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Aquaculture 189 (2000) 109–117

Aquaculture

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Early weaning of Atlantic cod (*Gadus morhua*) larvae onto a microparticulate diet

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Received 9 August 1999; received in revised form 17 February 2000; accepted 17 February 2000

Abstract

A microparticulate diet was introduced on 8, 15, 22, 29 and 29 days post-hatch (dph) (with *Artemia*). Larvae were weaned from rotifers directly onto the diet in the first four treatments. *Artemia* were used in the fifth treatment and were fed for a period of 10 days. The microparticulate diet was able to completely replace live prey long before metamorphosis and larvae were weaned by 8.5 mm standard length. With the earliest introduction of the microparticulate diet on 8 dph, we observed 35% survival through 71 dph (21 mm). The survival of larvae from the other treatments was not significantly different and ranged from 32.7% to 39.4%. Weaning time did not have a significant affect on growth of cod larvae, as there were no differences when introducing the microparticulate diet on 8 or 29 (without *Artemia*) dph. However, supplementation of *Artemia* for 10 days had a growth-promoting effect. Larvae were larger than individuals from the other four treatments beginning on 29 dph and continued throughout the experiment. By 71 dph, they were 24 mm and weighed 20 mg (dry weight). Successful culture of many finfish species is dependent on the use of live prey during the larval period. Early introduction of a microparticulate diet reduces the quantity of rotifers required and makes *Artemia* nonessential. This lowers production costs considerably by reducing the number of live animal cultures that must be maintained. However, until better diets are produced, careful consideration should be given to how early weaning affects production cost vs. growth rate of the larvae. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Cod; *Gadus morhua*; Microparticulate diet; Weaning

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PII: S0044-8486(00)00356-2

1. Introduction

The control of larva nutrition is a key element in the successful culture of marine fishes. The use of live food organisms has been employed in the culture of the early life stages of most marine larvae and is currently considered as obligatory for successful culture. Cultivation methods of live organisms such as *Brachionus* (rotifers) and *Artemia* (brine shrimp) are simple and well documented but their use is costly and unreliable. Le Ruyet et al. (1993) calculated that live prey represented only 1.6% of the total amount of dry matter consumed during the first 3 months of life, but represented 50% of the feed cost. *Artemia*, alone, represented 40% of the feed cost, or 80% of the live prey feeding cost. Similar costs were reported by Ehrlich et al. (1989) for early weaned smallmouth bass (*Micropterus dolomieui*). The reduction or elimination of live food organisms, especially *Artemia*, from the production cycle of marine fish would be economically advantageous.

A manufactured microparticulate diet suitable for the complete replacement of live food at the onset of exogenous feeding has not yet been developed for most larval finfish species. Weaning of Atlantic cod has traditionally been performed during or after metamorphosis (Howell, 1984; Bromley and Sykes, 1985; Rosenlund et al., 1993). Earlier introduction of a manufactured diet was observed to adversely affect survival and growth rates of cod larvae (Molvik et al., 1984; Garatun-Tjeldsto et al., 1987, 1989; Ottera and Oyvund, 1991; Ringo et al., 1991). Poor performance of manufactured diets to first-feeding larvae may be due to: (1) low ingestion rates due to low palatability or low residence time in the water; (2) low digestibility of the diet due to inadequate digestive enzyme activity; or (3) poor nutritional composition of the diet. And, as pointed out by Rosenlund et al. (1993), the feeding of manufactured diets can have profound negative effects on water quality parameters resulting in high larval mortality unrelated to its nutritional characteristics.

Holt (1993), Hart and Purser (1996) and Rosenlund et al. (1993) reported improved growth and survival of several marine fish larvae from the combined feeding of live and manufactured diets (co-feeding). Holt (1993) achieved 60% survival in red drum from hatch to metamorphosis on a commercial diet when supplied in combination with live food for the first 5 days. Previously, red sea bream had been successfully weaned only after 21–25 days post-hatch (dph). Hart and Purser (1996) achieved significantly higher survival in greenback flounder (*Rhombosolea tapirina*, Gunther) weaned prior to metamorphosis at 23 dph using a 10 day co-feeding regime than was achieved weaning after metamorphosis, after 50 dph.

In an earlier study, cod larvae accepted a microparticulate diet 8 days after first feeding (11 dph) and exhibited growth rates comparable to larvae weaned after 36 dph (unpublished data). However, poor survival was observed with early introduction of the diet, due to tank fouling and inadequate water quality. This paper presents the results of a study conducted to determine the earliest time Atlantic cod larvae can be weaned onto a commercially manufactured microparticulate diet employing a co-feeding strategy.

2. Materials and methods

2.1. Chemical analysis of the diet

A commercially available microparticulate diet was used in the feeding trial (Bio-Kyowa™, Kyowa Hakko Kogyo, Ohtemachi, Chiyoda-ku, Tokyo, Japan). Two size fractions were used during the experiment: Kyowa A-250 (< 250 μm) and Kyowa A-400 (250–400 μm).

The diets were analyzed for dry matter content, crude protein, fat and ash content. Dry matter content was determined by drying a sample of the feed for 24 h at 105°C. Prior to the analyses of protein, fat and ash, the feed was dried as above, ground with a mortar and pestle, redried for an additional 2 h, and stored in a desiccator until needed for analysis. Crude protein was analyzed according to AOAC (1984) as modified by Tecator™ Application Note 1981.10.05 (1981), using a Kjeltec Auto 1030 Analyzer. A copper catalyst was used during digestion and a mixture of boric acid (1%) with bromocresol green/methyl red indicator was used as the receiving solution. Fat was analyzed according to the method of Bligh and Dyer (1959). Ash content was measured after heating a feed sample at 600°C for 12 h in a Sybron/Thermolyne™ muffle furnace.

2.2. Eggs and incubation

Atlantic cod (*Gadus morhua*) eggs were provided by the National Marine Fisheries Service (NMFS) at the Narragansett laboratory in Rhode Island. They were disinfected with glutaraldehyde (200 mg/l) for 10 min and placed into 135-l incubators. Continuous aeration was applied to keep the eggs moving uniformly throughout the tanks. They were maintained at 6–8°C and kept in total darkness. Eggs were disinfected again 1 day prior to hatching and transferred to individual 22-l rearing tanks. Aeration was maintained to sustain constant motion of the larvae. Well water was used and the salinity was adjusted to 33–34 g/l using artificial sea salt (Forty Fathoms™). Water flow rates into the culture tanks were adjusted to 200 ml/min. Water temperature was increased at first feeding by 1°C day^{-1} until 10–11°C had been achieved. The larvae were kept on a photoperiod of 24 h light: 0 h dark. Lighting was increased to 22 lx 1 day after hatching.

2.3. Feeding trial

Five treatments ($n = 4$) were randomly assigned to 20 dark blue tanks (22 l). Each tank was stocked with 1200 larvae (55 larvae/l). First feeding began on 1 dph with the addition of rotifers (*Brachionus plicatilis*). The rotifers were enriched with DHA-Selco™ (Inve Aquaculture, Grantsville, UT, USA) and added to the tanks four times daily (8, 12, 16, and 20 h). The microparticulate diet was introduced on 8, 15, 22, and 29 dph for tanks in treatments 1, 2, 3, and 4, respectively (Fig. 1). Larvae in these treatments received no *Artemia* during the study. Larvae in treatment 5 were fed rotifers, *Artemia* (Great Salt Lake), and the microparticulate diet on 1, 26, and 29 dph, respectively. *Artemia* were enriched with DHA-Selco for 24 h prior to feeding to the larvae. During weaning, live food was co-fed with the microparticulate diet for 7 days. The quantity of live prey (250,000 rotifers $tank^{-1}$ $meal^{-1}$ or 75,000 *Artemia* $tank^{-1}$ $meal^{-1}$) was

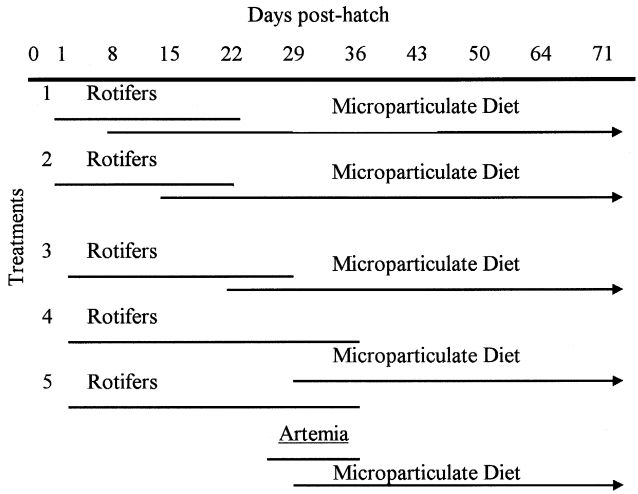

Fig. 1. Feeding regimes used for weaning trials. Overlap of lines indicates co-feeding periods.

reduced by 12.5% day $^{-1}$ over 7 days and then discontinued. The Kyowa 250-A diet was fed every 2 h either by Eheim™ automated feeders (Hawaiian Marine Imports, Houston, TX, USA) or by hand. The quantity delivered per feeding by the automated feeders was grossly controlled by manipulating the size of the opening of the feeding barrel and amounted to approximately 1 g/day. The tanks were cleaned daily by siphoning. Larvae were gradually switched over to the Kyowa 400-A diet when they attained an average size of 15 mm.

2.4. Sampling

One-hundred larvae were sampled after hatching to measure initial lengths and dry weights of the larvae. Twenty larvae from each of the 20 tanks ($n = 4$) were sampled on 8, 15, 22, 29, 36, 43, 50, 57, 64, and 71 dph. Images of the larvae and a calibrated scale were electronically saved at each sampling period using a Targa plus frame grabber (Media Cybernetics, Silver Springs, MD, USA). Standard lengths were measured to the nearest 0.1 mm using Image Pro Plus image analysis software (Truevision, Indianapolis, IN, USA). Length measurements were taken from the tip of the upper jaw to the end of the notochord. Larvae were rinsed with freshwater, freeze-dried, and measured to the nearest ± 2 μg using a Mettler-A20 microbalance (Mettler Toledo, Hightstown, NJ, USA). On 71 dph, all remaining larvae were counted and survival was calculated. Growth rates were calculated for each treatment from 1 to 71 dph.

2.5. Statistics

A completely random design was used in each of the experiments. Each treatment was randomly assigned to four experimental tanks ($n = 4$). Larvae were subsampled from all tanks during each sampling period to obtain tank averages that were used in

subsequent statistical computations. All statistical procedures used SAS/STAT™ software (SAS Institute, 1985). Treatments were compared to one another at each sampling time. Normality of the data (Shapiro and Wilk, 1965) and homogeneity of variance (Snedocor and Cochran, 1993) were tested to ensure that assumptions for analysis of variance were satisfied. Data expressed as percentages (survival and specific growth rate, SGR) were transformed (arcsin) before conducting analysis of variance. Length and dry weight data were analyzed using analysis of variance (Snedocor and Cochran, 1993). Differences due to weaning protocol were considered significant at $P < 0.05$. If the analysis of variance was shown to be significant, mean separation was determined using Duncan's multiple range test (Snedocor and Cochran, 1993).

3. Results

The chemical analysis confirmed similar nutrient contents of the commercial microparticulate A-250 diet (< 250 μm) and A-400 diet (250–400 μm). The diets contained 56.8% and 57.4% crude protein, 20.6% and 20.8% total fat and 9.3% and 9.2% ash, respectively.

During the co-feeding period, we observed little ingestion of the microparticulate diet when high quantities of live prey were available to the larvae. Instead, cod larvae selectively ingested rotifers and it was not until the abundance of live prey had declined that they initiated feeding on the microparticulate diet. When the microparticulate diet was introduced on 15, 22, and 29 dph, majority of the larvae were observed with inert diet in their guts at the end of the co-feeding period. Very little of the diet was ingested when introduced on 8 dph and only 20% of the larvae in this treatment was observed

Table 1
Survival, SGR and tank biomass of cod larvae[u]

Introduction of MPD[v] (dph[w])					
	8	15	22	29	29 + *Artemia*
Survival[x] to 71 dph					
Number	334	343	389	328	340
%	33.8 + 2.5	35.0 + 2.0	39.4 + 0.9	32.7 + 1.0	34.8 + 3.5
SGR[y] (%)	8.6 + .05[b]	8.2 + .05[c]	8.2 + .04[c]	8.4 + .1[bc]	9.0 + .1[a]
TB[z] (g/l)	0.23 + 0.01[b]	0.18 + 0.02[b]	0.21 + 0.01[b]	0.20 + 0.02[b]	0.30 + 0.02[a]

[u] Values are means ± standard error of four replicates. Means within a row with the same superscript are not significantly different ($P < 0.05$).

[v] MPD = microparticulate diet.

[w] dph = days post-hatch.

[x] Percent survival to 71 dph. Survival (%) = [Number of survivors at the end of the experiment/(Number of larvae at start − Number of larvae sampled)] × 100.

[y] SGR (% day^{-1}) = specific growth rate of cod larvae from 1 to 71 dph. SGR (% day^{-1}) = [ln final dry weight − ln initial dry weight)/days] × 100.

[z] TB (g/l) = tank biomass of cod larvae at 71 dph. TB (g/l) = Number of survivors per tank × Mean larval dry weight per tank.

with microparticulate diet in their intestine at the end of the co-feeding period (14 dph). Rotifers were offered for an additional 1 week at half ration to help these larvae make the transition to the inert diet.

Survival to 71 dph ranged from 32.7% to 39.4% (Table 1) and larvae attained a length ranging from 20 to 23 mm. Their fins were greatly differentiated and extensive pyloric ceca were present. There were no differences in length or dry weight between larvae fed the microparticulate diet 8 dph with a 14 day co-feeding period and those fed

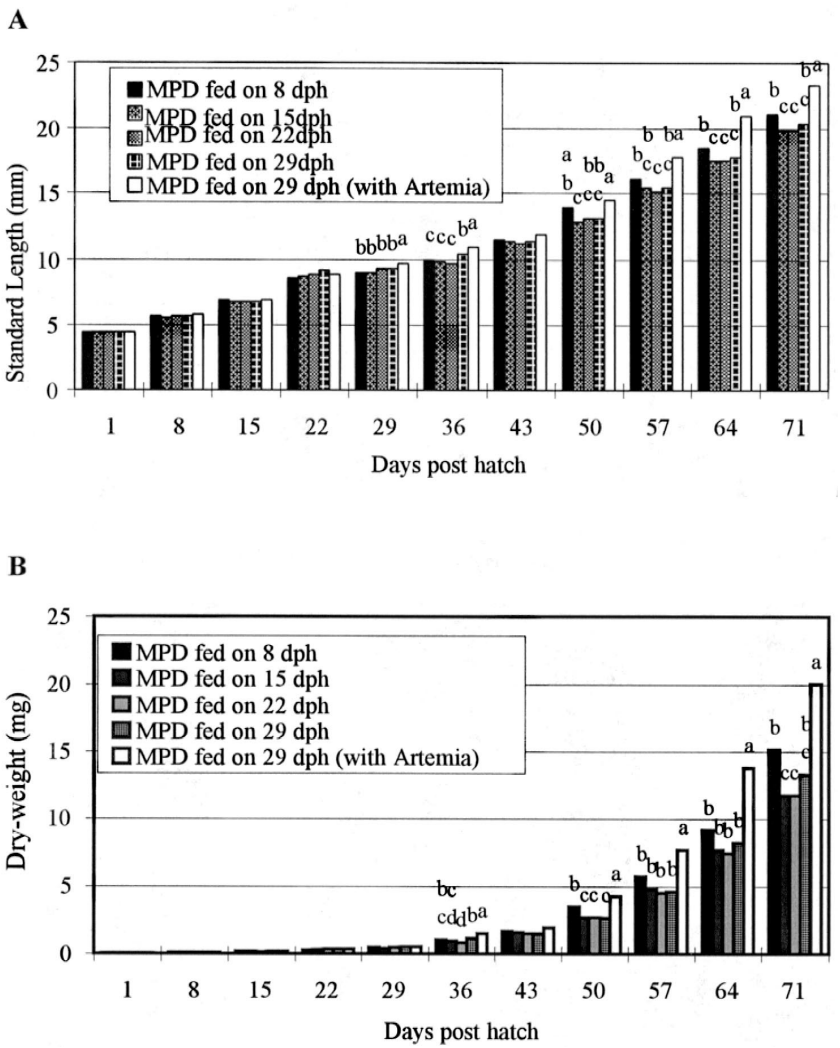

Fig. 2. The effect of weaning time on standard length (A) and dry weight (B) of cod larvae from 1 to 71 dph. Bars within each time interval with different letters were significantly different ($P < 0.05$). MPD: microparticulate diet.

the microparticulate diet 29 dph (no *Artemia*) with a 7-day co-feeding period. However, larvae fed the microparticulate diet on 8 dph with a 14-day co-feeding period were significantly ($P < 0.05$) larger than larvae receiving the diet on 15 and 22 dph with a 7-day co-feeding period. Larvae in treatment 5, receiving *Artemia* from days 26 to 35, were significantly ($P < 0.05$) longer than larvae from all other treatments by 29 dph (Fig. 2A). This trend continued and the larvae receiving *Artemia* were both significantly longer and heavier at the end of the experiment (71 dph).

The highest SGR (1–71 dph) was observed in larvae fed *Artemia* along with the microparticulate diet (9% day $^{-1}$) (Table 1). SGR for the other treatments ranged from 8.2% to 8.6% day^{-1} and followed the same trend that was observed for dry weight. There were no significant differences in tank biomass when the microparticulate diet was introduced on 8, 15, 22, or 29 dph without *Artemia* but tank biomass was significantly ($P < 0.05$) higher when the larvae were supplemented with *Artemia* (Table 1). Contrary to previous experiments, aggressive behavior and cannibalism were not observed in this feeding trial.

4. Discussion

The commercial microparticulate diet was effective for early weaning and supported growth through metamorphosis (> 20 mm) in all treatments. Larvae were completely weaned from live food onto a microparticulate diet by 22 dph (8.5 mm standard length), reducing the length of time that live prey were required. We believe this is the earliest reported weaning age for this species (Molvik et al., 1984; Garatun-Tjeldsto et al., 1987, 1989; Ottera and Oyvund, 1991; Ringo et al., 1991).

The introduction of the microparticulate diet on 8 dph followed by a 14-day co-feeding period resulted in better growth of cod larvae than introducing it on 15 dph followed by a 7-day co-feeding period. Even though live food was discontinued in both treatments on the same day (22 dph), differences in larval growth between the two treatments were detected by 36 dph and were still evident on 71 dph. Hart and Purser (1996) observed a similar phenomenon in weaning greenback flounder; while a short co-feeding period resulted in similar survival, a longer co-feeding period resulted in higher growth rates. The difference in growth rate observed between treatment 1 and treatments 2 and 3 may be explained by the shorter co-feeding period for the latter two treatments. The presence of the diet in the water column so soon after hatching may allow the larvae to better identify the microparticulate diet as a food item. Earlier recognition and ingestion of the diet could account for the enhanced growth rate that we observed when introducing the diet 1 week after first feeding (Rosenlund et al., 1993).

Cod larvae were successfully cultured through metamorphosis without using *Artemia*. This provides a large reduction in production costs because larvae can be weaned from rotifers directly onto the microparticulate diet. Although there were no differences in survival, the presence or absence of *Artemia* significantly influenced growth rates. Larvae in treatment 4 (no *Artemia*) attained a dry weight of 13 mg by 71 dph. Larvae from treatment 5, which were fed *Artemia* for 10 days (26–35 dph), but were otherwise treated identically to larvae from treatment 4, weighed 20 mg by 71 dph. The addition of

Artemia resulted in a 150% increase in body weight, compared to larvae given microparticulate diet without *Artemia*. Kolkovski et al. (1997b) reported an increase in the rate of assimilation and growth in European seabass larvae fed microparticulate diets with the co-feeding of *Artemia*. Kolkovski et al. (1997a) reviewed the possible mode of action of *Artemia* in enhancing the utilization of microparticulate diets in gilthead seabream larvae. Co-feeding of *Artemia* with microparticulate diets was observed to enhance ingestion rates of the inert diet by 120%. Moreover, microparticulate diets supplemented with various lipid and non-lipid extracts from *Artemia* significantly increased the assimilation of the inert diet by 10–20% in 22-day-old gilthead seabream.

Acknowledgements

Maine Agricultural and Forest Experiment Station external publication no. 2411. This work was supported by the University of Maine and University of New Hampshire Sea Grant College Program as well as the Maine Agricultural and Forest Experiment Station. We would like to thank Jacqueline Hunter for the technical help she provided and for producing the live food organisms necessary to conduct this research. Thanks also to undergraduates Trevor Davis and Eric Jefts for their help with this research.

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