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Effect of Weaning Strategies on Growth and Survival of Pikeperch, *Sander lucioperca*, Larvae

Uroš Ljubobratović[1,*], Balázs Kucska[2], Tibor Feledi[3], Vesna Poleksić[3], Zoran Marković[3], Mirjana Lenhardt[4], András Peteri[1], Shivendra Kumar[1], András Rónyai[1]

[1] Research Institute for Fisheries and Aquaculture, Naik Haki, Anna liget 8, H-5540, Szarvas, Hungary.
[2] Kaposvár University, Faculty of Agricultural and Environmental Sciences, H-7400 Kaposvár, GubaSándor u. 40, Hungary.
[3] University of Belgrade, Faculty of Agriculture, Department of Zootechnics, Nemanjina 6, Belgrade, Serbia.
[4] University of Belgrade, Institute for Multidisciplinary Research, Department of Natural Resources and Environmental Sciences, KnezaViseslava 1, Belgrade, Serbia.

* Corresponding Author: Tel.: +36.704 403133; Fax: +36.66 312142;
E-mail: u.ljubobratovic@gmail.com

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Abstract

The effects of different weaning strategies on survival, cannibalism and growth were investigated in pikeperch (*Sander lucioperca*) larvae. Two weaning strategies were tested with 15 days post hatch (DPH) larvae: Co-feeding and Supplementary feeding. Co-feeding was performed with *Artemia* nauplii and dry feed by the gradual reduction of nauplii either @ 25% day^{-1} or 14% day^{-1}, whereas supplementary feeding was performed with sudden weaning to exclusively dry feed while *Artemia* nauplii was the last meal of the day. Each strategy was tested in two time durations, for 4 and 7 days. Co-feeding weaning strategy seems to be more beneficial compare to supplementary feeding weaning strategy in response of lower mortality (%), higher growth and finally maximum total yield. Even though higher cannibalism was found in the larvae weaned for 7 days from 15-22 DPH but higher growth and survival in this group make this weaning strategy better than larvae weaned for 4 days from 15-19 DPH. Therefore, it is concluded that the co-feeding weaning strategy for 7 days from 15 to 22 DPH should be followed for successful larviculture of pikeperch.

Keywords: Cannibalism, larviculture, live food, pikeperch, weaning.

Introduction

Weaning of fish is considered as transition from live food to formulated feed. It is referred asthemost critical period in larviculture of many species, with special regard on the piscivorous fish (Kestemont *et al*., 2003). Live food contributes largely in various ways to improve survival and growth of larvae. Its movement and metabolite secretion have a major effect on the feeding response (Kolkovski *et al*., 1997a; Kolkovski *et al*., 1997b). Further, enzymes present in the live nauplii and its movement in the intestinal tract may lead to improved digestion of the formulated feed (Kestemont *et al*., 1996). The influence of live food on the cannibalism rate in larviculture of piscivorous species is still an inconclusive issue (Baras and Jobling, 2002; Kestemont *et al*., 2003). Hence, live food availability during weaning period could make an influence on the final success oflarviculture.

For the feasibility of larviculture, it is important to start weaning as early as possible, considering the fact that the majority of expenses in fingerling production are due to livefood supplementation (Person Le Ruyet *et al*., 1993; Cahu and ZamboninoInfante, 2001; Langdon, 2003).The initial studies on early weaning of pikeperch larvaereported poor results in terms of survival and growth (Ruuhijärvi *et al*., 1991; Schlumberger and Proteau, 1991; Proteau *et al*., 1993; Mani-Ponset *et al*., 1994). More recent studies using commercial diets have indicated significant improvements in survival and growth with appropriate larval diets and/or optimal weaning timing (Ostaszewska *et al*., 2005; Kestemont *et al*., 2007). It was reported that day 18 post-hatch at 20.0–21.2°C is the optimal time for starting the gradual transition to dry feed (Kestemont *et al*. 2007), whereas Hamza *et al*. (2007) found that starting the weaning from day 15 post-hatch at 19-20°C had no negative effect on the digestive tract and enzyme activity of larvae, resulting in the best larval performance. However, these studies did not investigate the effect of weaning procedure. Therefore, the aim of the present study was to investigate the effect of different weaning strategy on survival and growth of pikeperch larvae.

Materials and Methods

Experimental Facilities and Larval Rearing

Wild pikeperch breeders were stocked in the

wintering ponds and sampled just before spawning at Research Institute for Fisheries and Aquaculture (HAKI), Szarvas, Hungary. Breeders were propagated according to method described by Rónyai (2007). Hormonal treatment for females was consisted of two injections of carp pituitary extract in concentration 3 mg kg^{-1}each with 24 hours interval between injections. Males were injected with single injection of same preparation with concentration 2 mg kg^{-1}. Breeders were stocked in pairs (male and female) in the divided spawning compartments supplied with an artificial nest placed in the middle of the tank. After the first spawning was noticed, females were checked for ovulation after each two hours periods and when the ovulation was noticed, stripping was performed. Eggs from three females were obtained and eggs of each female were fertilized with milt of two males. Egg de-adhesion method was done with stirring the eggs in a milk solution for 60 minutes. After incubation and hatching, about 10,000 larvae (3 days old) were stocked in 250 L water volume grey coloured polyester tank connected to RAS. Initial larval density was 40 larvae L^{-1} in accordance with the suggestion of Szkudlarek and Zakęś (2007) for this age of larvae. In order to promote swim bladder inflation, surface of the water was cleaned with surface blower (Chatain and Ounais-Guschemann, 1990) having 30 cm diameter and placed in the middle of the tank. The surface inside the blower was cleaned 3 times per day. From day 4 post-hatch (4 DPH), larvae were fed with newly hatched *Artemia nauplii* (AF origin, INVE, Dendermonde, Belgium) exclusively till 10 DPH.

Experimental Design

Three hundred and forty 11 DPH pikeperch larvae (mean weight ±SD: 2.40±0.17 mg) were randomly distributed in four treatment groups in triplicate for experimental trial following a complete randomised design. The experimental rearing system was consisted of 12 uniform size dark green colour polypropylene circular tanks (22 L capacity) connected to RAS. The total volume of water in each tank was maintained at 20L throughout the experimental period. Initial stocking density in the trial was 17 larvae L^{-1}, which is in agreement with the recommendation of Szkudlarek and Zakęś (2007). Water temperature and dissolved oxygen were measured daily at 3 PM in all rearing tanks. Samples for analysis of nitrogen compounds (nitrite, nitrate and ammonium) were taken twice per weak (Monday and Thursday) at the outflow of tanks. The dissolved oxygen in recalculating water was maintained within 7 to 7.5 mg L^{-1}, the ammonium and nitrite in rearing water were kept below 0.1 mg L^{-1}. Water temperature in all experimental tanks was maintained at 22.6±0.6°C (mean ± SD). The flow rate in each tank was approximately 0.8 L min^{-1} with a slight aeration. A fluorescent light fixture (100-W neon lamp, white colour) was suspended above experimental tanks (80 cm above water surface) and the larvae were subjected to a constant photo-period (LD 14 h: 10h) throughout the experiments. Light was turned on at 7 AM followed by the 1 hour cleaning of the tanks and turned off at 9 PM after the cleaning of tank. Feeding started at 8 A Mandlast feeding was given at 8 PM. Siphoning of uneaten food and dead larvae from the tanks was started half hour after last feeding.

Experimental Diet and Feeding

The experimental diet used for the present weaning experiment was Perla Larve Proactive (Skretting, Hendrix Spa, Mozzecane, Italy) having 8.1% moisture, 62% protein, 11% fat, 10% ash and 0.8% fibre. Larvae (11 DPH) in all experimental tanks were fed with newly hatched *Artemia nauplii* in every two hours from 8 AM to 8 PM with feeding level of 700-800 nauplii $larvae^{-1}$ day^{-1} till 14 DPH. Then after, two weaning strategies were tested with 15 DPH larvae: Co-feeding and Supplementary feeding. Co-feeding was performed with *Artemia nauplii* and dry feed by the gradual reduction of nauplii either 25% day^{-1} or 14% day^{-1}, whereas supplementary feeding was performed with sudden weaning to exclusively dry feed while *Artemia nauplii* was the last meal of the day, which was given in excess. Different treatments were:

CF-19: Co-feeding of *Artemia nauplii* and dry feed with reduction of nauplii 25% day^{-1}(4 days weaning from 15 – 19 DPH);
CF-22: Co-feeding of *Artemia nauplii* and dry feed with reduction of nauplii 14% day^{-1} (7 days weaning from 15 – 22 DPH);
SF-19: Supplementary feeding for 4 days from 15 – 19 DPH;
SF-22: Supplementary feeding for 7 days from 15 – 22 DPH.

The schematic illustration of the weaning protocol is given in Figure 1. Feeding levels were fixed at 0.5 g $tank^{-1}$ day^{-1} during the weaning period and then after 1 g $tank^{-1}$ day^{-1} till the end of the experiment (27 DPH). During and after weaning period, feeding was performed in every two hours and three hours respectively from 8 AM to 8 PM. Each tank was cleaned twice a day and dead fish were counted. Uneaten food and faeces were siphoned out daily. At the end of experiment (27 DPH), all larvae were measured for individual body weight and total length, with the precision 0.01 mg and 0.1 mm, respectively.

Relevant Parameters

Survival, cannibalism and growth were calculated as follows:

$$\text{Counted mortality (\%)} = 100\ N_d \times N_i^{-1}$$

$$\text{Survival (\%)} = 100\ N_h \times N_i^{-1}$$

$$\text{Cannibalism (\%)} = 100\ (N_m \times N_i^{-1})$$

$$\text{Specific growth rate (SGR, \% day}^{-1}) = 100\ (\text{Ln}W_f - \text{Ln}W_i) \times \Delta T^{-1}$$

$$\text{Daily growth rate (DGR, mg day}^{-1}) = (W_f - W_i) \times \Delta T^{-1}$$

where, N_i, N_d, N_h and N_m represent the number of initially stocked, died, harvested and missing ($N_m = N_i - N_d - N_f$) fish, respectively; W_i and W_f – initial and final body weights (mg), respectively; ΔT- duration of the experiment (days).

Additionally, harvested yield (Y) at 27 DPH was calculated and was expressed as fish production per unit of water volume (g L^{-1}).

Statistical Analyses

Statistical analysis was based on one-way Analysis of Variance (ANOVA). Values describing probability (survival, cannibalism and mortality) were arcsine transformed prior to statistical analysis. Significant differences between treatments were estimated using a post-hoc Duncan's Multiple Range Test with a significance level at $P \leq 0.05$. Analyses were performed using SPSS 13.0 software (IBM, New York, NY, USA).

Results and Discussion

Transition of the embryo to exogenous feeding is the decisive event in the early stage of development of percid fish, because failure to accomplish first feeding results in mortality (Balon, 1984). Kestemont *et al*. (1995) suggested that the weaning age is directly related to the ontogeny of the species and to the development of the digestive system of the larvae. Further, it has been well explained that combined feeding of live and artificial diet, referred as co-feeding, enhances larval performance beyond that achieved by feeding either types of feed alone (Kanazawa *et al*., 1989; Holt, 1993; Abi-Ayad and Kestemont, 1994) and co-feeding also permits weaning in a shorter time (Person Le Ruyet *et al*., 1993). In the present study, highest ($P<0.05$) survival (43.8%) was obtained in the treatment group SF-19 (weaned with supplementary feeding for 4 days from 15-19 DPH), while the mean survival rate in other groups were 31.9%, 35.1% and 21.6% in treatment group CF-19, CF-22 and SF-22 respectively (Table 1). There was high cannibalism in one replication of treatment SF-19 and finally only 6 individuals were left. Therefore, we excluded this replication from the every analysis. Present result reflected that 7-days co-feeding strategy is more favourable compared to 4-days co-feeding strategy, which may be due to decline in the influence of feed attraction and feeding response with the age. Kolkovski *et al*. (1997a,

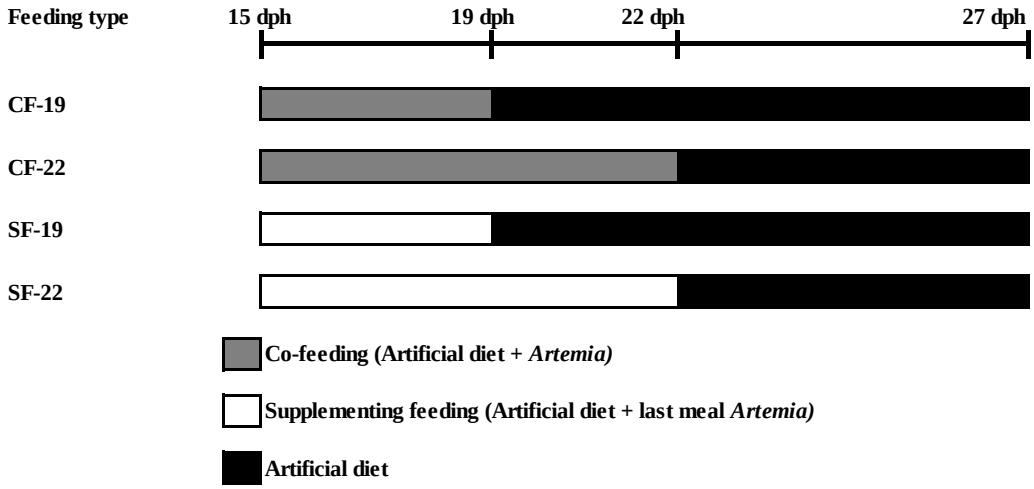

Figure 1. Schematic diagram of the weaning protocols.

Table 1. Effect of different weaning strategies on mortality, survival and cannibalism of pikeperch larvae

Parameters	CF-19	CF-22	SF-19(*)	SF-22
Counted mortality (%)	42.4±3.4^{a}	19.9±1.2^{c}	30.9±2.5$^{(b)}$	36.1±7.7^{ab}
Actual survival (%)	31.9±5.8^{ab}	35.1±6.4^{a}	43.8±2.9$^{(a)}$	21.6±5.9^{c}
Cannibalism (%)	25.8±9.1^{b}	45.0±5.2^{a}	25.3±5.4$^{(b)}$	42.4±6.8^{a}

Values (mean±SD) in the samerow with different superscript (a, b)are significantly different ($P \leq 0.05$).
(*) – two replications in the treatment.

1997b) also reported that influence of *Artemia nauplii* on the ingestion, digestion and assimilation of artificial diet during co-feeding may be due to remote influence on artificial diet ingestion by visual and chemical stimuli. The chemical stimuli originating from the metabolites secreted by the nauplii help the larvae to orientate towards the food and once attracted by the chemokinesis, visual stimulus coming from the movement of the prey is enabling the larvae to recognize the food which enhances the success of early feeding.

The larviculture mortality of pikeperch follows bimodal curve (Szkudlarek and Zakęś, 2007). First period of increased mortality is linked to the transition from endogenous to exogenous feeding and swim bladder inflation. However, the second period of increased mortality corresponds to occurrence of cannibalistic behaviour. In the present study, significantly increased ($P<0.05$) mortality during weaning procedure (Table 1) implies that this procedure enhances the cannibalistic behaviour from one side, but from the other side this mortality may be the consequence of inability of larvae to recognize the dry feed as a food. The lowest ($P<0.05$) counted mortality was found in CF-22 treatment whereas the highest ($P<0.05$) counted mortality was found in another co-feeding treatment CF-19 favours the importance of live/dry feed ratio during weaning. The mortality rate was significantly ($P<0.05$) higher in all treatment groups during weaning week compare to post weaning week (Figure 2). Average daily mortality assessment reflected that higher mortality was found during 18 to 22 DPH and the maximum mortality was found at 20 DPH in all treatment groups (Figure 3). Reduced mortality rate in the treatment weaned for longer period with co-feeding weaning strategy confirms increased feeding response and lower rate of starved larvae. Hence, longer weaning period with optimal dry/live feed ratio (i.e gradual live feed reduction) is needed in order to minimize the loss as a consequence of weaning procedure. Nevertheless, increased feed availability with usage of automatic feeders could be an important tool for higher weaning success.

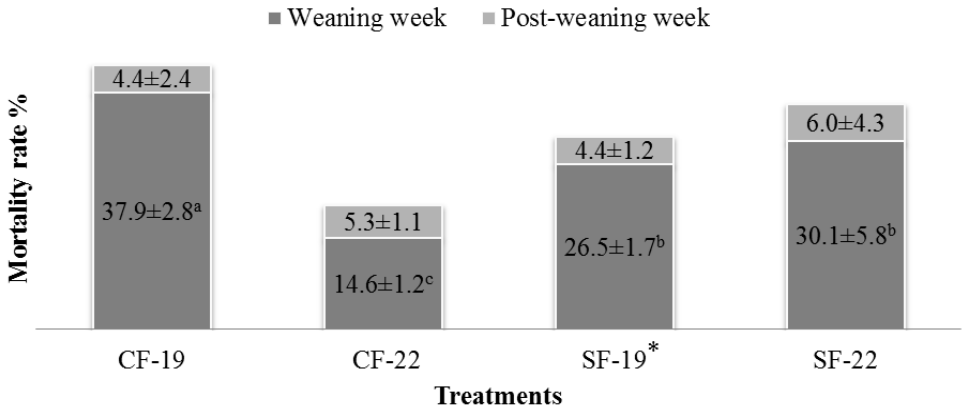

Figure 2. Mortality rates during the weaning and post-weaning week.
Treatment series (mean±SD) with different superscript (a, b, c) are significantly different ($P\leq0.05$)
* - two replications in the treatment

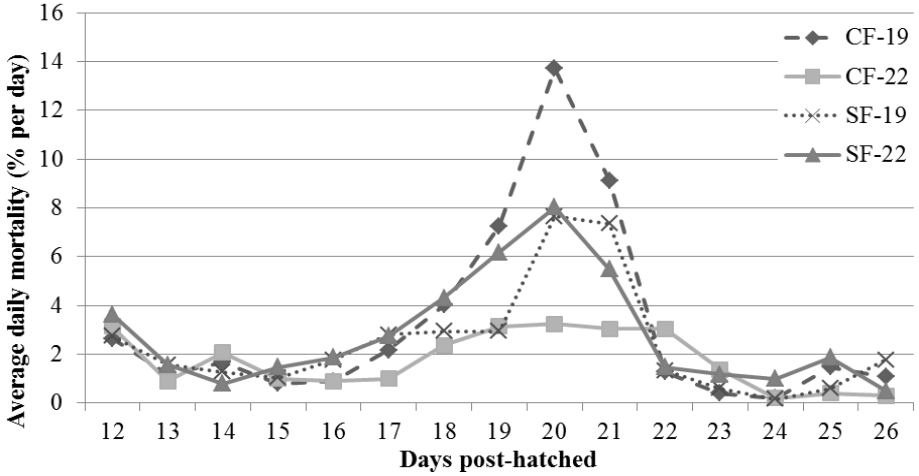

Figure 3. Daily mortality of pikeperch larvae during the experiment.

Cannibalism has severe impact in the culture of predatory fish species and highest cannibalism was reported during larval period (Kestemont *et al*., 2003). First sign of cannibalism in the present study was found on 15 DPH, which is in agreement with the result of Hamza *et al*. (2007). Effect of live food on cannibalism is not fully explained (Baras and Jobling, 2002; Kestemont *et al*., 2003).However, with respect to nutritional value of dry feed compared to live nauplii, Baras and Jobling (2002) pointed towards the importance of earlier weaning. They explained that the earlier weaned larvae have significantly higher growth compared to larvae still predating on nauplii solely becoming eventually the prey. This may be the explanation for our findings indicating that longer exposure to live food results in higher losses due to cannibalism (Figure 4). Significantly ($P<0.05$) highest losses by cannibalism was recorded in the CF-22 group (45%) which was similar to SF-22 group (42.4%), whereas the lowest ($P<0.05$) was in SF-19 group (25.3%) which was similar to CF-19 (25.8%) (Table 1). Similarly, influence of live food on cannibalistic behaviour of pikeperch larvae has been reported by Kestemont *et al*. (2007). Results of present study on cannibalistic behaviour of pikeperch larvae suggest that cannibalistic behaviour is inevitable characteristic of this culture. Similar observations have been made by Szkudlarek and Zakęś (2007). However, in the present study, increased mortality due to cannibalism took place earlier and had greater consequences. This might be explained by the different feeding strategies and photoperiod used in these two studies. In order to reduce the consequences of this inevitable phase of culture, authors of previously mentioned study as well as studies conducted on walleye (Summerfelt, 1996; Summerfelt *et al*., 2011) suggested often feeding with mechanical feeders, appropriate feeds to the fish satiation and 24:0 LD photoperiod. Finally, size grading of post larvae has been suggested as the effective tool for the reduced mortalities in this period (Szczepkowski *et al*., 2011).

The final mean weight of larvae ranged between 30.5 and 84.3 mg at the end of experiment (27 DPH) and was significantly influenced by different weaning strategies (Table 2). The range of mean weight in previous studies was 25 mg at 27 DPH (Kowalska *et al*., 2006) and 110 mg at 25 DPH (Szkudlarek and Zakęś, 2007). Higher mean weight found by Szkudlarek and Zakęś (2007) after 25 DPH was most probably due to better rearing conditions and /or use of more adequate dry feed. The positive effect of longer weaning period on growth performance (gain in body mass and SGR) in the present study (Table 2) suggests that either larvae utilize digestive enzymes from *Artemia nauplii* to facilitate the process of digestion (Dabrowski, 1984; Lauff and Hofer, 1984) or product of live prey (*Artemia nauplii*) autolysis may stimulate secretion of trypsinogen from the pancreas and / or activate gut zymogens (PersonLe Ruyet *et al*., 1993). Nevertheless, earlier mentioned positive effect of live feed on feeding response (Kolkovski *et al*., 1997a) may have a significant role as well.

The coefficient of variation (CV) in size is also the important parameter in the larviculture of predatory species, as it can lead to high rates of cannibalism, which has been pointed out by several authors (Kubitza and Lovshin, 1999; Baras and Jobling, 2002; Kestemont *et al*., 2003). High (ranged from 83.6% to 116.3%) and similar ($P>0.05$) CV for weight of larvae in the present study is in agreement with the result of Kestemont *et al*. (2007), who also found high CV values of weight for pikeperch larvae fed with marine starter feed. Similarly, Baras and Jobling (2002) found connection of suitability of dry

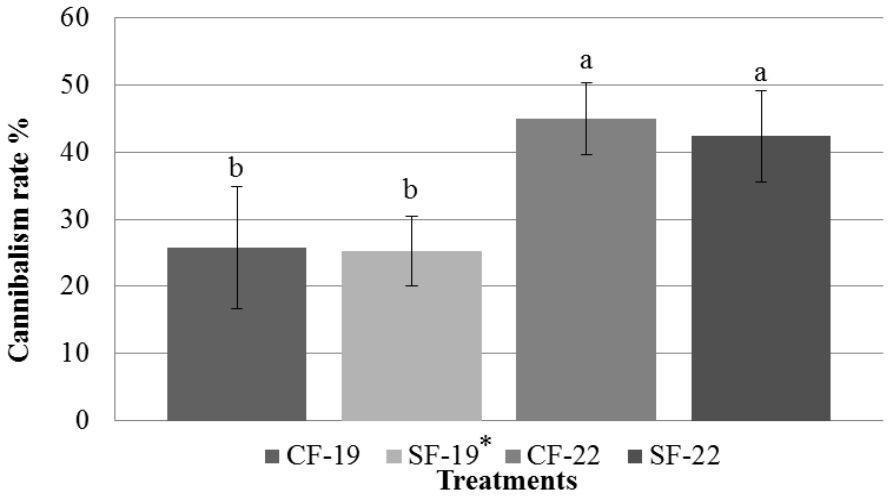

Figure 4. Differences in cannibalism rates (mean ± SD) between the treatments with different time of exposure to live feed. Treatment series (mean±SD) with different superscript (a, b) are significantly different ($P\leq0.05$)
* - two replications in the treatment

Table 2. Effect of different weaning strategies on the growth performance of pikeperch larvae

Parameters	CF-19	CF-22	SF-19(*)	SF-22
BW_I (mg)[a]	2.4±0.2	2.4±0.2	2.4±0.2	2.4±0.2
BW_F (mg)[b]	47.7±7.0[bc]	84.3±7.3[a]	30.5±3.4[(c)]	52.7±12.1[b]
SGR (% day^{-1})[c]	18.6±0.9[b]	22.2±0.6[a]	15.9±0.7[(c)]	19.2±1.6[ab]
DGR (mg day^{-1})[d]	2.8±0.4[b]	5.1±0.5[a]	1.8±0.2[(c)]	3.1±0.8[b]
Final length (mm)	17.6±1.1[bc]	21.4±0.4[a]	15.8±0.5[(c)]	17.8±1.2[b]
CV_{TL} (%)[e]	17.1±1.0[ab]	16.2±0.7[ab]	15.3±1.3[(b)]	19.9±3.2[a]
CV_{BW} (%)[f]	83.9±16.0	83.6±14.4	92.9±19.0	116.3±25.4
Y (g L^{-1})[g]	0.25±0.02[b]	0.50±0.09[a]	0.23±0.01[(b)]	0.19±0.04[b]

[a]BW_I:Initialbody weight; [b]BW_F: Final body weight; [c]SGR: specific growth rate; [d]DGR:daily growth rate; [e]CV_{TL}: coefficient of variation (100 SD $mean^{-1}$) for total length: [f]CV_{BW}: coefficient of variation (100 SD $mean^{-1}$) forbody weight, [g]Y: harvested yield
Values (mean±SD) in the same row with different superscript (a, b, c) are significantly different ($P \leq 0.05$).
(*) – two replications in the treatment.

feed with the occasion of cannibalistic behaviour. Therefore, more appropriate dry feed for pikeperch larvae could lead to reduction of this parameter and consequently lower the losses of cannibalism.

The results of this study showed that weaning for 7 days from 15-22 DPH support higher growth and survival of the pikeperch larvae compared to weaning for 4 days from 15-19 DPH. Along with that, co-feeding weaning strategy seems to be more beneficial compared to supplementary feeding weaning strategy in response of lower mortality (%), higher growth, (%) and finally maximum total yield. With respect to described feeding strategies of pikeperch larvae (FAO, 2012), it is concluded that for the technology based on first feeding with *Artemia* exclusively, the co-feeding weaning strategy for 7 days from 15 to 22 DPH should be followed for successful larviculture of pikeperch.

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