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STUDIES ON HEMOGLOBINS AND SERUM PROTEINS IN SPRAT FROM NORWEGIAN WATERS

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INTRODUCTION

Hemoglobin polymorphism in sprat, *Sprattus sprattus* (L.), has been described by WILKINS and ILES (1966), who found three hemoglobin patterns, called «type 1», «type 2», and «type 3» (outlined in Fig. 2). Although the sprat hemoglobin patterns corresponded closely to some of the length-assosiated hemoglobin patterns in herring, no assosiation between length and hemoglobin pattern could be found in sprat, and the authors concluded that «these patterns may represent the phenotypic expression of a complex genetic segregating mechanism in this species.»

In Norwegian waters sprat is abundant in the Oslofjord and in the fjords of western Norway as far as Stad. The main sprat fisheries take place in these districts, but also at the Skagerak coast off south-eastern Norway and in the Trondheimfjord sprat is abundant enough to be of importance to fisheries.

Although spawning occurs in some fjords of south-eastern and western Norway, the sprat in Norwegian waters evidently is recruited in part from spawning grounds in the Skagerak and the Kattegat (see DANNEVIG 1951 for references). A correlation between catches in western Norway and the thickness of coastal water (BAKKEN 1966) indicates that drift of eggs and larvae with the coastal current northward along the coast is of importance for the recruitment of sprat in western Norway. Results from vertebrae counts (DANNEVIG 1951) indicate, however, that different shoals of sprat, even from adjacent localities, may be of different origin.

Serological methods have been applied to the problem of the population structure of sprat in Norwegian waters, and this paper deals with provisional results obtained from electrophoretic studies of hemoglobins and serum proteins. Part of the results have been briefly dealt with in preliminary reports (MØLLER, NÆVDAL and VALEN 1966, NÆVDAL 1966).

MATERIAL AND METHODS

Blood was obtained from live sprat by cutting the tail, and collected in small glass tubes which were packed and sent on ice in thermos flasks

from the sampling localities to the laboratory. There the blood was centrifuged, and the serum pipetted off.

The erythrocytes were lysed by adding destilled water, and the hemolysate was centrifuged before electrophoresis. In the first few samples (no. 1—5), heparin was used as anticoagulant, but as it appeared that hemoglobin solutions could easily be prepared also from partly clotted blood, no anticoagulant was used for the rest of the samples.

The agar-gel electrophoresis described by SICK (1965) was applied for the sprat hemoglobins. The electrophoretic run lasted for 60 minutes. Most hemoglobin analyses were made within 24 hours after the blood had been collected, but some samples had to be stored (at about 2°C) for two days before analysis. The storage did not seem to influence seriously the technical quality of the results, except that weak components tended to become stronger after storing. Two samples (no. 20 and 24) were accidentally exposed to temperatures about 10°C for one or two hours. They showed to contain several specimens with hemoglobin components of high cathodic mobility. To see whether these types excisted *in vivo* or were produced under influence of increased temperatures, experiments were carried out with storage of hemoglobin specimens at room temperatures.

Sera were subjected to electrophoresis without any initial treatment. Most samples were analysed fresh (within two days after collection), but some were stored frozen for a few days or weeks. Storage did not seem to alter the electrophoretic mobility of the proteins, but the electrophoretograms tended to become weaker and more diffuse.

Serum proteins were analysed by combined starch-/agar-gel electroresis (SICK 1965, MØLLER 1966). For determination of transferrin types, electrophoretic runs of 90 minutes were applied.

Both hemoglobin- and serum-gels were fixed in a 5:5:1 solution of methanol, water and glacial acetic acid, dried at room temperature, and then stained. Hemoglobins were stained in Amidoblack 10 B. The serum protein bands were best made visible by staining with Nigrosine, but Amidoblack 10 B could also be used. Autoradiography according to GIBLETT, HICKMAN and SMITHIES (1959), modified for this type of electrophoresis by MØLLER (1966), was carried out for identification of transferrins. Staining with o-dianisidine (peroxidase activity) was applied to detect haptoglobin/hemoglobin complexes.

Sample localities are shown in Fig. 1 and are listed in Table I together with sampling dates and the number of specimens in each sample analysed for hemoglobins and serum proteins respectively. For several specimens low consentration of proteins in the sera prevented the determination of transferrin types. Therefore the number of specimens is

Fig. 1. Sampling localities of 28 blood samples of sprat from Norwegian coastal waters.

higher for hemoglobins than for transferrins in most samples. For sample no. 9, however, working stress at the laboratory prevented analyses of all hemoglobin specimens. Unreliable results from hemoglobin samples 20 and 24 are discussed later.

Lengths were measured for part of the material, and the age of the bulk of each sample was determined partly from size and partly from growth zones in the otholits.

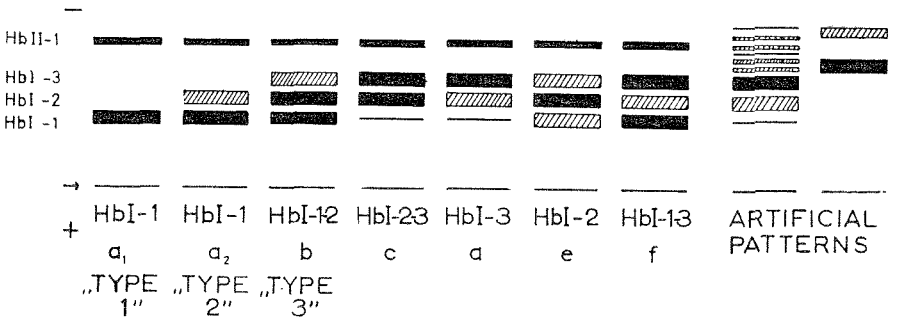
RESULTS AND DISCUSSION

A. BIOLOGICAL VARIATIONS

1. *Hemoglobin*

The technique used by WILKINS and ILES (1966) allows direct comparison of results, and the three hemoglobin patterns revealed by these authors also made up the greater part of the material from Norwegian waters. In a preliminary report (NÆVDAL 1966) these patterns were called a_1, a_2, and b respectively. Other patterns were called c, d, e and f. These designations have been retained as «working names», but for a

complete description of the sprat hemoglobin variations, a nomenclature similar to that used by SICK (1961, 1965) for cod hemoglobins, has been accepted.



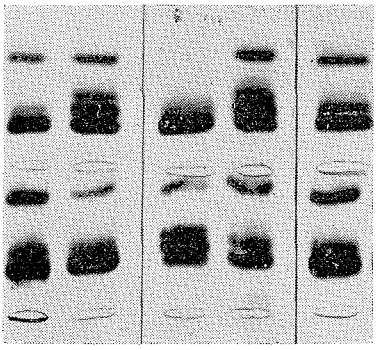


Fig. 2. Outline of hemoglobin patterns in sprat obtained by agar gel electrophoresis at pH 7.2, and photograph of electrophoretograms obtained by routine analyses. The two patterns at right were produced by heating of the specimens. Legend: Filled in bars: Strong bands. Hatched bars: Moderately strong bands. Single lines: Faint bands. Arrow indicates the point of application.

From left to right the photographed types are (in upper and lower row respectively): Hb I–1, Hb I–1–2, Hb I–1 (note the absence of the Hb II–group), Hb I–1–2, Hb I–1, Hb I–1, Hb I–2, Hb I–1 and Hb I–1.

The hemoglobin patterns (phenotypes) revealed by these studies, are outlined in Fig. 2, where also a photo of stained slides with some of the patterns is shown.

Most variations in hemoglobins were found in the slower moving group named Hb I. Three strong fractions were found to belong to this group, and these components were named Hb I–1, Hb I–2, and Hb I–3 in order of increasing cathodic mobility. One or two of these strong components were always present. All the six possible combinations were found, although some of the combinations were very rare.

Weak components were found at the positions where strong fractions were lacking. These weak components varied to some extent, and suggested several more groups of classification. However, the weak components tended to increase in strength upon storing, and it was not possible to analyse all samples immediately after sampling. Therefore classification according to weak components appeared to be less reliable, and was not applied. For the same reason, distinction between «type 1» and

«type 2» of WILKINS and ILES (1966), preliminary called a_1 and a_2 respectively (NÆVDAL 1966), was also omitted because these two patterns differ only in the presence or absence of one weak component at the position of Hb I - 2. However, the weak components were present also in fresh material, and therefore probably also excist *in vivo* like the minor hemoglobin components of cod (SICK 1965). The weak sprat hemoglobin components were designed Hb I–1', Hb I–2', and Hb I–3', respectively found at the positions of Hb I–1, Hb I–2, and Hb I–3.

The hemoglobin patterns (phenotypes) were named according to which of the three main components they contained. Thus the phenotype Hb I–1 comprises the component Hb I–1 only, phenotype Hb I–1–2 comprises Hb I–1 and Hb I–2, etc. The names of the six phenotypes follow from Fig. 2.

A group of hemoglobins of somewhat greater catodic mobility, were called Hb II. The major part of the specimens contained only one strong component, named Hb II–1, in this group. This component was sometimes accompanied by a weak component (named Hb II–1') at its cathodic side. The strength of this weak component increased upon storing, and it was rarely found in fresh specimens. Therefore it probably represents denaturation products. Another component was found at the anodic side of Hb II–1 in a few specimens. This component, called Hb II—2, seemed to be stable, and probably excisted also *in vivo*. In some specimens the Hb II-group of hemoglobin components seemed to be either absent or present as a very faint band only. This occurred also in fresh specimens, and had probably nothing to do with the preservation of the samples. The variations in the Hb II-group, however, were too rare to be of any use in population studies.

Prolonged storage of the samples in the refrigerator did not result in major changes in the hemoglobin patterns, except that the minor components became stronger. After four or five days the bands became diffuse, and the patterns could not be determined. Heating of the blood, however, resulted in «new» patterns. Among specimens which had been kept at room temperatures (about 20°C) for 20 hours before reanalysis two «new» patterns, outlined to the right in Fig. 2, were found. One of these patterns comprised several bands which might vary somewhat in relative intensity, some at positions of normal hemoglobin components and some with higher cathodic mobility. It might be confused with patterns Hb I–2–3 or Hb I–3, but the weak components clearly distinguish this pattern from the normal ones. The other pattern comprised two bands, none, however, at the positions of any of the normal components.

Storage of hemoglobin specimens at room temperatures for periods

of up to five hours caused no major essential changes in hemoglobin patterns, and even after 20 hours, only about half of the specimens were drastically affected.

Two samples from the Oslofjord (no. 20 and 24) contained several specimens which posessed one of the patterns which could be produced *in vitro*. These samples were exposed to a temperature of about 10°C for some hours before analysis because the ice in the thermos flasks had thawed at their arrival in the laboratory. Although considerably higher temperatures and longer time was required to produce the artificial patterns, the results of the hemoglobin analyses for these two samples seemed unreliable and were not considered.

For the greater part of the material, differences between patterns were clear, and the classification therefore fairly easy. The difference between patterns Hb I–2 and Hb I–2–3 might be less evident, and

Table 1. Distributions of hemoglobin patterns (phenotypes) in samples of sprat from western Norway and the Trondheim fjord.

Sample no.	Hemoglobin patterns						Numbers in sample	Frequency of pattern HbI–1
	HbI–1	HbI–1–2	HbI–2–3	HbI–2	HbI–2	HbI–1–3		
1	36	17	—	2	—	—	55	65.5
2	22	8	—	—	1	1	32	68.8
3	21	7	4	—	2	—	34	61.8
4	135	7	—	—	1	—	143	94.4
5	95	5	—	—	—	—	100	95.0
6	94	5	—	—	1	—	100	94.0
7	36	—	—	—	—	—	36	100.0
8	80	4	—	1	—	—	85	94.1
9	99	1	—	—	—	—	100	99.0
10	96	19	2	—	2	—	119	80.7
11	81	11	—	—	—	—	92	88.0
12	83	23	—	—	3	—	109	76.1
13	76	24	—	—	2	—	102	74.5
14	77	16	—	—	1	—	94	81.9
15	105	9	—	—	—	—	114	92.1
16	71	23	—	1	4	—	99	71.7
17	73	21	—	2	1	—	97	75.3
18	81	15	—	2	2	—	100	81.0
19	74	22	2	—	2	—	100	74.0
Total	1 435	237	8	8	22	1	1 711	83.9

the type determination of specimens with one of these patterns might accordingly be unreliable.

The material was separated into age-groups, and the hemoglobin variations were found in samples of the 0-group as well as in samples from older fishes. This supports the conclusion of WILKINS and ILES (1966) that hemoglobin patterns assosiated with age or length are not present in sprat.

Results of the hemoglobin analyses reported here support the hypothesis of genetic control (WILKINS and ILES 1966). The genetic system is still obscure, but some conclusions may be inferred from the population data presented in Table 1 and Table 2. These tables give the distributions of hemoglobin types in the analysed samples which gave reliable results.

Table 2. Distributions of hemoglobin patterns (phenotypes) in samples of sprat from south-eastern Norway.

Sample no.	Hemoglobin patterns						Numbers in sample	Frequency of pattern HbI–1
	HbI–1	HbI–1–2	HbI–2–3	HbI–2	HbI–2	HbI–1–3		
21	81	15	3	—	2	—	101	80.2
22	65	22	3	—	1	—	91	71.4
23	83	13	—	—	1	—	97	85.6
25	62	13	1	—	2	—	78	79.5
26	81	6	—	—	1	—	88	92.0
27	97	4	—	—	1	—	102	95.1
28	59	5	—	—	—	—	64	92.2
Total	528	78	7	—	8	—	621	85.0

From the distributions of phenotypes, it seems obvious that a hypothesis of three allelomorphic genes, each of which controls the strong fractions Hb I–1, Hb I–2 and Hb I–3, is not applicable. If so controlled Hb I–3 should have occurred more frequently in combination with Hb I–1 than demonstrated. However, it seems probable that the phenotype Hb I–1–2 with the strong fractions Hb I–1 and Hb I–2, represents heterozygotes with two alleles where each controls polypeptide chains in Hb I–1 and Hb I–2 respectively. Pattern Hb I–1 should then be the phenotypic expression of one of the homozygotes. The other homozygote should be expected to show a hemoglobin pattern with Hb I–2 as the only strong component. This phenotype presumably is represented by pattern Hb I–2, although the two weak fractions

Hb I–1' and Hb I–3' also occur in this pattern. With this assumption the distributions of the patterns (phenotypes) are in accordance with expected Hardy-Weinberg distributions of genotypes, and therefore the hypothesis may be correct.

It is not easy to fit the patterns Hb I–2–3, Hb I–3, and Hb I–1–3 into this hypothesis. However, transitional stages between patterns Hb I–2–3 and Hb I–2 have been noted. This finding suggests that fraction Hb I–2 is rather unstable and may be converted into Hb I–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 Hb I–2 and Hb I–2–3, perhaps also Hb I – 3, are phenotypical expressions of the same genotype. However, if this hypothesis is correct, some samples have an excess of hypotetical homozygotes compared to expected Hardy-Weinberg distributions. The genetic basis of pattern Hb I–1–3, and also of the variations in the weak components which occurred, still is unexplained, but the possibility exsist that unknown noninherited factors may influence the hemoglobin patterns.

The variations in the Hb II-groups are also unexplained. In specimens which evidently lack this group of hemoglobins, the synthesis of the hemoglobins may be inhibited by some genetical or nongenetical factors. The Hb II–2 component may be controlled by an allele to a gene controlling the normal Hb II–1 component. These alleles must be located to another locus than the genes which control the components in the Hb I-group, because no correlation between variations in the Hb I- and Hb II-groups can be discovered.

The hemoglobin variation in sprat is rather similar to variations in the achoveta, *Engraulis ringens,* and some other clupeoid fishes in Chile (Simpson and Simon Schlotfeldt 1966, Simon Schlotfeldt and Simpson 1966). In these species inter- and intraspecific variation in hemoglobin patterns were found, and there was also great variations among samples caught at different localities. The intraspecific variation did not appear to be ontogenetic, but the numbers and complexity of some of the hemoglobin patterns did not permit genetic interpretation.

2. *Transferrin*

The transferrins of sprat moved towards the anode at pH 9.0, but slower than any of the other serum proteins. By autoradiography, it was shown that three bands on the sprat serum electrophoretograms represented proteins with ironbinding properties. These three bands therefore are believed to represent transferrins, and they have been named

Tf A_1, Tf A_2, and Tf B_1 in order of decreasing anodic mobility. A fourth band of still lower anodic mobility was not tested, since sera in which it occurred were not available when the tracing experiments were made. Its strength and position conforms with the transferrin bands, however, and therefore it was interpreted as a rare transferring component and named Tf B_2.

One, two, or three of the transferrin bands were present in each specimen. The transferrin patterns (phenotypes) which were found, are outlined in Fig. 3, where also a photo of some typical electrophoretograms of sprat sera is reproduced.

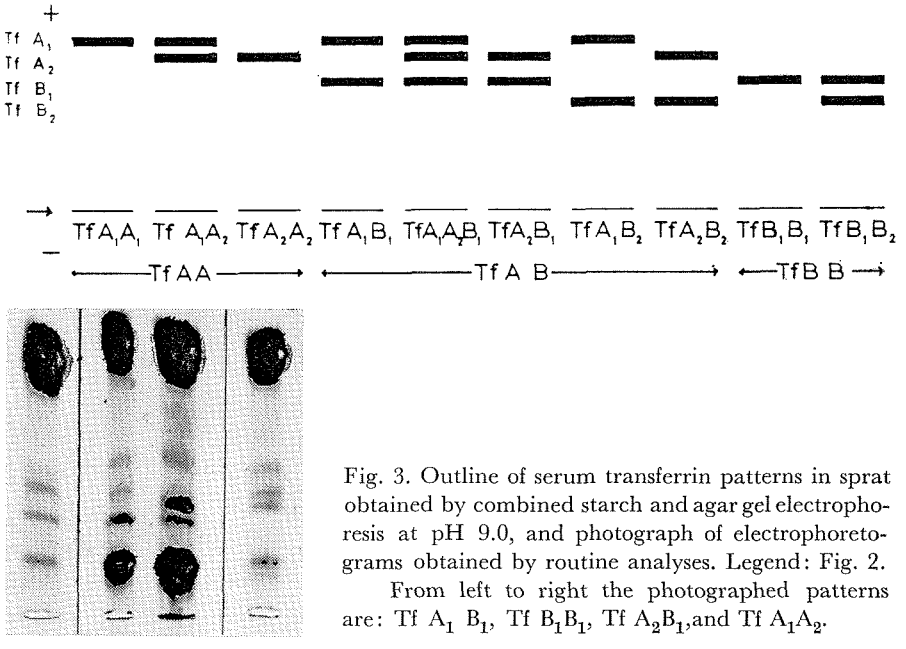


Fig. 3. Outline of serum transferrin patterns in sprat obtained by combined starch and agar gel electrophoresis at pH 9.0, and photograph of electrophoretograms obtained by routine analyses. Legend: Fig. 2.
From left to right the photographed patterns are: Tf A_1 B_1, Tf B_1B_1, Tf A_2B_1,and Tf A_1A_2.

The phenotypes got their designations according to the components they possessed, i. e. specimens with only Tf A_1, were said to belong to the phenotype Tf A_1A_1, those with both Tf A_1 and Tf A_2 to the phenotype Tf A_1A_2, those possessing both Tf A_1, Tf A_2, and Tf B_1 to the phenotype Tf $A_1A_2B_1$, etc. As shown in Fig. 3, 10 different phenotypes were found.

However, for routine analysis it proved to be difficult to distinguish between the bands Tf A_1 and Tf A_2, and therefore these two transferrins were combined and called Tf A. Tf B_1 and Tf B_2 were also combined as Tf B, since Tf B_2, which was found only in some of the samples and always at very low frequencies, did not seem to be of any value for population studies. Therefore the greater part of the material was separated into only three phenotypes, called Tf AA, Tf AB, and Tf BB.

In specimens which evidently belonged to single-component phenotypes, weak bands were often present at the positions of the other components. This was especially true for specimens belonging to phenotype Tf B_1B_1 which often had a faint band at the position of Tf A_1. Usually these components were too weak to cause any difficulty, but the faint bands varied in strength, and occasionally caused doubt about whether a specimen belonged to a double- or a single-component phenotype. However, cases of such hesitation were too few to have any great influence upon the distribution of the phenotypes and the calculated frequencies.

Transferrin variations in cod, *Gadus morhua,* have been found to be controlled by a series of polyalleles (MØLLER 1966). A similar hypothesis of co-dominant alleles each controlling one of four transferrin components, can not be accepted for the transferrin variations in sprat, because three strong transferrin components (Tf A_1, Tf A_2, and Tf B_1) occasionally were found in the same specimen. The transferrin variations in sprat therefore are more complicated than in cod and some other fishes which have been investigated (MØLLER and NÆVDAL 1966).

If the three main transferrin types, Tf AA, Tf AB, and Tf BB are considered alone, the hypothesis may be introduced that two allelomorphic genes, Tf^A and Tf^B control Tf A and Tf B respectively. The genotypes resulting from combinations of these alleles, may be called *Tf A/Tf A, Tf A/Tf B,* and *Tf B/Tf B* according to which of the alleles they possess. In Tables 3 and 4 the frequencies of the gene Tf^A, q^A, have been calculated from observed distributions of the three main transferrin types, and the observed distributions have been compared to expected Hardy-Weinberg distributions of genotypes. The tables show that for most samples there is good agreement between observed and expected distributions. Deviations have been tested by common χ^2-tests (except for a few samples where the numbers within some groups are too low, i.e. $n_{exp} < 5$).

The χ^2-values and the corresponding values for Probability show that the deviations between observed and expected distributions are not significant for any sample. The hypothesis of two allelomorphic genes therefore may be accepted to explain the main transferrin types. However, it has not yet been decided whether the subtypes are under genetical control. Tf B_1 and Tf B_2 may be controlled by two separate alleles at the actual locus, which then contain at least three alleles, Tf^A, Tf^{B1}, and Tf^{B2}, because both Tf B_1 and Tf B_2 have not been found together with Tf A in any specimen.

The fact that Tf B_2 has never been found alone also support the theory of three alleles at the actual locus, because the Tf B_2 components is so rare that the gene which eventually controls it, should not be expected

to be found in a homozygotous state. The Tf A_1 and Tf A_2 components possibly are controlled by two alleles at a second locus, which influence only these two components. However, the distributions of subgroups in samples which gave the best electrophoretograms, show that there are too few hypothetical heterozygotes (Tf A_1A_2) to fit such a system. It is also possible that the subtypes depend upon noninherited factors. Consequently no explanation of the subtype variations can be given at present.

The presence of weak transferrin components in addition to the stronger components has also been demonstrated in herring (NÆVDAL and HARALDSVIK 1966). The weak components do not necessarily exclude genetic control of the main transferrin types. However, the weak components vary in strength and are often absent, and therefore they indicate a more complicated genetic control by modifying genes or an influence by noninherited factors.

The main types, however, seem to satisfy the important claim that characteristics to be used in population studies should be genetically controlled. The corresponding gene frequencies therefore can be used to compare samples.

3. *Other serum proteins*

Outlines of some electrophoretograms of sprat sera containing some hemolysate as obtained by the combined starch-/agar-gel method at pH 9.0 are shown in Fig. 4. The hemoglobin types might also be detected by this method, which causes the Hb I-group to move towards the anode.

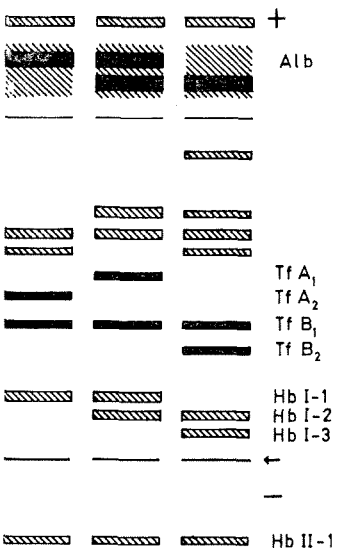


Fig. 4. Outline of sprat serum protein electrophoretograms obtained by combined starch and agar gel electrophoresis at pH 9.0. The position of the hemoglobin components are indicated. Legend: Fig. 2.

However, the agar-gel method is preferred for routine analyses of hemoglobins, since the types are revealed better and more rapid this way.

The position of the transferrin components in relation to the other serum proteins and the hemoglobins, is also shown in Fig. 4. The other serum proteins possessed a higher anodic mobility than the transferrins. In several proteins which were represented by more or less diffuse or strong bands on the electrophoretograms, individual variations were observed, and three of these patterns are shown. These bands, however, are too weak or diffuse for proper classification of the specimens, and they can therefore not be utilized in population studies.

Also in the albumin region individual variations were seen. However, the albumins did not show up as distinct bands which could be easily recognized on the electrophoretograms, and therefore also these variations were unsuitable for identification of populations. The relatively strong band which is shown in the anodic side of the main albumin components, was characteristic for sprat sera, and corresponding bands have not been found in other species (NÆVDAL and HARALDSVIK 1966, and unpublished data). Test for hemoglobin/haptoglobin complexes also gave remarkable results. Peroxidase activity was found only in the albumin region (except for the free hemoglobins), where strong, but diffuse staining with o-dianisidine was obtained. This does not agree with results for some other fish species, where well defined haptoglobin bands, not located in the albumin region, have been obtained (KOEHN 1966, NÆVDAL, unpublished data).

B. *GEOGRAPHICAL VARIATIONS*

Assuming that the hemoglobin variations are controlled genetically, attention can be turned to the geographical distribution of the samples. Frequencies (in per cent) of pattern Hb I–1 have been calculated as characteristic sample parameter and given for each sample in Tables 1 and 2.

The genetical control of the main transferrin types seems to be clear. The frequency of the gene Tf^A, q_A, has been used as characteristic parameter, and the calculated values are given in Tables 3 and 4.

Hemoglobin frequencies vary more than expected for random samples from a homogenous population, and the variation in q_A-values among samples is also considerable. However, the observed frequencies have been found from a limited and varying number of specimens within each sample, and therefore 95 % limits of confidence for the universal frequencies have been calculated.

Table 3. Observed distributions of transferrin types in samples of sprat from western Norway and the Trondheim fjord compared to expected Hardy-Weinberg distributions.

Sample no.		Transferrin groups			Numbers in sample	q_A	χ^2	Probability of worse fit
		Tf AA	Tf AB	Tf BB				
1	obs	6	24	18	48	0.38		
	exp	6.9	22.6	18.6	48.1		0.40	$0.5 < P < 0.7$
2	obs	1	7	11	19	0.24		
	exp	1.1	6.9	11.0	19.0			
3	obs	8	15	10	33	0.47		
	exp	7.3	16.4	9.3	33.0		0.24	$0.5 < P < 0.7$
4	obs	15	64	60	139	0.34		
	exp	16.1	62.4	60.5	139.0		0.12	$0.7 < P < 0.8$
5	obs	12	45	25	82	0.42		
	exp	14.5	40.0	27.6	82.1		1.30	$0.2 < P < 0.3$
6	obs	11	48	36	95	0.37		
	exp	13.0	44.3	37.7	95.0		0.70	$0.3 < P < 0.5$
7	obs	5	15	15	35	0.36		
	exp	4.5	16.1	14.3	34.9			
8	obs	12	47	27	86	0.41		
	exp	14.5	41.6	29.9	86.0		1.41	$0.2 < P < 0.3$
9	obs	14	60	61	135	0.33		
	exp	14.7	59.7	60.6	135.0		0.03	$0.7 < P < 0.8$
10	obs	29	54	32	115	0.49		
	exp	27.6	57.5	29.9	115.0		0.43	$0.5 < P < 0.7$
11	obs	21	44	19	84	0.51		
	exp	21.8	42.0	20.2	84.0		0.20	$0.5 < P < 0.7$
12	obs	14	29	18	61	0.47		
	exp	13.5	30.4	17.1	61.0		0.13	$0.7 < P < 0.8$
13	obs	8	45	34	87	0.35		
	exp	10.7	39.6	36.8	87.1		1.63	$0.2 < P < 0.3$

(cont.)

Table 3 continued.

Sample no.		Transferrin groups			Numbers in sample	q_A	χ^2	Probability of worse fit
		Tf AA	Tf AB	Tf BB				
14	obs	6	27	35	68	0.29		
	exp	5.7	28.0	34.3	68.0		0.07	$0.7 < P < 0.8$
15	obs	12	40	41	93	0.34		
	exp	10.8	41.7	40.5	93.0		0.21	$0.5 < P < 0.7$
16	obs	7	47	34	88	0.35		
	exp	10.7	40.0	37.2	87.9		2.79	$0.05 < P < 0.1$
17	obs	8	33	39	80	0.31		
	exp	7.7	34.2	38.1	80.0		0.07	$0.7 < P < 0.8$
18	obs	9	29	32	70	0.34		
	exp	8.1	31.4	30.5	70.0		0.35	$0.7 < P < 0.8$
19	obs	14	38	40	92	0.36		
	exp	11.9	42.4	37.7	92.0		0.97	$0.3 < P < 0.5$
Total	obs	212	711	587	1510	0.376		
	exp	213.5	708.6	588.0	1510.1		0.04	$0.8 < P < 0.9$

A diagram of the limits of confidence is shown in Fig. 5, where the samples are arranged in geographical order. The significance of the variations in frequencies are tested by standard χ^2-homogenity tests and t-tests («Student»s tests). For calculation of χ^2-values, the number of specimens of all hemoglobin types other than Hb I–1, were combined to get sufficiently high numbers within each class, i. e. $n_{exp} > 5$. An account of the statistical test is given in Table 5. The χ^2-homogenity test of the total samples demonstrated that all samples were not drawn from one homogenous population with regard to these characteristics.

When all samples from western Norway and the Trondheimfjord were compared to all samples from South-eastern Norway by t-tests (means) and χ^2-homogenity tests (distributions), it was demonstrated that significant differences exist in the distributions of transferrin types and in the frequencies of genes supposed to control them. Corresponding differences for the hemoglobins are not significant.

According to χ^2-homogenity tests, there are significant variations in distributions of hemoglobin types within both main groups of samples.

Table 4. Observed distributions of transferrin types in samples of sprat from south-eastern Norway compared to expected Hardy-Weinberg distributions.

Sample no.		Transferrin groups			Numbers in sample	q_A	χ^2	Probability of worse fit
		Tf AA	Tf AB	Tf BB				
20	obs	16	43	30	89	0.42		
	exp	15.7	43.4	29.9	89.0		0.01	$0.9 < P < 0.95$
21	obs	18	46	27	91	0.45		
	exp	18.4	45.0	27.5	90.9		0.04	$0.8 < P < 0.9$
22	obs	14	28	26	68	0.41		
	exp	11.4	32.9	23.7	68.0		1.54	$0.2 < P < 0.3$
23	obs	12	26	17	55	0.45		
	exp	11.1	27.2	16.6	54.9		0.10	$0.7 < P < 0.8$
24	obs	12	34	10	56	0.52		
	exp	15.0	28.0	12.9	55.9		2.60	$0.1 < P < 0.2$
25	obs	12	35	23	70	0.42		
	exp	12.3	34.1	23.5	69.9		0.04	$0.8 < P < 0.9$
26	obs	16	36	32	84	0.40		
	exp	13.4	40.3	30.2	83.9		1.07	$0.3 < P < 0.5$
27	obs	13	40	34	87	0.38		
	exp	12.6	41.0	33.4	87.0		0.05	$0.8 < P < 0.9$
28	obs	10	23	19	52	0.41		
	exp	8.7	25.2	18.1	52.0		0.42	$0.5 < P < 0.7$
Total	obs	123	311	218	652	0.427		
	exp	118.9	319.1	214.1	652.1		0.50	$0.3 < P < 0.5$

The distributions of transferrin types, however, varied significantly only within the group of samples from western Norway. It appears from Fig. 5 that several samples (a. o. no. 2, 4, 9, 14, 15, 17 and 18) have low values of q_A, and these samples probably account for both the significant difference between the group of samples, and the significant variations among the samples from western Norway.

The samples from south-eastern Norway may be separated into two main groups, namely samples from the Skagerak coast and from the Oslofjord. By comparing these two subgroups of samples, the tests showed

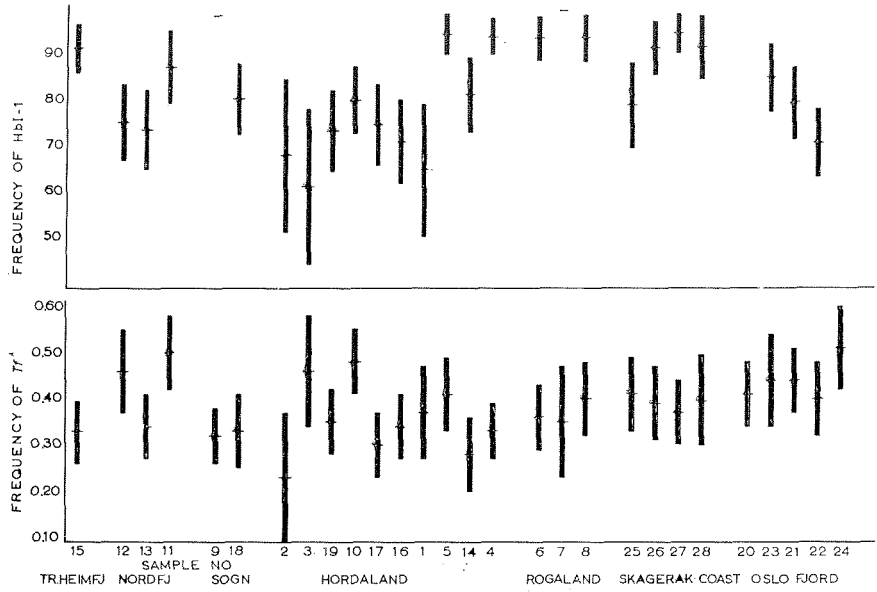


Fig. 5. Confidence intervals for the universal frequencies of Hb I–1 and for the gene Tf^A supposed to control the transferrins in the Tf A-group in sprat.

Horizontal lines mark the observed frequencies, and the vertical bars show the 95 % confidence limits. The samples are arranged in geographical order.

that the distribution of hemoglobin types vary significantly between the two subgroups of samples, whereas variation between the distributions of transferrin types is insignificant.

Further tests of the variations within each of the subgroups show that the distributions of hemoglobin types vary significantly within the samples from the Skagerak coast, but not within the samples from the Oslofjord. The distributions of transferrin types vary sligthly within both groups.

Such tests seems unnecessary for the samples from western Norway, which showed great variations, even between samples from adjacent areas (see Fig. 5). No marked geographical trend can be discovered in the variations of sample parameters, except that the samples from Rogaland all had a high percentage of Hb I–1 and nearly constant intermediate values of q_A.

The observed variations between samples are not easily interpreted. Attention may be drawn to the reliability of the type determinations. Uncertain determination of the transferrin types might be a source of error. However, cases of doubt have been tested by repeated analyses, and specimens which gave electrophoretograms of poor quality, have

Table 5. Statistical tests of significans of observed variations in distributions of hemoglobin and transferrin types of sprat.

	Hemoglobin types						Transferrin types					
	χ^2-homogenity tests			t-test of frequencies			χ^2-homogenity tests			t-tests of frequencies		
	χ^2	d. f.	P	t	d. f.	P	χ^2	d. f.	P	t	d. f.	P
Total samples	165.90	25	<0.001				88.03	54	>0.01 <0.02			
Difference between samples from western and south-eastern Norway	0.45	1	>0.5 <0.7	0.65	1.238	>0.5 <0.6	10.48	2	>0.001 <0.01	3.13	2 604	>0.001 <0.01
Total samples from western Norway	133.32	18	<0.001				66.98	36	>0.001 <0.01			
Total samples from south-eastern Norway	31.21	6	<0.001				11.43	16	>0.7 <0.8			
Difference between samples from the Oslofjord and the Skagerak coast	14.20	1	<0.001	3.76	574	<0.001	2.89	2	>0.2 <0.3	1.61	1 168	>0.1 <0.2
Total samples from the Oslofjord	5.76	2	>0.05 <0.10				9.87	8	>0.3 <0.5			
Total samples from the Skagerak coast	13.15	3	>0.001 <0.01				1.41	6	>0.2 <0.3			

been rejected. Consequently, the number of specimens for which the transferrin types have been correctly determined, is low in the present material, and such cases can not explain the observed variations.

Cases of doubt are even less for hemoglobin type determinations. However, the possibility do excist that unknown non-inherited factors may influence the hemoglobin patterns, but genetic factors seem to be responsible for at least the main hemoglobin types. The variations among samples, discussed here, are based upon the main types only, and therefore these variations should be reliable.

The most likely explanation for the variations among samples is, however, that the samples have been drawn from populations which differ in their gene pool, and between which a high degree of reproductive isolation excist. The present variations may have been caused either by recruitment from separate sprat populations in the Skagerak or Kattegat, or combined recruitment from these areas with recruitments from local spawning in the fjords. Analyses of sprat from the spawning grounds in Skagerak and Kattegat must be made before further conclusions can be drawn on this subject. The present data on serological characteristics correspond fairly well with results from vertebrae counts (DANNEVIG 1951).

SUMMARY

1. A total of 2 332 hemoglobins and 2 162 sera of sprat have been analyzed by agar-gel electrophoresis at pH 7.2 (hemoglobins) and combined starch-/agar-gel electrophoresis at pH 9.0 (serum proteins). The material comprises 28 samples collected at different localities at the Norwegian coast from the Trondheimsfjord to the Oslofjord.
2. The hemoglobins were separated into several weak and strong components. Six main patterns (phenotypes) of strong components could be distinguished.
3. Intraspecific variations were also found in the serum proteins. Most of these variations appeared as presence or absence of weak bands, which were too weak or diffuse to form the basis for proper classification of specimens. However, among the serum transferrins (identitified by Fe^{59}-autoradiography) a total of ten different phenotypes were found. In most samples, individual specimens could be classified into three main combined types only.
4. The hemoglobin types did not demonstrate any relation to length or age. A hypothesis of genetical control involving two (or more) allelomorphic genes is suggested, and the observed distributions of pheno-

types coincide fairly well with expected distributions according to this theory.

5. The three main combined transferrin types seemed to be controlled by two allelomorphic genes, but all the ten types recorded could not be explained by this theory.
6. Frequencies of the most common hemoglobin type (type Hb I–1) in per cent of total number of specimens within each sample, and frequencies of the gene Tf^{A} supposed to control one of the components in the three main transferrin types, have been selected as characteristic sample parameters.
7. Significant variations in sample parameters and distributions of hemoglobin and transferrin types were found among the samples. No marked geographical trend could be discovered, and in some cases great differences were found among samples from adjacent areas. This implies that among the sprat in Norwegian coastal waters there exist two or more populations of different genetic composition, with a high degree of reproductive isolation.

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APPENDIX

Table I. Number of specimens analyzed for hemoglobin and transferrin types in blood samples of sprat from western Norway and the Trondheim fjord. (1—19), and south-eastern Norway (20—28). Results from samples in brackets were unreliable, and have been omitted.

Sample no.	Locality and date		Hemoglobin	Transferrin	Age
1	Håvik i Fusa Hordaland	5.X.65	55	48	1-group
2	Fensfjorden, Hordaland	15.X.65	32	19	0-group
3	Fensfjorden, Hordaland	23.X.65	34	33	0-group
4	Førdespollen, Hordaland	1.IV.66	143	139	1-group
5	Håvik i Fusa, Hordaland	6.VI.66	100	82	1-group
6	Frafjord, Rogaland	13.VI.66	100	95	1-group
7	Selvikvåg, Rogaland	13.VI.66	36	35	1+2-group
8	Krokholmane, Idsøy, Rogaland	13.VI.66	85	86	1-group
9	Simlenes, Sogn	21.VI.66	100	135	1-group
10	Grøsvik, Osterfjorden, Hordaland	2.VIII.66	119	115	2-group
11	Ryssfjæra, Nordfjord	11.VIII.66	92	84	1-group
12	Blaksæter, Nordfjord	15.VIII.66	109	61	1-group
13	Utvik, Nordfjord	16.VIII.66	102	87	1-group

Sample no.	Locality and date	Hemoglobin	Transferrin	Age
14	Nå, Hardanger, Hordaland 14.X.66	94	68	1-group
15	Åsenfjord, Trondheimsfjorden 24.X.66	114	93	1-group
16	Stamnes, Osterfjorden, Hordaland 9.XI.66	99	88	0-group
17	Mostraumen, Osterfjorden, Hordaland 9.XI.66	97	80	0-group
18	Dalsøyra, Sogn 11.XI.66	100	70	0-group
19	Lindås, Hordaland 15.XI .66	100	92	1+2-group
20	Måkerøy, Oslofjorden 25.VIII.66	(87)	89	2-group
21	Son, Oslofjorden 25.VIII.66	101	91	2-group
22	Son, Oslofjorden 29.VIII.66	90	68	2-group
23	Slemmestad, Oslofjorden 29.VIII.66	97	55	2-group
24	Rørvik, Oslofjorden 30.VIII.66	(90)	56	2-group
25	Flødevigen, Aust-Agder 1.IX.66	78	70	0-group
26	Sandnesfjord, Aust-Agder 27.IX.66	88	84	0-group
27	Kattøya, Langesundfjorden 3.X.66	102	87	1-group
28	Smedholmen, Langesundfjorden 3.X.66	64	52	0-group