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Short- and long-term differences in growth, feed conversion efficiency and deformities in juvenile Atlantic cod (*Gadus morhua*) startfed on rotifers or zooplankton

Albert K Imsland[1,2], Atle Foss[3], Roland Koedijk[2], Arild Folkvord[2], Sigurd O Stefansson[2] & Thor M Jonassen[4]

[1]Akvaplan-niva Iceland Office, Kópavogur, Iceland

[2]Department of Biology, University of Bergen, High Technology Centre, Bergen, Norway

[3]Akvaplan-niva Bergen, Bergen, Norway

[4]Marine Harvest Norway AS, Bergen, Norway

Correspondence: A K Imsland, Akvaplan-niva Iceland Office, Akralind 4, 201 Kópavogur, Iceland, E-mail: albert.imsland@akvaplan.niva.no

Abstract

Growth, feed conversion efficiency and frequencies of skeletal deformities were studied in juvenile Atlantic cod (*Gadus morhua*) that had been startfed on either rotifers (rotifer group) or zooplankton (zooplankton group). After metamorphosis, the fish were reared at four constant temperatures (7, 10, 13, 16 °C) or moved successively from 16 to 13 and 10 °C (T-step, average 13.2 °C). The zooplankton group had a consistently higher growth rate at all the temperatures studied. Further, the zooplankton group had higher food intake (20%) and higher feed conversion ratio (1.65 vs. 1.31). In addition, a significantly higher incidence of skeletal deformities was found in the rotifer group (14.2%) compared with the zooplankton group (4.1%). After termination of the laboratory study, the fish were reared in sea pens under ambient conditions for 17 months. Final weights of the zooplankton group were consistently larger (between 12% and 14% larger depending on original temperature groups). To verify the growth results, we conducted a follow-up study where a single egg group was divided into two parts and fed either on rotifers or zooplankton. This study indicated similar growth differences as found in the first study. Overall, our data suggest that different startfeeding diets may be important for long-term growth, incidence of deformities and quality of juvenile cod. The use of zooplankton can greatly improve long-term growth and quality of cod juveniles. The study also highlights the advantage of using elevated temperatures in the juvenile phase as this will lead to significantly higher final weights in the adult stage.

Keywords: anatomic deformities, startfeeding methods, feed conversion efficiency, growth physiology, long-term growth

Introduction

Intensive aquaculture of cod (*Gadus morhua* L.) relies mainly on the use of rotifers (*Brachionus*. sp.) and *Artemia* for feeding through the early developmental stages (Brown, Minkoff & Puvanendran 2003; Shields, Irwin, Smith & McEvoy 2003). Much effort has been focused on the enrichment or bioencapsulation to increase the essential highly unsaturated n-3 fatty acid (n-3HUFA) levels to increase their nutritional value (Devresse, Leger, Sorgeloos, Murata, Nasu, Ikeda, Rainuzzo, Reitan, Kjørsvik & Olsen 1994; Olsen 1997; Yoshimatsu, Imoto, Hayashi, Toda & Yoshimura 1997). This procedure provides a stable source of larval nutrition as rotifers can be cultured, and dry cysts of *Artemia* are available, all year round. It is also possible to use only rotifers during first feeding and wean directly on dry feed (Baskerville-

Bridges & Kling 2000). Some hatcheries, however, rely on the use of natural zooplankton during a part of the year or synchronize their production cycle with the availability of zooplankton in the wild. There are, however, large differences in the nutritional value of rotifers and zooplankton (Evjemo & Olsen 1997; Mæland, Rønnestad, Fyhn, Berg & Waagbø 2000; Bell, McEvoy, Estevez, Shields & Sargent 2003). Based on nutrient content, copepod nauplii are assumed to be the most suitable live prey for first feeding of fish larvae (Evjemo & Olsen 1997). Rotifers can provide a useful method of essential fatty acid delivery whereas *Artemia* are poorer in this regard, especially at first feeding. Copepods are nutritionally beneficial due to their naturally high levels of the essential HUFAs, 20:5n-3 (eicosapentaenoic acid (EPA)) and 22:6n-3 (docosahexaenoic acid (DHA), Bell *et al.* 2003).

Differences in physiological and anatomical properties of fish juveniles fed different first feeding diets have been indicated in other species (e.g. Hamre, Opstad, Espe, Solbakken, Hemre & Pittman 2002). Little is known, however, for Atlantic cod, in particular, consequences for skeletal development. Hence, the aim of the present study was to characterize how different startfeeding diets affect subsequent growth potential and anatomical development of juvenile Atlantic cod reared at five different temperature regimes. After metamorphosis, the fish were reared at four constant temperatures (7, 10, 13, 16 °C) or moved successively from 16 to 13 and 10 °C (T-step, average 13.2 °C). Appetite, feeding rate and feed efficiency ratio (FCE) were analysed in a satellite study.

Materials and methods

Fish material

Zooplankton group

The eggs were obtained from two commercial cod hatcheries. The zooplankton group was obtained from a cod hatchery in western (59°50′N) Norway and transported to the facilities of the University of Bergen at the Bergen High Technology Centre, where they were incubated on 14 March. The broodfish were wild caught in the area around Bømlo (W-Norway) in 2003 and reared in 40 m^3 tanks at a simulated natural photoperiod and a temperature of 6–8 °C (sea water pumped from 160 m depth). The mean weight of the broodfish was approximately 7 kg (range 5–18 kg). The incubation temperature for the eggs was 6.8 °C. The eggs hatched on 28 March and the larvae were subsequently transferred to a 500 L tank with a constant temperature of 7.8 °C. The larvae were reared under a natural light regime for Bergen (LDN, adjusted once a week). The larvae was startfed on 21 March (larval size 4.5 mm) and fed once a day fresh-filtered natural zooplankton approximately 2000 zooplankton L^{-1} with gradually increasing size fraction from 80 to 1000 µm, but with an addition of Nannochloropsis-enriched rotifers during the first 4 days. The water in the tank was kept stagnant and 1 L of concentrated Nannochloropsis (Instant Algae® Premium 3600, Reed Mariculture, Campbell, CA, USA) was used as green water and added to the tank on a daily basis, consisting of approximately 96 billion cells L^{-1}. The water was mildly aerated and the oxygen level was maintained above 80% at all times. No surface skimming was applied. On 6 April, the larvae were transferred to a tank with a continuous water flow, at a constant temperature of 8.6 °C, and co-fed from 12 April with commercial formulated feed and natural zooplankton until 20 June. From 20 June, only formulated feed (Marin 030 and 050, Ewos A/S, Bergen, Norway) containing 60% protein, 12% fat and 12% carbohydrates was used. On 20 June 2003, the juveniles were brought to the Industrial and Aquatic Laboratory at the Bergen High Technology Centre and reared at 10 °C and a simulated natural photoperiod (LDN, 60°N). This light regime was used throughout the acclimation and experimental period. Survival from hatching to weaning was approximately 13% and the juveniles were not size sorted during the startfeeding and weaning period.

Rotifer group

The rotifer group originated from a cod hatchery in western Norway (61°40′N). The brood fish were wild caught in the area around Møre (W-Norway) in 2003 and reared in 70 m^3 tanks at a simulated natural photoperiod and temperature of 6–8 °C (sea water pumped from 100 m depth). The mean weight of the broodfish was approximately 15 kg (range 7–22 kg). The larvae protocol that was used was a modified intensive protocol (modified from Brown *et al.* 2003). The eggs were incubated at 7.0 °C, hatched on 25 March and were maintained at 8–10 °C (gradual increase) during the first weeks of development. The larvae were reared in a 20 000 L circular tanks, and 10–20 L of concentrated Nannochloropsis was used to create green water conditions. They were fed (size

Table 1 Mean weights (g) for juvenile cod fed either rotifers or zooplankton during the larval stage and subsequently reared at five different rearing temperatures during the juvenile stage

Variable			Laboratory trial						Sea pen rearing	
Temperature	Feeding group	*n*	9 September 2003	7 October 2003	29 October 2003	19 November 2003	12 December 2003	*n*	12 December 2003	29 June 2005
7 °C	Rotifers	44	8.4 (0.3)	12.0 (0.6)^{b}	15.7 (0.9)^{b}	20.4 (1.3)^{b}	26.8 (1.9)^{b}	9	36.5 (7.8)	1350 (106)^{a}
	Zooplankton	41	8.7 (0.3)	14.1 (0.6)^{a}	20.0 (0.9)^{a}	26.5 (1.3)^{a}	36.1 (1.9) a	8	43.1 (6.4)	1616 (87)^{b}
10 °C	Rotifers	50	8.4 (0.3)	14.3 (0.6)^{b}	20.8 (0.9)^{b}	28.6 (1.3)^{b}	39.0 (2.0)^{b}	12	47.7 (6.4)	1423 (88)
	Zooplankton	42	8.8 (0.4)	18.2 (0.5)^{a}	27.3 (0.9)^{a}	37.8 (1.3)^{a}	53.2 (1.8)^{a}	8	68.2 (6.7)	1632 (92)
13 °C	Rotifers	45	8.8 (0.3)	16.0 (0.6)^{b}	23.2 (0.9)^{b}	31.5 (1.4)^{b}	43.1 (2.0)^{b}	11	51.8 (5.5)^{b}	1529 (75)^{b}
	Zooplankton	43	9.1 (0.3)	20.0 (0.5)^{a}	31.4 (0.9)^{a}	45.5 (1.3)^{a}	66.9 (1.9)^{a}	9	71.2 (6.8)^{a}	1712 (92)^{a}
16 °C	Rotifers	45	8.6 (0.4)	15.3 (0.8)^{b}	22.8 (1.2)^{b}	29.3 (1.8)^{b}	39.1 (2.6)^{b}	6	42.9 (5.8)	1458 (78)^{b}
	Zooplankton	48	9.1 (0.4)	18.9 (0.8)^{a}	29.5 (1.3)^{a}	40.3 (1.9)^{a}	53.5 (2.7)^{a}	9	65.2 (6.4)	1720 (87)^{a}
T-step	Rotifers	42	8.9 (0.4)	16.8 (0.8)^{b}	24.0 (1.3)^{b}	33.6 (1.9)^{b}	42.7 (2.8)^{b}	14	46.3 (5.1)^{b}	1554 (69)^{b}
	Zooplankton	46	8.9 (0.4)	18.9 (0.8)^{a}	28.9 (1.3)^{a}	43.5 (1.9)^{a}	63.4 (2.5)^{a}	15	77.3 (5.0)^{a}	1825 (67)^{a}

Results are given as mean (standard error of mean); *n*, number of individually tagged fish of each startfeeding group at each temperature regime. Note that different analyses were performed for each temperature regime so in cases of significant ANOVAS (two-way ANOVA, $P < 0.05$), different letters indicate statistical differences, with 'a' as the highest value within each temperature regime (Student–Newmans–Keuls test, $P < 0.05$). The table includes data from temperature trial and from subsequent rearing under ambient conditions in sea pens.

at start-feeding approximately 5 mm) enriched (Nannochloropsis Instant Algae®) rotifers for 35 days (prey density 2000 rotifers L^{-1} for the first 10 days and then 4000 rotifers L^{-1}) and then co-fed for 10 days with commercial dry feed (Ewos Marin 030 and 050). During the rotifer feeding, the larvae were feed in three batches each day. The larvae were reared under constant 16 h light:8 h darkness (LD16:8). The water was mildly aerated and the oxygen level maintained above 80% at all times. No surface skimming was applied. After the weaning period, the juvenile was fed the same commercial feed as the zooplankton group described above. On 22 June, the fish were transported to the Industrial and Aquatic Laboratory (ILAB) at the Bergen High Technology Centre and reared under the same conditions as the zooplankton described above. The fish groups are hereafter referred to as either the zooplankton group or the rotifer group as determined by their first feeding diet. Survival from hatching to weaning was approximately 15% and the juveniles were not size sorted during the startfeeding and weaning period.

Experimental rearing conditions

After arrival at ILAB, the juveniles from the different producers were tagged with visible implant fluorescent elastomer tags (Northwest Marine Technology, Shaw Island, WA, USA) and thereafter the fish ($n = 1123$) from the two groups were mixed and distributed randomly into 10 rearing tanks. The 1 m^2 square, grey, covered fiberglass experimental tanks had a rearing volume of 400 L and a bottom outlet. Seawater with a salinity of 33.5‰ ($\pm$ 0.2‰) was pumped from 90 m depth. Water flow was set to 10 L min^{-1} for all experimental tanks. Oxygen saturation was measured several times each week in the effluent (i.e. bottom outlet) water of all tanks and was higher than 80% at all times. A 36 W fluorescent daylight tube integrated in the tank-cover provided light. Photon-irradiation measured at the bottom of the tanks was ca. 5 μmol m^{-2} s^{-1}. Before, and during, the experiment, the juveniles were fed a commercial formulated feed (Marin 10 and 20, Ewos A/S) containing 55% protein, 12% fat and 11% carbohydrate. Pellet size (2 and 3 mm) was adjusted during the experiment, depending on fish size, with an introduction of 3 mm pellets from 14 October.

The laboratory growth study was carried out from 8 September until 12 December 2003. On 25 August 2003, in preparation for the study, a subgroup of each fistfeeding group within each tank ($n = 41–50$ in each tank, $n_{total} = 446$, Table 1) was tagged intraperitoneally with Trovan® Passive Transponder tags (BTS Scandinavia, Åhus, Sweden) and gradually acclimated over 1 day (7 and 13 °C groups) or 3 days (16 °C and temperature-step groups (T-step group)) to the five experimental temperatures regimes of 7, 10, 13, 16 °C and T-step group. Each temperature regime consisted of two replicate tanks. The temperature-step regime was as follows: 16 °C from 9

September to 7 October; 13 °C from 8 October to 19 November; and 10 °C from 20 November to 12 December. This group is supposed to mimic temperature conditions found in Norwegian waters in late summer and autumn (Anonymous 2005). The mean rearing temperature in this group was 13.2 °C. The temperature in all groups was measured twice daily, and remained within ± 0.2 °C (SD) of that prescribed. All fish were anaesthetized (metacain, 0.05 g L^{-1}), and weighed individually (0.1 g) at 22–28 day's interval during the experiment. Feed was provided in excess for two 30 min periods daily (09:00–09:30 and 14:30–15:00 hours).

Feeding parameters

To measure food intake, feeding rate and feed conversion ratio, short-term (3 weeks) experiments were conducted after finishing the main laboratory experiment in January 2004. This experiment could only be performed at 13 °C. Untagged fish from each startfeeding group were distributed into four (i.e. two replicate tanks for each startfeeding group) 1 m^2 tanks ($N = 23$ in each tank). The fish were fed the same feed, and reared under the same experimental conditions as in the main experiment. Food was provided in excess twice daily (09:00–09:30 hours and 14:30–15:00 hours). Uneaten pellets were collected after each feeding (no later than 30 min after each feeding pulse) by filtering the outlet water with a fine mesh, and counted to estimate feed intake and feed conversion efficiency. Our observations showed that the amount of feed broken down was negligible in the short time from feeding to sampling and that this sampling method gave an accurate estimate of the amount of feed eaten.

Rearing in sea pens

On 15 January 2004, after termination of the temperature trial all individual fish ($n = 446$) were acclimatized to 10 °C and transported by truck to the production site of Marine Harvest at Smøla (W-Norway, 63°,31′) where the fish were reared at ambient temperatures (range 15 °C in August, 5 °C in March) in sea pens (40 m in diameter, 7 m deep, 1000 m^3 in volume). The fish were reared together with 7500 untagged cod in one sea pen. The fish were hand fed five times a week using a commercial formulated feed from Dana Feed (Dan-Ex 1562, containing 15% fat and 58% protein). On 29 June 2005, the weight of 101 tagged fish from the temperature study was recorded.

Anatomical deformity study

The anatomical study was performed before the temperature trial using two replicate tanks at 10 °C. The experiment started on June 24 when both groups had been weaned onto formulated feed and continued until September 1. Before the trial, we investigated the occurrence of anatomical differences in untagged fish. Ten fish were sampled every other week. The mean weights (± SE) at first sampling were 0.29 ± 0.03 and 0.25 ± 0.03 g, and the final mean weights were 9.45 ± 0.08 and 10.82 ± 0.08 g, for the zooplankton and the rotifer groups respectively. No size differences were found in the sampled fish (two-way nested ANOVA, $P > 0.15$, Power $(1 - \beta) > 0.65$). All sampled fish were anaesthetized and killed with an overdose of metacain dissolved in seawater. Deformities were noted and divided into two major groups. Firstly, fish were scored for externally visible deformities under a microscope: severe S-shaped spinal column, lack of operculum or fish with a deformed jaw resulting in an open mouth. Second, all other fish were checked for spinal or caudal deformities that were not externally visible, but only appeared under a light microscope after staining the fish with Alizarin red and Alcian blue. The fish were stained according to a modified method of Gavaia, Sarasquete and Cancela (2000). Following staining, spinal deformities, fused vertebrae, distorted haemal and neural arches and the bone development in the caudal complex of the fish were investigated ($n = 40$ vs. 43). The total number of externally deformed fish was calculated as a percentage of the total amount of sampled fish ($n = 120$ in each group). For the stained fish, the percentage was calculated as the amount of fish showing deformities in each group of stained fish.

Follow-up growth study

As the eggs in the growth trial described above came from two commercial producers, a follow-up study was performed with a single parental egg group divided into two parts and fed either rotifers or zooplankton. The eggs were obtained from a cod hatchery in western Norway (61°40′N, same hatchery as supplied the first rotifer group described earlier). Eggs from one spawning population were

collected with a few days' interval, and the first batch was incubated at 7 °C on site, whereas the second batch of eggs was transported to another commercial juvenile cod producer. The first batch hatched between 20 and 24 September 2004. Start feeding was performed at 10° with enriched rotifers. Rotifers were fed for 27 days, after which the fish were weaned on a commercial formulated feed (Marin 030 and 050, Ewos A/S, Bergen) containing 60% protein, 12% fat and 12% carbohydrates. The second batch hatched on 4 October 2004, and this group was first fed fresh-filtered natural zooplankton (gradually increasing size fraction from 80 to 1000 μm) and was later weaned on the same commercial formulated feed (Marin 030 and 050, Ewos A/S, Bergen) as the group fed rotifers (following the previously described protocol, except that the tank size was now 20 000 L for both larval groups). On 6 January 2005, both fish groups 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. The fish groups are hereafter referred to as either the zooplankton group or the rotifer group as determined by their first feeding diet. On 2 February 2005, 65 fish were tagged intraperitoneally with Trovan® Passive Transponder tags. The fish from the two groups were mixed and distributed randomly into two $1\,m^2$ square, grey, covered fiberglass experimental tanks with a rearing volume of 400 L and a bottom outlet. Seawater with a salinity of 33.5‰ (± 0.2‰) was pumped from a 90 m depth. Water flow was initially set to $3\,L\,min^{-1}$ for all experimental tanks, but was increased to $4\,L\,min^{-1}$ on 10 April due to increasing biomass in the tanks. Temperature (SD) averaged 12.4 (0.4) °C. A 36 W fluorescent daylight tube integrated in the tank-cover provided light. Photon-irradiation measured at the bottom of the tanks was approximately $5\,\mu mol\,m^{-2}\,s^{-1}$. Before, and during, the experiment, the juveniles were fed a commercial formulated feed (EWOS Marin 20), containing 55% protein, 12% fat and 11% carbohydrate (pellet-size 3 mm). Food was provided in excess from automatic feeders for 2 h daily (between 08:00–09:00 and between 14:00–15:00 hours), except for Saturdays (fed once) and Sundays (not fed). On 9 March 2005, the fish were anaesthetized (metacaine, $0.05\,g\,L^{-1}$), and weighed individually to the nearest 0.1 g. The initial mean weight (SD) was 20.8 (5.6) g and did not differ significantly between tanks. Subsequently, weights were recorded on days 24, 45 and 66.

Data analysis and statistical methods

Total feed consumption (C_T) was calculated as total feed supplied – total remaining feed. C_T was calculated on a daily basis and then summarized for each tank. Daily feeding rate ($F\%$) was calculated as

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

where C is feed consumption (g) in the period and B_1 and B_2 are fish biomass (g) on days t_1 (start) and t_2 (final) respectively. Feed conversion efficiency (FCE) was calculated as biomass gain per unit weight of feed consumed:

$$\mathrm{FCE} = (B_2 - B_1)/C$$

Specific growth rate (SGR) was calculated according to the formula of Houde and Schekter (1981):

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

where the instantaneous growth coefficient g is

$$g = (\ln\ W_2 - \ln\ W_1)(t_2 - t_1)^{-1}$$

and W_2 and W_1 are wet weight (g) at days t_2 and t_1 respectively.

All statistical analyses were performed with Statistica™ 6.0. The mortality and frequencies of anatomical deformities between the two startfeeding groups were tested using a χ^2 test (Zar 1996). To assess normality of distributions, a Kolmogorov–Smirnov test was used, and the homogeneity of variances was tested using Levene's F test. Two-way nested ANOVA (Zar 1996), where replicates are nested within the startfeeding groups, was applied to calculate the effect of different startfeeding scheme on mean weights and specific growth rates. Separate analyses were carried out for each temperature. For weight data of fish in the sea pen, we used a Fisher LSD unequal N test to locate differences as the number of fish was unequal between the groups. For the parameters where only group data at one temperature existed (FCE, $F\%$ and C_T), a one-way ANOVA (Zar 1996) was applied. Significant ANOVAs were followed by a Student–Newman–Keuls multiple comparison test to locate differences among treatments (Zar 1996). Individual growth trajectories were analysed using a growth curve analysis model (GCM, Chambers & Miller 1995), which is an extension of the multivariate repeated measurements analysis of the variance (MANOVA) model. The model equation of the GCM had the form:

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

where $\mathbf{Y}(n \times p)$ are the growth at age vectors $\mathbf{y} = (y_1, y_2, \ldots, y_p)$ for each p (age) measurements on n individual fish; $\mathbf{X}(n \times q)$ is the design matrix or the set of

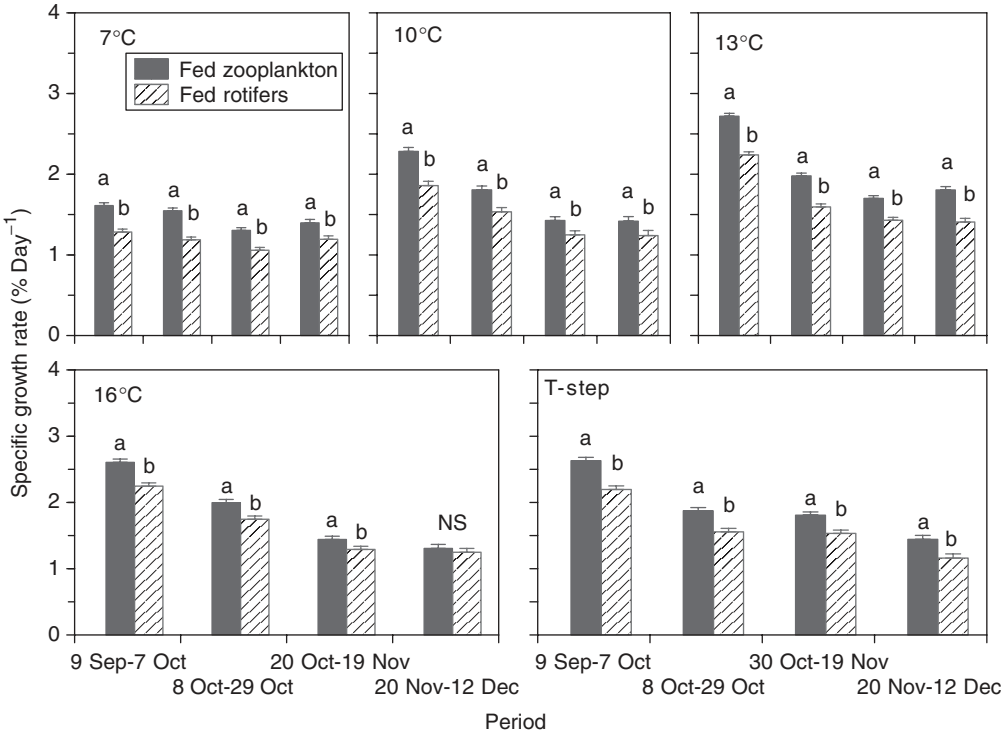

Figure 1 Mean specific growth rates of individually tagged Atlantic cod fed rotifers or zooplankton during the larval stage and reared at five temperature regimes during the juvenile period. Vertical whiskers indicate SEM. Different letters denote significant differences (Student–Newman–Keuls test, $P < 0.05$) within each temperature regime, $n = 41–50$ for each mean value.

extraneous variables measured for each individual, i.e., $q = \text{age}_p + \text{startfeeding}_i$ (i = fed rotifers, fed zooplankton); $\mathbf{B}(q \times p)$ is the matrix of parameters estimated using the model; and $\mathbf{E}(n \times p)$ is the matrix of deviations for each individual from the expected value of $\mathbf{Y} = \mathbf{XB}$.

Analyses were carried out for all temperatures combined and for each temperature separately. A significance level (α) of 0.05 was used if not stated otherwise. In cases with non-significant statistical tests, power ($1 - \beta$) analyses were performed using the PASS program package (Hintze 1996) using $\alpha = 0.05$.

Results

Mortality

Total mortality did not vary between the two groups ($\chi^2 = 0.9$, $P > 0.35$) and was 1.7% (four fish) in the zooplankton group and 2.9% (seven fish) in the rotifer group. Mortality occurred in all rearing units except for the T-step group (7 °C = 5 fish; 10 °C = 2 fish; 13 °C = 1 fish; 16 °C = 3 fish, $\chi^2 < 2.2$, $P > 0.20$), with no systematic trend.

Effect of startfeeding method on growth

The overall initial mean weight (SD) was 8.5 (0.3) g and did not differ (two-way nested ANOVA, Power $(1 - \beta) > 0.7$, Table 1) among the startfeeding groups. The zooplankton group had the highest final mean weight at all temperatures (two-way nested ANOVA, $P < 0.05$, Table 1). The final mean weights of the zooplankton group were between 26% and 36% higher than those of rotifer group. The largest size differences were seen at 13 °C and in the T-step group (Table 1). The mean individual growth trajectories were different at all temperatures between the two startfeeding groups (GCM, $\text{MANOVA}_{\text{Firstfeeding}}$, Wilk's lambda $(\Lambda)_{4,429} = 0.73$, $P < 0.001$, Fig. 1) throughout the study period. Significant differences were also found in growth-at-age trajectories of the startfeeding groups ($\text{MANOVA}_{\text{Firstfeeding} \times \text{Age}}$, Wilk's $\Lambda_{3,430} = 0.92$, $P < 0.001$, Fig. 1) from early September

Table 2 Feed consumption (C_T), daily feeding rate (F%) and feed conversion efficiency (FCE) of juvenile Atlantic cod fed either rotifers or zooplankton during the larval stage

Group	C_T (g ww)	F%	FCE
Fed rotifers	136.5 (4.2)[b]	1.36 (0.11)[b]	1.31 (0.04)[b]
Fed zooplankton	172.1 (10.5)[a]	1.46 (0.16)[a]	1.64 (0.13)[a]

Results are given as mean (standard error), $n = 2$ for each experimental group. Different letters denote significant differences (one-way ANOVA, $P < 0.05$) between temperature treatments. This trial was performed at 13 °C (see text for details).

onwards. The zooplankton group displayed higher growth rates at all temperatures at all times apart from the last date at 16 °C (two-way nested ANOVA, $P < 0.01$, Fig. 1). Growth declined at all temperatures except the 7 °C group between September and December. The overall growth rate was the highest at 13 °C and T-step (1.98, 1.96% vs. 1.65, 1.62% day^{-1}, for the zooplankton and the rotifer group respectively) and the lowest at 7 °C (1.41 vs. 1.14% day^{-1}, for the zooplankton and the rotifer group respectively).

Feed intake and feed conversion efficiency

Feed consumption (C_T) and daily feeding rate (F%), and FCE differed between two startfeeding groups (Table 2). The zooplankton group had higher feed consumption and feeding rate and food conversion efficiency (two-way nested ANOVA, $F_{1,4} > 9.2$, $P < 0.05$). Feed conversion efficiency differed between startfeeding groups (two-way nested ANOVA, $F_{1,4} = 8.0$, $P < 0.05$), as the zooplankton group had significantly higher FCE (1.65) than the rotifers group (1.31).

Growth in sea pens

No differences in growth rates were found between the startfeeding groups during the rearing period in sea pens (one-way ANOVA, $F_{1,95} = 2.0$, $P > 0.35$). The mean weight of the different startfeeding groups reared at different temperatures during the early juvenile stage differed after rearing the fish in sea pens under ambient conditions for 17 months (Student–Newman–Keuls test, $P < 0.05$, Table 1). The initial weight of these fish ($n = 101$) differed in December 2003 as the zooplankton fish was significantly larger in the 16 °C group and in the T-step group (Table 1), and these differences were largely maintained throughout the period of pen rearing. Of the prior temperature groups, the zooplankton T-step fish from the laboratory experiment was the largest (1.82 kg), followed by the zooplankton 13 °C fish (1.70 kg).

Table 3 Occurrence of the different types of deformities per group, containing operculum/jaw and spinal deformed fish that are externally visible and stained spinal, stained caudal that are only visible after staining the fish for cartilage and bone as a percentage of the total amount of fish sampled

Type of deformity	Rotifer group	Zooplankton group
Operculum/jaw	2.5% ($n = 3/120$)	2.5% ($n = 3/120$)
Spinal	6.7% ($n = 8/120$)	0.8% ($n = 1/120$)
Stained spinal	7% ($n = 3/43$)	5% ($n = 2/40$)
Stained caudal	7% ($n = 3/43$)	0% ($n = 0/40$)
Total % deformed	14.2%	4.1%

Anatomical deformities

Juveniles fed rotifers had a higher incidence of externally visible deformities than the zooplankton group ($\chi^2 = 4.0$, $P < 0.05$, Table 3). Spinal malformations were encountered in both groups but the rotifer group had 6.7% externally visible spinal deformities compared with 0.8% in the zooplankton group (Table 3). Most externally visible spinal deformities occurred at the posterior part of the vertebral column, just posterior to or right at the third dorsal fin (Fig. 2). The occurrence of jaw and operculum deformities was mostly the lack of an operculum or a malformation of the jaw resulting in 'gaping' fish. Both groups contained 2.5% of operculum- and jaw-deformed fish (Table 3).

After clearing and staining, the fish were checked again for spinal deformities. Deformities at the caudal end of the vertebral column (Fig. 3) occurred only in the rotifer group (7%, Table 3), whereas similar frequencies of spinal deformities were found among groups (Fig. 4, Table 3). The deformities consisted mainly of 'broken' vertebrae (Fig. 3b), fused centra, lack of neural or haemal arches or a deformed urostyle (Fig. 3b). Overall, the accumulated percentage of all deformities was higher in the rotifer group ($\chi^2 = 7.2$, $P < 0.01$). There was no fish with accumulated deformities, so none of them was counted twice.

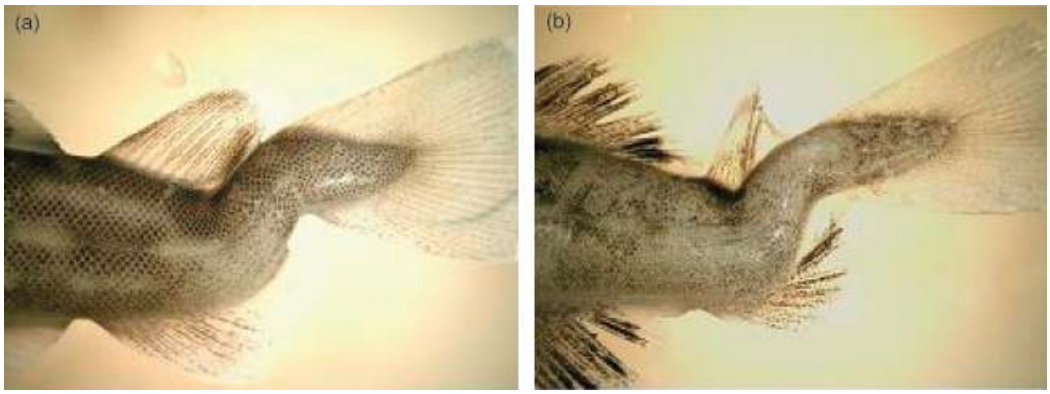

Figure 2 Externally visible spinal deformity in Atlantic cod at the posterior part of the vertebral column, (a) just posterior to the third dorsal fin and (b) right at the dorsal fin. This deformity was found in both startfeeding groups but was more common in the rotifer group (7% vs. 1%).

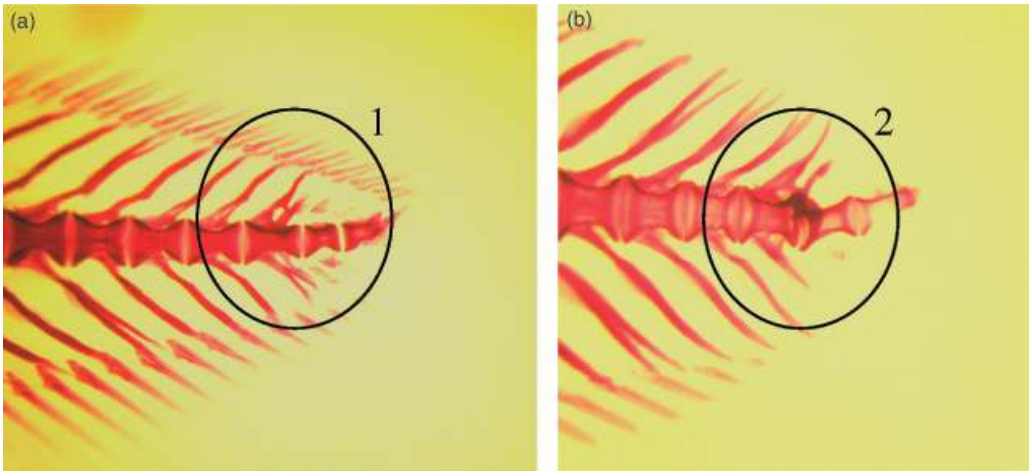

Figure 3 Normal (a) and deformed (b) vertebra in the caudal part of the vertebral column. The encircled part shows the normal developed caudal (1) and deformed caudal (2) end of the vertebral column. This type of deformation occurred exclusively in the rotifer group.

Growth in a follow-up study

The overall initial mean weight (SD) was 22.5 (0.6) g and did not differ (two-way nested ANOVA, Fig. 5a) among the startfeeding groups. The zooplankton group had a higher mean weight from late April onwards (Student–Newman–Keuls ANOVA, $P < 0.05$, Fig. 5a). The final mean weights of the zooplankton group were 17% higher than those of the rotifer group. The mean individual growth trajectories were different between the two startfeeding groups (GCM, $\text{MANOVA}_{\text{Firstfeeding}}$, Wilk's $\Lambda_{3,59} = 0.73$, $P < 0.001$, Fig. 5b) throughout the study period. The zooplankton group displayed higher growth rates in the first and second period and had 14% higher overall growth rates (two-way nested ANOVA, $P < 0.01$, Fig. 5b).

Discussion

Our data indicate that differences in growth, FCE and anatomical deformities in Atlantic cod juveniles are related to first feeding scheme of the larvae. Similar differences in growth as found in the temperature study were confirmed in the follow-up study where the eggs used to produce the juveniles came from one partental group. Current intensive production methods of Atlantic cod rely on live prey as rotifers and *Artemia*, although production protocols vary

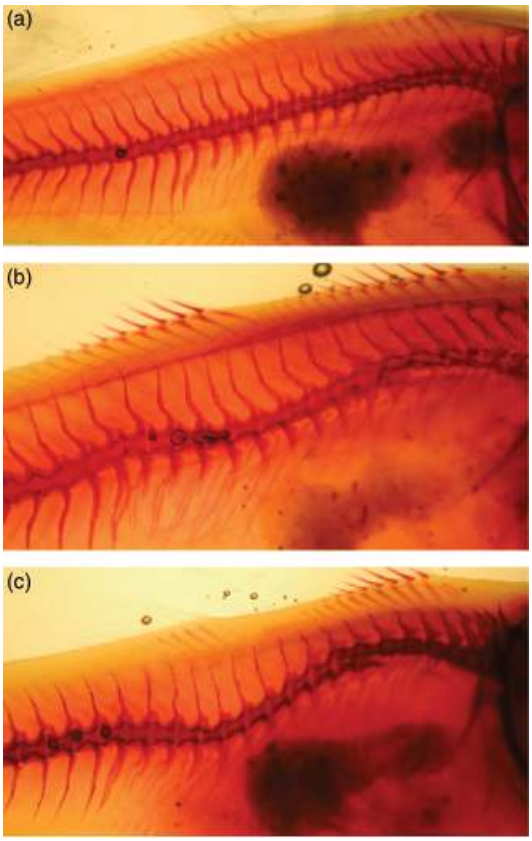

Figure 4 Normal developed (a) and deformed (b, c) spinal columns. These spinal deformities were observed in both startfeeding groups.

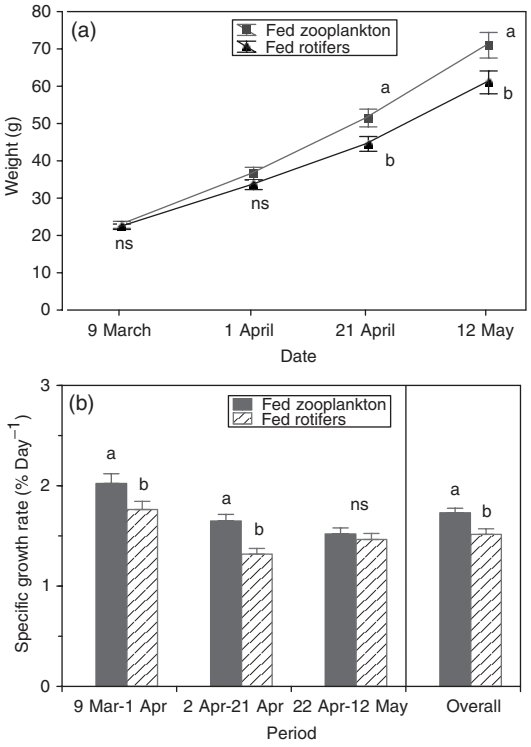

Figure 5 Follow-up study. Mean weights (a) and mean specific growth rates (b) for juvenile Atlantic cod fed either rotifers or zooplankton during the larval stage and subsequently reared at one constant temperature (12.4 °C) during the juvenile stage. Vertical whiskers indicate SEM. Different letters denote significant differences (Student–Newman–Keuls test, $P < 0.05$) between the two groups, $n = 31–32$ for each mean value. Eggs in this study came from one parental group divided into two parts and fed either rotifers or zooplankton.

from using only rotifers (Baskerville-Bridges & Kling 2000) to co-feeding with *Artemia* in various forms (Shields *et al.* 2003; Puvanendran, Salies, Laurel & Brown 2004). Using only rotifers may have both economic and practical implications (Le Ruyet, Alexandre, Thebaud & Mugnier 1993; Baskerville-Bridges & Kling 2000), but our data imply that feeding with rotifers only during the larval stage results in insufficient quality (measured as differences in growth capacity) of the juvenile fish. Our data show that replacing rotifers with zooplankton until metamorphosis may increase growth and food conversion during the whole juvenile stage, suggesting possible long-term dietary effects of different diets during the early life stages. These may have important consequences for growth, feed conversion and anatomical development throughout the animal lifecycle. Using zooplankton in the commercial production of cod larvae may, however, be difficult under intensive industrial conditions as zooplankton is only available during limited time of the year, whereas production of juveniles will occur on a year-round basis (Brown *et al.* 2003). Diseases and parasites may also be transferred with the zooplankton to the cod larvae (Karlsbakk, Otterlei, Høie & Nylund 2001), so many farmers have abandoned this production method. An alternative approach involves the development of better enrichment or bioencapsulation possibilities for the rotifers, resulting in better startfeeding and weaning diets. This will probably lead to increased juvenile quality as shown in culture of flatfish as better quality larval diets have greatly reduced the incidence of morphological deformities over recent years (Pittman, Jelmert, Næss, Harboe & Watanabe 1998; Shields, Gara & Gillespie 1999; Bolker & Hill 2000). With respect to enrichments of live feed, so far, there have not been any definitive studies aimed at comparing or evaluating the optimal live food enrichment

procedures for cod. Most studies have used rotifers enriched on commercial products. Brown *et al.* (2003) pointed out that cod larvae have requirements for HUFAs that might not be met by the commercial enrichment products that are available on the market or by specific development of enrichment specially designed for cod larvae.

Deformities occurred in both groups in the present study, but the rotifer group showed a three times higher overall percentage of deformed fish (14.2%) compared with the zooplankton group (4.1%). The most severely deformed fish were always found in the rotifer group. This fact, together with the significantly higher total number of deformed fish in this group, is a reason for concern. Earlier studies have tried to link deformities with dietary causes (Cahu, Infante & Takeuchi 2003; Fraser, Anderson & de Nys 2004), egg incubation temperature (Ørnsrud, Wargelius, Sæle, Pittman & Waagbø 2004) and other environmental factors (e.g. Divanach, Papandroulakis, Anastasiadis, Koumoundouros & Kentouri 1997; Sfakianakis, Koumoundourosa, Divanach & Kentouri 2004; Grotmol, Kryvi & Totland 2005). From the dietary studies, it is clear that especially vitamins seem to play an important role in the development of deformities in the spinal bones and operculum. Further, Cahu *et al* (2003) found that high dietary retinoic acid levels were associated with a higher incidence of bone deformities, such as vertebral curvature, central fusion and compression of vertebra in Japanese flounder larvae, *Paralichthys olivaceus*. In addition to malformations at the embryonic stage, vitamin A may also induce malformations in later life stages, especially in the vertebra (Ørnsrud *et al.* 2004). Our findings are partly in line with studies on Atlantic halibut, where developmental disorders have been attributed to different nutritional content of zooplankton and formulated feed (Hamre *et al.* 2002). Several environmental factors have been suggested as causes of various bone disorders. Grotmol *et al.* (2005) described a malformation that frequently occurs in the Atlantic cod in intensive culture. The malformation is characterized by a slight upward tilt of the head and an indented dorsal body contour at the transition between the head and the trunk. The latter is similar to what we found in this study. Grotmol *et al.* (2005) found the pathomorphological characteristics to be compatible with the notion that the notochord has been subjected to an upward mechanical force generated by a persistent increase in pressure between the swim bladder and the notochord during the period of development of the vertebral anlagen. The critical period for development of malformation seems to be the period from 18 to 36 days post hatch, during which the chordacentra and the neural arches are formed. They discussed two possible factors causing the malformation i.e. upwelling currents in tanks due to aeration and overfilling of the digestive tract resulting from overfeeding or sup-optimal feed delivery protocols. Both our groups were reared in tanks with aeration although different sizes of tank may have caused different upwelling currents. High water currents during the larval rearing have been linked to malformation in sea bass, *Dicentrarchus labrax* L. (Divanach *et al.* 1997), but in our study the water was stagnant during the first week of rearing and subsequently only mild currents were used in both groups. The other plausible explanation mentioned by Grotmol *et al.* (2005) relates the different feeding organisms to vertebral malformation. Our data are in line with these observations. Temperature has been shown to affect the development rate of the skeleton (Sfakianakis *et al.* 2004) and to be a crucial factor for the development of morpho-anatomical abnormalities, with generally an increasing incidence as temperature increases. In the present study, the temperatures applied in the larval protocols were nearly identical (rotifer group, eggs incubated at 7.0 °C, larvae reared at 8–10 °C (gradual increase); zooplankton group; eggs incubated at 6.8 °C, larvae reared at 7.8–8.6 °C (gradual increase), making temperature unlikely to be causing the differences seen in the anatomical malformation. Overall, it seems that dietary causes are more likely than environmental to have caused the morpho-anatomical seen in our study.

Both rotifer and zooplankton fish reared under the T-step and 13 °C regimes showed the highest final mean weight after being reared fro 17 months in pens. The T-step regime was designed to utilize the gradual decline in optimal temperature for growth ($T_{opt}G$) with increasing size (Björnsson, Steinarsson & Oddgeirsson 2001; Imsland, Foss, Folkvord, Stefansson & Jonassen 2006). Hansen and Falk-Petersen (2002) found that growth of spotted wolffish (*Anarhichas minor*) larvae up to 63 days after hatching (0.2–3 g) was the highest when larvae were moved from 12 to 10 and later to 8 °C, compared with being reared at constant temperatures. In Atlantic halibut (*Hippoglossus hippoglossus*, 160–400 g) reared at constant (11 and 14 °C) or switched (14 °C moved to 11 °C and vice versa) temperature regimes, Aune, Imsland and Pittman (1997) found that the growth rate was the highest in fish transferred from 14 °C to 11 °C. This

coincides with the $T_{opt}G$ for Atlantic halibut, which has been shown to decrease from 14.9 to 12.7 °C in the early juvenile stage (Jonassen, Imsland & Stefansson 1999). These findings are in line with results from the present experiment, but our data point to a potential long-term gain from rearing fish at T-steps during the juvenile period.

An important practical implication seen in our study is the advantage of rearing the fish at elevated temperatures during the juvenile period as size differences established at this stage can be manifested in the adult fish and can decrease production time significantly. In contrast, recent findings (Sæther 2005) have indicated that moving fish from different rearing temperatures (between 8 and 15 °C) to a low (3 °C) temperature will lead to growth relapse in all groups that will obliterate the short-term gain of rearing juvenile cod at elevated temperatures. In our study, the tagged fish were acclimatized to 10 °C before transport to production facility of Marine Harvest at Smøla, and moved to sea pens when the sea temperature was approx. 7 °C. Size differences after 17 months in sea pens were reduced, but still around 13% (see Table 1).

In nature, Atlantic cod larvae are poorly developed at hatching (approximately 4 mm), with a relatively small yolk sac, which is completely absorbed after 5–10 days. Norwegian Coastal cod feed and live pelagically for several weeks and settle in coastal areas in benthic environments at 4–5 cm length (Pedersen & Falk-Petersen 1992). Observations on growth of wild juvenile Atlantic cod indicate that a size of 15–20 g can be reached 5–9 months after hatching (i.e. the same age as current experimental material) (Asthorsson, Gislason & Gudmundsdóttir 1994; Björnsson *et al.* 2001). Compared with the much higher growth seen in the present experiment, this demonstrates that wild Atlantic cod seldom experience growing conditions where their maximum potential can be achieved. Björnsson *et al* (2001) pointed out the fact the benefits of selecting $T_{opt}G$ may be outweighed by other crucial factors such as feed availability, risk of starvation and predation risk.

In the present experiment, the two experimental groups were startfed in different ways and we have tracked both the short- and-long term effects of these different first feeding methods. It is, however, possible that different rearing technologies used by the two producers (e.g. tank design, tank hygiene, feeding regimes, etc.) may indirectly influence the growth and development of the larvae. However, it is not likely that such differences will be manifested in such large size and growth differences as those found in the present study, although this cannot be excluded. But, our follow-up study was performed with much more similar rearing technologies, and still we observed growth differences in the some order of magnitude as before, thus undermining different rearing technologies as causing the differences in growth and bone deformities seen in the original study. Another possible confounding effect is the different genetic background of the fish. The fish from both producers were spawned volitionally so it is uncertain whether the fish were siblings or not. Gjerde, Terjesen, Barr, Lein and Thorland (2004) estimated family breeding values for body weight for 51 full-sib cod families from two different regions (broodfish collected over 500 km apart) in Norway. Their data indicated that the standard deviation of mean predicted family value for body weight in juvenile Atlantic cod is approximately 10%. In our study, the final weights of the different groups (across temperatures) differed between 26% and 36% in December 2003. Gjerde *et al.* (2004) found SD of average full-sib family weight between two geographically separated groups to be 20% and 26%. Accordingly, although different genetic background of the fish may account for some of the differences seen, it cannot account for all size and growth differences seen between the two groups. To estimate how, and whether, different genetic variation between the groups (e.g. number of families) influenced the results, we performed a follow-up study at one constant temperature, where the experimental fish had the same genetic background (i.e. same parents). The result of this follow-up study (Fig. 5) confirmed the differences seen in the original study, and validated that the encountered differences in the temperature study are mostly related to dietary and nutritional origin. However, it is still possible that different numbers of families are being compared in the study material, so that the genetic component cannot be excluded as part of the explanation. To scrutinize the family component, a genetic screening of the study material is needed (e.g. microsatellites) so that each individual can be assigned to a specific family.

The zooplankton fish had higher feed consumption, daily feeding rate and feed conversion efficiency. To our knowledge, similar findings in the poststartfeeding phase have not been published before. Differences in the growth capacity of cod larvae fed rotifers and zooplankton have been indicated in the literature (Otterlei, Nyhammer, Folkvord & Stefansson 1999; Callan, Jordaan & Kling 2003; Hamre 2006). These differences are possibly linked to sub-optimal

nutrition, as there are many differences in nutrient composition between copepods in the zooplankton and rotifers (Hamre 2006). Our data show that such sub-optimal nutrition may have long-term consequences also for feeding behaviour and feed conversion. We can at present only speculate on these differences. Firstly, Grotmol *et al*. (2005) indicated that the overfilling of the digestive tract could lead to vertebral deformities in Atlantic cod larvae and that this was common in groups fed artificial diets (rotifers and *Artemia*). It is possible, thus, that the differences we found in the vertebral deformities can also be seem in the digestive tract leading to differences in feeding capacity. Secondly, differences in feeding behaviour may be of genetic origin. One published study can be found on cod where this aspect has been studied. Salvanes & Hart (2000) suggested a link between haemoglobin genotype and growth in Atlantic cod through feeding behaviour. They found indications of genotype dependent competitive performance as one haemoglobin genotype, the *Hb-I(2/2)*, had a higher motivation to feed, was a better competitor and ate a larger share of the meal than individuals with different genotypes. We propose that these behavioural, nutritional and genetic aspects should be considered in future research of different startfeeding groups.

In conclusion, the present study clearly demonstrates significant short- and long-term effects of startfeeding diet on growth dynamics and anatomical development. Growth, appetite and feed efficiency ratio were significantly improved in cod juvenile startfed with zooplankton and skeletal deformities were more frequent in groups startfed with rotifers compared with groups startfed with zooplankton. Our data suggest that different startfeeding methods may be important for production performance, incidence of deformities and quality of juvenile cod.

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