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The Propagation of Cod *Gadus morhua* L.

STOCK STRUCTURE OF COD IN THE MØRE AREA

J. Reisegg[1] and K.E. Jørstad[2]

1 Department of Fisheries Biology, University of Bergen, P.O. Box 1839, N-5011 NORDNES Norway
2 Institute of Marine Research, P.O. Box 1870, N-5011 NORDNES Norway

ABSTRACT

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Recently several research projects have involved studies of cod in the Møre area. Due to a common sampling programme a large number of individual fish have been classified for biological and genetical characters. The genetical study included analyses of mature fish, juveniles and eggs/yolksac larvae, and all samples were analysed for polymorphic proteins by using electrophoresis combined with selective staining methods. A significant amount of heterogeneity, expressed as variation in genotype distributions and allele frequencies, was observed among cod samples from this area, suggesting a rather complex stock structure.

Samples which were taken during spawning consisted of a mixture of Arctic and coastal cod. Some of these samples permitted comparisons between biological and genetic data. The correlation between otolith structure and the allele frequencies for the enzymes analysed was not as significant as in earlier studies on blood factors and blood proteins. Significant differences between the two groups of cod were detected for two proteins (lactate dehydrogenase and haemoglobin), confirming the results from earlier genetical studies of cod in this area.

The variation observed in different samples of coastal cod was very complex and difficult to interpret in terms of a reliable stock model. At present, however, the genetical data on spawning samples of coastal cod indicate a distinct stock in the Smøla region. In the Sunnmøre region the preliminary analyses of cod eggs taken on the main spawning ground at different times suggest more than one spawning

group. This new sampling approach seems to be very useful in studies of fish gene pools. With regards to the stock structure of coastal cod in this area, we feel that genetic analyses of cod eggs on locally distributed spawning grounds will provide the most reliable information.

INTRODUCTION

In spring the Arctic and coastal cod are found on the same spawning grounds from Møre and north along the Norwegian coast. Genetic studies on blood proteins (Frydenberg, Møller, Nævdal and Sick, 1965; Møller, 1968) and different blood groups (Møller, 1967) have shown that the two main groups of cod form genetically separate populations with limited gene flow. The genetic data (Møller, 1968) on cod in the Møre area also suggested difference between samples of caostal cod, and recently biological studies indicated a rather complex stock structure (Godø, 1977). Knowledge about what kind of mechanisms maintain the two groups as geneticall-ly distinct units is limited and the management of cod resources are at present based on classification of cod individuals according to otolith structure (Rollefsen, 1933).

During the last decade the technique of enzyme electrophoresis (Harris and Hopkinson, 1976) has been more widely applied, and population genetic studies have shown that most fish species seem to be divided into a number of genetically distinct populations (for review see Allendorf and Utter, 1979; Ferguson, 1980). It has been recommended (FAO, 1981) that such population units or stocks should be considered and managed as unique biological units.

As a result of the new methods available, the stock structure problems in the Møre area and the general interest in stock structure of cod, a genetical investigation programme was started as a part of the studies of cod in this area. The main purpose was to study the relationship between Arctic and coastal cod, focusing on potential genetical markers which could discriminate between the two groups. In addition, we wanted to identify local stocks of coastal cod.

In principle, two different sampling strategies can be chosen in population genetical studies of natural species and populations. The first one involves genetical analyses of a limited number of individuals and samples for as many different gene loci as possible. Such analyses include both monomorphic and polymorphic loci and give valuable information about the phylogenetic relationship between different species and populations (Nei, 1975).

In the second approach only polymorphic proteins are analysed, and this usually permits large scale screening of individuals and samples. Intraspecific variation is detected as statistically significant differences in genotype distributions and/or allele frequencies for the polymorphic enzymes analysed. In a pilot study, we screened a limited number of cod individuals to identify polymorphic enzymes of potential use in characterization of cod populations. In this report, however, we present results from a screening of a large number of fish only for those polymorphic enzymes which looked most promising. In addition to sampling of spawning fish, we extended the studies by including analysis of cod eggs occurring on different spawning grounds.

MATERIALS AND METHODS

The main area of the investigation is shown in Fig. 1 where the most important spawning grounds (Borgundfjord, Runde) are also indicated. The sampling programme in this area in different years is summarized in Table 1 and was carried out in cooperation with O.R. Godø of the Institute of Marine Research, Bergen between 1980-1982. Some of the samples of 0-group cod were collected on cruises organized by the Department of Fisheries Biology, University of Bergen.

A small piece of white muscle was cut from each fish from which biological data were also recorded. The tissue samples were frozen and analysed either onboard the research vessel

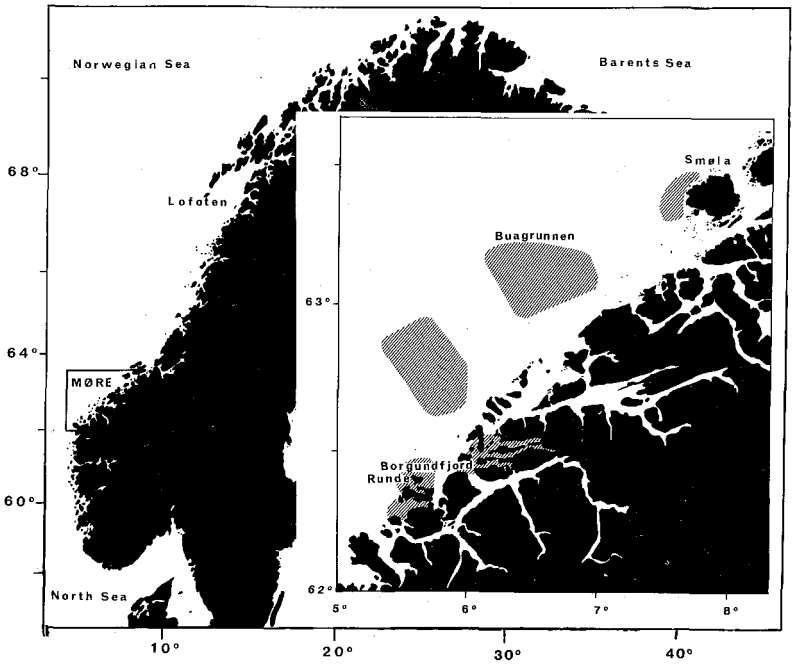

Fig. 1. Sampling localities in the Møre area.

or stored at low temperature (-80°C) until analysis in the laboratory.

Samples of eggs on the spawning grounds were collected by using Juday nets, and the eggs were carefully washed in cold seawater in order to separate them from other planktonic material. The eggs were kept alive in seawater at about 6-8°C and fresh seawater was added at one or two days intervals depending on egg density in the sample. When hatching occurred, samples of newly hatched yolksac larvae were analysed following the procedures described by Jørstad et al. (1980).

All tissue samples were analysed by horizontal starch gel electrophoresis using a histidine/citrate buffer system (Ward and Beardmore, 1977) followed by staining of different

enzymes (Harris and Hopkinson, 1976). Usually, the following enzymes were investigated (see Table 1): lactate dehydrogenese (LDH), isocitrate dehydrogenase (IDH), ∝-glycerophosphate dehydrogenase (GPD), phosphoglucose isomerase (PGI) and phosphoglucomutase (PGM). The different enzyme loci and alleles detected in natural populations of cod have been described elsewhere (Cross and Payne, 1978; Jørstad et al. 1980; Moth-Poulsen, 1982; Mork et al. 1982). In this study LDH and especially the *LDH-3* locus seemed to be most informative. A picture of a starch gel stained for this enzyme is shown in Fig. 2 demonstrating the three most common phenotypic banding patterns. This gel represents newly hatched cod larvae from one of the main spawning ground. Designation of enzyme loci and alleles follow the recommendations of Allendorf and Utter (1979).

TABLE 1

Sampling programme and polymorphic proteins analysed.

Year	Month	Stage of development	Protein loci
1980	March-April	spawners	*IDH-2, PGI-1, PGM, GPD*
	June	mature	*LDH-3, IDH-2, PGI-1, PGM, GPD*
	July-December	mature	*LDH-3, IDH-2, PGI-1, PGM, GPD*
1981	March-April	spawners	*LDH-3, IDH-2, PGI-1, PGM, GPD*
	June	mature	*LDH-3, IDH-2, PGI-1, PGM, GPD*
	August	0-group	*LDH-3, IDH-2, PGI-1, PGM*
1982	March-April	spawners	*LDH-3, IDH-2, PGI-1, PGM, GPD, HbI*
		egg/yolksac larvae	*LDH-3*
	August	0-group	*LDH-3, IDH-2, PGI-1, PGM*

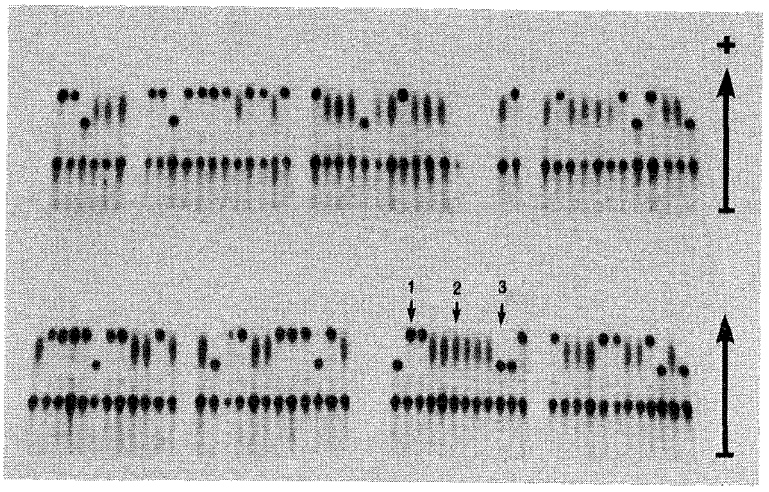

Fig. 2. Lactate dehydrogenase (LDH) zymogram of newly hatched cod larvae analysed by horizontal starch gel electrophoresis. Banding patterns of the most common *LDH-3* phenotypes are indicated. (1) *100/100* homozygote; (2) *70/100* heterozygote; (3) *70/70* homozygote.

In the haemoglobin analyses, included in the later part of the study, the methods described by Sick (1961) were applied with minor modifications. We preferred to increase the concentration of agar (2X) in the gel and the concentration of the electrode buffer (10X). For comparison with earlier studies we used the nomenclature used by Frydenberg et al. (1965).

Usually, genetic data are presented as genotype distributions and allele frequencies for the different loci and samples analysed. Due to the large amount of data obtained in this study, it was impossible to give a complete record of all observations. For this reason the data have been pooled into groups according to geographic distribution and biological information. For simplicity the tables only show the frequency of the most common allele for a polymorphic locus

(for example *LDH-3(100)*) and the number of individuals analysed. In the statistical treatment, however, we have used the actual number of genotypes present in each sample group. To detect heterogeneity within a sample group, we applied a test of homogeneity (G-test) described by Sokal and Rohlf (1969). Genotype distributions at more than one loci may be included in the test. The P values given in the various tables reflect the probability of drawing the samples from a genetically homogenous population of cod. Significant heterogeneity is observed for P values <0.05. In some cases the homogeneity tests were applied to compare different samples in a sample collection. When testing for deviation from Hardy-Weinberg's expectations, we applied the test described by Christiansen et al. (1976).

RESULTS

The total material consisted of about 6000 individuals, including analyses of 850 eggs from the spawning grounds. Only few of the sample groups were analysed for all proteins (see Table 1), and no significant variation was found for three of the polymorphic enzymes investigated. For these, the range of variation for the most common allele was as follows: *PGM(100)* 0.92 - 0.98; *IDH-2(100)* 0.97 - 1.00; and *GPD(100)* 0.94 - 1.00. Because of the low level of variation further details of these enzymes are not presented.

The analyses of *LDH-3*, *PGI-1* and *HbI* revealed significant variation and are given a more detailed treatment. About 60% of the analysed material consisted of samples taken on the main spawning grounds during spawning (Table 3, Table 4). In some of these samples, especially those from the 1982 season, biological and genetical characteristics could be compared. The material was grouped into Arctic and coastal cod according to otolith type and when the genetical data were compared for the two groups, the results given in Table 2 were obtained. The P values given were calculated from the actual

TABLE 2

Allele frequencies(q) and number of specimens analysed(n) in cod groups classified according to otolith structure.

Cod group	*PGI-1(100)* q / n	*LDH-3(100)* q / n	*HbI(1)* q / n
Arctic cod	.69 / 241	.57 / 230	.19 / 234
Coastal cod	.62 / 81	.72 / 77	.34 / 76
Test of homogeneity, P:	.137	.002	.006

Homogeneity test based on genotype distributions at three loci, P=0.0002

number of genotypes for the different loci. The differences between the two groups were significant for *LDH-3* (P=0.002) and *HbI* (P=0.006). By including all three loci in the homogeneity test, the estimated probability for the two groups of cod belonging to the same population was approximately 0 (P=0.0002). The frequencies of *PGI-1(100)* and *LDH-3(100)* for the Arctic cod were very close to the estimated values of Arctic cod in Lofoten, the Barents Sea and the Spitsbergen area (Jørstad, unpublished). The frequency of *HbI(1)* was higher than expected, and this observation is discussed in more detail below.

In Table 3 data on spawning samples consisting of 70-90% of Arctic cod are summarized. No significant variations were detected over three consecutive years in a large number of individuals. Note that the sensitivity of the statistical test is greatly increased with high numbers of individuals analysed and this faciliates detection of minor genetic differences. The low frequency of *HbI(1)* (0.13) agree with the earlier estimates in samples of Arctic cod (Møller, 1968).

The most extensive sampling was carried out on the main spawning grounds in the Sunnmøre area (Borgundfjord, Runde).

TABLE 3

Allele frequencies(q) and number of specimens analysed(n) of samples mainly consisting of Arctic cod.

Year	Month	Area	*PGI-1(100)* q / n	*LDH-3(100)* q / n	*Hbl(1)* q / n
1980	March	Sunnmøre	.66 / 721	.61 / 93	
	April	Sunnmøre	.65 / 472	.57 / 453	
1981	March	Sunnmøre	.69 / 464	.58 / 458	
	April	Sunnmøre(Runde)	.62 / 285	.59 / 191	
1982	March	Sunnmøre(Runde)	.66 / 158	.59 / 188	.13 / 184
	April	Sunnmøre(Runde)	.65 / 95	.59 / 93	.12 / 96
Test of homogeneity, P:			.445	.269	.842

Homogeneity test based on genotype distributions at three loci, P=0.431

TABLE 4

Allele frequencies(q) and specimens analysed(n) in samples of spawning coastal cod.

Year	Area	*PGI-1(100)* q / n	*LDH-3(100)* q / n	*Hbl(1)* q / n
1980	Sunnmøre	.67 / 542	.63 / 571	-
1982	Sunnmøre	.69 / 90	.53 / 90	-
	Smøla	.59 / 90	.62 / 90	.47 / 39
Test of homogeneity, P:		.246	.069	-

Homogeneity test based on genotype distributions at two loci, P=0.083

The Arctic cod constitute the greater fraction in these samples, and therefore the number of coastal cod sampled during the spawning season is low compared to Arctic cod. The results obtained for some samples of coastal cod are, however, shown in Table 4. Due to the existence of other local spawning grounds for coastal cod, it is clear that the material shown does not represent all the different groups of coastal cod present in the area. A high frequency of *HbI(1)* was found in the sample of coastal cod in the Smøla region and this result has been confirmed in the 1983 analyses. The coastal cod in this area, therefore, seem to constitute a genetically distinct population. Surprisingly, the sample of coastal cod taken in the Sunnmøre area in 1982 have a low frequency for *LDH-3(100)* compared with other groups of coastal cod and with the Arctic cod. Although not statistically significant, the observation suggests a more complicated stock structure in the southern part of the area.

TABLE 5

Allele frequencies(q) and the number of specimens analysed(n) in samples of coastal cod in the non-spawning season.

Year	Month	Area	*PGI-1(100)* q / n	*LDH-3(100)* q / n
1980	June	Smøla	.67 / 875	.62 / 672
	June/July	Buagrunnen	.65 / 404	.60 / 455
1981	June	Buagrunnen	.65 / 336	.64 / 336
	June/July	Sunnmøre	.71 / 119	-
	June	Sunnmøre	.60 / 170	.64 / 171
Test of homogeneity, P:			.0003	.130

Homogeneity test based on genotype distributions at two loci, P=0.431

The existence of different groups of coastal cod in the area is also supported by results obtained from samples of mature fish taken in the non-spawning season (Table 5). The table shows pooled data from several catches in different areas, and the heterogeneity detected in the *PGI-1* locus is mainly due to the samples drawn from the Sunnmøre area. Variations in allele frequency of *LDH-3(100)* from 0.55 to 0.69 (data not shown) have also been observed among different catches in this region. These observations have been made during the non-spawning season, and care should be taken when interpreting the variations seen in terms of sub-population structure. At present it is also unclear if such variation could be due to different feeding migrations, environmental preference or other behavioural differences.

In spite of few samples, the data on 0-group cod in the area (Table 6) agree with the other results from analyses of coastal cod.

TABLE 6

Allele frequencies(q) and number of specimens(n) analysed in samples of 0-group cod.

Year	Month	Area	*PGI-1(100)* q / n	*LDH-3(100)* q / n
1980	August	Borgundfjord	.66 / 115	.67 / 115
1982	"	Smøla	.67 / 190	.62 / 190
Test of homogeneity, P:			.463	.430

Homogeneity test based on genotype distributions at two loci, P=0.507

In 1982 eggs from the main spawning grounds were collected at different times during the spawning season. As shown in Table 7 the eggs taken late in April have a relatively low frequency of *LDH-3(100)*. A significant amount of heteroge-

neity existed (P=0.014) in the material. By performing a homogeneity test between early and late spawning, the probability that the eggs were drawn from the same population was calculated as low as 0.006. Unfortunately, no samples of spawning cod at this time were available, but the data point to the existence of a separate stock of coastal cod spawning in the second half of April in the southern part of the area.

TABLE 7

Allele frequencies(q) and number of specimens analysed(n) of cod eggs sampled on the spawning grounds in 1982.

Spawning season	Area	*LDH-3(100)* q / n
March-April (early spawning)	Inner Sunnmøre	.62 / 231
" "	Southern Sunnmøre	.62 / 190
" (late spawning)	Inner Sunnmøre	.55 / 176
" "	Southern Sunnmøre	.54 / 254

Test of homogeneity, P: .014
Homogeneity test between eggs
of early and late spawning: P= 0.006

DISCUSSION

Frydenberg et al. (1965) provided evidence for the existence of different stocks of coastal cod along the Norwegian coast. In more detailed investigations Møller (1967, 1968) found correlation between the otolith types of cod (Rollefsen 1933) and haemoglobin variants as well as blood factors, and interpreted the results as evidence for the Arctic and coastal cod being genetically distinct groups with little or no gene transfer.

In the present study, which included several tissue enzymes in the white muscle of cod, the main conclusion above

is supported by the results obtained for the *LDH-3* locus. Except for the *HbI* locus, the other proteins investigated show no significant differences between Arctic and coastal cod. At present, only a fraction of the spawning cod samples analysed permitted grouping of the genetical data according to otolith structure, but due to the low level of variation the chance of finding a discriminating genetical marker for one of these enzymes is very low.

The present classification of otoliths (Rollefsen, 1933) is based on visual inspection. Each otolith is grouped into one of five different types, of which three categories represent transition forms between typical Arctic and coastal cod otolith structure pattern. Because of the existence of transition forms in natural populations of cod, and preliminary results from otolith examination of offspring from crosses between Arctic and coastal cod in the laboratory (Godø, Institute of Marine Research, Bergen, personal communication, 1982), it is believed that otolith structure is more influenced by environmental factors rather than by genotype. For this reason the genetical data grouped according to otolith type must be interpreted carefully.

This grouping is necessary for comparisons with earlier results and the analyses of *HbI* give a close agreement with earlier work (Møller, 1968). For the pooled data in Table 3 the frequency of *HbI(1)* was 0.34 for coastal cod and 0.19 for Arctic cod. The first value is a result from pooled samples of coastal cod of different regions within the area and these possibly consist of several groups. Møller (1968), for example, found a frequency of 0.27 in the Sunnmøre area and 0.41 in the northern part of the area. The latter value agrees with our result for the coastal cod occurring in the Smøla area (0.47).

The observed frequency of *HbI(1)* for Arctic cod (0.19), classified according to otolith type, was higher than expected. This could be due to variations in the otolith classification methods discussed above. The mean value estimated from the spawning samples in 1982 mainly consisting of Arctic

cod (Table 3), however, was as low as 0.13 which is in the range reported for the main stock of Arctic cod (Møller, 1968).

Evidently, the frequency of *HbI(1)* for coastal cod in this area has not changed during the last 15 years, implying that no directional selection works on this polymorphism. Recently, Mork et al. (1983) have proposed a kind of selection model to explain the variation observed for *HbI* in samples of coastal cod in the Trondheimsfjord. Since the haemoglobin molecules are directly exposed to the environment, the different genotypes could be influenced by factors like temperature and oxygen concentration (Karpov and Novikov, 1980). We feel, at the present time, that studies under controlled conditions as well as extended field investigations are required to distinguish between alternative causes like natural selection, sample selection due to the gear used, environmental preference for different genotypes or differential migration behaviour. In any case, the interpretation of genetic data in terms of reproductive units or differentiated stocks should be based on analyses of spawning samples caught on the spawning grounds.

The data presented in this work do not satisfy the above requirements with regards to the stock structure of coastal cod in the area. Except for the sample of coastal cod in the Smøla area, the small number of samples from local spawning grounds limit the conclusions which can be drawn about the actual stock structure of coastal cod. With reference to the data on eggs sampled on the main spawning grounds, the methods used seem to be the most promising sampling approach in order to reveal stock structure of coastal cod. The data presented indicate variation on the microgeographic level, and the methods described should be applied in extended studies of local spawning grounds as well as sampling at different times during the spawning season.

With regards to the different strategies for revealing genetical variation between species and populations (see Introduction), genetical differences at the level observed in

this work could only be detected by analysing a large number of individuals. As reported here, large scale screening of individuals and samples can offer significant information about the actual stock structure of a fish species within a geographical region. None of the enzymes analysed in this work, however, is suitable as a general genetical marker for routine classification of Arctic and/or coastal cod. The variation observed our last group of cod, lead us to doubt the existence of such genetical markers for the cod stocks investigated.

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SECTION IV

Mass rearing and on-growing

CHAIRMAN

E. Egidius