Is individual variation in competitive performance of reared juvenile cod influenced by haemoglobin genotype?

Anne Gro Vea Salvanes & Paul J.B. Hart

SARSIA

Salvanes AGV, Hart PJB. 2000. Is individual variation in competitive performance of reared juvenile cod influenced by haemoglobin genotype? *Sarsia* 85:265-274.

To succeed in scramble competition for food an individual fish will have to have characteristics that allow it to respond rapidly to encountered prey. A trait such as metabolic rate, which has a positive effect on oxygen consumption and growth rate, is likely to be positively correlated with the traits that determine the speed of reaction. An important factor underlying metabolic rate may be the transport efficiency of oxygen from the gills to the respiring tissue and this is mediated by the structure of the haemoglobin molecule. In cod, two structures of this molecule exist due to polymorphism at the *HbI** locus. An individual cod may be homozygous (*HbI*1/1* or *HbI*2/2*) or heterozygous (*HbI*1/2*). Evidence exists in the literature that *HbI*2/2* fish have higher growth rate and earlier maturation and higher transport efficiency of oxygen at low temperature. However, no study has examined whether this could be associated with fish behaviour. In a study reported here we designed an experiment to test the hypothesis that fish with the *HbI*2/2* genotype have a higher motivation to feed and are better competitors than individuals with the other haemoglobin genotypes and that they will eat a larger share of the prey. We use prey capture success early in a feeding trial and the rank of the first prey taken, as proxy-variables for competitive performance. Randomly chosen one-year-old cod *Gadus morhua* L. in small groups were tested experimentally for individual responses to prey offered sequentially. We analysed the effect on competitive performance of haemoglobin genotype, group, fish size, sex, maturation status and unobserved effects using Components of Variance Analysis, which accounts for repeated observations from the same individuals. The most successful fish were usually among the first to feed and tended to possess haemoglobin genotype *HbI*2/2*. Other factors such as body size, sex, stage of maturation and group also had effects which may modify the effect set by genotype. Our results suggest that the link between *HbI** genotype and growth is through feeding behaviour and it supports the idea that fish with *HbI*2/2* genotype are better able to support an active metabolism. The results obtained are among the first on fish that show that variation in feeding behaviour could be under genetic control.

Anne Gro Vea Salvanes, University of Bergen, Department of Fisheries and Marine Biology, PO Box 7800, N-5020 Bergen, Norway. – Paul J.B. Hart, Department of Biology, University of Leicester, University Road, Leicester LE1 7RH, UK.
E-mail: anne.salvanes@ifm.uib.no – pbh@leicester.ac.uk

Keywords: Cod; feeding behaviour; haemoglobin genotype; competitive performance.

INTRODUCTION

To succeed in scramble competition for food an individual fish will have to have characteristics that allow it to respond rapidly to prey. Properties could include quick reactions, fast swimming and good vision. A trait such as metabolic rate, which has a positive effect on oxygen consumption and growth rate (e.g. Jobling 1994), is likely to be positively correlated with the traits determining speed of reaction. In salmonids, fish with higher metabolic rate have higher competitive performance and a faster growth than similar sized fish (Metcalfe & al. 1995; Nakano 1995; Cutts & al. 1998; Yamamoto & al. 1998). These inherent properties will be moderated by the state of the animal. Those recently fed will have lost motivation and fish with maturing gonads might be expected to require more food than those not maturing. These short-term variables will operate within the framework determined by the inherent properties of the fish.

An important factor underlying metabolic rate can be the transport efficiency of oxygen from the gills to the respiring tissue and this is mediated by the structure of the haemoglobin molecule (Weber 1990). In cod (*Gadus morhua* L.), two structures of this molecule exist due to polymorphism at the *HbI** locus. An individual cod may be homozygous (*HbI*1/1* or *HbI*2/2*), or it can be heterozygous (*HbI*1/2*) (Sick 1962). In cod, growth rate is reported to be related to these haemoglobin genotypes (Mork & al. 1984; Nævdal & al. 1992) with the *HbI*2/2* genotype having a faster growth rate and an earlier age of first spawning in the mid to northerly regions off Norway. The different forms of the haemoglobin molecule

differ in their efficiency as an O_2-carrier at low temperatures (Karpov & Novikov 1980; Brix & al. 1998) with the *HbI*2/2* genotype being a better oxygen carrier than the other two at temperatures below 15 °C. This difference is a likely reason for the better growth at lower temperatures of the *HbI*2/2* genotype. Taking into account these published results we hypothesised that individuals with the *HbI*2/2* genotype will have a higher motivation to feed than the other two genotypes and we predict that they will be better competitors than individuals with the other genotypes and will eat a larger share of the prey. We describe in this paper two experiments that were designed to test this hypothesis.

Because genotype alone may not be the only characteristic determining the competitive performance of individuals our experiments also took account of other candidate determinants of competitive performance such as sex, body size and state of maturity. Our data analysis has included also an account of the effects of unobserved individual variation on competitive performance through the use of appropriate statistical methods.

Through their lives, cod food ranges from planktonic copepods to fish. As we could not examine all food types we chose food characteristic for juveniles. These settle in the near-shore benthic habitat at 0-20 m depth when 4-5 months old, 4-5 cm long and < 5 g (Salvanes & al. 1994). Here they switch from zooplankton to feed on gobies (mainly *Gobiusculus flavescens*) and benthic crustaceans (Salvanes & Nordeide 1993). Gobies are major prey for cod in July-December and were therefore used as prey in the experiments.

MATERIAL AND METHODS

One-year-old fish taken at random from about 200 were divided randomly into six groups which were held separately in 500-litre tanks of seawater in a temperature and light controlled room. Groups consisted of 3 or 4 individuals. The water renewal was approximately 5 l min^{-1} and the water was circulating in the tank. The cod were hatchery reared and originated from second or third generation cod taken originally from the western Norway population. They were reared from a mixture of eggs naturally spawned by several groups of brood fish (60-100 individuals) in 1995 and 1996. Each year newly hatched larvae were released into a predator-free marine pond where they fed on natural zooplankton. In late June they were transferred to 500-litre tanks at the University of Bergen and housed under natural temperature and light conditions. They were transferred to continuous feeding using "Supra Marin" (Felleskjøpet Fiskefôr, Norway) formulated feed with pellet sizes increasing as fish grew in size.

For six weeks before the experiment started the fish were adapted to a daily light cycle, including dawn and dusk light levels, for 60°25'N, 5°20'E (Bergen) on 15 January and to constant temperature conditions (6.3 ± 0.05 °C). Experiment 1 was conducted in 1996 and Experiment 2 in 1997. Individual fish were assigned to weight categories as follows; 1: < 150g; 2: 151-180 g; 3: 181-250 g; 4: 251-330 g; 5: > 330g.

Individual dead gobies offered during each trial were recorded and numbered 1 to *n* as they were fed to the cod, *n* being the total number of gobies. The sequential deliveries of prey were randomised events. A new prey was always introduced after the previous one had been captured and swallowed by one of the fish. Prey which sank to the bottom were usually those offered late in a trial. To randomise within a tank, individual dead gobies were thrown by hand and one by one into random positions over the water surface so that neither the experimenter nor the fish had control over the landing positions of prey. To further reduce the possibility of carry-over effects between tanks (groups), the order in which tanks were treated on each day was varied. Also, each individual in a group had the same opportunity to take every prey introduced to a tank, as we did not observe any aggression or any sign of territorial defence during the experiments. Nor was there any evident sign of co-operation between individuals.

The fish in a tank were marked with either a red, blue, yellow or white spaghetti tag to allow the identification of the individual which took each prey, the information being recorded on a Dictaphone. We also recorded the elapsed time between the first and the last prey in a trial, making it possible to calculate average time spent per consumed prey.

Experiment 1: Uneven distribution of genotypes among groups

The fish in the first experiment belonged to three weight categories (3, 4, and 5) and there were four fish in each group. On the last day of the experiment, blood samples were taken from the 11 fish still alive so that haemoglobin (*HbI**) genotype could be determined by gel electrophoresis (McFarland 1977; Jørstad & Nævdal 1989). Because the genotypes were determined after the feeding experiment had terminated, they were not evenly distributed among the groups. In one group there were three *HbI*1/2* individuals and one *HbI* 2/2* genotype. In the second group there were two *HbI* 2/2* individuals and one each of the *HbI*1/1* and *HbI*1/2* genotypes. In the third group one individual had *HbI*1/2* genotype, two were *HbI*1/1* and the fourth had unknown haemoglobin genotype. This latter fish had jumped out of the tank three days before the experiment was terminated, and blood samples could not be taken.

We used a 6-week acclimation period during which

the fish were given the opportunity to learn the experimental procedure. Both in the acclimatisation and during the experimental period the fish were fed every other morning until they would accept no more prey (mean size 0.43 g), or they had taken the maximum offered to each tank (40g). After 3, 7, or 24 h, fish were again fed and offered a maximum of 20 g. Gobies were always offered sequentially. Hence, there are repeated observations for each individual for the period 24 October - 12 November 1996.

Experiment 2: Even distribution of genotypes among groups

The fish in this experiment belonged to weight categories 1, 2, and 3 and the haemoglobin (*HbI**) genotype was determined by gel electrophoresis before the fish were grouped. In each of three groups there was one individual of each of the three *HbI** genotypes. Both in the six-week acclimatisation period prior to the experiment and during the experiment the fish were fed gobies every other morning until they would accept no more prey (mean size 0.52 g). There was no fixed maximum number of prey given. In the middle of the day and after 7 h, fish were again fed until they accepted no more prey. There are repeated observations for each individual from the period 8-24 September 1997. During the 6-week acclimation period the fish were given the opportunity to learn the same experimental procedure as in Experiment 1.

DATA ANALYSIS

The aim of our experiment was to test the hypothesis that cod with the *Hb*2/2* genotype would be the most effective competitors. We used repeated observations of the same fish to benefit from the valuable information that can be gained from a panel-data[1] set (i.e. longitudinal data). To assess feeding performance of individuals we used 3 indices: capture success, first prey, and standardised prey number taken.

Analysing variation in prey capture success early in feeding trials

One index of competitive performance was the number of prey captured by each fish during encounter with the first 20 prey (referred to as *capture success*). This index was assumed to be distributed normally. Data sequences that were longer than 20 captures by all of the cod in a group were truncated and only the first 20 captures used in the statistical analysis. This was done to standardise the trials and to avoid bias which could have been caused by cod running out of prey before they were full. This was possible for some of the trials in Experiment 1. The summed weight of the first 20 prey delivered was always less than the maximum weight of prey offered to the group. A second index for competitive performance was the position in the sequence of prey offered where a cod took its first prey (denoted henceforth as *first prey*).

We analysed the effect on *capture success* of haemoglobin genotype, group, fish size, *first prey* category (see below), sex and maturation status (all fixed effects) using Components of Variance Analysis (CVA) (Winer & al. 1991; Diggle & al. 1994; Greene 1997) implemented by the software STATA6 (1999). When used as fixed effect *first prey* was categorised into values 1 (sequence positions 1-5), 2 (positions 6-10), and 3 (positions >10) and denoted *first prey category*. We used three categories for haemoglobin genotype, fish size and group and had two categories for sex and maturation status. The CVA accounts for repeated observations from the same individuals. Hence, the CVA is one statistical way out of the so-called "pseudo-replication problem" noted by Hurlbert (1984), Krebs (1989), and Barnard & al. (1999). The way the CVA deals with this is to incorporate other uncontrolled effects into a random variable (denoted here by *Fish*) which reflects any unobserved contributions to *capture success* variability deriving from each individual and which is stochastically determined because the individual fish being selected at random for the experiment. The *Fish* variable is specified with its own error which

[1] Panel-data means that one has repeated observations from the same individuals. Hence, there is dependency between repeated observations from an individual. This type of data are often denoted as "pseudo-replicates" by many scientists (Hurlbert 1984; Krebs 1989; Barnard & al. 1999) because repeated observations violates the assumption of independence among data points which underlies statistical analyses such as ANOVA, OLS-regression and the most common statistical approaches used in marine biology. It will therefore be incorrect to use these statistical methods on panel-data. As a clarification we emphasize two quotations: The first is by Krebs (1989; p 274, but see also p 287-288); "*Successive samples from a single experimental unit are clearly not independent samples and should not be analyzed as replicates but as repeated measures analysis of variance*". The second is by Hurlbert (1984; p 205); "*It should be remembered that repeated sampling of experimental units and the use of such data in statistical analysis can be quite proper in some circumstances. It is only the treating of successive data as if they were independent replicates of a treatment that is invalid*. Any statistical analysis of such data requires special estimation techniques such as Repeated Measures Anova, Components of Variance Analysis etc. (c.f. Winer & al. 1991; Diggle & al. 1994; Greene 1997). Alternatively, one could calculate the average for each individual and then apply e.g. classical statistical ANOVA (e.g. Barnard & al. 1999; p 68-69). However, averaging will omit the interesting information of individuals collected.

is assumed normally distributed. By definition the effect from the random variable is independent of the effect from the fixed effect variables (Winer & al. 1991; Diggle & al. 1994). All effects are tested against the variation in *Fish*. Fixed effects that are significant in a CVA have been adjusted for the fact that data consist of repeated observations. If the *Fish*-variation is significant and its large, then its evident that there are some systematic differences between the individuals that have not been captured by the specified fixed effects. If the *Fish*-variation is not significant, this shows that the fixed effects such as haemoglobin genotype, groups, fish size, sex, maturity state, have captured and explained most of the variance in *capture success* and have not left out any important factor.

We believe the assumptions underlying our statistical analysis have been met because: 1) The *Fish*-variable is specified as a stochastic or random variable, and not a fixed effect variable so as to capture potentially unspecified explained variance that is not accounted for by the fixed effects which could be correlated with the *Fish*-variable. Hence, all relevant variables that could affect the prey capture success have been considered. 2) The procedures used were the same during both experiments, including the acclimatisation periods. 3) Fish were not given food every day so as to reduce the possible state-dependent influence of hunger on *capture success*. This means that between observational days no state-dependent feeding motivation was expected. There might be a possibility of state-dependence within the feeding day with a lower feeding motivation on the second daily trial for those that took more prey in the first trial, and *vice versa*. This could reduce individual differences within day, but would generate a large variation within individuals which could generate significant unobserved effects from the CVA estimation. If so, this would suggest that not all relevant factors had been considered.

Analysing the distribution of first prey taken

We applied the Components of Variance Analysis (CVA) to the *first prey* index to test our hypothesis further. We examined the influence of haemoglobin genotype on *first prey* and also looked at the influence of group, fish size, sex, and maturation status. We also controlled for unobserved effects through the variable *Fish* as defined earlier.

Table 1. The explained sum of squares obtained from a Components of Variance Analysis when analysing the effect of up to six factors on 1) the *capture success* of the first 20 encountered prey and 2) the position in the sequence of prey offered of the first successful capture (*First prey*). Factors considered are haemoglobin genotype, fish size, group, *first prey category* (only for *capture success*), and unobserved causes of individual variation. The effects of these variables are examined with (A) and without (B) the addition of sex and maturation status as explanatory variables. *F*-values and *df*'s for each component are in parenthesis. R^2 refers to the adjusted R-squared. RSS is the residual sum of squares.

Experiment 1: 1996				
Dependent variable	*Capture success*	*Capture success*	*First prey*	*First prey*
Effects	*A*	*B*	*A*	*B*
*Hbl**	8.0 (P = 0.4) (F = 0.97, df = 2)	14.8 (P = 0.06) (F = 4.09, df = 2)	58.0 (P = 0.090) (F = 5.96, df = 2)	32.7 (P = 0.5) (F = 1.90, df = 2)
Group	49.8 (P = 0.019) (F = 6.04, df = 2)	53.4 (P = 0.002) (F = 14.75, df = 2)	86.1 (P = 0.055) (F = 8.86, df = 2)	36.1 (P = 0.4) (F = 2.10, df = 2)
Fish size	55.8 (P = 0.014) (F = 6.77, df = 2)	58.7 (P = 0.002) (F = 16.21, df = 2)	120.5 (P = 0.035) (F = 12.41, df = 2)	35.4 (P = 0.4) (F = 2.06, df = 2)
First prey category	33.2 (P = 0.052) (F = 4.02, df = 2)	33.02 (P = 0.009) (F = 9.12, df = 2)	-	-
Sex	-	10.0 (P = 0.046) (F = 5.54, df = 1)	-	0.04 (P = 0.9) (F = 0.01, df = 1)
Maturation status	-	18.62 (P = 0.013) (F = 10.29, df = 1)	-	2.07 (P = 0.7) (F = 0.24, df = 1)
Unobserved effects (Fish)	41.2 (P = 0.6) (F = 0.79, df = 10)	14.5 (P = 0.9) (F = 0.35, df = 8)	14.6 (P = 0.9) (F = 0.22, df = 3)	8.6 (P = 0.5) (F = 0.39, df = 1)
R^2	0.52	0.52	0.05	0.05
Model	348.2	348.2	256.3	256.3
RSS	145.8 (df = 28)	145.8 (df = 28)	823.5 (df = 37)	823.5 (df = 37)

Analysing the standardised prey number taken

The third index used was a rank. We calculated the average location in the sequence of prey eaten when *n* prey were offered and m eaten (m ≤ n) for each trial and for each fish using un-truncated data. We then standardised for the unequal numbers of prey introduced to each group of fish in each trial by assigning a rank to individual fish for each trial based on these average values. Rank 1 was assigned to the fish that had the smallest average, rank 2 to the second smallest, rank 3 to the third smallest and rank 4 to the individual that had the fourth smallest (or the largest) average value. There were only three possible ranks in Experiment 2. A small rank value therefore means that an individual took prey early in the sequence. We then calculate the average for an individual of each haemoglobin genotype. We refer to this rank as the *standardised prey number taken*.

RESULTS

Experiment 1

When controlling for the unobserved individual differences and taking account of repeated observations of individual fish using the Components of Variance Analysis we found that haemoglobin genotype, group composition, fish size, sex, maturity stage, and *first prey category* had an effect on the *capture success* (A and B; Table 1). The *HbI** effect was significant at 10 % level (P = 0.06) whereas the other effects were significant at 5 % level. The *HbI*1/1* genotype had lower success than the *HbI*1/2* and *HbI*2/2* genotypes, large fish had a higher success than did small fish, females had higher success than males and maturing individuals had higher success than those that did not show any sign of maturation at the end of the experiment (Fig. 1). Other unobserved individual differences (included in *Fish*) were highly insignificant (P = 0.6 for A and P = 0.9 for B; Table 1). Sex and maturation status did, however, not have an effect on the position in the sequence of the *first prey* (P = 0.9 for sex and P = 0.7 for maturation status; B Table 1). Exclusion of these insignificant effects from the analysis shows that the fish size had a significant effect on *first prey* (P = 0.035; Table 1, A) and there were indications that genotype (P = 0.09) and group composition (P = 0.055) also had effects although they were not significant at the 5 % level. The *HbI*1/1* genotype tended to take prey later in the sequence than the *HbI*2/2* and *HbI*1/2* genotypes and large fish took prey earlier than small fish (Fig. 1).

The *standardised prey number* taken was 2.75 ± 0.11 for *HbI*1/1*, 2.32 ± 0.14 for *HbI*1/2*, and 2.31 ± 0.13 for *HbI*2/2*. This suggest that the *HbI*2/2* and *HbI*1/2* took prey earlier in the sequence than the *HbI*1/1* genotype.

Table 1. (continued)

Experiment 2: 1997				
Dependent variable	*Capture success*	*Capture success*	*First prey*	*First prey*
Effects	*A*	*B*	*A*	*B*
*HbI**	168.7 (P = 0.0005) (F = 15.25, df = 2)	80.2 (P = 0.018) (F = 6.19, df = 2)	374.7 (P = 0.5) (F = 1.17, df = 2)	327.5 (P = 0.06) (F = 131.2, df = 2)
Group	64.1 (P = 0.017) (F = 5.79, df = 2)	11.4 (P = 0.4) (F = 0.88, df = 2)	35.1 (P = 0.9) (F = 0.11, df = 2)	198.4 (P = 0.08) (F = 79.5, df = 2)
Fish size	107.2 (P = 0.003) (F = 9.69, df = 2)	52.6 (P = 0.051) (F = 4.06, df = 2)	116.9 (P = 0.7) (F = 0.36, df = 2)	228.7 (P = 0.07) (F = 91.6, df = 2)
First prey category	68.3 (P = 0.014) (F = 6.17, df = 2)	61.9 (P = 0.035) (F = 4.78, df = 2)	-	-
Sex	-	1.7 (P = 0.6) (F = 0.26, df = 1)	-	317.7 (P = 0.04) (F = 254.5, df = 1)
Maturation status	-	0.03 (P = 0.9) (F = 0.00, df = 1)	-	-
Unobserved effects (Fish)	66.4 (P = 0.9) (F = 0.43, df = 12)	64.8 (P = 0.9) (F = 0.51, df = 10)	320.4 (P = 0.0002) (F = 10.11, df = 2)	1.23 (P = 0.8) (F = 0.08, df = 1)
R^2	0.33	0.33	0.41	0.41
Model	646.1	646.1	723.7	723.7
RSS	536.4 (df = 42)	536.4 (df = 42)	744.5 (df = 47)	744.5 (df = 47)

The cod used a similar time per consumed prey throughout the entire experimental period, and there was no difference between the groups. The average time (time ± SE) used per consumed prey was 6.4 ±0.50 s, 5.9 ± 0.40 s, and 7.10 ± 0.58 s for the three groups.

Experiment 2

When controlling for the unobserved individual differences and taking account of repeated observations of individual fish using the Components of Variance Analysis we found that haemoglobin genotype, group composition, fish size and *first prey category* all had an effect on the prey capture success (A; Table 1), whereas sex and maturity stage were not significant (B; Table 1). The *HbI*2/2* genotype had higher *capture success* than the *HbI*1/2* which in turn had higher success than the *HbI*1/1* genotype (Fig. 1). Small fish had slightly higher *capture success* than large fish, although differences were small (Fig. 1). Other unobserved individual differences (*Fish*) were highly insignificant (P = 0.9; Table 1). In the test of effects on *first prey*, the genotype, group composition and fish size had no significant effects when excluding sex and maturity stage as factors (*first prey* A; P > 0.5), whereas the highly significant unobserved effects (*first prey* A; P = 0.0002; Table 1) suggest that there were some other causes not accounted for. This other cause turned out to be sex (*first prey* B, P = 0.04; Table 1) as the unobserved effects then became insignificant (P = 0.8) when sex was included as a factor in CVA. This inclusion also reduced the P-values for the haemoglobin genotype, group and fish size to be in the range 0.06-0.08 (Table 1) and the result support the trend along the x-axis of panel "genotype" of Fig. 1. The *HbI*2/2* genotype took their first prey earlier in the sequence than did the *HbI*1/1* and *HbI*1/2* genotypes, and males took their first prey much earlier than females (Fig. 1). We could not test the effect of maturity stage as there were insufficient degrees of freedom, but Fig. 1 suggest that individuals that had begun to mature by the end of the experiment took prey earlier than those that were immature. Nevertheless, the results indicate that maturation had an indirect effect since 5 out of 6 males had begun to mature whereas none of the 3 females had. The observed sex effect (Table 1) may thus be interpreted as an effect of maturation.

The *standardised prey number* taken was 2.06 ± 0.12 for *HbI*1/1*, 2.52 ± 0.09 for *HbI*1/2*, and 1.57 ± .012 for *HbI*2/2*. This suggests that an average individual with the haemoglobin genotype *HbI*2/2* feeds earlier than the other two genotypes. This supports the trend in Fig. 1.

The average time (time ± SE) used per consumed prey was around four times longer in Experiment 2 than in Experiment 1, and was 24.5 ± 2.1 s, 22.1 ± 2.1 s, and 29.3 ± 24.9 s, respectively in groups 1, 2, and 3. Except for 4 trials in one of the groups, the average time spent per prey was similar through the entire period of Experiment 2.

DISCUSSION

Our hypothesis was that individuals with the *HbI*2/2* genotype will have a higher motivation to feed, will be better competitors and will eat a larger share of the prey than individuals with *HbI*1/2* and *HbI*1/1* genotypes. The results we have obtained are in agreement with these expectations (Table 1, Fig. 1). In both experiments the *HbI*2/2* genotype had the highest *capture success*. In Experiment 1 the high *capture success* was shared with heterozygous individuals, whereas in Experiment 2 the *HbI*2/2* genotype had higher *capture success* than both *HbI*1/2* and *HbI*1/1* genotypes. The haemoglobin effect on *first prey* taken showed the same trend among the genotypes but was, however, less clear as it was not significant at 5 % level but at 10 % as 0.05 < P < 0.08.

The results also show that other factors are important. These are body size, sex, stage of maturation, and group. In Experiment 1 the large fish had higher *capture success* and took prey earlier than small fish whereas in Experiment 2 the reverse was true and small fish also tended to take prey slightly earlier than large fish. Females and individuals that had begun to mature had higher *capture success* than males and immature fish in Experiment 1 whereas the difference was less clear in Experiment 2. In this there were no differences in capture success between the sexes, but males tended to take prey earlier than females.

Our results agree with previously published relationships between haemoglobin genotype and growth. The field data on cod, presented in Mork & al. (1984), and the data on hatchery cod in Nævdal & al. (1992) demonstrate that *HbI*2/2* individuals had the highest growth. Moreover, Karpov & Novikov (1980) and Brix & al. (1998) show that the haemoglobin in cod having this genotype has higher efficiency as an O_2-carrier at low temperatures. The haemoglobin molecules that these genotypes possess are determined by characteristics inherited from their parents. The molecules have a structure that results in a higher oxygen uptake and transport efficiency at low temperatures and high oxygen availability compared with the *HbI*1/1* genotype (Weber 1990). Our results suggest that the link between *HbI** genotype and growth is through feeding behaviour, supporting the idea that fish with *HbI*2/2* genotype support a more active metabolism. These fish would be expected to have higher food demand and more active behaviour to service such needs.

Studies on Atlantic salmon (Metcalfe & al. 1992, 1995; Cutts & al. 1998) and masu salmon (*Oncorhynchus masu*

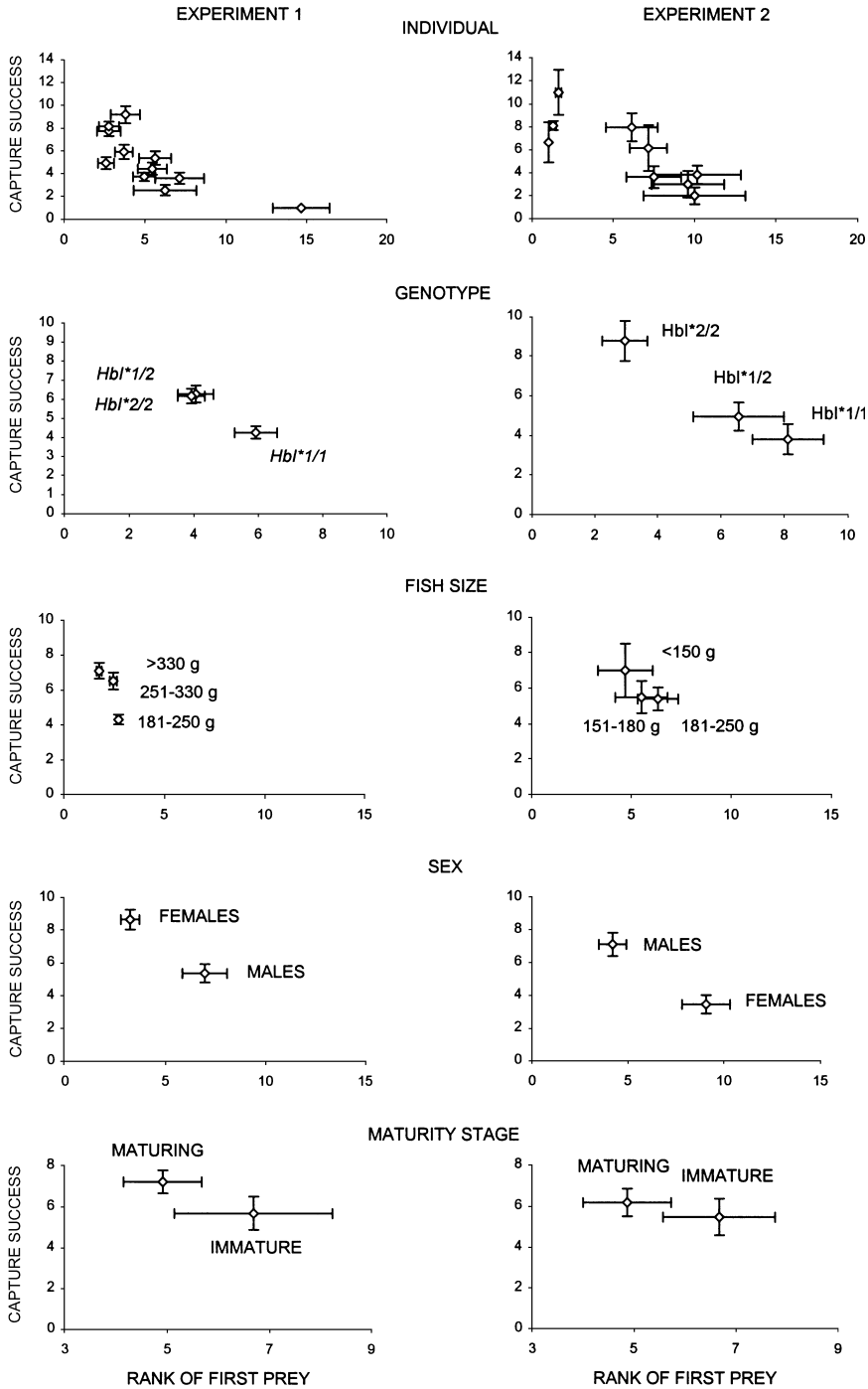

Fig. 1. Capture success versus rank of first prey for individual fish, haemoglobin genotype, fish size, sex, and maturity stage. Average values are shown. Bars refer to SE.

Brevoort) (Nakano 1995; Yamamoto & al. 1998) have shown that metabolic rate is correlated with aggression, which in these territorial species determines success during feeding. Fish with high residual metabolic rate had high status, obtained more food and grew faster to a large size. In salmonids aggressiveness is an inherited trait (Taylor 1990; Dunbrack & al. 1996), a trait which Cutts & al. (1998) demonstrated to be linked to individual differences in physiology when they controlled for size. Our results suggest that a relationship between feeding success and physiology possibly also exists in cod, although there is no association with aggression. Our results suggest that haemoglobin structure, which sets the physiological basis of an individual's metabolic efficiency, has an effect upon competitive performance in a situation when prey have to be competed for.

We have assumed that the reaction we observed in the experimental period was the response of individuals in groups of fish that were well versed in the experimental protocol and that there was no learning during the experimental period. This contrasts with results of Steingrund & Fernö (1997) who reported improved foraging skills of cod with increasing experience. These authors tested the fish during a day–long learning period and after only a three-day acclimation period to the experimental tanks during which time no feeding occurred. At the onset of their feeding experiment, fish were thus new to the experimental protocol. We did not include the learning period in our experiment as we put the emphasis on the effect of haemoglobin genotype and other relevant causes influencing individual variation in feeding behaviour. We controlled for learning using a prolonged acclimatisation period of six weeks during which fish were adapted to the experimental protocol. It is interesting to note that fig. 4A & B in Steingrund & Fernö (1997) shows a large increase in foraging skills from the first to the second trial within a day, followed by a much smaller change. Based on these considerations, and on the fact that cod spent similar times per consumed prey throughout the entire experimental period within each of our experiments, we conclude that our assumption of no learning during the experimental period is justified.

The higher time spent per prey in Experiment 2 might be due to differences in the total amount of prey given in each trial. In Experiment 2 fish were always fed until they did not take more prey (until satiation) and it was observed (although not quantified) that close to satiation the fish tended to devote a longer time to each prey. This would increase mean time spent per prey. In Experiment 1 this was slightly different as the fish were fed until they took no more prey or had taken the maximum on offer. As it turned out the fish took the maximum prey offered in most trials (87 %), so they were less close to satiation than the fish of Experiment 2. It was therefore not unexpected that the fish on average spent a shorter time per prey in Experiment 1 than in Experiment 2.

We have also assumed that the cod is a scramble competitor. We observed no interactions between individuals that could have been construed as showing a hierarchy. We did not systematically record possible co-operative behaviour, although co-operation could be important for a shoaling species (Mestertongibbons & Dugatkin 1992). One-year-old coastal cod are generally not considered to shoal. However, due to the space limitations experienced by our experimental fish, the possibility exists for developing co-operation between the individuals during foraging, but if this was so it was not evident from our data. The individuals that we observed responding first to encountered prey during the course of a trial were later identified in our analysis as the most successful and we believe that behavioural interactions ameliorating the effects of competition did not develop to bias our results.

In common with many experimental behavioural studies our results are limited by the small number of fish used and by the use of an experimental environment that differs from the natural habitat (e.g. Kadri & al. 1996; Steingrund & Fernö 1997; Barber & Ruxton 1998). Consequently the results may not be regarded as generally valid for all types of habitats or populations. The individual fish had smaller space available than they otherwise would in nature. As we were interested in the effects of genetic differences on competitive performance, our need to control and standardise the environment dictated that we use experimental tanks of manageable size for observations of events at the individual level, and to standardise the temperature and light conditions. Our results showed that haemoglobin genotype can be an important characteristics for one-year-old cod and that it is linked to competitive performance. This effect was clearly significant and *was not* masked by other unobserved effects (confounding factors) that potentially could correlate with it. We expect that similar results would be found among wild cod if tested under similar conditions as in our experiments and we expect similar mechanisms to operate in cod in nature, although more experiments are required to test this.

A strong reason for believing that the genetic effects we have demonstrated are important is the fact that inherited traits affecting fish behaviour are well documented. For example sex differences in schooling behaviour have been reported for Trinidadian guppies (*Poecilia reticulata*) (Magurran & Seghers 1994b; Griffiths & Magurran 1998) and sex differences in the courtship behaviour in cod and other gadoids (Brawn 1961; Hislop 1984; Hutchings & al. 1999). Our study is one of the first on fish that suggests an inherited behav-

iour which is related to a genetic marker other than sex. The reported superior competitive ability of the cod with *HbI*2/2* genotype, when unobserved individual differences are controlled for, is likely to have been due to an inherent and rapid response to food, as cod did not attempt to prevent other fish from feeding.

Although we cannot draw strong conclusions about wild cod from our study there are direct implications for the aquaculture of cod. It is well known that farmed fish that start the same length soon develop a large variation in size. Grading by weight or length is used to reduce the effects of this size hierarchy on growth rates. Our results show that size alone is unlikely to be the sole cause of the observed difference. The growth rate differences in farms could be a direct result of physiological variation caused by genetic differences. The size grading of fish may just be sorting out those with high metabolic rates from those with low and the groups with large fish should eventually come to be composed of higher proportions of the *HbI*2/2* genotype than grader groups containing small fish.

ACKNOWLEDGEMENTS

AGVS wish to forward a special thank to professor Trygve Nilsen at the Dept. of Mathematics, Section of Statistics, University of Bergen for advice in the statistical analysis and to Jens Krause for valuable comments to the manuscript. We thank Linda Anett Hansen, Are Jacobsen, and Frank Midtøy for technical assistance, Gunnar Nyhammer for the fish, Torild Johansen for help with gel electrophoresis, and Albert Imsland and five anonymous referees for comments to an earlier version of the paper. AGVS appreciated the discussions on cod behaviour with Charlotte Hemelrijk and Alexander Skolnick. PH's visits to Bergen were funded by grants from the Research Council of Norway, the TMR Programme of the EU (Contract ERBFMGECT950013), and the Royal Society of London. AGVS was funded by the Research Council of Norway (Contract Number 113726/122).

REFERENCES

Barber I, Ruxton GD. 1998. Temporal prey distribution affects the competitive ability of parasitized sticklebacks. *Animal Behaviour* 56:1477-1483.

Barnard C, Gilbert F, McGregor P. 1999. *Asking questions in biology. Design, analysis and presentation in practical work*. Harlow: Addison Wesley Longman. 157 p.

Brawn VM. 1961. Reproductive behaviour of the cod. *Behaviour* 18:177-197.

Brix O, Forås E, Strand I. 1998: Genetic variation and functional properties of Atlantic cod haemoglobins: introducing a modified tonometric method for studying fragile haemoglobins. *Comparative Biochemistry and Physiology* 119A:575-585.

Cutts CJ, Metcalfe NB, Taylor AC. 1998. Aggression and growth depression in juvenile Atlantic salmon: the consequences of individual variation in standard metabolic rate. *Journal of Fish Biology* 52:1026-1037.

Diggle PJ, Liang K-Y, Zeger SL. 1994. *Analysis of longitudinal data.* Oxford: Clarendon Press. 253 p.

Dunbrack RL, Clarke L, Bassler C. 1996. Population level differences in aggressiveness and their relationship to food density in a stream salmonid (*Salvelinus fontinalis*). *Journal of Fish Biology* 48:615-622.

Greene WH. 1997. *Econometric Analysis. 3rd edition*. New Jersey: Prentice-Hall. 1072 p.

Griffiths SW, Magurran AE. 1998. Sex and schooling behaviour in the Trinidadian guppy. *Animal Behaviour* 56:689-693.

Hislop JRG. 1984. A comparison of the reproductive tactics and strategies of cod, haddock, whiting and Norway pout in the North Sea. In: Potts GW, Wootton RJW, editors. *Fish reproduction. Strategies and tactics.* London: Academic Press. p 311-329.

Hurlbert SH. 1984. Pseudoreplication and the design of ecological field experiments. *Ecological Monographs* 54:187-211.

Hutchings JA, Bishop TD, McGregor Shaw CR. 1999. Spawning behaviour of Atlantic cod, *Gadus morhua*: evidence of mate competition and mate choice in a broadcast spawner. *Canadian Journal of Fisheries and Aquatic Sciences* 56:97-104.

Jobling M. 1994. *Fish bioenergetics*. London: Chapman & Hall. 309 p.

Jørstad KE, Nævdal G. 1989. Genetic variation and population structure of cod, *Gadus morhua* L., in some fjords in northern Norway. *Journal of Fish Biology* 35 Suppl A:245-252.

Kadri S, Huntingford FA, Metcalfe NB, Thorpe JE. 1995. Social interactions and the distribution of food among one-sea-winter Atlantic salmon (*Salmo salar*) in a seacage. *Aquaculture* 139:1-10.

Karpov LK, Novikov GG. 1980. The haemoglobin alloforms in cod (*Gadus morhua* L.), their functional characteristics and distribution in the populations [translated from Russian]. *Voprosy Ichtiologii* (T 20) 6:823-827.

Krebs CJ. 1989. *Ecological methodology*. New York: Harper & Row. 654 p.

Magurran AE, Seghers BH. 1994. Predator inspection behaviour covaries with schooling tendency amongst wild guppy, *Poecilia reticulata*, populations in Trinidad. *Behaviour* 128:121-134.

McFarland G. 1977. The staining of starch gels with Coomassie Blue G250 perchloric acid solution. *Experentia* 33:166-168.

Mestertongibbons M, Dugatkin LA. 1992. Cooperation among unrelated individuals - evolutionary factors. *Quarterly Review of Biology* 67:267-281.

Metcalfe NB, Taylor AC Thorpe JE. 1995. Metabolic rate, social status and life history strategies in Atlantic salmon. *Animal Behaviour* 49: 431-436.

Metcalfe NB, Wright PJ, Thorpe JE. 1992. Relationships between social status, otolith size at first feeding and subsequent growth in Atlantic salmon (*Salmo salar*). *Journal of Animal Ecology* 61:585-589.

Mork J, Giskeødegård R, Sundnes G. 1984. The haemoglobin polymorphism in Atlantic cod (*Gadus morhua* L.); genotypic difference in somatic growth and in maturing age in natural populations. *Flødevigen Rapportserie* 1; The propagation of cod *Gadus morhua* L.:721-732.

Nævdal G, Folkvord A, Otterlei E, Thorkildsen S. 1992. Growth rate related to genotype of 0-group cod at three environmental temperatures. *Sarsia* 77:71-73.

Nakano S. 1995. Individual differences in resource use, growth and emigration under the influence of a dominance hierarchy in fluvial red-spotted masu salmon in a natural habitat. *Journal of Animal Ecology* 64:75-84.

Salvanes AGV, Giske J, Nordeide J. 1994. Life-history approach to habitat shifts for coastal cod, *Gadus morhua* L. *Aquaculture and Fisheries Management* 25 Suppl 1:215-228.

Salvanes AGV, Nordeide J. 1993. Dominating sublittoral fish species in a west Norwegian fjord and their trophic links to cod (*Gadus morhua* L.). *Sarsia* 78:221-234.

Sick K. 1961. Haemoglobin polymorphism in fishes. *Nature* 192:894-896.

StataCorp. 1999. *Stata Statistical Software*: *Release 6.0.* College Station, Texas: Stata Corporation.

Steingrund P, Fernö A. 1997. Feeding behaviour of reared and wild cod and the effect of learning: two strategies of feeding on the two-spotted goby. *Journal of Fish Biology* 51:334-348.

Taylor EB. 1990. Variability in agonistic behaviour and salinity tolerance between and within two populations of juvenile chinook salmon, *Onchorynchus tshawytscha*, with contrasting life histories. *Canadian Journal of Fisheries and Aquatic Sciences* 47:2172-2180.

Weber RE. 1990. Functional significance and structural basis of multiple haemoglobins with special reference to ectothermic vertebrates. In: Truchot J-P, Lahlou B, editors. *Comparative Physiology. Volume 6. Animal nutrition and transport processes. 2. Transport, respiration and excretion: Comparative and Environmental Aspects*. Basel: Karger. p 58-75.

Winer BJ, Brown, DR, Michels KM. 1991. *Statistical principles in experimental design*. New York: McGraw-Hill, Inc. 1057 p.

Yamamoto T, Ueda H, Higashi S. 1998. Correlation among dominance status, metabolic rate and otolith size in masu salmon. *Journal of Fish Biology* 52:281-290.

Accepted 27 October 1999 – Printed 29 September 2000
Editorial responsibility: Jarl Giske