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Larval rearing environment influences the physiological adaptation in juvenile Atlantic cod, *Gadus morhua*

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Abstract Juvenile Atlantic cod, *Gadus morhua*, that had been fed rotifers as larvae (R group) had significantly lower growth rates (SGR) at high-ambient ammonia (UIA = 115–120 μg l^{-1}) than did juveniles that had been first fed with natural zooplankton (Z group). Overall specific growth rates (SGRs) were 5–11% higher in the Z group at the control ammonia (UIA = 1–2 μg l^{-1}) treatments. An interaction between larval rearing history, oxygen and ammonia levels was found, as SGR decreased with decreasing oxygen levels at high ammonia in the R group, while the SGR in the Z group were less affected by hypoxia. At high-ambient ammonia, the SGRs were 5% (mild hyperoxia, 101–104% O_2 in effluent water), 28% (normoxia, 83–88% O_2 in effluent water) and 86% (hypoxia, 57–69% O_2 in effluent water) higher in the Z group, compared to the R group. The present findings indicate that larval rearing environment could influence the adaptability to environmental changes and growth performance during later juvenile stage in cod. These findings have implications for the optimization of Atlantic cod culture.

Keywords Atlantic cod (*Gadus morhua*) · Larval diet · Rotifers · Zooplankton · Ammonia · Oxygen · Growth

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Introduction

Intensive aquaculture of Atlantic cod, *Gadus morhua*, relies mainly on the use of rotifers, *Brachionus* sp., for feeding through the early developmental stages (Imsland et al. 2006). Much effort has been focused on the enrichment if the rotifers to increase their nutrient value and to improve the larval production (Park et al. 2006; Garcia et al. 2008). However, zooplankton as a larval diet can provide a better scope for growth and development in Atlantic cod (Imsland et al. 2006; Koedijk et al. 2010a, b), compared to enriched forms of rotifers or *Artemia*. The quality of a juvenile fish is in essence determined by the successful metamorphosis from a larva into a juvenile fish (Koven 2003), which stresses the importance of optimal nutrition during this critical life stage.

First-feeding prey can have a profound effect on growth rates and developmental success of marine fish larvae. In Atlantic halibut, *Hippoglossus hippoglossus*, larvae an increased pigmentation rate and increased eye migration were observed when the larvae were fed different stages of wild copepodites, *Eurytemora* sp. and *Centropages* sp., rather than enriched *Artemia* (Hamre et al. 2002). Also, Atlantic cod juveniles showed a lower incidence of deformities when fed zooplankton rather than enriched rotifers (Imsland et al. 2006). The higher overall developmental success in marine fish that were fed zooplankton instead of *Artemia* or rotifers has been suggested to be caused by a higher concentration of docosahexaenoic acid (DHA) in zooplankton (Shields et al. 1999; Hamre et al. 2002).

Long-term effects of rearing environment or larval diet on the physiological adaptation during the later juvenile stage have, however, remained largely unstudied. This study aims at determining the effects of early rearing environment on the response to differences in water quality during the juvenile stage of Atlantic cod, with oxygen and ammonia as environmental variables. Dissolved oxygen (DO) and ammonia are likely to differ in high-density aquaculture systems. Dissolved oxygen, besides feed and temperature, is the most important factor controlling fish growth in intensive aquaculture. Use of additional oxygen can increase the carrying capacity of a fish culture system, as DO is generally the first growth limiting factor (Jobling 1994). Further, both cod and spotted wolffish, *Anarhichas minor*, showed a lower sensitivity to ammonia under hyperoxic conditions compared to normoxic conditions suggesting an interactive effect of oxygen and ammonia on growth (Foss et al. 2003; Remen et al. 2008). Recent findings indicate that cod juveniles can adapt to high unionized ammonia nitrogen (UIA-N) concentrations when exposed to chronic high-ambient ammonia (Foss et al. 2004; Remen et al. 2008).

The direct differences in larval growth, metamorphosis, or developmental disorders such as malpigmentation (McEvoy et al. 1998; Bell et al. 2003; Hamre et al. 2007), or skeletal deformities (Imsland et al. 2006) are often being described as caused by inadequate feeding during the larval stage. We hypothesize that physiological boundaries in the capacity to adapt to environmental changes are to a certain extent also being established during the larval period. Since the quality of cod juveniles for use in aquaculture is thought to be higher when first fed with zooplankton (Imsland et al. 2006), we hypothesize that these fish can adapt to a wider range of ammonia and oxygen conditions compared to fish that were first fed with enriched rotifers. The aim of this study was, therefore, to investigate whether larval rearing environment has an effect on the adaptive capacity towards changes in water quality in the later juvenile period.

Materials and methods

Fish material and experimental rearing conditions

Two groups of eggs of Atlantic cod were obtained from a cod hatchery in western Norway with three-day interval (61°40′N). The brood fish were wild caught in the waters off Møre (W-Norway) in 2003 and reared in 70 m^3 tanks at simulated natural photoperiod and temperature of 6–8°C (sea water pumped from 100 m depth, filtered and UV treated). The mean weight of the brood fish was approximately 15 kg (range 7–22 kg). The first batch of eggs was incubated on site, whereas the second batch of eggs was transported to another commercial juvenile cod producer where they were incubated at the same temperature (8°C). Incubating and rearing at two separate sites was done to be able to benefit from two different first-feeding techniques (enriched rotifers (R) versus wild caught zooplankton (Z)), while tank size, temperature and larval density were similar. The first batch (R group) hatched on 24 September 2004 after an incubation period of 13 days. First feeding was done at 8–10°C with rotifers (*Brachionus plicatilis*, average size $\approx$ 260 μm) enriched with *Nannochloropsis* (Instant Algae® Premium 3600, Reed Mariculture, Campbell, CA, USA) after which the fish were weaned on a commercial formulated feed (Marin 030-050, Ewos A/S, Bergen, Norway) containing 60% protein, 12% fat and 12% carbohydrates. A co-feeding period of 10 days was applied from 25 to 35 days post hatch (dph). The second batch (Z group) hatched on 27 September 2004 after 13 days of egg incubation and was first fed on freshly filtered natural zooplankton. In the first 20 days, the size fraction ranged from 80 to 500 μm, mean size $\approx$ 210 μm and increased to 80–1,000 μm from 20 to 25 dph (mean size $\approx$ 460 μm). Zooplankton was mainly containing copepod nauplii (predominantly consisting of *Temora* sp.). Prey densities were similar for both fish groups at 2,000–5,000 prey l^{-1} until 12 dph, increasing to 8,000–9,000 prey l^{-1} until 25 dph when formulated feed was introduced and the amount of live prey was decreased over a period of 10 days. Prey density was measured and adjusted twice daily in both groups. Both groups were reared at a light regime of 16 h light/ 8 h dark (LD16:8), at the same density of 50 larvae per l in circular tanks (20,000 l). The water was mildly aerated and oxygen level kept above 80% at all times. 'Green water technique' was used (*Nannochloropsis*, 100 cells μl^{-1}). Flow through was applied in both groups from 5 or 6 dph, with 10–20% exchange per day increasing to 150–200% per day at weaning. The salinity was 34 ppt at both locations. On 6 January 2005, all fish were transported to the Industrial and Aquatic Laboratory at the Bergen High Technology Centre and reared at 10°C and simulated natural photoperiod (LDN, 60°N). This light regime was used throughout the acclimation and experimental period.

To be able to observe the growth performance of individual fish, a total of 258 (R group, age 132 dph, mean weight ± SEM, 15.8 g ± 0.4) and 275 (Z group, age 129 dph, 16.4 g ± 0.5) juveniles were tagged intraperitoneally with Trovan® Passive Transponder tags (BTS Scandinavia, Sweden); on 2 February 2005, the juveniles were then distributed randomly into 12 (1 m^2) square, covered fibreglass experimental tanks with a rearing volume of 420 l. Efforts were made to ensure equal size distributions and initial size range (min–max) was 10.7–56.3 g for the R group and 11.4–59.2 g for the Z group. Additional untagged fish were distributed randomly to each tank to a total initial biomass per tank of 3.4–3.7 kg m^{-3} ($n = 60$–64 in each tank). The seawater had a salinity of 34‰, and the temperature was 12°C throughout the experiment. Water flow was initially set to 3 l min^{-1} for all experimental tanks and was increased to 4 l min^{-1} on 10 April due to increasing biomass. A 36 W fluorescent daylight tube integrated in the tank-covers provided light. Photon-irradiation measured at the bottom of the tanks was approximately 5 μmol m^{-2} s^{-1}.

Table 1 Experimental conditions for the six treatments during the study period (64 days). Results are given as means ± standard deviation of two replicate tanks per treatment. *n* denotes the number of fish per group within each treatment

Treatment	*n* (R)	*n* (Z)	O_2 (%)	TAN (mg l^{-1})	UIA-N (μg l^{-1})	pH	T (°C)
Hyperoxia-control	40	46	100.5 ± 8.6	0.30 ± 0.06	2.0 ± 0.4	7.7	12.4 ± 0.4
Hyperoxia-high	45	46	104.0 ± 9.1	17.80 ± 3.32	115 ± 21.8	7.8	12.3 ± 0.3
Normoxia-control	44	45	82.7 ± 3.7	0.17 ± 0.02	1.0 ± 0.2	7.8	12.5 ± 0.7
Normoxia-high	42	48	87.5 ± 4.9	17.60 ± 3.00	115 ± 20.1	7.8	12.4 ± 0.3
Hypoxia-control	44	47	57.0 ± 6.4	0.29 ± 0.06	2.0 ± 0.4	7.7	12.5 ± 0.3
Hypoxia-high	43	43	68.2 ± 7.2	18.50 ± 2.07	120 ± 16.6	7.8	12.3 ± 0.4

TAN total ammonia nitrogen, *UIA-N* un-ionized ammonia nitrogen

Prior to, and during the experiment, the juveniles were fed a commercial formulated feed (EWOS Marin 20, EWOS AS, Bergen), containing 55% protein, 12% fat and 11% carbohydrate with pellet size 3 mm. Because the current experiment was part of a feed efficiency experiment for which waste feed was collected, the pellet size was kept the same throughout the experiment. Food was provided in excess from automatic feeders for 2 h daily (0800–0900 and 1400–1500).

Experimental design

The experiment was set up as a 2 × 2 × 3 factorial design with both fish groups represented within each tank (individuals or groups traceable by individual tags) and replicate tanks nested within treatments. Treatments consisted of a hypoxic (57–69% O_2 in effluent water), a normoxic (83–88% O_2 in effluent water) and a mild hyperoxic group (101–104% O_2 in effluent water), in combination with two levels of un-ionized ammonia: high (115–120 μg l^{-1}) and control (1–2 μg l^{-1}, Table 1). Hypoxic conditions were obtained by partially replacing the dissolved oxygen with nitrogen gas (N_2), which was continuously injected into a packed aeration column where the inflowing water passed over a large polyethylene surface. Hyperoxic conditions were obtained by adding pure oxygen to the water in the header tanks. Elevated ammonia levels were obtained by adding a concentrated solution of NH_4Cl (100 g NH_4Cl l^{-1} dissolved in fresh water) by electromagnetic metering pumps (Iwaki Co. Ltd, Tokyo, Japan) into the header tanks. Total ammonia nitrogen (TAN) was measured daily from outlet water (100 ml, replicated) with an ammonia gas sensing combination electrode (Thermo Orion, Model 95-12) connected to an ion analyzer (Thermo Orion, EA™ 920). The fraction of un-ionized ammonia nitrogen was calculated as described by Remen et al. (2008). The pH was 7.78 (±0.07, SE) and did not differ between treatments.

The growth trial lasted for 9 weeks, from 9 March to 12 May 2004. Wet weight (WW) and total length (L) were measured on anaesthetized (Metacain, 0.05 g l^{-1}), individually tagged fish, at approximately 3-week intervals on day 0, 23, 43 and 64. In order to measure total tank biomass, wet weight was also measured for untagged fish at each sampling date.

Growth parameters

All growth estimates in the present study were based on individually tagged fish, whereas results on feed conversion efficiency and daily feed consumption were based on the total

biomass per tank. Specific growth rate (SGR) was calculated according to the formula of Houde and Schekter (1981).

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

where the instantaneous growth coefficient g is given as follows: $g = (\ln W_2 - \ln W_1)(t_2 - t_1)^{-1}$ and W_2 and W_1 are wet weight (g) on days t_2 and t_1, respectively.

Relative condition factor (CF) was defined as follows:

$$\mathrm{CF} = 100\left(W\left(0.004357 \cdot L^{-3.26}\right)\right)$$

where W is the weight of the fish and L the corresponding total length. The factor 3.26 was calculated for all individually marked fish as being the slope of the least-squares regression of $\log W \times \log L$ and gave the overall equation: $\log(W) = 3.26 \log(L) - 2.36$. The intercept correction induced the total average to be close to 1.0.

Feeding efficiency

Total feed consumption (C_T), daily feeding rate (F %) and feed conversion efficiency (FCE) were calculated on tank level (ammonia and oxygen levels) during the experimental period. Feed that was not consumed was flushed out of the tank by the normal water flow through a bottom outlet, collected and pellets were counted manually to obtain the amount of uneaten feed as a fraction of the fed amount. Daily feeding rate (F %) was calculated as follows:

$$F\,\% = 100\,[C_\mathrm{T}/\,((B_1 + B_2)/2)]\,(t_2 - t_1)^{-1}$$

where C_T is feed consumption (g) in the period and B_1 and B_2 are fish biomass (g) on days t_1 and t_2, respectively.

Feed conversion efficiency (FCE) was calculated as biomass gain per unit weight of feed consumed:

$$\mathrm{FCE} = (B_2 - B_1)\,C_\mathrm{T}^{-1}.$$

As the R and Z groups were mixed in each tank, feed parameters in these groups were estimated separately in a 14 days satellite experiment. After termination of the main growth experiment, a random group of tagged fish from both first-feeding diets ($n = 30$ per first-feeding diet) were reared in four tanks (300 l, two replicates per group), where FCE and F % were measured. All fish were from the control ammonia treatment at normoxia. Temperature was kept at 12°C, the same as during the main experiment. Tank biomass (1,970–2,070 g) and mean initial weight of the fish (66–69 g) did not differ between tanks or first-feeding groups in the satellite experiment.

Statistical analysis

All statistical analyses were performed using SPSS 14.0.2. To assess normality of distributions, a Kolmogorov–Smirnov test (Zar 1996) was used, and homogeneity of variances was tested using Levene's F test (Brown and Forsythe 1974). Mortality percentages for both groups were arcsin transformed to assure homogeneity of variance and normal distribution of data. A four-way Model III nested MANOVA (Johnson and Wichern 1992) where replicate tanks were nested within ammonia and oxygen was applied to test for

overall differences in specific growth rates, with Wilks' Lambda (Λ) multivariate test to observe overall trends over time. The equation of the full nested model had the form:

$$X_{ijklm} = \mu + \alpha_i + \beta_j + \gamma_k + \delta_{l(ij)} + \alpha\beta_{ij} + \alpha\beta\gamma_{ijk} + \varepsilon_{ijklm}$$

where: μ, is the general level; α_i, is the treatment effect for oxygen$_i$; β_j, is the treatment effect for ammonia$_j$; γ_k, is the effect for first feeding diet$_k$; $\delta l_{(ij)}$, is the replicate factor (here: tank$_l$) nested within oxygen α_i and ammonia β_j; $\alpha\beta_{ij}$, is the interactive effect between oxygen α_i and ammonia β_j; $\alpha\beta\gamma_{ijk}$, is the three way interaction term; ε_{ijkl}, is the model error term.

Significant ANOVAs were tested for individual differences using Student–Newman–Keuls multiple comparisons test. Full model ANOVAs were reduced when replicate- or interaction terms were non-significant, when testing for main effects. Growth analyses were performed with a two-way analysis of covariance, (ANCOVA) with the initial weight as covariate, followed by a comparison of main effects with Bonferroni's confidence interval adjustment. A significance level (α) of 0.05 was used for all tests.

Results

Mortality

Overall mortality was significantly higher in the R group (13.8%) compared to the Z group (3.2%) (two-way ANOVA, $P < 0.02$). No trends were observed in mortality among treatments ($P > 0.60$).

Effect of first feeding on growth performance

The two first-feeding groups (R and Z) responded the same way to differing oxygen levels (three-way, type III, MANOVA$_{(group \cdot oxygen)}$, Wilks' lambda $(\Lambda)_{6,\ 950} = 0.98$, $P > 0.25$). However, the overall response to ammonia was found to be highly significant between the R and Z groups (three-way, type III, MANOVA$_{(group \cdot ammonia)}$, Wilks' lambda $(\Lambda)_{6,\ 950} = 0.98$, $P < 0.001$), with a significantly higher SGR at high un-ionized ammonia concentration in the Z group compared to the R group. This effect of first-feeding diet was most apparent under hypoxic-high conditions (Fig. 1). Under these conditions, mean SGR of the R group increased from -0.07 to 0.29% day^{-1}, while mean SGR of the Z group increased from 0.31 to 1.1% day^{-1} (Fig. 1). At control ammonia, higher SGRs were found in the Z group compared to the R group (Fig. 1). A decreasing SGR over time and with increasing size was observed in the three O_2 concentrations at control ammonia, while an increasing trend was observed at high-ambient ammonia. This pattern was similar in the R and Z groups.

Within the R and Z groups no initial weight difference was found among treatments (one-way ANOVA, $P > 0.75$ and $P > 0.20$ for the zooplankton and R group, respectively, Fig. 2). The Z group reached higher final weights compared to the R group (two-way ANCOVA, $P < 0.05$) in the hyperoxia-control ammonia and normoxia-control ammonia treatments, as well as in the hypoxia-high treatment.

Relative condition factor was higher overall in the Z group than in the R group, except in the hypoxic-control ammonia treatment and normoxic-high ammonia treatment, where the two groups did not differ (Fig. 3). High ammonia decreased relative condition mainly

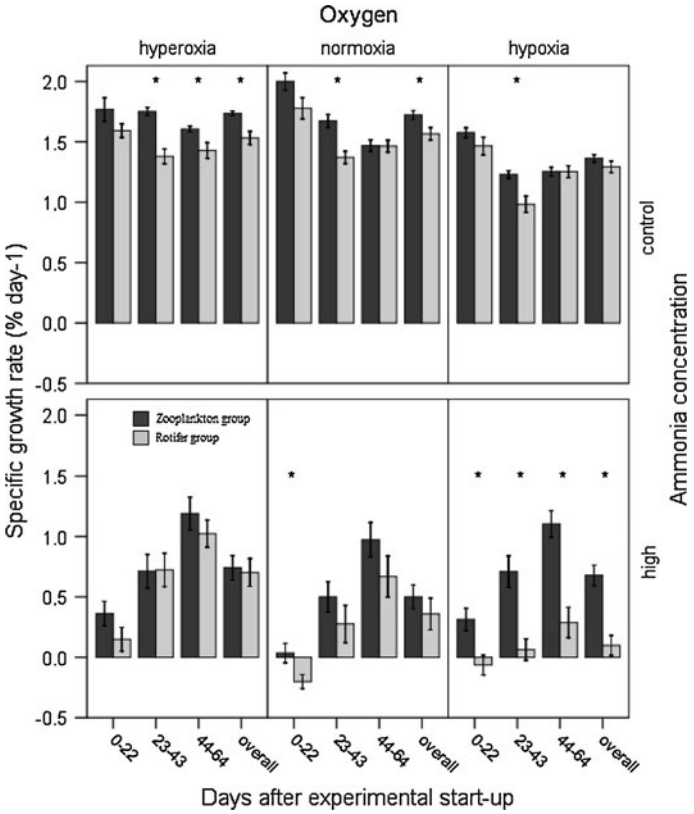

Fig. 1 Specific growth rates (SGR) of two groups of juvenile Atlantic cod reared at six combinations of oxygen and ammonia at three consecutive measuring periods and the overall experimental period. *Vertical whiskers* indicate standard error of mean (SEM). *Asterisks* show significant differences between groups within time period (two-way ANCOVA). n (R) = 40–45; n (Z) = 43–48 per treatment

during the first experimental period (0–23 days), after which the reduction in condition diminished. The Z group showed no further reduction in condition under high ammonia conditions from 43 days onwards, in contrast to the R group. At the control ammonia level, oxygen had a profound influence as both R and Z groups as both showed reduced condition under hypoxia, while a smaller decrease was observed at normoxia and hyperoxia. An overall interactive effect was found between ammonia and first-feeding diet, this was related to a higher condition in the Z group at both control and high-ambient ammonia (three-way, type III $\text{MANOVA}_{(\text{group}\cdot\text{ammonia})}$, Wilks' lambda $(\Lambda)_{4,\ 474} = 0.95$, $P < 0.001$) as well as an interactive effect between oxygen and ammonia ($\text{MANOVA}_{(\text{oxygen}\cdot\text{ammonia})}$, Wilks' lambda $(\Lambda)_{8,\ 948} = 0.84$, $P < 0.001$) which was related to higher condition factor with increased oxygen levels at high ammonia.

Feed consumption and feed conversion efficiency

High-ambient UIA-N concentrations resulted in a reduction in feed conversion efficiency (FCE), total feed consumption (C_T) and daily feeding rate (F) (Table 2). Oxygen level had

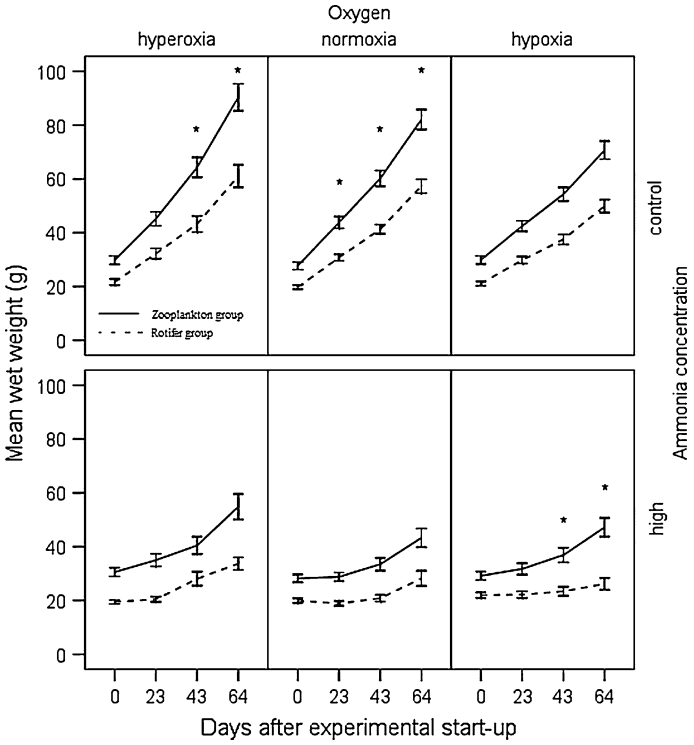

Fig. 2 Mean weight (g) for two groups of juvenile Atlantic cod reared at six combinations of oxygen and ammonia. *Whiskers* indicate standard error of mean (SEM). *Asterisks* indicate a significant difference between the two fish groups within treatment and date (two-way ANCOVA), with initial weight being covariate). *n* (R) = 40–45; *n* (Z) = 43–48 per treatment

no effect on FCE, but the total feed consumption increased significantly with increasing oxygen concentration at the control ammonia level (Table 2). Under high ammonia concentrations, hyperoxia significantly increased the total feed consumption.

When the feeding efficiency in the R and Z groups was determined in a separate 14 days experiment after termination of the oxygen-ammonia experiment, higher feed consumption (28%) and higher FCE (14%) were observed in the Z group compared to the R group (Table 3) together with a significantly higher final mean weight (Table 3).

Discussion

Present results suggest that a long-term effect of larval rearing method on the physiological capacity of juvenile cod to adapt to high NH_3 and low O_2 and subsequent growth performance at later life stages. The juveniles in the rotifer (R) group seem to have compromised physiological ability to withstand high NH_3 levels. Fish that were first fed on zooplankton (Z) rather than enriched rotifers showed higher specific growth rates during the juvenile stage across all treatments and performed significantly better under the most unfavourable environmental conditions. The difference in adaptive performance was most profoundly visible under the unfavourable environmental condition with high

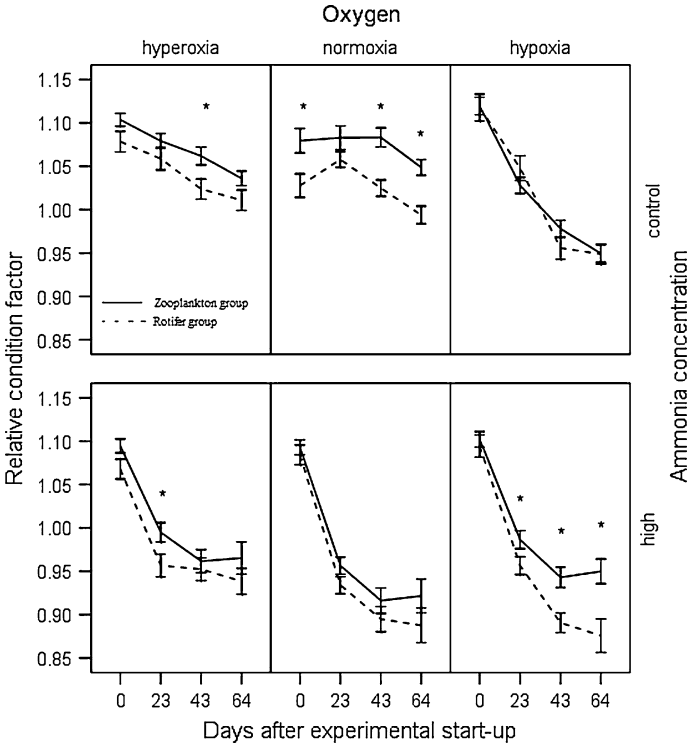

Fig. 3 Mean relative condition factor (CF) of Atlantic cod juveniles reared at six combinations of oxygen and ammonia. *Whiskers* indicate standard error of mean (SEM). n (R) = 40–45; n (Z) = 43–48 per treatment. *Asterisks* show significant differences between groups within time period and treatment (two-way ANCOVA)

Table 2 Food conversion efficiency (FCE), total feed consumption (C_T) and daily feeding rate (F %) in Atlantic cod juveniles reared at different oxygen and ammonia concentrations

Treatment	FCE	C_T (g)	F (%)
Hyperoxia-control	0.94 ± 0.09^{a}	$2{,}302 \pm 48.0^{a}$	1.85 ± 0.03^{a}
Hyperoxia-high	0.62 ± 0.12^{ab}	944 ± 13.4^{d}	1.06 ± 0.01^{c}
Normoxia-control	0.94 ± 0.05^{a}	$2{,}100 \pm 160.8^{b}$	1.85 ± 0.15^{a}
Normoxia-high	0.27 ± 0.12^{b}	692 ± 52.7^{e}	0.91 ± 0.03^{cd}
Hypoxia-control	0.85 ± 0.09^{a}	$1{,}684 \pm 34.9^{c}$	1.54 ± 0.03^{b}
Hypoxia-high	0.35 ± 0.19^{b}	671 ± 12.4^{e}	0.85 ± 0.03^{d}

Results are given as means ± standard deviation. Different letters in the same column denote significant differences between treatments (Student–Newman–Keuls multiple comparison, $P < 0.05$)

environmental ammonia combined with hypoxia. At the hypoxic-high ammonia treatment, the Z group displayed an overall 38% increase in weight, compared to 16% in the R group. Atlantic cod has a capacity to withstand and adapt to relatively high levels of ambient unionized ammonia over an extended time period (Foss et al. 2004; Remen et al. 2008). The Z group was initially less affected by high UIA-N levels and showed increased SGR after exposure to UIA-N faster than the R group, suggesting more rapid acclimation in the

Table 3 Mean values for initial weight (W_I), final weight (W_F), total feed consumption (C_T), feeding rate (F), specific growth rate (SGR) and feed conversion efficiency (FCE), for two fish groups during a 14-day period

	n	W_I (g)	W_F (g)	C_T (g)	FCE	F (%)
R group	30	66.6 ± 1.3	74.2 ± 0.1[b]	455 ± 29[b]	0.50 ± 0.06	0.65 ± 0.05[b]
Z group	30	68.1 ± 1.2	80.5 ± 1.3[a]	636 ± 18[a]	0.58 ± 0.02	0.86 ± 0.04[a]

Results are given as means ± standard deviation. Different letters in the same column denote significant differences between the two fish groups (one-way ANOVA, $P < 0.05$)

Z group. The tolerance to hyperoxic conditions is generally high in marine fish species (Person-Le Ruyet et al. 2002; Foss et al. 2003; Remen et al. 2008), but even though the mild hyperoxic and hypoxic conditions (at control ammonia level) in the present study did not affect feed conversion efficiency, the feed consumption decreased significantly with decreasing oxygen levels. Mild hyperoxia (120% or 150% air saturation) for a period of 94 days did not cause significant difference in feed consumption or growth in juvenile Atlantic halibut compared to fish exposed to normoxia (100% air saturation in water outlet) (Thorarensen et al. 2010). This is in line with our findings where no differences were found in final weight between hyperoxic and normoxic conditions in the Z group, although the fish in the present study only experienced a relatively mild hyperoxia. This result was, however, not consistent between the two first-feeding groups, as the R group had a significantly higher final weight when exposed to hyperoxia compared to normoxia, thus benefiting from the increased oxygen level. The R group also showed a clear decrease in SGR with decreasing oxygen levels under high UIA-N levels, while the Z group performed similarly between all three oxygen levels at high UIA-N. This indicates a higher sensitivity towards hypoxia or hyperoxia in the fish that were first fed on enriched rotifers compared to the fish that had been fed zooplankton as a first-feeding diet.

Direct effects of first-feeding diet have previously been shown to affect growth performance in turbot larvae (Conceição et al. 1997), and a lower incidence of deformities in Atlantic cod was observed when larvae were first fed natural zooplankton (Imsland et al. 2006). In general, studies performed on different species of flatfish have shown that the successful completion of metamorphosis is strongly linked to first-feeding diet (Estevez et al. 1997; Næss and Lie 1998; Hamre et al. 2002; Bell et al. 2003). These findings have been related to a higher docosahexaenoic acid (22:6n-3; DHA) content and DHA/EPA (eicosapentaenoic acid; 20:5n-3) ratio (McEvoy et al. 1998). Nutritional differences between enriched rotifers and natural zooplankton are largely related to a higher content of the fatty acids DHA and EPA in copepods (Evjemo et al. 2003; Drillet et al. 2006; van der Meeren et al. 2008), and our results suggest that these early dietary differences influence the physiological response to dissolved oxygen and ammonia in juvenile Atlantic cod.

Even though no genetic profile was made for the two groups, it is likely that the two fish groups originate from the same spawning group, since the observed spawning interval (2–3 days) corresponds with known spawning intervals for cod in captivity (Kjesbu et al. 1996). However, as the two first-feeding groups are from two different egg groups from the same spawning group, genetic effect, e.g. different family composition of the two groups, cannot be ruled out as confounding factor. In a recent study (Imsland et al. 2011), body weight variation in Atlantic cod was retrospectively fractionized according to different environmental and genetic sources. The study shows that first-feeding method and environmental manipulation explain nearly 90% of the body weight variation during early

juvenile phase. The genetic effect only accounted for around 2% of body weight variation during the early juvenile (<100 g) rearing period, whereas it had a large impact on growth variation during long-term rearing at ambient conditions (Imsland et al. 2011). For the present study, this means that the environmental manipulation (oxygen and ammonia concentrations) will dominate the observed growth variation during the trial period, whereas the genetic effect can be disregarded as a confounding variable during this period.

In the present study, the largest performance difference between the R and Z groups occurred in the treatment where high ammonia was combined with hypoxia, suggesting that zooplankton fed fish are less susceptible to the interactive effect between high ammonia and hypoxia. Remen et al. (2008) showed a reduced ability of cod to maintain homoeostasis when hypoxia was combined with high ammonia due to increased gill ventilation as a reaction to hypoxia, leading to a relatively higher effective exposure to ammonia. These findings, and those of the current study, may indicate different efficiency of the gills between the R and Z fish groups, but additional research is needed to clarify this.

The Z group showed a significantly higher feed consumption and feeding rate compared to the R group, together with a higher, albeit non-significant, feed conversion efficiency. In line with current findings, Imsland et al. (2006) found significant differences in feed consumption, feeing rate and feed conversion efficiency for Atlantic cod juveniles (60–80 g) that had been first fed on enriched rotifers or zooplankton. These differences may have played a part in the higher overall growth capability seen for the Z group. A recent study (Amberg et al. 2008) revealed a higher increase in the oligopeptide transporter PepT1 expression in the digestive tract of Atlantic cod larvae when they had been first fed with zooplankton compared to those first fed with enriched rotifers. Since PepT1 is thought to facilitate the absorption of di- and tri-peptides (Amberg et al. 2008), a higher digestive capacity would then be expected in the Z group, which may help to explain the differences seen in feeding parameters between the R and Z groups.

Conclusion

The present results suggest that first-feeding diet significantly influences juvenile growth and may influence the subsequent successful adaptation to differences in water quality during the juvenile period. Differences in larval nutrition resulted in profound differences in the response to high environmental ammonia levels. Further research is needed to define which factors during early development primarily affect the adaptive response to environmental stressors.

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