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A retrospective approach to fractionize variation in body mass of Atlantic cod *Gadus morhua*

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Eggs of a single spawning batch from wild-caught Norwegian Atlantic cod *Gadus morhua* were hatched and first fed on either natural zooplankton or enriched rotifers *Brachionus plicatilis* during the larval period. Juvenile *G. morhua* (initial mass 14·2 g) from the two first-feeding groups were then reared for 3 months under a variety of temperature (10 and 14° C) and salinity (15 and 32) combinations. All fish were individually tagged and microsatellite markers were used in a multiplex to trace the pedigree of all fish and body mass variation analysed according to different environmental and genetic sources. After the termination of the laboratory trial, the fish were transferred to land-based tanks and later to sea pens and reared at ambient conditions for 26 months until they were harvested in March 2009. Growth gain from the larval and juvenile periods was persistent during the 26 months of sea pen ongrowing. The final mass of the zooplankton group was 12% higher compared to the *B. plicatilis* group. Similarly, rearing under a temperature of 14° C and salinity of 15 during the initial 3 month period during the early juvenile stage resulted in 7–13% larger size at harvesting compared to the other three temperature and salinity combinations. The study indicates that the first-feeding method and temperature and salinity manipulation explain nearly 90% of the body mass variation explained by the model. The genetic effect (measured as body mass variation within the families studied) only accounted for *c*. 2% during the initial rearing period, whereas it has a large effect on growth variation (30%) during the long-term rearing at ambient conditions. Sex proportion and final maturation did not differ between family groups, and no interaction between sex and family group was seen.

Key words: environmental manipulation; growth; long-term effects; pedigree.

INTRODUCTION

Atlantic cod *Gadus morhua* L. experiences a wide range of environmental conditions in its distribution area (Brander, 1995; Suthers & Sundby, 1999). Variable and changing environmental conditions may affect growth and mortality and generate

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recruitment variability (Buckley *et al*., 2004; Baumann *et al*., 2006). Many attempts to relate variation in recruitment to environmental conditions, however, have failed because of the interactions of covarying factors (*e.g.* fish size, temperature, food availability and genetic effects), which makes it difficult to quantify the extent of covariation and the effect of each factor separately. Another way is to study such relationships under controlled and standardized environmental conditions in the laboratory and then track possible long-term effects when the fish are reared under ambient conditions. The study of Hanson (1996) indicated that juvenile *G. morhua* can be found in shallow water during summer, but migrate into deeper water during autumn and winter. This could be related to juvenile thermoregulative behaviour of the deep-water *G. morhua*, where the fish avoid the low winter temperatures in shallow water. Little is known about the potentially positive growth effects of salinity on *G. morhua* juveniles although Lambert *et al*. (1994) found that growth rates were highest for *G. morhua* maintained in intermediate salinity conditions (14). In this study, the effect of near-optimum temperature (14° C) and near-optimum salinity (15) on the short and long-term growth in *G. morhua* was investigated.

Analysis of variation in microsatellite loci often reveals population differences in exploited species at small geographical scales, which can subsequently be integrated into fisheries management. Although various methods can be used for assessing stock structure (Jónsson, 1996; Jónsdóttir *et al*., 1999; Imsland & Jónsdóttir, 2003), genetic analyses are the most informative and economical method. Microsatellite loci have revealed a high level of structure in *G. morhua* populations within the Atlantic Ocean (O'Leary *et al*., 2007). Recently, Jakobsdóttir *et al*. (2006) and Skírnisdóttir *et al*. (2008) reported 27 new microsatellite loci suitable for population genetics, genetic monitoring and kinship as well as pedigree tracing in *G. morhua* farming. Twenty of these new microsatellites can be managed in only two large multiplex assays systems, which is a major advantage. Larger number of samples and smaller DNA quantities can then be genotyped at reasonable cost and time. The manipulation of a large number of samples is also minimized and therefore, the risk of handling errors. In the present study, these multiplex markers were utilized to trace the pedigree of group of individually tagged *G. morhua* reared under different nutritional and environmental conditions. The study material consisted of fish from a single mass spawning of multiple broodfish, but by grouping the material into different families, size variation in adult fish could be traced to its original, *e.g.* environmental and genetic, sources.

The purpose of this study was to investigate the possible short- and long-term effect of different first-feeding methods, rearing salinities and temperatures and family on final body size of *G. morhua* at harvest and to fractionize body mass variation into different environmental and genetic sources.

MATERIALS AND METHODS

FISH MATERIAL, REARING CONDITIONS AND FIRST-FEEDING STUDY

Eggs from a single mass spawning batch (number of fish in spawning tank; $n_{\text{total}} = 80$, $n_{\text{females}} = 32$, $n_{\text{males}} = 48$) of *G. morhua* (multiple spawners, egg collected at tank surface) were incubated at a commercial *G. morhua* hatchery in western Norway (61° 40′ N) and hatched on 9 April 2006. The broodfish were caught wild in the area around Bømlo (west

Norway) in 2003–2005 and reared in 40 m^3 tanks under simulated natural photoperiod and temperature of 6–8° C (sea water pumped from 160 m depth). The mean mass of the broodfish was *c*. 7 kg (range 5–18 kg). Hatched larvae were transported to the Bergen High Technology Centre and were randomly distributed over four 1 m^3 green fibreglass tanks. Larvae in two tanks were fed with enriched rotifers *Brachionus plicatilis* (R group) and larvae in the other two tanks were fed natural zooplankton (Z group). Larvae were reared as part of a large-scale research project (Koedijk *et al*, 2010). Briefly, larval densities were 14 larvae l^{-1} and prey densities were kept at 2000–3000 prey l^{-1} by feeding three times daily. Natural zooplankton (consisting mainly of calanoid nauplii, gradually increasing size fraction from 80–250 μm, until 17 days post hatch (dph) to 80–400 μm, from 18 dph until weaning) was automatically filtered from the sea and transported to the experimental facilities on a daily basis. Rotifers were enriched (40–60% oil emulsion, 30–50% protein and 2–3% vitamins) and transported daily from a commercial *G. morhua* hatchery. All larvae were weaned onto commercial dry feed from 36 dph onwards (EWOS Marin 20, EWOS AS; www.ewos.com) containing 55% protein, 12% fat and 11% carbohydrates.

LABORATORY STUDY

Juvenile fish from both first-feeding groups (mean ± S.E. mass 14·2 ± 0·4 g) were anaesthetized (metacain, 0·05 g l^{-1}) and individually tagged intraperitoneally with passive transponder tags (Trovan; www.trovan.com; $n_{total} = 282$) and exposed to full (32) or reduced (15) salinity in combination with two temperatures (10 or 14° C) in duplicate tanks for a period of 95 days (25 September to 18 December 2006). All fish were individually weighed at the start and the termination of the laboratory trial. Both groups were equally represented within each of the eight experimental tanks (1 m^2 square, grey, covered fibreglass tanks with a rearing volume of 400 l). At the termination of the laboratory trial, a fin-tissue sample was taken from each fish and preserved in ethanol until genetic analysis. Water flow was set to 10 l min^{-1} for all experimental tanks. Oxygen saturation was measured daily in the effluent (*i.e.* bottom outlet) water of all tanks and was >80% at all times. The fish were reared at fixed 20 L (including twilight): 4D during the laboratory trial. A 36 W fluorescent daylight tube integrated in the tank cover provided light. Photon irradiation measured at the bottom of the tanks was *c*. 5 μmol m^{-2} s^{-1}. During the experiment, the juveniles were fed a commercial dry diet (Marin 10 and 20, Ewos AS; 55% protein, 12% fat and 11% carbohydrate; gross digestible energy 20·4 MJ kg^{-1}). Feed was provided in excess for 2 h daily (0800 to 0900 and 1400 to 1500 hours). Pellet size (2 and 3 mm) was adjusted during the experiment, depending on fish size, with an introduction of 3 mm pellets from October. A detailed description of the growth and feed utilization during the laboratory trial can be found in Koedijk *et al*. (2010).

The experiment described has been approved by the Norwegian Animal Research Authority (NARA) and registered by the Authority. The experiment was conducted in accordance with the laws and regulations controlling experiments on live animals in Norway, *i.e.* the Animal Protection Act of 20 December 1974, No. 73, chapter VI sections 20–22 and the Animal Protection Ordinance concerning Biological Experiments in Animals of 15 January 1996.

LONG-TERM STUDY

After the termination of the laboratory trial, all fish were pooled, acclimated to 10° C and transported by lorry on 22 February 2007 to the ongrowing facilities at the Institute of Marine Research, Austevoll (west Norway; 60° 04′ N). Here, the fish were held in land-based open tanks (3 m diameter, 15 m^3) with a mean temperature of 9° C, salinity of 34 and reared under natural photoperiod until 13 December 2007 when they were transferred to a sea pen (5 m in diameter, 5 m deep, 125 m^3 in volume) at the same facility. Here, the fish were reared at ambient temperatures (range of mean temperatures, maximum 16° C in August, minimum 5° C in March) and natural photoperiod. The fish were fed using a commercial formulated feed from Dana Feed (Dan-Ex 1562, containing 15% fat and 58% protein; www.biomar.com) from an automatic feeder. The fish were individually weighed in May 2007 and again at harvest on 11 March 2009, where both total body mass (M_T) and gonad mass (M_G) were measured.

DNA EXTRACTION

DNA was isolated using Chelex 100 Resin (Walsh *et al.*, 1991). Polymerase chain reactions (PCR) were performed in a 10 μl volume containing 3 μl of 1/10 diluted DNA, 200 μm of each dNTP, 1× TEG buffer (100 mM Tris–HCl, pH 8·8; 500 mM KCl; 15 mM $MgCl_2$; 1% Triton X-100), 0·6 U TEG polymerase (Matis-Prokaria, Taq comparable; www.matis.is), 0·03–0·075 μl of the labelled forward (100 μM) and 0·03–0·075 μl of the reverse primer (100 μM). Polymerase chain reactions were performed on PTC-225 Peltier (MJ Research; www.mjresearch.com) and Tetrad2 Peltier (Bio-Rad; www.bio-rad.com) thermal cyclers as follows: initial denaturation step of 4 min at 94° C followed by 30 cycles of 50 s at 94° C, 50 s at 55° C and 2 min at 72° C, final elongation step of 7 min at 72° C.

DNA MICROSATELLITES AND GENOTYPING

Tissue samples from *G. morhua* were genotyped at 10 microsatellite loci (*PGmo38*, *PGmo61*, *PGmo87*, *PGmo49*, *PGmo94*, *PGmo124*, *PGmo100*, *PGmo134*, *PGmo71* and *PGmo74*) (Jakobsdóttir *et al.*, 2006; Skírnisdóttir *et al.*, 2008). Samples were analysed on an ABI PRISM 3730 sequencer using the GeneScan-500 LIZ size standard, and genotyping was performed with GeneMapper 4.0 (Applied Biosystems; www.appliedbiosystems.com).

FAMILY ANALYSIS

Levels of genetic relatedness were calculated for pairs of individuals within the group using the programme Kinship 1.3.1 (Goodnight & Queller, 1999). The programme was used to investigate possible sibling relationships between individuals in the population. This software carries out maximum likelihood tests of pedigree relationships between pairs of individuals in a population. It uses genotype information for single-locus, codominant genetic markers (such as DNA microsatellite loci). Two hypothetical pedigree relationships are used, a primary hypothesis and a null hypothesis, and the programme calculates likelihood ratios comparing the two hypotheses for all possible pairs in the data set.

DATA ANALYSIS AND STATISTICAL METHODS

All fish were weighed individually to the nearest 0·1 g at the start and at the termination of the laboratory experiment (after 95 days) and to nearest 1 g on 10 May 2007 and 11 March 2009. Specific growth rate (G) was calculated according to the formula of Houde & Schekter (1981): $G = 100(e^g - 1)$, where $g = (\ln W_2 - \ln W_1)\ (t_2 - t_1)^{-1}$ and W_2 and W_1 is wet mass (g) at days t_2 and t_1, respectively. The gonado-somatic index (I_G) was calculated as: $I_G = 100 M_G\ M_T^{-1}$.

A Kolmogorov–Smirnov test (Zar, 1984) was used to assess the normality of distributions. The homogeneity of variances was tested using the Levene F-test (Zar, 1984). In the laboratory trial, a nested ANOVA (where the replicates are nested within the experimental variables) was applied to calculate the effect of different first-feeding regimes (two-way nested ANOVA), families (two-way nested ANOVA), salinities and temperatures (three-way nested ANOVA) on mean masses. Significant ANOVAs were followed by a Student–Newman–Keuls multiple comparison test to locate differences among treatments (Zar, 1984). Individual G trajectories were analysed using a growth curve analysis model (GCM; Chambers & Miller, 1995; Imsland *et al.*, 2007), which is an extension of the multivariate repeated-measurements analysis of variance (MANOVA) model. The model equation of the GCM for the temperature data had the form: $Y(n \times p) = X(n \times q)B(q \times p) + E(n \times p)$, where $Y(n \times p)$ are the growth-at-age vectors $y = (y_1, y_2, \ldots, y_p)$ for each p (age) measurements on n individual fish; $X(n \times q)$ is the design matrix or the set of extraneous variables measured for each individual, *i.e.* $q = \text{age}_p + \text{temperature}_i$ ($i = 10$ and 14° C) + salinity_j ($j = 15$ and 32) + family_k ($k = \text{F1}, \ldots, \text{F9}$); $B(q \times p)$ is the matrix of parameters estimated by the model; $E(n \times p)$ is the matrix of deviations for each individual from the expected value of $Y = XB$.

The contribution of the different independent variables (*i.e.* first-feeding regime, temperature and salinity in laboratory trial and different family membership) was estimated using the

VEPAC (variance estimation and precision) programme in STATISTICA (www.statsoft.com). In short, the programme will estimate the fixed effects design and random effects design separately, but incorporate the variance of random effects in the tests of the parameters of the linear model and related statistics (Searle *et al*., 1992; Demidenko, 2004). The model equation of the mixed model had the form: $y = X\beta + Z\gamma + \varepsilon$, where y is the vector of individual body masses, X is a design matrix that accounts for all fixed effects (first-feeding method, salinity and temperature) and β is the unknown vector of parameter estimates for fixed effects; Z is a design matrix that accounts for all random effects (here, individual variation and different families) and γ is the unknown vector of parameter estimates for random effects; ε is the vector of unknown random error which is no longer required to be independent or homogeneous. In this model, the variance components for both the random and fixed effects are estimated with a restricted maximum likelihood estimate (RML) procedure (Searle *et al*., 1992; Demidenko, 2004).

RESULTS

FAMILY ALLOCATIONS

The 10 microsatellite loci multiplex genotype scoring (Fig. 1) was analysed with a uniform error value of 0·02 (the default value for uniform error rate in the programme). All the individually tagged fish ($n = 282$) could be assigned into nine families (F1 to F9) with family size varying between 23 and 39 individuals (Table I). The proportion of the two first-feeding groups was similar within all nine families (37–42% from the Z group *v*. 58–63% from the R group).

SHORT-TERM EFFECT ON GROWTH

The overall initial mean ± S.E. mass was $14{\cdot}2 \pm 0{\cdot}4$ g and did not differ [three-way nested ANOVA, power $(1 - \beta) > 0{\cdot}7$, $P > 0{\cdot}05$; Table I] among experimental groups. At the termination of the laboratory trial, mean masses of the first-feeding groups differed significantly (two-way ANOVA, $P < 0{\cdot}05$), as the final mean masses of the Z group was 12% higher than those of the R group.

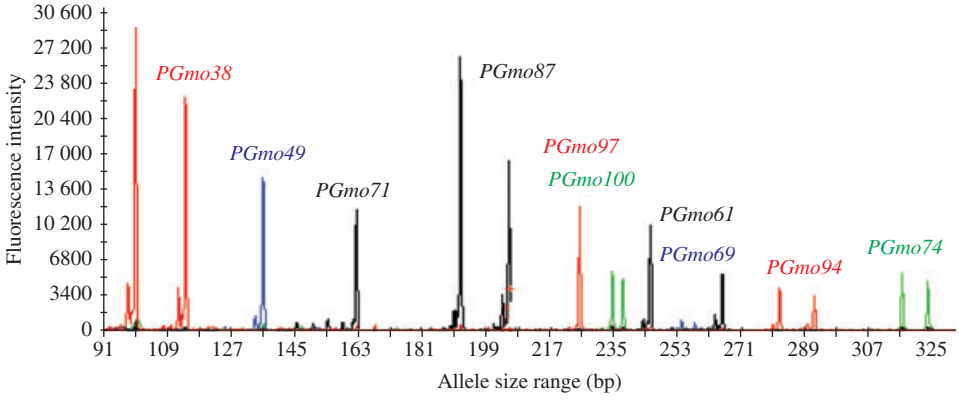

FIG. 1. Electropherogram from a single multiplex reaction of *Gadus morhua*. The name of each loci is shown at each respective peak. The *y*-axis displays fluorescence intensity in arbitrary units.

TABLE I. Mean ± S.E. total body mass (W1–W4) for individually tagged *Gadus morhua* from nine different families (F1–F9), fed either zooplankton or rotifers during the larval period, combined after weaning and reared under different salinities (15 or 32) and temperatures (10 or 14° C) for 95 days in the laboratory and then at ambient conditions in sea pens for 790 days. Mean mass information is pooled for the sexes. Different superscript lowercase letters indicate statistical differences (Student–Newman–Keuls multiple comparison test, $P < 0{\cdot}05$) between the experimental groups, with 'a' as the highest value. Separate tests were performed for first-feeding regime, temperature–salinity regime and families

		n	W1 (g)	W2 (g)	W3 (g)	W4 (g)
First-fed						
	Zooplankton	165	14·1 ± 0·4	63·6 ± 1·8^{a}	234·2 ± 5·4^{a}	2380·3 ± 65·5^{a}
	Rotifers	117	14·4 ± 0·5	53·7 ± 2·3^{b}	196·2 ± 7·8^{b}	2106·6 ± 72·8^{b}
Temperature–salinity						
	10° C–32	79	14·8 ± 0·6	51·6 ± 2·4^{c}	200·4 ± 7·3^{b}	2250·5 ± 86·9
	10° C–15	57	14·0 ± 0·5	59·6 ± 2·2^{b}	220·6 ± 9·4^{b}	2210·1 ± 79·0
	14° C–32	84	14·5 ± 0·5	56·7 ± 2·3^{bc}	209·4 ± 9·1^{b}	2336·3 ± 88·9
	14° C–15	62	13·8 ± 0·7	68·0 ± 2·8^{a}	248·2 ± 9·8^{a}	2482·3 ± 95·6
Family (F)						
	F1	39	15·1 ± 0·8	59·8 ± 3·4	226·0 ± 13·4	2438·1 ± 133·3^{ab}
	F2	23	15·5 ± 1·0	64·6 ± 4·4	207·3 ± 19·4	1775·0 ± 150·4^{b}
	F3	34	14·2 ± 0·9	55·2 ± 4·7	222·8 ± 18·1	1933·3 ± 123·3^{b}
	F4	35	14·0 ± 1·1	60·4 ± 5·3	209·0 ± 18·3	2461·5 ± 154·2^{ab}
	F5	31	15·6 ± 1·9	61·4 ± 5·1	236·2 ± 16·3	2575·4 ± 146·9^{a}
	F6	26	12·8 ± 0·8	57·5 ± 5·0	227·2 ± 11·8	2790·5 ± 193·2^{a}
	F7	29	15·8 ± 1·7	61·7 ± 7·4	223·8 ± 15·9	2333·3 ± 108·5^{ab}
	F8	33	15·0 ± 0·8	58·9 ± 3·3	211·4 ± 11·7	2244·2 ± 96·2^{b}
	F9	32	14·4 ± 1·3	68·9 ± 6·1	258·9 ± 17·8	2300·0 ± 149·5^{ab}

W1, 25 September 2006; W2, 18 December 2006; W3, 10 May 2007; W4, 11 March 2009.

Significant differences were also found at the termination of the temperature–salinity trial (Table I). Final mean mass in December 2006 of the 14° C-15 group was between 12 and 24% higher than those of the other groups. The relative difference between mean mass at both salinities was similar at both temperatures, hence no significant interaction between temperature and salinity on size was found (three-way nested ANOVA, $P > 0{\cdot}05$).

No differences in initial or final mean mass of different families were seen during the laboratory trial (two-way nested ANOVA, $1 - \beta = 0{\cdot}72$, $P > 0{\cdot}05$; Table I) and no family-based effect on individual growth trajectories was observed [GCM, $\text{MANOVA}_{\text{FAMILIES}}$, Wilk's $\Lambda_{6,272} = 0{\cdot}53$, $P > 0{\cdot}05$].

LONG-TERM EFFECT ON GROWTH

Mean growth trajectories at the individual level were different [GCM, $\text{MANOVA}_{\text{FIRSTFEEDING}}$, Wilk's Λ, d.f. $= 6,272$, $P < 0{\cdot}05$] between the Z and R first-fed *G. morhua*. Significant differences were also found in growth-at-age trajectories of the first-feeding groups ($\text{MANOVA}_{\text{FIRSTFEEDING}\times\text{AGE}}$, Wilk's Λ, d.f. $= 6,272$, $P < 0{\cdot}001$) from September 2006 to March 2009. The mean mass of fish start fed on Z or R during the early juvenile stage differed after 26 months of open tanks on land and sea pen rearing at ambient conditions (Student–Newman–Keuls test, $P < 0{\cdot}05$; Table I).

Mean individual growth trajectories of fish reared at different temperatures, and salinities during the early juvenile stage were different during the long-term rearing (GCM, $\text{MANOVA}_{\text{TEMPERATURE}}$, Wilk's Λ, d.f. $= 7,145$, $P < 0{\cdot}001$; GCM, $\text{MANOVA}_{\text{SALINITY}}$, Wilk's Λ, d.f. $= 7,145$, $P < 0{\cdot}05$). Significant differences were also found in growth-at-age trajectories of the temperature groups ($\text{MANOVA}_{\text{TEMPERATURE}\times\text{AGE}}$, Wilk's Λ, d.f. $= 6,146$, $P < 0{\cdot}001$) and a tendency towards significance for the salinity groups ($\text{MANOVA}_{\text{SALINITY}\times\text{AGE}}$, Wilk's Λ, d.f. $= 6,146$, $P > 0{\cdot}05$) from September 2006 to March 2009. The mean mass of fish reared at different temperatures and salinities during the early juvenile stages differed in May 2007 with the 14° C-15 being larger than the other groups (Student–Newman–Keuls test, $P < 0{\cdot}05$; Table I). The 14° C-15 group was still 7–13% larger compared to the other groups at the termination of the trial. Overall, the 14° C group was 12% larger at termination compared to the 10° C, whereas the two salinity groups did not differ (Table I).

A family-based growth effect was seen during the long-term rearing trial (GCM, $\text{MANOVA}_{\text{FAMILIES}}$, Wilk's Λ, d.f. $= 6,272$, $P < 0{\cdot}05$) and the final mean mass of the different families varied highly (Student–Newman–Keuls test, $P < 0{\cdot}05$; Table I).

VARIANCE COMPONENT ANALYSIS OF GROWTH

The restricted maximum likelihood (REML) based variance component analysis of the body mass at the termination of the laboratory trial [Table II(a)] and at the termination of the long-term trial [Table II(b)] revealed two different variance estimations. The size variation at the end of the laboratory trial was largely explained by the different start-feeding method [16·7%, $P < 0{\cdot}01$; Table II(a)], rearing salinity (30·8%, $P < 0{\cdot}01$) and rearing temperature (39·7%, $P < 0{\cdot}001$), whereas the different families only explained 2·1% ($P > 0{\cdot}05$) of the observed size variation. In contrast to

TABLE II. Variance component analysis for body mass of juvenile *Gadus morhua* from nine different families, first fed with either zooplankton or rotifers and reared at different salinities (15 or 32) and temperatures (10 or 14° C) and reared together for 95 days in (a) the laboratory and (b) later reared at ambient conditions in land-based tanks and sea pens for 790 days

	d.f. numerator	d.f. denominator	*F*-value	*P*-value	Explained variance (%)
(a) Laboratory trial					
Families	8	278	0·81	>0·05	2·1
First-feeding method (FM)	1	278	6·59	<0·001	16·7
Salinity	1	278	12·15	<0·001	30·8
Temperature	1	278	15·66	<0·001	39·7
Families × salinity	8	278	0·44	>0·05	1·1
Families × temperature	8	278	1·32	>0·05	3·3
FM × temperature	1	278	1·03	>0·05	2·6
Salinity × temperature	1	278	0·44	>0·05	1·1
Families × salinity × temperature	8	278	0·97	>0·05	2·5
(b) Long-term rearing					
Families	8	278	3·54	<0·001	30·1
FM	1	278	1·99	>0·05	16·9
Salinity	1	278	2·00	>0·05	17·0
Temperature	1	278	1·91	>0·05	16·2
Families × salinity	8	278	1·50	>0·05	12·7
Families × temperature	8	278	0·15	>0·05	1·3
FM × temperature	1	278	0·27	>0·05	2·3
Salinity × temperature	1	278	0·20	>0·05	1·7
Families × salinity × temperature	8	278	0·21	>0·05	1·8

this, different families explained 30·1% ($P < 0{\cdot}01$) of the growth variation during the long-term trial [Table II(b)], whereas different start-feeding regimes, juvenile rearing salinity and temperature each explained *c*. 16% of observed variation ($P > 0{\cdot}05$). With one exception (families × salinity during the long-term rearing, $P > 0{\cdot}05$), interactions between the different explanatory variables had only marginal effect on the observed size variation during both the laboratory ($P > 0{\cdot}05$) and the long-term ($P > 0{\cdot}05$) trial.

MATURATION

No differences in gonad maturation (I_G) between any of the families were seen at W4 ($P > 0{\cdot}05$). Not all the fish could be sexed based on inspection of the gonads, but for those possible no differences in sex proportion between family groups were seen (χ^2-test, d.f. = 8, $P > 0{\cdot}05$). The I_G was significantly higher in the females at W4, whereas no interaction between sex and family group was seen ($P > 0{\cdot}05$).

DISCUSSION

Differences in size have been shown to persist in groups of wild fishes during short growth trials [*Menidia menidia* (L.), Billerbeck *et al*., 2000; *Menidia peninsulae*

(Goode & Bean), Yamahira & Conover, 2003], whereas this is the first experiment where growth persistence is studied on an individual level for nearly 3 years and observed body mass variation fractioned into environmental and genetic components.

This is also the first published trial applying a panel of new DNA microsatellites recently isolated for *G. morhua* (Jakobsdóttir *et al*., 2006; Skírnisdóttir *et al.,* 2008) where they are used for parentage and family assignment. By applying these genetic tools, improvements in the overall success of parentage studies in *G. morhua* should be possible, particularly in complex situations such as mass spawning tanks (as in the present study). Present findings indicate significant effects of rearing environment during early life stages and significant effects between families during the long-term rearing at ambient conditions. The current study differs from previous studies (Gjerde *et al*., 2004; Kolstad *et al*., 2006) by combining fixed environmental and dietary effects with random family effects and retrospectively analysing the growth response both during and after the environmental manipulation. Kolstad *et al*. (2006) investigated body mass, spinal deformity and sexual maturity at 2 years of age and at harvest in 51 full-sib families of *G. morhua* reared at four locations along the coast of Norway. Heritability estimates for body mass ($h^2 = 0{\cdot}64 \pm 0{\cdot}12$, mean $\pm$ S.E.) at harvest indicated promising potential for the improvement by selective breeding. All traits showed a significant interaction effect of family group by location. As in the present study, Kolstad *et al*. (2006) found significant relations between early juvenile mean mass and mass at harvest and suggested that juvenile size could be a selection criterion for harvest mass. For aquaculture production, the possible benefit would be that early juvenile size at 40–80 dph can be utilized as a management tool for the ongrowing phase. For *G. morhua*, there is still debate about the extent of genetically based growth differences (Imsland & Jónsdóttir, 2002). The present findings and those of Gjerde *et al*. (2004) and Kolstad *et al*. (2006) indicate that the genetic component has an important effect on the growth of *G. morhua*, but that the magnitude of the parentage effect is dependent on rearing environment and life stage.

The different growth performance of the *G. morhua* families during the long-term rearing indicates a significant genotype (G) by environment (E) (G $\times$ E) interaction. Significant G $\times$ E interactions, measured through reaction norms: different strains or genotypes reared in different environments, for growth have been found in many marine species including turbot *Psetta maxima* (L.) (Imsland *et al*., 2000), Atlantic halibut *Hippoglossus hippoglossus* (L.) (Jonassen *et al*., 2000) and *G. morhua* (Imsland *et al*., 2005*a*). Studies estimating genetic correlations between the same trait in different environments are less numerous and results highly variable (Hanke *et al*., 1989; Stefansson *et al*., 1990; Saillant *et al*., 2006; Dupont-Nivet *et al*., 2007). In the present study, a long-term family $\times$ salinity interaction was seen [Table II(b)]. This can indicate the possibility of family-based selection for adaptation to lower salinities. Generally speaking, G $\times$ E interactions seem to depend on the traits, populations, species and environments studied and are still difficult to predict. It is, however, a key point when setting up a breeding programme in species distributed over a wide range of environments and large geographical area like the *G. morhua*.

The current study and those of Imsland *et al*. (2006*a*, 2007) have indicated that environmental-related growth differences in 0 year group of *G. morhua* are mirrored in size differences at harvest several years later. In the current study, this effect was most pronounced for fish first fed on zooplankton and later reared at 14° C

and 15. Although the experimental interval encompassed only a brief period of the life cycle, growth differences persisted throughout the entire juvenile period up to adult sizes. The current trial is the first one where the relative importance of the dietary effect is verified. Dietary-induced growth and survival differences between groups fed natural zooplankton or enriched forms of *B. plicatilis* or *Artemia* sp. have been reported for several fish species, including *H. hippoglossus* (Næss *et al*., 1995; Hamre *et al*., 2002), *P. maxima* (Conceição *et al*., 1997), Asian sea bass *Lates calcarifer* (Bloch) (Rajkumar & Kumaraguru Vasagam, 2006) and *G. morhua* (Imsland *et al*., 2006*a*; present study). These differences have been suggested linked to the nutritional contents of the feed, but whether the specific dietary polyunsaturated fatty acids (PUFA) levels and PUFA ratios (Evjemo & Olsen, 1997; Benitez-Santana *et al*., 2007), amino acid availability (Conceição *et al*., 1997) or micronutrient composition (Hamre *et al*., 2008) individually or interactively cause larval growth differences is yet largely indecisive.

During the laboratory trial, an interactive effect between salinity and temperature on growth in *G. morhua* juveniles was seen. Similar salinity × temperature interaction has been previously observed in juvenile *P. maxima* (Imsland *et al*., 2001) and in spotted wolffish *Anarhichas minor* Olafsen (Magnussen *et al.,* 2008). In all three studies, the interaction resulted in higher optimal temperature at reduced salinities. It is therefore likely that different species could react similarly to reduced salinity in combination with varying temperatures. The relative mean mass differences established during the laboratory trial, diminished for the first-feeding, temperature and salinity groups during the long-term rearing. This is probably an effect of size dependent growth (Folkvord, 2005; Imsland *et al.,* 2006*b*) as the smaller fish will display higher growth, independently of previous rearing conditions, in the initial stages of the long-term trial.

The temperature (T) and salinity (S) treatment applied in the current study had a significant short-term effect and although the effect was not significant during the long-term trial the original temperature and salinity treatment still accounted for 34% of the body mass variation seen at the termination of the trial. The optimum $T \times S$ combination for growth found here (*i.e.* T of 14° C and S of 15) may be reflected in the habitat preference of the *G. morhua*. Robichaud & Rose (2004) reviewed a century of tagging studies with *G. morhua* and found that of all *G. morhua* groups investigated 41% could be categorized as sedentary, 20% as dispersers and 38% as homers. Robichaud & Rose (2004) found that it was the sedentary *G. morhua* groups that had lower maximum historical biomass, confirming that migration begets abundance. This effect would mostly be attributed to the secondary effect of migration as migrating *G. morhua* can better exploit the seasonal changes in productivity. The optimum $T \times S$ regime found in the present study mimics the mechanism of behavioural thermoregulation where juveniles may seek environmental conditions maximizing their fitness (Michalsen *et al*., 1998; Kristiansen *et al*., 2001). In some species, juveniles seek out a temperature close to their optimal temperature for growth (Wildhaber & Crowder, 1990), whereas other species seek to maximize their feeding efficiency. Björnsson *et al*. (2001) postulated that *G. morhua* migrations around Iceland are, at least partly, driven by feeding and optimal foraging mechanism, *i.e.* seeking out temperatures that maximizes feeding efficiency.

Important practical implication of the current findings for the aquaculture sector is the advantage of rearing the fishes at different first-feeding regimes and temperatures

during the juvenile period as size differences established at this stage are maintained in the adult fishes. Similar findings have been observed for wild juvenile north-east Arctic *G. morhua* where significant size correlations are documented between year-classes, and the basis for these relative size differences are formed during the first half year of life (Ottersen *et al*., 2002). In the present study, the tagged fish were acclimated to 10° C before being transported to the ongrowing site in western Norway and moved to sea pens when sea temperature was *c*. 7° C. Size differences after 26 months in sea pens were reduced, but the general picture persisted that differences in the juvenile stage can be traced up to the adult stage.

It should be noted that the biological material used in the present study reflects one restricted geographical location as it is constructed from individuals originated from the coast of western Norway. Recent studies clearly indicate a significant genetic variation and local selection in relation to temperature and salinity conditions at spawning grounds (Moen *et al*., 2008; Nielsen *et al*., 2009); also, previous studies of haemoglobin polymorphism suggested the presence of an important genetic component in relation to temperature and growth (Nævdal *et al*., 1992; Imsland *et al*., 2005*b*). Nielsen *et al*. (2009) findings suggest that marine fishes are not only isolated into local populations, even on relatively small geographical scales, but that these populations can indeed follow semi-independent adaptive evolutionary trajectories shaped by selection by their local environments including temperature and salinity conditions at spawning grounds at spawning time. This clearly points to interesting future research avenue where the retrospective body mass variation approach is used to investigate the growth variation in different populations of *G. morhua*.

In conclusion, the present study shows that environmental-related growth differences in 0 year group of *G. morhua* are partly mirrored in size differences at harvest several years later. The study indicates that diet and environmental manipulation during first-feeding explain nearly 90% of the observed growth variation during the early juvenile period. The genetic effect only accounts for *c*. 2% during this period, whereas it has great effect on growth variations (30%) during long-term rearing at ambient conditions. This might indicate that less genetic variation is to be expected in an important trait such as early growth potential. These findings could increase the understanding of size variation in natural and cultured stocks of *G. morhua*. Better understanding of the mechanism behind size variation can have wide range applications for fisheries and culture of the species.

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