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GENETIC VARIABILITY AT ISOZYME LOCI IN HADDOCK (*Melanogrammus aeglefinus*) FROM NORWEGIAN FJORD AND COASTAL WATERS

by

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ABSTRACT

A population genetic study of stock structure was conducted on 2964 haddock from 32 locations in Norwegian fjord and coastal waters from the Varanger Fjord to the Møre coast as well as a sample from the Swedish west coast. The samples were collected during research vessel cruises in 1992, 93 and 94. Allele frequencies on eight polymorphic isozyme loci (*IDHP-1**, *LDH-1**, *LDH-2**, *LDH-3**, *MDH-1**, *PGM-1**, *PGI-2**, *SOD-2**) were calculated from starch gel electrophoresis data.

Generally the results indicated little genetic differentiation in haddock from these waters, only 0.6% of the total gene diversity in the material was due to differences between locations. However a heterogeneity in the allele frequencies of *LDH-2** was discovered (P=0.039). The frequency of the *LDH-2*100* allele showed a highly significant decrease southwards along the coast (P<0.001). The steepest decline seemed to be at the Møre and Trøndelag coast (i.e. approx. 63°N). Since no sign was found indicating environmental selection at *LDH-2**, these results may indicate restrictions to the gene flow between southern and northern haddock in the East Atlantic.

INTRODUCTION

Haddock (*Melanogrammus aeglefinus*) is a bottomdwelling marine teleost with a discontinuous east-west distribution in the North Atlantic. In the Northwest Atlantic it is found from Cape Hatteras to Newfoundland and in the Northeast Atlantic from Portugal to Iceland, Spitsbergen and Novaja Zemlja. The most important East Atlantic spawning grounds are off the coast of mid- and northern Norway, the North Sea, at the Faroes, and south-west of Iceland. 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. The haddock is commercially very important in the North Atlantic, and annual catches constitute several hundred thousand tons.

Early studies of haddock population structure, based on morphometrics and meristics (Martin 1953, Templeman 1953, and Grosslein 1962 in the West Atlantic; Raitt 1936, Fraser 1958, and Lee 1974 in the East Atlantic), concluded with the existence of distinct stocks at different banks. Due to high mortality of tagged specimens and hence low recapture rates, tagging experiments have not been very successful in haddock. Results from such studies are thus not available as a control of results from morphometric studies (pers. comm: O. R. Godø, Institute of Marine Research, Bergen, Norway). Also, population genetic studies on haddock have been few. Zwanenburg *et al.* (1992) investigated haddock stock structure on West Atlantic banks by means of mtDNA polymorphisms. No significant heterogeneity between banks was revealed. In the East Atlantic Child (1988) found no evidence of genetic structuring of North Sea haddock based on isozyme analysis (*LDH-2** and *PGI-2**). Using transferrin allele frequencies, however, Jamieson & Birley (1989) concluded with different stocks east and west of the Greenwich median. They also reported distinct stocks at the Faroes and at the Rockall Bank.

In North Norwegian and Barents Sea waters, where most of the material in this study was collected, an early study of morphometrics conducted by Awerinzev (1927) concluded with the existence of at least two distinct stocks; one western and one eastern. Also Raitt (1936) claimed that the Barents Sea is likely to support a separate haddock stock. Sætersdal (1952), on the other hand, found no evidence of population subdivision in a study of vertebrae numbers, brood stock strength and growth patterns along the Norwegian coast and in the Barents Sea.

The objective of this study was to investigate the genetic population structure of haddock by means of tissue enzyme polymorphisms. The study particularly focused on fjord and coastal waters of Northern Norway.

MATERIALS AND METHODS

Sample collection

Most samples were collected by bottom trawl on cruises in 1992, 1993 and 1994 in the fjords and coastal waters of Northern Norway. These cruises were parts of a coastal resource study program run by The Norwegian Institute of Fisheries and Aquaculture, Ltd., Tromsø, Norway, which also provided technical assistance during sampling and performed age determinations by otholith readings. Two samples were obtained from commercial catches (Table 1).

Tissue samples (muscle and liver) were cut immediately after catch and frozen in individually numbered plastic bags. For each specimen length, weight, sex and gonad maturity stage were recorded and otholiths were collected for age determination. The samples were kept at -82°C until analysis.

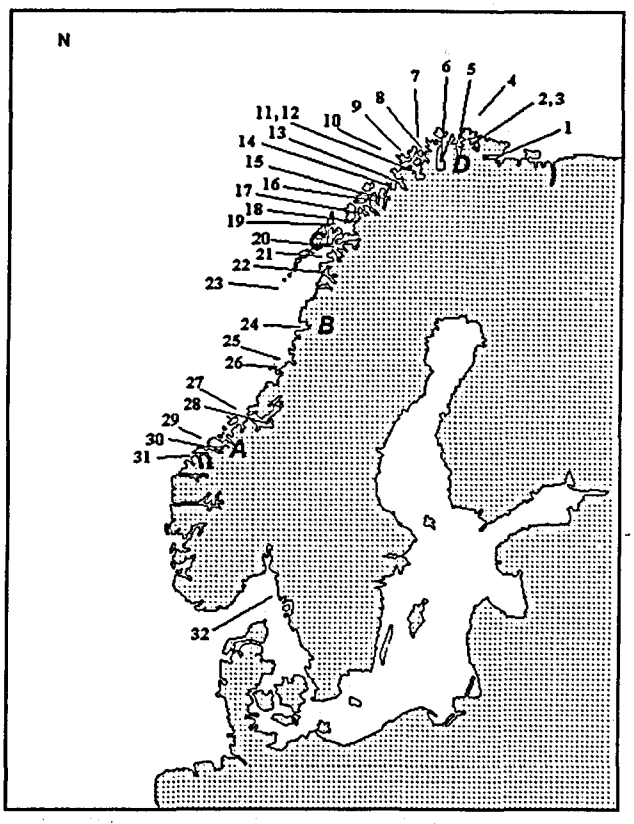

FIGURE 1: Haddock sampling sites in 1992, 1993 and 1994. A: Møre, B:Nordland, C: Lofoten, D: Finnmark.

Electrophoresis

Tissue extracts for enzyme electrophoresis were prepared by mincing equal amounts of muscle and liver tissue in an equal amount of destilled water. The homogenate was then centrifuged at 10.000 g for 10 min. Care was taken to keep sample temperature below 4°C during all stages of preparation. Horizontal starch gel electrophoresis was performed as described by Allendorf *et al.* (1977). The two buffer systems applied are described by Ridgway *et al.* (1970) and Clayton & Tretiak (1972). Enzyme staining was performed according to Allendorf *et al.* (1977) with one exception: To improve banding intensity the pH was increased to 9.0 when staining for dehydrogenases (Mork 1990). Only polymorphic loci were included in this study. The enzymes stained for were the following (scored loci in parenthesis):

Isocitrate dehydrogenase, E.C. 1.1.1.42 (*IDHP-1**)
L-lactate dehydrogenase, E.C. 1.1.1.27 (*LDH-1**, *LDH-2**, *LDH-3**)
Malate dehydrogenase, E.C. 1.1.1.37 (*MDH-1**)
Phosphoglucoisomerase, E.C. 5.3.1.9 (*PGI-2**)
Phosphoglucomutase, E.C. 5.4.2.2 (*PGM-1**)
Superoxid dismutase, E.C. 1.15.1.1 (*SOD-2**)

The genetic interpretation of banding patterns followed Allendorf *et al.* (1977). Loci, genotypes and alleles are abbreviated according to Shaklee *et al.* (1990).

Data analysis

The biological and electrophoretic data were analysed using «Statgraphics Plus 1.0» (STSC, Inc.), «BIOSYS 1.7» (Swofford and Selander 1981), «Chirxc» (Zaykin & Pudovkin 1993) and various in-house software for genetic data analysis (Mork 1992). Where no χ^2 value is attached, P-values in the text refer to output from the exact tests by Zaykin & Pudovkin (1993).

TABLE I. Haddock sampling sites: Abbreviation, position, vessels, date of catch and number of fish in each sample. Trawl type: "Harstadtrål", mesh width: 35mm with a fine mesh innernet in the trawl bag. Exceptions for samples 29 and 32 which were caught with bottom trawl, 100 and 70-120 mm mesh width respectively.

Sample	Position	ICES Fisheries Statistical Area	Vessel/date	Trawl station number	Number of fish
1	70°07'N, 28°47'E (Varangerfjorden)	I	R/V «Johan Ruud» 92.08.26	1318	84
2	70°27'N, 28°28'E (Tanafjorden)	I	R/V «Johan Ruud» 92.08.31	1369	73
3	70°33'N, 28°17'E (Tanafjorden)	I	R/V «Johan Ruud» 92.09.01	1375	100
4	71°12'N, 28°30'E (Outer Tana)	I	R/V «Johan Ruud» 92.09.01	1384	100
5	70°32'N, 26°58'E	I	R/V «Johan Ruud» 92.09.03	1403	32
	70°29'N, 26°59'E		R/V «Johan Ruud» 92.09.03	1405	17
	70°23'N, 26°32'E (Laksefjorden)		R/V «Johan Ruud» 92.09.03	1407	51
6	70°11'N, 25°04'E (Porsangen)	IIa	R/V «Johan Ruud» 92.09.06	1420	100
7	71°06'N, 23°20'E (Rolvsøy west)	IIa	R/V «Johan Ruud» 92.09.11	1503	90
8	70°57'N, 24°36'E (Revsbotn)	IIa	R/V «Johan Ruud» 92.09.12	1518	100
9	70°43'N, 22°15'E (Åfjord)	IIa	R/V «Johan Ruud» 92.09.15	1567	67
10	70°51'N, 21°13'E (Nygrunn)	IIa	R/V «Johan Ruud» 92.09.15	1570	86
11	69°57'N, 23°09'E (Altafjorden)	IIa	R/V «Johan Ruud» 92.09.13	1534	100
12	70°07'N, 23°19'E (Altafjorden)	IIa	R/V «Johan Ruud» 92.09.13	1538	96
13	69°51'N, 21°58'E (Kvænangen)	IIa	R/V «Johan Ruud» 92.09.22	1672	100
14	69°52'N, 20°58'E	IIa	R/V «Johan Ruud» 92.09.30	1822	81
	69°48'N, 20°57'E (Nordreisa)		R/V «Johan Ruud» 92.09.30	1826	19
15	69°17'N, 19°56'E (Lyngenfjorden)	IIa	R/V «Johan Ruud» 92.09.30	1810	100
16	69°34'N, 19°43'E (Sørfjord)	IIa	R/V «Johan Ruud» 92.09.27	1760	100
17	69°17'N, 18°39'E	IIa	R/V «Johan Ruud» 92.10.03	1875	35
	69°17'N, 18°38'E (Malangen)		R/V «Johan Ruud» 92.10.03	1876	65
18	69°27'N, 18°05'E (Solbergfjorden)	IIa	R/V «Michael Sars» 93.08.01	284	96
19	68°25'N, 15°57'E (Vågsfjorden)	IIa	R/V «Michael Sars» 93.08.06	324	96
20	68°12'N, 15°53'E (Inner Vestfjorden)	IIa	R/V «Michael Sars» 93.08.16	414	96
21	67°56'N, 15°34'E (Sagfjorden)	IIa	R/V «Michael Sars» 93.09.08	587	76
22	67°18'N, 15°07'E	IIa	R/V «Michael Sars» 93.09.05	568	28
	67°19'N, 15°07'E (Skjerstadfjorden)		R/V «Michael Sars» 93.09.05	570	47
23	67°33'N, 12°23'E (outer Vestfjorden)	IIa	R/V «Michael Sars» 93.08.14	389	96
24	66°16'N, 13°35'E (Ranafjorden)	IIa	R/V «Michael Sars» 93.09.02	543	96
25	65°13'N, 12°44'E (Vegafjorden)	IIa	R/V «Michael Sars» 93.09.01	528	96
26	65°18'N,11°58'E (Torghatten)	IIa	R/V «Michael Sars» 94.09.22	502	100
27	63°54'N, 08°54'E (Frohavet)	IIa	R/V «Michael Sars» 94.10.03	581	96
28	63°32'N, 10°32'E	IIa	R/V «Michael Sars» 94.10.09	627	16
	63°32'N, 10°47'E (Åsenfjord)		R/V «Michael Sars» 94.10.09	628	86
29	63°07'N, 06°47'E (Buagrunnen)	IIa	"Vevang trål" 94.03.09	-	87
30	62°34'N, 07°44'E (Romsdalsfjorden)	IIa	R/V «Michael Sars» 94.10.12	642	96
31	62°26'N, 06°05'E (Vartdalsfjorden)	IIa	R/V «Michael Sars» 94.10.17	683	98
32	58°25'N, 11°10'E (Smögen)	IIIa	"Wardö" 94.04.13	-	64

RESULTS

A detailed survey of the biological data of the material is given by Eliassen *et al.* (1994 a,b).

The following variant alleles were found on the eight loci:

IDHP-1:*	**25, *55, *129*
LDH-1:*	**-200, *75, *138*
LDH-2:*	**192, *262*
LDH-3:*	**72, *113*
MDH-1:*	**56, *144*
PGI-2:*	**43, *60, *75, *104*
PGM-1:*	**-140, *-150, *15*
SOD-2:*	**60, *80, *135*

The samples were in Hardy-Weinberg equilibrium at all loci with few exceptions: Sample 9 (Åfjord) showed an excess of heterozygotes at *PGI-2** (P=0.045). Sample 17 (Malangen) deviated from Hardy-Weinberg equilibrium at *LDH-2** because of the occurrence of the two rare genotypes *LDH-2*192/262* and *LDH-2*262/262* (P=0.004). When a chi-square test with pooling of the rare alleles was performed no significant deviation was found.

No significant linkage disequilibrium was found. The allele distribution varied randomly between age groups. No correlation between sex and allele distribution was found. Length at age varied independently of genotype at all loci scored.

The samples did not differ much in allele frequencies. Only 0.6% (as indicated by Fst analysis) of the total genetic variation was due to differences between samples. A dendrogram (UPGMA) based genetic distances (D of Nei (1972)) showed no apparent correlation between geographic proximity and the clustering in the dendrogram.

The northernmost samples (Finnmark and Troms counties) were genetically very homogeneous except for sample 11 (Altafjord, Table 1), which stood out somewhat from the rest, even from a sample taken on a very nearby location in the same fjord on the same day. Despite the impression of an overall genetic homogeneity among all samples, there were certain geographic trends. The sample from the Swedish coast (no. 32) stands out from the rest by having extreme frequencies of five out of thirty alleles, which is far more than would be expected assuming a random distribution (P<0.001). The largest heterogeneity in allele frequencies between samples was detected at *LDH-2** (χ^2=82.98, df=62, P=0.039). The samples that contributed the most to the overall χ^2 value were sample no. 30 (the Romsdalsfjord) which had a high *LDH-2*192* frequency, and sample 32 (the Swedish west coast) which had a high *LDH-2*262* frequency. The *LDH-2*100* frequency seemed to decrease towards the south. This tendency of higher *LDH-2*100* frequencies in the northern samples and lower in the southern was shown to be highly significant by a linear regression, P<0.001 (Fig. 3). One might expect that the significance of the regression is heavily influenced by the low allele frequencies in samples 28 - 32. There is, however, still a significant slope when those samples are excluded from the test (P=0.042).

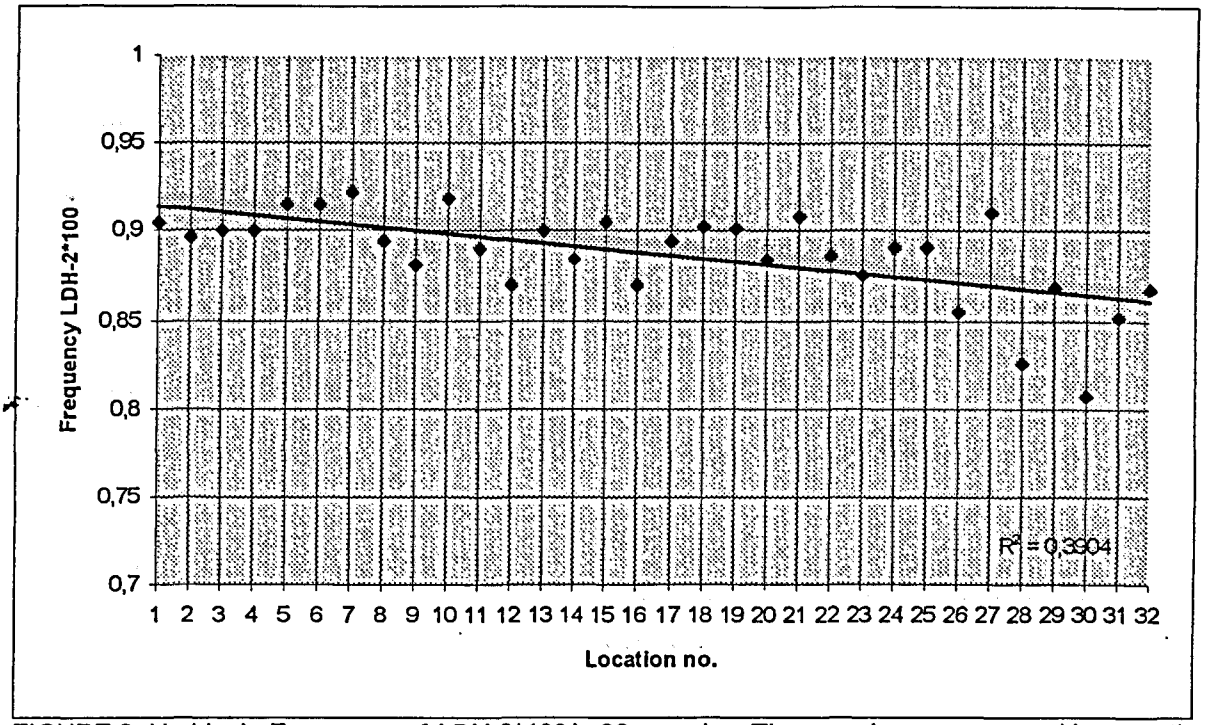

FIGURE 3: Haddock. Frequency of *LDH-2*100* in 32 samples. The samples are arranged in a north-east to south-west order (cf. Fig. 1). The corresponding decrease in allele frequency is significant ($P<0.001$). R^2 is the determination coeffesient.

DISCUSSION

The general result of this study of eight polymorphic isozyme loci was that the haddock of Norwegian fjords and coastal waters is little genetically differentiated. Only 0.6% of the total genetic variation was due to between-sample variation. Similar investigations of other gadoides has shown similar results: In cod (Mork *et al.* 1985) and blue whiting (Mork & Giæver 1993) throughout their distribution ranges 2.1% and 1.7%, respectively, of the total genetic variation was found to be between-location variation. In both species fish from the fringes of the distribution ranges deviated mostly. In this study the outmost parts of the haddock distribution range are represented by the north-eastern samples. Ripe haddock is rarely found on the Finnmark coast during spawning season and maturing haddock is believed to migrate to spawning grounds mainly in the Lofoten area (pers. comm. Jan Sundet, the Norwegian Institute of Fisheries and Aquaculture Inc., Tromsø, Norway). Also, haddock eggs and larvae stay pelagic and adrift with the currents for a long period. In this study the Finnmark haddock appeared genetically similar to that of the Troms and Lofoten area. If the Barents Sea contains one eastern and one western haddock stock, as claimed by Awerinzev (1927), they are either genetically similar or one of the stocks is not represented in our material.

Despite the extensive genetic homogeneity of the material, the Swedish sample (no. 32) stood out, having extreme allele frequencies in more cases than expected by chance. The allele frequencies of *LDH-2** and *PGI-2** in the southern areas are very similar to those found in the North Sea by Child (1988). The Møre coast appeared to make a transition zone with respect to *LDH-2*100* frequencies, while the samples from the Nordland coast showed greater similarity to those taken further north.

When relating genetic data to the individuals' biological data no obvious signs of selection were found. These observations supports a hypothesis that the decrease in LDH-2*100 frequency from north-east to south-west is caused by limitations to the gene flow between areas.

ICES has chosen to practice separate haddock management reccomendations in the areas north and south of 62°N (ICES fishery areas I, IIa and IIb, and areas IVa, b and c separately). This separation is based on the Fisheries Statistical areas only, rather than biological criteria (pers. comm. Knut Sunnanå, Institute of Marine Research, Bergen, Norway). The genetic results in this study may lend some support to this managemental regime. The allele frequencies in the Swedish sample showed greater similarity to that reported by Child (1988) in the North Sea (ICES Fisheries statistical area IVa and IVb) than to those further north. The samples from the Møre coast formally belong to area IIa, but from the results in this study, their *LDH-2*100* frequencies recembled the frequencies of southern haddock. Firm conclusions on this matter must await the results from further sampling and analysis of haddock from the southern and western part of the East Atlantic.

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