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Variations in growth in haemoglobin genotypes of Atlantic cod

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Abstract

In the present paper are described the growth properties of three different haemoglobin genotypes of juvenile Atlantic cod *(Gadus morhua)* reared at 7, 10, 13 and 16 °C. In addition one group was reared under "temperature steps" i.e. moved successively from 16 to 13 and 10 °C. The genotype *Hb-I(2/2)* displayed the overall highest growth rate in the temperature range 13–16 °C, whereas the *Hb- I(1/1)* genotype showed the highest overall growth at the lowest temperature (7 °C). Accordingly, we found a significant interaction between genotype and temperature. The differences in growth were largest when cod were reared under the temperature step regime where the *Hb-I(2/2)* genotype displayed 17 and 24% higher growth than *Hb-I(1/1)* and *Hb-I(1/2)*, respectively. Optimal temperature for growth ($T_{opt.}G$) varied between the genotypes with the genotype *Hb-I(1/2)* displaying the highest (mean ± SE) $T_{opt.}G$ (14.5 ± 1:0.8 °C) and *Hb-I(1/1)* the lowest (12.5 ± 0.2 °C). The biological significance of this link between biochemical genetic variation and physiological properties might be the influences on growth pattern, ultimate size and age at first maturity.

Introduction

In Atlantic cod haemoglobin is characterised by three different and common genotypes called *Hb-I(1/1)*, *Hb-I(1/2)* and *Hb-I(2/2)*. Mark et al. (1984a,b) and Nævdal et al. (1992) indicated haemoglobin dependent growth rate in Atlantic cod, although in other studies no such dependence has been found (Jørstad and Nævdal 1994; Glover et al. 1997). If present haemoglobin dependent growth rates might be correlated with differences in functional properties of the haemoglobins as Karpov and Novikov (1980) and Brix et al. (1998) have reported specific temperature dependence of oxygen dissociation curves for cod haemoglobins. A similar system has been reported for turbot, *Scophthalmus maximus* Rafinesque and in both species the *Hb-I(2/2)* genotype has the highest oxygen affinity at all tested temperatures, followed by *Hb-I(1/2)* and *Hb-I(1/1)*. For both species temperature sensitivity of O_2 binding for haemoglobin is low but increases with increasing pH, and it has been hypothesised that the temperature insensitive haemoglobins might be an adaptation to variable temperature conditions in the distribution area of the species.

Fish typically show a rapid increase in relative growth rate as the temperature rises, passing through a peak at optimum temperature ($T_{opt.}G$) and falling rapidly at temperatures beyond $T_{opt.}G$ (cf. Brett 1979; Cuenco et al. 1985; Imsland et al. 1996; Jonassen et al. 1999). A common finding in studies examining the relationship of temperature and size on growth is that $T_{opt.}G$

decreases as fish size increases (Imsland and Jonassen 2001). To utilise this mechanism to improve growth, fish would have to be reared in ''temperature steps'' i.e. a group of fish has to be moved to successively lower temperature following changes in fish size. In the present study we try to mimic different this ''temperature-step'' method by rearing one group of fish at three different temperatures, i.e. 16 °C followed by 13 and 10 °C.

Nævdal et al. (1992), Jørstad and Nævdal (1994) and Glover et al. (1997) have investigated the association between haemoglobin genotype and growth at two or three constant temperatures. However, at present no studies have systematically investigated optimum growth properties of the haemoglobin genotypes over a wide range of temperatures covering sub-, near- and super-optimal temperature range for juvenile cod (e.g. Björnsson et al. 2001). Hence, the aim of the present study was to characterise growth potential of different haemoglobin genotypes in Atlantic cod at early juvenile stages reared at five different temperature regimes. Four regimes consisted of constant temperatures between 7 and 16 °C and one regime was designed to follow the Topt.G for juvenile cod between 10 and 70 g. More specifically, we wanted to investigate whether optimal temperature for growth varies between genotypes and whether growth differences (if any) are more pronounced at optimal temperature than at sub- and super-optimal temperatures.

Materials and methods

Fish material and rearing conditions

The eggs were obtained from Sagafjord Seafarm AS, Stord, Norway and transported to the facilities of the University of Bergen were they were incubated. The broodfish were wild caught in the area around Bømlo (W-Norway) in 2003 and reared in 40 m^3 tanks at simulated natural photoperiod and temperature of 6–8 °C (sea water pumped from 160 m depth). The mean weight of the broodfish was approximately, 7 kg (max 18–min 5 kg).

The eggs hatched on 28 March and the larvae were subsequently transferred to a 500 l tank with a constant temperature of 7.8 °C. The larvae were reared under continuous light, fed fresh filtered natural zooplankton (gradually increasing size fraction from 80 to 1000 μ) and weaned on a commercial formulated feed (Marin 030 and 050, Ewos A/S, Bergen, Norway) containing 60% protein, 12% fat and 12% carbohydrates. On 20 June 2003 the juveniles were brought to the Industrial and Aquatic Laboratory at the Bergen High Technology Centre and reared at 10 °C and simulated natural photoperiod (LDN). This light regime was used throughout the acclimation and experimental period.

The fish (N = 220) were distributed randomly into 10 rearing tanks (see below). The fish used in the present study were reared together with 90–100 cod juveniles of the same size in each tank, as part of another study. This paper, however, focuses on results from the 220 fish described above. The growth study was carried out from 8 September until 12 December 2003. The 1 m^2 square, grey, covered fiberglass experimental tanks had a rearing volume of 400 l and a bottom outlet. Seawater with a salinity of 33.5‰ (± 0.2‰) was pumped from 90 m depth. Water flow was set to 10 l min^{-1} for all experimental tanks. Oxygen saturation was measured weekly in the effluent (i.e. bottom outlet) water of all tanks and was higher than 80% at all times. A 36 W fluorescent daylight tube integrated in the tank-cover provided light. Photon-irradiation measured at the bottom of the tanks was ca. 5 μmol m^{-2} s^{-1}. Prior to, and during the experiment, the juveniles were fed a commercial formulated feed (Marin 10 and 20, Ewos A/S) containing 55% protein, 12% fat and 11% carbohydrate. Food was provided in excess for 2 h daily (0800–0900 and 1400–1500). Pellet size (2 and 3 mm) was adjusted during the experiment, depending on fish size with an introduction of 3 mm pellets from 14 October.

Experimental design

On 25 August 2003 the fish were tagged intraperitoneally with Trovan® Passive Transponder tags, and gradually acclimated over one day (7 and 13 °C groups) or 3 days (16 °C and temperature-step groups) to the five experimental

temperatures regimes of 7, 10, 13, 16 °C and temperature-step group (T-step group). Each temperature regime consisted of two replicate tanks. The temperature step regime was as follows: 16 °C from 9 September to 7 October; October 13 °C from 8 October to 19 November; 10 °C from 20 November to 12 December. The mean rearing temperature in this group was 13.2 °C. The temperature was measured twice daily, and remained within ±0.2 °C (SD) of that prescribed. All fish were anaesthetised (metacain, 0.05 g l^{-1}), weighed individually (0.1 g) at 22–28 days interval during the experiment (Table 1). Specific growth rate (SGR) was calculated according to the formula:

$$\mathrm{SGR} = (e^{g} - 1)100,$$

where $g = (\ln(W_2) - \ln(W_1))/(t_2 - t_1)$ and W_2 and W_1 are individual weights at days t_2 and t_1, respectively. The initial mean weight (SEM) was 9.1 (0.5) g on 8 September, and did not differ significantly (three way nested ANOVA, Power $(1 - \beta) > 0.6$) between genotypes or tanks (Table 1). The experiment was terminated on 12 December 2003.

Analysis of haemoglobin genotypes

After termination of the growth experiment blood (0.2 ml) for genetic analysis was sampled from the caudal vessels and kept on ice until analysed. All samples were analysed by 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 gels (Agar Noble, Difco laboratories, Detroit, Michigan, USA).

Statistical methods

All statistical analyses were performed with STATISTICA™ 6.0. To assess normality of

Table 1. Mean weights (g) for cod of different haemoglobin genotypes at the four rearing temperatures. Results are given as mean (standard error of mean)

Temperature (*T*):	Genotypes (G):	n	Variable	9 September	7 October	29 October	19 November	12 December
			Temperature (*T*):	NS	$P < 0.01$	$P < 0.01$	$P < 0.01$	$P < 0.01$
			Genotypes (G):	NS	NS	NS	$P < 0.05$	$P < 0.05$
			Interaction (TxG):	NS	NS	NS	$P < 0.05$	$P < 0.05$
7 °C	*Hb-I(1/1)*	17		8.9 (0.7)	14.4 (1.5)	20.7 (2.5)	28.5 (3.4)	37.9 (4.0)[a]
	Hb-I(1/2)	19		9.2 (0.6)	14.5 (1.4)	20.3 (2.3)	26.0 (3.3)	31.4 (4.4)[a,b]
	Hb-I(2/2)	6		8.8 (1.4)	14.3 (2.2)	19.2 (3.4)	21.1 (4.5)	27.5 (4.9)[b]
10 °C	*Hb-I(1/1)*	21		8.8 (0.6)	16.7 (1.3)	25.3 (2.2)	35.0 (3.3)	48.6 (4.2)
	Hb-I(I/2)	17		10.0 (0.7)	19.0 (1.5)	28.3 (2.5)	38.8 (3.3)	51.9 (4.4)
	Hb-I(2/2)	7		10.3 (1.0)	19.4 (2.1)	28.9 (3.1)	39.9 (4.1)	52.7(6.4)
13 °C	*Hb-I(1/1)*	20		8.6 (0.5)	18.4 (1.4)	28.1 (2.3)	40.6 (3.4)	60.4 (4.0)
	Hb-I(1/2)	16		9.2 (0.7)	19.4 (1.5)	29.2 (2.5)	40.4 (3.3)	56.6 (5.4)
	Hb-I(2/2)	7		8.6 (1.0)	19.1 (2.2)	29.6 (3.2)	43.8 (4.3)	64.9 (6.3)
16 °C	*Hb-I(1/1)*	12		9.7 (0.8)	20.0 (1.7)	31.2 (2.9)	42.6 (4.4)	54.7 (5.7)
	Hb-I(I/2)	24		8.9 (0.6)	18.9 (1.2)	29.6 (2.1)	40.5 (3.1)	54.2 (4.0)
	Hb-I(2/2)	7		10.1 (1.0)	19.8 (2.3)	30.4 (3.5)	40.8 (4.4)	56.6 (6.1)
T-step group	*Hb-I(1/1)*	23		10.0 (0.5)	20.8 (1.3)	31.6 (2.1)	44.4 (3.2)[a,b]	60.7 (44)[a,b]
	Hb-I(1/2)	18		8.8 (0.7)	17.6 (1.4)	27.4 (2.4)	40.3 (3.3)[b]	54.9 (4.4)[b]
	Hb-I(2/2)	6		8.6 (1.6)	19.5 (2.4)	31.9 (3.5)	49.9 (4.7)[a]	72.6 (6.3)[a]

n = number of fish of each genotype at each temperature. Results (three way nested ANOVA, $P < 0.05$) for the effects of temperature and genotypes, as well as the interaction between these effects are given for each date. In cases of significant ANOVAs different letters indicate statistical differences with "a" as the highest value within each temperature regime (Student–Newmans–Keuls test, $P < 0.05$). Non-significant results are marked NS

distributions a Kolmogorov–Smirnov test was used, and homogeneity of variances was tested using the Levene's F test. Three-way nested ANOVA (Zar 1984), was applied to calculate the effect of different genotypes on mean weights and SGRs at the different temperature regimes, where genotypes and temperatures are nested within the replicates. Significant ANOVAs were followed by a Student–Newman–Keuls multiple comparison test to locate differences among treatments (Zar 1984). Individual growth trajectories were analysed using a growth curve analysis model (GCM, Chambers and Miller 1995) which is an extension of the multivariate repeated measurements analysis of variance (MANOVA) model. The model equation of the GCM had the form:

$$\mathbf{Y}(n \times p) = \mathbf{X}(n \times q)\mathbf{B}(q \times p) + \mathbf{E}(n \times p),$$

where $\mathbf{Y}(n \times \mathrm{p})$ are the growth at age vectors $\mathbf{y} = (y_1, y_2, \ldots, y_p)$ for each p (age) measurements on n individual fish; $\mathbf{X}(n \times q)$ is the design matrix or the set of extraneous variables measured for each individual, i.e., $q = \mathrm{age}_p + \mathrm{genotype}_i + \mathrm{temperature}_j$; (i = *Hb-I(1/1), Hb-I(1/2)* and *Hb-I(2/2)*), (j = 7, 10, 13, 16 °C and T-step); $\mathbf{B}(q \times p)$ is the matrix of parameters estimated by the model; $\mathbf{E}(\mathrm{n} \times \mathrm{p})$ is the matrix of deviations for each individual from the expected value of $\mathbf{Y} = \mathbf{XB}$.

Analyses were made for all temperatures combined and for each temperature separately. Genotypic specific growth was also analysed with a parabolic regression (Zar 1984) where SGR was regressed against temperature for all genotypes. The regression was made using the combined growth rates of all tagged fish of each genotype at each temperature. Optimal temperatures for growth ($T_{opt.}G$) for each haemoglobin genotype were calculated as the zero solution to the first derivative of the parabolic regression equations, i.e. the solution of $\mathrm{SGR} = aT^2 + bT + c$ where SGR = specific growth rate, T = temperature, and a, b and c are constants determined by the regression. Asymptotic standard error of mean (SEM) for $T_{opt.}G$ was calculated based on individual growth data. A significance level (α) of 0.05 was used if not stated otherwise. In cases with non-significant statistical tests, power ($1-\beta$) analysis were performed using the PASS program package (Hintze 1996) using $\alpha = 0.05$.

Results

Haemoglobin genotypes

Based on the electrophoretic pattern the juveniles were grouped into the three haemoglobin genotypes: *Hb-I(1/1), Hb-I(1/2)* and *Hb-I(2/2)* and interpreted as the two homozygotes and the heterozygote in a two allele system. The number of each genotype were as follows; 93 *Hb-I(1/1),* 94 *Hb-I(1/2),* and 33 *Hb-I(212)* (Table 1). The observed distributions appeared to be in accordance with expected Hardy–Weinberg distributions ($P > 0.3$), supporting the earlier interpretation of segregation of two alleles controlling the haemoglobin types in cod.

Growth

At 7 °C the *Hb-I(1/1)* had the highest and the *Hb-I(2/2)* the lowest mean weight in December. Conversely in the T-step group the *Hb-I(2/2)* genotype had the highest mean weight from 19 November (three way nested ANOVA, $P < 0.05$, Table 1). Consequently a significant interaction between genotype and temperature on weight was found from November onwards (Table 1). No significant differences in mean weight were found between the *Hb-I(1/1)* and *Hb-I(1/2)* genotypes at any temperature or period.

Mean individual growth trajectories varied significantly between genotypes (GCM, MANOVA $_{\mathrm{GENOTYPES}}$, Wilk's lambda $(\Lambda)_{8,396} = 0.69$, $P < 0.001$) with the genotype *Hb-I(2/2)* displaying highest overall mean growth of the three genotypes (1.85, 1.72 and 1.81% for *Hb-I(2/2), Hb-I(1/1)* and *Hb-I(1/2),* respectively). Growth also varied among temperatures (MANOVA $_{\mathrm{TEMPERATURES}}$, Wilk's $\Lambda_{16,605} = 0.53$, $P < 0.001$), with the overall highest growth rates for all genotypes seen in the T-step group (*Hb-I(1/2)* and *Hb-I(2/2)* or at 13 °C (*Hb- I(1/1)*) and the lowest at 7 °C (Figures 1 and 2). Overall the interaction between genotypes and temperature tended towards significance ($\mathrm{MANOVA}_{\mathrm{GENOTYPE} \times \mathrm{TEMP}}$, Wilk's $\Lambda_{32,732} = 0.82$, $P = 0.10$). When growth rates were studied separately for each temperature (Figure 1) mean individual growth trajectories were found to vary at 13 °C, 16 °C and in the T-step group. At these temperature regimes the *Hb-I(2/2)* genotype displayed significantly higher growth

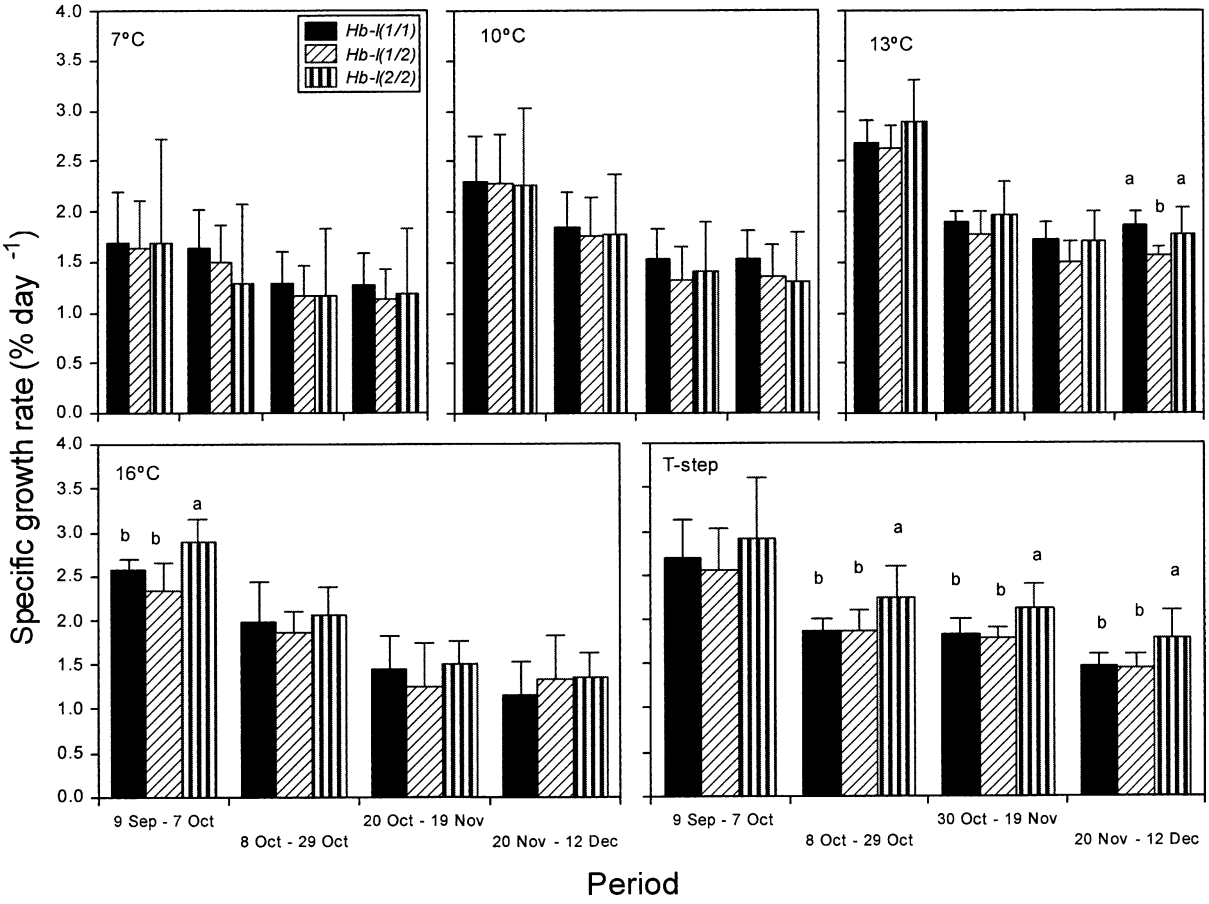

Figure 1. Genotype specific mean growth rates (±SEM) of individually tagged cod reared under five temperature regimes. Different letters denote significant differences (Student–Newman–Keuls test, $P < 0.05$) within each temperature regime.

rates compared to the two other genotypes (Student–Newman–Keuls test, $P < 0.05$, Figure 1).

The optimal temperature for growth (Topt.G) varied among the genotypes (Figure 2). The parabolic regressions showed that the *Hb-I(1/1)* had the lowest Topt.G (mean ± SE) (12.5 ± 0.2 °C) whereas the Topt.G for the *Hb-I(2/2)* and *Hb-(1/2)* genotypes were found to be 13.1 ± 0.2 °C, and 14.5 ± 0.8 °C, respectively.

Discussion

Few studies have assessed the underlying individual genetic growth factor (Forsberg 1995; Imsland et al. 1998) although it is axiomatic that individuals vary in growth (e.g. Rosenberg and Haugen 1982; Imsland et al. 1996b, 1998). Imsland et al. (1998) suggested, based on investigations of individual growth trajectories, that the individual growth trait X_i is stochastic with some kind of "memory", i.e. the relative growth rate of individual (i) is to some extent correlated with its relative growth rate in the previous period. The fact that there is a positive correlation between imminent growth rates (Imsland et al. 1998) suggests that the individual growth factor X_i is inherent. The genotype dependent growth reported in the present study supports this view. For cod, initial trials (Gjerde et al. 2004) indicate that the heritability for body weight is large, and that there is a substantial additive genetic variation of growth rate in cod. However, traditional methods of selective breeding for genetic improvement depend on estimation of heritability factors and breeding value based on parent/offspring or sib correlations (cf. Falconer 1989). The breeding technique for cod makes collection

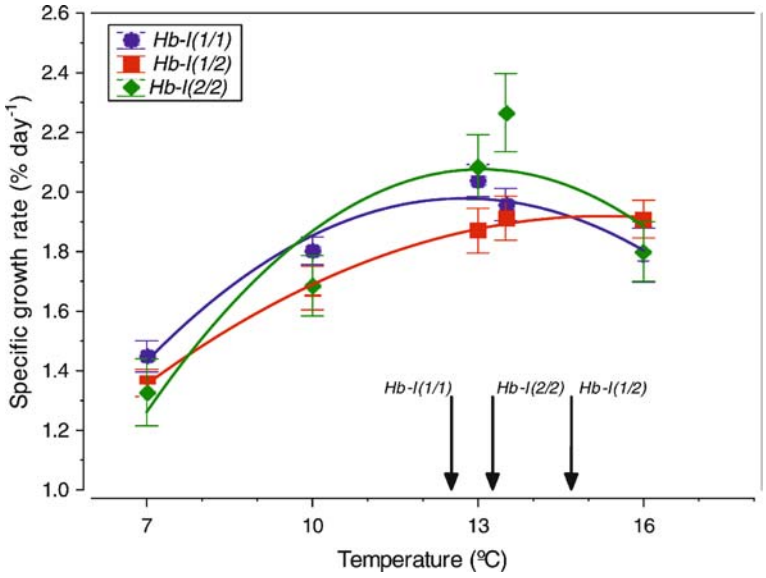

Figure 2. Overall mean growth rates of the three haemoglobin genotypes of juvenile cod plotted against temperature. The lines represent the least squares second order polynomial fit to the data: $SGR = aT^2 + bT + c$ where SGR = specific growth rate, T = temperature, and a, b and c are constants determined by the regression. Vertical lines indicate standard error of mean. Arrows mark optimal temperature for growth of the different genotypes. *Hb-I(1/1);* $SGR = -0.016T^2 + 0.420T - 0.717$; *Hb-I(1/2);* $SGR = -0.008T^2 + 0.245T + 0.029$; *Hb-I(2/2);* $SGR = -0.021T^2 + 0.560T - 1.629$.

of family data difficult and expensive (Brown et al. 2003); hence the need for the application of correlated traits or quantitative trait loci (QTL) for effective selective breeding for improved aquaculture production. The first step is to reveal individual genetic variation, and then to test for co-variation between genotype and physiological traits of productive value e.g. growth. The genotype dependent growth rates as found by Mork et al. (1984a,b) and Nævdal et al. (1992), may be correlated with differences in functional properties of the haemoglobins. Karpov and Novikov (1980) found that in cod, the Hb-I(2/2) molecule was the most efficient oxygen carrier at low temperatures while the Hb-I(1/1) molecule had similar advantages at higher temperatures. In contrast, in the present study Hb-I(1/1) showed the highest mean growth at the lowest temperature, and Brix et al. (1998) found the Hb-I(2/2) molecule to be more efficient oxygen carrier at all temperatures (10, 15 and 20 °C) and pH (7.5 and 8.0) combinations (except at 20 °C/8.0) investigated. Studies on Atlantic salmon and Arctic charr indicate that homozygotes of the trypsin isozyme allele 92 (*TRP-2*(*92*/*92*)) have better growth than other TRP genotypes (Torrissen 1991; Torrissen and Shearer 1992), and that this relates to higher protein efficiency ratio of *TRP-2 (92/92)* homozygotes. Previous studies on turbot (Imsland et al. 1997, 2000; Samuelsen et al. 1999) have indicated a genotypic dependent difference in oxygen affinity where the *Hb-I(2/2)* has the highest oxygen affinity in the temperature range 10–19 °C. Accordingly it may be postulated that these two physiological factors are to some extent correlated as the genotype with highest affinity also displays the highest overall growth.

Genotype dependent differences in physiological performance seen in earlier studies on Atlantic cod (Karpov and Novikov 1980; Nævdal et al. 1992) may indicate differential selection pressure on the controlling genes. However, such selection pressure may be balanced by other factors. 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 to the *Hb-I(2/2)* genotype. However, it was rather surprising that *Hb-I(1/1)* displayed the highest growth rate at the lowest temperature. This is in contrast to Karpov and Novikov (1980) who indicated that *Hb-I(2/2)* is best adapted to

cold water, and in most area the *Hb-1*2* allele is found at highest frequencies in the northern parts of the cod's distribution (except around the Faeroe Islands), indicating that this allele represents a cold water adaptation. It may also indicate that the genotype dependent variation in growth rate is not connected to temperature, but rather to other physical factors. Genotypic growth properties (Imsland et al. 1997, 2000), oxygen affinity (Imsland et al. 1997; Samuelsen et al. 1999) and genotype dependent 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 1999). Hence, lower age at first maturity of the *Hb-I(2|2)* genotype may be balanced by increased risk of predation as maturing fish need to forage more than immature fish to fulfil higher energy demands. Whether, there exist a similar balancing system in cod is still unsolved. However, Salvanes and Hart (2000) compared the competitive performance of cod of the three main Hb-I genotypes. Randomly chosen one-year-old cod were tested for individual responses to prey offered sequentially, and they 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 exist a link between genotypic growth and feeding behaviour. Albeit the clear, but not consistent, differences in physiological and behavioural properties of the cod haemoglobin genotypes (Karpov and Novikov 1980; Nævdal et al. 1992; Salvanes and Hart 2000, present study) no clear indication of directional selection on haemoglobin genotype distribution has been seen when comparing early analyses of cod haemoglobins in Norwegian waters (Frydenberg et al. 1965; Møller 1968) with corresponding analyses in recent years (Jørstad and Nævdal 1989; Gjøsæter et al. 1992; Dahle and Jørstad 1993; Fyhn et 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 and Nævdal 1989).

Since the fish in the present study, in contrast to most wild-living cod (Björnsson 1999; Dutil and Lambert 2000), were fed in excess and grew fast, the genotype dependent growth rate variation may reflect differences in metabolic capacity rather than metabolic efficiency. This is supported by the results on oxygen affinity (Brix et al. 1999), showing that the *Hb-I(2|2)* binds dissolved oxygen in the water more efficiently, thus increasing the metabolic capacity (Fonds et al. 1992) of the fish. A better utilisation of the oxygen supply, i.e. higher oxygen affinity may lower the energy spent in metabolism so that more energy can be channelled into growth, i.e. increased metabolic capacity (Brett 1979; Fonds et al. 1992), which is in accordance with the findings in the present study. The fact that the relative differences in growth between the genotypes were largest at near-optimal, optimal and super-optimal temperatures (Figure 2) suggests that this co-variation between haemoglobin genotypes and growth may be of some value in future breeding programme on cod as genotypes may be selected based on environmental conditions at different rearing sites.

According to Björnsson et al. (2001) the Topt.G for 12, 29 and 109 g juvenile cod is 16.2, 13.2 and 10 °C, respectively. The T-step group in the present study follows in part this rearing scheme. Overall the growth in the T-step group was similar to that found in the 13 °C group, and higher than in the 10 and 16 °C (Figure 1, Table 1), but the genotypes responded differently to this rearing scheme. The *Hb-I(2|2)* displayed very high growth with this rearing method. This may in part be explained by the fact that this genotype seems to have physiological properties e.g. high oxygen affinity (Brix et al. 1999) and better growth (Nævdal et al. 1992) suited for temperatures around the Topt.G, whereas the *Hb-I(1|1)* has better comparative growth at lower temperatures (Figure 2).

Apart from the *Hb-I(1|1)* genotype the optimal temperatures for growth (Topt.G) of the fish in the present study are in line with those reported earlier for juvenile cod (Björnsson et al. 2001). Variation in Topt.G between genotypes has been reported for turbot (Imsland et al. 2000) and it has been postulated that this mechanism might be an adaptation to variable temperature conditions in the distribution area of the species. Atlantic cod is the major demersal fish resource distributed on the continental shelves and banks on both sides of the North Atlantic Ocean (review Imsland and Jónsdóttir 2003)

distributed in a variety of temperature conditions (e.g. Brander 1995). Genotypic adaptation to environmental gradient as indicated in the present study is, therefore, a possible evolutional mechanism to increase the overall fitness of the species. Further, it can be speculated if the indicated differences in physiological properties of the haemoglobin genotypes may not be view as a balanced polymorphism. The apparent lower: oxygen affinity (Brix et al. 1998), competitive performance (Salvanes and Hart 2000) and growth (Nævdal et al. 1992; present study) of the *Hb-I(1/1)* genotype could thus be a mechanism to insure a constant proportion of "slow growers" and thus later maturating fish. Such a mechanism could be seen as "safe guarding mechanism" to insure the species fitness in its changeable environment.

In conclusion, growth of juvenile cod varied between haemoglobin genotypes with *Hb-I(2/2)* showing the highest growth of the three genotypes, whereas no differences were found between the *Hb-I(1/1)* and *Hb-I(1/2)* genotypes. The biological significance of this relationship between biochemical genetic variation and physiological properties might be variation in growth pattern, ultimate size and age at first maturity.

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