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DESCRIPTION OF POLYMORPHISMS AT TISSUE ENZYME LOCI IN EAST ATLANTIC HADDOCK (*Melanogrammus aeglefinus* L.): TISSUE MANIFESTATION, ELECTROPHORETIC CONDITIONS AND ESTIMATES OF BASIC POPULATION GENETIC PARAMETERS.

by

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ABSTRACT

A set of samples covering a large part of the latitudinal range of haddock (*Melanogrammus aeglefinus*) in the East Atlantic were used in a study of the general level of genetic variability in the species. A total of 351 specimens were collected from northeastern Norway (100), northern Norway (100), Norwegian west coast (87), and Swedish west coast (64). Electrophoretic analyses of 14 tissue enzymes revealed 10 reliably scorable loci. Variant alleles were found in nine of these (*G3PDH-3*, IDHP-1*, LDH-1*, LDH-2*, LDH-3*, PGI-1*, PGI-2*, PGM*, SOD-2**). Four were polymorphic according to the 99% criterion ($P_{0.99}$ = 0.40) (*G3PDH-3*, LDH-2*, PGI-2*, PGM**). The expected average heterozygosity per locus was 0.084, and the effective number of alleles per locus varied from 1.01 (*LDH-3**) to 1.93 (*PGI-2**) with a mean of 1.14. Details of electrophoretic conditions, staining procedures, and tissue manifestation of the loci are described.

key words: haddock; *Melanogrammus aeglefinus*; isozymes; fisheries management; population genetics; starch gel electrophoresis.

INTRODUCTION

The demersal gadoid haddock (*Melanogrammus aeglefinus* Linnaeus, 1758) is found on both sides of the North Atlantic; from New Foundland to Cape Hatteras on the western side, and from Novaja Zemlja to Portugal on the eastern (Scott and Scott 1988). Important East Atlantic spawning grounds are found off the coast of mid- and northern Norway, in the North Sea, at the Faroes, and south-west of Iceland. The eggs and larvae are pelagic. Specimens usually reach sexual maturity at an age of 3 - 4 years (30 - 40 cm length) in the North Sea and 4 - 8 years (40 - 65 cm length) in North Norway (Svetovidov 1948). The haddock is commercially very important in the North Atlantic, and annual catches constitute several hundred thousand tons.

Both biologically and with respect to geographic distribution, the haddock resembles the Atlantic cod, and spawning in these two gadoid species often occurs at the same grounds and at the same time of the year. Also their total abundance in the Atlantic are of comparable scales. Given these similarities in biology, it is of interest to compare their general level of genetic variability. While estimates of basic population genetic parameters like average heterozygosity per locus, percentage of polymorphic loci, and effective number of alleles have been estimated for the Atlantic cod (Mork *et al.* 1982; 1985), there is currently no such information available for the haddock in the literature.

This paper reports from a study designed to provide such information for haddock in the East Atlantic.

MATERIALS AND METHODS

Sample collection

Samples were collected in two fjords in Northern Norway (Varangerfjorden and Malangen; bottom trawl by research vessel «Johan Ruud» in autumn 1992), on the coast of mid-Norway (Buagrunnen; commercial fishing vessel in mars 1994), and on the Swedish west coast (Smögen, commercial fishing vessel in april 1994). Biological data (length, weight, sex) were recorded for each specimen. Tissue samples (muscle, liver, and heart) were cut out and frozen immediately after catch. Otholiths were collected for age readings. Details of the samples are given in Table 1 and Fig. 1.

Table 1. Haddock sample information. N = number of fish analysed

Location	Station code	Position	Vessel	N	Date	Trawl type and mesh width
Varangerfjorden	1318	70°07' N 28°47' E	RV "Johan Ruud"	100	26.08.92	Bottom trawl (35 mm)*
Malangen	1875	69°18' N 18°39' E	RV "Johan Ruud"	35	03.10.92	Bottom trawl (35 mm)*
	1876	69°18' N 18°38' E	RV "Johan Ruud"	65	03.10.92	Bottom trawl (35 mm)*
Buagrunnen		63°07' N 06°47' E	"Vevang Trål"	87	09.03.94	Bottom trawl (100 mm)
Smögen		58°25' N 11°10' E	"Wardö"	64	13.04.94	Bottom trawl (70-120 mm)

* With a 10 mm mesh insert in the cod-end.

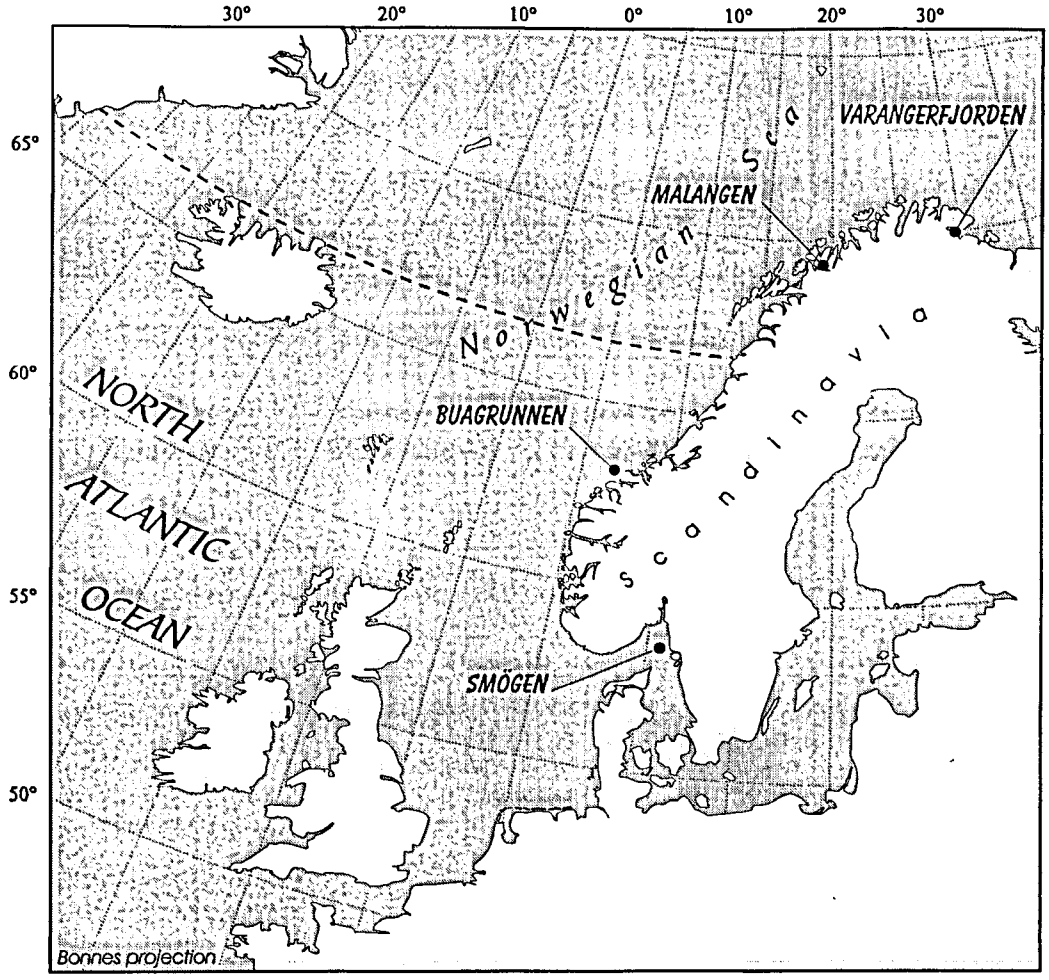

Figure 1. Haddock sampling locations.

Tissue extract preparation

Muscle, liver, and heart tissues were separately homogenized in equal amounts of destilled water and centrifuged (10 min at 10 000 g, 4°C). The supernatant was used for electrophoresis.

Electrophoresis

Two buffer systems were used; the discontinuous system by Ridgway *et al.* (1970) (RW) and the continuous system by Clayton and Tretiak (1972) (AC). 10 mm thick starch gels (11%) were moulded directly onto ceramic cooling plates. Tissue extract samples were applied to the gel in filter paper (1x12 mm) inserted along a slot cut 4 cm from the cathodic electrode. The filter papers were removed after 15 minutes run at 0.08 W/cm^2, whereafter the wattage was raised to 0.10 W/cm^2. Electrophoresis was completed after 2 hrs for the RW-system and 3.5 hrs for the AC-system.

Staining

After electrophoresis, the gel was sliced into 1 mm slices which were incubated in the respective staining solutions and left in the dark (37°C) until sufficiently strong bands had developed. In general, the stain recipes followed Hillis and Morris (1990) with the following modifications: except for SOD, the staining solutions were modified by the replacement of phenazine methosulphate (PMS) with phenazine ethosulphate (PES), and the pH in the staining solutions was raised to 9.0 when staining for dehydrogenases (Mork 1990).

Interpretation of banding patterns

The genetic interpretation of banding patterns followed the principles outlined by Allendorf *et al.* (1977). The nomenclature of loci, genotypes and alleles accords to Shaklee *et al.* (1990). If more than one buffersystem are listed in Table 2, the first one was used in this study, but both are usable.

Polymorphism at *LDH-2** and *PGI-2** have previously been described by Child (1988), and variant alleles at *LDH-2** in this study follow his designations. *PGI-2** showed a much better differentiation of variant alleles in buffersystem AC than in RW. Because of this advantage, buffersystem AC was prefered to RW, and hence the abbreviation of the most frequent variant allele at *PGI-2** was abbreviated *PGI-2*75* which corresponds to Childs *PGI-2*96.*

It should be noted that the *LDH-2*192* can be difficult to distinguish from the *LDH-2*262* when using the RW-system, and *LDH-2*192* from *LDH-2*100* when using the AC-system (see Fig. 2). Due to this problem, both buffersystems were employed to score this locus, which proved to be easy, reliable and very effective.

Enzymes stained for:	E.C. number	Abbreviation
Alcohol dehydrogenase	E.C. 1.1.1.1	ADH
Aspartate aminotransferase	E.C. 2.6.1.1	AAT
Creatine kinase	E.C. 2.7.3.2	CK
Fumarate hydratase	E.C. 4.2.1.2	FUMH
Glycerol-3-phosphate dehydrogenase	E.C. 1.1.1.8	G3PDH
Glucose-6-phosphate isomerase	E.C. 5.3.1.9	PGI (GPI)
Hexokinase	E.C. 2.7.1.1	HK
L-Iditol dehydrogenase	E.C. 1.1.1.14	IDDH (SDH)
Isocitrate dehydrogenase	E.C. 1.1.1.42	IDHP
L-Lactate dehydrogenase	E.C. 1.1.1.27	LDH
Malate dehydrogenase	E.C. 1.1.1.37	MDH
Malate dehydrogenase phosphate	E.C. 1.1.1.40	MDHP (Me)
Phosphoglucomutase	E.C. 5.4.2.2	PGM
Superoxide dismutase	E.C. 1.15.1.1	SOD

RESULTS

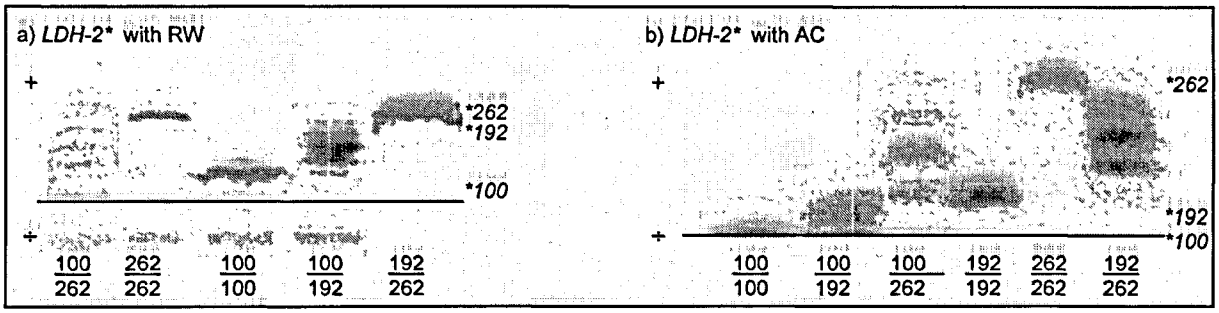

Figure 2. Phenotypes at *LDH-2** as revealed by the buffersystem of a) RW (Ridgway *et al.* 1970) and b) AC (Clayton and Tretiak 1972). The lines indicate the application slot.

Electrophoretic analysis in a total of 14 tissue enzymes gave 10 readable loci. In nine of these (*G3PDH-3*, IDHP-1*, LDH-1*, LDH-2*, LDH-3*, PGI-1*, PGI-2*, PGM*, SOD-2**) variants were found of which four were polymorphic, $P_{0.99} = 0.4$ (*G3PDH-3*, LDH-2*, PGI-2*, PGM**) and two of these again were also inside the 95% criterion, $P_{0.95} = 0.2$ (*LDH-2*, PGI-2**).

The main results from the genetic analysis are compiled in Table 2. ADH, CK, FUMH and HK did not stain specifically or clear enough to permit any conclusions about their genetic control and are not included in the table. ADH did not show any activity at all. Activity on these gels was shown to be exclusively due to LDH background staining. CK, FUMH, and HK showed little or no activity. Staining for 12 hrs (over night) at 37°C gave weak bands, but these were shown to be LDH, too. The observed banding patterns and tissue expression for the other enzymes were assigned to loci which we here treat in three groups: monomorphic, polymorphic (99% criterion), and uninterpreted.

Monomorphic loci (*IDHP-1*, LDH-1*, LDH-3*, MDH-1*, PGI-1** and *SOD-2**)

IDHP-1, LDH-1*, LDH-3*, MDH-1*, PGI-1** and *SOD-2** can be scored with buffersystems RW and AC. *IDHP-1*, LDH-3*, PGI-1**, and *SOD-2** resolved better in RW than AC, while for MDH good resolution was only obtained in AC. With exception of MDH, variants were found at all these loci. At both *IDHP-1** and *PGI-1** some variant alleles revealed by only one of the buffer systems.

Polymorphic loci (*G3PDH-3*, LDH-2*, PGI-2** and *PGM**)

G3PDH-3, LDH-2*, PGI-2** and *PGM** all gave strong staining and good resolution. *G3PDH-3*, LDH-2** and *PGM** showed best activity in muscle, while *PGI-2** showed best activity in heart and liver. *LDH-2*, PGI-2** and *PGM** showed good activity and resolution in both buffersystems, but for *PGI-2** there was a much better differentiation of the variant alleles with the AC system, which made interpretation much easier and safer than with the RW system.

Uninterpreted loci (*AAT-1**, *AAT-2**, *G3PDH-1**, *G3PDH-2**, *IDDH**, *MDH-2** and *MDHP**)

Two loci were tentatively designated for AAT (*AAT-1** and *AAT-2**). Neither of them seemed to have any variation. Both expressed too low activity and resolution for routine use. IDDH seemed to have genetic variation, but bad resolution and low differentiation of potential alleles excluded the separation of homozygotes and heterozygotes in this tetrameric enzyme. Strong LDH activity was observed when staining for IDDH. Thus when staining for this enzyme it could be scored for LDH on the same gel. *MDH-2** showed best activity in muscle and stained only in AC. The activity was very low, but this locus also seemed to have genetic variation. Its bands were partly covered by *MDH-1** (which has very strong activity), which made the interpretation of the locus very difficult. *MDHP** showed best activity in heart, and somewhat less in muscle tissue. The activity was very variable between individuals; from very good to no activity at all. Two other loci which showed variation were *G3PDH-1** and *G3PDH-2**, with best activity in liver and heart respectively. Both showed good resolution, but the activity was very low. It seems that the products from these two loci degrade faster than for *G3PDH-3**. *SOD-1** was expressed very weakly and is not considered usable in population surveys.

Table 2. Enzymes stained for, locus designation, tissues with best activity and resolution, preferred buffer system, and alleles found. Abbr.: M = muscle, L = liver, H = heart.

Enzyme	Locus	Best tissue	Buffer system	Alleles	Comments
AAT	*AAT-1**	M	RW	-	Cathodic mobility
	*AAT-2**	L	RW	-	Anodic mobility
G3PDH	*G3PDH-1**	L	RW	*33, 100, 170*	Low activity.
	*G3PDH-2**	H	RW	*100, 112*	Low activity
	*G3PDH-3**	M	RW	*95, 100, 104*	
IDDH	*IDDH**	L	RW	-	See text
IDHP	*IDHP-1**	L	RW	*73, 100, 124*	
	*IDHP-2**	M	AC	-	Low resolution and activity
LDH	*LDH-1**	L	AC, RW	*-225*, -100, 75, 138*	
	*LDH-2**	M	RW, AC	*100, 192, 262*	
	*LDH-3**	H (M)	RW	*72, 100, 113*	
MDH	*MDH-1**	L	AC	*100*	
	*MDH-2**	M	AC	-	Low activity
MDHP	*MDHP**	H (M)	RW	-	Low activity
PGI	*PGI-1**	M	RW	*76, 84, 100, 116*	
	*PGI-2**	H/L	AC	*38, 60, 75, 100*	
PGM	*PGM**	M	AC, RW	*-150, -100, 15*	
SOD	*SOD-1**	M	RW	-	Almost invisible
	*SOD-2**	L	RW, AC	*60, 80, 100, 135*	

* A minus "-" before the allele indicate cathodic migration.

Genetical results

χ^2– Goodness-of-fit tests indicated that all samples were in HW-equilibrium with one exception; a significant heterozygote deficiency was found at *LDH-2** in the sample from Malangen (P=0.004). One individual with the genotype 262/262 contributed with 85.5 % of the chi-square. No deficiency from the HW-equilibrium was found in the total material. A contingency table test for heterogeneity of allele frequencies between samples did not give a significant χ^2.

The F_{ST} (Wright 1951, 1965) gave indications of very little heterogeneity between the samples. $F_{ST} = 0.003$ indicate that almost all the variation found in the material (99.7 %) was allocated within samples.

Table 3. Allele frequencies, number of fish in each sample, observed and expected heterozygosity (H_{obs} and H_{exp}) in all samples and the total sample at each locus, and the effectiv number of allels (n_e).

	Allele frequency							
G3PDH-3*	*100*	*95*	*104*		N	H_{obs}	H_{exp}	n_e
Varangerfjorden	.980	.020			100	.040	.039	1.04
Malangen	.990	.005	.005		100	.020	.020	1.02
Buagrunnen	.971	.029			87	.057	.056	1.06
Smögen	.969	.031			64	.063	.061	1.06
TOTAL	.979	.020	.001		351	.043	.042	1.04
IDHP-1*	*100*	*124*	*73*		N	H_{obs}	H_{exp}	n_e
Varangerfjorden	.995	.005			100	.010	.010	1.01
Malangen	.990	.005	.005		100	.020	.020	1.02
Buagrunnen	.994	.006			87	.011	.011	1.01
Smögen	.984	.016			64	.031	.031	1.03
TOTAL	.991	.007	.001		351	.017	.017	1.02
LDH-1*	*-100*	*-225*	*138*	*75*	N	H_{obs}	H_{exp}	n_e
Varangerfjorden	.985	.010	.005		100	.030	.030	1.03
Malangen	.990	.005		.005	100	.020	.020	1.02
Buagrunnen	.994	.006			87	.011	.011	1.01
Smögen	1				64			1.00
TOTAL	.991	.006	.001	.001	351	.017	.017	1.02
LDH-2*	*100*	*192*	*262*		N	H_{obs}	H_{exp}	n_e
Varangerfjorden	.905	.060	.035		100	.190	.176	1.21
Malangen	.895	.085	.020		100	.180	.191	1.24
Buagrunnen	.868	.103	.029		87	.218	.235	1.31
Smögen	.867	.063	.070		64	.234	.239	1.31
TOTAL	.886	.078	.036		351	.202	.208	1.26
LDH-3*	*100*	*72*	*113*		N	H_{obs}	H_{exp}	n_e
Varangerfjorden	.995	.005			100	.010	.010	1.01
Malangen	.995		.005		100	.010	.010	1.01
Buagrunnen	.994	.006			87	.011	.011	1.01
Smögen	1				64			1.00
TOTAL	.996	.003	.001		351	.009	.009	1.01
PGI-1*	*100*	*76*	*84*	*116*	N	H_{obs}	H_{exp}	n_e
Varangerfjorden	.990			.010	100	.020	.020	1.02
Malangen	.995	.005			100	.010	.010	1.01
Buagrunnen	.989	.006	.006		87	.023	.023	1.02
Smögen	1				64			1.00
TOTAL	.993	.003	.001	.003	351	.014	.014	1.01
PGI-2*	*100*	*75*	*38*	*60*	N	H_{obs}	H_{exp}	n_e
Varangerfjorden	.625	.370	.005		100	.530	.472	1.90
Malangen	.575	.420		.005	100	.470	.493	1.97
Buagrunnen	.592	.408			87	.425	.483	1.94
Smögen	.609	.391			64	.500	.476	1.91
TOTAL	.600	.397	.001	.001	351	.481	.482	1.93
PGM*	*-100*	*-150*	*15*		N	H_{obs}	H_{exp}	n_e
Varangerfjorden	.995	.005			100	.010	.010	1.01
Malangen	.980	.015	.005		100	.040	.039	1.04
Buagrunnen	.983	.011	.006		87	.034	.034	1.04
Smögen	.984	.016			64	.031	.031	1.03
TOTAL	.986	.011	.003		351	.028	.028	1.03
SOD-2*	*100*	*80*	*60*	*135*	N	H_{obs}	H_{exp}	n_e
Varangerfjorden	.990	.005	.005		100	.020	.020	1.02
Malangen	.995		.005		100	.010	.010	1.01
Buagrunnen	1				87			1.00
Smögen	.984	.008		.008	64	.031	.031	1.03
TOTAL	.993	.003	.003	.001	351	.014	.014	1.01

DISCUSSION

The quantitative estimates of genetic variability in this study were based on 10 loci considered potentially usable in population genetic surveys. Depending on the criterion used for polymorphism (95% or 99%) the frequence of polymorphic loci in the present material was $P_{0.95} = 0.20$ and $P_{0.99} = 0.40$. The expected average heterozygosity per locus based on allele frequencies was estimated at $H = 0.084$ (Table 4). These results seems to be comparable with those for cod (0.082; Mork *et al.* 1982), which were based on 30 loci in cod from a fjord in mid-Norway (Table 4). A later study of the same species (Mork *et al.* 1985), covering most of the species range and based on 19 loci, estimated the heterozygosity at $H = 0.071$.

In Table 4, the here obtained measures of genetic variability in haddock are listed together with corresponding values obtained for other gadoid fishes. The haddock characteristies for P and H seem to be intermediate among gadoids.

Table 4. Genetic variability measures in five different gadoids; haddock, cod, whiting, saithe and blue whiting

	Polymorphism		Heterozygosity		
	$P_{0.99}$	$P_{0.95}$	H	Source:	Comments:
Haddock:	0.20	0.40	0.084	This paper	10 loci
Cod:	0.20	0.30	0.082	Mork *et al.* 1982	30 loci
Whiting:	0.30	0.50	0.164	Forthun and Mork 1997	10 loci
Saithe:	0.20	0.50	0.036	Forthun and Mork 1997	10 loci
Blue whiting:	0.12	-	0.043	Mork and Giæver 1995	25 loci

The majority of these loci have not been previously described in the open scientific literature. They may provide a basis for analysis of the genetic population structure of this economically very important species.

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