Flødevigen rapportser., 1, 1984. ISSN 0333-2594
The Propagation of Cod *Gadus morhua* L.

GENETIC ANALYSES OF COD IN NORTHERN NORWAY

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ABSTRACT

Jørstad, K.E., 1984. Genetic analyses of cod in Northern Norway. In: E. Dahl, D.S. Danielssen, E. Moksness and P. Solemdal (Editors), The Propagation of Cod *Gadus morhua* L. Flødevigen rapportser., 1, 1984: 745-760.

Several polymorphic proteins have been used in studies of cod stock structure in northern Norway. Samples of Arctic cod spawning in Lofoten in the spring of 1981 were analysed for enzyme polymorphism by starch gel electrophoresis. The genotype distributions and allele frequencies estimated for spawning cod were consistent with corresponding results for eggs and yolksac larvae sampled in Lofoten and the Vesterålen area in May. Investigations of postlarvae in July, however, revealed samples which differed significantly from the samples of Arctic cod analysed earlier the same year. Most surprisingly, a postlarval sample from Eidsfjord, close to the main hatching area of Arctic cod in Vesterålen, differed in biological as well as genetic characters. Similar observations were made in 1982, when postlarvae were found in high numbers in the Vesterålen - Andfjord area. Samples of spawning cod were collected from several fjords in 1982. Especially, the sample drawn from Porsangerfjord differed from Arctic cod giving evidence for a genetically distinct stock of coastal cod in this area. This conclusion was also confirmed by analysis of postlarvae and juveniles found in the same area. Haemoglobin analysis in spring 1983 supported the existence of several genetically differentiated groups of coastal cod stocks in the area investigated.

INTRODUCTION

Cod is one of the most abundant fish species occurring in oceanic, coastal as well as fjord marine environments. Traditionally, the most important fishery in northern Norway is based on cod resources. Catches of Arctic cod taken in the Lofoten area in the spawning season, account for the largest quantities. However, the amounts of coastal cod taken during a year also constitute a significant fraction of the total quantities of cod caught in the area.

In addition to the main spawning area in Lofoten, several other spawning grounds for Arctic cod exist. With regards to the coastal cod, hundreds of local spawning grounds are known. Genetic studies of cod populations have revealed a complex stock structure in this area, and the relationship between coastal and Arctic cod is important for management reasons. Genetic differences between the two cod types have been indicated from analyses of blood proteins (Frydenberg et al., 1965; Møller, 1968), as well as blood factors (Møller, 1967, 1969).

At present, new methods used in population genetic studies (Allendorf and Utter, 1979; Harris and Hopkinson, 1976) are available and have revealed a large amount of genetic variation within and between species and populations. The results also show that most fish species are divided into several intraspecific units or stocks which should be managed as separate resource units (FAO, 1981).

Due to the increased fishing pressure and the recruitment problems of cod, short term management of cod resources and preservation of genetic resources (Smith and Chesser, 1981; Frankel and Soulé, 1981) must be based on realistic stock models. As pointed out recently (Ihssen et al., 1981; FAO, 1981) such models ought to be based on biological as well as genetic information about the fish species in question. The fundamental data for identification of local resources of coastal cod may be provided by genetic studies.

MATERIALS AND METHODS

The main sampling programme, which is summarized in Table 1, has been carried out in cooperation with a number of research projects at the Institute of Marine Research. These include biological studies on eggs and yolksac larvae (P. Solemdal), abundance of postlarvae and geographical distribution (H. Bjørke) and studies on coastal cod (A. Hylen, T. Jakobsen) in the northern part of Norway.

TABLE 1

Sampling programme and polymorphic loci investigated. The proteins analysed was PGM, PGI, LDH and haemoglobin.

Year	Month	Stage of development	Protein loci			
1981	March-April	spawners (Lofoten)	*PGM*	*LDH-3*	*PGI-1*	
	May	egg/yolksac larvae	*PGM*	*LDH-3*	*PGI-1*	
	July	postlarvae	*PGM*	*LDH-3*	*PGI-1*	
1982	March-April	spawners	*PGM*	*LDH-3*	*PGI-1*	
	May	egg/yolksac larvae	*PGM*	*LDH-3*	*PGI-1*	
	July	juveniles	*PGM*	*LDH-3*	*PGI-1*	
	July	postlarvae	*PGM*	*LDH-3*	*PGI-1*	
1983	March-April	spawners	*PGM*	*LDH-3*	*PGI-1*	*Hbl*
	May	juveniles	*PGM*	*LDH-3*	*PGI-1*	*Hbl*
	May	egg/yolksac larvae	*PGM*	*LDH-3*	*PGI-1*	

The spawning stock of Arctic cod was sampled on the spawning grounds in the Lofoten area (Fig.1) in March-April 1981. The fish were caught by using purse seine and biological data (length, sex, age, otolith type etc.) on each fish

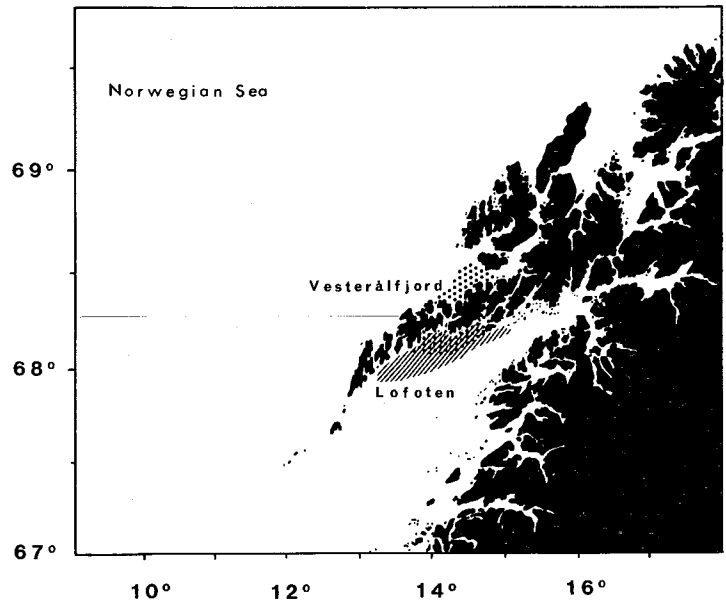

Fig. 1. Sampling areas in 1981 of Arctic cod (▨ March - April) and eggs/yolksac larvae (⁘ May).

were recorded. This sample consisted of about 500 cod individuals and they were classified as Arctic cod according to otolith typing (Rollefsen, 1933). During the biological sampling a small piece of white muscle were cut from each cod specimen and frozen as soon as possible. The tissue samples were kept at low temperature until analysed in the laboratory.

As indicated in Fig. 1, the eggs and yolksac larvae from the Arctic cod spawning were distributed close to the Lofoten Islands. In addition, a substantial fraction of the larvae analysed were sampled in the Vesterålfjord area.

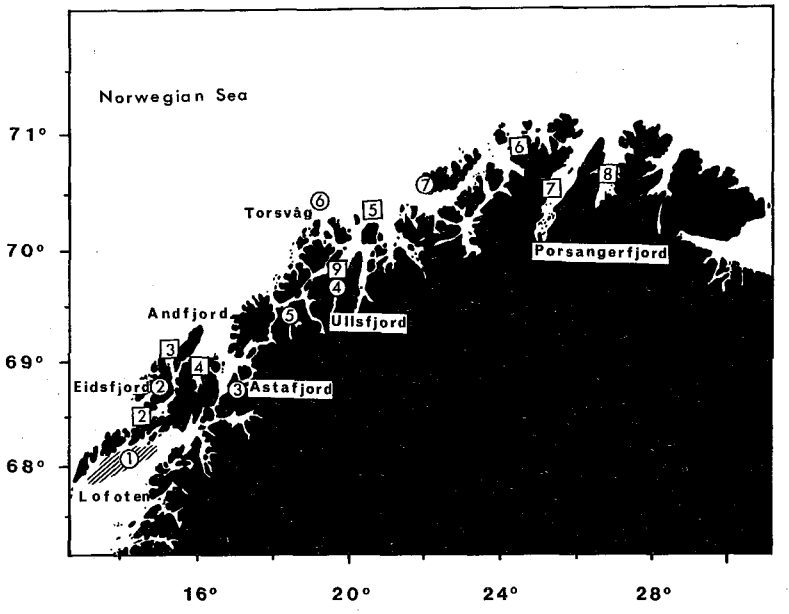

Fig. 2. Sampling localities of postlarvae. ◯: 1981, (for details see Table 3), □: 1982, (for details see Table 4), (▨ : main spawning area of Arctic cod).

The eggs and yolksac larvae were sampled by a Juday net, and the eggs were kept alive in seawater until hatching. Newly hatched yolksac larvae were sampled and analysed by electrophoresis according to Jørstad et al. (1980).

Collection of postlarvae samples were carried out in July by using pelagic trawl. Fig. 2 shows the localities on the coast and fjords where larvae were found in relatively high numbers. Individual samples of white muscle were frozen until analysed on the research vessel or in the laboratory. The postlarvae found in the open sea have not been included in this paper.

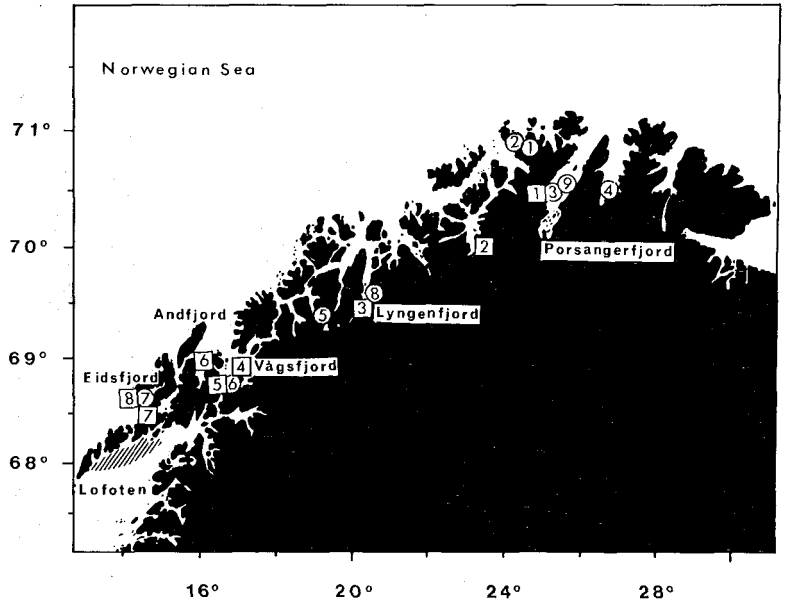

Fig. 3. Sampling localities of coastal cod. ◯: 1982, (for details see Table 5), □: 1983, (for details see Table 6). (▨ : main spawning area of Arctic cod).

The sampling localities of coastal cod are shown in Fig. 3. Samples of juvenile cod were caught by using shrimp trawl (July 1981, May 1982), and gill nets and Danish seine were used when sampling on the spawning grounds of coastal cod during the spawning season.

In 1983 haemoglobin analyses were incorporated into the investigation programme. Blood samples were collected from individual fish at the spawning grounds, transported by air to Bergen and analysed in the laboratory by agar electrophoresis (Sick, 1961).

All samples of white muscle were analysed for polymorphic enzymes by starch gel electrophoresis (Gordon, 1975) followed by selective staining of enzymes (Harris and Hopkinson, 1976). The enzymes investigated (Table 1) were lactate

dehydrogenase (*LDH*), phosphoglucose isomerase (*PGI*) and phosphoglucomutase (*PGM*).

Designation of enzyme loci and alleles followed the recommendations of Allendorf and Utter (1979), and the polymorphic systems present in natural populations of cod are described elsewhere (Cross and Payne, 1978; Jørstad et al. 1980; Moth-Poulsen, 1982; Mork et al. 1982). In the haemoglobin analyses we used the nomenclature of Frydenberg et al. (1965).

In the statistical treatment a G-test described by Sokal and Rohlf (1969) was used. All tests are based on the actual number of genotypes in the samples, and the P values in the different tables give the probability of the sample collection being drawn from a genetically homogenous population. In addition, tests of homogeneity were performed between the different samples within each sample collection. The limit of heterogeneity within a sample group and/or between two different samples is defined as $P<0.05$.

RESULTS

For simplicity, only the frequency of the most common allele for each enzyme loci (for example *LDH-3(100)*) are given in the tables together with the number of individuals analysed. No significant variation was observed for the *PGM* locus, and therefore data for this enzyme have been omitted in the tables.

The results from the analyses of the reference sample of the Arctic cod stock are shown in Table 2. More than 90% of the spawners taken in Lofoten this year were Arctic cod and several year classes were present in the material. The sampling design permitted comparisons between biological and genetic data, and no significant differences were detected for any enzyme loci between the two sexes, different year classes or length groups. The distribution of genotypes for all loci were consistent with Hardy-Weinberg's expectations,

tested as described by Christiansen et al. (1976). As expected, the genetic data for the spawning fish in March-April corresponded very closely to the data obtained from analyses of eggs and yolksac larvae found in the same area in May. Further, no differences were detected between the two hatching areas (Lofoten, Vesterålfjord) indicating that all larvae belonged to the same gene pool.

TABLE 2

Allele frequencies (q) of the most common allele at the *LDH-3* and *PGI-1* loci and number of specimens analysed (n) in samples of Arctic cod. The sampling areas are shown in Fig. 1.

Month	Area	Stage of development	*LDH-3(100)* q / n	*PGI-1(100)* q / n
March-April	Lofoten	spawners	.60 / 497	.70 / 511
May	Lofoten	egg/yolksac larvae	.57 / 749	.68 / 635
May	Vesterål-fjord	egg/yolksac larvae	.58 / 407	.67 / 403
Homogeneity tests based on genotype distributions:			P = .77	P = .447

The different localities where postlarvae were found on the coast and in the fjords in 1981 are indicated in Fig. 2. A dense concentration of larvae were found in the open sea N of Torsvåg. These larvae are probably offspring of the Arctic cod and are therefore omitted in the figure. Table 3 indicates that the samples of postlarvae constitute very heterogenous material. As shown, several samples have a relatively high value for *LDH-3(100)*, and the tests of homogeneity for the sample group give P(*LDH-3*) = 0 and P (*PGI-1*) = 0.019 respectively. A test of homogeneity (based

on genotype distributions at the *LDH-3* locus) between the reference sample of Arctic cod and the sample from Eidsfjord and the sample from Astafjord revealed significant differences (P=0.001; P=0.029). These two samples also differed from the samples taken on the coast (Table 3, sample no. 6 and 7) which corresponded more closely to the Arctic cod sample (P=0.37; P=0.98). Significant differences in length distribution and mean length were also detected when comparing these samples with postlarvae found in the open sea (Jørstad, unpublished). Most interesting the sample from Eidsfjord consisted of small larvae with a mean length of 18.7 mm compared to 28.9 mm for larvae in the open sea.

TABLE 3

Allele frequencies (q) of the most common allele at the *LDH-3* and *PGI-1* loci and number of specimens analysed (n) in samples of postlarvae in July 1981. Spawners of Arctic cod in Lofoten are used as a reference sample. The sample numbers correspond to the locality numbers ① - ⑦ in Fig. 2.

Sample no.	Locality	*LDH-3(100)* q / n	*PGI-1(100)* q / n
1	Lofoten, control	.60 / 497	.70 / 511
2	Eidsfjord	.69 / 138	.71 / 137
3	Astafjord	.65 / 47	.70 / 56
4	Ullsfjord	.62 / 25	.72 / 25
5	Malangen	.73 / 21	.64 / 21
6	Torsvåg	.56 / 89	.64 / 91
7	Breivikbotn	.58 / 239	.70 / 239
Homogeneity tests based on genotype distributions:		P = 0	P = 0.019

The analyses of postlarvae in 1982 (Fig. 2, Table 4) con-

firmed the genetic heterogeneity (P=0) of samples collected earlier from the coast and the fjords. In this year, the heterogeneity was mainly due to the sample from Andfjord and Porsangerfjord which both have a high frequency of *LDH-3(100)*. The values were 0.67 and 0.69 respectively. Especially, the sample from Andfjord differed (P=0) from the sample of eggs/yolksac larvae sampled in May of same year, which have been used in Table 4 as a reference sample. Comparison between the control sample and the postlarvae from Porsangerfjord revealed a P value of 0.09 which is only close to significance. This is possibly due to the low number of individuals analysed in the postlarval sample.

TABLE 4

Allele frequencies (q) of the most common allele at the *LDH-3* locus and number of specimens analysed (n) in samples of postlarvae in July 1982. Sample no. 1 is a sample of eggs/yolksac larvae taken in May of the same year. The other samples correspond to the locality numbers [2] - [9] in Fig. 2.

Sample no.	Locality	*LDH-3(100)* q / n
1	Lofoten-Vesterålfjord	.56 / 149
2	Hadselfjord	.63 / 168
3	Gavelfjord	.65 / 142
4	Andfjord	.67 / 175
5	Arnøy	.59 / 160
6	Refsbotn	.57 / 192
7	Porsangerfjord	.69 / 54
8	Laksefjord	.57 / 41
9	Ullsfjord	.61 / 82

Homogeneity test based on genotype distributions: P = 0

Samples mainly consisting of coastal cod were collected in 1982 (Fig. 3), and the results of the genetic analyses of *LDH-3* locus are shown in Table 5. The samples taken in March are spawners, and they are therefore more informative with respect to stock structure interpretations compared with the juvenile cod samples in July. The two samples taken in Porsangerfjord have a relatively high frequency of *LDH-3(100)*. When testing the samples of coastal cod together with the reference sample of Arctic cod used earlier, the probability of all samples belonging to the same population was as low as 0.001. Homogeneity tests (based on genotype distributions) between the samples in Table 4 and the reference sample of Arctic cod show that both samples from Porsangerfjord were significantly different (sample no.3, P=0.001; sample no.9, P=0.005) from the Arctic cod sample. Thus, the genetic data clearly indicated a genetically distinct stock of coastal cod

TABLE 5

Allele frequencies (q) of the most common allele at the *LDH-3* locus and number of specimens analysed (n) in samples of coastal cod in 1982. The sample numbers correspond to the locality numbers ① - ⑨ in Fig. 3.

Sample no.	Month	Stage of development	Locality	*LDH-3(100)* q / n
1	March	spawners	Refsbotn	.59 / 93
2	"	"	Refsbotn	.60 / 94
3	"	"	Porsangerfjord	.71 / 94
4	"	"	Laksefjord	.62 / 66
5	"	"	Balsfjord	.57 / 42
6	July	juveniles	Vågsfjord	.59 / 54
7	"	"	Vesterålfjord	.57 / 42
8	"	"	Lyngenfjord	.64 / 73
9	"	"	Porsangerfjord	.68 / 61

Homogeneity test based on genotype distributions: P = 0.013

TABLE 6

Allele frequencies (q) of *HbI(1)* and number of specimens analysed (n) in samples of coastal cod in spring 1983. The sample numbers correspond to the locality numbers [1] - [8] in Fig. 3.

Sample no.	Month	Stage of development	Locality	*HbI(1)* q / n
1	March	spawners	Porsangerfjord	.29 / 93
2	March	spawners	Altafjord	.25 / 90
3	March	spawners	Lyngenfjord	.28 / 29
4	May	mature	Bygdenfjord	.25 / 85
5	May	juveniles	Vågsfjord	.26 / 57
6	May	juveniles	Andfjord	.28 / 81
7	May	mature	Hadselfjord	.20 / 81
8	May	juveniles	Eidsfjord	.27 / 24
Homogeneity test based on genotype distributions:				P = .58

in the Porsangerfjord with Olderfjord as a local spawning ground (Jakobsen, Institute of Marine Research, Bergen, personal communication 1982).

Table 6 shows the results from the haemoglobin analyses which were incorporated in the study in 1983. The sample from Porsangerfjord showed the highest frequency (0.29) of *HbI(1)*, and the sample from Hadselfjord the lowest one (0.20). A test of homogeneity indicated no significant variation in the sample collection (P=0.58).

DISCUSSION

The *LDH-3* locus seemed to be the most informative of the different enzyme loci analysed. The significant variations observed at this locus between Arctic cod and samples of coastal cod, as well as within samples of coastal cod, offer

valuable information on cod stock structure in this area. These results contrast with earlier studies (Jamieson, 1975; Mork et al. 1980) which concluded that the allele frequencies at the *LDH-3* locus seemed to be almost uniform throughout the species' range. Recently, several other studies (Moth-Poulsen, 1982; Jørstad et al. 1981) have supported evidence for significant variation at this locus.

As expected, we observed close agreement between the data obtained from the spawning stock of Arctic cod and that of the eggs/yolksac larvae found in the same area. These data point to the possibility of analysis of eggs/yolksac larvae on the spawning ground to identify genetically different spawning groups. The actual allele frequencies for the different loci analysed both in the spawning cod samples and the samples of eggs/yolksac larvae correspond to similar data on Arctic cod samples from the Barents Sea and the Spitsbergen area (Jørstad, unpublished). The data on yolksac larvae drawn from the Vesterålen area are also in accordance with the present knowledge of egg and larval drift from the main spawning grounds of Arctic cod (Ellertsen et al. 1981).

The haemoglobin data obtained from samples of coastal cod in 1983 agree very closely with earlier studies (Møller, 1968, 1969). Møller estimated the frequency of *HbI(1)* of coastal and Arctic cod in Lofoten to be 0.25 and 0.12, respectively. By including his data on coastal cod with the presented data (Table 6) a heterogeneity test revealed no significant variation (P=0.67). In contrast, performing a similar test with his data on Arctic cod, the difference was significant (P=0).

The most divergent population of coastal cod detected in this study was the stock occurring in Porsangerfjord. Biological studies on this cod stock have shown limited migration within the area, and unique growth characteristics and age composition compared to other coastal cod stocks (T. Jakobsen, Institute of Marine Research, Bergen, personal communications 1982). When including haemoglobin data on Arctic cod as described above, significant differences was observed for

two loci (*LDH-3*, *HbI*). Thus, we consider this cod to be a genetically distinct stock unit compared to the Arctic cod.

The data also indicate several stock units of coastal cod in the Vågsfjord - Andfjord - Eidsfjord area, but insufficient data from spawning samples in these areas prevents confirmation. The existence of a local stock in the inner part of Eidsfjord has been supported by egg surveys in May during recent years, showing high abundance of eggs and yolksac larvae in the bottom of the fjord.

As discussed by Allendorf and Phelps (1981), the choice of sampling strategy depends on what kind of information is required. In this work, however, the importance of an extensive sampling programme is clearly documented by the data obtained from the postlarvae surveys. Without a great number of sampling stations in the different fjords, valuable information on coastal cod stocks might not be available. As shown here, the strategy involving adequate sampling of early developmental stages of fishes lifecycle seems to be very useful, and supports the experiences from genetic studies on other fish species (Jørstad and Nævdal, 1981).

ACKNOWLEDGEMENTS

The work has received financial support from Norwegian Council for Fisheries Research.

I appreciate the technical assistance of Ole I. Paulsen, the typing work of Ingrid Mjeldheim and Anne S. Mjeldheim and the critical comments and linguistic suggestions of Gunnar Nævdal and Karin Pittman.

I will also thank the staff at the Institute of Marine Research, especially Karsten Hansen and Per I. Halland, who have been involved in the collection of a large number of samples.

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