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EFFECT OF FEEDING FREQUENCY ON THE GROWTH OF THE EUROPEAN MUDMINNOW LARVAE (*UMBRA KRAMERI* WALBAUM, 1792) REARED IN CONTROLLED CONDITIONS

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Abstract

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Effects of feeding frequency were examined on European mudminnow larval growth (initial average total body length: 7.5 mm) under controlled rearing conditions. Two treatments were set in 3 replicates: „Group A": fed with *Artemia salina* nauplii four times day^{-1} and "Group B": fed with *Artemia* nauplii six times $^{-1}$ day. At the end of the 21-day-long examination period significant differences ($p<0.05$) were found in the total length between the two groups. Average final total lengths were the followings in the groups: "A" 15.5 mm, „B" 16.6 mm. Average live weights of the fish in experimental groups reached 34.4±2.4g, and 44.4±1.4g at the end of the trial in groups A and B, respectively. At the end of the trial period larvae were suitable for stocking into natural waters.

Key words: *Umbridae, Artemia*, stocking size

Abbreviations: A – Artemia feeding group; A4× – four times feeding day-1; A6× – six times feeding day-1; BM – body mass; BM_t – final body mass (mg); BM_0 – initial body mass (mg); DGL – Daily Growth in Length (mm day^{-1}); exp. – experiment; ind. – individual; F – feeding; S – survival rate; SD – standard deviation; t – the duration of the experiments (days); T - water temperature (°C); TL – total length; TL_0 – initial total length (mm); TL_t – final total length (mm)

Introduction

European mudminnow (*Umbra krameri*, Walbaum 1792) is an endemic fish species of Danube and Dniester rivers. It is a typical stagnophil species (Wilhelm, 2003) and a sudden extinction of the globally endangered fish (Simić, 2007). It is on the Red List of many European countries (Hungary - Bankovics, 1990; Austria - Hacker, 1983; Slovenia - Povž, 1992; Croatia - Mrakovčić et al., 2006; Ukraina - Serbaka, 1994; Slovakia - Baruš, 1989, Bulgaria - Velkov et al., 2004) and is listed on the Annex II of the European Union Council Directive on the Conservation of natural habitats and of wild fauna and flora, the Appendix II of the Bern Convention. It is categorized as "vulnerable" on the IUCN Red List due to its isolated population's consisting of only a few individuals and thus it is assumed that it may go extinct locally. The species is known to have been extirpated from many of its original habitats. It is estimated that mudminnow populations have declined by more than 30 % in the past 10 years (Freyhof, 2011). The main reason for this decline is considered to be habitat destruction, especially channelization followed by the destruction of river and stream floodplains (Wanzenböck, 1996). Recently, the invasive and aggressive Rotan (*Perccottus glenii*, Dybowski, 1877) supplants *U. krameri* in Hungarian waters (Specziár, 2010).

Systematic stockings of mudminnow into adjacent streams, canals and still waters might help to develop self-

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sustaining populations of *U. krameri* in places where the species disappeared or occurs only sparsely. The best method for the maintenance of populations would be the preservation of a variety of suitable micro-habitats. Furthermore, artificial propagation of mudminnow could also help to increase its stocks (Bíró and Paulovits, 1995). Though propagation, embryo and larva development of European mudminnow has been studied by a number of papers (Bohien, 1995; Kováč, 1995; 1997) no data are available about larval rearing in intensive systems. Our aim was to carry out artificial propagation and rearing of *U. krameri* for the maintenance of natural stocks. Probably a most promising way to improve the generally low efficiency of larvae stocking of *U. krameri*, similarly to other species, would be to use juvenile fish reared under controlled conditions prior to stocking into natural waters.

A principal aim of this study was to investigate the effect of feeding frequency of mudminnow larvae on growth and mortality within controlled conditions.

Materials and Methods

Broodstock

On the 2nd April 2010, three known Hungarian populations of European mudminnow [(strictly protected Lake Bábtava (in village Csaroda), Stream Csaronda (in village Lónya), Stream Gőgő-Szenke (in village Nagyszekeres)] were fished by electric fishing equipment. In the first two sites only invasive Rotan was caught, though Sallai (2004) reported about remarkable populations in these places. In the third site 15 individuals were collected (8 females, 7 males; BL: 71.2±13.8 mm; BW 7.9±4.6 g, water temperature: 12.0°C). All individuals were transported to the Fish Laboratory of the Department of Aquaculture, Gödöllő and stocked in a 700 L volume tank. Water temperature was maintained at 14.0°C and the photoperiod was close to natural.

Reproduction, embryo and non-feeding larva development

4-5 days after stocking the broodstock reproduced successfully in a natural way. One female was managed to be stripped (75 eggs/female: SL: 59 mm, BW: 4.45 g, fertilisation rate 98.7%, swollen egg diameter: 1.79±0.12, n=25). Eggs from the tank (~ 1200) were collected by a net and incubated in 7×1.5 L plastic tanks. Hatching time is shown in Table 1. On the 8-14 (12.9±1.9°C) days after hatching larvae started to feed exogenously depending on the hatching time. In the experiment 1-day-old feeding larvae were used.

Culture facilities of feeding experiment

Larvae were reared for 21 days in 1.33 L tanks (11×13×7cm) of a recirculation system (2 000 L filtration and buffer system). Recirculation system refilled groundwater from water well with three weeks from start of experiment. Water flow in each tank was maintained at 0.2-0.5 mL s^{-1}. A total number of 240 larvae were stocked in six plastic tanks. Fish were divided into two experimental groups (stocking density: 40 individuals/ tank (1.33 L); 30 ind./L,); Group "A" (*Artemia salina* 4×) were fed with *Artemia* nauplii four times per day (9.00, 12.00, 15.00, 18.00 h) and Group "B" (*Artemia* 6×) were fed with *Artemia* nauplii six times per day (6.00, 9.00, 12.00, 15.00, 18.00, 21.00 h) for 21 days. Each treatment groups were stocked in three replicates. Nutritional information: *Artemia* nauplii size 525±88 µm, 1 mL incubation water contained 8.5±1.7 mg *Artemia* nauplii (wet weight), which contained 414±108 individuals. Feeding rates were the followings: 1st day: 2 mL/feeding(F); 2nd day: 3 mL/F; 3-4th day: 6 mL/F; 5-7th day: 12 mL/F; 8-14th day: 18 mL/F; 15-21st day: 24 mL/F. Fish tanks were cleaned from faecal waste and dead fishes were replaced and recorded every day after the last feeding.

Water quality

Temperature was monitored twice a day at 06.00 and 18.00 hours. Dissolved oxygen was measured every third day using an oxygen meter (WTW 340i, MERCK). Nitrite–N and Nitrate-N were also determined (Macherey-Nagel VISOCOLOR ECO test kit) together with pH and conductivity

Table 1
Hatching rhythm of eggs (*n = 263; hatching rate = hatched larvae / fertilized eggs ×100 - was 84.6 %)

Day after spawning (13.7±0.4 °C in the first 5 days, then 11.9±1.1 °C)	Ratio of hatched larvae, %*
10	0.8
11	18.6
12	33.1
13	43.3
14	0.8
15	3.4

Table 2
Water quality parameters in the experimental system (mean±SD)

Temp., °C	pH	O_2, mg L^{-1}	NO_2^-, mg L^{-1}	NO_3^-, mg L^{-1}	Conductivity, µS cm^{-1}
15.4±2.8 °C	8.58±0.13	8.69±0.42	0.02±0.01	8±0.82	1720±40.82

by using pH and conductivity meter (VOLTCRAFT PH-100 ATC; VOLTCRAFT LWT-01) four times (0^{th} day, 7^{th} day, 14^{th} day, 21^{st} day) during the experimental period (Table 2).

Data analysis

Every week total body length of all fish in each group was measured and recorded by using digital photos and Image J software (National Institutes of Health). At the end of the experiment each group was weighed (due to animal welfare considerations fish were measured in water and in groups since it was a sensitive species) by a Sartorius scale (±0.01 g). The following parameters were calculated:

Condition factor = $100 \times w_t / (l_t)^3$, where w_t and l_t were the final body weights in grams and body length in cm.

Daily growth in length was expressed as DGL = $(TL_t - TL_0)t^{-1}$ where TL_t is the final total length (mm), TL_0 is the initial total length (mm) and t (days) is the duration of the experiments.

Statistical analyses were carried out with SPSS 7.5 for Windows (1996). Independent t-test was used to compare data of growth rate and Kruskal-Wallis test for comparing mortality within the groups.

Results

Growth rates of length of Group "B" (*Artemia* 6 ×) differed significantly from Group "A" (*Artemia* 4 ×**)** right from the first week to the end of the experimental period (Figure 1). Mean length ranged from 11.1 mm to 17.9 mm in Group "A" and 13 mm to 18.7 mm in Group "B" at the end of the experiment. Average body weight in Group "A" was smaller than in Group "B", however, these differences were not significant at the end of the experiment (P>0.05). The reason for that was that there was no possibility to measure the individual weight of fish so only feeding groups were compared (as a result there were not enough sample numbers). Average growth rates/day of length and condition factors was also similar (Table 3). Mortality was below 5-7 % (Figure 3.). Length frequency distributions of both groups were normal (Figure 2).

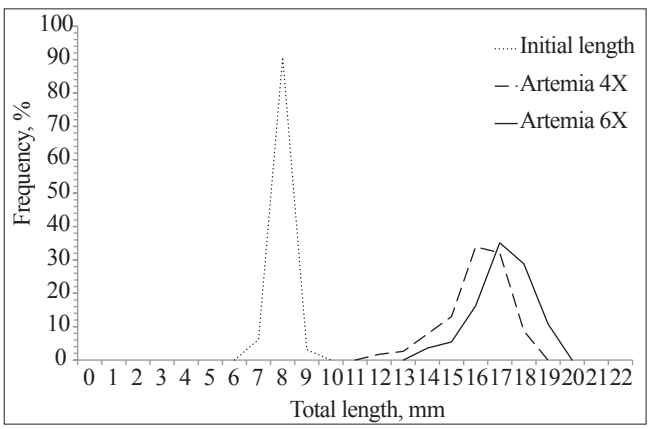

Fig. 1. Total length histograms of experimental groups at the beginning and end of the experiment

Discussion

Feeding frequency experiments resulted in the expected effects: European mudminnow fed six times a day (Group "B") grew faster than those fed four times a day (Group "A"). It is evident that higher growth rate depends on both higher and more frequent food supply (Wolnicki et al., 2003; Başçınar et al., 2007). Moreover, the preference of *Artemia* nauplii for salinity – they can live in fresh water for about 4-5 hours before they die – also affects their consumption. An *U. krameri larva* - similarly to *Esox lucius* larvae - does not prey for unmoving organisms. In *Artemia* 6× groups larvae could meet with living *Artemia* nauplii for a longer period of time (feeding time: 6.00-21.00 h) than *Artemia* 4× groups (feeding time: 9.00-18.00 h). *U. krameri* is a tipical diurnal predator so they do not prey during the night. Remains of *Artemia* was rarely observed on the bottom of the tanks so the amount of *Artemia* nauplii was very close to the limit of *ad libitum* feeding during the experiment.

Compared to growth data of other marsh indicator fish species (*Tinca tinca, Carassius carassius, Misgurnus fossilis, Scardinius erythrophthalmus*) reared under intensive conditions and fed by *Artemia* nauplii, the growth of mudminnow larvae was close to *T. tinca* in this experiment but it has to be noted that *U. krameri* was reared in lower temperature than already mentioned species (Table 4). For example *Carassius*

Table 3
Summarised results of *U. krameri* larvae during the experiment (mean±SD, * group statistics)

	Artemia 4× (Group "A")	*Artemia* 6× (Group "B")	Significant differences (P=)
Initial length, mm	7.5±0.3		-
Final length, mm	15.5±1.2	16.6±1.1	<0.001
*Daily growth rate, mm day^{-1}	0.38±0.03	0.43±0.01	0.184
*Final weight, mg	34.4±2.4	44.4±1.4	0.292
*K factor at the end of exp.	0.92±0.17	0.97±0.02	0.628
*Survival rate, %	95±2.5	93.3±3.8	0.583

gibelio larvae grew two times faster at 28 °C than at 20 °C (Kestemont, 1995). But our reared larvae had significant larger growth rate comparing to Kováč (1995) data, who reported about *U. krameri* larvae early development rearing in aquaria (average temp. 15.8 °C, min-max: 11 - 20.8 °C). Larvae total length was from 10.5-11.31 mm (n=3) at the age of 43 days from onset of exogoneous feeding, while our fish reached 11.1 – 18.9 mm total length under 21 days. Mortality rate of *U. krameri* was close to mortality rates of other fish species. Compared to other predator fish species no cannibalism was observed among mudminnow larvae. Other investigations are needed to reveal the growth rate of *U. krameri* in intensive conditions in larger size as well. At the end of experiment 236 *U. krameri* juveniles were introduced into two sites (a natural water originating from broodstock and a Pilot Demonstration Area;) which was part local action plans (Freyhof, 2011; Tatár et al., 2012; Bajomi et al., 2013).

Conclusion

This is the first results of *U. krameri* rearing under controlled conditions by feeding Artemia. Feeding frequency experiments resulted in the expected effects: European mudminnow fed six times a day grew faster than those fed four times a day. At the

Table 4
Summarized data about growth rates of different marsch indicator fish species feeding on *Artemia* in laboratory conditions

Species	TL_0, mm	BM_0, mg	TL_t, mm	BM_t, mg	DGL, mm day^{-1}	T, days	T, °C	Source
Umbra krameri	7.5	-	15.5	34.4	0.38	21	15.4	Present study 4× times feeding day^{-1}
	7.5	-	16.6	44.4	0.43	21	15.4	Present study 6× times feeding day^{-1}
Misgurnus fossilis	7.3	-	25.4	116.2	1.21	15	24	Demény et al. (2009)
Tinca tinca	4.82	0.47	12.8	24.4	0.53	15	28	Wolnicki and Górny (1995b)
	4.53	-	17.6	88.8	0.65	20	28	Wolnicki et al. (2003), 24 h feeding
	4.53	-	16.5	67.9	0.60	20	28	Wolnicki et al. (2003), 18 h feeding
	4.53	-	13.5	31.7	0.44	20	28	Wolnicki et al. (2003), 12 h feeding
	4.93	0.45	21.68	87.9	0.67	25	25	Mamcarz et al. (2011)
Scardinius erythrophthalmus	5.7	0.9	18.9	68.5	0.66	20	25	Wolnicki et al. (2009)
Carassius carassius	5.49	0.9	17.77	60.73	0.59	21	25	Żarski et al. (2011), Exp. I. (50 ind. l^{-1})
	5.49	0.9	16.70	51.83	0.53	21	25	Żarski et al. (2011), Exp. I. (100 ind. l^{-1})
	5.49	0.9	16.55	52.69	0.53	21	25	Żarski et al. (2011), Exp. II. (100 ind. l^{-1})
	5.6	-	15.2	39.8	0.46	21	24.5	Demény et al. (2012) (50 ind. l^{-1})
	6.3	-	18	68.9	0.56	21	25.2	Demény et al. (2012) (50 ind. l^{-1})
	6.9	-	17.5	64.5	0.52	21	25.2	Demény et al. (2012) (50 ind. l^{-1})

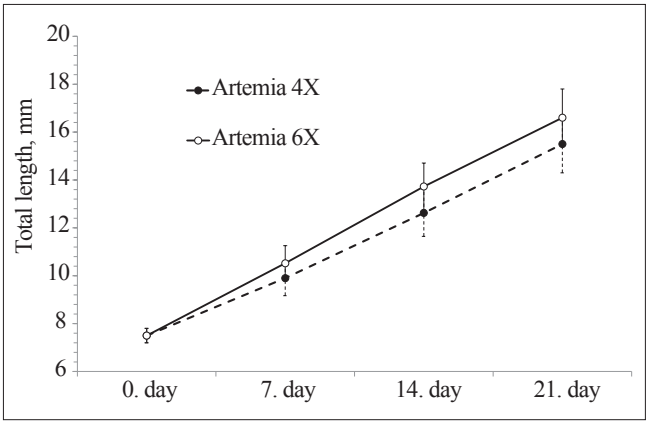

Fig. 2. Growth as mean±SD length of experimental fish

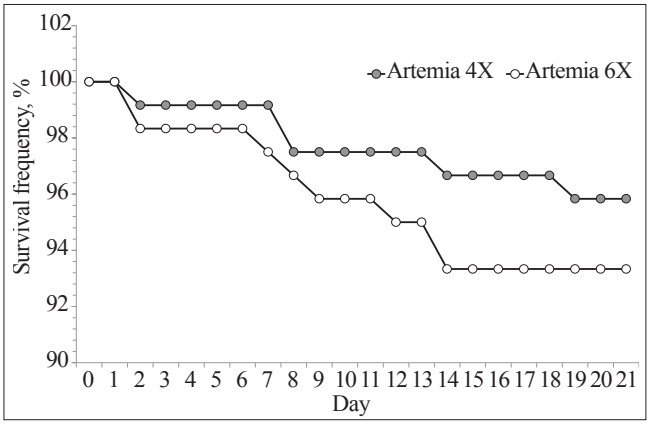

Fig. 3. Survival frequencies of feeding groups

end of experiment 3 weeks old *U. krameri* juveniles (11.1 – 18.9 mm) were suitable for stocking into natural waters.

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