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The influence of first-feeding diet on the Atlantic cod *Gadus morhua* phenotype: survival, development and long-term consequences for growth

R. M. Koedijk*†, A. Folkvord*, A. Foss‡, K. Pittman*, S. O. Stefansson*, S. Handeland§ and A. K. Imsland*‖

*Department of Biology, University of Bergen, High Technology Centre, P.O. Box 7803, 5020 Bergen, Norway, ‡Akvaplan-niva Bergen Office, P.O. Box 2026 Nordnes, 5817 Bergen, Norway, §SagaFjord Seafarm, Stord, Norway and ‖Akvaplan-niva Iceland Office, Akralind 4, 201 Kópavogur, Iceland

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Atlantic cod *Gadus morhua* larvae reached four-fold (at low larval density) to 11 fold higher body mass (high larval density) at 50 days post hatch (dph) when fed zooplankton rather than enriched rotifers. A short period (22–36 dph) of dietary change affected larval growth positively if changed from enriched rotifers to natural zooplankton and negatively if prey type changed *vice versa*. Overall survival did not differ between the two larval groups at low larval density, but at high density the rotifer group had a higher overall survival (10·8% *v*. 8·9%). Long-term growth was affected significantly by larval diet in favour of the zooplankton diet; juveniles reached a 23% higher mass in a 12 week growth period. No difference in growth performance was found between juveniles fed natural zooplankton during the larval period for 36, 22 or 14 days, but all these juveniles performed significantly better compared with the rotifer-fed group. These findings suggest that optimal diet during a short period in the larval period can result in improved growth in both the larval and juvenile period. Improved rotifer quality may, therefore, hold a large potential for growth improvement in this species.

Key words: *Brachionus plicatilis*; larval nutrition; ontogeny; zooplankton.

INTRODUCTION

Wild zooplankton as a larval diet can provide a better scope for growth and development in cold-water marine species (Imsland *et al*., 2006) compared with enriched forms of rotifers *Brachionus plicatilis* or *Artemia* sp. Studies in mesocosms, ponds or extensive production systems all produced larvae that outperformed intensively reared larvae (Blom *et al*., 1994; Folkvord *et al*., 1994; van der Meeren *et al*., 1994). Pigmentation rate in Atlantic halibut *Hippoglossus hippoglossus* (L.) (McEvoy *et al*., 1998; Næss & Lie, 1998; Hamre *et al*., 2007) or the occurrence of deformities in Atlantic cod *Gadus morhua* L. juveniles (Imsland *et al*., 2006) could both be

†Author to whom correspondence should be addressed. Tel.: +31 683200973; fax: +47 55584450; email: roland.koedijk@bio.uib.no

influenced by first-feeding diet. Extensive systems differ from intensive aquaculture production systems in two main ways: first, the food source consists of wild zooplankton instead of enriched rotifers or *Artemia* sp., and second, the larval density is generally lower compared with intensive systems. The present aquaculture production of *G. morhua* in Norway relies on the use of enriched rotifers as a first-feeding diet due to the predictability and relative stability of a year round, on-site rotifer production, in contrast to unwanted seasonal, size or quantity-related fluctuations of zooplankton. To improve rotifer quality, enrichment procedures have been established (Dhert *et al*., 1993, 2001; Lie *et al*., 1997), in which the main focus has been to increase the level of key essential polyunsaturated fatty acids such as 20:5*n*-3 (eicosapentaenoic acid, EPA) and 22:6*n*-3 (docosahexaenoic acid, DHA). These unsaturated fatty acids are found in high concentrations in wild zooplankton (Evjemo & Olsen, 1997; Bell *et al*., 2003; van der Meeren *et al*., 2008), which is why they are believed to be especially important in diets for marine fish larvae (Shields *et al*., 1999; Evjemo *et al*., 2003; Cutts *et al*., 2006; Hamre & Harboe, 2008). If extensive aquaculture systems can produce *G. morhua* larvae with growth rates up to 22% per day (van der Meeren *et al*., 1994), it is important to understand how larval growth is being suppressed under culture conditions. Accordingly, this research investigated the effects of prey type, larval density and tank environment on growth performance and developmental success of *G. morhua* larvae. Furthermore, the study evaluated the effect of first-feeding diet and larval density on general growth performance, mortality and developmental success of *G. morhua* larvae during later stages of development. The hypothesis was that larvae at high and low densities perform better when fed zooplankton rather than enriched rotifers, and that this effect will also be manifested in the juvenile stage.

For this study, one egg batch was used to exclude genetic effects. Larvae were reared at two densities with natural zooplankton or enriched rotifers as a first-feeding diet until weaning at 36 days post hatch (dph). Additionally, a diet change experiment was conducted to investigate the effect of a short period of dietary change on subsequent development and growth performance. A third experiment was conducted to determine the persistent effects of larval diet on growth.

MATERIALS AND METHODS

FISH MATERIAL AND EXPERIMENTAL DESIGN

Eggs of *G. morhua* were incubated at a commercial hatchery in western Norway (61° 40′ N) at 7° C and hatched on 9 April 2006. At 1 dph, the yolk-sac larvae were transported to the Bergen High Technology Centre, where they were acclimated before they were randomly divided over 10 experimental tanks (1 m × 1 m, with a rearing volume of 300 l) and two additional tanks (0·6 m × 0·6 m) with a rearing volume of 130 l. The initial temperature was 8° C increasing to 9° C towards the end of the experimental period. The light regime was fixed to 16L:4D with 4 h twilight. The initial fish density was 14 larvae l^{-1} (in eight tanks) and 81–83 larvae l^{-1} in the four high-density tanks (including the two 130 l tanks). Groups are hereafter referred to as low and high-density groups. A schematic overview of the experimental setup is given in Fig. 1. All tanks were stagnant systems, with a gentle airflow at the bottom of the tank to ensure proper water circulation. Tanks were provided with 2 l freshly cultured algae (*Rhodomonas* sp. and *Isochrysis* sp.) and were siphoned on a daily basis. Water removed by siphoning was replaced with UV filtered sea water.

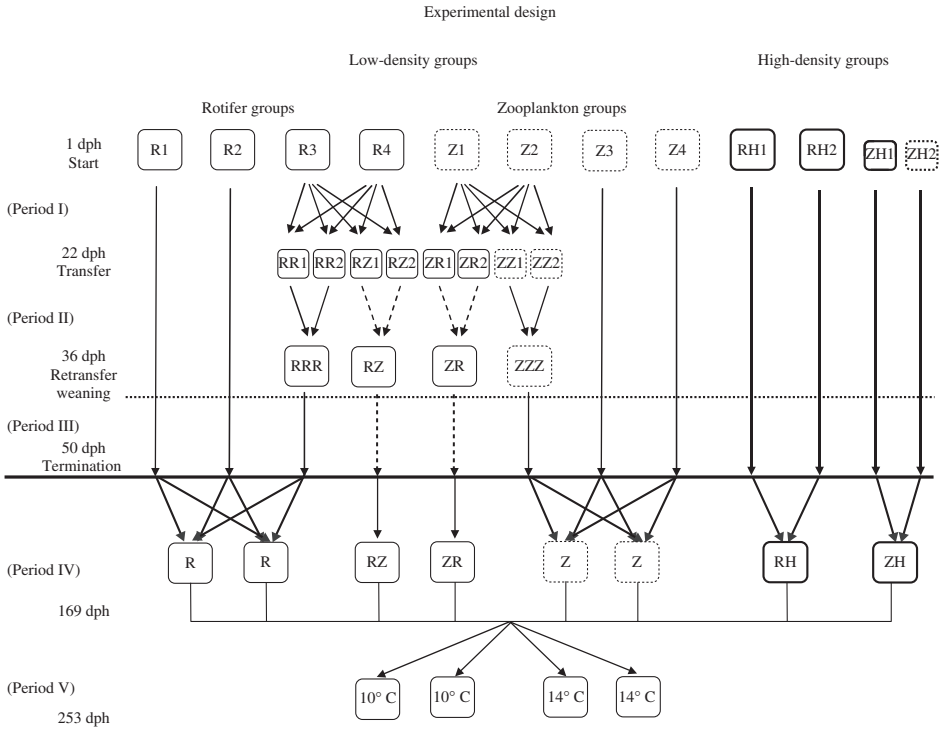

FIG. 1. Schematic overview of the experimental design. The larval experiment consisted of two larval rearing densities: 14 larvae l^{-1} (eight tanks) or 62 larvae l^{-1} (four tanks). Feed types are enriched rotifers (R); zooplankton (Z); diet change from rotifers to zooplankton (RZ) at 22 days post hatch (dph); *vice versa* (ZR). Period I lasted from 1 to 22 dph, period II lasted from 22 to 36 dph and period III from 36 to 50 dph. During period III, all larvae were weaned onto the same formulated feed. Period IV was an intermediate period in which early juveniles were regrouped according to first-feeding diet. Before period V, all juveniles were individually passive integrated transponder (PIT) tagged and divided over four tanks with 10 or 14° C.

The larval feeding experiment lasted from 1 until 50 and consisted of three subperiods (Fig. 1). From 1 to 22 dph (period I), the larvae were fed either rotifers or zooplankton. At 22 dph (start of period II), the larvae from four tanks (two rotifer-fed and two zooplankton-fed) were collected, counted and subdivided over eight new tanks with a rearing volume of 130 l. The remaining groups were continued without change (Fig. 1). Each new tank was stocked with 800 larvae (six larvae l^{-1}), similar to the remaining density in the unchanged low-density tanks when mortality until 22 dph is taken into account (six to eight larvae l^{-1}). Rotifer-fed larvae were distributed over four tanks in which the larvae in two tanks remained on rotifers (RR group), and the larvae in the two other tanks changed diet from rotifers to zooplankton (RZ group). Accordingly, for the zooplankton-fed larvae, two tanks remained on zooplankton (ZZ group), while the other two tanks changed diet from zooplankton to rotifers (ZR group).

At 36 dph (start period III), the larvae from the transfer tanks were counted and the replicate transfer groups were combined. The amount of live prey was reduced by 20% per day and larvae were weaned onto formulated feed (Ewos AgloNorse Standard, containing 59% protein, 21% fat, 10% ash and 1% fibre; www.ewos.com). Dry feed was continuously administered to the larval rearing tanks by automatic belt feeders during light period, and all tanks were switched to flow-through.

During the larval experiment, live prey were added three times per day (*ad libitum*) from 2 dph onwards at a prey density of 2000 prey l^{-1}, increasing to 3000 prey l^{-1} (low-density tanks), and at an initial 5000 prey l^{-1}, increasing to 8000 prey l^{-1} in the four high-density tanks to accommodate for grazing.

Zooplankton was filtered from the fjord at the Espegrend Marine Biological Station and was transported to the High Technology Centre, Bergen, on a daily basis. The size fraction used was 80–250 μm (10 to 26 April) increasing to 80–400 μm (27 April to 15 May). Before feeding, the zooplankton was rinsed and the density was increased. The zooplankton fraction consisted primarily of calanoid nauplii (*Temora* sp.). On average, 8–12% of the zooplankton fraction that was fed to the larvae consisted of non-edible organisms. This fraction was dominated by polychaete larvae.

Enriched rotifers were obtained from a commercial hatchery in western Norway and were transported to the High Technology Centre on a daily basis. Rotifers *B. plicatilis* L strain: 150–320 μm were cultivated in four circular rearing tanks (2500 l). To suppress the inhibitory effects of ammonia, waste particles and opportunistic bacteria coexisting with rotifers, all tanks were supplied with UV-treated, marine deep water from 160 m depth, which was filtered over three filters in series (60, 10 and 1 μm). Wastewater was removed continuously (300% water exchange per day). During the experimental period, temperature and salinity were measured daily (YSI-multimeter 550, YSI; www.ysi.com) and were kept at *c*. 25° C (range ± 0·5° C) and 35 (range ± 0·1). Oxygen was supplied constantly in each tank through diffusers and was regulated manually (80–120%). Rotifers were enriched at the commercial hatchery with 2–3 h enrichment with a mixture containing lipids (40–60%), protein (30–50%) and vitamins (2–3%) before and during transport to the experimental facilities. For detailed fatty acid composition of the prey items, see A. Folkvord, R. Koedijk, O. Grahl-Nielsen, S. Meier, B. Rydland Olsen, G. Blom & A. Imsland, unpubl. data. In short, zooplankton contained relatively high *n*-3 fatty acids DHA (docosahexaenoic acid) and EPA (eicosapentaenoic acid) while rotifers contained relatively high *n*-6 fatty acids. Rotifers were rinsed upon arrival and acclimatized to the rearing temperature for at least 0·5 h before feeding. Oxygen concentrations remained >85% during transport, and rotifers arrived in good condition. Both rotifers and zooplankton were administered to the rearing tanks three times daily.

SAMPLING

At 1 dph, a common sample was taken (150 larvae) before the larvae were randomly distributed over the experimental tanks. Hereafter, samples were taken twice weekly between the start (1 dph) and termination of the experiment (50 dph) from the unchanged groups, and once per week for the eight transfer tanks in period II. Each sample consisted of 10 larvae per tank. Larvae were individually photographed using a dissecting microscope (Leica MZ95; www.leica.com) connected to a digital camera (Olympus camedia C-5060, wide zoom; www.olympus-global.com). Larvae were rinsed with fresh water and frozen in individually marked microcentrifuge tubes in liquid nitrogen. Standard length (L_S) was measured from the tip of the snout to the end of the notochord, and myotome height (H_M) was measured posterior to the anus. Measurements were performed on the pictures using ImageJ software (Abramoff *et al*., 2004) after calibrating the software for the used magnification and number of pixels.

Survival counts were made by counting larvae at 1 dph (stocking of tanks), 22 dph (transfer), 36 dph (retransfer) and 50 dph (termination).

CONDITION FACTOR

The individual condition factor was based on the measured L_S and H_M. A regression between L_S and H_M was fitted, which was used to calculate the difference between individually observed and population mean values for condition factor (residual, y). The residual function had the following form: $y = H_M - [(1{\cdot}68 \ln L_S) - 3{\cdot}93]$. The average residual value is 0, since this represents the overall mean value of all larvae. Positive values represent larvae with above average condition factors, while negative values represent below average performing larvae.

WATER QUALITY

pH (LF 330/set, WTW; www.WTW.com), dissolved oxygen (Oxi 330i, WTW) and salinity (Cond 315i, WTW) were measured daily. Oxygen levels remained >80% saturation, and salinity was stable. Total ammonium nitrogen (TAN) was measured weekly with an ammonia gas sensing combination electrode (model 95-12, Thermo Orion; www.orion.com) connected to an ion analyser (Thermo Orion, EA 920). Mean ± S.D. CO_2 concentration was measured (NIVA AS; www.niva.no) at 22 dph, just before transfer, to establish the maximum value reached during this experiment, and this varied from 3·8 ± 0·4 mg l^{-1} in the low-density groups to 6·5 ± 0·5 mg l^{-1} in the high-density groups.

PERIOD IV

After the larval experiment was finished at 50 dph (Fig. 1), the differences in size between the rotifer, the zooplankton and the diet change groups were reduced by adjusting the water temperature of the groups, rearing the zooplankton and diet change groups at 10° C, and the rotifer-fed fish at 14° C for a period of 10 weeks (period IV, Fig. 1) in 500 l grey fibreglass covered tanks at the same light regime as during the larval period. Six weeks before the juvenile growth experiment started, all fish were acclimated to 12° C. All groups were fed in excess from automatic feeders with the same diet (EWOS Marin 2, 5 and 10, containing 58% protein and 12% fat) from weaning onwards, with increasing pellet size to accommodate increase in fish size. A total of 254 fish (66 R, 52 RZ, 68 ZR and 68 Z fish) were individually tagged intraperitoneally [Trovan passive integrated transponder (PIT) tags; www.rfidsystems.co.uk] between 4 and 6 weeks before the start of the experiment.

JUVENILE PERFORMANCE, PERIOD V

Period V consisted of a juvenile growth experiment and lasted for 3 months from 25 September (169 dph) to 18 December 2006 (253 dph) and consisted of four fish groups from the previous larval experiment (R, Z, RZ and ZR groups, as previously described). During the experiment, all fish were fed in excess from automatic feeders with EWOS Marin 20, containing 55% protein and 12% fat. Water temperatures were stable at 10 or 14° C, with two replicate tanks per temperature. Each fish group was present in each of the four tanks and additional untagged fish were added to a total initial mean ± S.D. biomass of 4·1 ± 0·0 kg m^3 per tank with 170–173 fish per tank with an initial mean ± individual mass of 11·9 ± 5·5 g. Water flow rates were 4 l min^{-1} and were similar between tanks. Total length (L_T) and body mass (M_W) were measured on individually tagged, anaesthetized fish (Metacain, 0·05 g l^{-1}) at 3 week intervals during a total experimental period of 3 months.

STATISTICAL METHODS

Statistical analyses were performed using SPSS 14.0 (www.spss.com). Data were tested for normality with a Kolmogorov–Smirnov test (Zar, 1996). Homogeneity of variance was tested with Levene's test (Brown, 1974). Larvae were individually dried at 37° C overnight after which their dry mass was measured (M_D). A three-way nested ANOVA was used to test for significant differences in L_S and M_D and had the following model: $Y = \mu + \alpha_i + \beta_j + \gamma_{k(ij)} + \alpha_i\beta_j + \varepsilon$, where α_i is the factor for prey type (rotifers or zooplankton), β_j for density (16 or 64 larvae l^{-1}) and $\gamma_{k(ij)}$ is the replicate factor (tank), nested in both prey type and density. If no interaction or tank effect was found, the model was reduced and main effects were tested. Performance data were $\log_{10}$ transformed to increase homogeneity of variances. A Student–Newman–Keuls *post hoc* test was applied to identify differences between treatments.

Water quality variables were tested using a two-way ANOVA with food type and density as factors. To assess individual growth trajectories in juvenile fish, a model III MANOVA (Johnson & Wichern, 1992) was used to test for overall differences in specific growth rates (g), [calculated from $g = 100\ (\ln M_{D2} - M_{D1})\ (t_2 - t_1)^{-1}$, where M_{D1} and M_{D2} are dry masses on days t_1 and t_2] with Wilks' lambda (Λ) multivariate test for overall trends over time. All tests were performed with a significance level of $\alpha < 0{\cdot}05$.

RESULTS

OVERALL INFLUENCE OF PREY TYPE AND DENSITY ON GROWTH

There were no differences in larval size between tanks, first-feeding groups or between the two densities at initiation of exogenous feeding at 4 dph (two-way ANOVA, $P > 0{\cdot}05$, Table I). L_S eventually increased faster in larvae fed with zooplankton at both densities (two-way ANOVA, $P < 0{\cdot}05$, Table I), with mean L_S of zooplankton-fed larvae being significantly higher at both densities when compared with rotifer-fed larvae at 50 dph. No difference in larval L_S was found between the two densities within the zooplankton-fed group, but within the rotifer group a significantly higher L_S at low density from 22 dph was found (Table I).

SURVIVAL

Average survival at low larval density was higher in the rotifer-fed larvae (63·9%) compared with the zooplankton-fed larvae (45·4%) during the first 36 days. Weaning started at 36 dph, after which the zooplankton group showed overall higher survival between 36 and 50 dph (56·8%, *v.* 41·2% for the rotifer group, Table II). Total survival during the first 50 days did not differ between groups.

At high rearing densities, the same pattern was observed with high initial survival (1–36 dph) in the rotifer group (72% *v.* 19·3% in the zooplankton group; Table II). From 36 to 50 dph, mean survival was significantly higher in the zooplankton group (41·5–51·2% *v.* 4·4–25·9% in the rotifer group). Within the rotifer group, a large variation in survival between tanks was observed. On average, there was no difference between first-feeding groups in total survival. Larvae that changed diet from 22 to 36 dph all showed high survival rates (Table III).

FIRST FEEDING (PERIOD I)

Period I lasted from hatching until 22 dph. During this period, prey species and larval density had a significant effect on L_S and mass of the larvae, where low-density groups showed a higher mean mass compared with high-density groups (two-way ANOVA, $P < 0{\cdot}001$, Fig. 2). Within densities, no differences in masses were found between diets during this first period (two-way ANOVA, $P > 0{\cdot}05$).

TRANSFER AND DIET CHANGE (PERIOD II)

Period II lasted from 22 until 36 dph. There was no difference in mass between larvae-fed different diets at low density at 22 dph (Fig. 3). An aliquot of larvae from the low-density treatments was used for the diet change experiment. Larvae that changed diet from rotifers to zooplankton at 22 dph increased in mass faster than larvae that were continued feeding on rotifers (Student–Newman–Keuls ANOVA, $P < 0{\cdot}05$, Fig. 3). Conversely, in larvae changed from zooplankton to rotifers, a significant reduction in growth was observed when compared with the respective unchanged group (Student–Newman–Keuls ANOVA, $P < 0{\cdot}05$, Fig. 3). The transfer control groups that were transferred to another tank but did not experience a change diet showed insignificant growth differences at both prey types when compared with

TABLE I. Mean ± S.D. standard length (L_S) of *Gadus morhua* larvae-fed enriched rotifers or zooplankton during three consecutive periods at two densities (14 or 81–83 larvae l^{-1}). Different lowercase letters indicate significant differences between the groups within a sampling date (Student–Newman–Keuls, *post hoc* test, $P < 0{\cdot}05$)

Variable		L_S (mm)											
		Period I					Period II				Period III		
		Days post hatch											
Prey type	Density	1	4	11	15	18	22	25	29	32	36	43	50
Rotifers	14	4·6 (0·2)	4·8 (0·3)	5·6 (0·5)	6·2 (0·8)^{a}	6·7 (0·7)^{a}	7·5 (0·8)^{a}	8·0 (0·7)^{a}	8·6 (0·9)^{b}	8·9 (0·7)^{ab}	9·9 (0·9)^{b}	10·5 (1·2)^{b}	12·2 (1·6)^{c}
	83	4·6 (0·2)	4·8 (0·3)	5·5 (0·6)	5·9 (0·6)^{ab}	6·8 (0·8)^{ab}	7·0 (0·9)^{b}	7·7 (1·0)^{b}	8·0 (1·0)^{c}	8·2 (1·4)^{c}	8·7 (1·9)^{c}	9·7 (1·2)^{c}	9·2 (1·2)^{d}
Zooplankton	14	4·6 (0·2)	4·9 (0·2)	5·5 (0·5)	5·9 (0·7)^{ab}	6·5 (0·8)^{ab}	7·5 (1·0)^{a}	8·4 (1·3)^{a}	9·8 (1·5)^{a}	10·1 (1·8)^{a}	12·3 (2·3)^{a}	14·3 (1·8)^{a}	19·5 (2·9)^{a}
	81	4·6 (0·2)	4·7 (0·3)	5·4 (0·5)	5·7 (0·5)^{b}	6·2 (0·6)^{b}	6·8 (0·6)^{b}	7·5 (1·0)^{b}	9·5 (1·7)^{a}	9·7 (2·0)^{b}	12·1 (2·3)^{a}	14·2 (1·6)^{a}	17·6 (2·2)^{b}

TABLE II. Survival of *Gadus morhua* in 12 larval rearing tanks (replicate rearing units). Survival was determined at 22 days post hatch (dph) for 2 R (larvae-fed enriched rotifers) and 2 Z (larvae-fed zooplankton) rearing units since larvae from these tanks were used for the diet change experiment. For the other tanks, survival is given for three periods: 1–36 dph, 36–50 dph and the total over the entire experimental period. Larvae were weaned onto commercially formulated feed at 36 dph

				Survival (%)			
Prey type	Density larvae·l^{-1}	Tank	*n*	1–22 dph	1–36 dph	36–50 dph	Total
Rotifers	14	1	4303	43·1			
Rotifers	14	2	4249	57·7			
Rotifers	14	3	4362		62·1	38·3	23·8
Rotifers	14	4	4549		65·6	44·0	28·9
Rotifers	81	5	24 999		72·5	4·4	3·2
Rotifers	81	6	24 996		71·5	25·9	18·5
Zooplankton	14	7	4108	61·9			
Zooplankton	14	8	4123	61·3			
Zooplankton	14	9	4138		44·5	53·9	24·0
Zooplankton	14	10	4156		46·3	59·7	27·7
Zooplankton	81	11	10 548		21·3	41·5	8·8
Zooplankton	81	12	10 228		17·3	51·2	8·9

n, the total number of larvae stocked in the tank at 1 dph.

the unchanged groups (Student–Newman–Keuls ANOVA, $P > 0{\cdot}05$, Fig. 2). Mean mass at 36 dph (after a 14 day period of diet change) was found to be 44·5% lower when the larvae were changed from zooplankton to enriched rotifers compared with the (transfer control) group that remained on zooplankton throughout. In the dietary change group from enriched rotifers to zooplankton for the same period, a 41·5% increase in final mass was found compared with the control (unchanged) group.

TABLE III. Survival of *Gadus morhua* during a diet change experiment from 22 to 36 days post hatch (dph). Prey type (22–36 dph) is the prey type fed to the larvae during this period. Prey type (1–22) is the previously fed prey. Tank refers to the separate rearing units and reflects the prey types fed to the larvae (R, rotifers; Z, zooplankton)

Prey type (1–22 dph)	Prey type (22–36 dph)	Tank	*n*	Density (larvae l^{-1})	Survival (%) (22–36 dph)
Rotifer	Rotifer	RR1	804	5	93·5
Rotifer	Rotifer	RR2	801	5	96·6
Rotifer	Zooplankton	RZ1	804	5	86·3
Rotifer	Zooplankton	RZ2	808	5	93·7
Zooplankton	Rotifer	ZR1	806	5	78·7
Zooplankton	Rotifer	ZR2	817	5	78·6
Zooplankton	Zooplankton	ZZ1	796	5	92·7
Zooplankton	Zooplankton	ZZ2	796	5	96·1

n, the stocked number of larvae at onset of the dietary change period (22 dph).

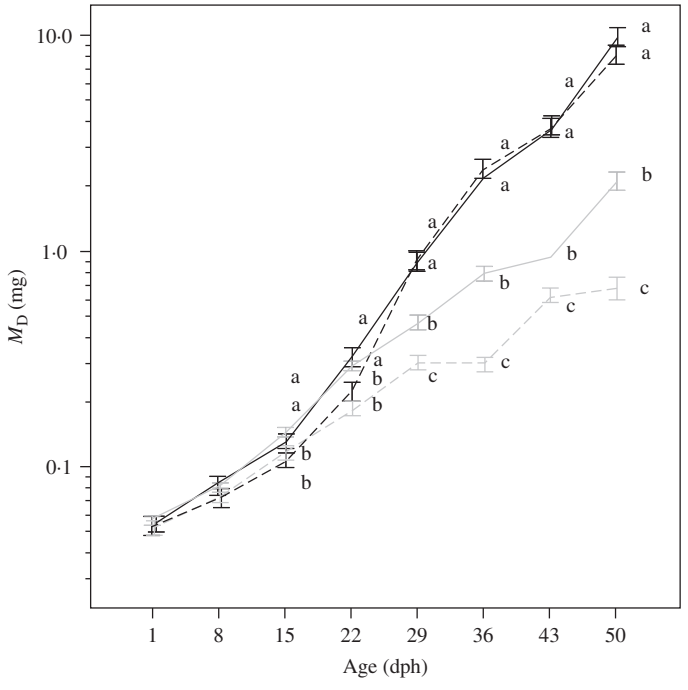

FIG. 2. Mean ± S.E. dry mass (M_D) for four groups of larval *Gadus morhua* at weekly intervals: larvae-fed zooplankton (Z; ——, – – –) and enriched rotifers (R; ——, – – –); low-density groups (14 larvae l^{-1}; ——, ——) and high-density groups (62 larvae·l^{-1}; – – –, – – –). Larvae were fed on either zooplankton or enriched rotifers until 36 dph, after which all larvae were weaned onto formulated feed. Different lowercase letters denote significant difference among groups within each sampling date (Student–Newman–Keuls *post hoc* test, $P < 0{\cdot}05$).

WEANING (PERIOD III)

Period III lasted from 36 to 50 dph, during which all larvae were weaned onto formulated feed. Larvae first fed on zooplankton were larger than rotifer-fed larvae at the start of weaning (36 dph, two-way ANOVA, $P < 0{\cdot}05$), and this difference persisted throughout weaning. Within the Z group, no rearing density effect was found up to termination of the experiment at 50 dph. In the R group, an effect of rearing density was found throughout period III (two-way ANOVA, $P < 0{\cdot}05$, Fig. 2), and larvae reared at high density had lower masses than those reared at low density.

Larvae from the zooplankton group increased in mass significantly faster during and after weaning compared with rotifer-fed larvae [ANCOVA, with mass at start of weaning (36 dph) used as covariate, $P < 0{\cdot}05$].

SIZE DISTRIBUTION

Even though there was little variation in initial size of larvae at 1 dph, both relatively small as well as relatively large larvae within the rotifer group increased in size evenly between 1 dph and 8 dph [Fig. 4(a)]. The larvae from the zooplankton group performed differently. Here, the 20% smallest larvae did not gain mass during this first week, while the larger the larvae were at onset of first feeding, the bigger

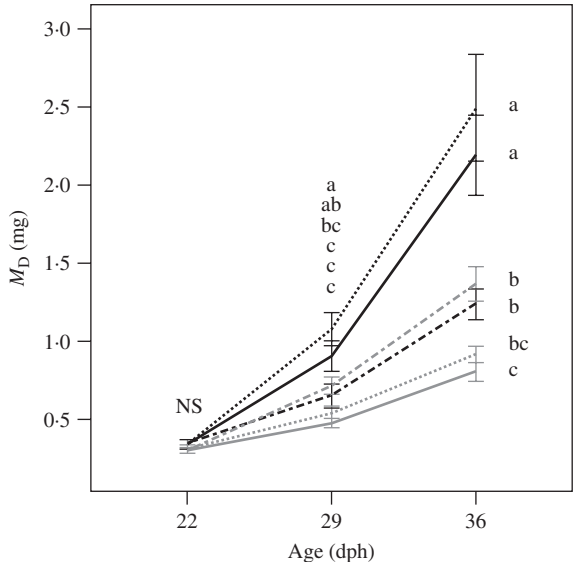

FIG. 3. Mean ± S.E. dry mass (M_D) for six groups of *Gadus morhua* larvae reared at six different first-feeding regimes: Larvae-fed zooplankton (Z, ——) or enriched rotifers (R, ——) from 1 days post hatch (dph) onwards; larvae that changed tanks at 22 dph but did not change diet (transfer controls) (ZZ, ... and RR, ...); larvae that changed diet at 22 dph from zooplankton to enriched rotifers (ZR, - - -) and from enriched rotifers to zooplankton (RZ, - - -). Different lowercase letters denote significant difference among groups within each sampling date (Student–Newman–Keuls *post hoc* test, $P < 0{\cdot}05$).

the size increase was during the first week [Fig. 4(b)]. In general, the size increase between the 20% smallest larvae and the 20% largest larvae was smaller in the group that was first fed with rotifers [Fig. 4(a)], indicating a more uniform size distribution and growth compared with the larvae that were fed with zooplankton [Fig. 4(b)], which generally had a larger difference between the small and large fraction of larvae within the first-feeding group.

Between 36 and 43 dph, at the onset of weaning, mass increase was slowed within the rotifer group across the full size range [Fig. 4(a)]. Within the zooplankton group a different response was observed, since here the 50% smaller fish of the population apparently increased in size, while a reduction in growth was found in the larger size fraction [Fig. 4(b)]. Between 43 and 50 dph, larvae from the zooplankton group increased in mass uniformly again.

CONDITION FACTOR

Larvae that were fed with zooplankton had overall higher mass at size at both rearing densities (one-way ANOVA, $P < 0{\cdot}001$). The zooplankton-fed larvae were always above the total average of mass at size, and the rotifer-fed larvae were always below average. These effects lead to positive residuals in the H_M and L_S regression function and subsequent positive residuals of the zooplankton group v. negative residuals in the rotifer group, which is a detailed measure for the mass at size comparison between the two groups within each density. The mean residual

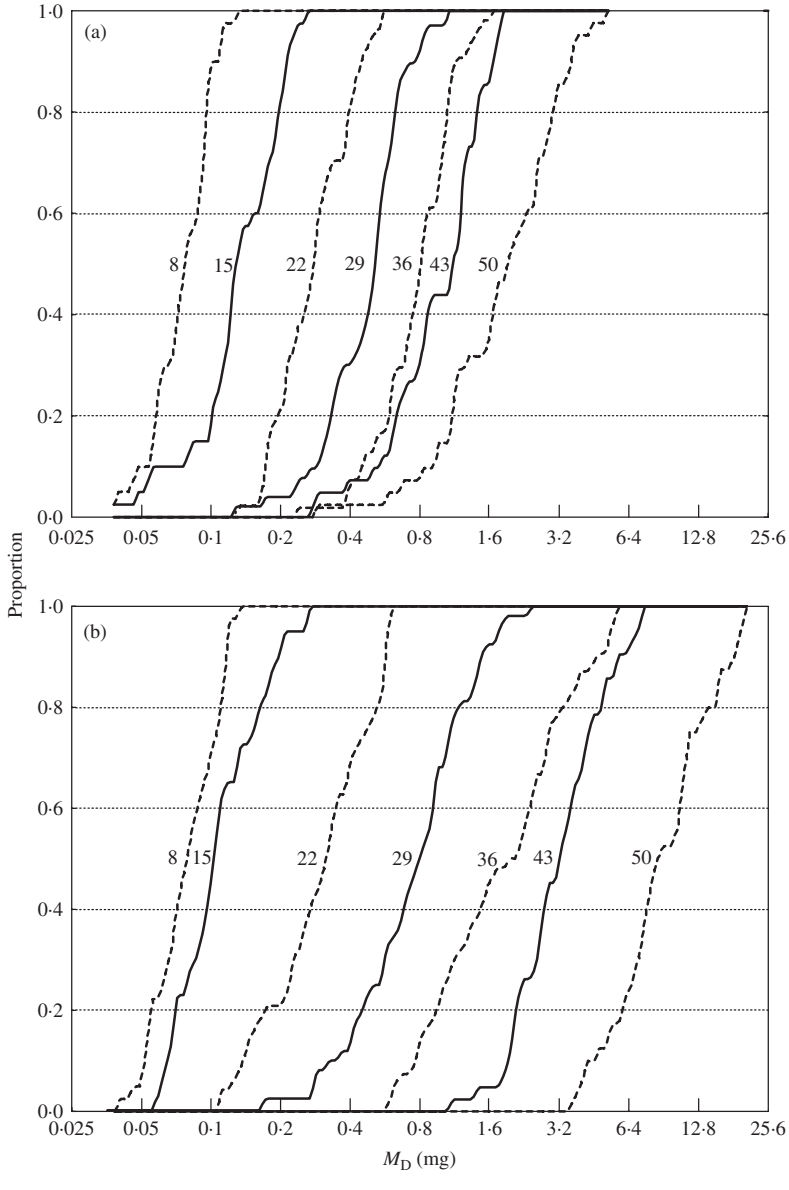

FIG. 4. Cumulative size distributions for dry mass (M_D) at age (days post hatch, dph) in *Gadus morhua* larvae-fed (a) enriched rotifers or (b) zooplankton measured at weekly intervals between 1 and 50 dph. Each line represents the proportion of larvae at a certain mass at age, indicating the size variation at date. Numbers represent the age (dph) of the larvae at the representative lines. Larvae were fed on enriched rotifers or zooplankton until weaning at 36 dph.

difference between the two groups was significantly different between the two first feeding diets at both densities (ANOVA; low density, $P < 0{\cdot}01$; high density, $P < 0{\cdot}01$). The mean residual difference of 4·7% at low density and 8·3% at high density

would correspond with a 6·1% difference in mass at L_S at low density and 10·4% difference at high density between the two groups.

WATER QUALITY

Mean ± S.D. unionized ammonia-N increased from undetectable levels at onset to 14·4 ± 5·0 μg l^{-1} at 22 dph in the high-density rearing tanks of the zooplankton group. The high-density rotifer group showed a 50% lower value at 22 dph (7·34 ± 1·35 μg l^{-1}). At low densities, the same difference was observed. Within the zooplankton group, a maximum value of 8·72 ± 2·19 μg l^{-1} was found compared with 4·05 ± 0·39 μg l^{-1} in the rotifer group.

Carbon dioxide concentrations were higher (two-way ANOVA, $P = 0{\cdot}001$) at high densities compared with low densities but did not differ between diet groups (two-way ANOVA, $P = 0{\cdot}693$).

LONG-TERM EFFECTS OF FIRST-FEEDING DIET

During period V, increased temperature exacerbated group differences: the higher the growth rate the bigger the difference between the groups (Fig. 5). The greater scope for growth was obtained in groups that had received zooplankton at any time during the larval period.

Specific growth rates were higher in all groups and showed a lower variability at high temperature. Regardless of prey type during the larval period, individual growth trajectories did not differ among groups when reared at low temperature (two-way, type III, MANOVA, Wilks' lambda, $P > 0{\cdot}05$, Fig. 6). At high temperature, growth rates were higher, and fish that had been fed with enriched rotifers during the larval period showed significantly lower growth rates during the entire experimental period, or during a short period in their larval development (two-way, type III, MANOVA, Wilks' lambda, $P \leq 0{\cdot}001$, Fig. 6). No differences in growth rates or final body mass were found between fish that had either entirely or partly been fed with zooplankton during the larval stage.

DISCUSSION

GROWTH PERFORMANCE

This experiment revealed major growth effects as determined by first-feeding diet in both the larval and juvenile periods of *G. morhua*. This result stresses the importance of diet during early development on long-term growth, as well as the potential for diet improvement in commercial aquaculture of this species. Nutritional contents and limitations of live prey (Hamre *et al*., 2008), as well as nutritional differences between enriched rotifers and zooplankton have been well documented (van der Meeren *et al*., 2008). Even though nutritional contents can vary widely across prey species, enrichment procedures, geographical location or season, the main trend is that zooplankton contains higher levels of DHA and EPA compared with enriched forms of rotifers or *Artemia* sp. Two different enriched *Artemia* sp. diets with similar DHA contents fed to *G. morhua* larvae (37–59 dph) resulted in significantly different mean g (10·4 or 6·9% day^{-1}) (Garcia *et al*., 2008*a*). A similar result was reported

for different rotifer enrichment methods where a diet containing the highest DHA content did not result in the highest *g* in *G. morhua* larvae (Garcia *et al*., 2008*b*). Enriched rotifers, however, differ from natural zooplankton not only nutritionally (van der Meeren *et al*., 2008) but also in size, and size variability, energetic content, swimming speed, behaviour (Beck & Turingan, 2007) and general appearance. It is likely that all these different aspects, individually as well as interactively, have contributed to larval feeding behaviour, larval growth performance and developmental success.

Larvae fed with zooplankton or copepods as first-feeding diet showed higher growth rates, resulting in final body mass on average six-fold higher at time of weaning at high larval density, up to 11 fold higher post-weaning (50 dph), when compared with rotifer-fed larvae. Growth in fish larvae is highly dynamic (van der Meeren & Næss, 1993) and reacts according to the availability, suitability and digestibility of prey (van der Meeren & Næss, 1993; Hamre *et al*., 2008). Little is known about the energetic reserves in *G. morhua* larvae, but different lipid storage strategies have been suggested during early development of fishes (Sabatés *et al*., 2003) depending on species and nutrient availability. But, because of the dramatic size increase

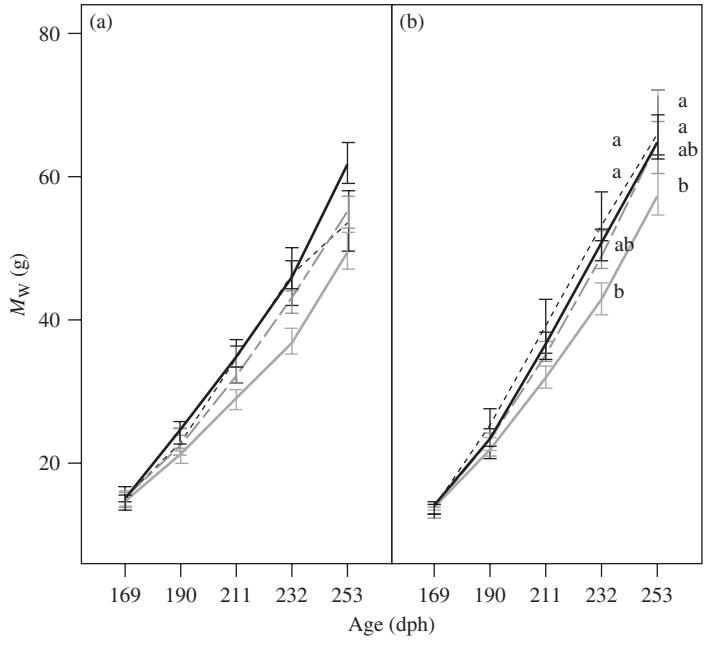

FIG. 5. Mean ± S.E. wet mass (M_W) for four groups of *Gadus morhua* juveniles reared at (a) 10 or (b) 14° C at age (days post hatch, dph). Groups differ in having received either zooplankton during the earlier larval period (Z, ——) or enriched rotifers (R, ——). Larvae from the RZ group (.....) had been fed with enriched rotifers from 1 to 22 dph, after which they received zooplankton from 22 to 36 dph. The ZR group (.....) received zooplankton between 1 and 22 dph, after which they changed to enriched rotifers between 22 and 36 dph. All larvae had been weaned onto the same formulated feed at 36 dph onwards. The juvenile growth experiment started at 169 dph and lasted for 3 months. Different lowercase letters denote significant difference among groups within each sampling date (Student–Newman–Keuls *post hoc* test, $P < 0{\cdot}05$).

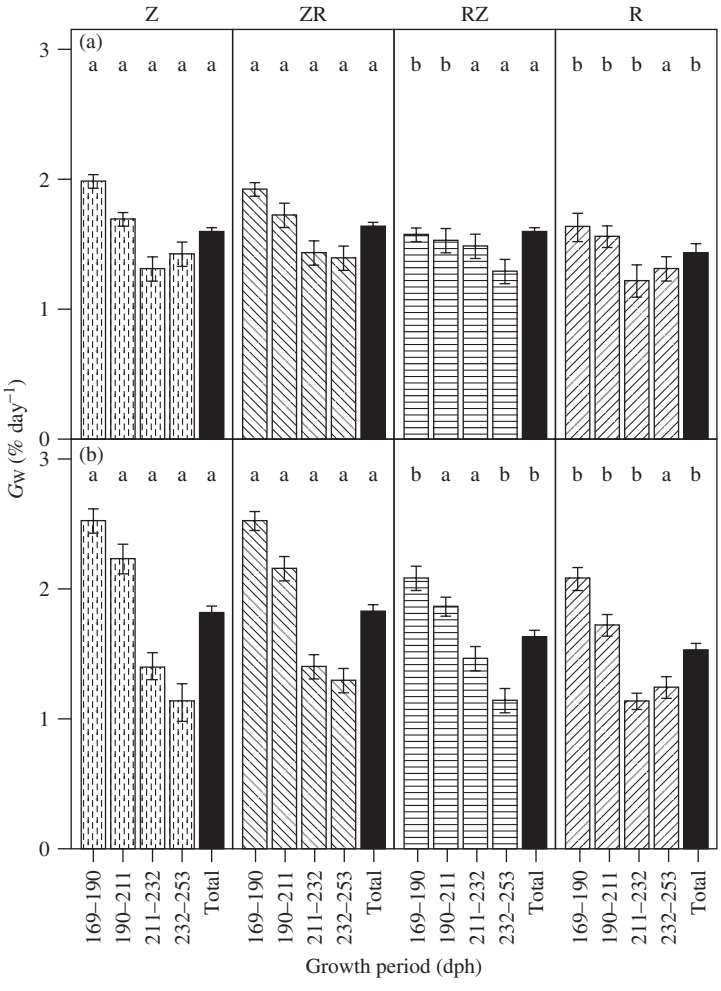

FIG. 6. Mean ± S.E. mass-specific growth rates (G_W) of four groups of individually tagged juvenile *Gadus morhua* reared at either (a) 10 or (b) 14° C, during four consecutive periods. Each period lasted for 21 days, starting at 169 days post hatch (dph). Total, G_W for the entire experimental period. The groups differ in having been fed with either zooplankton as a first-feeding diet (Z) or enriched rotifers (R). Fish from the RZ group had been fed with enriched rotifers during their larval period, from 1 to 22 dph, after which they received zooplankton from 22 to 36 dph. The ZR group changed from zooplankton during 1 and 22 dph to enriched rotifers from 22 to 36 dph. All fish had been weaned onto the same formulated feed at 36 dph onwards. Different lowercase letters indicate significant differences between groups within each period ($P < 0{\cdot}05$).

during the larval period and consequent need for adequate nutrients, it is likely that even a short period of nutrient deficiency, or availability of prey, could result in disrupted growth or development. Nutritional differences, environmental differences, prey size or larval selection are all likely to have individually or interactively caused the growth differences between the two groups in the current study. Prey size was probably one of these factors, which could have led to the sharp growth increase

in larvae that changed diet from rotifers to zooplankton at 22 dph since the size of prey increased from 150 to 320 μm to 400 μm. An increase in prey size at 20 dph has been shown to affect somatic growth positively in *G. morhua* larvae (van der Meeren & Moksness, 2003), which shows the effect of a size-related preference at different ontogenetic stages. This then indicates that rotifers are not optimal for larval nutrition from *c*. 20 dph onwards. How much the size difference or size variation between the rotifers and the zooplankton is being accounted for in this growth effect, however, is yet unclear.

No growth difference was observed between rotifer or zooplankton-fed larvae before 29 dph, at either density. It seems that prey type is not a major issue up to a certain larval size (7–7·5 mm L_S). Given the fact that the larvae increased in body mass up to six times during this specific period, it was expected that optimal prey size would increase accordingly (Ward & Coburn, 2008), which would then have led to increased growth in the zooplankton group, which was not observed until later. Differences in live prey species can lead to early differentiation of larval growth (Grageda *et al*., 2008), which can be due to purely nutritional factors (Garcia *et al*., 2008*a*, *b*, *c*). The present findings show a lack of growth differences between the first-feeding groups until 29 dph. This effect has also been found in *H. hippoglossus* (Hamre *et al*., 2002; Sæle *et al*., 2003), where no growth difference was found during the first 45 days of feeding either zooplankton or enriched *Artemia* sp. This lack of growth difference in early larval development could be due to different developmental strategies or priorities. *Hippoglossus hippoglossus* shows a distinct metamorphosis during this period when fed enriched *Artemia* sp. rather than zooplankton (Hamre *et al*., 2002; Sæle *et al*., 2003). This could indicate that *G. morhua* larvae can prioritize the process of size increase as compared to flatfishes. Based on the present results, this implies that *G. morhua* development can be divided into early development with relatively low exogenous nutrient requirements (1 to 22 dph) followed by rapid development for which a wider or more balanced variety of nutrients are required (22 to 36 dph).

The diet transfer period was during the period of fast growth and leads to a sharp growth increase in the group that changed diet from rotifers to zooplankton, while the opposite change reduced growth significantly. Even though there were no effects of prey type between the groups up to 22 dph, it is possible that the cumulative effects of suboptimal nutrition became apparent by 29 dph either through direct or indirect effects on organogenesis. Shields *et al*. (2003) studied the effect of a diet change from rotifers to *Artemia* sp. in *G. morhua* larvae at 5, 15 or 25 dph, which resulted in high mortality when changed at 5 dph, and had no lasting size effect in groups that changed diet at 15 or 25 dph (Shields *et al*., 2003). They recommended feeding rotifers until the completion of metamorphosis. The present results, however, indicate a persistent growth disadvantage when rotifers are offered beyond 22 dph compared with zooplankton. Weaning onto microparticulate diets at 22 dph could be an alternative option. This can be done successfully as early as 8 dph in *G. morhua* (Baskerville-Bridges & Kling, 2000*a*), but survival at early weaning was reduced compared with feeding live prey (Baskerville-Bridges & Kling, 2000*b*), and optimal nutritional compounds of microparticulate diets are possibly not yet optimized (Blair *et al*., 2003; Fletcher *et al*., 2007).

The present results show homogenous growth patterns in rotifer-fed larvae, while a larger size variation was found in the zooplankton-fed group. The smallest fish in the

zooplankton group benefited most from weaning and showed a rapid mass increase at weaning, while the proportion of large fish showed reduced growth during weaning. Due to the homogeneity of size in the rotifer group, this effect was not observed and overall growth suppression was observed at onset of weaning.

MORTALITY

Survival was different between the two first-feeding diets, up to weaning, and was higher in the rotifer-fed group (63·9 *v*. 45·4%), which may have been an effect of higher bacterial load in the zooplankton group. Total survival at 50 dph, however, showed no differences (26·4 *v*. 25·9%). Some studies have previously reported survival to be unaffected by prey type in *H. hippoglossus* fed rotifers or *Artemia* sp. (Hamre *et al*., 2002) or in larval haddock *Melanogrammus aeglefinus* (L.) fed rotifers or *Artemia* sp. (Blair *et al*., 2003). Survival, however, has also been found to be significantly higher in *H. hippoglossus* fed copepods rather than *Artemia* sp. (Shields *et al*., 1999), indicating large variations in survival between studies or rearing environments. Overall, survival rates in the current study (R = 10·8% and Z = 8·9%) were below those reported in comparable studies of *G. morhua* as reported survival differed between 32·7 and 39·4% at 71 dph (Baskerville-Bridges & Kling, 2000*b*) or 15 and 40% (Folkvord *et al*., 1994).

At high density, survival at 50 dph was low in both feeding groups, which may be related to unsuccessful weaning rather than high density, because 41·6% survival up to 44 dph has previously been reported at high densities (300 larvae l^{-1}) (Baskerville-Bridges & Kling, 2000*c*). Survival was positively correlated with DHA levels in larval feed (Cutts *et al*., 2006) or omega 6DPA in the larval diet (Garcia *et al*., 2008*a*), which could have influenced the results in the present study too.

Stagnant systems up to weaning led to elevated ammonia concentrations at high densities, and thus, water quality could have contributed to the higher mortality within these groups. Except for one tank, the highest mortality occurred in the period after weaning, when all tanks had been switched to flow-through, and water quality could not have been a limiting factor. General disruption of the established balance between microbial communities within the tanks might then possibly have contributed to induced larval mortality (Olafsen, 2001).

CONDITION

Besides the general growth advantage for larvae-fed zooplankton rather than enriched rotifers, a significantly higher condition factor was observed in these larvae. A similar effect was found in *G. morhua* larvae-fed diets with variable DHA:EPA ratios (Galloway *et al*., 1999), where the high-ratio diet enhanced growth rates and increased body mass, but did not affect larval length. This was further explained by a 25% difference in cross-sectional area of white muscle fibres at 8·5 mm length (Galloway *et al*., 1999). The difference in relative condition between the two first-feeding groups was most apparent when larvae were reared at high densities. Since growth and mass differed most between the groups at high density, this could suggest that this condition factor is growth rate related, suggesting that length (skeletal) growth is being prioritized over muscle deposition, in line with Galloway *et al*. (1999).

PERSISTING GROWTH EFFECTS

Besides faster growth and increased mass at size during the larval period, a long-term effect of first-feeding diet was observed, resulting in significantly higher growth rates in juveniles that had been first fed with zooplankton rather than enriched rotifers. This was first suggested in *G. morhua* (Imsland *et al*., 2006), where growth rates were higher in juveniles fed zooplankton during the larval period. Optimal larval diet has further been linked to juvenile growth and survival after weaning in sole *Solea senegalensis* Kaup (Canavate & Fernandez-Diaz, 1999). In the present study, no differences were found in juvenile growth rates between fish that had been first fed solely with zooplankton or only during a short period in their larval development. Only the group that had been fed with enriched rotifers throughout the larval period showed significantly lower growth rates. The superiority of copepods as a larval feed may be mainly due to the availability of HUFAs supplied as pre-formed phospholipids (Bell *et al*., 2003), as well as the naturally high concentration of DHA and EPA (Hamre *et al*., 2002). The fact that juvenile fish (10–70 g) performed equally well if they had been fed zooplankton in their larval period for 36 days or only for 14 days (22–36 dph) suggests that there is a critical time window between 22 and 36 dph in which optimal larval nutrition is required to attain full growth capacity during both their late larval and early juvenile periods.

The present findings agree with previously reported results where zooplankton or rotifers were fed to marine fish larvae, which resulted in better developed and faster growing larvae when fed zooplankton (Shields *et al*., 1999; Evjemo *et al*., 2003; Cutts, 2003), but are unique in reporting a long-term growth effect according to larval nutrition in larvae from a single egg batch. The lack of a diet-induced growth difference until 29 dph needs further investigation, as does the cellular and molecular basis for juvenile growth plasticity. The potential for growth improvement in *G. morhua* aquaculture could be large with minor adjustments of feeding regime. More research needs to identify nutrients and critical periods in early ontogeny for optimal exploration of commercially interesting teleost species.

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