

Larval rearing of burbot (*Lota lota* L.) using *Brachionus calyciflorus* rotifer as starter food

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Summary

Burbot *Lota lota* L. is one of the endangered freshwater fish species in western Europe for which the development of controlled larval rearing procedures could produce enough material for stock enhancement. The suitability of the freshwater rotifer *Brachionus calyciflorus* as a start food for larviculture of burbot was investigated. After yolk-absorption, the larvae were stocked in 40-L tanks under different feeding conditions: clear water rearing conditions with rotifers (*Brachionus calyciflorus*) for 10 days (R), green water conditions (*Chlorella* sp.) with rotifers offered for 10 days (MALR), green water conditions (*Chlorella* sp.) for 3 days followed by clear water in combination with rotifer feeding for 7 days (AL3R), and clear water conditions with *Artemia* nauplii offered for 10 days (Art). After the 10-day feeding, all groups received *Artemia* nauplii up to 35 days post-hatching. Larval survival was counted at day 10 and at the end of the 35-day rearing experiment. At day 35, a significant survival difference was noted between the groups where rotifers were supplemented with algae vs only *Artemia*. At the end of the experiment, the highest survival rate (69.20%) was obtained with larvae receiving only algae in the first 3 days of feeding. Lowest survival rate (24.90%) was obtained with larvae receiving only *Artemia* for 35 days. This indicates that smaller prey are essential for burbot at first feeding. Larval length and wet weight were measured at the time of mouth opening, at days 7, 10, and 21, and at the end of the experiment (day 35). On day 35, mean length of the larvae varied significantly between the treatments. However, the final wet weight of the larvae did not vary significantly between the treatments.

Introduction

The burbot, *Lota lota* L., is a member of the family of Gadidae (cod), but differs from most cod in that it usually passes its entire life in freshwater (Scott and Crossman, 1973). This species is one of the rare or endangered freshwater fish species in many European countries (Maitland and Lyle, 1991). The causes of its decline remain unknown. Habitat loss through changes in river design and management, pollution and climatic warming may have resulted in population decline of the fish. Potential measures such as stocking or introduction of young fish in rivers, lakes and reservoirs have been suggested for the protection of fish populations. An essential prerequisite for any stocking or reintroduction programme would be the rearing of large numbers of the fish in captivity.

While numerous works abound outlining the various aspects of the life history of adult burbot (e.g. Bailey, 1972), relatively

little research has been devoted to the larval stage of the species. Few reports have been published on larval culture of burbot (Steiner et al., 1996; Kujawa et al., 1999, 2000). At the yolk sac stage, they measure 3–4 mm (Pokorny and Adamek, 1997). Small prey are needed to fulfil the demand of burbot larvae in the early period of exogenous feeding. In a field study, Ghan and Sprules (1993) found that the larvae started to feed on zooplankton 5 days after hatching; of 25 burbot larvae, only two larvae contained zooplankton prey in their stomach at the first feeding. These authors indicated that the first food items taken by larval burbot were rotifers. In a laboratory study, Vachta (1990) reported that the larvae first ate phytoplankton and did not switch to copepod nauplii until the third day of exogenous feeding.

Rotifers are the most important live food organisms for use as starter food for rearing small fish larvae (Watanabe et al., 1983; Lim and Wong, 1997; Awa' ss and Kestemont, 1998; Lubzens et al., 2001). Contrary to established techniques for mass production of the marine rotifer, *Brachionus plicatilis*, the potential application of freshwater rotifer *B. calyciflorus* in fish larviculture has not been fully developed. To date, it is restricted to only a few freshwater fish species such as gudgeon (*Gobio gobio* L.), perch (*Perch fluviatilis* L.) (Awa' ss, 1991; Awa' ss et al., 1992), and sunshine bass (*Morone chrysops* - *M. saxatilis*) (Ludwig, 1994).

The aim of the present research was to develop a suitable method for larval rearing of burbot (*L. lota*) under controlled conditions using *B. calyciflorus* as a starter food.

Materials and methods

The experiment was conducted at the fish culture centre at Linkebeek, part of the Institute for Forestry and Game Management, Ministry of the Flemish Community, Belgium. Cultures of the microalgae (*Chlorella* sp.) were started by agar plating techniques, upscaled to test tubes, and then to Erlenmeyer flasks of 500 ml. The content of the flasks was used to inoculate 5-L bottles, which, in turn, were used to seed 20-L carboys and then to plastic bags of 60-L and finally in four tanks of 1000-L each. Dechlorinated filtered (0.45 µm) tap water was used for algal cultures. Microalgae were fertilized with ES (basal medium) (Provasoli et al., 1957) and Walne (Walne, 1956) media. Temperature was maintained at 25°C.

Rotifer *B. calyciflorus* was cultured using adaptation of the techniques described by Lavens and Sorgeloos (1996). Rotifer resting eggs were incubated in centrifuge tubes of 50-ml containing prefiltered and dechlorinated tapwater. The tubes were exposed to 1000 lux artificial light for hatching of the

rotifers. Upon hatching, the rotifers were fed microalgae (*Chlorella* sp.). Thereafter, the cultures were upscaled to 500-ml Erlenmeyer flasks, then to 15-L bottles, and after 1 week the 15-L bottles were used for the inoculation of rotifers on a larger scale. Total ammonia levels in the rotifer cultures were generally kept below 5 mg L⁻¹ by water exchange. Rotifers were added to the fish tanks each morning and their concentration adjusted to the desired level (10 ml⁻¹) the following morning.

Hatching of *Artemia* cysts was performed according to standard methods developed in the Laboratory of Aquaculture and Artemia Reference Centre (Lavens and Sorgeloos, 1996). Newly-hatched *Artemia* nauplii were used for feeding the larvae.

Newly-hatched burbot larvae were obtained from a semi-industrial hatchery (Czech Republic). Upon arrival, the larvae were acclimatized to experimental conditions for 3 days in the holding tray, using springwater exposed to UV light. Water temperature was constant during rearing (10°C). Dissolved oxygen ranged between 7.9 and 9.6 p.p.m.

After yolk-absorption, the larvae were stocked at random in the rearing tanks each containing 40-L of spring water using a flow-through system. The water flow through each tank was similar and constant (0.5 l min⁻¹). Water was gently aerated with a single airstone. Stocking density in the rearing tank was kept at 2800 larvae per tank (70 individuals L⁻¹). Initial larval total length (mean ± SD) and average wet body weight were 4.39 ± 0.28 mm and 0.36 mg, respectively. Each day, just before feeding, bottom debris was siphoned from every tank.

The experiment was performed under different feeding conditions: clear water rearing conditions with rotifers (*B. calyciflorus*) fed at a density of 10 organisms per ml for 10 days (R), green water conditions (*Chlorella* sp.) with rotifers (10 ml⁻¹) offered for 10 days (MALR), green water conditions for 3 days followed by clear water in combination with rotifers (10 ml⁻¹) for 7 days (AL3R), and *Artemia* nauplii (4 ml⁻¹) offered for 10 days (Art). After 10 days of feeding with rotifers, all groups were given solely *Artemia* nauplii up to 35 days post-hatching (Table 1). There were three replicates per group. The number of fish was obtained by direct counting. Growth parameters (length and wet weight) were measured on days 0, 7, 10, 21 and 35 post-hatching. Fish length was measured to the nearest 0.1 mm with a binocular equipped with an ocular micrometer. For length measurement, 20 larvae were collected randomly from each replicate. Survival of the larvae was recorded by counting the fish in the tank on day 10 and at the end of the experiment.

Data were analysed with a computerized statistical program (S-Plus, 2000). Survival data were arcsine-transformed before analysis. Only data collected on day 10 (switching of food

items) and day 35 (the end of experiment) were subjected to statistical analysis. The analysis of variance (ANOVA) was performed to determine any significant difference among treatments. Significant differences between treatments were determined by Tukey's multiple range test (P < 0.05).

Results

Larval survival was counted at day 10 and at the end of the 35-day rearing experiment. No significant difference (P > 0.05) was observed in larval survival at day 10 among the different groups (Table 2). However, a significant difference (P < 0.05) in survival rate was noticed among the groups at the end of the experiment. The survival rate in group Art (clear water + *Artemia*) was significantly lower than ALR (green water + rotifer) and AL3R (green water for 3 days) after 35 days. Other groups were not significantly different from each other. At the end of the experiment, the highest survival rate (69.20%) was obtained with the larvae receiving only algae in the first 3 days of feeding. Average survival rate of the larvae cultured in green water condition for 10 days was 61.22%. The survival rate of the larvae receiving rotifers in clear water condition was lower (39.20%) compared with the other two groups receiving rotifers. Lowest survival rate (24.90%) was obtained with the larvae receiving only *Artemia* during 35 days.

On day 10, mean size of the larvae receiving rotifers in green water condition (treatment ALR) was significantly (P < 0.05) higher than in the *Artemia*-fed fish group. A similar observation was detected on day 35 (P < 0.05). The larvae cultured in green water conditions for 10 days had the largest size (9.45 mm total length) after 35 days of culture (Table 3).

On day 10, the mean weight of the rotifer-fed larvae for 10 days (R and ALR) was not significantly different (P > 0.05). On day 10, average wet weight of larvae receiving rotifers supplemented with algae was significantly (P < 0.05) higher than in the group fed on *Artemia* (Table 4). However, final wet weight of the larvae did not vary significantly among the groups at the end of the experiment (P > 0.05).

Discussion

Although all larvae in different groups were fed *Artemia* after 10 days, survival of the larvae receiving rotifers in green water condition was significantly better than the group fed solely on *Artemia* during the experiment. A low survival of burbot larvae fed on *Artemia* was also shown by Kujawa et al. (1999). The high survival rate achieved in the groups of larvae fed on rotifers could be influenced by the quality of the starter food. These findings may suggest that quality of the starter food is crucial to the later developmental stages of burbot larvae. According to Awar'ss (1991), *B. calyciflorus* fed

Table 1
Feeding regimes of burbot larvae raised in different experimental groups

| Treatment group | Feeding regimes (days) | | |
|-------------------------------------|--|------------------------------------|---|
| | Algae (10 ⁵ cells ml ⁻¹) | Rotifers (10 ml ⁻¹) | <i>Artemia</i> (4 ml ⁻¹) |
| R (clear water + rotifer) | | D1–D10 | D11–D35 |
| ALR (green water + rotifer) | D1–D10 | D1–D10 | D11–D35 |
| AL3R (3 days green water + rotifer) | D1–D3 | D4–D10 | D11–D35 |
| Art (clear water + <i>Artemia</i>) | | | D1–D35 |

Table 2
Survival rate of larvae counted on day 10 and at the end of the experiment (Mean ± SD). Different superscript letters within a column indicate significant difference (p < 0.05)

| Treatment group | Day 10 | Day 35 |
|-------------------------------------|---------------------------|----------------------------|
| R (clear water + rotifer) | 98.40 ^a ± 0.63 | 39.20 ^b ± 6.60 |
| ALR (green water + rotifer) | 97.93 ^a ± 0.60 | 61.22 ^a ± 13.23 |
| AL3R (3 days green water + rotifer) | 98.86 ^a ± 0.3 | 69.20 ^a ± 8.25 |
| Art (clear water + <i>Artemia</i>) | 98.53 ^a ± 0.55 | 24.90 ^b ± 10.32 |

| Treatment group | Day 7 | Day 10 | Day 21 | Day 35 |
|-------------------------------------|-------------|--------------------------|-------------|--------------------------|
| R (clear water + rotifer) | 5.02 ± 0.22 | 5.13 ^b ± 0.54 | 7.03 ± 0.9 | 8.33 ^b ± 0.49 |
| ALR (green water + rotifer) | 4.98 ± 0.26 | 5.55 ^a ± 0.46 | 7.51 ± 0.70 | 9.45 ^a ± 0.50 |
| AL3R (3 days green water + rotifer) | 4.70 ± 0.20 | 4.84 ^b ± 0.46 | 6.74 ± 0.59 | 8.49 ^b ± 0.19 |
| Art (clear water + <i>Artemia</i>) | 4.45 ± 0.24 | 4.96 ^b ± 0.44 | 7.05 ± 0.84 | 8.87 ^b ± 0.09 |

Table 3
Length (mm) of burbot larvae measured on days 7, 10, 21 and 35 of the experimental course (mean ± SD). Different superscript letters within a column indicate significant difference ($p < 0.05$)

| Treatment group | Day 7 | Day 10 | Day 21 | Day 35 |
|-------------------------------------|-------------|--------------------------|-------------|--------------------------|
| R (clear water + rotifer) | 0.53 ± 0.04 | 0.74 ^a ± 0.04 | 1.86 ± 0.40 | 3.86 ^a ± 0.22 |
| ALR (green water + rotifer) | 0.52 ± 0.03 | 0.77 ^a ± 0.10 | 2.20 ± 0.30 | 4.67 ^a ± 0.63 |
| AL3R (3 days green water + rotifer) | 0.45 ± 0.01 | 0.58 ^b ± 0.05 | 1.62 ± 0.16 | 4.08 ^a ± 0.12 |
| Art (clear water + <i>Artemia</i>) | 0.42 ± 0.02 | 0.56 ^b ± 0.06 | 1.87 ± 0.20 | 4.16 ^a ± 0.14 |

Table 4
Wet weight (mg) of burbot larvae measured on days 7, 10, 21 and 35 of the experimental course (mean ± SD). Different superscript letters within a column indicate significant difference ($p < 0.05$)

Dictyosphaerium chlorelloides algae was abundant with highly unsaturated (n-6) fatty acids (mainly 18:2n-6). Awar^{ss} (1991) found a high survival of gudgeon (*G. gobio*) larvae that received *B. calyciflorus* rotifer cultured on algae. In this respect, Watanabe et al. (1983) and Dhert et al. (2001) described the importance of the transfer of fatty acids and other nutrients through the algae-rotifers-larvae food chain.

A significantly higher mean length and wet weight of the larvae cultured in green water condition in comparison with larvae fed on *Artemia* was detected on day 10, indicating that feeding rotifers along with algae accelerated fish larvae growth.

In the present study, a green mushy mass was observed in the gut of burbot larvae offered *Chlorella* sp. in the first 3 days. Vachta (1990) observed that the burbot larvae in the first days of feeding preferred only phytoplankton. Filter feeding on algae by drinking activity and using the visceral arches as a trap was also reported for cod larvae (Mangor-Jensen and Adoff, 1987; van den Meerem, 1991). Positive effects of adding microalgae to the larval tanks are well documented (Naas et al., 1992; Reitan et al., 1993, 1994). Feeding on algae during early developmental stages may provide the larvae with essential nutrients, may act as an initiator for the digestive system, or may have an effect on the microflora of the larvae (Howell et al., 1998). This requires an increased knowledge about mechanisms of algal-larval interaction at the first feeding stages.

The present results clearly demonstrate the potential use of *B. calyciflorus* as a food for growing burbot larvae. *B. calyciflorus* rotifers can be cultured in freshwater with many of the techniques developed for the brackishwater *B. plicatilis* (Park et al., 2001). As the mass production of unicellular algae is labour-intensive and expensive, optimum methods must be developed for the mass culture of *B. calyciflorus* rotifer when using an artificial diet.

In conclusion, the results indicate that burbot larvae in the first feeding period need live food of small size and that algae may play an important role during this period. However, even if rotifers are an adequate starter diet for the first larval stage of the burbot, they must be replaced after 7–8 days by larger animals such as *Artemia*.

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