International Council for the Exploration of the Sea **ICES CM 1998/O:76**

Population studies on roughhead grenadier, *Macrourus berglax* L., from the North Atlantic

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Abstract

Samples of the roughhead grenadier, *Macrourus berglax* L., were taken from several locations on East and West Greenland waters and the Norwegian Sea. The samples were screened by starch-gel electrophoresis to reveal polymorphic systems to be applied in studies of the genetic structure of the species. A number of isozyme systems are described. Seven enzyme loci (*GP3DH-1**, *GPI-1**, *GPI-2**, *LDH-1**, *LDH-2**, *MDH** and *PGM**) displayed sufficient activity and allele variation to be used in the routine work. Of those seven polymorphic loci, the *GPI-1** and *PGM** loci displayed great variation. Test of accordance between observed distributions of phenotypes and expected Hardy-Weinberg distributions as well as tests of heterogeneity among sampling sites, were performed using chi-square tests. No genetic differences within the three main areas (West-, East- Greenland and the Norwegian Sea) were revealed, while significant differences were found among those areas showing that the roughhead grenadier in the North Atlantic is composed of different stock units with their own gene pools rather than belonging to one panmictic population.

Key words: gene pool, genetic structure, heterogeneity, isozyme, polymorphic, roughhead grenadier, stock unit.

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Introduction

Roughhead grenadier, *Macrourus berglax* L., is an abundant deepwater species widely distributed in moderate to great depths (Parson 1976; Moyle *et al.* 1988). It is typical demersal species with a wide distribution across the North Atlantic (Figure 1).

Roughhead grenadier is found in the Labrador sea (Mombeck 1984; Sahrhage 1982; Savvatimsky 1984), Baffin Bay (Forest *et al.* 1978; Savvatimsky 1984), between Greenland and the Azores on the Mid-Atlantic ridge (Sahrhage 1986), from Denmark Strait to Jan Mayen (Magnusson1978; Sahrhage 1986 and the references therein), to the west and north-west of Iceland (Magnusson 1978; 1979), further east in the water between Iceland and the Faeroe Islands (Sahrhage 1986 and the references therein), and west of the British Isles, Ireland and Norway (Bakken *et al.* 1975; Hognestad & Vader 1979; Eliassen & Lorentsen 1982; Eliassen 1983; Eliassen & Breiby 1983). Occurrence of the species has also been reported in a number of the deep basins in the Barents Sea (Sahrhage 1986 and the references therein), between Bear Island and Svalbard (Geistdoerfer 1979), and as far as the Kola Peninsula (Savvatimsky 1989).

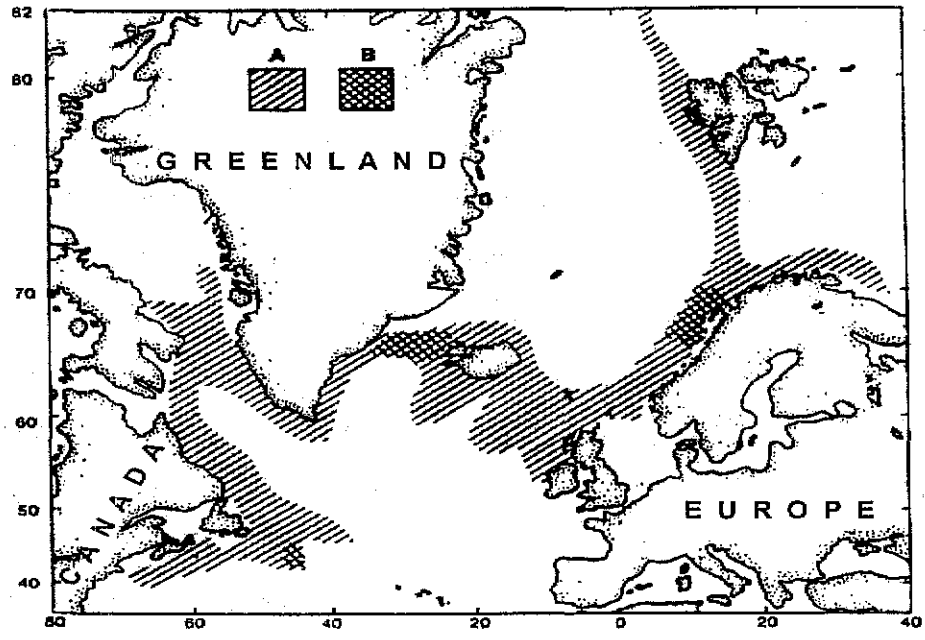


Figure 1. (A) Distribution of roughhead grenadier (*Macrourus berglax* L.) and (B) areas of the largest aggregations (Savvatimsky 1989)

Investigations on roughhead grenadier have been carried out since 1969, together with those of commercial fish done in parallel (P.I. Savvatimsky, Polar Research Institute, Murmansk, Russia, pers. comm.). In the late seventies the species was extensively studied in the North

Atlantic (Magnusson 1977; Eliassen & Breiby 1983; Savvatimsky 1989). The Norwegian fishery statistics include data on the catches around Norway. The stock size of roughhead grenadier can not be precisely estimated, since research and fishing gears do not cover the total range of fish's vertical distribution (Savvatimsky, pers. comm.). The growing interest in utilizing "new" resources has focused on the roughhead grenadier as one potential species for commercial harvest (Savvatimsky 1989; 1992). Bottom trawls and particularly longline fisheries are expected to be a promising method of utilizing the resource in the North Atlantic (Savvatimsky 1992).

The use of electrophoretic techniques has made it possible to investigate the genetic structure of natural populations. To illustrate the genetic structure of the roughhead grenadier population, an electrophoretic analysis of genetic variation has carried out on samples from different geographic areas in the North Atlantic (Figure 1; Table 1). The goals of the present investigation were to reveal genetic variation and to apply the most useful traits for studies of possible population structure of the species. The investigation was based on following hypothesis: the roughhead grenadier consists of a single panmictic population in the North Atlantic.

Material and methods

Sample collection

During the period August 1991-June 1996, 649 individual samples were collected on board research vessels at fifteen sampling locations within different fishing grounds on the shelf and continental slope off Greenland and Norway (Figure 2). Four samples were collected from Baffin Bay and Davis Strait off the coast of West Greenland, five sample units were from Irminger and Dohrnbank off the coast of East Greenland and four samples were from Storegga and Lofoten at the Norwegian Sea (Figure 2; Table 1). The sample size was ranged from two to 260 specimens. Therefore, for statistical analyses, small samples from adjacent localities were amalganated, such as an overview of the material is given in Table 1.

After capture, small pieces (approximately 1 cm^3) of liver and white muscle near the dorsal fin were removed from each fish. All tissue samples were stored in Microwelles at -20 °C on board the ship until they were transferred to an ultra freezer (-80 °C) at the laboratory for further processing. From some localities the fish were frozen whole and the sampling was done after thawing at the laboratory.

Electrophoresis

Two or three hours before electrophoresis, the samples were thawed. The samples were prepared by adding a few drops of distilled water to each compartment and disrupt the tissue cell by ultrasonic waves. The tissue enzymes were analyzed by both starch-gel electrophoresis (two buffer systems were assessed for their ability to accurately resolve polymorphic enzymes: i) Histidin-citrate pH 7.0 (Harris & Hopkinson 1976) and ii) Tris-citrate-borate pH 8.6 (Selander *et al.* 1971)) and Isoelectric Focusing (Anon. 1990) on ampholine polyacrylamide gel (pH range 3.5-9.5).

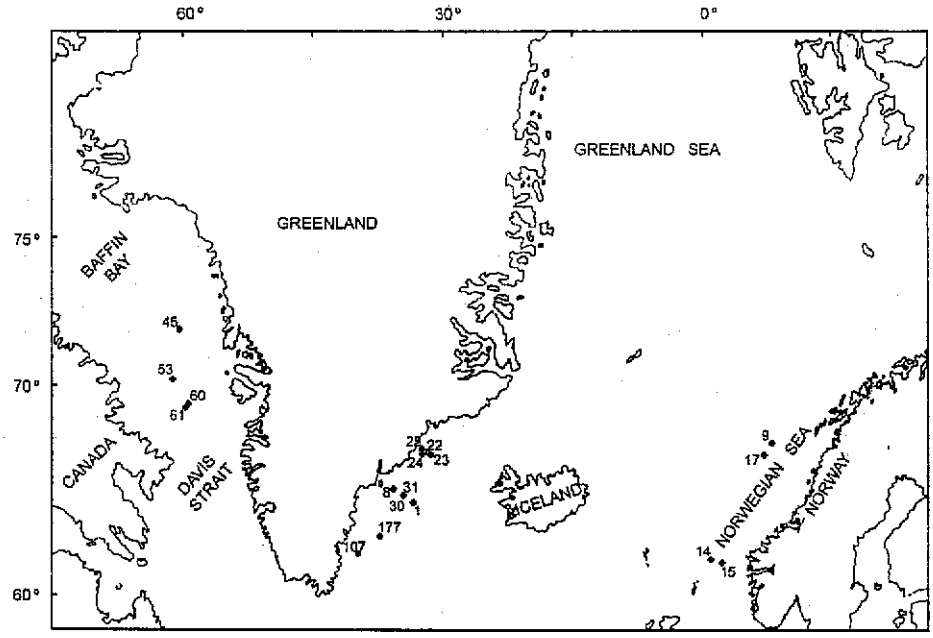


Figure 2. Polar projection showing locations where roughhead grenadier were sampled around Greenland and the Norwegian Sea from 1991 to 1996. For exact positions see Table 1

The homogenates samples were incubated in horizontal starch-gels (Harris & Hopkinson 1976; Murphy *et al.* 1990), 8 mm thick (using 12% starch Sigma potato starch, no S54501) and measured 260X180X8 mm. The gels were cooled to +4 °C during electrophoresis. Each gel was sliced horizontally five or six times and each slice was stained for a particular enzyme system using procedures based on protocols of Murphy *et al.* (1990). The enzyme patttern was immediately interpreted and photographed after staining and incubation.

Isoelectric focusing (IEF) was performed on ampholine polyacrylamide gel pH range 3.5-9.5, and the gel measured 245x110x1 mm. The used gel was *"Ampholine® PAGplate, Precast polyacrylamide gels for analytical isoelectric focusing"* from Pharmacia Biotechnology AB

(manual), Uppsala, Sweden. Samples were prepared using the same procedure as for SGE. Two filter papers (24.5x6x1 mm) were placed along the two longer sides of the gel as a bridge between gel and electrodes. Electrode solutions were used according to the instructions of Ampholine® PAGplates, Pharmacia Biotechnology (Anon. 1990). Only non-specific esterase was screened by IEF and it was stained by the same method as that used for SGE.

Genetic interpretation and standardized nomeclature

The nomenclature and designations of the alleles followed the protocol recommended by the Genetic Nomenclature Committee of the American Fisheries Society's Fish Genetics Section, laid out by Shaklee *et al.* (1990). The ten enzyme systems (15 loci) assayed, their abbreviations, the optimal buffer system used, and the number of loci and alleles consistently scorable are given in Table 2.

Table 1. *Macrourus berglax*. Collection sites, with sample designation abbreviations in parentheses. Dates of collection are based on information from field samplers, fishing mehtod (L=longline, BT=bottom trawl), depth range in meters (based on estimation from ecco depth). Sample size (N) of muscle (M) and liver (L) are shown.

Location of sampling areas	Station number	Position Latitude; longitude	Collection data (Dd, mm, yy)	Fishing method	Ecco depth (m)	N	
						M	L
East Greenland							
Dohrnbank01 (dhr.b.01)[1]	30	65°27′N 31°20′W	10.09.91	L	628-389		
	31	65°27′N 31°24′W	10.09.91	L	600-394	260	95
	1	65°26′N 31°17′W	21.06.94	L	594-446	44	-*
	8	65°29′N 31°26′W	21.06.94	L	519-559	23	-*
Dohrnbank02 (dhr.b.02)[2]	22	67°08′N 30°35′W	07.09.91	L	750-535		
	23	67°07′N 30°25′W	07.09.91	L	548-371		
	24	67°09′N 30°28′W	08.09.91	L	540-375	33	-*
	25	67°10′N 30°37′W	08.09.91	L	341-512	5	-*
Dohrnbank-Irminger (dhr.irm.)[3]	177	64°08′N 36°02′W	29.08.93	L	796-467	96	-*
	107	62°19′N 40°25′W	10.07.94	L	830-465	15	-*
West Greenland							
Baffin Bay (bf.b.)[4]	45	71°35′N 60°26′W	24.08.93	L	651-563	19	19
	53	70°28′N 60°47′W	27.08.93	L	439-388	43	43
Davis Strait (dv.st.)[5]	60	69°12′N 58°40′W	29.08.93	L	465-322	26	26
	61	69°07′N 58°41′W	29.08.93	L	445-322	18	18
Norwegian sea							
Storegga (nr.s.01)[6]	14	62°20′N 01°05′E	24.06.95	BT	585	43	12
	15	62°21′N 01°29′E	24.06.95	BT	530	12	-*
Lofoten (nr.s.02)[7]	9	67°46′N 09°47′E	28.06.96	BT	638	12	-*
	17	67°19′N 08°48′E	30.06.96	BT	596	2	-*
						651	213

*: No liver tissue available

[1-7]: for the statistical analyses, some samples from adjacent localities were amalganated;

[1] Dohrnbank01= dhr.b01 [2] Dohrnbank02= dhr.b.02 [3] Dohrnbank-Irminger= dhr.irm.

[4] Baffin Bay = bf.b. [5] Davis Strait=dv.st. [6] Storegga= nr.s.01 [7] Lofoten= nr.s.02

Data analyses

Statistical analyses were carried out using the BIOSYS package program (Swofford & Selander 1981).The Levene's (1949) correction for small sample size was employed in the chi-square analyses of deviation from Hardy-Weinberg equilibrium. The exact significance probabilities were calculated to test deviations from expected Hardy-Weinberg proportions. To test the heterogeneity in allele frequencies between samples, Wright's F-statistics and contigency chi-square analysis were carried out (Swofford & Selander 1981). The genetic distance between samples was calculated using the unbiased genetic distance coefficient of Nei (1978).

Results and Discussion

Of the ten enzymes examined, five consistenly produced variable isoenzyme patterns which are likely to be controlled by seven loci. The number of presumptive loci which could be accurately discerned amounted to fifteen. *Alcohol dehydrogenase (ADH*;* 1.1.1.1*)*, and *Sorbitol dehydrogenase (SDH*:* 1.1.1.14*)* were rejected because they showed no activity. Further, *Creatine phosphokinase (CPK*;* 2.7.3.2*)*, and *Esterase (EST-1*, EST-2*;* 3.1.1.-*)* were resolved poorly. However, seven polymorphic loci for the following proteins were found to be consistently interpretable and were scored for all sample units; *Glycerol-3-phosphate dehydrogenase (G3PDH-1*;* 1.1.1.8*)*, *Glucose-6-phosphate isomerase (GPI-1*, GPI-2*;* 5.3.1.9*)*, *L-Lactate dehydrogenase (LDH-1*, LDH-2*;* 1.1.1.27*)*, *Malate dehydrogenase (MDH*;* 1.1.1.37*)*, and *Phosphoglucose mutase (PGM*;* 5.4.2.2*f)*. Three monomorphic loci for the following proteins were found for *Glycerol-3-phosphate dehydrogenase (G3PDH-2*;* 1.1.1.8*)* and *Isocitrate dehydrogenase (IDHP-1*, IDHP-2*;* 1.1.1.42*)*.

Activity was only observed in skeletal muscle extracts while liver tissues extract failed to give adequate staining for all enzymes. The isoenzyme pattern for the five variable enzymes are displayed in Figure 4. An account of the results is given in Table 2 together with an overview of the results of the analyses of each enzyme. The polymorphic enzymes are briefly discribed below.

Glycerol-3-phosphate dehydrogenase (G3PDH). A strong pattern of activity was observed in muscle tissue for this dimeric protein. Best resolutions were found in SGE using Histidin-citrate pH 7.0 buffer system (Table 2). Three zones of G3PDH activity were observed, which appeared to be coded by two different loci and an interlocus dimer; *G3PDH-1**, *G3PDH-2** and G3PDH-1/2 (intermediate heterodimer zone) (Figure 3). *G3PDH-1** locus expressed with one common

allele; **100* and one rare; **110*. The **100/110* genotype was very rare, occurring only three times (Figure 1; Table 3). *G3PDH-2** locus was fixed for the same allele in almost all specimens, as can be seen in Figure 3, although one specimen showed inactivity, indicating a "null allele". The presence of "null allele" may lead to an apparent excess of phenotypic homozygote (Utter *et al.* 1987) (Table 3).

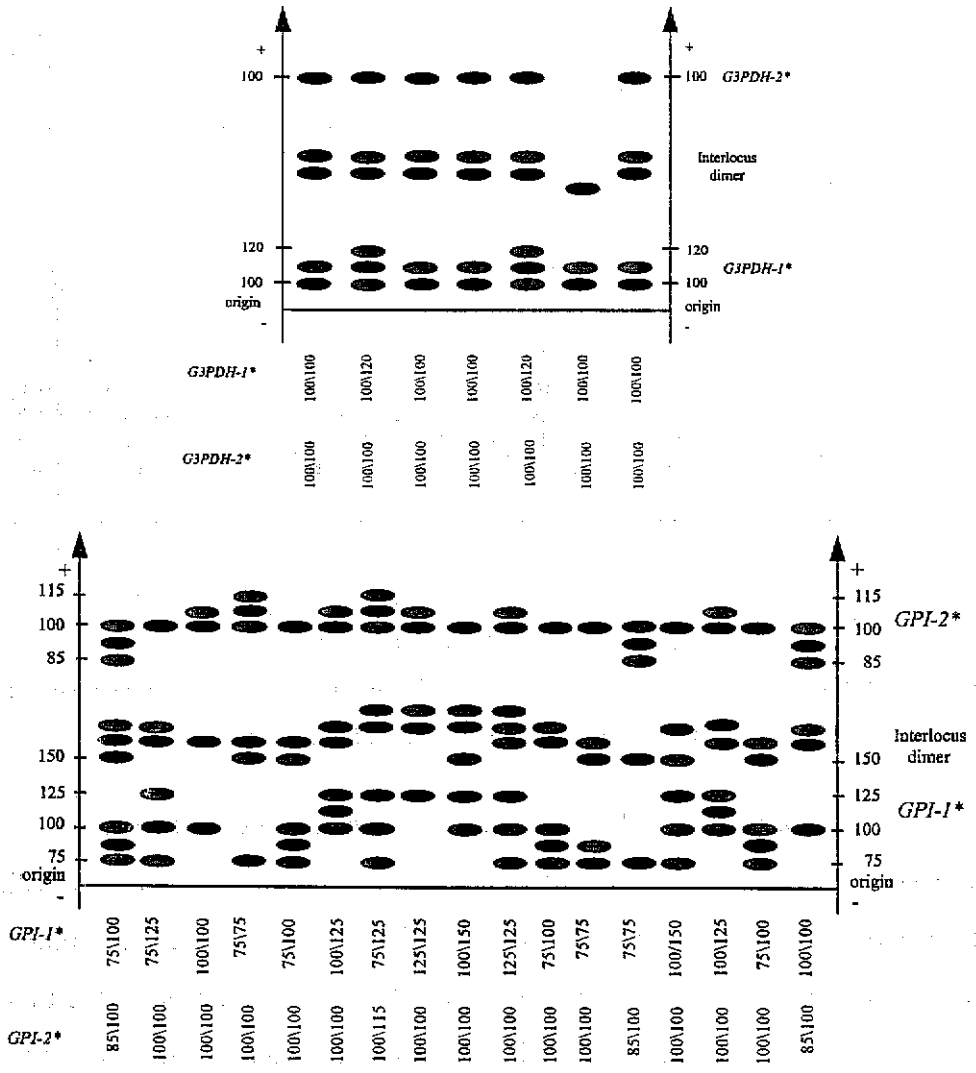
Glucose-6-phosphate isomerase (GPI). The zymograms demonstrated the dimeric nature of this enzyme by the presence of three zones. The least anodic zone had a single- and triple-banded phenotypes (Utter *et al.* 1987) and was interpreted as representing a polymorphic locus; *GPI-1**. The most anodic zone was also presumably coded by another polymorphic locus; *GPI-2**. Single, double, or triple bands of intermediate mobility were associated with specific phenotypes of *GPI-1** and *GPI-2** and were interpreted to be interlocus hybrid heterodimeric. All observed

Table 2. *Macrourus berglax.* Enzymes and enzyme commission numbers (EC), quaternary structure of their functional proteins, number of loci scored, number of alleles, buffer systems used for electrophoresis, activity and technical quality of buffer systems and estimated numbers of loci. Protocols for routine screening of electrophoretic variation at fifteen polymorphic isozyme loci (Horizontal starch gel (SGE) electrophoresis; HC discontinuous Histidin-citrate, pH 7.0(I); TCB discontinuous Tris-citrate-borate, pH 8.6(II); and Isoelectric (IEF) Focusing pH 3.5-9.5(III)). Activity and technical quality code: *= unclear, **= faint, ***= intermediate, moderate, ****= abundant.

Enzyme	Quaternary structure	Loc.des.[1]	No.all.	Buffer (pH)	Activity and technical quality Muscle	Liver[2]
Alcohol dehydrogenase (1.1.1.1)	dimeric	*ADH**	-	II	*	-
Creatine phosphokinase (2.7.3.2)	dimeric	*CPK**	1	I	**	*
Esterase (3.1.1.-)	monomeric	*EST-1**	1	III	**	*
		*EST-2**	2?			
Glycerol-3-phosphate dehydrogenase (1.1.1.8)	dimeric	*G3PDH-1**	2	I	***	*
		*G3PDH-2**	1			
Glucose-6-phosphate isomerase(5.3.1.9)	dimeric	*GPI-1**	4	I, II	****	**
		*GPI-2**	3			
Isocitrate dehydrogenase (1.1.1.42)	dimeric	*IDHP-1**	1	I, II	***	**
		*IDHP-2**	1			
L-Lactate dehydrogenase (1.1.1.27)	tetrameric	*LDH-1**	3	I, II	****	**
		*LDH-2**	3			
Malate dehydrogenase (1.1.1.37)	dimeric	*MDH**	3	I	***	**
Phosphoglucomutase (5.4.2.2f)	monomeric	*PGM**	6	I, II	****	**
Sorbitol dehydrogenase (1.1.1.14)	tetrameric	*SDH**	-	I	*	*

[1]the estimated number of loci are based only on muscle analyses.
[2]Liver tissues extract failed to geve adequate staining for the enzymes and was uncleared for frequency calculations.

patterns could be revealed by the different buffers used; Histidin-citrate pH 7.0 and Tris-citrate/borate pH 8.6 (Table 2) but the quality of the staining was improved by using the Histidin-citrate buffer system and thus the separation amongst the two loci and the interlocus hybrid (intermediate) zone was made clearer. In general, this enzyme was found to be highly polymorphic. The degree of variation at the *GPI-1** locus was much greater than that of the *GPI-2** locus (Table 3). The *GPI-1** locus appeared to be controlled by four alleles, **75*, **100*, **125* and **150*, with a similar variation among the specimens, while three alleles were observed at *GPI-2*; **85*, **115* and **100* where the last one was the most frequently allele (Table 3).*GPI-1*100* allele was found to occur at frequencies ranging from 0.500 at bf.b. to 0.737 at dhr.b.02. The second most frequent allele was the **75* with a frequency ranging from 0.250 at dhr.b.02 to 0.460 at bf.b. (Table 3). The interlocus hybrid zone (GPI-1/2) almost overlapped the *GPI-1** locus and occasionally made difficulties for interpretation (Figure 3).



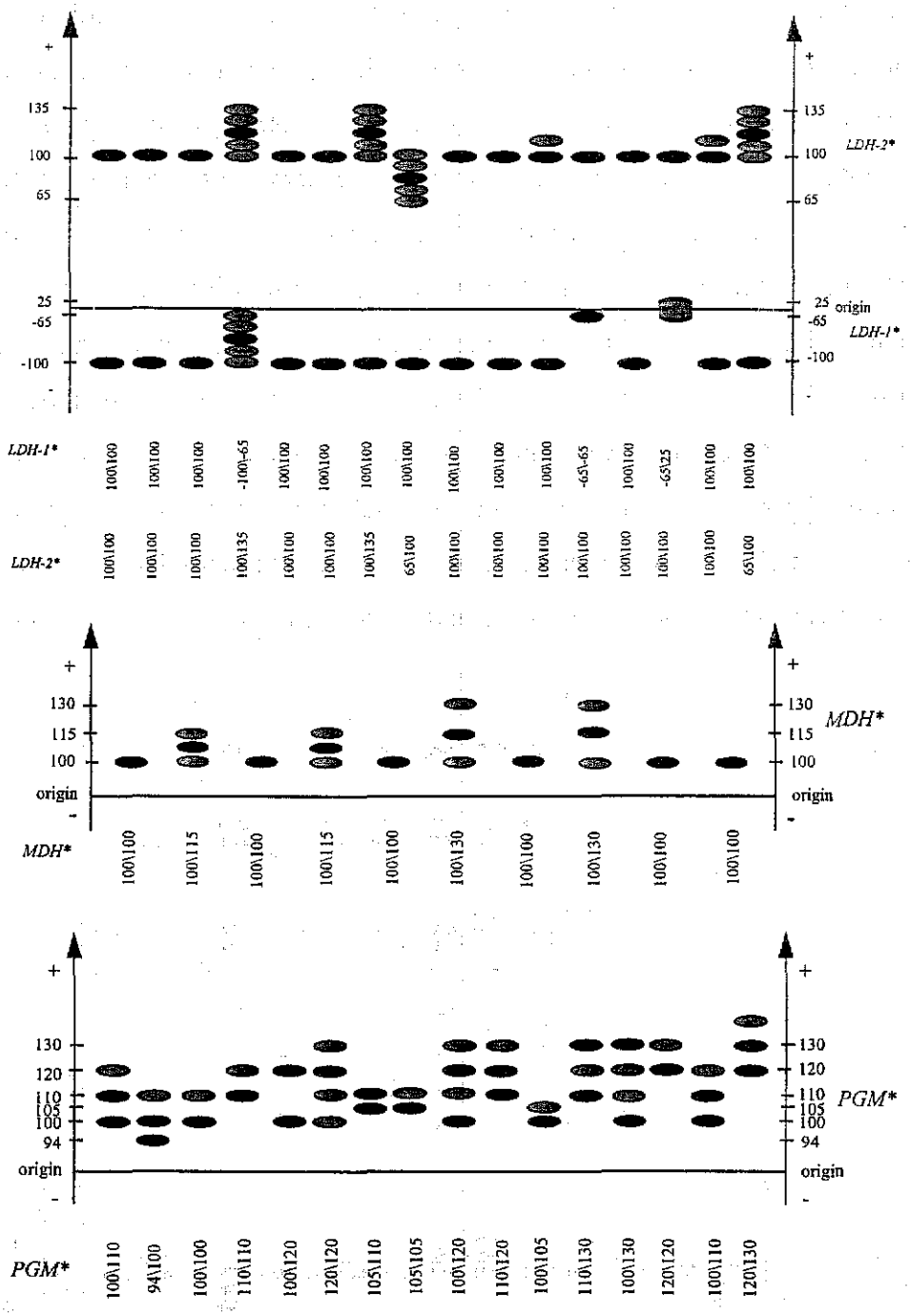


Figure 3. Diagrammatic summary of isozyme patterns for the five variable enzymes revealed in muscle tissues by starch-gel electrophoresis. Origin: denotes the point of origin of migration

L-Lactate dehydrogenase (LDH). The observed patterns could be explained as controlled by two loci, *LDH-1** and *LDH-2**, and the intermediate heteropolymer zone (LDH-1/2). All the observed paterns showed the same migration in the different buffers used; Histidin-citrate pH 7.0 and Tris-citrate/borate pH 8.6 (Table 2). A polymorphism was indicated in the more cathodic group, *LDH-1** locus, with component migration towards the cathode. Genetic control by three alleles

was assumed; *-*100*, *-*65* and **25*. In the more anodic group, *LDH-2** locus, a genetic control by three alleles is expressed; **65*, **100* and **135* (Figure 3). At *LDH-1** the most common allele, *-*100* was at high frequency (0.964-0.995), while the rare **25* allele appeared only once at a very low frequency (0.002) at the dhr.b.01 site (Table 3).

Malate dehydrogenase (MDH). The observed patterns agreed with those expected for dimeric molecules of which the peptides are controlled by allelic control. All the observed patterns were revealed clearly in Histidin-citrate pH 7.0 buffer system (Table 2). All individuals gave good clear banding patterns although the staining was weak. The *MDH** locus is assumed to be controlled by three alleles; **100*, **115* and **130* (Figure 3). The two latter alleles appeared only in dhr.b.01 sample (Table 3). The staining products appeared to represent a polymorphic pattern with the common allele at high frequency (Table 3).

Phosphoglucomutase (PGM). A single PGM isoenzyme was found (Figure 3). High activity was observed in both SGE and IEF. All the observed patterns were visualized under both buffer systems; Histidin-citrate (HC) pH 7.0 and Tris-citrate/borate (TCB) pH 8.6 (Table 2). However, the TCB buffer gave largely diffuse bands, whereas the HC buffer gave discrete and clear resolution, making the latter the preferred system. The banding pattern conformed to a model of a single locus (*PGM**) encoding a monomeric structure. The locus was represented by six alleles, with the most common allele represented by **100*. Variants were **94, *105, *110, *120* and **130* (Figure 3). Determination of phenotypes was complicated by the occurrence of so called "satellite bands" in some form of strong shadowing. Similar satellite bands have been reported in other fish species (Purdom *et al.* 1976; Fairbairn 1981; Nedreaas & Nævdal, 1989). Phenotypic variation was observed in all specimens, although many rare alleles were found throughout the sampling areas. The rare **105* variant showed an observation that appeared only twice at the sites of dhr.b.01 and dhr.irm., while the other rare variant **94* appeared only once at the site of dv.st.

Distribution

Allele frequencies for all regular (*G3PDH-1**, *GPI-1**, *GPI-2**, *LDH-1**, *LDH-2**, *MDH**, *PGM** loci) samples are shown in Table 3. The lowest amount of variation was observed for the two dimeric enzymes controlled by the glycerol-3-phosphate dehydrogenase (*G3PDH-1**) and malate dehydrogenase (*MDH**) loci. *G3PDH-1** only twice showed any variation (dhr.b.01

and nr.s.01; 0.008 and 0.036, respectively) (Figure 3; Table 3). Likewise, *MDH** only in dhr.b.01 sample showed any variation. *MDH*115* and **130* appeared once (Table 3).

For the tetrameric enzyme, lactate dehydrogenase (*LDH-1** and *LDH-2**) loci showed a low degree of genetic variation at all sampling sites with a monomorphic stage at the dv.st. site at the *LDH-2** locus. The *LDH-1*25* and *LDH-2*65* were rare; appeared only once each in one sample (dhr.b.01) site (Table 3).

Table 3. Allelic frequencies at seven polymophic loci for the seven sampling locations. The most common allele at each locus arbitrarily assigned of **100*; mobilities of other alleles are relative to the common allele. For a definition of station abbreviations see Table 1. Allele designations based on mobilities are given in the left-hand column. Number of specimens in parentheses.

Locus	Allele (N)	dhr.b.01 (327)	dhr.b.02 (38)	dhr.irm. (111)	bf.b. (62)	dv.st. (44)	nr.s.01 (55)	nr.s.02 (14)
*G3PDH-1**	**100*	0.992	1.000	1.000	1.000	1.000	1.000	0.964
	**110*	0.008	-	-	-	-	-	0.036
*GPI-1**	**75*	0.387	0.250	0.347	0.460	0.398	0.373	0.286
	**100*	0.569	0.737	0.595	0.500	0.568	0.509	0.607
	**125*	0.037	0.013	0.050	0.040	0.023	0.118	0.071
	**150*	0.008	-	0.009	-	0.011	-	0.036
*GPI-2**	**85*	0.009	-	0.014	-	-	0.009	-
	**100*	0.910	0.947	0.887	0.984	0.920	0.900	1.000
	**115*	0.081	0.053	0.099	0.016	0.080	0.091	-
*LDH-1**	*-*100*	0.989	0.987	0.995	0.968	0.966	0.991	0.964
	*-*65*	0.009	0.013	0.005	0.032	0.034	0.009	0.036
	**25*	0.002	-	-	-	-	-	-
*LDH-2**	**65*	0.002	-	-	-	-	-	-
	**100*	0.930	0.921	0.941	0.944	1.000	0.927	0.893
	**135*	0.069	0.079	0.059	0.056	-	0.073	0.107
*MDH**	**100*	0.994	1.000	1.000	1.000	1.000	1.000	1.000
	**115*	0.003	-	-	-	-	-	-
	**130*	0.003	-	-	-	-	-	-
*PGM**	**94*	-	-	-	-	0.011	-	-
	**100*	0.670	0.776	0.694	0.694	0.705	0.664	0.607
	**105*	0.008	-	0.005	-	-	-	-
	**110*	0.242	0.184	0.225	0.145	0.170	0.255	0.321
	**120*	0.070	0.026	0.072	0.153	0.114	0.064	0.071
	**130*	0.011	0.013	0.005	0.008	-	0.018	-

The glycose-6-phosphate isomerase (*GPI-1**, *GPI-2**) loci and phosphoglucomutase (*PGM**) locus showed abundant genetic variation (Table 3); *GPI-1*75* occurred frequently

besides the common allele **100*. *GPI-1*150* at lower rate in the East Greenland compared to nr.s.02 site (0.036). This allele had lower appearance in the dhr.b.01, dhr.irm. and dv.st. sites (0.008-0.011) and much higher at the nr.s.02 site (0.036). The products of the other locus (*GPI-2**) showed lower heterozygosity and *GPI-2*100* occurred at frequencies between 0.887 and 1.000, while *GPI-2*85* appeared at low frequencies (0.009-0.014) in the dhr.b.01, dhr.irm. and nr.s.01 sites, respectively (Table 3). The polymorphism of *PGM** was exposed at all sample locations. *PGM*110* was the most common allele (0.145-0.321) besides *PGM*100* (0.607-0.750). *PGM*120* appeared at very low frequency at the dhr.b.02 site (0.026), somewhat higher at the sites of dhr.b.01, dhr.irm. and the Norwegian Sea (0.064-0.072) and considerably higher at the sites of West Greenland (0.114-0.153). The product of *PGM*94* only appeared once (dv.st.) at a frequency of 0.011 and parallel the product of *PGM*105* were also appeared at low appearance (0.05-0.08) and only at the dhr.irm. and dhr.b.01, respectively sites (Table 3).

A chi-square goodness of fit to test for accordance between observed and expected do Hardy-Weinberg distributions revealed no significant deviations for most sample units (Table 4) when pooling rare alleles as recommended (Swofford & Selander 1981; Chakraborty & Leimar 1987). In some samples, however, some discrepancies were observed (Table 4). The sample unit (dhr.b.01) did not appeared to be in Hardy-Weinberg equilibrium with respect to distribution of Ldh-genotypes (χ^2=29.670; *d.f.*=1; *P*=0.000<0.005) (Table 4). This outcome is probably overestimated by the occurence of one specimen (*LDH-1*-65/25*) with very low expectance and

Table 4. Test of accordance between observed and expected frequencies, with pooling of rare alleles (Swofford & Selander 1981; Chakraborty & Leimar 1987) and chi-square test for deviation from Hardy-Weinberg equilibrium. Obs.=observed frequencies, Exp.=expected frequencies, χ^2 =chi-squares value, *d.f.*=degrees of freedom and *P*=significance probability; N.s.=not significant, *=*P*=<0.10, **=*P*<0.05, ***=*P*<0.005

	*GPI-1**		*GPI-2**		*LDH-1**		*LDH-2**		*MDH**		*PGM**	
Sampling sites	χ^2	*P*	χ^2	*P*	χ^2	*P*	χ^2	*P*	χ^2	*P*	χ^2	*P*
Dohrnbank01 (dhr.b.01)	0.005	N.s.	3.156	*	29.670	***	1.829	N.s.	0.009	N.s.	2.691	N.s.
Dohrnbank02 (dhr.b.02)	1.530	N.s.	0	N.s.	0	N.s.	0	N.s.	0	N.s.	9.083	***
Dohrnb.-Irminger (dhr.irm.)	4.071	**	1.709	N.s.	0	N.s.	0	N.s.	0	N.s.	2.729	*
Baffin Bay (bf.b.)	0.101	N.s.	0	N.s.	0	N.s.	0	N.s.	0	N.s.	6.593	N.s.
Davis Strait (dv.st.)	3.586	*	0	N.s.	0	N.s.	0	N.s.	0	N.s.	2.715	*
Storegga (nr.s.01)	1.020	N.s.	0.612	N.s.	0	N.s.	0	N.s.	0	N.s.	0.004	N.s.
Lofoten (nr.s.02)	1.175	N.s.	0	N.s.	0	N.s.	0	N.s.	0	N.s.	1.175	N.s.

therefore it is not emphasized in the present investigation. Likewise, the very low probability values for *PGM** at dhr.b.02 and among *GPI-1** of dhr.irm., can be explained by the occurrence

of a very low expected frequencies of some genotypes. If the calculated χ^2 is biased (Zar 1984) it could be due to the expected values are very small and consequently the statistic test does not follow the χ^2 distribution. Probability level of 0.05<P<0.10 is to be expected due to samples variation (type I error) (Sokal & Rohlf 1995).

The *GPI-1** and *PGM** loci displayed most variation in the calculations for heterozygosity deficiency or excess, ranging -0.427 to 0.257. Positive values of D indicate an excess of heterozygotes and negative values indicate a deficiency of heterozygotes. All the other loci (*G3PDH-1**, *GPI-2**, *LDH-1**, *LDH-2** and *MDH**) showed small deviations (Table 5). Value for *LDH-1** in the Dohrnbank01 (dhr.b.01) sample unit is probably overestimated.

Table 5. Coefficients for heterozygote deficiency or excess in the nine sampling areas. Probability levels resulting from tests for homogeneity among loci and localities are shown in the two first rows. Obs. het.= observed heterozygotes, Exp. het.=expected heterozygotes, D= Selanders D-statistic for the excess of deficiency of heterozygotes.

Locus	Sampling area	Obs. het.	Exp. het.	D	Sampling area	Obs. het.	Exp. het.	D
*G3PDH-1**	Dohrnbank01	5	4.969	0.006				
*GPI-1**	(dhr.b.01)	168	172.069	-0.024				
*GPI-2**		59	54.247	0.088				
*LDH-1**		6	6.945	-0.136				
*LDH-2**		46	42.899	0.072				
*MDH**		4	3.988	0.003				
*PGM**		174	159.815	0.089				
*GPI-1**	Dohrnbank02	12	15.187	-0.210	Dohrnb.-Irminger	67	58.385	0.148
*GPI-2**	(dhr.b.02)	4	3.840	0.042	(dhr.irm.)	25	22.584	0.107
*LDH-1**		1	1.000	0.000		1	1.000	0.000
*LDH-2**		6	5.600	0.071		13	12.294	0.057
*PGM**		8	13.960	-0.427		46	51.606	-0.109
*GPI-1**	Baffin Bay	31	33.569	-0.077	Davis Strait	29	23.069	0.257
*GPI-2**	(bf.b.)	2	1.984	0.008	(dv.st.)	7	6.517	0.074
*LDH-1**		4	3.902	0.025		3	2.931	0.024
*LDH-2**		7	6.659	0.051				
*PGM**		21	29.650	-0.292		16	20.540	-0.221
*G3PDH-1**	Storegga				Lofoten	1	1.000	0.000
*GPI-1**	(nr.s.01)	30	32.633	-0.081	(nr.s.02)	6	7.889	-0.239
*GPI-2**		11	10.083	0.091				
*LDH-1**		1	1.000	0.000		1	1.000	0.000
*LDH-2**		8	7.486	0.069		3	2.778	0.080
*PGM**		28	27.220	0.029		6	7.593	-0.210

Contingency chi-square analysis, however, indicated heterogeneity in the total material of roughhead grenadier (Table 6). The randomizations of the data for the loci that are responsible for the significance also support it.

Table 6. Contingency chi-square table for the inter-area with respect to the seven loci for the nine sampling sites. χ^2= chi-squares value test, *d.f.*=degrees of freedom and *P*=significant probability

Locus	No. of alleles	χ^2	*d.f.*	*P*
*G3PDH-1**	2	10.092	6	0.121
*GPI-1**	4	36.195	18	0.006
*GPI-2**	3	15.536	12	0.213
*LDH-1**	3	10.828	12	0.544
*LDH-2**	3	8.889	12	0.712
*MDH**	3	3.975	12	0.984
*PGM**	6	42.998	30	0.059
Total		128.513	102	0.039

Test for heterogeneity of the genotypic distributions did not reveal any significant variation among samples within the three main areas (West-, East- Greenland and the Norwegian Sea) were found (Table 7) indicating panmictic population within these areas.

Table 7 Contingency chi-square analysis for intra-area with respect to two most variable polymorphic loci, *GPI-1* and *PGM*. χ^2 =chi-squares value, *d.f.*=degrees of freedom and *P*=significance probability: N.s.=not significant, *=*P*=<0.10, **=*P*<0.05, ***=*P*<0.005

*GPI-1**

Area	No. of alleles	χ^2	*d.f.*	*P*
East Greenland	4	9.776	6	N.s.
West Greenland	4	2.800	3	N.s.
Norwegian Sea	3	5.259	3	N.s.

*PGM**

Area	No. of alleles	χ^2	*d.f.*	*P*
East Greenland	5	5.850	8	N.s.
West Greenland	5	2.929	4	N.s.
Norwegian Sea	4	1.011	3	N.s.

The amount of genetic differentiation based upon the most variable loci, *GPI-1** and *PGM** loci, among the three areas (West-, East- Greenland and the Norwegian Sea) is quite large and significant (Table 8). Some alleles were absent from some of the sampling areas (Table 3); but since these alleles are also rare in those areas in which they are present, it is probable that they would not be observed unless larger sample sizes were used. Genetic distance values showed very little differentiation among the sampling areas. The largest genetic distance value of 0.004 is quite

small and is within the expected range of distances between "local races" of the species (Nei 1987). Given the overall small distance values, an estimate of the relationship among the

Table 8. Contingency chi-square analysis for inter-area with respect to two most varaible polymorphic loci, *GPI-1** and *PGM**. χ^2 =chi-squares value, *d.f.*=degrees of freedom and *P*=significance probability; N.s.=not significant, *=*P*=<0.10, **=*P*<0.05, ***=*P*<0.005

*GPI-1**

Area	No. of alleles	χ^2	*d.f.*	*P*
East- West Grennland	4	3.439	3	N.s.
East Greenland-Norwegian Sea	4	13.715	3	***
West Greenland-Norwegian Sea	4	9.011	3	**
All the areas	4	18.394	6	**

*PGM**

Area	No. of alleles	χ^2	*d.f.*	*P*
East- West Grennland	6	21.591	5	***
East Greenland-Norwegian Sea	5	1.976	4	N.s.
West Greenland-Norwegian Sea	5	11.072	4	**
All the areas	6	25.520	10	***

sampling sites would be "non-informative" due to sampling error (Nei 1987). The degree of genetic differentiation among the sampling areas, as measured by F_{ST} between 0.004 to 0.025, mean 0.018, (Table 9) indicate moderate differentiation (Hartl 1980).

Table 9. Summary of the three *F*-statistics, F_{IS}, F_{IT}, and F_{ST}, at all fifteen loci for the seven sampling sites. F_{IS},= genetic variance within populations, F_{IT}= genetic deviation in total population (whole set of samples), F_{ST}= deviation due to differences in gene frequencies between populations.

Locus	F_{IS}	F_{IT}	F_{ST}
*G3PDH-1**	-0.032	-0.006	0.025
*GPI-1**	0.018	0.038	0.020
*GPI-2**	-0.090	-0.064	0.024
*LDH-1**	-0.017	-0.009	0.008
*LDH-2**	-0.084	-0.068	0.015
*MDH**	-0.005	-0.001	0.004
*PGM**	0.143	0.155	0.014
Mean	0.043	0.059	0.018

Conclusion

The contigency chi-square test among the sampling sites revealed significant heterogeneity at the polymorphic locus *GPI-1** (Table 6) and for all loci combined.

The homogeneity tests revealed no variation among samples within any of the three main areas (Figure 2; Table 7).

Pairwise comparison (Table 8) using contigency chi-square test for the most variable polymorphic loci (*GPI-1** and *PGM**) showed that the frequency distributions differed significantly between sampling areas. This is contradicting the hypothesis of panmictic population in the North Atlantic.

The foregoing analysis shows that the roughhead grenadier population in the North Atlantic does not share the same gene pool in the West-, East- Greenland waters and the Norwegian Sea, indicating that the species does not form a single genetically homogeneous stock but is composed of different stock units with their own gene pools.

References

Anon., 1990. Instruction for *"Ampholine® PAGplate, Precast polyacrylamide gels for analytical isoelectric focusing"*. Pharmacia Biotechnology AB, Uppsala, Sweden, 34p

Bakken, E., J. Lahn-Johannesen, & J. Gjøsæter, 1975. Demersal fish on the continental slope off Norway.*Coun. Meet. int. Coun. Explor.* Sea, F:**29:**1-15

Chakraborty, R., & O. Leimar, 1987. Genetic variation within a subdivided polulation. Genetic distance and molecular philogeny, In*Population genetics and Fishery management* (Nils Ryman and Fred Utter, edt). University of Washington, Washington, D.C., pp 89-120

Eliassen, J.-E., 1983. Distribution and abundance of roundnose grenadier*(Coryphaenoides rupestris* Gunnerus) (Gadiformes, Macrouridae) in nothern and mid-Norway. *Coun. Meet. int. Coun. Explor. Sea,* G:**43:**1-24 (mimeo)

Eliassen, J.-E. & A. Breiby, 1983. Forsøksfiske etter isgalt og blåkveite langs Trænaegga, Nordland fylke i 1983*Rep. Ser. B:Ressurbiologi 1983*, institutt for fiskerifag, University of Tromsø, Norway, **6:**1-13 [in norwegian]

Eliassen, J.-E. & E. Lorentsen, 1982. Toktrapport fra forsøksfiske etter isgalt og blåkveite utenfor Nordland og Tromsø i juni og juli 1982*Rep. Ser. B:Ressurbiologi 1982*, institutt for fiskerifag, University of Tromsø, Norway, **6:**1-9 [in norwegian]

Fairbairn, D.C., 1981. Biochemical genetic analysis of population differentiation in Greenland halibut (*Reinhardtius cynoglossus*) from the North-West Atlantic, Gulf of St. Lawrence and Bering Sea. *Canad. J. Fish. Aquat. Sci.*, **38:**669-677

Forest, A., J.P. Minet, & J.B. Perodou, 1978. Results of groundfish survey on Baffin Island shelf. *ICNAF Res. docum. 78/VI/45*, 10p

Geistedoerfer, P., 1979. Recherches sur l'alimentation de *Macrourus berglax* (Macrouridae, Gadiformes). *Ann. Inst. Oceanograph.*, **55:**135-144

Harris, H. & D.A. Hopkinson, 1976. Handbook of Enzyme Electrophoresis in Human Genetics Oxford: North Holland Publishing CO

Hartl, D.L., 1980. Principles of population genetics. Sinauer Associates, Inc. Sunderland, Massachussetts.

Hognestad, P.T., & V. Vader, 1979. Saltvannsfiskene i Nord Norge, *Universitet i Tromsø*, Tromura, **6:**74p [in norwegian]

Levene, H., 1949. On a matching problem arising in genetics. *Ann. Math. Stat.*, **20:**91-94

Magnusson, J.V., 1977. Some notes on the spawning habits of Macrouridae at Iceland. *ICES*, CM 1977/F:49, 9p

- 1978. On the distribution and spawning grounds of the roughhead grenadier*(Macrourus* berglax Lacepéde) west of Iceland. *ICES*, CM 1977/G:36, 10p
- 1979. Icelandic investigations on grenadier fish (*Coryphaenoides rupestris* and *Macrourus berglax*) in 1977. *ICES Ann. biol.*, **34:**223-225

Mombeck, F., 1984. Untersuchung der Grundfischbestäende vor Labrador (Investigation of benthic fish stocks near Labrador). *Inf. Fischwirtsch*, **31(1)**, pp 3-9 [in german]

Moyle, P.B. & J.J. JR., Cech, 1988. Part II: 21. Bristlemouths, Codfishes and Deepsea Predators.*FISHES. An Introduction to Ichthyology* (Moyle P. B., Cech J. J., JR). University of California, Davis, 264p

Murphy, R.W., J.W. Jr. Sites, D.G. Buth, & C.H. Haufler, 1990. Proteins I: Isozyme electrophoresis.*Molecular systematics* (David M. Hills and Craig Moritz, edt). Sinauer Associates, Inc. Publishers, Sunderland, Massachusetts, U.S.A., pp 45-126

Nedreaas, K. & G. Nævdal, 1989. Studies of Northeast Atlantic species of redfish (genus*Sebastes*) by protein polymorphism.*J. du Conc. Intern. de Explorat. de la Mer,* **46:**76-93

Nei, M., 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics*, **89:**583-590

Parsons, L.S., 1976. Distribution and relative abundance of roundnose, roughhead and common grenadiers in the North West Atlantic*ICNAF Select. Pap.*, **1:**73-88

Purdom, D.E., D. Thompson & P.R. Dando, 1976. Genetic analysis of enzyme polymorphisms in plaice (*Pleuronectes platessa*).*Heredity*, **37:**193-206

Sahrhage, D., 1982. Erforschung der Grundfischbestäende vor Labrador (Research of benthic fish stocks near Labrador). *Inf. Fischwirtsch*, **29(1):**4-13

- 1986. Mitteilungen aus dem Institstut fuer Seefischerei der Bundesforschungsanstalt fuer Fischerei. Commercially important grenadiers of the North Atlantic. *Canad. Transl. Fish. and Aquat. Sci.*, Hamburg 37, 81p

Savvatimskiy, P.I., 1984. Biological aspects of roughhead grenadier (*Macrourus berglax*) from longline catches in the eastern Grand Bank area, 1982. *NAFO Scien. Counc. Studies*, **7:**45-51

- 1989. Investigations of roughhead grenadier (*Macrourus berglax* L.) in the Northwest Atlantic, 1967-83*NAFO Scien. Counc. Studies*, pp 59-75

- 1992. Severnyj makrurus (*Macrourus berglax* L.) potentsial'nyj ob'ekt tralovogo i yarusnogo promysla v Severnoj Atlantike (The roughhead grenadier *Macrourus berglax*, a potential resource for trawl and longline fisheries).*Studies of Biological resources in the North Atlantic #Issledovaniya Bioresursovsevernoj Atlantiki.* Savvatimskij, P.I. Pinko 1992, pp 45-67 [in russian]

Selander, R.K., M.H. Smith, S.Y. Yang, W.E. Johnson & J.R. Gentry, 1971. Biochemical polymorphism and systematics in the genus *Peromyscus*. I. Variation in the old-field mouse (*Peromyscus polionotus*). Studies in genetics VI. The university of Texas publication **7103:**49-90

Shaklee, J.B., F.W. Allendorf, D.C. Morizot, & G.S. Writt, 1990. Gene nomenclature for protein-coding loci in fish.*Transact. Amer. Fish. Soc.*, **119:**2-15

Sokal, R.R. & F.J. Rohlf, 1995. Biometry, 3rd ed, W.H. Freeman company, New York, 887p

Swofford, D.L. & R.B. Selander, 1981. BIOSYS-1 (Release 1.7).*A computer program for the analysis of allelic variation in genetics.* Users manual. Department of Genetics and Development, University of Illinois 63p+Appendices

Utter, F., P. Aebersold, & G. Winans, 1987. Interpreting Genetic Variation-Detected by electrophoresis.*Population genetics and Fishery management* (Nils Ryman and Fred Utter, edt). University of Washington, Washington, D.C., pp 21-45

Zar, J.H., 1984. Biostatistical analysis, 2nd ed., Prentice Hall, inc., Englewood Cliffs, New Jersey