



Effects of diet transition regimen on survival, growth and lipid composition of intensively reared Atlantic cod, *Gadus morhua*, larvae

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Received 26 November 2001; accepted 3 November 2002

Abstract. Replicated groups of Atlantic cod were reared for up to 40 days in 100 l tanks stocked at a density of 75 eggs l⁻¹. Larvae were transferred from rotifers, *Brachionus plicatilis*, to either fresh-hatched or enriched *Artemia* nauplii on each of days 5, 15 and 25 post-hatch (ph). Rotifers were progressively withdrawn over a 5 day period. The type of *Artemia* offered (fresh-hatched, enriched) did not affect survival or growth rates at any of the 3 transfer ages. Larvae transferred to *Artemia* from day 5 ph suffered a high incidence of swimbladder over-inflation and high mortality during metamorphosis (< 1% survival to day 36 ph). Cod in the day 15 and day 25 transfer groups did not differ significantly in weight-specific growth rate or size on day 40 ph (mean standard length 13.8 mm, dry weight 3.8 mg). Highest mean survival rates to day 40 ph (18.1%) and lowest mortality following transfer to nursery tanks were also observed in the day 25 transfer groups. Fish that received *Artemia* from day 5 ph contained *circa* twice as much total lipid per unit body weight and had a 30% higher triacylglycerol (TAG) content compared to all other groups. Ratios of the essential fatty acids docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA) and arachidonic acid (ARA) also differed according to age-at-transition. DHA:EPA ratio exceeded 1 only in cod transferred to *Artemia* on day 25 ph. Based on these findings, it is recommended that intensively reared Atlantic cod should continue to receive rotifers until completion of metamorphosis.

Key words: *Artemia*, Cod larvae, Growth, Intensive rearing, Lipids, Rotifers, Survival, Swimbladder

Abbreviations: s – second; h – hour; d – day; ph – post-hatch; l – liters; UV – ultra-violet; ppt – parts per thousand; ml – milliliter; μ m – micrometer; cm – centimeter; g – gram; TAG – triacylglycerol; EFA – essential fatty acids; DHA – docosahexaenoic acid; EPA – eicosapentaenoic acid; ARA – arachidonic acid

Introduction

Farming of Atlantic cod, *Gadus morhua*, is an emerging commercial activity in salmon farming regions of the north Atlantic, with large scale juvenile production primarily taking place in intensive hatcheries. This reliance on intensive larviculture techniques marks a shift from the extensive cod production methods pioneered in Norway (van der Meeren and Naas 1997) and reflects the dominance of intensive hatcheries in European marine fish farming (Shields 2001).

The adaptability of cod to intensive rearing methods was initially demonstrated by Howell (1984) and confirmed by Rosenlund et al. (1993), although rearing protocols were not optimized in these studies. Subsequent research examined the effects of physical environmental conditions (Puvanendran and Brown 1998; Steinarsson and Björnsson 1999; Otterlei et al. 1999; van der Meeren and Jørstad 2001), stocking density (Baskerville-Bridges and Kling 2000a) and dietary variables (Puvanendran and Brown 1999; Baskerville-Bridges and Kling 2000b) on the survival and growth rates of cultured Atlantic cod larvae.

Baskerville-Bridges and Kling (2000b) demonstrated that Atlantic cod larvae can be reared in clear water and weaned directly from rotifers to a specialized microparticulate diet, without incorporating an intermediate *Artemia* phase. This experimental protocol may be applicable for commercial scale cod production, provided that system hygiene can be maintained and there is sufficient cost benefit in replacing *Artemia* with microdiets. Alternatively, commercial cod hatcheries have the option of retaining *Artemia* in the feeding sequence and using conventional weaning diets.

Cod larvae are sufficiently large to accept *Artemia* from an early developmental stage, however there is no published data on optimal rotifer-*Artemia* transition regimen for this species. In our own pilot rearing trials, cod larvae transferred to *Artemia* around d10 ph performed well until metamorphosis, at which stage high mortality was encountered (unpublished observations). This mortality was associated with over-inflation of the swimbladder, reminiscent of swimbladder stress syndrome (SBSS) in other fish species (Johnson and Katavic 1984; Kolbeinshavn and Wallace 1985; Bagarinao and Kungvankij 1986) and characterized behaviorally by the fish swimming on their sides and backs at the water surface, unable to feed. An experiment was therefore conducted to define an appropriate rotifer-*Artemia* transition regimen for intensively reared Atlantic cod and to establish whether there is a dietary basis for the abnormal swimbladder phenomenon.

Materials and methods

Source of experimental animals

The experiment was carried out at Seafish Aquaculture's Marine Farming Unit, Ardtoe, Scotland. Fertilized Atlantic cod eggs were obtained from a natural spawning population maintained under ambient photoperiod and temperature. The captive population of 7 male and 7 female cod was held in a 10 m diameter circular tank (operating volume 150,000 L) and fed *ad libitum* with a moist diet containing low temperature fish meal, minced herring and squid. Eggs were collected from the tank outflow and surface-disinfected by immersion in a 0.02% solution of peroxyacetic acid for 45 s, before stocking into the experimental rearing tanks.

Experimental protocol

Disinfected cod eggs were stocked into 24 × 100 l black cylindrical polyethylene rearing tanks (height 70 cm, diameter 50 cm), at a density of 75 l⁻¹. Water temperature was maintained at 8.8 ± 0.2 °C and salinity at 34.2 ± 0.1 ppt. Continuous overhead lighting at an intensity of *circa* 100 lux at the water surface was provided via PAR 38 tungsten lamps. Each tank received continuous aeration by means of a single airstone. Fifty percent of water volume was exchanged per day, using a 5 µm-filtered, UV-sterilized water supply. Microalgae (*Nannochloris* sp) was added once a day to each tank to maintain a density of *circa* 1 million cells ml⁻¹. Hatching was completed 5 d after stocking (experiment day 0), at which time 6 experimental feeding regimens were assigned at random (4 replicate tanks per group), as illustrated in Figure 1.

Enriched rotifers (*Brachionus plicatilis*) were first presented to the cod larvae on d1 ph. Rotifers were applied at a density of 1.5 ml⁻¹ during first-feeding, increasing to 10 ml⁻¹ by day 12 ph. The experimental groups were transferred from rotifers to *Artemia* ("EG" grade, Inve Aquaculture NV, Dendermonde, Belgium) on d5, d15 or d25 ph. On each occasion, larvae were either transferred directly to 16 h-enriched *Artemia* (groups EN5, EN15 and EN25) or *via* freshly-hatched nauplii (groups FH5, FH15 and FH25). An initial prey density of 1 *Artemia* ml⁻¹ was applied, increasing to 5 ml⁻¹ by day 18. Numbers of residual prey were checked twice daily and new prey added to obtain the required density. Both rotifers and *Artemia* were enriched using the *Schizochytrium*-based product, Algamac 2000™ (Aquafauna Biomarine Inc, Hawthorne, California, USA). Fatty acid compositions of the rotifer and *Artemia* prey are presented in Table 1.

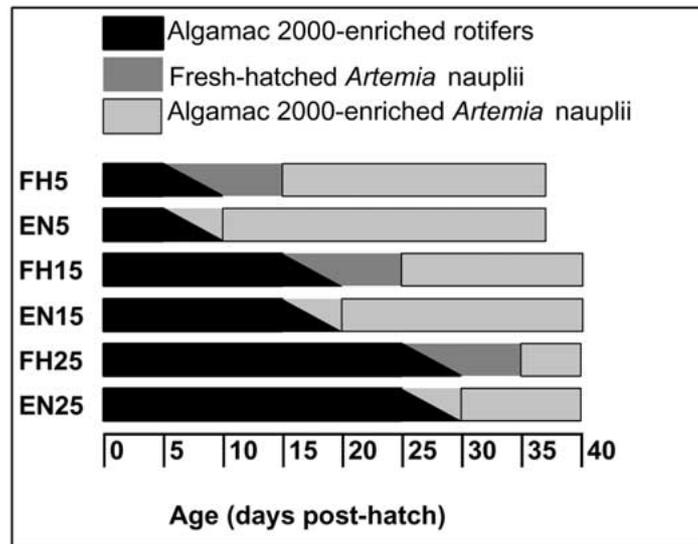


Figure 1. Experimental diet transition regimen applied to Atlantic cod larvae.

Groups FH-5 and EN-5 were terminated on d36 ph in response to high mortality rates, while the remaining treatments were retained until d40 ph. Surviving cod from the d15 and d25 transition groups were pooled into 4 nursery tanks on d 40 ph and survival rate quantified 24 h post-transfer.

Sampling

Four larvae per tank were collected for length measurement and gut contents analysis 24 h after each diet transfer. Surviving fish from each tank were enumerated at the end of the experiment and 20 fish sampled for length and weight measurement. Survival rates were calculated on the basis of percentage of eggs stocked. Weight-specific growth rates (SGR, % day⁻¹) were calculated according to the formula:

$$\text{SGR} = \frac{\ln(\text{final dry weight}) - \ln(\text{initial dry weight})}{\text{days}} \times 100$$

This is equivalent to instantaneous growth rate (g), as defined by Ricker (1958).

All sampled larvae were killed by anesthesia in 3-aminobenzoic acid ethyl methane sulfonate (MS222, Sigma Chemical, Poole, UK). Standard length in mm (tip of upper jaw to end of notochord) was measured using an Olympus SZ60 dissecting microscope with ocular micrometer. For weight measurement, larvae were rinsed in distilled water, frozen in pre-weighed 1.5 ml

Table 1. Fatty acid compositions (weight percent of total lipid) for rotifers and *Artemia* fed to Atlantic cod larvae.

Fatty acid/diet	Algamac 2000 TM -enriched rotifers	Unenriched <i>Artemia</i> nauplii	Algamac 2000 TM -enriched <i>Artemia</i> nauplii
14:0	6.9 ± 0.1	1.1 ± 0.2	1.8 ± 0.6
16:0	26.9 ± 1.2	14.2 ± 2.4	14.9 ± 0.5
18:0	2.5 ± 0.2	4.7 ± 0.9	4.4 ± 0.1
Total saturates ¹	37.3 ± 1.5	20.4 ± 3.7	28.6 ± 0.7
16:1 (n-7)	0.0 ± 0.0	5.4 ± 0.6	3.5 ± 0.1
18:1 (n-9)	5.1 ± 0.3	22.0 ± 3.9	15.5 ± 0.8
18:1 (n-7)	2.1 ± 0.1	9.7 ± 0.4	5.8 ± 0.3
20:1 (n-9)	1.5 ± 0.1	0.4 ± 0.0	0.3 ± 0.2
24:1	0.4 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Total monoenes ²	10.2 ± 0.9	38.0 ± 6.3	25.6 ± 0.9
18:2 (n-6)	2.3 ± 0.1	6.2 ± 0.2	4.5 ± 0.2
20:2 (n-6)	0.2 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
18:3 (n-6)	0.3 ± 0.0	0.2 ± 0.0	0.0 ± 0.0
20:3 (n-6)	0.6 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
20:4 (n-6)	3.4 ± 0.2	1.2 ± 0.0	2.1 ± 0.1
22:5 (n-6)	9.1 ± 1.1	0.0 ± 0.0	3.1 ± 0.4
Total (n-6)	15.9 ± 1.2	7.4 ± 0.5	9.6 ± 0.3
18:3 (n-3)	0.3 ± 0.1	22.4 ± 1.4	19.3 ± 0.8
18:4 (n-3)	0.2 ± 0.0	2.4 ± 0.0	2.1 ± 0.1
20:3 (n-3)	0.1 ± 0.1	0.3 ± 0.1	0.3 ± 0.3
20:4 (n-3)	1.1 ± 0.0	0.4 ± 0.0	0.4 ± 0.3
20:5 (n-3)	4.6 ± 0.1	5.3 ± 0.0	5.0 ± 0.1
22:5 (n-3)	2.4 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
22:6 (n-3)	26.5 ± 1.2	0.0 ± 0.0	9.1 ± 1.2
Total (n-3)	35.1 ± 1.2	30.7 ± 1.4	36.1 ± 1.0
Total PUFA ³	52.5 ± 2.4	39.3 ± 2.1	45.7 ± 1.3
DHA ⁴ /EPA ⁵	5.7	0.0	1.8
EPA/AA ⁶	1.4	4.6	2.4

¹Includes 15:0, 20:0 and 22:0.

²Includes 20:1 (n-11), 20:1 (n-7), 22:1 (n-11) and 22:1 (n-9).

³PUFA, polyunsaturated fatty acids.

⁴DHA, 22:6 (n-3), docosahexaenoic acid.

⁵EPA, 20:5 (n-3), eicosapentaenoic acid.

⁶AA, 20:4 (n-6), arachidonic acid.

Table 2. Survival rate, size and weight-specific growth rate of Atlantic cod larvae transferred from rotifers to fresh-hatched or enriched *Artemia* on d5 ph.

Parameter	Feed group	
	FH5	EN5
Standard length at transition (mm)	5.5 ± 0.5	5.5 ± 0.5
Dry weight at transition (mg)	0.12 ± 0.01	0.12 ± 0.01
% Feeding incidence on <i>Artemia</i> , 24h post-transfer	0	0
% Survival to d36 ph	0.6 ± 0.4 ^a	0.9 ± 0.3 ^a
Standard length (mm) d36 ph	11.1 ± 1.9 ^a	12.0 ± 0.3 ^a
Dry weight (mg) d36 ph	1.4 ± 1.4 ^a	1.4 ± 0.1 ^a
SGR d5-d36 ph	7.1 ± 3.3 ^a	7.8 ± 0.9 ^a

Values are means ± standard error of 4 tank replicates. Values within a row having the same superscript are n.s.d. at $p < 0.05$.

plastic microcentrifuge tubes (10–20 larvae per tube), freeze-dried for 24 hrs (Edwards Micro Modulyo, Edwards High Vacuum International, Sussex, UK) and weighed using a Sartorius H110 electronic balance. Ten cod per tank were analyzed for total lipid content, lipid class composition and fatty acid composition of total lipid using methods described by Shields et al. (1999).

Data analysis

Percent data were arc-sin transformed before analysis. Results for the FH5 and EN5 treatments (end point d36 ph) were compared using Student's t-test, while 2 way analysis of variance was applied to data from the d15 and d25 groups (end point d40 ph). Statistical tests were carried out using Minitab software, Release 9 (Minitab Inc, USA). A significance level of $p < 0.05$ was applied throughout.

Results

Cod larvae receiving *Artemia* from d5 ph did not ingest any nauplii within 24 h of diet transition (Table 2) and only 27% of sampled larvae contained nauplii after 48 h (data not shown). In contrast, circa 80–90% of sampled larvae in the d15 and d25 transfer groups had ingested freshly hatched or enriched nauplii 24 h after introduction (Table 3).

Larvae transferred to *Artemia* on d5 ph suffered a high incidence of swim-bladder over-inflation, beginning circa d20 ph (data not shown). Affected fish had a grossly distended abdomen and swam on their sides or back, at the water

Table 3. Survival rate, size and weight-specific growth rate of Atlantic cod larvae transferred from rotifers to fresh-hatched or enriched *Artemia* on d15 or d25 ph.

Parameter	Feed group			
	FH15	EN15	FH25	EN25
Dry weight (mg) d5 ph	0.12 ± 0.01	0.12 ± 0.01	0.12 ± 0.01	0.12 ± 0.01
Standard length at transition (mm)	8.1 ± 0.3	8.1 ± 0.3	9.8 ± 1.1	9.8 ± 1.1
% Feeding incidence on <i>Artemia</i> , 24h post-transfer	87.5 ± 25.0	87.5 ± 25.0	87.5 ± 25.0	81.2 ± 23.9
% Survival to d40 ph	6.6 ± 4.7 ^a	8.0 ± 2.1 ^a	18.7 ± 4.9 ^b	17.5 ± 2.2 ^b
Standard length (mm) d40 ph	14.3 ± 1.0 ^a	13.4 ± 0.7 ^a	13.5 ± 0.6 ^a	14.2 ± 1.1 ^a
Dry weight (mg) d40 ph	4.0 ± 0.8 ^a	3.4 ± 1.7 ^a	3.6 ± 1.3 ^a	4.0 ± 1.1 ^a
SGR, d5-d40 ph	10.0 ± 0.5 ^a	9.3 ± 1.2 ^a	9.6 ± 1.0 ^a	9.9 ± 0.9 ^a
% Survival, 24hr after nursery transfer	83.9	75.9	95.9	97.4

Values are means ± standard error of 4 tank replicates. Values within a row having the same superscript are n.s.d. at $p < 0.05$.

surface. Subsequent mortality in both the FH5 and EN5 groups resulted in lower cumulative survival rates (< 1%) than all other groups (Tables 2 and 3), as a result of which the d5 groups were terminated on d36, rather than d40 ph. The surviving cod were also substantially smaller in size (mean dry weight on d36 ph, 1.4 mg) compared to those from the d15 and d25 groups, weighed on d40 ph.

There were no statistically significant differences between the end-point size or weight-specific growth rates of larvae transferred to *Artemia* on d15 or d25 ph (Table 3). Mean dry weight was 3.7mg and weight-specific growth rate 9.7% d⁻¹ (pooled data). However, larvae receiving *Artemia* from d15 ph had significantly lower mean survival rates (13.4%, 14.3%) than those transferred on d25 (18.7%, 17.5%). Furthermore, fish from the d15 groups suffered higher mortality following transfer to nursery tanks on d40 ph (Table 3). The type of *Artemia* offered during diet transition (fresh-hatched versus enriched) did not significantly affect survival rate, size or weight-specific growth rate at any of the 3 transfer ages (Tables 2 and 3).

Surviving cod from the d5 transfer groups had a greater total lipid content and contained a higher proportion of triacylglycerol (TAG) than cod from all other groups (Figures 2a, b). For cod in the d15 and d25 transfer groups, there were no significant effects of age-at-transition, or *Artemia* type on total lipid content or percentage TAG on d40 ph. However, ratios of the essential fatty acids (EFAs) docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA) and arachidonic acid (ARA) differed according to age-at-transition

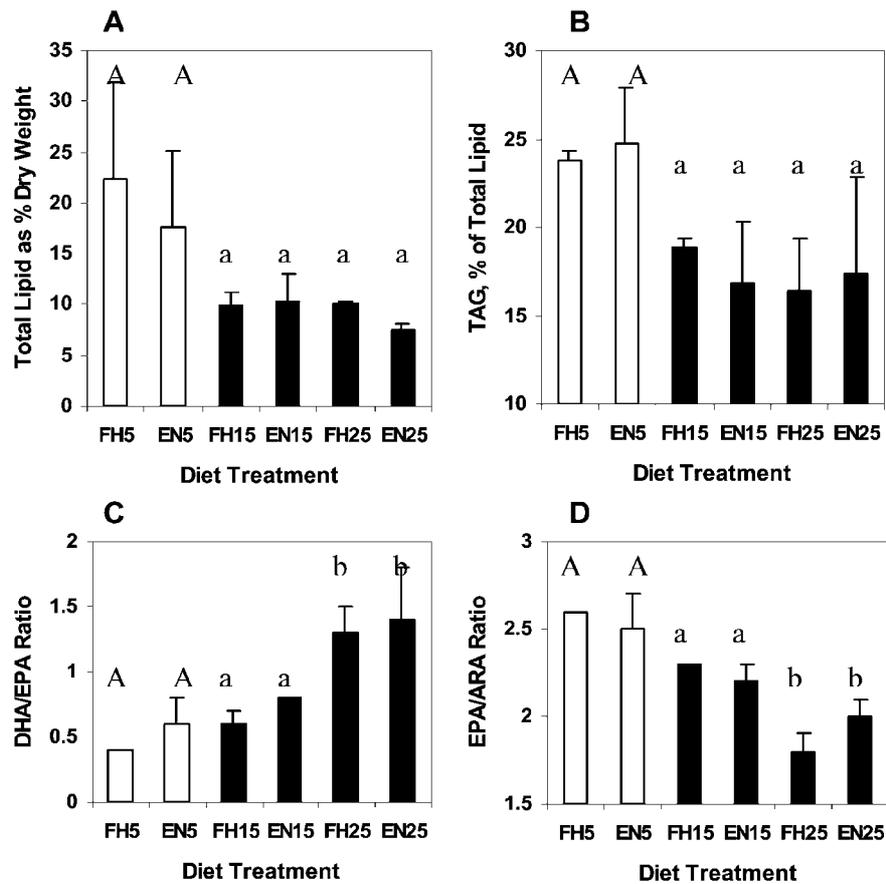


Figure 2. Effect of cod diet transition regimen on (A) total lipid content, (B) percent triacylglycerol, (C) DHA/EPA ratio and (D) EPA/ARA ratio. Values are means \pm standard deviation of 4 tank replicates. White bars = fish sampled d36 ph, black bars = fish sampled d40 ph. Upper case superscripts refer to FH5 and EN5 treatments only.

(Figures 2c, d). DHA/EPA ratios increased with age-at-transition, while EPA/ARA ratios decreased, reflecting the relative quantities of these EFAs in *Schizochytrium*-enriched rotifers and *Artemia* (Table 1). The type of *Artemia* (fresh-hatched or enriched) offered during diet transition did not significantly affect cod lipid composition at any of the 3 transfer ages.

Discussion

A positive relationship has been demonstrated between age-at-transfer from rotifers to *Artemia* and rearing performance of intensively cultured Atlantic

cod, *Gadus morhua*. Although cod larvae were able to ingest *Artemia* from as early as d7 ph (FH5 and EN5 groups), these larvae suffered a high incidence of swimbladder over-inflation and high mortality during metamorphosis. The surviving fingerlings were also smaller in size than those in all other groups. Treatment effects were less dramatic between the d15 and d25 transfer groups, although there was still a clear benefit of withholding *Artemia*, in terms of higher cumulative survival rate and reduced incidence of swimbladder over-inflation. Metamorphosed cod in the d25 groups also suffered less mortality 24 h after transfer from the experimental larvae rearing tanks.

Whole body lipid composition at the end of the experiment varied according to the fish's nutritional history. Larvae receiving *Artemia* from d5 ph contained approximately twice as much total lipid per unit body weight and had a 30% higher TAG content compared to those in all other groups. It is possible that high dietary lipid input was detrimental to larvae in the d5 transfer groups, as reported for *Artemia*-fed larvae of *Scophthalmus maximus* (Padros et al. 1993) and *Dentex dentex* (Crespo et al. 2001), based on observations of intestinal steatosis and epithelial degeneration. Histological examination of affected cod would be required to check whether or not that was the case in this study. Based on the current understanding of EFA requirements in marine fish larvae (Sargent et al. 1999; Izquierdo et al. 2000), we also conclude that tissue ratios of DHA:EPA:ARA were less favorable among cod in the d5 and d15 transfer groups.

Previous research has suggested that sub-optimal environmental conditions (temperature, salinity, water depth) may induce stress-related swimbladder abnormalities in cultured fish (Johnson and Katavic 1984; Bagarinao and Kungvankij 1986), a phenomenon referred to as SBSS. This study is the first to present evidence that swimbladder over-inflation is influenced by the nutritional status of fish reared under uniform environmental conditions. Recognizing the interaction between stress tolerance and nutritional status of fish (Pickering 1998), it is possible that cod from the d25 groups were best able to withstand sub-optimal environmental conditions in the experimental rearing tanks. Further research is recommended to investigate the role of nutrition in ameliorating SBSS among intensively cultured fish larvae.

In considering the effect of age/developmental stage on the ability of cod larvae to digest and assimilate *Artemia*, we suggest that maturation of the digestive tract was critical to the better performance of fish in the d15 and d25 groups. This might also account for uniformly high survival rates (> 30% of larvae stocked) among groups of co-fed cod larvae that received rotifers until at least day 22 ph (Baskerville-Bridges and Kling 2000b). While there is little published information on digestive enzyme ontogeny in Atlantic cod, the available histological, biochemical and gene expression studies point to

onset of stomach functionality no earlier than *circa* day 17 ph (Kjørsvik et al. 1991; Pedersen and Falk-Petersen 1992; Morrison 1993; Murray et al. 2001). Although timing of functionality is likely to vary according to growth rate, the ages quoted in these studies align well with our current observations on cod rearing performance in relation to feeding regimen.

The growth parameters recorded in the current study are within the range of previously published values for cod reared intensively using rotifers and *Artemia*. End-point size (standard length, dry weight) in the d15 and d25 groups was comparable to values recorded by Baskerville-Bridges and Kling (2000a, b), for cod reared at 10–11 °C. Puvanendran and Brown (1998, 1999) documented smaller mean standard lengths among comparable-aged cod reared at 8 °C in glass aquaria, while Zhao et al. (2001) recorded intermediate growth rates in the same experimental system, at 11–15 °C.

Such growth variability highlights the need to apply size-specific feeding protocols for cod larvae, rather than relying on age as the basis for diet transition. While growth is influenced by a diversity of biotic and abiotic variables, temperature has been shown to strongly influence cod larval growth rate over the range of naturally experienced temperatures (Otterlei et al. 1999; Steinarsson and Björnsson 1999). The current growth rates, obtained at relatively low rearing temperature (8.8 °C), would thus be expected to increase substantially at higher temperatures, subject to appropriate increases in dietary input.

In selecting an appropriate intensive feeding regimen for commercial scale Atlantic cod larviculture, we conclude that *Artemia* should be withheld until completion of metamorphosis. No advantage is gained by offering freshly-hatched nauplii during the rotifer-*Artemia* transition and it is recommended that cod larvae be fed enriched *Artemia* nauplii directly.

Acknowledgements

The authors would like to thank Peter Harvey, Kay Robins and Jon Sherwood for their invaluable technical assistance during the feeding experiment. Thanks are also due to Carlos Mazorra and Gordon Bell for contributing rotifer fatty acid compositions. This study was funded by a grant awarded to Seafish Aquaculture by Highlands and Islands Enterprise, Scotland.

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