of the marinus type.
Some specimens possessed morphological characteristics both of marinus and mentella types. This was expecially evident at one locality on the Norwegian coast (sample 8) ehere the greater part of the specimens were recorded as such "intermediates" (Smestad, unpublished). However, with no exceptions all these specimens showed the marinus hemoglobin pattern.
The analysed specimens varied in length from about 15 to 40 cm, and both hemoglobin patterns were observed among the smaller as well as among the greater specimens. Consequently, no indications of ontogenetic variation in hemoglobin patterns were found in redfish.

The hemoglobin pattern could be recognized also after freezing and thawing of the hemolysate, but the clearest patterns was obtained with fresh material. No _post mortem_ variation, except that the total patterns became diffuse, could be observed after prolonged storage in the refrigerator.

The distributions of the two different patterns in the samples from the Barent 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 Barent Sea and near the Norwegian coast. Both were found together in the same hault, 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).

HEMOGLOBIN TYPES OF REDFISH FROM ICELANDIC WATERS

Similar _mentella_ and _marinus_ hemoglobin patterns occurred among redfish samples from Icelandic waters as in the samples from the Barent 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 sample10 and 19 specimens of sample 11 which were supposed to be _S. viviparus_ from their backward directed preopercular spines, all 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 the 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 differensiation of the fishes showing the different hemoglobin types was nearly impossible, even for the greater specimens.

Four specimens, two in each of sample 9 and 10, showed a hemoglobin pattern with both the component of _mentella_ ond _marinus_ pattern, Fig. 2. Morphologically these four specimens could not be distinguished from the _marinus_ type, but because the pattern indicates hybridization between individuals with different hemoglobin types, this pattern tentatively was called the "hybrid" pattern. The fishes which showed this pattern ranged in length from 36 to 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 hemoglobins usually are species specific, illustrated by a.o. Tsuyuki _et al._ (1968) who described species specific electrophoretic patterns of hemoglobins of 28 species Pacific Ocean Scorpaenidae. Intraspecific variations of hemoglobin patterns, controlled by co-dominant alleles, have been described for several species (see de Ligny 1969 for references). In other species, a.o. salmon (Kock, 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 hemoglobin components after prolonged storage of samples have been described for several species, but these usually variations are found in the minor hemoglobin components (Sick 1965, Møller and Nævdal 1969, Tsuyuki _et al._ 1968, Nævdal 1968).

Neither ontogenetic variation nor _post mortem_ changes can account for the observed patterns of redfish hemoglobins, because the two common patterns were observed in fishes ranging from less than 10 cm to more than 40 cm in length, and 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 co-dominant 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 unlikly that hemoglobin 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 redfosh really belong to different species, each with their own hemoglobin 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 hemoglobins 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 teory is supported by the fact that the four specimens with the "hybrid" hemoglobin 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 Barent 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 repressent 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 a.o. Sindermann (1961) and Yanulov (1962a,b) in their studies of parasites and meristic charateristics.

The fact that Sebastes viviparus showed the marinus hemoglobin pattern has no effect upon this conclusion. Sebástes viviparús doubtless is a "good" species (Andriiashev 1954, Trout 1961), and two related species may have similar hemoglobin structure, while others differ widely.

SUMMARY AND CONCLUSION

Hemoglobins of 225 specimens of redfish from the Barent Sea and 357 specimens from Icelandic waters were analysed by electrophoresis to search for genetic differences between the morphological marinus and mentella types. Two main hemoglobin 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 hemoglobin pattern. Morphological "intermediates" showed the marinus pattern in the Barent Sea, while some specimens which showed the mentella hemoglobin pattern but could not be separated morphologically from the marinus type were recorded in the Iceland-Faeroe Ridge area. Four specimens showed a hemoglobin pattern which indicated hybridization between individuals with differnt hemoglobin pattern.

The results indicate that Sebastes mentella Travin is a species distinct from 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.

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Table I. Distributions of hemoglobin patterns in redfish from the Barents Sea and Icelandic waters.

Sample no, locality and date of sampling	Depth m	mentella-pattern	marinus-pattern	others	Sum
1 71°15'N, 20°00'E					
Nov. 7, 70	200	—	30	—	30
2 75°40'N, 16°50'E					
Nov.10, 70	350	40	—	—	40
3 76°30'N, 13°50'E					
Nov.12, 70	200	6	24	—	30
4 76°58'N, 12°30'E					
Nov,12, 70	400	20	—	—	20
5 73°00'N, 33°45'E					
Nov.17, 70	218	2	18	—	20
6 71°00'N, 34°42'E					
Nov.19, 70	200	6	19	—	25
7 71°00'N, 29°06'E					
Nov.19, 70	218	5	55	—	60
8 71°20'N, 26°40'E					
Nov.20, 70	150	—	100	—	100
Total, Barents Sea		79	246	—	225
9 64°20'N, 12°36'W					
Aug.10, 71	236	9	27	2	38
10 63°20'N, 25°08'W					
Aug.13, 71	300	—	158	2	160
11 65°38'N, 26°47'W					
Aug.16, 71	280	3	156	—	159
Total, Icelandic waters		12	341	4	357

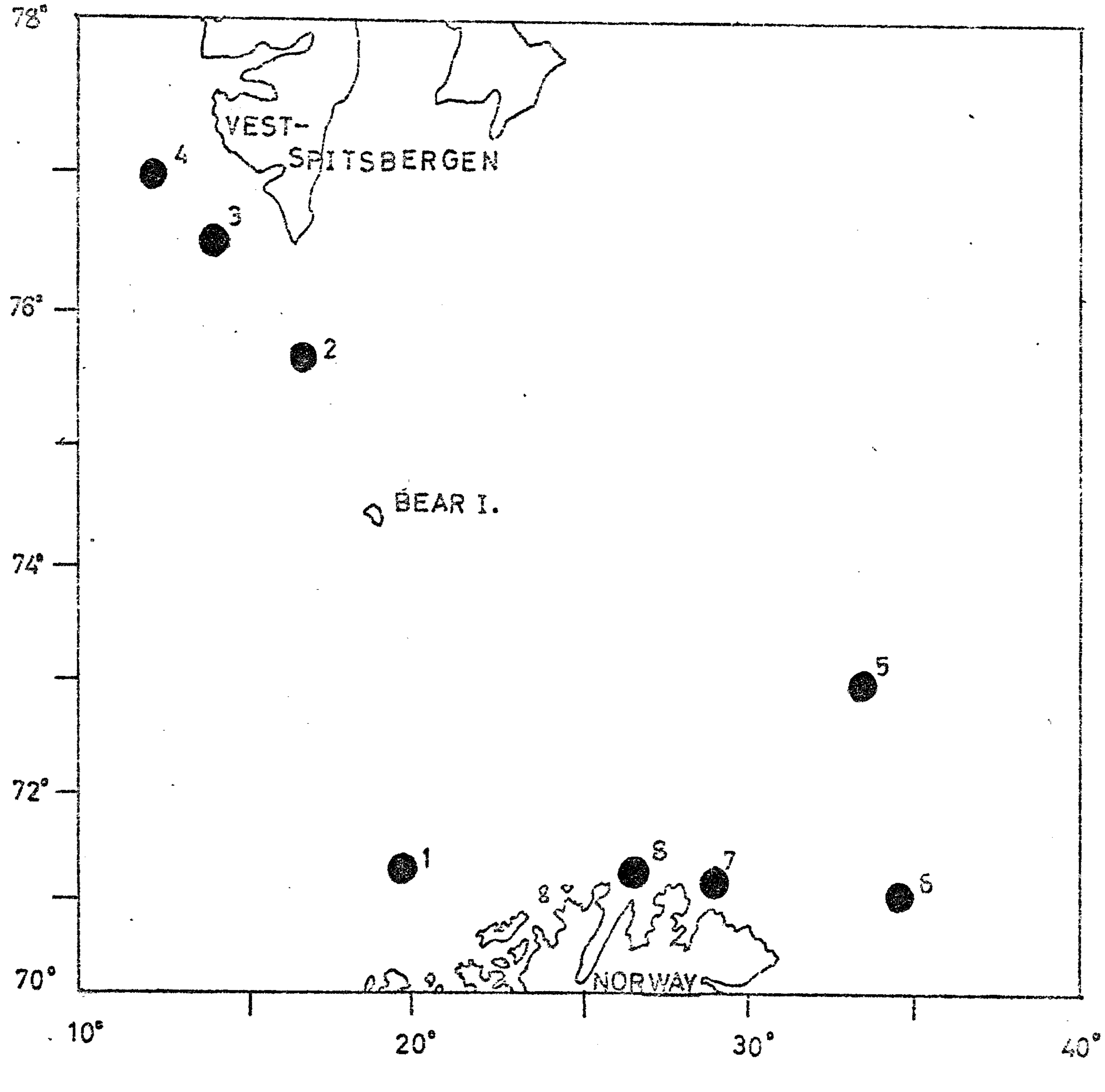

Fig. I. Sampling localities of redfish in the Barents Sea.

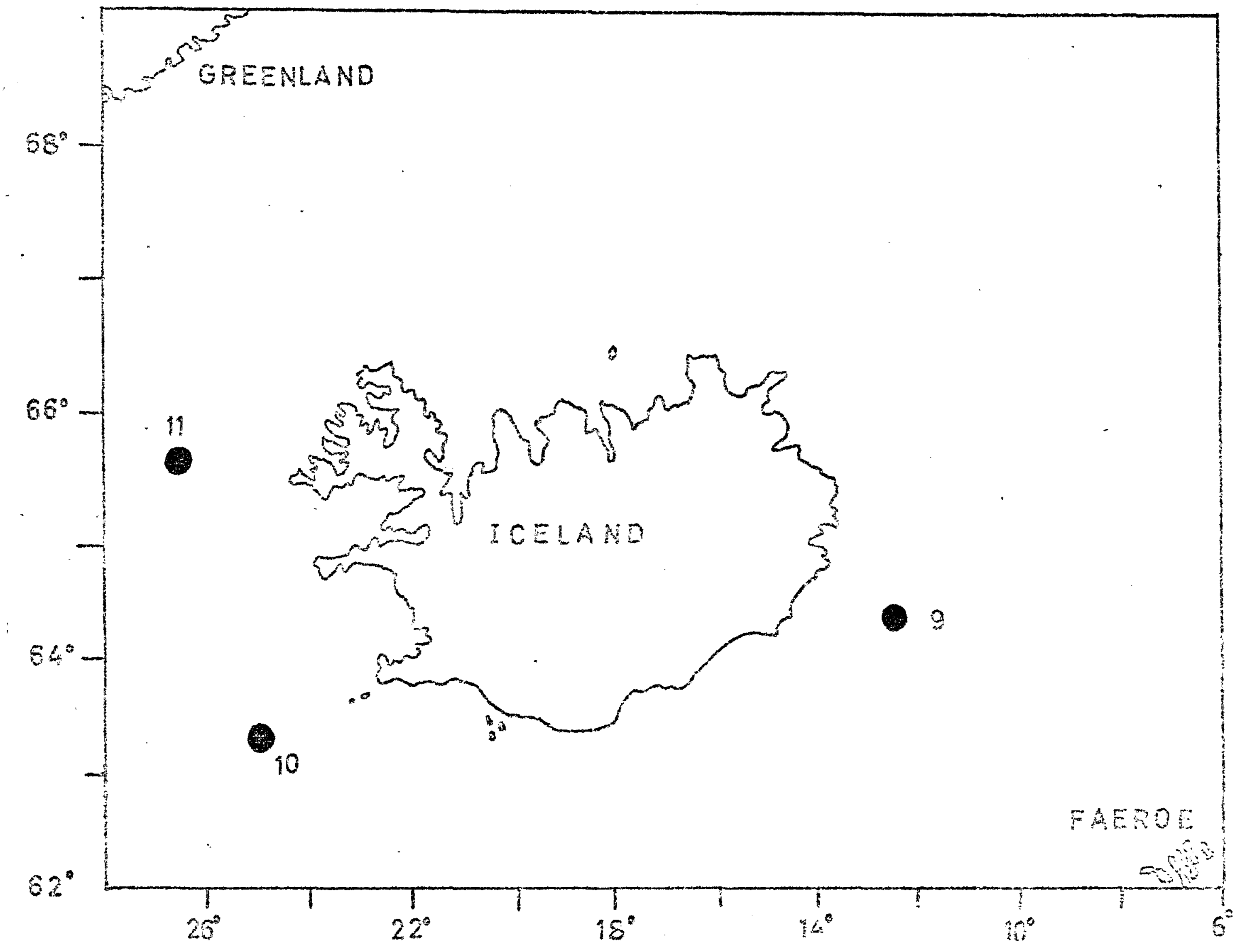

Fig. II. Sampling localities of redfish in Icelandic waters.

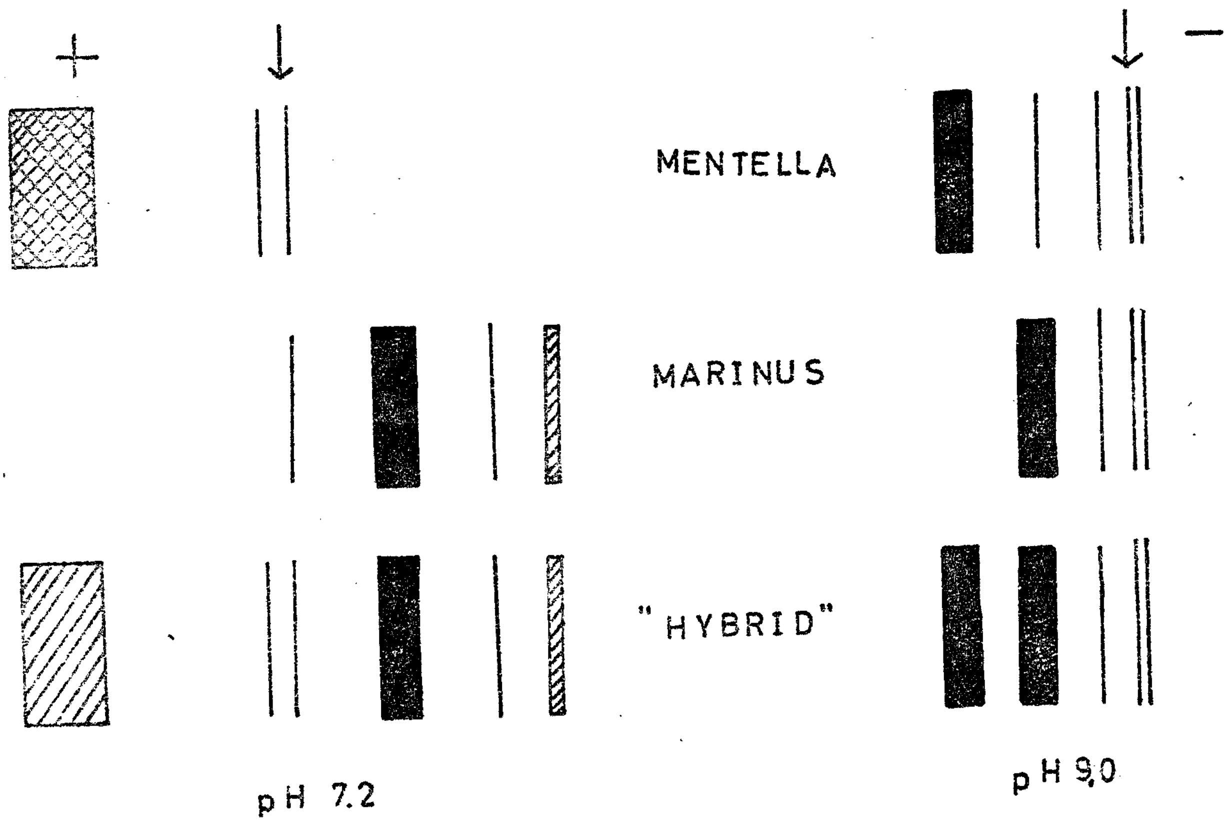

Fig. III. Outline of hemoglobin patterns in redfish obtained by agar gel electrophoresis at pH 7.2 and combined starch and agar gel electrophoresis at pH 9.0. Legend: Filled in bars: Strong bands. Hatched bars: Moderately strong bands. Single lines: Faint bands. Arrows indicate the points of application.