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Common carp fry survival during salinity stress test: effect of feeding regime - short communication

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ABSTRACT

Experiments were carried out on common carp (*Cyprinus carpio* L.) larvae in a recalculating aquatic system for a period of 21 days. Four feeding variants with different transition times from live (nauplii of *Artemia salina*) to artificial feed (Biomar larviva) were examined. The greatest average total length (25.72 ± 3.37 mm) and weight (213.81 ± 105.52 mg) was achieved by common carp larvae fed over the whole period with nauplii of the brine shrimp (group D). A significantly higher ($P<0.05$) final bodyweight was reported in group D in comparison with groups A and B. Survival in groups B, C and D was high (81.53, 84.14, 80.66% respectively) but not in group A (32.44 - 43.36%) with the earliest transition to artificial feed. A salinity stress test was carried out on the final day of rearing. Common carp fry survival in the salinity stress test was analyzed using the Kaplan - Meier test. High significant differences ($P<0.001$) have only been reported between feeding treatments at 14‰ salinity level.

Key words: fish larvae, rearing system, salinity stress test

Introduction

Larval fish culture is one of the riskiest phases of freshwater fish culture and the main obstacle to higher production of commercial fish in a three-year cycle on common carp fish farms in Croatia. In nature, survival and success of the larva is mainly dependent on avoidance of predators, or feeding conditions (FELDLITE and MILSTEIN, 1999; JIRÁSEK

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and MAREŠ, 2001a), with only a few larvae surviving through metamorphosis. The highest losses, which could be from 50 - 90% in mud pond production conditions, occur in the larvae to fry growth period, i.e. in a month old fry (MATIĆ and JURAKIĆ, 2006; KUMAR et al., 2012; JELKIĆ et al., 2012; GJURČEVIĆ et al., 2012). This fact can later cause a significant lack of common carp fingerlings on the market. Practice has indicated that changes in larval rearing technology, applying controlled production and feeding plankton to the larvae, have increased the common carp larval survival rate from 30% to 80-90% during short rearing (BARR et al., 2007). The rearing of most cyprinid larvae requires live food in sufficient quantities, and naulii of *Artemia salina* is commonly used as the source of live feed (SORGELOOS et al., 2001). Since the development of commercial fish culture, the demand for artemia cyst has gradually increased over the years (SORGELOOS et al., 2001) and it has been followed by an increase in price. The process of preparing live feed 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 costs in marine aquaculture.

The necessary control in the early rearing stages, based on artificial food, is considered to be one of the key problems for common carp aquaculture. Recent research conducted on common carp with the complete replacement of live with artificial feed (CHARLON and BERGOT, 1984; ESCAFFRE et al., 1997; CARVALHO et al., 1997; CAHU et al., 1998; REGENDA et al., 2003) provided a good starting point. The technology of rearing cyprinid larvae in controlled conditions lacks a satisfying starter-feed that would replace live zooplankton (JIRÁSEK and MAREŠ, 2001b).

Since the beginning of the 1990's, significant attempts have been made to solve the problem of larval starter feeds, primarily in marine production. Nevertheless, there is no commercial starter feed for carp larvae yet available on the market. Thus, attempts are being made to reduce the dependence of common carp larvae on live feed in practice by switching to artificial feed at various times. Under controlled culture conditions, the success rate for fish larvae is much higher due to the regulated food supply and absence of predators, but even under such conditions the mortality rate is high and can vary between batches. The persistent variations in the quality of the larvae and their unpredictable future have led to the development to different tests to assess the quality of the reared larvae or fry. One commonly applied test is the salinity stress test, which has been developed to detect subtle differences in the physiological condition of larval fish between treatment groups in nutritional studies, when no differences exist in survival and growth (DHERT et al., 1992; ASHRAF et al., 2010).

The first goal of the present study was to determine the effect of different transitions from live to artificial feed on a commercial (large) scale. The second goal was to determine differences in the resistance of such reared larvae using a simple salinity stress test.

Materials and methods

The rearing of common carp larvae (*Cyprinus carpio* L.) took place in a recirculation aquatic system on a fish farm in the town of Donji Miholjac (Croatia) from the beginning of the acceptance of exogenic feeding (third day post hatch) over the following 21 days. The rearing was carried out in 12 conical flow-through tanks. Each tank had a usable volume of 500 liters. The indoor recirculating system was equipped with a submerged upflow biofilter with a settling tank for solids removal, UV irradiation, oxygen injection and degassing chamber. During carp rearing, the water flow was maintained at 8 liters per minute. Common carp larvae were obtained from the artificial reproduction of the Nasice - Szarvas broodstock line in the same recirculation aquatic system. The starting mean individual weight of larvae was 2.36 ± 0.14 mg and 6.76 ± 0.03 mm total length (TL). The initial density of the stock was 5500 larvae per tank. As the diet we used nauplii of the brine shrimp (*Artemia salina*) nauplii (Koral, Russia, crude protein 60% and fat 24%) as live food for larvae and Biomar larviva Start 300 (BioMar, France, crude protein 72%, fat 8%) as artificial dry food. Artemia nauplii were hatched in a cone shaped hatcher under controlled conditions according to the manufacturer's instructions. Group D was fed over the entire period of rearing with brine shrimp nauplii; group A was started for 4 days with *A. salina* nauplii, followed by 6 day of co-feeding and then fed with Biomar larviva. Groups B and C were started for 8 and 12 days respectively with *A. salina*, followed by 6 days of co-feeding, and then fed with Biomar larviva (Table 1).

Table 1. Feeding treatment during common carp larvae rearing

Feeding treatment	Group A	Group B	Group C	Group D
Live feed	FD1 - FD4	FD1 - FD8	FD1 - FD12	FD1 - FD21
Co-feeding period	FD5 - FD10	FD9 - FD14	FD13 - FD18	
Artificial feed	FD11 - FD21	FD15- FD21	FD19 - FD21	

FD - feeding day

The individual group regimes were repeated 3 times. Larvae were fed live freshly-hatched artemia nauplii. Feeding was performed manually every 90 minutes from 7.00 to 22.00 hours. The tanks were lit daily for 16 hours. Larvae were fed with artemia according to the suggestions of BRYANT and MATTY (1980) and for dry feed, according to the suggestions of BRYANT and MATTY (1981). Survival was monitored every day by removing dead larvae from the tanks. The water temperature was established at 27 ˚C and was monitored continuously by an automatic heating system. Dissolved oxygen in the water and water pH were measured three times a day (WTW oxi 330i, WTW pH 330i). The content of ammonia, nitrites and nitrates was measured three times a day by a multiparameter photometer Hanna 83200. The average water temperature was 27.64 ± 1.74 °C, pH 8.42 ± 0.05, dissolved oxygen 6.95 ± 0.22 mg/L, total ammonia 0.08 ±

0.10 mg/L (TAN), nitrite 0.12 ± 0.06 mg/L (NO_2^-) and nitrate 22.02 ± 12.75 mg/L (NO_3^-) during the larval rearing period. On the last day of rearing a representative samples (n = 30 larvae) was taken from individual groups from each replication. These larvae were first euthanized with clove oil (*Eugenia caryophyllata*), then individually weighed and total length was measured.

For survival, body mass and length, the means and standard deviations were calculated within the feeding groups. The significance of differences among the feeding groups was tested using one-way ANOVA, followed by the Tukey's highly significant difference (HSD) multiple comparison procedure, to identify which treatments differed. Statistical analysis was done using SPSS 16. Survival percentages during rearing were normalized using arcsine transformation (SOKAL and ROHLF, 1995).

After the 21st feeding day carp fry were exposed to 8, 10, 12, 14 and 16‰ salinity. There were 10 fish per feeding treatment with three replicates (n = 30) exposed to each salinity level (n = 120). The survival was monitored during 2 h exposure (ASHRAF et al., 2010). Dead fry were removed and counted every 5 minutes. Survival curves for common carp fry in different levels of salinity stress were derived using the Kaplan - Meier test. Survival time was defined as follows: carp fry deaths were considered as events and survival time was the time to the event from the beginning of the salinity test stress at a given salinity level, measured in minutes. Survival between feeding treatments was analyzed using the Log Rank test.

Results

The greatest average total length (TL = 25.72 ± 3.37 mm) and weight (b.w. = 213.81 ± 105.52 mg) were reached by larvae fed over the whole period with brine shrimp (*A. salina*) nauplii (group D). The shortest average length (TL = 22.46 ± 2.63 mm) and minimum weight (b.w. = 163.36 ± 58.72 mg) were found in group B. The body weight and total length of larvae were not statistically different between groups A and B and between groups A and C. Also, there was no statistically significant difference in body weight between groups C and D (Table 2).

Table 2. Average body weight (b.w.), total length (TL) and survival (S) in common carp fry at the end of experiment (21 days from the start of exogenic feeding)

Feeding treatment	B.w. (mg)	TL (mm)	Survival (%)
Group A	167.62 ± 75.19^{ab}	23.41 ± 3.27^{ab}	38.14 ± 5.49
Group B	163.36 ± 58.72^{a}	22.46 ± 2.63^{a}	81.53 ± 6.63^{a}
Group C	198.79 ± 87.07^{bc}	24.23 ± 3.17^{b}	84.14 ± 7.72^{a}
Group D	213.81 ± 105.52^{c}	25.72 ± 3.37	80.66 ± 8.02^{a}

Data are expressed as mean ± SD. Different letters in column indicate difference between means ($P<0.05$)

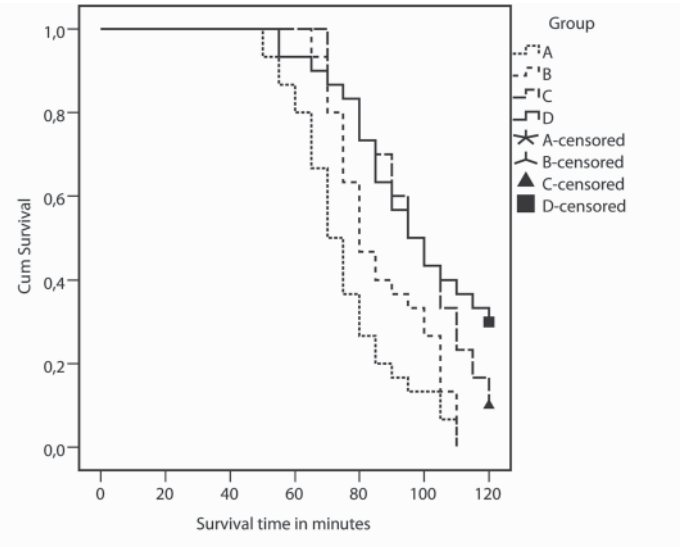

Fig. 1. Kaplan-Meier survival curve for common carp fry exposed to 14‰ salinity

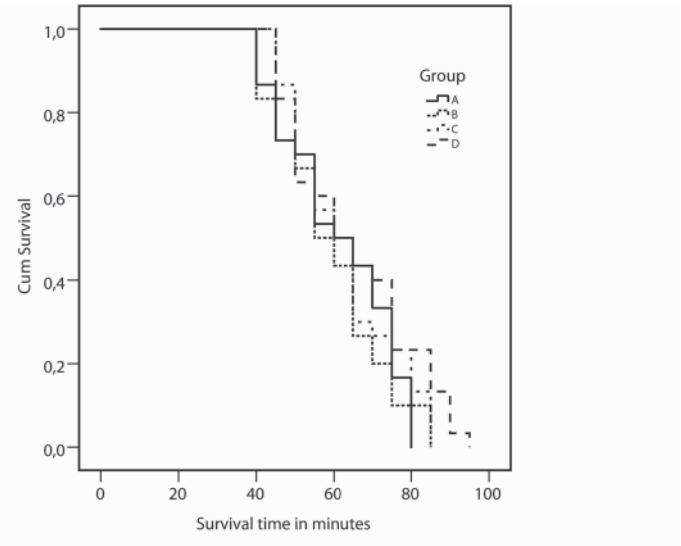

Fig. 2. Kaplan-Meier survival curve for common carp fry exposed to 16‰ salinity

Survival in almost all the experimental groups was high except in group A (32.44 - 43.36%) with the earliest transition to artificial feed. The only statistically significant difference in the percentage of survival was observed between group A and the other groups. The Log Rank test showed a high statistically significant difference ($P<0.001$) between feeding treatments at 14‰ salinity (Fig. 1). At lower salinity levels (8, 10, and 12‰) and 16‰ salinity (Fig. 2), there were no statistically significant differences between feeding treatments.

Discussion

Unlike predatory fish larvae, which require continuous feeding, for most cyprinid species, 13 - 15 h feeding a day is sufficient to obtain satisfactory growth rates of larvae (WOLNICKI et al., 2003). All four feeding treatments achieved a satisfactory body weight in just 21 days of rearing. Similar results, in final body weight of carp larvae, were achieved by ALAMI - DURANTE et al. (1991) and ESCAFFRE et al. (1997) only on own mixtures, while BIŁAS et al. (2012) in first 20 days of rearing achieved much higher (281 - 455 mg) body weights with different transitions from live to artificial feed. DABROWSKI (1984a) recommended a rearing period of 4 days with live feed of common carp before transition from live feed, and few researches showed that common carp larvae can be reared only on artificial feed (ALAMI-DURANTE et al., 1991; ESCAFFRE et al., 1997; CAHU et al., 1998). But, larvae reared sole on artificial feed achieved lower average body weight compared to larvae reared on live feed (DABROWSKI 1984a; SHARMA and CHAKRABARTI, 1999; REGENDA et al., 2003). This research has also demonstrated that larvae reared only on live feed achieved greatest body weight (Group D). Considering the limits of the presently available commercial dry feeds in rearing cyprinid larvae, DEMENY et al. (2012) stated that initial rearing on live feed and then a gradual transition to dry feed is still mandatory. This is linked to the alimentary canal that is not fully formed at this stage which relies on exogenous enzymes for digestion that are consumed along with the prey (DABROWSKI, 1984a; 1984b). Survival of carp larvae during 21 days of rearing was satisfactory (around 80%) except in group A, which had an average survival of 38%. A common carp larvae achieves good survival even when it's reared exclusively on artificial feed **(**SHARMA and CHAKRABARTI, 1999; ESCAFFRE et al., 1997; REGENDA et al., 2003). The impact of adequate feed and its quality on survival of carp larvae is obvious. If feed is not appropriate for that developmental stage, larval mortality is much higher (CAHU et al., 1998; PRZYBYŁ et al., 2006). Different rearing conditions have been used for rearing of common carp larvae under controlled conditions, such as using live feed (SHARMA and CHAKRABARTI, 2000; KORWIN-KOSSAKOWSKI, 2008; SCHLECHTRIEM et al., 2004) or using own mixtures of artificial feed (CHARLON and BERGOT, 1984; ALAMI-DURANTE et al., 1991; ESCAFFRE et al., 1997; CAHU et al., 1998; PRZYBYŁ et al., 2006) while others used combinations of live and artificial feed (REGENDA et al., 2003; BIŁAS et al., 2012).

However, no author tested the effect of different feeding treatments on resistance of larvae.

Larval survival and growth depends on environmental factors and adapting ability to ever-changing environment conditions (KOEDIJK et al., 2012). Larger larvae that grow more rapidly can consume larger plankton and avoid predation more easily (SOGARD, 1997). Therefore, larval feeding in intensive larviculture should not be exclusively focused on high survival rates, but also provide a high resistance to stress with high body weight and total length. Carp fry, 24 days post hatch, had high survival rates at salinity level of 8, 10, and 12‰, regardless of the feeding treatments. Considering that the common carp is tolerant to salinity level of 8‰ at 30 °C for longer time (KASIM, 1983), result achieved by salinity stress test indicates that at lower salinity levels (8-12‰) the transition time from live to dry feed doesn't have an effect on survival rate. At extreme salinity level (16‰) for common carp fry, no significant level was detected among feeding treatments ($P = 0.201$) and none individual survived 120 minutes. The physiological stress for common carp fry was too big to be affected just with different feeding treatment. But under 14‰ salinity level, prolonged feeding with live feed resulted with longer fry survival including 10% survival rate in group C and 30% in group D.

In practice, this enables the manufacturer to adjust the resistance of common carp fry to the outdoor conditions. If the changing weather is expected at the time of pond rearing, a longer feeding with live feed will provide a better resistance of fry and consequently better survival rate. Or otherwise, if it is expected a stable weather, earlier transition to artificial feed will reduce involvement of manpower and lower the production costs.

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SAŽETAK

Istraživanja su provedena na ličinkama šarana (*Cyprinus carpio* L.) u uzgojnim uvjetima recirkulacijskog sustava u trajanju 21 dan. Promatrana su četiri hranidbena režima s različitim prijelazima sa žive hrane (naupliji artemije, *Artemia salina*) na umjetnu hranu (Biomar larviva). Najveću prosječnu dužinu tijela (25,72 ± 3,37 mm) i masu tijela (213,81 ± 105,52 mg) ostvarile su ličinke šarana hranjene čitavo vrijeme nauplijima artemije (*A. salina*) u skupini D. Značajno signifikantna (P<0,05) završna tjelesna masa utvrđena je u skupini D u odnosu na skupine A i B. Najveći postotak preživljavanja utvrđen je u skupinama B, C i D (81,53; 84,14; 80,66 %), dok je u skupini A s najranijim prijelazom na umjetnu hranu postotak preživljavanja znatno manji (32,44 - 43,36 %). Test na salinitetni stres proveden je zadnjeg dana uzgoja. Preživljavanje mladunaca šarana tijekom salinitetnog stresa analizirano je Kaplan-Meierovim testom. Statistički značajna razlika (P<0,001) utvrđena je između hranidbenih režima samo pri salinitetu od 14‰.

Ključne riječi: riblja ličinka, uzgojni sustav, salinitetni stres