

EARLY FEEDING OF NEW ARTIFICIALLY PROPAGATED FISH: WEATHER LOACH, *Misgurnus fossilis*, LARVAE

HRANIDBA LIČINAKA UMJETNO RAZMNOŽAVANIH ČIKOVA (*Misgurnus fossilis*)

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ABSTRACT

For the advantage of new rearing technologies, the influence of dry artificial diet (Artificial Plankton) and natural diet (fresh hatched artemia nauplii) on growth and survival of weather loach (*Misgurnus fossilis*) larvae was studied from the first feeding onwards. Altogether 250 weather loach larvae of individual weight of 2.5 ± 0.3 mg and total length of 5.4 ± 0.6 mm were grown in each aquarium at a water temperature of 17.7 ± 2.3 °C over a period of 28 days. Larvae were fed ad libitum live artemia nauplii (Artemia salina PREMIUM, Sanders, USA) and artificial starter microdiet with natural profile similar to zooplankton (Artificial Plankton 000, Alma, Germany). All treatments were run in triplicate. In weather loach larvae fed the dry diet, high mortality (97%) occurred during the second week of feeding, and the experimental feeding this diet was stopped consequently, while feeding of artemia nauplii continued successfully until the day 28. Growth performance of artemia fed larvae was characterised by 8.4 mm and 15.8 mg, 12.4 mm and 24.8 mg, 18.7 mm and 81.7 mg and 23.4 mm and 140.7 mg on the days 7, 14, 21 and 28, respectively. Both length and weight growth rates of dry diet fed larvae were significantly lower than of those fed natural diet and reached 7.3 mm ($P < 0.001$) and 12.5 mg ($P < 0.05$) and 8.8 mm ($P < 0.001$) and 11.3 mg ($P < 0.001$) on average on the days 7 and 14, respectively. Based on the results achieved with dry diet application, the early stages of weather loach appeared to utilize only endogenous reserves without ability to digest the dry artificial diet. This phenomenon has already been described in other stomachless fish species such a carp. More research focused on the activities of digestive enzymes in weather loach larvae is required to assess this presumption.

Key words: fish larvae, *Misgurnus fossilis*, feed, artemia, dry diet, artificial diet, growth, survival

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INTRODUCTION

Weather loach (*M. fossilis* L.) is classified among endangered species according to the Czech Nature Protection Act No. 114/1992. This fish species has no commercial value, nevertheless its endangered status induced pressure on its rearing under controlled conditions for restocking and fish biodiversity conservation purposes. The first step for the intensive rearing of weather loach under commercial conditions was already done, as the possibility of controlled reproduction of weather loach by means of carp pituitary treatment was already proved (Geldhauser, 1992, Kouřil et al., 1996, Adámková-Stibranyiová et al., 1999). The second prerequisite for industrial scale rearing consists in the feeding of early stages as the releasing of free swimming embryos is connected with the high losses in a natural environment. The early feeding of weather loach larvae with size graded live natural zooplankton in aquarium culture was successfully done and the cladoceran *Chydorus sphaericum* was a highly preferred food item of the weather loach larvae (Kouřil et al., 1996). Using the natural zooplankton in larval rearing is often connected with a risk of diseases and low availability for farmers. In order to overcome these inconveniences, the present study was focused on the use of *Artemia* nauplii for early feeding of weather loach larvae. Freshly hatched artemia nauplii are generally used as a nutritionally adequate and much more convenient alternative to natural zooplankton in intensive fish culture. The replacement of live food source in weather loach larvae feeding by more practical dry microdiet was also studied.

MATERIAL AND METHODS

Fish rearing

Weather loach larvae of individual weight 2.5 ± 0.3 mg and total length 5.4 ± 0.6 mm were placed after initiation the exogenous nutrition (resorption the yolk sac) in amount of 250 individuals into 6 static 15 L aquaria. The aquaria were supplied by riverine surface water, which was aerated and replaced partly every day. Dissolved oxygen concentration amounted to 9.5 ± 0.2 mg.l⁻¹ and

water temperature 17.7 ± 2.3 °C during the experimental feeding. The larvae were fed two diets for 28 days. Each treatment was tested in triplicate and the representative samples of 10 to 30 individuals (according to age) from each aquarium were taken for the total length and weight assessments every 7 days. All fish in aquarium were counted every 7 days and the production parameters (growth rate, survival rate, weight gain and specific growth rate) were calculated.

Diet

Weather loach larvae were fed in two treatments, live artemia nauplii (*Artemia salina* PREMIUM, Sanders, USA) and dry synthetic larval/starter microdiet with a natural profile similar to zooplankton (Artificial plankton 000, Alma, Germany; protein 50%, lipids 10 %, particle size 0.050 - 0.125 mm). Larvae were fed *ad libitum*, three and two times during the first (1 to 14 days) and second (14 to 28 days) period of feeding, respectively.

Data processing

Specific growth rate (SGR) was calculated according to formula $SGR = 100 \times (\ln \text{ final mean individual weight} - \ln \text{ initial mean individual weight}) / \text{days of feeding} (\% \cdot \text{day}^{-1})$.

The primary data were subjected to t-test for significant differences at the $P < 0.001$ and $P < 0.05$ level.

RESULTS

Survival rates

As evident from Table 1, the survival rate of weather loach larvae after the first seven days of feeding ranged from 85.9 % in the dry diet treatment to 96.3 % in case of artemia fed larvae. The critical period for survival of larvae came in period between 7th and 14th day of exogenous feeding, when the survival rates in fish groups fed artemia was found to be 30.9 % on average, while

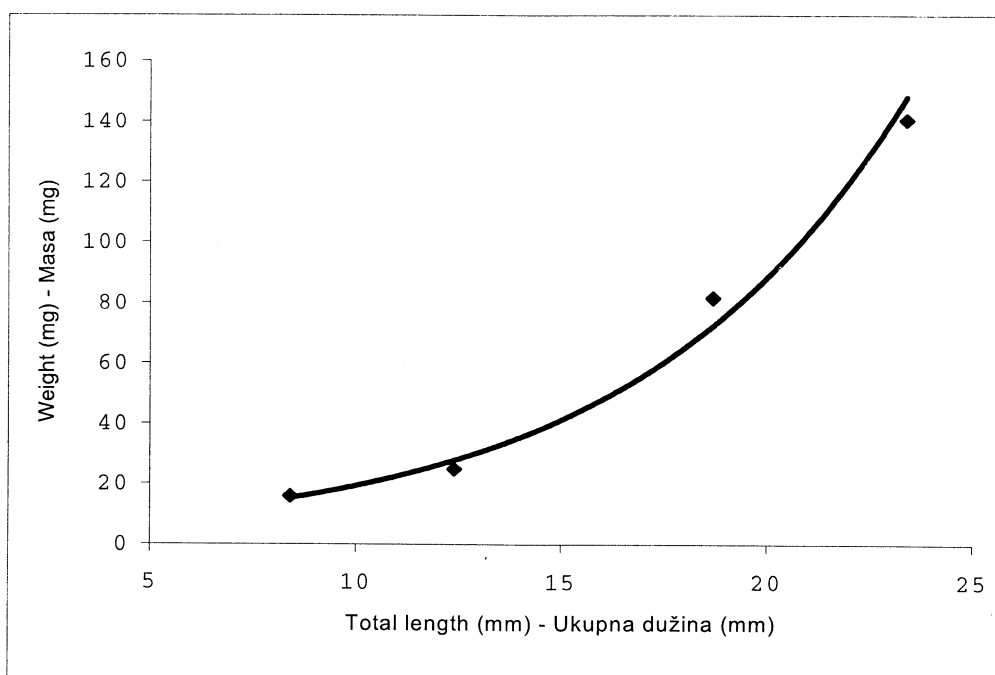
in fish groups on dry diet only 3.1 %. Due to the high mortality, the further using dry diet was stopped and only feeding artemia nauplii continued. Over the third week of feeding, an equal larval survival rates of 40 % in all replicates were registered. During the last week (day 21 to 28), survival rate amounted to 95.8 % on average.

Growth

The results of length and weight growth rates of weather loach larvae from the first feeding onwards are summarized in Table 2 and the relation between length and weight growth of larvae is shown in Graph 1.

Graph 1. Length-weight relationship of loach larvae from upset of exogenous feeding to 28 days

Grafikon. 1. Odnos dužine i mase ličinki čikova od prelaska na vanjsku hranidbu do 28 dana



The growth of larvae fed artemia diet during the first 7-day period was 8.4 mm on average in total length and 15.8 mg in weight. Significantly lower growth performance was found with the dry diet and amounted to 7.3 mm in total length ($P < 0.001$) and 12.5 mg in individual weight ($P < 0.05$). The critical period between 7th and 14th day of feeding led to highly significant differentiation in both dietary treatments applied. Larvae fed artemia diet continued in increasing tendency in growth parameters (12.4 mm, 24.8 mg), while in dry diet fed larvae, the negligible length growth (8.8 mm) was registered but weight growth appeared to be loss-making (11.3 mg). At the end of the 28-day trial, the total length of 23.4 mm and mean

individual weight of 140.7 mg was achieved by weather loach larvae fed artemia.

The maximum weight gain was observed at the initial period of feeding of early larvae with artemia diet when amounted 71.3 %. day^{-1} (Table 3). Over the following period (7th - 14th day of feeding), the weight gain was negative in all aquaria due to the increased fish mortality. The highest specific growth rate (26.1 %. day^{-1}) was registered with artemia treatment at the beginning of feeding. With increasing age of fish larvae, the SGR coefficient in artemia fed larvae declined on 7.8 %. day^{-1} at the end, whereas in case of dry diet fed larvae, the decline from 22.7 %. day^{-1} during the first week of culture to -1.6 %. day^{-1} at the second week was registered.

Table 1. Survival rate (mean \pm SD, %) of weather loach (*Misgurnus fossilis*) larvae when fed by artemia nauplii and artificial dry starter microdiet**Tablica 1. Preživljavanje ($\bar{x} \pm$ SD, %) ličinki čikova (*Misgurnus fossilis*) hranjenih s artemia nauplii i suhim mikrostarterom**

Day of feeding Dan hranidbe	Diet - Hrana	
	Artemia - Artemia nauplii	Artificial plankton - Umjetni plankton
7	96.3 \pm 1.6	85.9 \pm 4.2
14	30.9 \pm 19.6	3.1 \pm 4.5
21	40.0 \pm 0.0	-
28	95.8 \pm 7.2	-

Table 2. Length (mm) and weight (mg.ind⁻¹) growth of weather loach (*Misgurnus fossilis*) larvae when fed by artemia nauplii and artificial dry starter microdiet**Tablica 2. Dužina (mm), masa (mg .ind⁻¹) i rast ličinaka čikova (*Misgurnus fossilis*) hranjenih artemiom nauplii i umjetnim suhim mikrostarterom**

Day of feeding Dan hranidbe	Diet - Hrana			
	Total length - Ukupna dužina		Weight - Masa	
	Artemia Artemia nauplii	Artificial plankton Umjetni plankton	Artemia Artemia nauplii	Artificial plankton Umjetni plankton
0	5.4 \pm 0.6		2.5 \pm 0.3	
7	8.4 \pm 0.8	7.3 ^{**} \pm 0.4	15.8 \pm 1.2	12.5 [*] \pm 0.7
14	12.4 \pm 1.2	8.8 ^{**} \pm 1.2	24.8 \pm 3.3	11.3 ^{**} \pm 4.0
21	18.7 \pm 1.9	-	81.7 \pm 1.5	-
28	23.4 \pm 3.9	-	140.7 \pm 6.1	-

* $P < 0.05$, ** $P < 0.001$ **Table 3. Weight gain (%.day⁻¹) and SGR (%.day⁻¹) of weather loach (*Misgurnus fossilis*) larvae when fed by artemia nauplii and artificial dry starter microdiet****Tablica 3. Prirast (%.dan⁻¹) i SGR (%.dan⁻¹) ličinaka čikova (*Misgurnus fossilis*) hranjenih artemiom nauplii i umjetnim suhim mikrostarterom**

Day of feeding Dan hranidbe	Diet - Hrana			
	Weight gain - Prirast		SGR - SGR	
	Artemia Artemia nauplii	Artificial plankton Umjetni plankton	Artemia Artemia nauplii	Artificial plankton Umjetni plankton
7	71.3 \pm 7.8	46.0 \pm 6.4	26.1 \pm 1.1	22.7 \pm 0.9
14	-7.2 \pm 4.7	-13.8 \pm 0.5	6.4 \pm 1.9	-1.6 \pm 4.1
21	4.8 \pm 2.9	-	17.1 \pm 2.2	-
28	9.3 \pm 1.5	-	7.8 \pm 0.4	-

DISCUSSION

The use of freshly hatched artemia ensures good survival of weather loach larvae during the first weeks of culture. The mean weight growth rate achieved after 28 days of their initial feeding was double to triple to that obtained with feeding live zooplankton (55.4 mg), as reported by Kouřil et al. (1996), whereas the difference in length growth rate of larvae was lower (23.4 mm versus 19.5 mm). The high survival rate (95.8 %) achieved at the final week (day 21 to 28) of experiment could be influenced by equipment of static aquaria by further effective water filtration, including the higher amount of supplemental fresh water.

Very low survival rates (3.1%) including zero in one replicate after two weeks of feeding the artificial plankton demonstrated, that the dry diet is not acceptable for feeding of early stages of weather loach under controlled conditions. It seems that the submitted dry feed was not digested by early stages of weather loach. It led to the decline of individual weight (contrary to artemia fed larvae) accompanied by high mortality (97 %) after consumption of endogenous reserves just between 7th and 14th day of feeding. This problem could be explained in the context with the knowledge about the nutrition of earliest stages in carp development (Kaushik, 1986, Szlaminska, 1982). Both weather loach and carp are fish without stomach.

Stomachless fish species such a carp do not secrete either hydrochloric acid or pepsinogen for protein digestion but proteolytic enzyme can be detected in small quantities in the intestine. The activity of alkaline proteases in the digestive tract of carp larvae is very low during the first few days following resorption of the yolk sac as the pancreatic tissue is not fully developed. About 14 days after start of swimming and feeding activity (at water temperatures of 20-23°C), a noticeable secretion occurs, accompanied by a rise in the proteolytic activity in the intestine to about five times higher than at the start (Ostroumova and Dementeva, 1981). In common carp larvae fed

exclusively dry diets, high mortality occurred in most cases (Dabrowski and Poczyczynski, 1988). Dabrowski (1984) found, that the transfer of four cyprinids larvae from the zooplankton diet directly to the dry diet is possible at the individual wet weight of 5-6 mg. This could explain the problems associated with the utilization of dry feed for early feeding of weather loach larvae when compared with the natural food. The support of this presumption will require further study with focus on the activities of digestive enzymes in weather loach larvae intestine.

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SAŽETAK

U cilju unapređenja nove tehnologije uzgoja ličinaka i mladunaca čikova (*Misgurnus fossilis*) korištene su dvije različite hrane. U prvom tretmanu korištena je suha umjetna hrana (Artificial Plankton), a u drugom prirodna hrana (*Artemia nauplii*). Svaki od dva tretmana ponavljan je tri puta. Kao kriterij kakvoće hrane praćen je rast i preživljavanje ličinaka i mladunaca od početka hranidbe do 28 dana starosti. U svaki akvarij nasadeno je po 250 ličinaka čikova prosječne individualne mase 2,5 mg +/- 0,3 mg i ukupne dužine 5,4 +/- 0,6 mm. Temperatura vode u akvarijima tijekom cijelog istraživanog razdoblja koje je trajalo 28 dana iznosila je 17;7 +/- 2,3° C. U prvom tretmanu ličinke su hranjene po volji s artemijom nauplii (Artemija salina Premium, Sanders, USA), a u drugom tretmanu također po volji umjetnom mikrohranom sličnom zooplanktonu (Artificial Plankton 000, Alma, Njemačka). Ličinke hranjene suhom umjetnom hranom tijekom drugog tjedna uzgoja imale su visok mortalitet, a iznosio je 97%. Zbog toga je pokus u kome je korištena suha umjetna mikrohrana prekinut. U drugom tretmanu gdje su ličinke hranjene artemiom nauplii pokus je uspješno nastavljen do 28. dana. Rast i masa ličinaka koje su hranjene s artemiom 7., 14., 21. i 28 dana iznosile su: 8,4 mm i 15,5 mg; 12,4 mm i 24,8 mg; 18,7 mm i 81,7 mg; 23,4 mm i 140,7 mg. Tempo rasta ličinka hranjenih suhom umjetnom hranom iznosio je 7.3 mm (P<0,001) i 12,5 mg (P<0,05); 8,8 mm (P<0.001) i 11,3 mg (P<0,01) 7. i 14. dana uzgoja. Ličinke čikova hranjene suhom industrijski pripremljenom hranom koristile su samo endogene rezerve bez mogućnosti probave dodatne hrane. Ovaj fenomen je već opisan kod nekih ribljih vrsta (šaran). Da bi se ova pretpostavka potvrdila i kod ličinaka čikova potrebna su istraživanja njihovih probavnih enzima.