International Council for the
Exploration of the Sea

C.M. 1966
Sardine Committee
J : 7

HAEMOGLOBINS IN SPRAT FROM NORWEGIAN WATERS, STUDIED BY AGAR-GEL ELECTROPHORESIS

by
Gunnar Nævdal
Institute of Marine Research, Bergen

Introduction

The sprat in Norwegian waters evidently is recruited in part from spawning grounds in Skagerak and Kattegat, but some spawning may also take place in the fjords of South-Eastern and Western Norway (Bjerkan 1930, Dannevig 1951, Bakken 1966). The results of vertebrae counts (Dannevig 1951) indicate that different shoals of sprat, even in the same locality, may be of different origin.

In the present study an attempt have been made to use frequencies of Mendelian characteristics to investigate the population structure of sprat. Haemoglobins and serum proteins have been studied by electrophoresis, and this report deals with the preliminary results from the haemoglobin analyses.

Sprat haemoglobin polymorphism has been described by Wilkins and Iles (1966), who found three haemoglobin patterns, called "type 1", "type 2", and "type 3" (outlined in Fig. I). Although the sprat haemoglobin patterns closely correspond to some of the length-assosiated haemoglobin patterns in herring, no assosiation between length and haemoglobin type could be found in sprat, and Wilkins and Iles (1966) concluded that "these three patterns may represent the phenotypic expression of a complex genetic segregating machanism in this species".

Material and methods

Blood was obtained from live sprat by cutting the tail. The blood was centrifuged, serum pipetted off, and the cells were lysed by destilled water. Most analyses were made within 24 hours after the blood had been collected, but in some cases the samples had to be stored (at about

2°C) for two days before the analyses could be carried out. This did not seem to have any serious influence upon the technical quality of the results, except that weak components which were present, tended to become stronger after storing.

The electrophoretic technique used for cod haemoglobins (Sick 1965) was applied. Because the same technique was used by Wilkins and Iles (1966), the results could be directly compared. The haemoglobins were stained in Amidoblack 10 B.

The numbers of specimens, date of sampling, and sampling localities are listed in Table I and Table II. Length measurements were taken for part of the material. The age of the principal part of each sample was determined partly from the size and partly from growth zones in the otholits.

Results and discussion

The haemoglobin patterns (phenotypes) which were revealed by these studies, are outlined in Fig. I. The patterns preliminary designed $\underline{a_1}$, $\underline{a_2}$, and b, correspond to the haemoglobin patterns called "type 1", "type 2", and "type 3" by Wilkins and Iles (1966). The other patterns, called c, d, e, and f, posessed other variants of the three strong haemoglobin fractions called 1, 2, and 3 in order of inceasing cathodic mobility. The latter patterns all occurred at low frequencies (f occurred in one specimen only). The distribution of the haemoglobin patterns are shown in Table I and Table II. If the weaker components are also taken into consideration, the specimens can be classified in several more groups. However, because the weak components seemed to increase somewhat upon storing, and because it has not been possible to analyse all samples immediately after sampling, this classification appears to be less reliable, and has been omitted. For the same reason, distinction between the patterns $\underline{a_1}$ and $\underline{a_2}$ was omitted, and these two patterns are lumped together under the heading "a" in the tables.

Two samples from the Oslofjord contained specimens which posessed other patterns, some of which consisted of several bands of high cathodic mobility. Unfortunately, these samples were exposed to temperatures about 10°C for one or two hours before analysis. The new bands may have been produced by the heating, and therefore these two samples are omitted from the present report. However, experiments to produce such patterns from the normal ones, have not been successful.

The differences between the main patterns were in most cases clear, and classification therefore was fairly easy for the greater part of the specimens. However, the difference between patterns c and e often was less evident, and therefore the type-determination of specimens posessing one of these patterns is somewhat unreliable.

Also in the fast component (fraction 4, Fig. I) some variation was observed.

In a few specimens this component was totally absent or appeared only as a very faint band. Some specimens posessed a component of lower cathodic mobility than fraction 4, while this component was still present. Others posessed a component of higher cathodic mobility than fraction 4. The latter variety occurred only in some specimens of the patterns c or e. The variations in fraction 4, however, seemed to be too rare to be utilized in population studies.

The material was separated into age-groups, and the heamoglobin variation was found in samples of the 0-group as well as in samples of older fish. This support the conclusion of Wilkins and Iles (1966) that length-assosiated haemoglobin patterns are not found in sprat.

The results of the analyses reprted here also support the hypothesis of genetic control (Wilkins and Iles 1966). The genetic system still is obscure, but some conclusions may be inferred from the population data presented in Table I and Table II.

From the distributions of the phenotypes it seens obvious that a hypothesis of three allelomorphic genes, controlling the strong fractions 1, 2, and 3, is not applicable. In that case fraction 3 would have occurred more frequently in combination with fraction 1; i.e. pattern f should have been much more frequent.

However, it seems probable that phenotype b with the strong fractions 1 and 2, represents heterozygotes which posess two alleles controlling polypeptide chains in fractions 1 and 2 respectively. Pattern a should then be the phenotypic expression of one of the homozygotes. The other homozygote should be expected to show a haemoglobin pattern with fraction 2 as the only strong component. This phenotype presumably is represented by pattern e, although in this pattern fractions 1 and 3 occur as weak bands. With this assumption the distributions of the patterns (phenotypes) are in good accordance with the expected Hardy-Weinberg distribution of genotypes. Therefore the hypothesis may be correct. It is not easy to fit the patterns c, d, and f into this hypothesis. However, transitional stages between patterns c and e have been noted. This suggest that fraction 2 is rather unstable and may be converted into fraction 3 by environmental factors (*in vivo* or *in vitro*) or by modifying genes which perhaps act upon the recombination of polypeptide chains. Thus it seems possible that patterns c and e, and possibly also d are phenotypic expressions of the same genotype. The genetic basis of pattern f, and also of the weak components which occur, is still unexplained, but the possibility excists that some unknown noninherited factors may influence the haemoglobin patterns.

Assuming that the variations are under genetical control, attention can be turned to the geographical distributions of the samples. Frequencies of pattern a have been calculated as sample parameters and are given for each sample in Table I and Table II. These values vary more than should be expected if the samples were taken at random from a homogenous population, but no marked geographical trend can be discovered. The

lowest values were found in samples 1, 2, and 3 which consist of 0-group sprat from the central parts of Western Norway, while the highest values were found in samples 6, 7, and 8 from localities further south on the coast, and in sample 9 from a northern locality (Sogn). The samples from the Oslofjord and the Skagerak coast all show values similar to the values of some of the samples from Western Norway. The preliminary results of this study therefore indicate that the sprat in Norwegian waters is not homogenous, but consist of several populations. This agrees with results from vertebrae counts (Dannevig 1951). It is, however, necessary to analyse samples from the spawning areas in the Skagerak and the Kattegat before further conclusions can be drawn. Also other polymorphic characteristics, as for instance serum proteins, of which analyses are under way, may become useful in this connection.

References

Bakken, E. 1966. Influence of hydrographical and meteorological factors on catch and recruitment strength of the sprat stock in Western Norway. FiskDir. Skr. Ser. HavUnders., 14 (2) (in press).

Bjerkan, P. 1930. Fluctuations in the stock of young sprat of the west coast of Norway and its relation to the sprat population as a whole. Rapp. P. - v. Réun. Cons. perm. int. Explor. Mer, 65 : 152-181.

Dannevig, G. 1951. Sprat from Norwegian waters. An analysis of vertebrae counts. FiskDir. Skr. Ser. HavUnders., 9 (12) : 1-22.

Sick, K. 1965. Haemoglobin polymorphism of cod in the Baltic and the Danish Belt Sea. Hereditas, 54 : 19-48.

Wilkins, N.P. & Iles, T.D. 1966. Haemoglobin polymorphism and its ontogeny in herring (Clupea harengus) and sprat (Sprattus sprattus). Comp. Biochem. Physiol., 17 : 1141-1158.

Table I. Distribution of haemoglobin patterns in samples of sprat from Western Norway.

Sample no., locality, and date	Haemoglobin pattern						Total	Frequency of pattern a	Age
	a	b	c	d	e	f			
1 Håvik i Fusa, Hordaland. 5.10.65	36	17	-	2	-	-	55	65.5	1-group
2 Fensfjorden, Hordaland. 15.10.65	22	8	-	-	1	1	32	68.8	0-group
3 Fensfjorden, Hordaland. 23.10.65	21	7	4	-	2	-	34	61.8	0-group
4 Førdespollen, Hordaland. 1.4.66	135	7	-	-	1	-	143	94.4	1-group
5 Håvik i Fusa, Hordaland. 6.6.66	95	5	-	-	-	-	100	95.0	1-group
6 Frafjord, Rogaland. 13.6.66	94	5	-	-	1	-	100	94.0	1-group
7 Selvikvåg, Rogaland. 13.6.66	36	-	-	-	-	-	36	100.0	1+2-group
8 Krokholmane, Idsøy, Rogaland. 13.6.66	80	4	-	1	-	-	85	94.1	1-group
9 Simlenes, Sogn. 21.6.66	99	1	-	-	-	-	100	99.0	1-group
10 Grøsvik, Osterfj. Hordaland. 2.8.66	96	19	2	-	2	-	119	80.7	2-group
11 Ryssfjæra, Nordfjord. 11.8.66	81	11	-	-	-	-	92	88.0	1-group
12 Blaksæter, Nordfjord. 15.8.66	83	23	-	-	3	-	109	76.1	1-group
13 Utvik, Nordfjord. 16.8.66	76	24	-	-	2	-	102	74.5	1-group

Table II. Distribution of haemoglobin patterns in samples of sprat from South-Eastern Norway.

Sample no., locality, and date	Haemoglobin pattern						Total	Frequensy of pattern a	Age
	a	b	c	d	e	f			
14 Son, Oslofjorden. 25.8.66	81	15	3	-	2	-	101	80.2	2-group
15 Son, Oslofjorden. 29.8.66	65	22	3	-	1	-	91	71.4	2-group
16 Slemmestad, Oslofjorden. 29.8.66	83	13	-	-	1	-	97	85.6	2-group
17 Flødevigen, Aust-Agder. 1.9.66	62	13	1	-	2	-	78	79.5	0-group

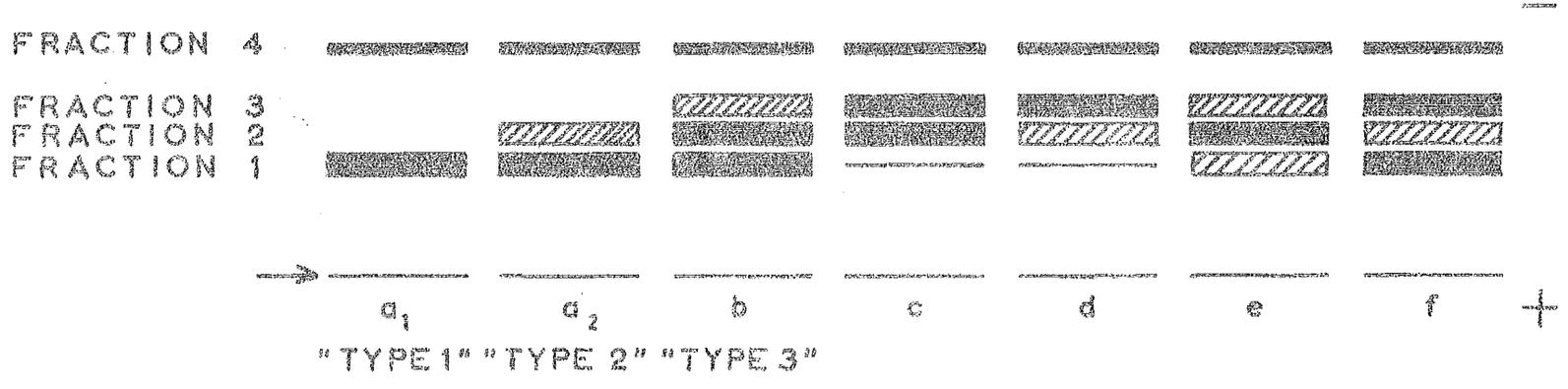

Fig. 1. Haemoglobin patterns in sprat obtained by agar-gel electrophoresis at pH 7.2. Arrow indicate the point of application.