

# COMPARISON OF BLOOD PROTEINS FROM EAST AND WEST ATLANTIC POPULATIONS OF *HIPPOGLOSSOIDES PLATESSOIDES*

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## ABSTRACT

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Hemoglobins, serum proteins and serum esterase of *Hippoglossoides platessoides* from the eastern and western part of the North Atlantic were analyzed by gel-electrophoresis. Great variety were observed among specimens, and a few rare phenotypes were found only among representatives of one of the two areas. Most phenotypes, however, were found both in the east and west Atlantic samples, although they occurred at different frequencies. The observed differences give no basis for regarding the east and west Atlantic populations as separate species.

## INTRODUCTION

NORMAN (1934) divided the species *Hippoglossoides platessoides* (Fabricius) in the North Atlantic into two subspecies, each with its own geographical range: *H.p. limandooides* (Bloch) in northwestern Europe and *H.p. platessoides* (Fabricius) in North America. He points out, however, that the European and American forms, called long rough dab and American plaice respectively, intergrade in areas where their ranges overlap. Specimens from Iceland and Spitzbergen, for example, approach the American subspecies in depth of body, number of scales, etc.

*H. platessoides* is only lightly exploited but may be regarded as a potential fish resource both on the east and west side of the North Atlantic. For management purposes criteria for distinction between possible stock units will be of significance.

The purpose of the investigations reported here has been to study the relation between the two subspecies by use of characteristics of some blood proteins.

## MATERIAL AND METHODS

An account of the collected material is given in Table 1.

Samples of the fish were selected to cover the entire size range. Bloods were obtained by cutting the tail or drawn by syringe from the heart.

Table 1. Samples of *Hippoglossoides platessoides* analyzed for blood protein variations.

Sample no	Locality and date	Length range, cm	Specimens analyzed		
			Hemoglobin	Serum proteins	Serum esterase
1	St. Margaret's Bay, N.S., Canada	Apr '68 28-53			14
2	—	Oct '68 29-59	73	73	73
3	59°20'N 04°00'E, North Sea	Aug '68 12-20	60	10	51
4	72°00'N 30°00'E, Barents Sea	May '70 25-40		90	90

Samples from Canada were shipped by air to the Institute of Marine Research, Bergen and received within two days. Sample 1 was sent as whole bloods, but this caused lysis of the blood cells, and the sample was suitable only for analyses of serum esterase. In sample 2 sera were separate from the cells before shipping, and both sera and cells were received in good conditions.

Sample 3 was collected onboard a trawler and sent to the Institute where it was received the next day. Sample 4 was collected onboard R.V. "Johan Hjort". This sample had to be kept in the deep freeze until the ship returned to Bergen, and because fish hemoglobins withstand freezing poorly, only sera were analyzed.

Hemoglobins were analyzed by agar-gel electrophoresis at pH 7.2 as described by SICK (1965). Sera were analyzed by the combined starch and agar-gel electrophoresis described by MØLLER (1966) and stained for general protein patterns by Nigrosin and for esterase activity by  $\alpha$ -naphthylacetate with Fast Blue BB Salt as dye coupler.

Hemoglobins were analyzed fresh while sera were analyzed both fresh and after being kept in the deep freeze for several months.

## RESULTS

## HEMOGLOBINS

The observed hemoglobin patterns are outlined in Fig. 1 A.

Two strong and one weak band, pattern 1, were found for all specimens, except four which had individual patterns. Patterns 2 and 3 were found in the sample from the North Sea while 4 and 5 were found in sample 2 from Canadian waters.

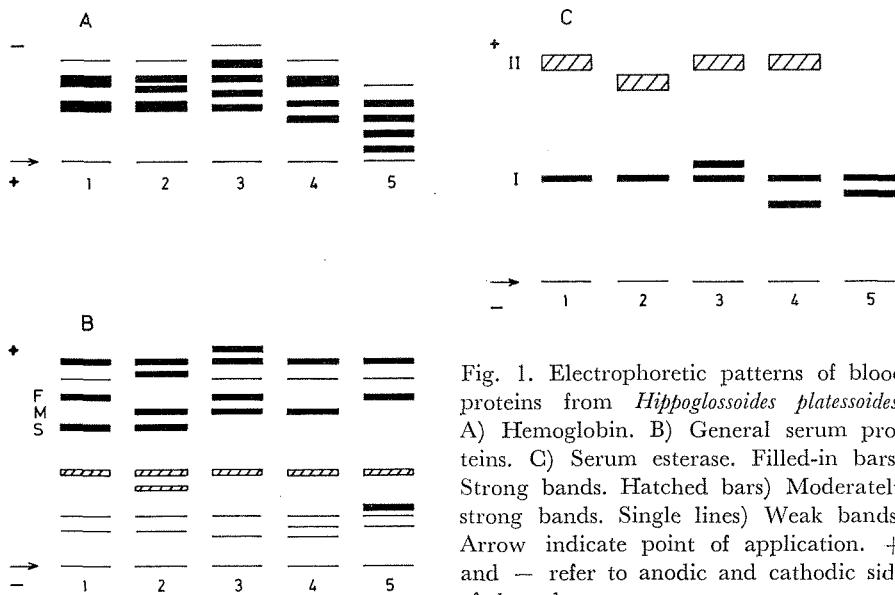


Fig. 1. Electrophoretic patterns of blood proteins from *Hippoglossoides platessoides*.  
 A) Hemoglobin. B) General serum proteins.  
 C) Serum esterase. Filled-in bars) Strong bands. Hatched bars) Moderately strong bands. Single lines) Weak bands. Arrow indicate point of application. + and - refer to anodic and cathodic side of the gel.

The observed variation may be genetically controlled, but this hypothesis can not be tested on the present material due to the scarcity of other phenotypes than the "normal" one.

#### GENERAL SERUM PROTEINS

Some typical patterns of general serum proteins are outlined in Fig. 1 B. A high degree of variation among individual specimens was found within all samples.

The proteins of highest anodic mobility, the albumins, were seen as a single band in most specimens, sometimes with a weak postalbumin at its cathodic side. Double albumins, patterns 2 and 3, occurred at low frequencies both in samples from the west and east Atlantic.

At least three strong bands, called F(ast), M(iddle) and S(low), occurred at the cathodic side of the albumins. In all specimens one or two of these bands were seen, indicating control by three (or more) allelic genes. However, these bands were not always clear enough to permit calculations of frequency distributions of the phenotypes, and thus the hypothesis of genetic control could not be tested. The S band occurred at considerably lower frequency in the samples from the east compared to the west Atlantic.

Individual variations, probably genetically controlled, were observed in several groups of weak components with low anodic mobility.

Due to the weakness of the bands, grouping of the individuals on the basis of their variations was impossible.

In the sample from the Barents Sea one strong component occurred in some specimens; pattern 5. This band may represent the "ripe female protein" noted in other species (NÆVDAL 1969, TSUYUKI and ROBERTS 1966).

#### SERUM ESTERASE

The patterns of esterase activity are outlined in Fig. 1 C.

In the Canadian samples two main zones of esterase activity, called I and II, occurred. In two specimens the II band was lacking. In the samples from the east Atlantic the II band was observed in only four specimens. When the II band occurred, it was found at the same position as in the Canadian samples, except in one specimen where it was found to possess somewhat lower anodic mobility; pattern 2.

Three different variations of double I bands were found; patterns 3, 4 and 5. Three specimens of sample 2 and one specimen in each of samples 3 and 4 showed an extra band at the anodic side of the normal I band. Extra bands at the cathodic side of the normal I band were seen at two positions. The slower moving band, pattern 4, was found in one specimen of sample 1 and five specimens in each of samples 3 and 4. An extra band of somewhat higher mobility, pattern 5, was only found in two specimens of sample 4.

A genetic system of four alleles would explain the observed variation of the I band, but scarcity of the variants, hypothetical heterozygotes, prevents this hypothesis from being tested by population data.

#### DISCUSSION

Two populations are said to be conspecific when they are actually or potentially inbreeding (MAYR, LINDSLEY and USINGER 1953). The populations of *H. platessoides* from the east and west Atlantic are geographically isolated and consequently not actually inbreeding. The problem of their conspecific nature therefore is reduced to determine whether they are potentially inbreeding.

In the present study the genetic basis of the observed variation has not been worked out in details. But no indications of growth dependent variation were found, and sex dependent variation was only indicated in one serum protein of low anodic mobility. The possibility exists that modifications caused by factors other than genetic may account for part of the observed variations. However, the genetic basis of the protein structure (PEACOCKE and DRYSDALE 1965) strongly signify that analyzes of charac-

teristics of the proteins are useful for discrimination of the genotype of individuals and populations.

The east and west Atlantic populations did not differ to a great extent. In the characteristics studied here some hemoglobin and serum esterase I phenotypes were found among representatives of one population only. All these phenotypes were rare, and analyses of greater material would possibly show that they exist also in the other population.

The greatest difference was found in the esterase II component which was lacking in most specimens from the east Atlantic. However, some specimens contained this component as well as it infrequently was lacking in the west Atlantic samples. The varied occurrence of esterase II is thus merely a difference in frequency distribution of phenotypes. Such differences were also observed in distributions of some esterase I components and serum protein components.

The two subspecies, as established by NORMAN (1934), differ in some morphological and physiological characters. In European waters (Clyde area) the maximum length is about 30 cm at 6 years of age (BAGENAL 1955) while it is about 68 cm and 26 years in Canadian waters (POWLES 1965). The fecundity or rate of egg production in relation to size and age is remarkably similar (PRITT 1964), but the females of the European form mature at much smaller size and at much younger age than the American form.

Such physiological variance are commonly found between fish subspecies and correspond to the differences observed in the present investigation of blood characteristics. These differences are of a type which should be expected between conspecific populations rather than between species.

The results of the present study have not excluded that the east and west Atlantic populations of *H. platessoides* is potentially inbreeding, and until greater differences in their genotypes are revealed, they should be regarded as conspecific.

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