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New variants of the haemoglobins of turbot (*Scophthalmus maximus*): possible use in population genetics studies and aquaculture

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Blood samples from turbot, *Scophthalmus maximus*, were analysed using agar gel electrophoresis and isoelectric focusing (IEF) to reveal individual and population variation in haemoglobin genotypes. In total, 394 individuals were sampled and analysed from five localities (Iceland, west Norway, southwest Norway, Kattegat and the Baltic Sea). A new haemoglobin polymorphism of turbot was documented using IEF electrophoresis (pH 5.5–8.5), and, in addition to the three genotypes seen in earlier studies, six subgroups are described. Significantly different genotype distributions were found between the samples from Iceland and west Norway on one hand and southwest Norway, Kattegat and Baltic on the other hand. Also, the frequencies of the *Hb-I* subgroups varied among the sampling sites. For instance, the subgroup *Hb-I(1/1)e* was absent in the samples from southwest Norway and Kattegat, whereas the subgroup *Hb-I(1/1)d* was almost absent in samples from Iceland and west Norway but common in the samples from southwest Norway and Kattegat. The results indicate that the turbot in northern European waters belong to more than one panmictic population.

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INTRODUCTION

Turbot, *Scophthalmus maximus*, is a widely distributed species, ranging from North Africa and the Mediterranean in the south (Morgan 1956) to Icelandic Sea/western Norwegian Sea in the north (Jónsson 1992) including the Baltic Sea (Steffensen & Bagge 1990). This wide distribution indicates that this species as such is adapted to a wide range of environmental conditions. However, it is largely unknown whether the species is plastic, with the ability to acclimatize to different environments, or whether it consists of population units which are genetically adapted to specific environmental conditions.

Studies on the population structure of turbot have been hampered by a lack of suitable genetic markers. Blanquer & al. (1992) reported low allozyme variation in turbot and apparently low genetic diversity. This study was, however, inconclusive with respect to population structure because it was based on a low number of fish from the different sites and samples were only taken from a part of the total distribution area of turbot. However, previous studies (Imsland & al. 1997a and references therein) have shown that the haemoglobin polymorphism in turbot is a more variable character than allozymes (Blanquer & al. 1992; Bouza & al. 1994), and is therefore expected to be more suitable for stock discrimination. Furthermore, previous studies have indicated that physiological factors in turbot are to some extent correlated with haemoglobin genotypes, as the haemoglobin genotype with the highest oxygen affinity (*Hb-I(2/2)*) in turbot (Samuelsen & al. 1999) displays the highest growth (Imsland & al. 1997a, 2000a) and the lowest age at first maturity (Imsland 1999).

In cod, *Gadus morhua*, substantial variation is found in the frequency distributions of the main haemoglobin genotypes (Jamieson & Jónsson 1971; Jamieson 1975; Gjøsæter & al. 1992; Dahle & Jørstad 1993; Nordeide & Pettersen 1998). Fyhn & al. (1994) described subgroups within the main groups of cod haemoglobins based on isoelectric focusing (IEF), and the frequency distributions of subgroups could be used to distinguish between Arcto-Norwegian and Norwegian coastal cods. The existence of such subgroups in other species is not entirely known, but could offer additional markers for use in population studies. As in turbot, haemoglobin genotype-dependent growth rate is indicated (Mork & al. 1984a, b; Nævdal & al. 1992), where the genotype with the highest growth (*Hb-I(2/2)*) also has the highest

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Table 1. Position, time of sampling and sample size of turbot analysed for haemoglobin polymorphism.

Location	Position	Time period	Sample size
South coast Iceland	63°30–35′N 21–22°W	July 1998	59
West coast Norway	59°50′–60°N 4–5°E	October 1994	68
Southwest coast Norway	57°–58°30′N 4–8°E	August 1996	93
Kattegat	56°50′–57°N 10–11°E	May 1996	84
Baltic Sea	54°50′–56°N 17–20°E	September 1997	90

oxygen affinity (Brix & al. 1998) and the highest competitive performance (Salvanes & Hart 2000).

In the present paper, observations on genotype frequency distributions in turbot from Icelandic waters, the Norwegian coast, from the Kattegat, and the Baltic Sea are presented. In addition, subgroup variations in the haemoglobins are described and preliminary observations on subgroup frequencies are given. The results are discussed in relation to application in studies of natural turbot populations, and the possible use of haemoglobin variations as correlated traits in turbot breeding.

MATERIAL AND METHODS

Blood samples from turbot were collected along the Norwegian coast (two sampling sites: west Norway n = 68; southwest Norway n = 93), in Kattegat (n = 84), from the Baltic (n = 90) and from the southwest coast of Iceland (n = 59) (Table 1).

All samples were analysed using agar gel electrophoresis (AGE) and the method described by Sick (1961) was applied with modifications (Jørstad 1984). Smithies buffer, pH 8.6 was used as an electrode buffer, and diluted 1:1 with distilled water for the gel buffer. A 2% agar concentration was used in the gels. The samples were run at 200 V (20 mA) for 70 min. The gels were stained in Brilliant Blue G Quick stain in perchloric acid and then destained by diffusion (14% acetic acid, 7% methanol) overnight.

IEF was carried out on all samples, apart from the Baltic sample, in accordance with the instruction manuals using pre-cast slab gels (Ampholine PAG plates, Pharmacia, pH 5.5–8.5) with modifications described by Fyhn & al. (1994). Initially, different treatment methods for the blood to be analysed with IEF were investigated. Freezing the samples (−80 °C), after adding 0.5 ml of glycerol to each individual sample, gave the best resolution of the haemoglobin components. After thawing the samples were centrifuged and the glycerol removed. Frozen and thawed samples displayed identical haemoglobin patterns as fresh samples. The gels were pre-focused for 30 min. After pre-focusing, filter papers containing haemolysate were applied to the anodic side of the gel. The filter papers were removed after 10 min and the gel was run for 90 min. The same staining procedure was used as for AGE and the haemoglobin components were identified manually using a transmitted light. Both AGE and IEF study the proteins coded by DNA. In the former method a constant pH is applied, but in the latter a pH gradient is applied so that each protein component can be studied at its isoelectric point (i.e. where the protein is neutral). These two methods are used in combination when studying both main and subcomponents of proteins, as in the present study.

Population pairwise F_{ST} statistics were calculated for all sample units using the ARLEQUIN 1.1 computer package, and the significance was tested with permutation (1000 times; Schneider & al. 1997). The pairwise F_{ST} can be used as short-term genetic distances between populations (Schneider & al. 1997) where the null distribution under the hypothesis of no difference between the populations is obtained by permuting haplotypes between populations. The p value of the test is the proportion of permutations leading to a F_{ST} value larger than or equal to the observed one. It can be shown that the F_{ST} statistic is χ^2 distributed with $r - 1$ degrees of freedom where r is the number of populations studied (Weir 1996). The genetic structure of the sampling sites was analysed using an analysis of molecular variance (AMOVA) framework (Weir & Cockerham 1984). The test is analogous to a regular analysis of variance (ANOVA), but is based on an analysis of variance of discrete (binomial, multinomial) gene frequencies. In this test, sampling sites were divided into two groups, based on the results from the F_{ST} test: group 1 (Iceland and west Norway), and group 2 (southwest Norway, Kattegat, Baltic Sea). The significance of the variance components associated with the different possible levels of genetic structure (among groups, among samples within groups, within individuals) was tested using non-parametric permutation procedures (Excoffier & al. 1992). Allele frequencies were bootstrapped 1000 times and Nei's (1972) genetic distances based on the allele frequencies were

Table 2. Observed and expected (in parentheses) phenotypic distributions and allelic frequencies of the *Hb-I* locus analysed with agar gel electrophoresis in different turbot populations.

		Sampling sites				
	Genotype	Iceland	West Norway	Southwest Norway	Kattegat	Baltic Sea
Hb-I	*Hb-I(1/1)*	24 (23.0)	27 (26.5)	62 (62.5)	60 (59.3)	67 (67.4)
	Hb-I(1/2)	26 (27.7)	31 (31.9)	29 (27.5)	21 (22.6)	21 (21.0)
	Hb-I(2/2)	9 (8.3)	10 (9.6)	2 (3.0)	3 (2.1)	2 (1.6)
N		59	68	93	84	90
G-statistic		0.19*	0.05*	1.21*	0.46*	0.01*
Allele frequency	*Hb-I(1)*	0.625	0.625	0.820	0.840	0.865
	Hb-I(2)	0.375	0.375	0.180	0.160	0.135

N, sample size.
G-statistic for deviations from expected Hardy–Weinberg phenotypic distributions.
*$p > 0.50$.

calculated using the SEQBOOT and GENDIST programs in the PHYLIP package (Felsenstein 1993). To study the similarities of the phenotypic characters under study (main and subgroups of turbot haemoglobin) a UPGMA dendrogram of the bootstrapped Nei's genetic distance matrix was constructed in the NEIGHBOR program in PHYLIP. Bonferroni corrections (Johnson & Field 1993) of the significance level ($\alpha = 0.05$) were applied when testing for significant departures from Hardy–Weinberg expectations (global test and for each sample unit) and for the significance of F_{ST} values.

RESULTS

AGE

When analysing the haemoglobins by AGE, three different electrophoretic patterns were found. These were *Hb-I(1/1)*, *Hb-I(1/2)* and *Hb-I(2/2)* (most anodic), and, in accordance with earlier descriptions (Manwell & Baker 1967; Imsland & al. 1997a), interpreted as the two homozygotes and the heterozygote in a two allele system. The homozygotes were represented by patterns which consisted of one strong and several weak haemoglobin bands, while the heterozygote was represented by three strong (one hybrid band) and several weak bands. The G-test did not reveal any significant difference between observed and expected Hardy–Weinberg distributions ($p > 0.50$) in any sample.

The *Hb-I** allele frequencies were significantly different ($\chi^2_2 = 14.88$, $p < 0.01$, Table 2) among the samples, indicating that different populations were sampled. Observed allele frequencies from Iceland and west Norway were identical but different from corresponding frequencies from the other locations (Table 2). Accordingly the samples were grouped into two groups: group 1 (Iceland and west Norway) and group 2 (southwest Norway, Kattegat and the Baltic Sea) and the molecular variance analysed with an AMOVA model (Table 3). A significant difference ($p < 0.05$) was observed between these two group. Also,

Table 3. Analysis of molecular variance (AMOVA) and hierarchical F-statistics (fixation indices) for the turbot analysed with agar gel electrophoresis in the present study. The genetic structure was analysed at the individual level so that the within individual variance was a test for global departure from Hardy–Weinberg equilibrium.

Source of variation	d.f.	Sum of squares	Variance component	Variance explained by AMOVA model	F_{XY}
Among groups	1	4.24	0.07*	12.9	0.120
Among samples within groups	3	1.26	−0.001	−0.9	−0.009
Within sampling sites	390	64.57	0.165**	88.0	0.128
Total	394	68.91	0.188	100	

Group 1 = Iceland, west Norway; group 2 = southwest Norway, Kattegat, Baltic Sea.
*$p < 0.05$, **$p < 0.01$.
d.f. = degrees of freedom. For calculation of d.f. see Schneider & al. (1997).
Note that the F-statistic estimators in the AMOVA model are random variables and can take either positive or negative values (Long 1986), negative values indicating an excess of heterozygotes (Long 1986; Excoffier & al. 1992). Such negative estimates should be interpreted as zero (Long 1986) in the AMOVA model, i.e. the variance explained by among sampling sites within groups is zero in the present study.

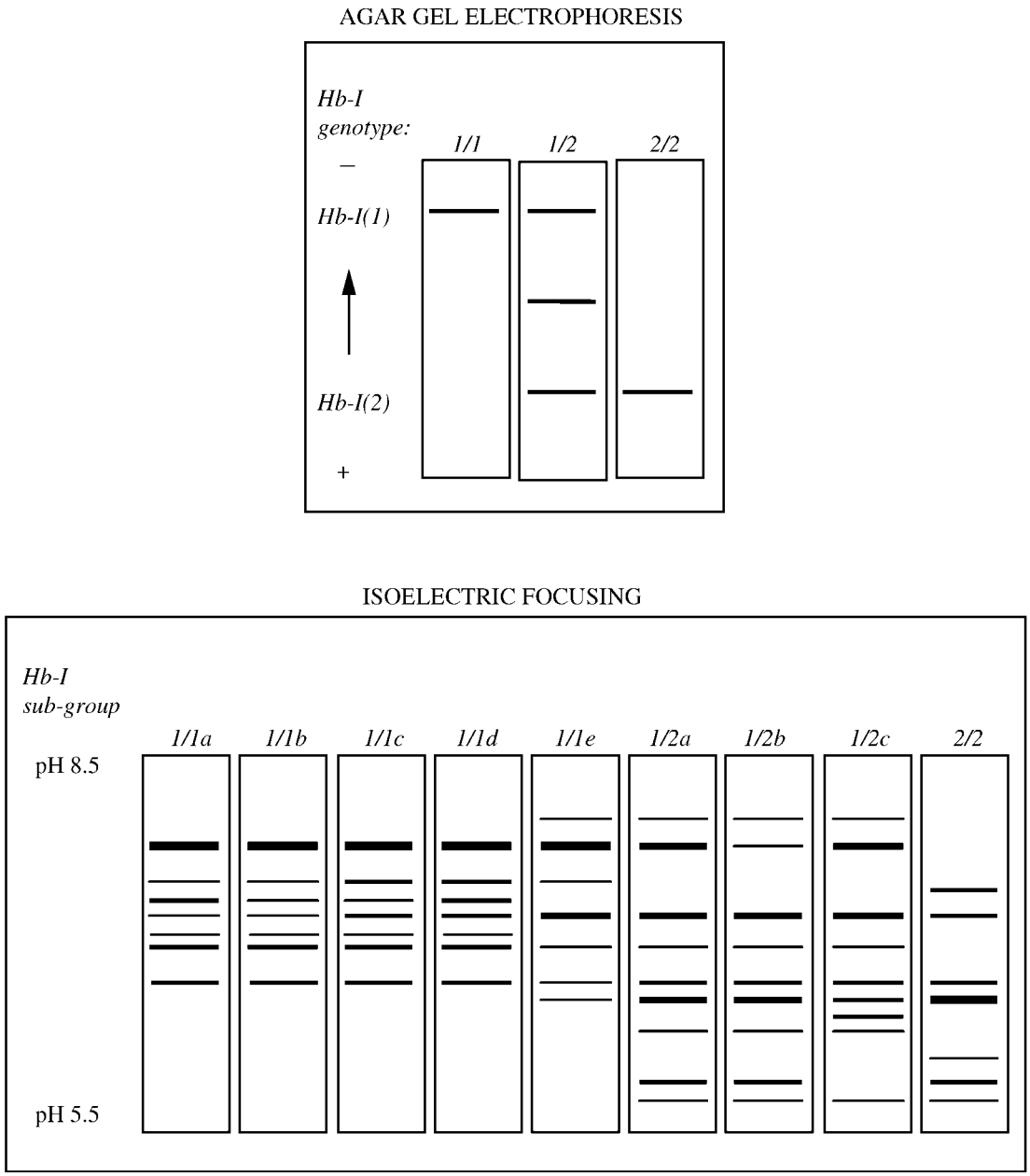

Fig. 1. Schematic representation of the patterns of components from agar gel electrophoresis and isoelectric focusing of haemoglobins of turbot. For agar gel electrophoresis the origin is at the anode (+) and the protein migrates towards the cathode (−), as indicated by the arrow. For isoelectric focusing the origin is approximately 2 cm from the anode side (i.e. at pH 5.5) and the proteins migrate towards their isoelectric point on the pH gradient.

the hierarchical F-statistics (Table 3) indicated a population differentiation and 12.9% of the total allelic variance was due to differences between the two groups (Table 3). Individual genetic variation in the AMOVA was significant, indicating that the total material (all samples compiled) was not in Hardy–Weinberg equilibrium ($p < 0.05$). Because fewer heterozygotes than expected were found when the total material was

Table 4. Frequencies of the different haemoglobin subgroups in samples of turbot from four different locations and analysed by isoelectric focusing. Note that some individual samples and the total sample from the Baltic Sea could not be identified using the isoelectric focusing method.

	Sampling sites			
Phenotype	Iceland	West Norway	Southwest Norway	Kattegat
Hb-I(1/1)a	0.18	0.14	0.30	0.29
Hb-I(1/1)b	0.11	0.14	0.14	0.15
Hb-I(1/1)c	0.07	0.08	0.07	0.15
Hb-I(1/1)d	0.02	0.02	0.14	0.13
Hb-I(1/1)e	0.09	0.08	0.00	0.00
Hb-I(1/2)a	0.21	0.30	0.22	0.13
Hb-I(1/2)b	0.11	0.06	0.07	0.08
Hb-I(1/2)c	0.07	0.04	0.03	0.05
Hb-I(2/2)	0.14	0.14	0.03	0.02
	56	50	58	84

pooled, this indicates that the samples were drawn from more than one population (Wahlunds effect).

IEF

On IEF gels, haemoglobins of the genotypes *Hb-I(1/1)* and *Hb-I(2/2)* displayed seven bands (Fig. 1), whereas the *Hb-I(1/2)* genotype displayed nine bands (Fig. 1). Furthermore, in *Hb-I(1/1)* and *Hb-I(1/2)* the position and strength of the band components on the IEF gels varied highly. No variation was found in the *Hb-I(2/2)* genotype. Accordingly, the *Hb-I(1/1)*, *Hb-I(1/2)* and *Hb-I(2/2)* genotypes could be subgrouped by the position and the strength of the IEF band patterns into nine subtypes. These subgroups are named: *Hb-I(1/1)a*, *Hb-I(1/1)b*, *Hb-I(1/1)c*, *Hb-I(1/1)d*, *Hb-I(1/1)e*, *Hb-I(1/2)a*, *Hb-I(1/2)b*, *Hb-I(1/2)c*, and *Hb-I(2/2)*. Here the index "a" is used for the most common subgroup, "b" for the second most common, etc. The different subgroups are outlined in Fig. 1. The appearance of sub-bands was consistent in repeated IEF runs of fresh, stored, or frozen erythrocytes in all samples.

Samples from the Baltic Sea could not be analysed by IEF but significant differences were found in the frequencies of the sub-band types between the four other sampling sites (Tables 4, 5). In accordance with allele frequency differences in the sub-band polymorphism at the *Hb-I* locus (Table 4) the population pairwise F_{ST} test revealed significant differences ($p < 0.05$) between the Icelandic sample vs. southwest Norway and Kattegat, and between the west Norway sample vs. the Kattegat sample site (Table 5). The bootstrapped UPGMA dendrogram constructed using Nei's (1972) genetic distances (Fig. 2) illustrated a clear

Table 5. Population pairwise $_{ST}$ estimates among the four sampling sites analysed for sub-band structure by isoelectric focusing. Permuting the individuals between the sampling sites tested the significance of the pairwise F_{ST} values.

	Iceland	West Norway	Southwest Norway	Kattegat
Iceland	-	-	-	-
West Norway	−0.00030	-	-	-
Southwest Norway	0.02434*	0.01256	-	-
Kattegat	0.02632*	0.02400*	−0.00595	-

One thousand permutations were performed for all pairs of sampling sites.
*$p < 0.05$ (Bonferroni correction for simultaneous tests).

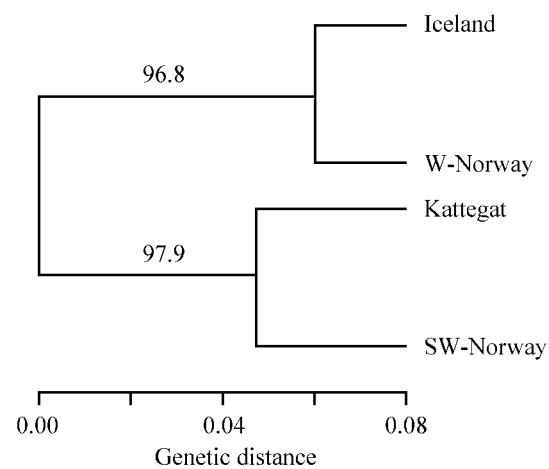

Fig. 2. A UPGMA dendrogram of Nei's (1972) genetic distance matrix among the four turbot populations analysed with isoelectric focusing in the present study (i.e. the Baltic Sea sample is not included). Values on the nodes represent the percentage of bootstrap samples (n = 1000).

differentiation between Iceland and west Norway on the one hand and Kattegat and southwest Norway on the other.

DISCUSSION

The studies of Aneer & Westin (1990), Déniel (1990), Iglesias & Rodríguez-Ojea (1994), and Bergstad & Folkvord (1997) indicate that turbot do not undergo long migrations, but is a stationary species. However, different spatial distribution between juveniles and adults is seen, as only large fish migrate to colder areas (Aneer & Westin 1990; Iglesias & Rodríguez-Ojea 1994), which may be partly explained by decreasing temperature sensitivity and a downshift in temperature optimum with size (Imsland & al. 1996), whereas this might also be a strategy to reduce predation risk by hiding from enemies. The lack of long migration, together with the fact that this species is found in different environments (among others different salinities), makes it reasonable to believe that turbot in European waters belong to more than one population.

Based on differences in genotype and subgroup frequencies (Tables 2–5) we suggest that turbot in north European waters consist of more than one population. Such genetic stock differentiation has previously been reported for several other flatfish species, e.g. witch flounder, *Glyptocephalus cynoglossus* (Fairbairn 1981), Pacific halibut, *Hippoglossus stenolepis* (Grant & al. 1984), Pacific black halibut, *Reinhardtius matsuurae* (Diakov 1998), Dover sole, *Solea solea* (Exadactylos & al. 1998), and Atlantic halibut, *Hippoglossus hippoglossus* (Foss & al. 1998). However, very little is known about the population genetics of turbot, although Blanquer & al. (1992) reported low allozyme variation in turbot and apparently low genetic diversity. Recent studies using polymorphic DNA microsatellites have indicated genetic differentiation between turbot populations (M.Ö. Stefánsson, Marine Research Institute, Reykjavík, Iceland, pers. comm.). Here, six microsatellite loci were studied and based on differences in allele frequencies heterogeneity was demonstrated in all intersample comparisons (samples from Iceland, Norway, Ireland, Baltic Sea, Kattegat) apart from the Baltic Sea and Kattegat. This is line with our findings on the haemoglobin locus. DNA microsatellite studies have also indicated genetic differentiation between wild and farmed turbot populations (Coughlan & al. 1998), and it was presumed to be caused by genetic drift in the hatcheries and that this drift could cause a considerable loss of genetic heterogeneity over a period of only a few generations. The fact that our study indicates that there is no significant difference in allele frequencies of turbot from Iceland and western Norway is odd, given the long geographical distance between these samples. This could be a sample artefact or it may be due to some mixing during the planktonic stage of egg and larvae. However, this difference is only based on one locus and must be treated with caution. Overall it is reasonable to expect that turbot belong to multiple populations, although further research is clearly warranted.

Recent studies have indicated that the polymorphism of the main haemoglobin components in turbot is correlated with physiological performance as one of the genotypes [*Hb-I(2/2)*] displays higher oxygen affinity (Samuelsen & al. 1999), better growth (Imsland & al. 1997a, 2000a), and lower age at first maturity (Imsland 1999). The present data have indicated a subdivision of turbot in northern European waters, but the differences in physiological performance between genotypes seen in turbot (Imsland & al. 1997a, 2000a; Samuelsen & al. 1999), as well as in earlier studies on Atlantic cod (Karpov & Novikov 1980; Nævdal & al. 1992), may indicate differential selection pressure on the controlling genes. However, this selection pressure may be balanced by other factors. It has been argued (Policansky 1983) that fish with access to abundant food and stable conditions for development mature at their earliest opportunity. Furthermore, it is generally accepted that some genetically determined developmental threshold values (e.g. size, energy stores) must be exceeded to initiate and sustain the maturation process (Thorpe 1989; Rowe & al. 1991). Imsland (1999) investigated age at first maturation among the haemoglobin genotypes of turbot. For both sexes fewer fish of the *Hb-I(1/1)* and *Hb-I(1/2)* genotypes matured at 2 years of age compared with the *Hb-I(2/2)* genotype. Genotypic growth properties (Imsland & al. 1997a, 2000a), oxygen affinity (Imsland & al. 1997a; Samuelsen & al. 1999) and genotypic age at first maturation (Imsland 1999), may imply a balanced polymorphism of the haemoglobin in turbot. As the *Hb-I(2/2)* genotype mature at an earlier age, this would lead to a lower ultimate size, as turbot grow slowly after reaching maturity (Imsland & al. 1997b). Hence, a lower age at first maturity of the *Hb-I(2/2)* genotype may be balanced by an increased risk of predation as maturing fish need to forage more than immature fish to fulfil higher energy demands.

Salvanes & Hart (2000) compared the competitive performance of cod of the three *Hb-I* genotypes. Randomly chosen 1-year-old cod were tested for individual responses to prey offered sequentially and found that the most successful fish were usually among the first to feed and tended to possess haemoglobin

genotype *Hb-I(2/2)*. Their findings indicate that there might exist a link between genotypic growth and feeding behaviour. However, direct observation of natural selection is very difficult (Nævdal 1994). Albeit the clear, but not consistent, differences in physiological and behavioural properties of the cod haemoglobin genotypes (Karpov & Novikov 1980; Nævdal & al. 1992; Salvanes & Hart 2000), no clear indication of directional selection on haemoglobin genotype distribution has been seen when comparing early analyses of cod haemoglobins in Norwegian waters (Frydenberg & al. 1965; Møller 1968) with corresponding analyses in recent years (Jørstad & Nævdal 1989; Gjøsæter & al. 1992; Dahle & Jørstad 1993; Fyhn & al. 1994). This does not disprove the action of selection forces, but it does show that the stability of the gene frequencies is high enough to use these frequencies as genetic population markers in cod (Jørstad & Nævdal 1989). Wells (1990) pointed out that one could not assume automatically that variation in haemoglobin is the result of natural selection. Selection acting on one trait may cause changes in other traits if the traits are genetically linked. Furthermore, Ferguson (1995) argued that selection coefficients of the order of 1% or 5% are important in terms of evolution over hundreds of generations but, by statistical definition alone, will not be detectable in the time scale in which most studies are undertaken and will not seriously bias the practical use of markers.

Although the genetic background of the sub-bands is still unknown, they probably represent real variation in turbot populations. Similar sub-bands have been documented in the haemoglobins of Atlantic cod (Fyhn 1991; Fyhn & al. 1994), and suggested as a marker for Norwegian coastal cod vs. Arctic cod populations as sub-bands were not found in cod from the Barents Sea or the Faeroe Bank. In their study, Fyhn & al. (1994) documented a total of five subgroups of which two were characterized by sub-bands. Husebø (1996) reported a high variation in the sub-bands of cod haemoglobins from Kattegat and suggested at least five new subgroups only represented in that area and she described a cline in the frequencies of the subgroups as higher frequencies in west and south Norway compared with Lofoten and north Norway. Brix & al. (1998) investigated the oxygen affinity of four of the haemoglobin genotypes found in cod [*Hb-I(1/1)*, *Hb-I(1/2)*, *Hb-I(2/2)*, and *Hb-I(2/2b)*]. They found the highest oxygen affinity for *Hb-I(2/2b)* suggesting that this type is a more efficient oxygen binder at high temperatures, supporting the results of Fyhn & al. (1994) and Husebø (1996) that this type is more restricted to coastal and warmer water and thus a better marker of the coastal cod population. In the present study (Table 5) the *Hb-I(1/1)e* subgroup was absent in the samples from southwest Norway and Kattegat but were found in turbot from Iceland (5/56) and west Norway (4/50). In contrast, the *Hb-I(1/1)d* phenotype was almost absent in samples from Iceland and west Norway but common in samples from southwest Norway (8/58) and Kattegat (11/84). This might suggest that the sub-band polymorphism could be used as a population marker in turbot and that the different subgroups represent adaptation to specific environmental conditions (here temperature). The sub-band polymorphism in turbot could also be linked to other environmental conditions, e.g. oxygen conditions, as differences are found in oxygen affinity between the three main haemoglobin genotypes in turbot (Imsland & al. 1997a; Samuelsen & al. 1999), and between the main groups and one subgroup in Atlantic cod (Brix & al. 1998). However, as long as the inheritance mode of this system is unsolved, utilization of the subgroups as population markers should be done with caution.

If more than one panmictic population exists in Europe it may be reasonable to believe that they may develop different life history strategies, as turbot from the southern part of the species range experience a different temperature regime and photoperiod compared with fish from the northern part of the species range (temperature data obtained from ICES oceanographic database, Copenhagen, Denmark). Imsland & al. (2000b, 2001a, b, c) found that food intake, food conversion efficiency and growth of juvenile turbot were higher in high latitude strains (Iceland, Norway) than in lower latitude (France and Scotland) strains when fish originating from different locations were reared together under common environmental conditions. Those findings may indicate local adaptation of turbot, but it may be discussed if local adaptation will be reflected in neutral genetic markers. Non-neutral markers (e.g. haemoglobin) may be a better tool for understanding local adaptation of fish populations than neutral markers, because interpopulation variation in the latter group must have been formed by coincidences (genetic drift).

The fact that there seems to be a correlation between physiological traits (Imsland & al. 1997a, 2000a; Imsland 1999; Samuelsen & al. 1999) and haemoglobin polymorphism in turbot might be utilized in aquaculture production. Imsland & al. (2000a) suggested that genotype-dependent growth rate variation between the haemoglobin genotypes might reflect differences in metabolic capacity rather than metabolic efficiency. This is supported by results on oxygen affinity (Imsland & al. 1997a; Samuelsen & al. 1999), showing that *Hb-I(2/2)* binds dissolved oxygen in the water more

efficiently, thus increasing the metabolic capacity of the fish. This suggests that the co-variation between haemoglobin genotypes and growth may be of some value in future breeding programmes and Imsland (1999) and Imsland & al. (2000a) suggested that the haemoglobin variations may represent quantitative trait loci in turbot.

The conclusion from the present study is that turbot are subdivided into two or more populations in northern Europe. To obtain a more complete picture of the structure of this species, samples from other parts of Europe must be collected and additional markers have to be included in the studies, for instance microsatellites. The frequencies of the subgroups reported here should also be studied on samples from other parts of the species distributions, and preferentially detailed studies should be carried out to reveal the genetic background and to see whether this system can be used as a correlated trait to select for improved growth rate of turbot. A fruitful way to continue this research might be to conduct controlled experiments where performance (growth, feeding behaviour, etc.) and environmental factors (e.g. temperature, oxygen, photoperiod, predation risk, food availability) are studied simultaneously for the haemoglobin genotypes.

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