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STUDIES ON HAEMOGLOBIN GENOTYPES IN TURBOT AND THEIR RELATION WITH GROWTH

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ABSTRACT

Turbot, *Scophthalmus maximus*, were kept in tanks and weighed every month for about two years. Haemoglobins of 90 untagged and 52 individually Pit tagged fish were analysed by agar gel electrophoresis. A polymorphic system was found in the haemoglobins. The electrophoretic patterns indicated a dimer structure of the proteins. Based on the observations the fish were classified into three phenotypes, supposed to represent three genotypes named; *Hb-I(1/1)*, *Hb-I(1/2)* and *Hb-I(2/2)*. The phenotype specific growth rate of the fish were analysed using weight data of the individually tagged fish. In addition the final weights and lengths of the untagged fish were analysed for differences in mean size of the phenotype groups.

INTRODUCTION

Turbot is one of the marine fish species found suitable for aquaculture. Problems regarding spawning (Devauchelle et al. 1988) and rearing in the early stages (Iglesias et al. 1987; Martinez-Tapia and Fernández-Pato 1991) have been the issue in many earlier studies. These studies have shown that juvenile turbot has its maximum growth rate around 16-19°C. However, Imsland et al. (in prep) in a study with individually Pit tagged turbot reported a individually variation in growth rate of fish of

the same size and reared at the same temperature. As the fish used in that study were all siblings or half-siblings, the authors point out the possibility that underlying genetic variation could to some extend explain the differences in individually growth rate.

Biochemical genetic variations of commercially important fishes have now been studied for three decades (review: Smith et al. 1990). The biological significance of biochemical genetic variation is though still at large unknown. However, in three recent studies on cod, *Gadus morhua*, Mork et al. (1984a,b) and Nævdal et al. (1992) have reported genotype growth differences releated to haemoglobin polymorphism in cod.

Traditional methods of selective breeding for genetic improvement depend on estimation of heritability factors and breeding value based on parent/offspring or sib correlations. The breeding technique for turbot makes collection of family data difficult and expensive, and therefore application of corrrelated traits are looked for. The first step is to reveal individual genetic variation, and then to test out possible covariation between genotype and productive traits. In the present report haemoglobin polymorphism is described, and preliminar observations on genotype dependent growth rate are presented. The results are also discussed in relation to application in studies of natural turbot populations.

MATERIAL AND METHODS

The fish used in the present originated from pooling of eggs from one female and sperm from two males. After hatching (July 15, 1991) the larvae were fed natural zooplankton until after metamorphosis, and then fed commercial dry diet. In October 1991 the fish were transported to the Industrial Laboratory at the Bergen High Technology Centre. From November 7, 1991 to June 19, 1992 the fish was reared at two water temperatures, 10°C and 19°C.

On January 17, 1992 the fish at 19°C were individually tagged with PIT tags. In June 1992 the temperatures were lowered from 10°C to 7°C and from 19°C to 16°C. At both temperatures the fish were fed in excess until the termination of the experiment.

The fish on 16°C were weighed every month (Feb. 1992 - Aug. 1992) and later every other month (Aug. 1992 - Jun. 1993). The experiment was terminated in June 1993. Specific growth rate (SGR) was calculated according to the formula

$$SGR = (e^{g} - 1)*100$$

where $g = (\ln(w_2) - \ln(w_1))\ (t_2 - t_1)^{-1}$ and w_2 and w_1 are individual weights at times t_2 and t_1, respectively. The SGR results were analysed with a two-way ANOVA with repeated measurements. Separate analysis were done for each temperature period. Blood samples were collected in May 1994.

The fish from 10°C were reared at 7°C from June 1992 until April 1994. In April blood samples from each fish were collected. Individual length and weight were recorded. The final length and weight was analysed using a one-way MANOVA.

For analysis of haemoglobins, the method described by Sick (1961) was applied with modifications (Jørstad 1984). Smithies buffer, ph 8.6 (Smithies 1959) was used as electrode buffer, and diluted 1:1 with destilled water for gel buffer. A homogeneity test, the G-test (Zar 1984), tested for differences in genotype distributions and for conformance with expected Hardy-Weinberg distributions.

RESULTS

When analysing the haemoglobins three different electrophoretic patterns where found. The three types are called type *Hb-I(1/1)*, type *Hb-I(1/2)*, type *Hb-I(2/2)* [most anodic], and interpreted as the two homozygotes and the heterozygote in a two allele system. The probable homozygotes were represented by patterns which consisted of one strong and several weak haemoglobin bands, while the probable heterozygote was represented by three strong and several weak bands. The middle strong band ("hybride zone") indicates a dimeric structure of the protein. Observed allele frequencies are presented in Table 1. The G-test did not reveal heterogeneity in the material ($P < 0.05$), and the observed distributions were in accordance with expected Hardy-Weinberg distributions, implying that the interpretation is correct.

In the period when the fish were reared at 19°C the phenotype *Hb-I(2/2)* had the lowest overall growth rate (Table 2). After lowering of the temperature to 16°C the phenotype *Hb-I(2/2)* showed the highest growth rate (Table 3). At both temperatures the growth rates were similar for *Hb-I(1/1)* and *Hb-I(1/2)*. The effect of phenotype were not significant at 19°C ($P = 0.71$, two-way ANOVA with repeated measurements). But, although not significant at 16°C the growth rate of the phenotypes tended towards significance ($F = 1.76$, $P = 0.18$, two-way ANOVA with repeated measurements).

At the lower temperature regime the phenotype *Hb-I(2/2)* showed both the highest final mean weight and length (Table 4). When both variables were tested simultaneously the *Hb-I(2/2)* was significantly larger than the other phenotypes ($P < 0.01$, one-way MANOVA). The mean weights and lengths of the phenotype *Hb-I(1/1)* and *Hb-I(1/2)*. were similar and not significantly different (one-way MANOVA).

Table 1. Allele frequencies in blood samples of turbot reared under two environmental temperature regimes.

Temp. regime	*Hb - I*	
(°C)	*1*	2
10 → 7	0.69	0.31
19 → 16	0.60	0.40

Table 2. Specific growth rate (SGR) in % d^{-1} of haemoglobin phenotypes in PIT tagged turbot reared at 19°C. s.d. = standard deviation, n = numbers.

	Hb-I(1/1)			*Hb-I(1/2)*			*Hb-I(2/2)*		
	SGR	s.d.	n	SGR	s.d.	n	SGR	s.d.	n
Feb 14 - Mar 13	1.70	0.34	19	1.74	0.24	24	1.56	0.26	9
Mar 14 - Apr 23	1.31	0.40	19	1.28	0.27	24	1.20	0.46	9
Apr 24 - May 31	0.58	0.23	19	0.57	0.23	24	0.62	0.25	9

Table 3. Specific growth rate (SGR) in % d^{-1} of haemoglobin phenotypes in PIT tagged turbot reared at 16°C. s.d. = standard deviation, n = numbers.

	Hb-I(1/1)			*Hb-I(1/2)*			*Hb-I(2/2)*		
	SGR	s.d.	n	SGR	s.d.	n	SGR	s.d.	n
Jun 1 - Jun 30	0.67	0.26	19	0.52	0.20	24	0.71	0.25	9
Jul 1 - Jul 31	0.97	0.22	19	0.98	0.26	24	1.04	0.26	9
Aug 1 - Sep 30	0.63	0.18	19	0.58	0.20	24	0.68	0.14	9
Oct 1 - Nov 30	0.47	0.20	19	0.48	0.21	24	0.67	0.12	9
Dec 1 - Jan 31	0.21	0.11	19	0.18	0.11	24	0.23	0.09	9
Feb 1 - Apr 26	0.34	0.19	19	0.34	0.19	24	0.37	0.14	9

Table 4. Mean weight (w) in grams and lengths (l) in cm of haemoglobin phenotypes in turbot reared under 10°C and 7°C. s.d. = standard deviation, n = numbers.

	w	s.d.	n	l	s.d.	n
Hb-I(1/1)	524.2	109.1	41	27.8	1.7	41
Hb-I(1/2)	513.3	125.6	43	28.3	2.2	43
Hb-I(2/2)	621.6	162.8	6	30.1	2.6	6

DISCUSSION

We have reported a previously unreported polymorphic system in the haemoglobin of turbot. As the system reported resembles the polymorphic haemoglobin system of cod (Sick 1961) a similar nomenclature was tentatively used.

The results obtained from growth studies indicate an association between haemoglobin phenotype and growth at low temperature (10°C and 7°C). Although not statistically significant the data indicated the same association at 16°C. At both these temperature regimes the phenotype *Hb-I(2/2)* showed the highest growth rate, whereas the reverse was found on 19°C. The results from 16°C and 10/7°C are in accordance with the results of Mork et al. (1984a,b) and Nævdal et al. (1992). These authors found that the genotype *Hb-I(2/2)* in cod grew on average faster than both *Hb-I(1/1)* and *Hb-I(1/2)*. However, in contrast to the results of Nævdal et al. (1992) the phenotype *Hb-I(2/2)* did not perform best at high (19°C) temperature.

Because the numbers of broodfish used to produce the present fish population, the calculated allele frequencies are only indicative of the corresponding frequencies of the natural populations from which the broodfish were sampled. However, with the reservation of that the haemoglobin types are not influenced by strong natural selection, the observed polymorphism may be used as a tool for studies on the population structure of turbot. Earlier studies have revealed very low degree of genetic variation in this species (Blanquer et al. 1992).

The preliminary conclusion from these studies is that an association between haemoglobin phenotype and growth in turbot seems to be at hand at low and moderate temperatures. This system must be further studied in detail, to see whether it can be used a correlated trait to select for improved growth rate of turbot at low and moderate temperatures.

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