

REARING CARP LARVAE (*Cyprinus carpio*) IN CLOSED RECIRCULATORY SYSTEM (RAS)

D. Jelkić¹, A. Opačak¹, I. Stević¹, S. Ozimec¹, J. Jug-Dujaković²,
R. Safner³

Summary

Postembryonic rearing of carp larvae in closed recirculatory system was conducted in 2009 at the fish farm Ribnjak LLC, Donji Miholjac, Croatia. The research was conducted in two test groups (A and B with three iterations in each) with a control group (C). Test group A (3 tanks x 250 l) consisted of 150 000 larvae (density of 200 larvae·l⁻¹), test group B (3 tanks x 500 l) consisted of 600 000 larvae (density of 400 larvae·l⁻¹), and the control group (C) was a mud fish pond T-6 which was stocked by 800 000 larvae·ha⁻¹ under standard production conditions. In this research, basic physical and chemical water parameters were controlled (temperature, oxygen, pH, total ammonia and nitrites). Initial measuring of carp larvae total length (TL) was conducted prior to their placement into tanks (N=120). On the fourth, sixth, eighth and tenth day of research 20 larvae (N=140) were taken out of every tank as well as out of control group and measured. Feeding with live feed began on the third day after hatching (larval TL 6.00±0.36 mm). Ten minutes after feeding live feed to larvae for the first time, 20 larvae (N=120) were taken out of every tank and a high portion of larvae that accepted live feed (89.17±3.76%) was determined by a magnifying glass. Feeding artificial feed began on the seventh day after the hatching. After ten minutes, a high portion of larvae who accepted artificial feed (96.67±2.58%) was determined. Since the end of the research, the determined length increment (ITL) per day was 0.41±0.04 mm, a very high survival rate was established (group A: 96%, group B: 93%). Feeding frequency was four times a day in five-hour intervals (at 06:00, 11:00, 16:00 and 21:00 hours). The research was terminated after ten feeding days due to deteriorating condition of zoohygienic filter. The total of 3807 g of live feed and 1080 g of artificial feed was used.

Key words: carp larvae, live and artificial feed, recirculatory system

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- 1 Dinko Jelkić, dipl.ing., assistant, e-mail: djelkic@pfos.hr (corresponding author); dr.sc. Anđelko Opačak, full professor, e-mail: andelko@pfos.hr; dr.sc. Ivan Stević, full professor (retired); dr.sc. Siniša Ozimec, assistant professor, e-mail: sozimec@pfos.hr, Faculty of Agriculture in Osijek, Department of game, fishery and beekeeping;
 - 2 Dr.sc. Jurica Jug Dujaković, full profesor, University of Dubrovnik;
 - 3 Dr.sc. Roman Safner, full professor, e-mail: rsafner@agr.hr; Faculty of Agriculture, University of Zagreb, Department of Fisheries, Beekeeping and Special Zoology

INTRODUCTION

Carp rearing has become more complex and precarious due to various ecological changes, climate changes being the most prominent one. The main obstacle to higher production of commercial fish in a three-year cycle on carp fish farms are large losses of fish per unit. The highest losses, which could go from 50 - 90% in mud pond production conditions, occur in the larvae to fry growth period, i.e. in a month old fry. This fact can later cause a significant lack of carp fingerlings on the market. High losses per unit of fish in the growth period in which carp fry has not yet reached individual mass of $1\text{ g}\cdot\text{pc}^{-1}$ are a consequence of inadequate ecological (water temperature fluctuations, lack of oxygen, high level of organic pollution, large zooplanktons, natural predators e.g. birds, frogs, snakes etc.) or feeding conditions (Feldlitz and Milstein, 1999, Jirásek and Mareš, 2001). Practice has indicated that the changes in larval rearing technology, applying controlled production and feeding plankton to the larvae increased carp larval survival rate from 30% to 80-90% during 12 days of experiment (Barr et al., 2007). As the growth and development of hatched larvae in endogenous feeding phase are dependent on the yolk-sack (Heming and Buddington, 1988), the transition to the exogenous phase is a critical stage in larval ontogenesis (Shimma et al., 1977). Up until the beginning of the 80s, it was generally thought that carp larvae cannot be fed artificial by starter-feed if live zooplankton was not fed them first (Bryant and Matty, 1981). Recent research conducted by complete replacement of live with artificial food (Charlon and Bergot, 1984; Escaffre et al., 1997; Carvalho et al., 1997; Cahu et al., 1998, Yúfera et al., 1999), provide a good starting point for further development of first feeds for carp larvae and support the views of Appelbaum and Dor (1978) that carp larvae can in fact be reared exclusively on artificial feed from the beginning of the exogenous feeding phase. The necessary control in the early rearing stages based on artificial food is considered to be the key problem and a drawback to carp aquaculture. The technology of rearing larvae in controlled conditions lacks a satisfying starter-feed that would replace live zooplankton (Jirásek and Mareš, 2001). The process of preparing live feed (decapsulation and incubation of artemia), is time-consuming and requires a high level of expertise and organization. Person-Le Ruyet et al., (1993) estimate that the expenses of feeding live feed amounts to 79% of total fry production cost. Since the beginning of the 90s, significant attempts have been made to solve the problem of larval starter feeds, primarily in marine production. Never the less, commercial starter feed for carp larvae has not yet available on the market. Thus, the dependence of small larvae, such as carp larvae, on live feed is trying to be reduced in practice by switching to artificial feed at various times. The purpose of this research was to determine the survival rate and the length increments of carp larvae in the early acceptance (7. day post hatch) of artificial feed while decreasing the amount of live zooplankton (*Artemia nauplii*).

MATERIALS AND METHODS

2.1. Experimental design

Carp spawning, egg incubation and postembryonic rearing of carp larvae were conducted in 2009 in closed recirculatory system at the fish farm Ribnjak LLC, Donji Miholjac, Croatia. Carp larvae were obtained by controlled spawning of selected Našice carp female fish. The number of larvae in the research was determined by using a volumetric method with $\pm 5\%$ precision. The research was conducted in two test groups (A and B with three iterations in each) with a control group (C). Test group A (3 tanks x 250 l) consisted of 150 000 larvae (density of 200 larvae·l⁻¹), test group B (3 tanks x 500 l) consisted of 600 000 larvae (density of 400 larvae·l⁻¹), and the control group (C) was a mud fish pond T-6 which was stocked by 800 000 larvae·ha⁻¹ under standard production conditions.

2.2. Recirculating system and water quality parameters

Feeding treatment lasted 10 days in an indoor recirculating system equipped with biofilter without solids removal mechanism, UV irradiation, oxygen injection and degassing chamber. During research, the water flow was maintained at 4 and 8 l·min⁻¹, depending on the tank volume. Basic physical and chemical water parameters were controlled during research (temperature, oxygen, pH, total ammonia and nitrites) (Table 1). In the ponds and tanks during larvae stocking was 19.8 °C. Water temperature in the tanks was gradually increased and maintained to 24 °C in the course of three days. The indoor larvae were exposed to the light during the entire research for 15 hours by combining natural and artificial light.

2.3. Larval management and feeding

Feeding larvae with live food, *Artemia nauplii* (*Artemia salina*, INVE, AF kind), began three days after hatching. The larvae were fed exclusively with *Artemia nauplii* for four days. The feeding was conducted manually, four times a day (at 6 and 11 a.m. and 16 and 21 p.m. hour). Feeding with artificial feed (BioMar Larviva Start 300) began seven days after hatching. The transition from live to artificial feed was done in two days (25% of artificial feed on the seventh and 50% on the eighth day after hatching). From the eighth day after hatching onwards carp larvae were fed only with artificial feed for the remaining meals. Intensity of feeding was adjusted to the larval density. During the research control group larvae (pond T-6) were fed only on available natural food.

2.4. Samples collection and data analysis

Initial measuring of carp larvae total length (TL) was conducted prior to their placement into tanks (N=120) with 0.1 mm precision. On the fourth, sixth, eighth and tenth day after hatching 20 larvae (N=140) were taken out of every tank and control group

and measured. Ten minutes after feeding live feed to larvae for the first time, 20 larvae (N=120) were taken out of every tank and a high portion of larvae that accepted live feed (89.17±3.76%) was determined by a magnifying glass. By the same method, the portion of larvae that accepted artificial feed on the seventh day after hatching was also determined. The number of surviving larvae at the end of the research was determined by means of volumetric method. For statistical data processing, the program SPSS, version 16.01. (SPSS Inc, Chicago), was used.

The daily total length increments (DITL), mm day⁻¹) was calculated as follows:

$$DI_{TL} = (TL_f - TL_i) t^{-1} \quad (1)$$

where TL_f and TL_i were final and initial larval total length (mm), and t was the rearing time (in days).

The size variation of larvae was defined by the coefficient of variation (CV, %) of total length and was calculated as:

$$CV_{TL} = 100 (SD TL^{-1}) \quad (2)$$

where SD is standard deviation and TL is the average larvae total length (mm).

Feed acceptance (FA, %) was calculated as:

$$FA = 100 (N_{FA} N_{Total}^{-1}) \quad (3)$$

where N_{FA} is number of fish which had food in the intestine and N_{Total} is number of fish in taken sample.

Larval survival (S, %) was calculated as:

$$S = 100 N_f N_i^{-1} \quad (4)$$

where N_i and N_f were the total number of fish (individuals) at the start and finish of rearing.

RESULTS AND DISCUSSION

As early as the first feeding of larvae with *Artemia* nauplii, it was noted that larvae instinctively catch moving food with great success (Table 2). Appelbaum (1976), states that in their first days, carp larvae mainly use their sense of sight. Therefore good lighting of the tanks is crucial, because only after the ontogenesis has finished larvae start using their mechanical and chemical receptors (Appelbaum and Riehl, 1997). During the feeding of carp larvae with artificial feed, no behavioral changes were noted. Larvae learned how to catch floating particles in water, thus the acceptance of artificial feed was at a very high level. Carp larvae were able to swallow artificial feed (Ø 300 µm), as Dabrowski and Bardega (1984) and also Hasan and Macintosh (1992) suggested.

Group A (0.45 ± 0.04 mm day⁻¹) had the largest DI_{TL} ; however, these results were below the expected 0.485 mm day⁻¹, which Escaffre et al., (1997) managed to obtain in 14 days by feeding the carp larvae with 85% mixture of beef liver and yeast. Cahu et al., (1998) had better results of growth length: 0.475 mm day⁻¹ during 20 days, by feeding carp larvae with hydroisolate of fish protein and yeast. Due to the combined feeding with

live zooplankton and commercial feed, a faster growth rate of those larvae compared to the larvae fed exclusively with prepared artificial feed, was expected.

Table 1. Physicochemical properties of water in recirculatory system during the experiment (mean \pm SD)

Tablica 1. Fizikalno-kemijska svojstva vode u recirkulacijskom sustavu tijekom pokusa ($\mu \pm$ SD)

Day of experiment Dan pokusa	Water temperature (°C) Temperatura vode (°C)	O ₂ mgL ⁻¹	pH	NH ₃ /NH ₄ ⁺ (mgL ⁻¹)	NO ₂ ⁻ (mgL ⁻¹)
1	20.50 \pm 0.42	14.38 \pm 1.23	8.18 \pm 0.01	0.4 \pm 0.14	0.0
2	22.18 \pm 0.54	14.68 \pm 2.34	8.18 \pm 0.01	0.3	0.0
3	24.30 \pm 0.92	11.73 \pm 0.62	8.19 \pm 0.01	0.3	0.0
4	24.76 \pm 0.47	10.86 \pm 1.29	8.17 \pm 0.03	0.3	0.0
5	24.63 \pm 0.84	10.43 \pm 1.97	8.18 \pm 0.01	0.8	0.5
6	24.60 \pm 0.38	10.00 \pm 1.45	8.13 \pm 0.04	0.8	0.3
7	24.16 \pm 0.68	8.79 \pm 0.72	8.14 \pm 0.08	0.8	0.4 \pm 0.14
8	24.86 \pm 0.39	9.07 \pm 0.63	8.11 \pm 0.04	0.7 \pm 0.17	0.4 \pm 0.3
9	25.43 \pm 0.13	9.21 \pm 0.25	8.01 \pm 0.06	0.8	0.87 \pm 0.12
10	25.65 \pm 0.65	9.46 \pm 1.58	7.99 \pm 0.03	0.8	0.95 \pm 0.07
10 days average Prosjek 10 dana	24.04 \pm 1.59	10.96 \pm 2.48	8.12 \pm 0.08	0.65 \pm 0.22	0.41 \pm 0.37

Table 2. The growth and survival rate (%) according to the test group (TL_{fi} - final and initial larval total length; DI_{TL} - daily total length increments; CV - coefficient of variation; FA - feed acceptance; S - larval survival)

Tablica 2. Rast i preživljenje (%) prema pokusnoj skupini (TL_{fi} - završna i početna totalna duljina ličinki; DI_{TL} - ukupni dnevni porast u duljinu; CV - koeficijent varijacije; FA - prihvaćanje krmiva; S - preživljenje ličinki)

Parameter Parametar	Group A Skupina A	Group B Skupina B	Group C Skupina C
TL _i (mm)	6.00 \pm 0.36	6.00 \pm 0.36	6.00 \pm 0.36
TL _f (mm)	10.52 \pm 0.41	9.68 \pm 0.10	10.18 \pm 1.00
DI _{TL} (mm day ⁻¹)	0.45 \pm 0.04	0.37 \pm 0.01	0.42 \pm 0.04
Initial CV (%)	6.00	6.00	6.00
Final CV (%)	8.88	2.71	9.52
FA (%) artemia	88.33 \pm 2.89	86.67 \pm 7.64	-
FA (%) artificial feed	93.33 \pm 2.89	95.00 \pm 5.00	-
S (%)	95.67 \pm 1.15	93.33 \pm 1.53	11.54 *

* survival determined on 36 DPH old larvae

One of the reasons for faster growth can be found in the larval density per tank. Escaffre et al., (1997) worked with the density of 85 larvae·l⁻¹ (510 larvae in 6 l tank), Cahu et al., (1998) a somewhat less: 77 larvae·l⁻¹ (2.700 larvae in 35 l tank), while in this research the density of 200 larvae·l⁻¹ and 400 larvae·l⁻¹ was used. Significant statistical difference in the daily total length increments of Group C and Group A (Figure 1) was not determined. Considering the available natural feed and water temperature variations in the pond, faster growth in controlled conditions was expected. All of the above indicates that, in this research, the necessary conditions in utilizing the genetic potential at larval stage were not ensured.

Although carp larvae accept inert feed rather well, the length increments do not indicate that it was a suitable replacement for zooplankton at such an early stage. Thus Albrecht et al., (1977) state that the best growth rate was obtained in the group that transferred to artificial food 21 days after hatching. Also, many authors state that even the best results achieved by combined feed are still below the results obtained by feeding zooplankton exclusively (Dabrowska et al., 1979, Kouřil and Hamačková, 1982). At the beginning of exogenous feeding, carp larvae have a short intestine (about 50% of body length) which has the same diameter in all parts (Stroband and Dabrowski, 1981; Groza, 1984). As the food goes through short intestine quickly, there is less time for food to be processed and nutrients to be absorbed. The observed successes of feeding carp larvae exclusively artificial inert food point to the low individual mass compared to the mass of larvae fed with zooplankton.

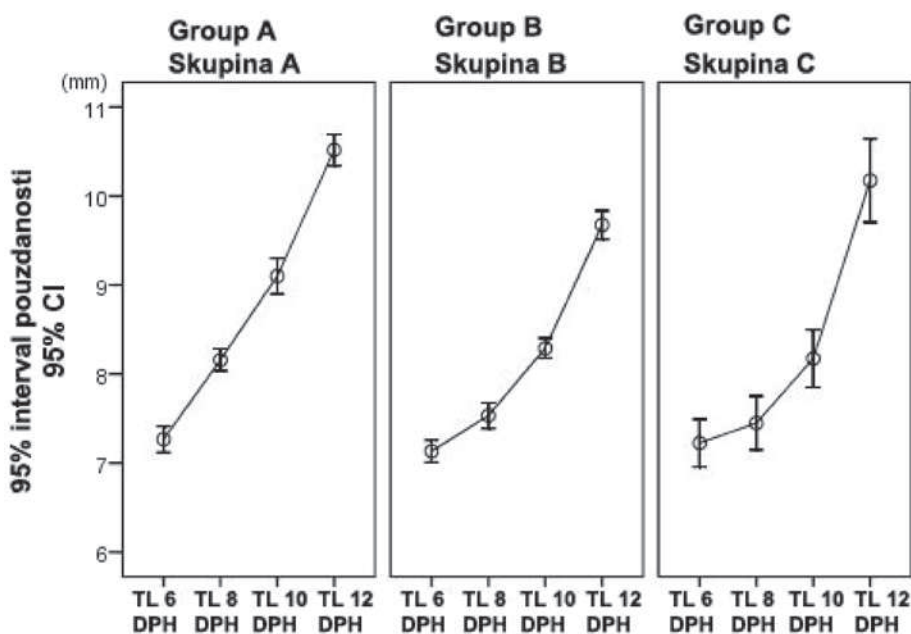


Figure 1. Dynamics of larval growth (total length) (DPH, days post hatch)

Slika 1. Dinamika prosječne totalne dužine ličinki šarana (DPH, dani nakon izvaljenja)

CONCLUSION

Reducing the amount of Artemia in favor of the artificial feed before the double loop formation in the indigestive tract has not proven justified. It does not mean that the larvae cannot be fed with artificial feed, but that their growth rate will not be the same as in natural conditions. For mass commercial production of larvae, where specific growth rate and larval mass are important, with the lack of commercial feed for young carp larvae, starter feeding with Artemia is mandatory.

The consequence of lesser growth rate achieved in this research was the combination of the following factors:

- Lower water temperature during research (24.04 ± 1.59 °C), which was below optimal temperature for carp larvae (26 - 28 °C).
- Deteriorating water conditions: reasons for potential slow growth could be found also in the increased ammonia and nitrites (0.65 ± 0.22 ; 0.41 ± 0.37). For carp, Koltai et al. (2002) determined that longer exposure to ammonia concentrations of 0.1 mgL^{-1} with the presence of high nitrites concentrations of 0.43 mgL^{-1} cause 100% mortality.
- Too early transition to artificial feed, while the indigestive tract of carp larvae is still unable to absorb the nutrients from the artificial feed.

Sažetak

UZGOJ LIČINKI ŠARANA (*Cyprinus carpio*) U RECIRKULACIJSKOM SUSTAVU (RAS)

D. Jelkić¹, A. Opačak¹, I. Stević¹, S. Ozimec¹, J. Jug-Dujaković²,
R. Safner³

Postembrionalni uzgoj šarnskih ličinki u zatvorenom recirkulacijskom sustavu proveden je 2009. godine na ribnjačarstvu Ribnjak d.o.o., Donji Miholjac. Istraživanja su provedena u dvije pokusne skupine (A i B s po tri ponavljanja) s kontrolnom skupinom (C). Pokusnu skupinu A (3 tanka x 250 l) činilo je ukupno 150.000 ličinki (gustoća $200 \text{ ličinki} \cdot \text{l}^{-1}$), pokusnu skupinu B (3 tanka x 500 l) činilo je ukupno 600.000 ličinki (gustoća $400 \text{ ličinki} \cdot \text{l}^{-1}$), a kao kontrola (C) služilo je ribnjak T-6 u standardnim proizvodnim uvjetima nasaden s $800.000 \text{ ličinki} \cdot \text{ha}^{-1}$. Tijekom istraživanja kontrolirani su osnovni fizikalno-kemijski parametri vode (temperatura, kisik, pH, ukupni amonijak i nitriti). Inicijalno

1 Dinko Jelkić, dipl.ing., asistent, e-mail: djelkic@pfos.hr (corresponding author); prof. dr.sc. Anđelko Opačak, e-mail: andelko@pfos.hr; prof. dr.sc. Ivan Stević (u mirov.); dr.sc. Siniša Ozimec, asistent, e-mail: sozimec@pfos.hr, Poljoprivredni fakultet u Osijeku;

2 Prof.dr.sc. Jurica Jug Dujaković, Sveučilište u Dubrovniku;

3 Prof.dr.sc. Roman Safner, e-mail: rsafner@agr.hr; Agronomski fakultet Sveučilišta u Zagrebu, Zavod za ribarstvo, pčelarstvo i spec. zoologiju

mjerenje totalne dužine (TL) šaranskih ličinki obavljeno je prije stavljanje u tankove (N=120). Na 4., 6., 8. i 10. dan istraživanja iz svakog tanka i kontrolne skupine izmjereno je po 20 ličinki (N=140). Hranjenje sa živom hranom započelo je treći dan nakon izvaljenja (TL ličinki $6,00 \pm 0,36$ mm). Deset minuta nakon prvog hranjenja živom hranom, iz svakog tanka uzeto je po 20 ličinki (N=120) i pod povećalom utvrđen visoki udio ličinki koje su prihvatile živu hranu ($89,17 \pm 3,76\%$). Hranjenje umjetnom hranom započelo je sedmi dan nakon izvaljenja. Nakon desetominutnog vremena utvrđen je visok udio ličinki koje su prihvatile umjetnu hranu ($96,67 \pm 2,58\%$). Utvrđeni dnevni dužinski rast na kraju istraživanja (ITL) iznosio je $0,41 \pm 0,04$ mm dnevno. Po završetku istraživanja procjenjen je vrlo visok stupanj preživljenja (skupina A: 96%, skupina B: 93%). Učestalost hranidbe bila je 4 puta dnevno u intervalima od 5 sati (u 6, 11, 16, 21 sat). Istraživanje je prekinuto nakon 10 dana hranidbe zbog pogoršanih zoohigijenskih uvjeta filtera. Ukupno je u istraživanju utrošeno 3.807 g žive hrane i 1.080 g umjetne hrane.

Ključne riječi: ličinke šarana, živa i umjetna hrana, recirkulacijski sustav

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