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# STUDIES ON BLOOD PROTEINS OF MACKEREL

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## INTRODUCTION

The mackerel, *Scomber scombrus*, spawns in the northeastern North Sea and Skagerak, and another spawning area is located south of Ireland. Tagging experiments (BOLSTER 1962, 1965, REVHEIM, personal communication) have shown that interchange of individual mackerel takes place between the two main spawning areas, but it is still not known whether the two main spawning stocks consist of two (or more) separate population units or whether the mackerel within these areas should be regarded as one single unit.

The present paper deals with studies on hemoglobins, serum proteins and serum esterase for the purpose of identifying characteristics to be applied on the population problems of mackerel.

The results of the studies on serum esterase have been dealt with in a separate report (JAMIESON, DE LIGNY and NÆVDAL 1969) where also the results of the present study have been compared to corresponding results of samples from the southern North Sea and the waters south of Great Britain and Ireland.

## MATERIAL AND METHODS

Sampling and treatment of mackerel samples were made as described for herring and sprat (NÆVDAL 1968, 1969). No anticoagulant was used because the mackerel blood clotted only partly, and hemolysate could easily be prepared. The hemoglobins were analysed fresh, but because of hard working stress at the laboratory at certain periods, some serum samples had to be stored in the deep freeze for some weeks before the analyses could be carried out.

The hemoglobins were analysed by the agar gel electrophoresis described by SICK (1965) for 60 minutes. The sera were analysed in combined starch and agar gel electrophoresis (SICK 1965, MØLLER 1966). For separation of serum proteins and esterase components 90 minutes were found to be sufficient. When analysing esterase, three sera were

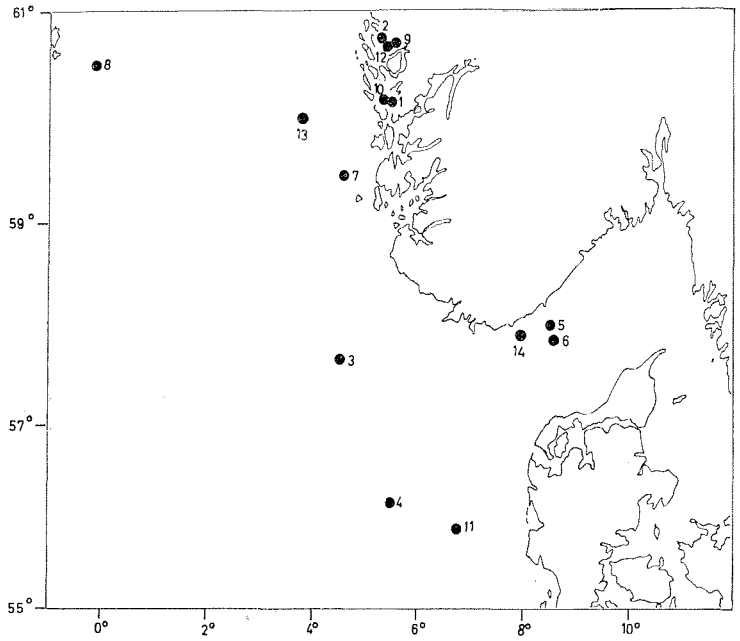



Fig. 1. Sampling localities of blood samples of mackerel.

run on each slide, the middle one being a reference serum of known type.

Staining of proteins and esterase bands, and identification of transferrins and haptoglobins were carried out as for similar studies on sprat (NÆVDAL 1968, 1969). Only samples collected in 1967 and later were analysed with respect to esterase phenotypes.

Sampling localities, date of sampling and other data of the samples are listed in Table I. Fig. 1 shows the sampling localities.

To see whether the results of the analyses of esterase phenotypes obtained by the present method corresponded to the results by starch gel electrophoresis, sera have been exchanged with other laboratories, and the electrophoretograms have been carefully compared (JAMIESON *et al.* 1969).

## RESULTS AND DISCUSSION

### *HEMOGLOBINS*

The hemoglobins of the mackerel moved towards the cathode in agar gel at pH 7.3 with a mobility in the range of several other fish species, for instance herring and sprat (WILKINS and ILES 1966), cod (SICK 1965) and some other gadoids (MØLLER and NÆVDAL 1969). The hemoglobin patterns found in mackerel are shown in Fig. 2.

The Hb component of lowest cathodic mobility, named HbII, was

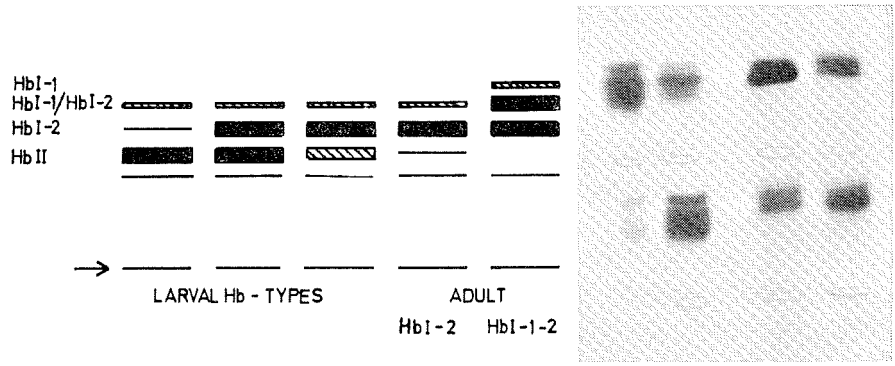



Fig. 2. Outline of hemoglobin patterns in mackerel by agar gel electrophoresis at pH 7.2 and photograph of electrophoretograms from routine analyses. The left part of the photograph are patterns of 0-group mackerel, and the right part are patterns from adult. Filled in bars: strong bands. Hatched bars: moderately strong bands. Single lines: faint bands. Arrow indicates the point of application.

found in samples 1 and 2 of the 0 group. Also in these samples the HbII component varied in strength. In some specimens HbII was the only strong component (left pattern in Fig. 2) while it was nearly absent in others. Sample 12, which also represented the 0-group, showed only weak traces of the HbII component. This sample, collected in October, contained fishes of about 23 cm in length (extremely high growth rate) while samples 1 and 2 represented fishes of 10—12 cm in length. In older fishes only faint traces of the HbII component were seen. Although several length stages were not represented in the present material, it is evident that the ontogenetic variation of the HbII component depend on growth or length rather than on age. However, the ontogenetic variation is less pronounced than in salmon, *Salmo salar*, (KOCK, EVANS and BERGSTRØM 1966) and herring, *Clupea harengus*, (WILKINS and ILES 1966) where it was found to persists up to significantly older stages in the fish's life.

In mackerel, one year and older, two strong components were found. The component of highest cathodic mobility, named HbI-1, was only present in a few specimens while the other, named HbI-2, was present in all specimens analysed. Thus two phenotypes, named HbI-1-2 (containing both components) and HbI-1 (containing only the component HbI-1) were found. Weak components were seen at the cathodic side of HbI-1 and HbI-2 (named HbI-1′ and HbI-2′ respectively). The HbI-1′ and HbI-2′ components may correspond to weak components of the same designation in cod, *Gadus morhua*, (SICK 1965), sprat, *Sprattus sprattus*, (NÆVDAL 1968) and other fishes (MØLLER and NÆVDAL 1969). They surely increased upon storing, but were also

present in completely fresh material, and HbI-2′ was found in the hemoglobin pattern of 0-group mackerel even when HbI-2 was seen only as a faint band. Often HbI-2′ was stronger in the 0-group than in older fishes.

As a hypothesis the components HbI-1 and HbI-2 are controlled by two co-dominant alleles, *HbI*$^1$ and *HbI*$^2$. The expected genotype represented by a phenotype with HbI-1 as the only strong component was not found, but because of the scarsity of the hypothetical gene *HbI*$^1$ it should not be expected in the present material. Accordingly, both ontogenetic and genetically controlled variations seem to be present in mackerel hemoglobins. The genetically controlled variation may potentially be used for studies on population units, but in mackerel one of the hypothetical hemoglobin-controlling genes, *HbI*$^1$, is so rare that the variation is of little value for this purpose.

*SERUM PROTEINS*

All serum proteins moved towards the anode in combined starch and agar gel electrophoresis at pH 9.0. The electrophoretograms obtained are outlined in Fig. 3.

In front of the albumins was seen a moderately strong component, followed at its cathodic side of a weaker one. By the present method intranspecific variations could not be detected in these components or in the albumins.

The transferrins were located near the albumins at their cathodic side. This high anodic mobility of transferrins conforms with the mobility of transferrins of some flat fishes (DE LIGNY 1967) while the transferrins of several other fishes have been found to possess a much lower anodic mobility (MØLLER 1966, MØLLER and NÆVDAL 1967).

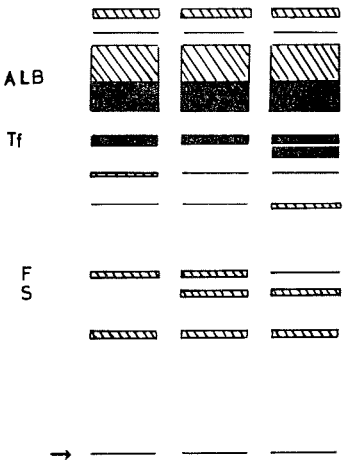



Fig. 3. Outline of serum protein patterns of mackerel by combined starch and agar gel electrophoresis at pH 9.0. Legend: see Fig. 2.

In most adult specimens only one band representing transferrins were found, but in a few specimens another strong band occurred at its catodic side. (Fig. 3). This rare band could not be tested autoradiographically since sera in which it occurred were not available when the tracing experiments were made. Its strength and position, however, suggests that it represents a rare transferrin component which may be

genetically controlled, but it is too rare to be utilized for studies on population units.

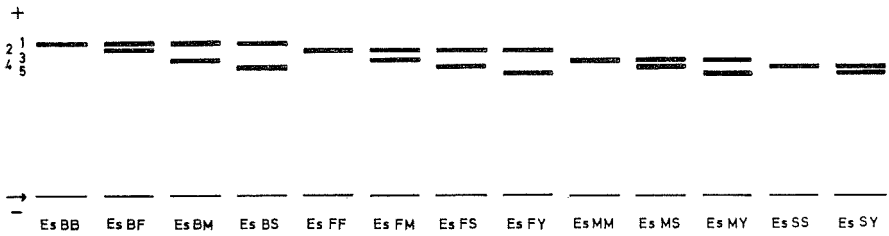
Another component was frequently seen at the anodic side of the common transferrin band in sera of 0-group mackerel and sometimes also in sera of older but immature fishes. The relative intensity of the two bands varied, and in some specimens the cathodic component (the common transferrin in adults) was nearly absent. Evidently these variations were related to ontogeny and therefore of no use for population unit studies.

Behind the transferrins two or more components were located. These components varied both in location and strength, and were often diffuse and very weak, but occasionally strong. These variations were not clear enough to form the basis of proper classification of the specimens.

One or both of the moderately strong components of unknown nature, tentatively named F and S, occurred in each specimen. Consequently three phenotypes, named FF, FS and SS, might be distinguished. Presence of faint bands at the positions of the lacking ordinary F or S band (Fig. 3), however, complicated the classification of the specimens, and the phenotypes did not seem to be completely stable because the intensity of the faint bands varied among repeated analyses of the same sera. A hypothesis of control by two allelomorphic genes was introduced and gene frequencies and expected Hardy-Weinberg distributions were calculated. In most samples there was fairly good accordance between observed and expected distributions, and gene frequencies ($q_F$) showed approximately the same values. However, samples 11 and 12, both from young mackerel, showed considerably lower $q_F$ values, and sample 12 also showed slight accordance between observed and expected distributions. Because it seems unlikely that the samples from young mackerel should represent other population units than the rest of the samples, and because of the unstability of the phenotypes, frequencies of the present characteristics are doubtful as parameters for studies of population units.

The band located at the cathodic side of the component named S, represent haptoglobins. It increased in strength when hemoglobins were added to the sera, and it stained with o-dianisidine. The free hemoglobins also moved towards the anode by the present method, but with a mobility lower than any of the serum proteins.

Although variations in several groups of serum proteins of mackerel have been observed by the present methods, none of these variations seem at present to be useful for studies of population units of this species.



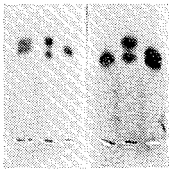



Fig. 4. Outline of serum esterase patterns in mackerel by combined starch and agar gel electrophoresis at pH 9.0, and photograph of electrophoretograms from routine analyses. From left to right the photographed patterns are: Es FM, Es FS, Es MM, Es SS, Es FS, and Es MS. Legend: see Fig. 2.

*SERUM ESTERASE*

Several components which represented esterase activity could be distinguished. But only small differences in electrophoretic mobility were observed among some of the components, and therefore they could not all form the basis of proper classification of the specimens. For this reason they were combined into five main components named Es B, Es F, Es M, Es S and Es Y in order of decreasing anodic mobility. One or two of these main components were present in each specimen, and the phenotypes got their designations according to the components they possessed, i.e. specimens with only Es B were said to belong to the phenotype Es BB, specimens with both Es B and Es F to the phenotype Es BF, etc. Of the 15 possible combinations, 13 were found. The observed phenotypes are outlined in Fig. 4.

A hypothesis of control by a series of polyalleles was introduced to explain the esterase variation. The alleles were named $Es^B$, $Es^F$, etc. according to the esterase component they were supposed to control. Table 1 gives observed distributions of esterase phenotypes together with calculated gene frequencies and expected Hardy-Weinberg distributions.

Significant deviations between observed and expected distributions were found in samples 5, 6, 7 and 14 where a clear excess of hypothetical homozygotes was observed. Also in the other samples, except sample 11, an excess of homozygotes was noted, but the overall accordance between observed and expected distributions was rather good.

The samples which showed the greatest deviation between observed and expected distributions were all sampled from adult fishes near spawning or in spawning condition. However, this tendency was not clear because for instance sample 12, which was sampled from the 0-group, also showed considerable deviation from expected distribution.

Table 1. Distributions of esterase phenotypes in mackerel samples from the North Sea and the Norwegian coast. obs: observed distributions, exp: expected Hardy-Weinberg distributions.

| Sample no. | Esterase phenotypes | | | | | | | | | | | | | No. | Gene frequencies | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | BB | BF | BM | BS | FF | FM | FS | FY | MM | MS | MY | SS | SY | | $q_B$ | $q_F$ | $q_M$ | $q_S$ | $q_Y$ |
| 4 obs | — | — | — | — | 7 | 20 | 5 | — | 19 | 4 | — | — | — | 55 | — | .355 | .564 | .082 | — |
| exp. | — | — | — | — | 6.9 | 22.0 | 3.2 | — | 17.5 | 5.1 | — | 0.4 | — | | | | | | |
| 5 obs. | — | — | 2 | — | 18 | 27 | 6 | — | 40 | 18 | — | 1 | — | 112 | .009 | .308 | .567 | .116 | — |
| exp. | 0.0 | 0.6 | 1.1 | 0.2 | 10.6 | 39.1 | 8.0 | — | 36.0 | 14.7 | — | 1.5 | — | | | | | | |
| 6 obs. | — | 1 | 2 | — | 32 | 60 | 6 | 2 | 84 | 24 | 2 | 3 | — | 216 | .007 | .308 | .593 | .083 | .009 |
| exp. | 0.0 | 0.9 | 1.8 | 0.3 | 20.5 | 78.9 | 11.0 | 1.2 | 76.0 | 21.3 | 2.3 | 1.5 | 0.3 | | | | | | |
| 7 obs. | — | — | 6 | 1 | 33 | 59 | 14 | — | 109 | 15 | 1 | 4 | — | 242 | .014 | .287 | .618 | .079 | .002 |
| exp. | 0.0 | 1.9 | 4.2 | 0.5 | 19.9 | 85.8 | 11.0 | 0.3 | 92.4 | 23.6 | 0.6 | 1.5 | 0.1 | | | | | | |
| 8 obs. | — | — | — | — | 3 | 11 | 3 | — | 16 | 5 | 1 | — | — | 39 | — | .256 | .628 | .103 | .013 |
| exp. | — | — | — | — | 2.6 | 12.5 | 2.1 | 0.2 | 15.4 | 5.0 | 0.6 | 0.4 | 0.1 | | | | | | |
| 9 obs. | — | 1 | 2 | — | 11 | 24 | 7 | 2 | 46 | 11 | — | — | 1 | 105 | .014 | .267 | .614 | .090 | .014 |
| exp. | 0.0 | 0.8 | 1.8 | 0.3 | 7.5 | 34.4 | 5.0 | 0.8 | 39.6 | 11.6 | 1.8 | 0.9 | 0.3 | | | | | | |
| 10 obs. | — | — | 2 | 1 | 11 | 30 | 5 | — | 39 | 9 | 1 | 1 | — | 99 | .015 | .288 | .606 | .086 | .005 |
| exp. | 0.0 | 0.9 | 1.8 | 0.3 | 8.2 | 34.6 | 4.9 | 0.3 | 36.4 | 10.3 | 0.6 | 0.7 | 0.1 | | | | | | |
| 11 obs. | — | 1 | 3 | — | 7 | 44 | 4 | — | 33 | 11 | — | 1 | 1.1 | 105 | .019 | .300 | .590 | .086 | .005 |
| exp. | 0.0 | 1.2 | 2.4 | 0.3 | 9.5 | 37.2 | 5.4 | 0.3 | 36.6 | 10.7 | 0.6 | 0.8 | 0.1 | | | | | | |
| 12 obs. | — | 1 | 4 | — | 12 | 24 | 3 | — | 42 | 12 | — | 2 | — | 100 | .025 | .260 | .620 | .095 | — |
| exp. | 0.1 | 1.3 | 3.1 | 0.5 | 6.7 | 32.2 | 4.9 | — | 38.4 | 11.8 | — | 0.9 | — | | | | | | |
| 13 obs. | 1 | 2 | 10 | — | 41 | 96 | 18 | — | 95 | 31 | 1 | 3 | 3.1 | 299 | .024 | .331 | .548 | .094 | .003 |
| exp. | 0.2 | 4.8 | 7.9 | 1.3 | 32.8 | 108.5 | 18.6 | 0.6 | 89.8 | 30.8 | 1.0 | 2.6 | 0.2 | | | | | | |
| 14 obs. | 1 | 2 | 6 | 1 | 17 | 33 | 10 | 1 | 65 | 9 | 1 | 1 | — | 147 | .037 | .272 | .609 | .075 | .007 |
| exp. | 0.2 | 3.0 | 6.6 | 0.8 | 10.9 | 48.7 | 6.0 | 0.6 | 54.5 | 13.4 | 1.3 | 0.8 | 0.1 | | | | | | |

Sampling of mixed populations, selection against heterozygotes (*negative heterosis*), or methodical error may be reasons for the deviations between observed and expected distributions. However, excistence of populations with different value of gene frequencies was not indicated by the results of any of the analysed samples. Negative heterosis has been observed in some cases of protein polymorphism (MANWELL and BAKER 1969), but should be expected to affect all samples to the same degree. Methodical error might rise because the two bands of the hypothetical heterozygotes sometimes appeared very different in strength. In specimens of generally weak esterase activity the weaker band might be overlooked, and heterozygotes might be classified as homozygotes. However, all specimens were analysed at least twice, and weak bands were carefully looked for. Therefore it seems improbable that this type of error could account for the observed deviations.

Thus no satisfactory explanation of the deviations between observed and expected distributions in some of the samples can be given. The hypothesis of genetical control, however, is supported by the comparatively good accordance in the other samples. In some tuna species genetically controlled variations in serum esterase were found (SPRAGUE 1967, FUJINO and KANG 1968). The resemblance between the esterase variations of tuna and of mackerel also supports the introduced hypothesis. Consequently, the hypothesis seems reasonable, and the observed gene frequencies may be regarded as characteristic sample parameters.

No marked differences in observed gene frequencies were observed among the samples, and the results of the present study give no reason to believe that more than one population unit of mackerel inhabit the area from which samples were collected. The gene frequencies did not deviate clearly from corresponding results of samples from the southern North Sea and the area south and west of Ireland (JAMIESON *et al.* 1969), and therefore the studies on serum esterase have given no indications of separate population units in the North Sea and west and south of the British Islands.

## SUMMARY

1. 14 samples of mackerel, about 1800 specimens, from the North Sea and the Norwegian Coast were analysed by agar gel electrophoresis (hemoglobins) and combined starch and agar gel electrophoresis (serum proteins and serum esterase).
2. Hemoglobin variations related to ontogeny appeared during the first year of the mackerel's life. In mackerel one year and older normally one strong hemoglobin component occurred. Two strong components

were observed in some specimens (probably heterozygotes) but too infrequently to be used for studies on population units.

3. In the serum proteins were found extensive variations, but the greater part of the variations occurred in very weak bands, or the observed phenotypes were somewhat unstable. Normally one transferrin band was seen, but also a double band pattern (probably heterozygote) were observed although too infrequently to be used in studies of population units.
4. Five esterase components could be clearly distinguished, and a few more were indicated. Each of the components seemed to be controlled by one gene in a series of polyalleles, although some samples showed a significant excess of observed homozygotes according to this theory.
5. No significant variations in gene frequencies between the samples were found. The results also were approximately in accordance with corresponding results from the southern North Sea and the areas south of the British Islands.

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Table I. Number of specimens analyzed for hemoglobins, serum proteins and serum esterase in blood samples of mackerel from the Norwegian coast and the North Sea.

| Sample no. | Locality and date | Indications of sample | Hemoglobin | Serum proteins | Esterase |
|---|---|---|---|---|---|
| 1 | Håvik i Fusa, Hordaland, 5 Oct. 65 | 0-group | 33 | 30 | — |
| 2 | Fensfjorden, Hordaland, 15 Oct. 65 | 0-group | 28 | 25 | — |
| 3 | 57°40′N, 04°30′E, North Sea, 25 May 66 | Adult, near spawning | 99 | 88 | — |
| 4 | 56°10′N, 05°30′E, North Sea, 25 Aug. 67 | Mixed | 85 | 40 | 55 |
| 5 | 57°58′N, 08°14′E, North Sea, 28 May 68 | Adult, near spawning | 120 | 103 | 120 |
| 6 | 57°55′N, 08°20′E, North Sea, 29 May 68 | Adult, near spawning | 225 | 207 | 215 |
| 7 | 59°27′N, 04°40′E, North Sea, 12 June 68 | Adult, spawning | 274 | 177 | 242 |
| 8 | 60°31′N, 00°05′E, North Sea, 20 June 68 | Immature | 40 | 30 | 39 |
| 9 | Stall, Austfj., Hordaland, 1 July 68 | Adult, spawning | 110 | 75 | 105 |
| 10 | Vernøy, Bjørnefj., Hordaland, 13 July 68 | Adult, spawned | 100 | 99 | 88 |
| 11 | 55°50′N, 06°45′E, North Sea, 9 Aug. 68 | Immature | 90 | 70 | 105 |
| 12 | Asgard, Austfj., Hordaland, 12 Oct. 68 | 0-group | 100 | 99 | 100 |
| 13 | 60°00′N, 03°50′E, North Sea, 24 April 69 | Mixed, mainly immature | 300 | 272 | 299 |
| 14 | 57°56′N, 08°00′E, North Sea, 10 June 69 | Adult, near spawning | 200 | — | 147 |