International Council for
the Exploration of the sea

C.M. 1981/H:65
Pelagic Fish Committee

ENZYME POLYMORPHISM OF SPRAT FROM NORWEGIAN WATERS - PRILIMINARY RESULTS

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ABSTRACT

To study the population structure of sprat in Norwegian coastal and offshore waters, search for polymorphism in muscle enzymes was undertaken in autumn 1980. Preliminary results show that the enzymes malate dehydrogenase (MDH), lactate dehydrogenase (LDH), phosphoglucomutase (PGM), phosphoglucose isomerase (PGI) and isolitrate dehydrogenase (IDH) are polymorphic. -glycero-phosphate dehydrogenase (GPD) showed great individual variation but so far the genetic basis of the variation is not revealed. In population work MDH and PGI seem most promising.

INTRODUCTION

Polymorphism in blood proteins of sprat, Sprattus sprattus, from Norwegian waters was studied 10-15 years ago by Nævdal (1968, 1970). The main purpose of the investigation was to study the population structure of the sprat in these waters, and heterogenety in frequency distributions of types of hemoglobins and serum transferrins were observed among samples from inshore Norwegian waters.

The main problems for these early studies were the small quantities of blood in the individual sprat, and thus the proteins were easily denaturated. It was also necessary to have the sprat alive to get blood samples. To overcome these difficulties and continue the study, search for polymorphism in soluble enzymes in muscle extract was initiated in autumn 1980. The present report deals with preliminary results from this study.

MATERIAL AND METHODS

Totally seven samples, representing 636 individuals were analysed. The samples are listed in Table 1. Two samples represent the North Sea sprat, one sample was collected in Kattegat and the rest were collected in inshore Norwegian waters.

The fish were frozen on the research vessels and kept frozen until thawed at the laboratory one day prior to analysis. Small piecies of muscle was cut off and homogenized, and after centrifugation, the supernatants were analysed by starch-gel electrophoresis. In these preliminar studies the technique used for corresponding analysis of herring, was directly applied. Until now, no modifications or adaptations of the technique for analysis of sprat have been carried out. The details of electrophoresis and the staining (methods) will be published separately (Jørstad, in preparation).

The gels were cut horizontally in slices, and each were stained separately for the following enzymes:

lactate dehydrogenase (LDH)

malate dehydrogenase (MDH)

phosphoglucomutase (PGM)

phosphoglucose isomerase (PGI)

isocitrate dehydrogenase (IDH)

-glycerophosphate dehydrogenase (GPD)

aspartate aminotransferase (AAT)

The zone controlled by the most common allele in each presumed genetic system was named 100. Other zones named according to their anodic moblilty relative (in percent) to the most common one. Teh phenotypes were named according to the zones they contained, for instance 100/100 (presumed homozygote) and 70/100 (presumed heterozygote).

RESULTS

MDH MDH was described by Koval (1976). Analysis of different tissuss (data not shown) indicate, however, that three loci are involved in the controle of MDH activity in sprat. The enzyme which predominate in white muscle, was at the present time named MDH-3. In the present study four phenotypes, probably representing two common and one rare alleles, were seen for this loci. The phenotypes are outlined in Figure 1a. The three common phenotypes probably represent the same system as described by Koval (1976).

The distributions of gene frequencies are shown in Table 2. The frequencies of the allele 150 are similar to the frequencies of the gene Mdh^A of Koval and Feldman (1976).

LDH Observed phenotypes of LDH are outlined in Figure 1b. The general pattern of sprat is very similar to the pattern of herring as described originally by Odense, Allen and Lenny (1976), and also in sprat variations both in A chanis (LDH-1) and B chains (LDH-2) were observed. The variation in the A chains, however, is not very well revealed by the present methode because of the low moblility of the A_4 molecules at this pH.

Distributions of the gene frequencies are shown in Table 2 for LDH-1 and LDH-2 separately. In each system only one allele is common; the other alleles were found at low frequencies and only represented as heterozygotes.

PGM In PGM one common and two rare phenotypes were found, Figure 1c. The phenotypes -30/100 and 100/200 probably represent heterozygotes for rare alleles with very low exectance to be found in a homozygotoes state.

PGI For this enzyme two loci were detected, and PGI-2 was strongly expressed in muscle tissue. In the samples in question totally eight phenotypes were seen, (Figure 11), some with very low frequencies. (Also one or two more were indicated, but they are tentatively lumped with the others). The phenotypes were explained by segregate of six alleles, but only two of them were found as homozygotes. The observed distributions were in good accordance to expected Hardy-Weinbergs distributions based on this hypothesis. Distributions of gene frequencies in the analysed samples are shown in Table 2.

IDH IDH activity seems to be under control of at least two different loci, called IDH-1 and IDH-2. In both systems one common and one or two rare alleles seems to be represented. The rare alleles were only found as presumed heterozygotes. The IDH-2 enzyme, however, was most strongly expressed in eye tissue and thus of limited use of this polymorphism in the present study.

GPD This enzyme showed extensive variation in electrophoretic patterns, but by the present methode it was difficult to group the patterns into well defined phenotypes. The stained zones of enzyme activities were often weak, and probably there were also some interference with other enzymes.

AAT This enzyme seemed to be monomorphic by the present methode. However, some broad and diffuse bands imply variation which may be revealed by a modified technique.

DISCUSSION

The present investigation is only the first approach for using enzyme polymorphism in studies of population structure of sprat in Norwegian waters. Koval and Feldman (1978) found heterogenety among samples of sprat from different areas of the North Sea by MDH and esterase polymorphism, and Aps and Tanner (1979) found similar differences in the Baltic Sea.

Polymorphism seems to be very extensive in sprat. This was shown already in the sixties (Nævdal 1967, 1970) and by Veldre and Veldre (1979) in blood proteins, and also in the present study extensive polymorphism is revealed. Although the genetic control of blood protein polymorphism were sometimes obscure, the genetic control of the polymorphism seen in muscle enzymes seems to be quite clear. As observed, the distribution of phenotypes are in accordance with the expected distribution. The variation observed in GPD needs further investigation.

The variations between samples in the present investigation are inconspicuous. Some heterogeneity among samples are indicated because the difference between some of the samples are close to statistical significance. According to investigation on drift of eggs and larvae a considerable part of the sprat in Norwegian fjords are recruited from spawning grounds in the Kattegat and possibly from the North Sea (Dannevig 1951, Bakken 1966). Spawning also occur in some fjords both on the southeast and west coast. The early investigations on blood proteins was in accordance with this theory, showing that the main part of the samples from the coast were very similar to the offshore samples, while some samples showed characteristics of their own. We will try to confirm these results vy further analyses of samples from the coastel waters, and if possible draw conclusion about the relative importance of the recruitment from the local spawning to the sprat fishery.

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Table 1. List of samples of sprat for analysis of enzyme polymorphism.

Sample no.	Locality	Area	Number in sample	Characteristics of sample	Fishing gear	Date of sampling
1	N 54°15' E 02°30'	North Sea	96	0-group	Mid water	Dec. 28,1980
2	N 54°15' E 03°30'	- " -	96	- " -	trawl	" 11, "
3	N 57°25' E 11°40'	Kattegat	96	- " -	- " -	Nov. 13, "
4	Jondal, Hardanger	Norwegian coast	66	0-group	- " -	" 10, "
5	Gulosen, Tr.heimsfjorden	- " -	96	- " -	- " -	" 19, "
6	Nordrana, Helgeland	- " -	96	- " -	- " -	" 24, "
7	Namsenfjorden,Trøndelag	- " -	96	- " -	- " -	" 21, "

Table 2. Allele frequencies of enzyme variants expressed in white muscle of sprat (Sprattus sprattus L.).

Sample No.	No.of fish analysed	LDH-1			LDH-2		MDH-3			PGI-2				
		70	90	100	100	130	0	100	150	50	80	100	160	200
1	96	-	0.010	0.990	0.995	0.005	-	0.750	0.250	0.026	-	0.927	0.047	-
2	96	-	0.010	0.990	1.000	-	0.010	0.750	0.240	0.031	-	0.911	0.052	0.005
3	96	-	0.005	0.995	1.000	-	0.005	0.719	0.276	0.026	-	0.932	0.042	-
4	60	-	0.008	0.992	1.000	-	-	0.695	0.305	0.030	-	0.917	0.053	-
5	96	0.005	0.036	0.953	1.000	-	0.005	0.776	0.219	0.036	0.010	0.896	0.052	-
6	96	-	0.031	0.969	1.000	-	0.010	0.698	0.292	0.031	-	0.932	0.031	-
7	96	-	0.021	0.979	1.000	-	-	0.766	0.234	0.047	-	0.917	0.036	-

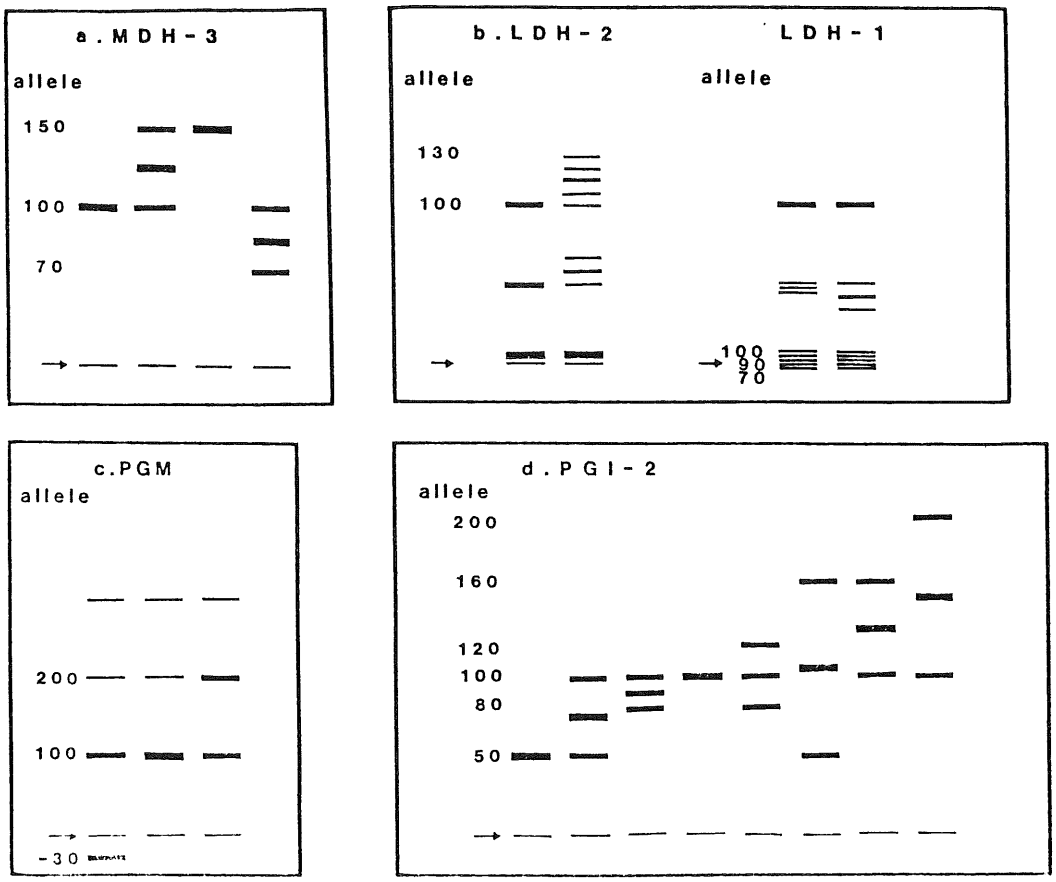

Fig. 1 Outlines of phenotypes for different enzyme loci expressed in the white muscle of sprat. Arrows indicate the point of application before electrophoresis.