

## SECTION III

## Population structure

GENETIC STUDIES ON EGGS, LARVAE AND 0-GROUP  
OF THE ARCTIC COD STOCK

by

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## ABSTRACT

Genetic analyses have been performed on samples of spawning cod (March), a large number of eggs and yolk sac larvae (May, postlarvae (July) and 0-group cod (August-September). The distribution of eggs and larvae at different stages as well as biological and genetic data were in accordance with earlier observations on larval drift from the main spawning grounds. Some samples drawn from localities on the coast or in fjords differed, however, significantly from samples in the open sea. The genetic analyses of these samples, as observed in analyses of the LDH-3 locus, confirm the existence of locally spawning groups of coastal cod. The genetic data are discussed in relation to stock structure of cod, the significance of genetic variation within stocks and the problem of conservation of genetic resources.

## INTRODUCTION

The early genetic studies on cod (Sick, 1961; Sick, 1965a,b; Frydenberg et al., 1965; Møller, 1968) suggested a complex population structure for this species. In these investigations the genetic variation seen in blood proteins was used as well

as immunogenetic methods (Møller, 1967). At present, however, polymorphic enzymes expressed in different tissues are more applied in studies of population structure of fish species (Allendorf and Utter, 1979).

Extensive studies of natural populations have revealed a large amount of genetic variation (Powell, 1975). Genetic differentiated populations of valuable fish species represent unique biological resources (Soulé, 1981) which should be managed according to the genetic characteristics of the population, environmental preference, migration behaviour and general life history.

A number of studies (for review see Soulé, 1980) also suggest that the amount of genetic variation within a population plays a key role both for short term fitness and long term evolution. Due to these observations, conservation genetics (Frankel and Soulé, 1981) predict that as much genetic variation as possible should be preserved within populations and species.

With respect to the north-east arctic cod, proper management of this important stock should be based on genetic data as well as biological information. Genotype distributions and allele frequencies for a large number of loci are needed for identification of subpopulation structures. To evaluate possible genetic effects due to increased fishing pressure, environmental changes and pollution, genetic baseline information is essential. Impacts on the gene pool are likely to take place in the early stages of the development. For this reason genetic data at different stages during the recruitment process will provide basic knowledge for detection of unwanted changes in the gene pool in the future.

#### MATERIALS AND METHODS

The sampling programme in different years and stages of development are summarized in Table 1 and the sampling areas are shown in Fig. 1 and Fig. 3. As a reference sample we have used arctic cod caught on the spawning ground in Lofoten in March-

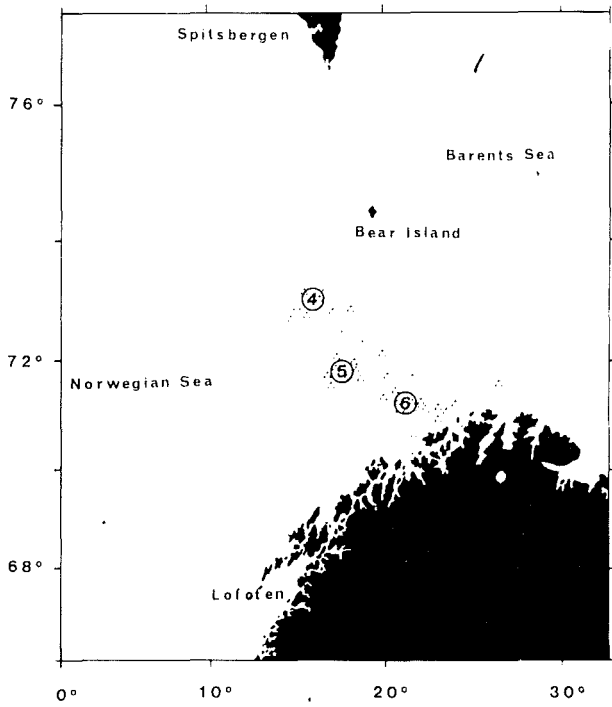
April 1981. From these fish, samples of white muscle were taken from each individual fish on which biological information (length, sex, maturity, age, otolith type) also were recorded. The tissue samples were kept frozen until analyses on the research vessel or in the laboratory.

Table 1. Sampling program in different years.

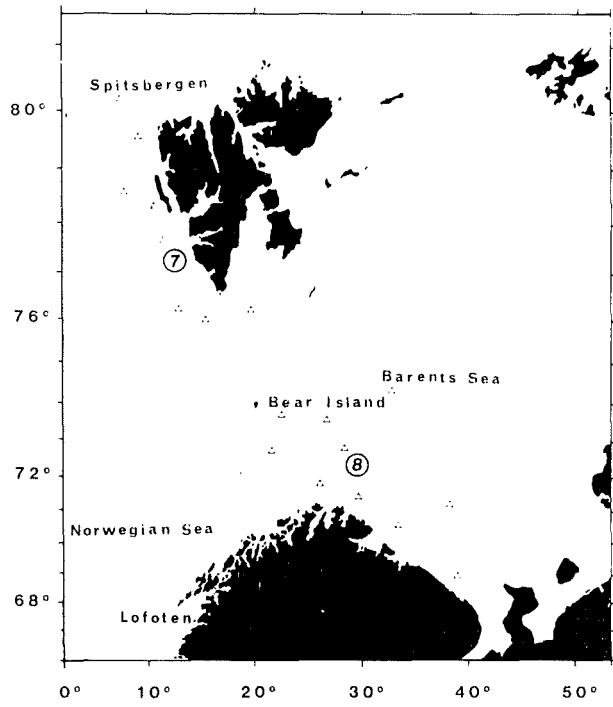
Year	Month	Stage of development	Area	Research vessel
1981	March	Spawners	Lofoten	"Djupaskjær"
	May	Eggs, yolk sac larvae	Lofoten-Vesterålsfjord	"M. Sars"
	July	Postlarvae	Norwegian Sea	"J. Ruud"
	August-September	0-group	Barents Sea-Vest-Spitsbergen	"J. Hjort" "G.O. Sars" "M. Sars"
1982	May	Eggs, yolk sac larvae	Lofoten-Vesterålsfjord	"J. Hjort"
	July	Postlarvae	Norwegian Sea	"J. Ruud"
	August-September	0-group	Barents Sea-Vest-Spitsbergen	"J. Hjort" "G.O. Sars" "M. Sars"

Samples of eggs and yolk sac larvae were collected in May in the Lofoten and Vesterålen area by using Juday nets. Eggs in early stages were kept alive in seawaters and incubated at 6°C until hatching. The samples were then analysed on the boat following the methods described by Jørstad, Solberg and Tilseth (1980).

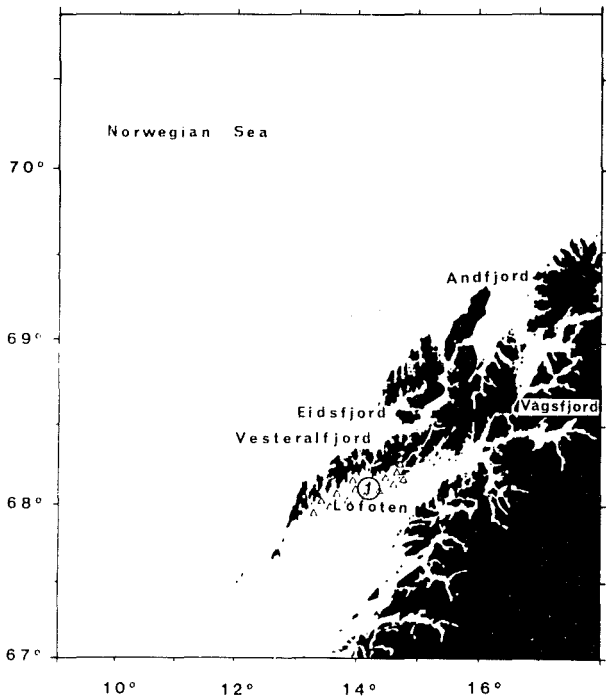
In July the most concentrated areas of postlarvae were found in the open sea north-west of Torsvåg-Sørøya. Only the main larvae distributions which have been taken in the open sea are indicated in the figures. In August-September the 0-group is found distributed in the area Bear Island-Vest-Spitsbergen and in the Barents Sea. The postlarvae and 0-group cod were



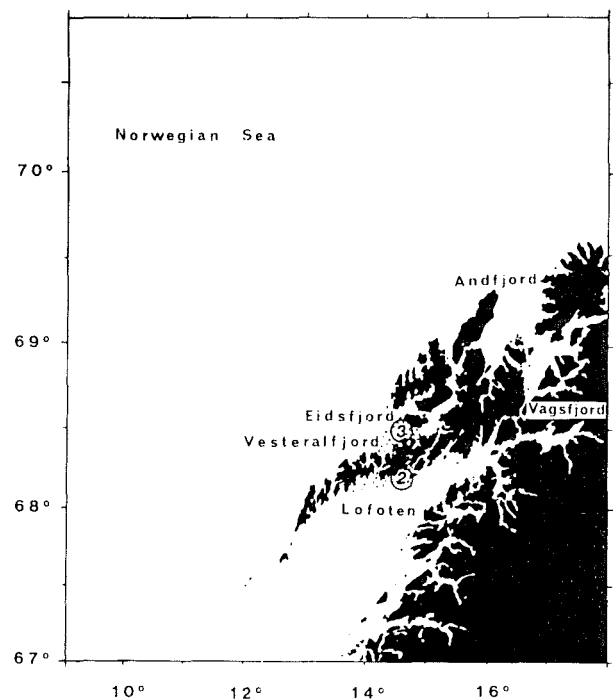
c. Postlarvae



d. O-group



a. Spawning stock



b. Eggs, yolk sac larvae

Fig. 1. Sampling areas in 1981.

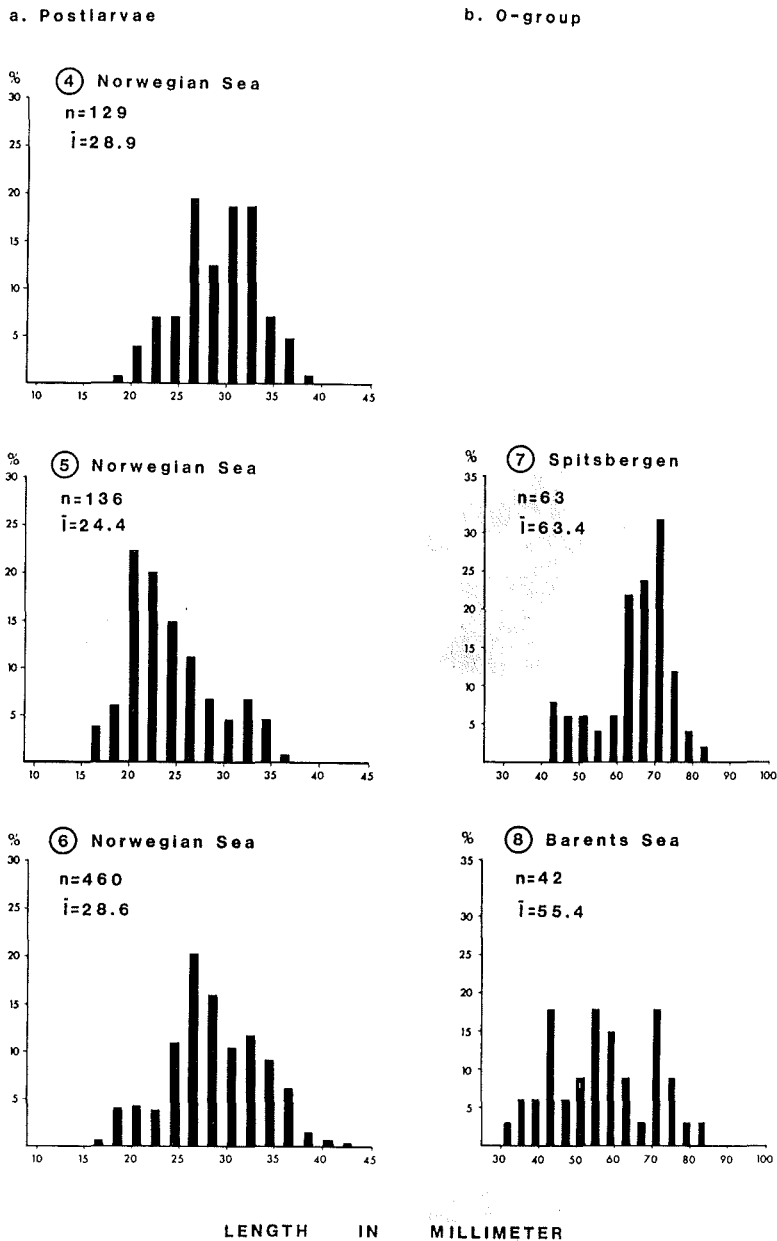
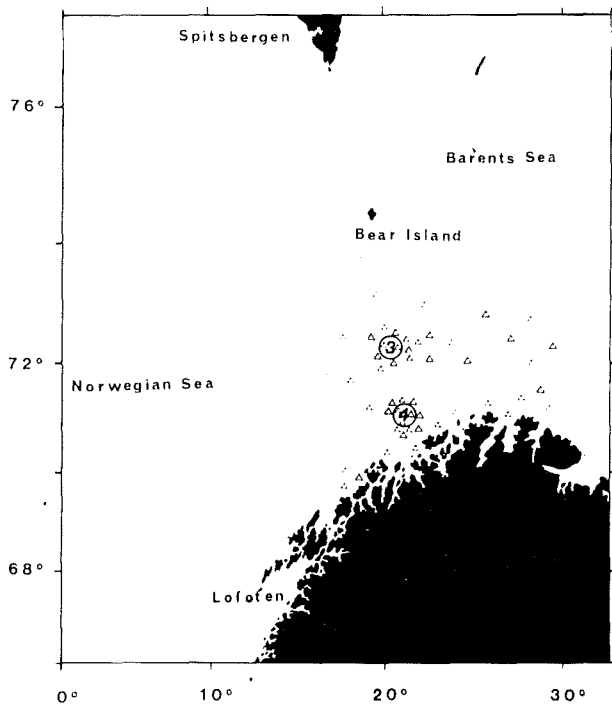
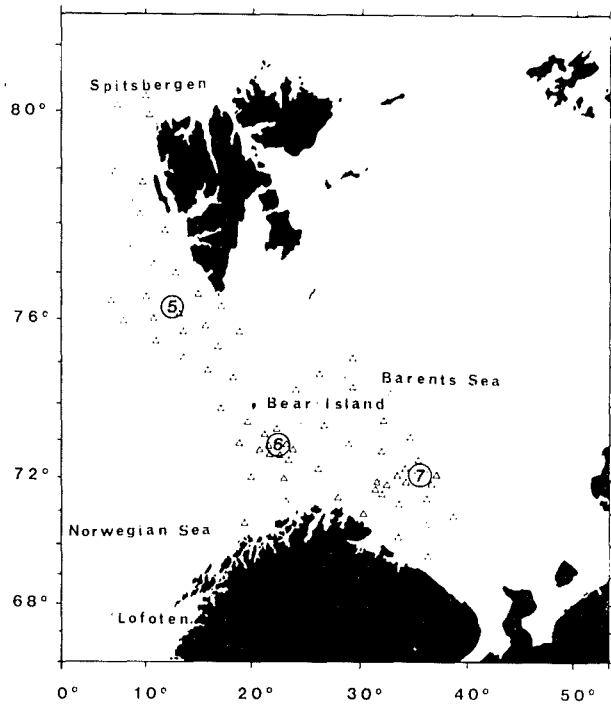


Fig. 2. Length distribution of postlarvae and 0-group in 1981.

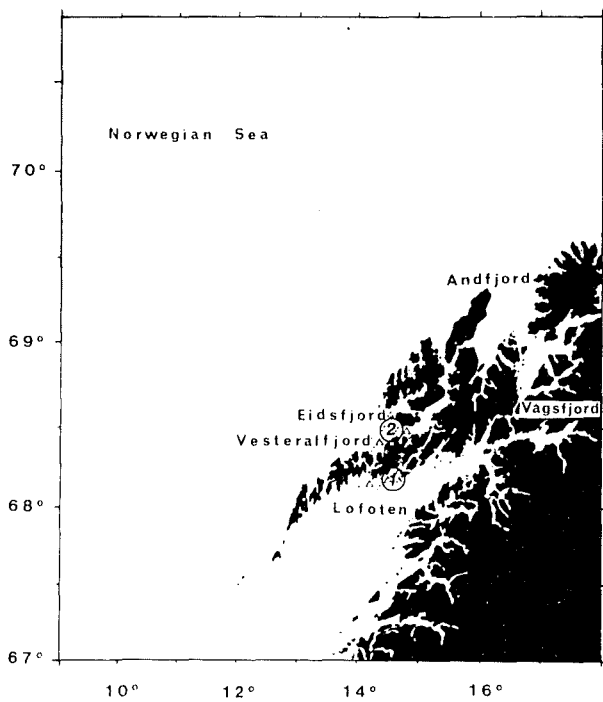
sampled by pelagic trawl and the main areas in open sea are indicated in Fig. 1 and Fig. 3. In addition to tissue samples for genetic analyses, the length of each fish was recorded, and the length distributions of postlarvae and 0-group cod in different years and for the most concentrated areas are given in Fig. 2 and Fig. 4.



b. Postlarvae



c. O-group

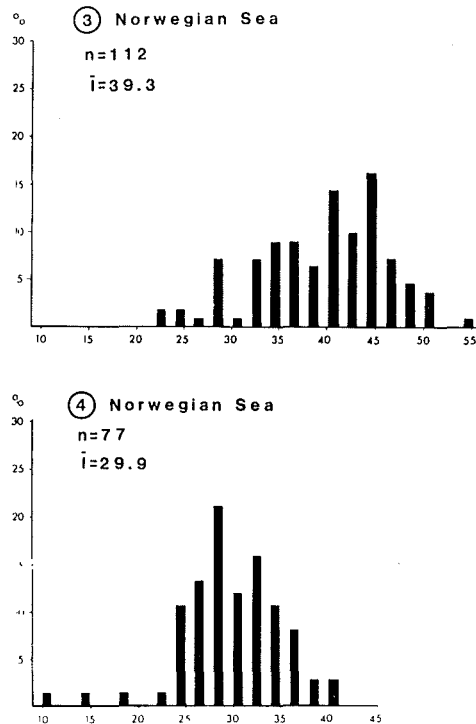


a. Eggs/yolk sac larvae

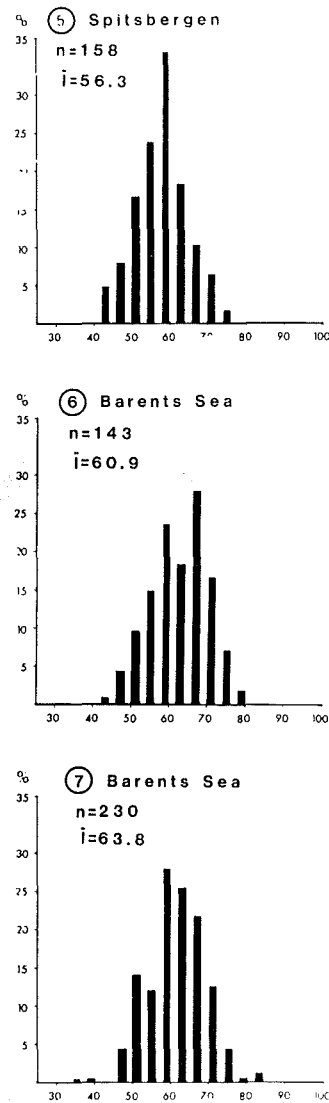
Fig. 3. Sampling areas in 1982.

A limited number of sampling stations in the fjords were also included in the sampling programme of postlarvae. Two fjord localities are shown in Fig. 5, and the length distribution of

a. Postlarvae



b. 0-group



LENGTH IN MILLIMETER

Fig. 4. Length distributions of postlarvae and 0-group in 1982.

these larvae samples are given in Fig. 6. For comparison, data from control samples are included.

All samples were analysed by using starch gel electrophoresis and selective staining methods (Harris and Hopkinson, 1976). Several polymorphic enzymes were investigated. These included lactate dehydrogenase (LDH), phosphoglucose isomerase (PGI) and

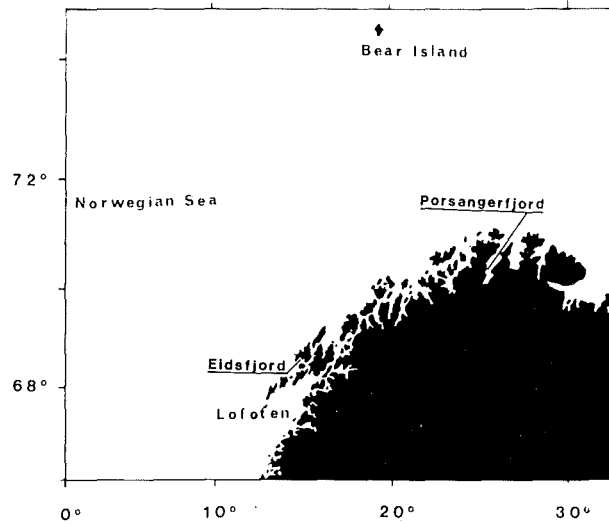


Fig. 5. Fjord localities of postlarvae.

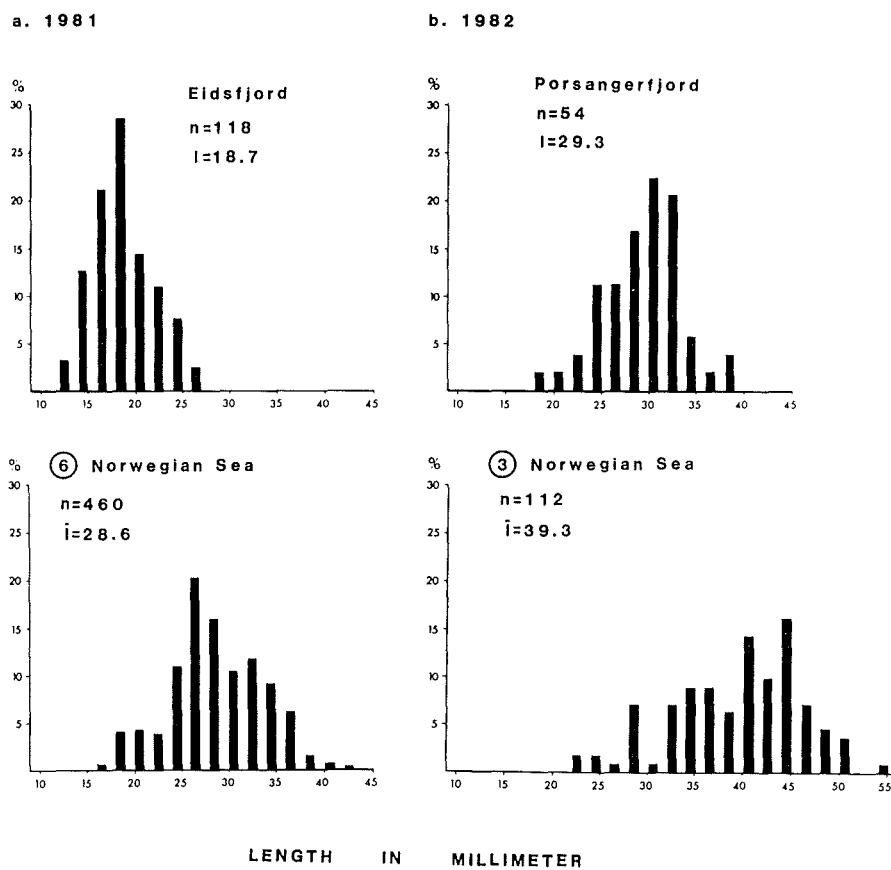


Fig. 6. Length distributions of fjord samples of postlarvae.



phosphoglucomutase (PGM). The different loci and alleles have been described elsewhere (Cross and Payne, 1978; Jørstad *et al.*, 1980, Mork *et al.*, 1982). Designation of enzyme loci and alleles followed the recommendations of Allendorf and Utter (1979). In this paper, only the results obtained from analyses of the LDH-3 locus are presented.

In the statistical comparisons between different areas and stages of development a G-test described by Sokal and Rohlf (1969) were used. The P-values given in some of the tables are the probability obtained from a G-test asking if the sample collection is genetically homogeneous. The test is based on the distribution of genotypes in the different samples, and significant heterogeneity is obtained for P-values  $< 0.05$ .

## RESULTS

The results from the analyses of the 1981-material are summarised in Table 2. For the LDH-3 locus three alleles, designed 70, 100 and 150 were found in all samples. Some rare heterozygotes in LDH-2 locus were also observed but are not considered here.

The catches of cod in Lofoten during the spawning season consist of a mixture of arctic and coastal cod. In Table 2 only individuals classified as arctic cod according to otolith structure (Rollefsen, 1933) have been used and constitute about 90% of all cod specimens in the samples which were analysed for genetic characters. In this reference sample of arctic cod several year classes were present, and due to the sample design, comparisons between biological and genetic data could be performed. No significant differences were found, however, between the two sexes, different year classes or length groups. The distribution of genotypes were also consistent with Hardy-Weinberg's expectations tested as described by Christiansen *et al.* (1976).

As shown in Table 2 the allele frequencies were nearly identical for the spawning stock in Lofoten and the eggs and yolk sac

Table 2. Allelfrequencies of LDH-3 in different samples in 1981.

Sample no.	Month	Stage of development	Area	No. of fish	Allele		
					70	100	150
1	March	Spawners	Lofoten	383	.410	.582	.008
2	May	Eggs/yolk sac larvae	Lofoten	749	.407	.588	.005
3	May	Eggs/yolk sac larvae	Vesterålsfjord	407	.425	.572	.002
4	July	Postlarvae	Norwegian Sea	232	.412	.586	.002
5	July	Postlarvae	Norwegian Sea	100	.450	.550	.000
6	July	Postlarvae	Norwegian Sea	383	.410	.582	.008
7	Aug.-Sept.	0-group	V.Spitsbergen	69	.406	.587	.007
8	Aug.-Sept.	0-group	Barents Sea	109	.367	.628	.005

Homogeneity test based on distribution of genotypes:  $P = .132$

larvae found a month later in Lofoten-Vesterålen. No genetic differences seem to exist between the two main hatching areas.

With respect to the postlarvae sampled in July in the areas shown in Fig. 1, no variation in allele frequencies were detected. In addition, the analyses of 0-group cod in the Barents Sea and Spitsbergen area in August-September reveal no significant variation. As the data indicate, no significant differences were found in the total material analysed, covering the spawning stock of arctic cod in Lofoten, different stages of development, different sampling time and geographic distribution. A homogeneity test based on the distribution of genotypes in the different samples in Table 2, revealed no heterogeneity ( $P=0.132$ ) within the sample collection. When performing a test between the different samples, one sample of postlarvae (sample no. 4) seemed to be different ( $P=0.02$ ) from the 0-group sample from Spitsbergen (sample no. 7). The allele frequencies were approximately identical for the two samples, but a closer examination showed that the distribution of genotypes in the 0-group sample differed significantly from the

values expected from Hardy-Weinberg's law. This is possibly the reason for the observed heterogeneity between those two samples.

A similar picture was obtained in the analyses of the 1982-material summerized in Table 3. This year the spawning stock of arctic cod in Lofoten was not sampled, but the data on eggs and yolk sac larvae found in May in Lofoten and Vesterålen corresponded well to the data on the spawning stock the preceding year as well as data on juvenile and mature fish sampled in Barents Sea and Spitsbergen (Jørstad, unpublished). As shown in Fig. 3, a similar distribution of postlarvae and 0-group cod was observed except for the 0-group this year was much more abundant in the Barents Sea compared to 1981.

Table 3. Allelfrequencies of LDH-3 in different samples in 1982.

Sample no.	Month	Stage of development	Area	No. of fish	Allele		
					70	100	150
1	May	Eggs/yolk sac larvae	Lofoten	79	.430	.563	.006
2	May	Eggs/yolk sac larvae	Vesterålsfjord	70	.443	.550	.007
3	July	Postlarvae	Norwegian Sea	63	.396	.590	.015
4	July	Postlarvae	Norwegian Sea	112	.375	.607	.018
5	Aug.-Sept.	0-group	V.Spitsbergen	162	.407	.590	.003
6	Aug.-Sept.	0-group	Barents Sea	145	.434	.562	.003
7	Aug.-Sept.	0-group	Barents Sea	230	.448	.550	.002

Homogeneity test based on distribution of genotypes:  $P = .172$

As seen from Table 3, testing for homogeneity in the sample group showed no heterogeneity ( $P=0.172$ ). A homogeneity test between the samples suggested that one of the postlarvae samples (sample no. 4) was different from the 0-group cod found in the Barents Sea (sample no. 7). As discussed above, also this difference is possibly due to the uneven distribution of

genotypes in the two samples. In addition, the postlarvae sample was taken near the coast and could be mixed with other more locally spawning groups of cod.

The length distributions of postlarvae and 0-group for the two years are shown in Fig. 2 and Fig. 4. The postlarvae taken in the open sea have a larger mean length compared to samples taken near the coast. Further, the postlarvae were significantly larger in July 1982 compared to the larvae found at the same time in 1981. In spite of this difference, the 0-group sampled in August-September have similar mean length for the two years. Significant differences were detected between the samples from the Barents Sea and the Spitsbergen area. In 1981 the 0-group cod found at Spitsbergen have a mean length of 63.4 mm compared to 55.4 mm in the Barents Sea, while the largest 0-group cod in 1982 was found in the Barents Sea.

In contrast to the genetic uniformity observed in the spawning stock of arctic cod, the different stages of development and the geographic distribution as described above, some fjord samples of postlarvae (Fig. 5) have different allele frequencies and genotype distribution. The two samples shown are both taken from fjord localities and have a relatively high frequency (0.69) of LDH-3(100) allele.

In Table 4 the fjord samples are compared to reference samples of arctic cod and the homogeneity test demonstrated a very heterogeneous sample group ( $P=0$ ). Homogeneity tests between the samples showed that both samples were different from the spawning stock in Lofoten (Eidsfjord,  $P=0.003$ ; Porsangerfjord,  $P=0.04$ ).

As shown in Fig. 6, both samples clearly differed in length distribution and mean length compared to postlarvae found in the open sea. The differences in geographic distribution and biological as well as genetic characters suggest other spawning groups than the arctic cod stock. The observation give evidence for the existance of genetic distinct stocks of coastal cod in the area. This conclusion have been further supported

Table 4. Allelfrequencies of LDH-3 in two fjord samples of postlarva

Sample no.	Year	Month	Stage of development	Area	No. of fish	Allele		
						70	100	15
1	1981	March	Spawners	Lofoten	356	.397	.598	.00
2	1982	May	Eggs/yolk sac larvae	Lofoten	79	.443	.550	.00
3	1981	July	Postlarvae	Eidsfjord	138	.304	.692	.00
4	1982	July	Postlarvae	Porsangerfjord	54	.306	.694	.00

Homogeneity test based on distribution of genotypes:  $P = 0$

by analyses of cod sampled in the Porsangerfjord during the spawning season (Jørstad, 1983).

#### DISCUSSION

Due to the extensive sampling programme focused on the north-east arctic cod stock it was possible to carry out detailed genetic studies at different stages of development for several year classes of this important cod stock. The genetic data so far demonstrate a very close agreement between the spawning stock in March and eggs/yolk sac larvae found in the main hatching areas in May. These data document the correlation between the spawning population and offspring. Further, this observation points to the possibility of sampling and analyses of eggs and yolk sac larvae on or near spawning grounds for identification of genetically differentiated spawning groups. As indicated, such methods offer a valuable tool in studies of stock structure of fish in general.

The data presented are obtained from samples which covered a large geographic area as well as different stages of development. With the exception of two samples of postlarvae, which deviations can be explained by some other reason, all the samples taken in the open sea at any stages in 1981 and 1982 are very similar in allele frequencies and phenotype distribu-

tion of the LDH-3 locus. This locus have been informative with respect to stock structure of cod in other area (Moth-Poulsen, 1982; Jørstad, 1983) as well as this study (e.g. coastal cod groups). The results obtained are in accordance with the present recruitment model of this cod stock concerning spawning area, main hatching area and drift/distribution of postlarvae/0-group cod to the feeding area in the Barents Sea and the Spitsbergen area.

The length distributions of 0-group cod in these two areas are different in the two years investigated. This is possibly due to the differences in the geographic distribution of postlarvae in July. In 1981 the largest postlarvae were found SW of Bear Island and far from the Norwegian coast. This larvae are likely to follow a more western drifting route. As expected, this year the 0-group cod in Spitsbergen area have a significantly higher mean length compared to the Barents Sea. In 1982, however, the largest postlarvae were found SSE of Bear Island and more close to the Norwegian coast compared to the distribution in 1981. These larvae are more likely to be distributed in the more eastern area in the Barents Sea. It must be pointed out, however, that the length data shown only represent the fish which have been analysed for genetic characters and do not reflect the total material of 0-group in the areas.

The genetic data presented suggest no differences between the two main areas in which the 0-group cod were distributed. In this work, however, data from only one polymorphic locus have been presented and a definite conclusion about possible sub-population structure of the arctic cod in this area cannot be made. Investigations including larger number of polymorphic loci will possibly offer more detailed information. With regards to the importance of this cod stock, the recruitment problems during the last years and the problems of conservation of genetic resources in fish (FAO/UNEP 1981), basic information of the genetic structure of this stock is necessary for both short time management and for long term preservation of this fish resource.

As recommended by the FAO expert team on conservation of genetic resources in fish, genetic monitoring programmes should be established for important fish stocks to detect any genetic changes due to overexploitation and/or pollution. For the north-east arctic cod stock genetic monitoring on the early stages and during the recruitment process is very desirable.

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