Short communication

Larval rearing of chub, *Leuciscus cephalus* (L.), using decapsulated *Artemia* as direct food

By A. Shiri Harzevili, D. De Charleroy, J. Auwerx, I. Vught and J. Van Slycken

Fish Culture Centre, Institute for Forestry and Game Management, Ministry of the Flemish Community, Linkebeek, Belgium

Summary

Little is known about the larviculture of the chub, *Leuciscus cephalus* (L.), an endangered cyprinid species endemic to European flowing waters. The use of decapsulated *Artemia* cysts as food for chub larviculture was investigated. After 3-day feeding with the rotifer *Brachionus calyciflorus*, the larvae were fed on different diets: (i) dried decapsulated *Artemia* cysts, (ii) *Artemia* nauplii, (iii) rotifers for seven more days and then *Daphnia* collected from a pond, and (iv) an artificial diet. After a 24-day rearing period, the highest survival rate was obtained with the larvae receiving decapsulated *Artemia* cysts. Feeding of the larvae with an artificial diet resulted in a significantly lower survival rate compared with the other groups. At the end of the experiment, the larvae fed on *Artemia* nauplii yielded a significantly higher mean length compared with the other groups. Feeding an artificial diet resulted in a significantly lower average weight and mean length gain compared with the other groups.

Introduction

Chub, *Leuciscus cephalus* (L.), is a cyprinid species living in European flowing waters and listed as an endangered species by the International Union for the Conservation of Nature and Natural Resources (IUCN, 1994). Little is known about the larviculture of chub (Ç alta, 2000; Kujawa et al., 2000).

The brine shrimp *Artemia* is widely used as a live food organism for many larval fish cultured in intensive systems. However, increased demand for good quality *Artemia* cysts and recent fluctuations in world harvests have sharply increased prices. As a result, attention is again concentrating on new alternative diets to *Artemia* nauplii.

Poor quality *Artemia* cysts might represent a potential alternative to *Artemia* nauplii. The outer layer of the *Artemia* cyst is non-digestible by predator organisms, but this outer layer can be quickly removed with hypochlorite treatment, a procedure called decapsulation. Decapsulated *Artemia* cysts have been successfully fed to fish larvae (Verreth et al., 1987; Vanhaecke et al., 1990; Pector et al., 1994). As decapsulated embryos have more energy content than newly hatched nauplii (Lé ger et al., 1986), they are potentially more nutritious for feeding. Several other advantages of decapsulated *Artemia* cysts are listed in Pector et al. (1994).

The aim of the present study was to investigate the suitability of decapsulated *Artemia* cysts for chub larvae during their early feeding stage.

Materials and methods

Chub eggs were obtained from broodfish held at the Fish Culture Centre (Linkebeek, Belgium) using an adapted technique described by Woynarovich and Horvá th (1980) for cyprinid fish. Fertilized eggs were incubated on an artificial substrate using aerated and dechlorinated tapwater at 18°C. Larvae hatched after 4 days of incubation. Five days after hatching, the larvae were fed *ad libitum* with the rotifer, *Brachionus calyciflorus*, for 3 days. Thereafter, the larvae were exposed to different feeding conditions: (i) decapsulated *Artemia* cysts, (ii) freshly hatched *Artemia* nauplii (4 $ml^{)1}$), (iii) rotifers for 7 more days and then *Daphnia* collected from a pond, or (iv) a granulated trout starter diet. The larvae were stocked at random in aquaria each containing 20 L of dechlorinated tapwater using a flow-through system. The water exchange in each aquarium was constant (300 ml $min^{)1}$). Water was gently aerated with a single air stone. The stocking density was kept at 200 individuals per aquarium (10 larvae $L^{)1}$). The initial larval total length (mean ± SD) and average wet body weight were 8.52 ± 0.28 mm and 3.53 mg, respectively. Each day just before feeding, bottom debris was siphoned from each aquarium. There were three replicates per treatment. Growth parameters (length and wet weight) were measured on days 0, 7, 14 and 24 of the experimental period. Fish length was measured with a binocular microscope equipped with an ocular micrometer. For length measurements, 10 larvae were randomly collected from each replicate. Survival of the larvae was recorded by counting the fish in the aquaria on day 7, day 14 and at the end of the experiment.

Cysts were decapsulated following the methodology outlined in Bruggeman et al. (1980). Fresh stocks of cysts were prepared everyday. Decapsulated cysts were distributed on a 100 lm screen in a <5 mm thick layer and dried at 35°C for 24 h. The quantity of decapsulated cysts per larva was estimated according to Vanhaecke et al. (1990).

The granulated diet was prepared from a trout starter food. This starter food (0.3–0.5 mm) was crushed with a mortar and sieved to a particle size of <200 lm. From day 12 onwards, starter food of 0.3–0.5 mm was used. Larvae were fed with this artificial diet *ad libitum*.

Data were analysed with a computerized statistical program (S-Plus, 2000). Analysis of variance was performed to determine any significant differences among the treatments. Significant differences among treatments were determined by Tukey's multiple range test ($P < 0.05$).

Treatment group	Day 7	Day 14	Day 24
Dry decapsulated cysts	10.62 ± 0.43^{a} (10)	13.83 ± 0.82^{a} (10)	17.82 ± 0.81^{b} (10)
Artemia nauplii	10.79 ± 0.44^{a} (10)	14.21 ± 0.90^{a} (10)	19.70 ± 1.03^{a} (10)
Rotifer + *Daphnia*	9.70 ± 0.69^{b} (10)	12.64 ± 1.23^{a} (10)	16.46 ± 1.13^{b} (10)
Artificial diet	9.20 ± 0.37^{b} (10)	10.30 ± 0.50^{b} (10)	11.71 ± 1.06^{c} (10)

Different superscript letters within a column indicate significant difference ($P < 0.05$). Numbers of larvae are in parentheses.

Table 1
Length (mm) of chub larvae measured on days 7, 14 and 24 of the experiment (mean ± SD)

Treatment group	Day 7	Day 14	Day 24
Dry decapsulated cysts	7.20 ± 0.07^{a} (10)	18.44 ± 0.27^{a} (10)	46.42 ± 1.26^{b} (10)
Artemia nauplii	7.26 ± 0.37^{a} (10)	20.73 ± 0.23^{a} (10)	57.52 ± 4.11^{a} (10)
Rotifer + *Daphnia*	4.61 ± 0.33^{b} (10)	12.45 ± 1.37^{b} (10)	30.56 ± 4.15^{c} (10)
Artificial diet	4.26 ± 0.25^{b} (10)	7.11 ± 0.60^{c} (10)	10.92 ± 1.40^{d} (10)

Different superscript letters within a column indicate significant difference ($P < 0.05$). Numbers of larvae are in parentheses.

Table 2
Wet weight (mg) of chub larvae measured on days 7, 14 and 24 of the experiment (mean ± SD)

Results

On day 7 and day 14, the mean sizes of larvae fed freshly hatched *Artemia* nauplii and decapsulated cysts were not significantly different ($P > 0.05$) (Table 1). However, a significantly better growth in *Artemia* nauplii-fed larvae was observed at the end of the experiment. Both diets produced significantly faster growth (in terms of total length) than the artificial diet. After 24 days of culture, the larvae fed on the artificial diet had the lowest mean length (Table 1).

On day 7 and day 14, the mean weights of the larvae (Table 2) fed on freshly hatched *Artemia* nauplii and decapsulated cysts were not significantly different ($P > 0.05$). However, a significantly better growth (in terms of wet weight) in *Artemia* nauplii-fed larvae was observed at the end of the experiment. The larvae fed on the artificial diet had the lowest wet weight.

No significant difference was observed in larval survival on day 7 among the treatments fed *Artemia* nauplii, decapsulated cysts and rotifers (Table 3). The survival rate of the larvae fed on the artificial diet was significantly lower compared with the latter groups (Table 3). At the end of the experiment, no significant difference was observed between the survival of larvae fed on *Artemia* nauplii and those on decapsulated cysts, although in the latter fed it was slightly higher.

Discussion

The use of decapsulated *Artemia* cysts as a direct food source in early larval nutrition of marine and freshwater fish, and marine shrimp has been previously suggested (Verreth et al., 1987; Vanhaecke et al., 1990; Pector et al., 1994; Ribeiro and Jones, 1998). The authors concluded that initial feeding of the larval fish with decapsulated cysts appeared to be suitable for complete replacement of live *Artemia* nauplii and might support acceptable survival when offered solely or incorporated into artificial feeds. However, they argued that the main problem using decapsulated cysts as a direct food source was their fast sedimentation in water and lack of motility.

In the present study, dried decapsulated cysts exhibited a positive effect on growth and survival of chub larvae. After 2 weeks, the mean larval size was similar when fed *Artemia* nauplii and decapsulated cysts. At the end of the experiment, however, mean length and wet weight of the larvae was larger when fed on *Artemia* nauplii in comparison to decapsulated cysts. During long-term feeding (beyond 14 days post-hatching), decapsulated cysts did not result in as much growth in chub larvae as did live nauplii, perhaps because of the limited size of the decapsulated cysts. A similar finding was also reported by Vanhaecke et al. (1990).

In the present study, poor growth and survival of chub larvae was obtained with the artificial diet in comparison with other treatments. According to Ribeiro and Jones (1998), better performance of prawn post-larvae fed on dried decapsulated cysts appeared to be related to retention of the nutritional value after rehydration in water, thus leaching very low levels of soluble protein and carbohydrates in comparison with the artificial diets. Pector et al. (1994) also observed high water stability of decapsulated cysts and fast growth for African catfish (*Clarias gariepinus*) as opposed to artificial diets. Slow growth and high mortality of larvae fed on artificial diets may be related to the absence of a stomach and low digestive capacity at the beginning of their development. Kujawa et al. (2000) found low survival and growth rates of chub larvae fed on artificial diets, and suggested that the transfer of chub larvae from live food to artificial diet should start after 12 days of exogenous feeding. In most cyprinid larvae fed exclusively on artificial diets, high mortality and poor growth occurred in most cases (Dabrowski and Poczycznski, 1988; Wolnicki and Gó rny, 1995; Wolnicki and Myszkowski, 1999).

In conclusion, the results of the present work demonstrate that dried decapsulated *Artemia* cysts appear to be a suitable food for the early developmental stage of chub larvae.

Treatment group	Day 7	Day 14	Day 24
Dry decapsulated cysts	90.0 ± 4.2^{a}	85.3 ± 1.1^{a}	79.3 ± 6.7^{a}
Artemia nauplii	92.5 ± 9.2^{a}	76.7 ± 9.7^{a}	71.6 ± 6.9^{a}
Rotifer + *Daphnia*	87.5 ± 6.4^{a}	66.5 ± 3.5^{a}	62.3 ± 6.0^{a}
Artificial diet	58.8 ± 3.9^{b}	38.5 ± 7.8^{b}	18.0 ± 7.1^{b}

Different superscript letters within a column indicate significant difference ($P > 0.05$).

Table 3
Survival rate (%) of chub larvae counted on day 7, 14 and at the end of experiment (day 24) (mean ± SD)

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Author's address: Alireza Shiri Harzevili, Fish Culture Centre, Institute for Forestry and Game Management, Ministry of the Flemish Community, Dwersbos 28, B-1630 Linkebeek, Belgium.
E-mail: alireza.shiriharzevili@lin.vlaanderen.be