

EX SITU PROTECTION OF THE EUROPEAN MUDMINNOW (*UMBRA KRAMERI* WALBAUM, 1792): SPAWNING SUBSTRATE PREFERENCE FOR LARVAE REARING UNDER CONTROLLED CONDITIONS

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Abstract: Captive breeding programs of endangered fish species, such as the European mudminnow *Umbra krameri*, are essential for population restoration. To improve captive spawning and larvae rearing under controlled conditions, two experiments were carried out. In the first, the spawning substrate preference was tested in triplicate, where five different types of artificial surface were provided for mudminnow pairs: (i) sand, (ii) artificial plants, (iii) gravel, (iv) sand + artificial plants and (v) gravel + artificial plants. All fish preferred the gravel + artificial plant combination, which indicates that this type of surface could be the most appropriate for spawning in captivity. In the second trial, three feeding protocols were tested in triplicate under controlled conditions. In the first treatment fish were fed exclusively with *Artemia* nauplii; in the second treatment fish were fed with *Artemia* for the first ten days then *Artemia* was gradually replaced with dry feed; for the third group the transition period started after 5 days of *Artemia* feeding. Although the survival rate of larvae could be maintained at a high level in some of the feeding protocols, a strong decrease in the growth rate was obvious in all diets containing dry food, which means that live food is essential for the first three weeks of mudminnow larvae rearing.

Key words: *Umbridae*; captive breeding; endemic species; conservation; larvae diet

INTRODUCTION

European mudminnow (*Umbra krameri*) is an endemic species of the middle and lower Danube and Dniester river basins (Bănărescu, 1964), inhabiting shallow lakes and backwaters with very low oxygen concentrations. The Habitats Directive [92/43/EEC] of the European Union lists the European mudminnow under Annex II because its conservation requires the designation of Special Conservation Areas and it is categorized as “vulnerable A2c” by the International Union for Conservation of Nature due to an estimat-

ed population size reduction of over 30% during the last ten years. Habitat fragmentation and loss because of river regulation, drainage of wetlands and pollution are the main reasons for the presumably irreversible decline of the species (Freyhof, 2011). As mudminnow can move between backwaters exclusively during the time of floods, a lack of extended long-lasting floods has isolated the populations, and therefore subsequent inbreeding and genetic drift may additionally put the species at risk. Therefore, beside restoration of their habitats, e.g. by local excavation of the clogged sections of drainage channels (Pekárik et

al., 2014), the maintenance of the genetic diversity of the species by *ex situ* breeding programs is required.

Captive breeding of mudminnow, including spawning and larvae rearing in aquaria, as well as its subsequent reintroduction to alluvial wetlands has been reported since the 1990s (Bohlen, 1995; Kovác, 1995, 1997; Kovác et al., 1996; Tatár et al., 2010; Müller et al., 2011; Bajomi et al., 2013). In contrast, experimentation on large-scale larvae production under controlled conditions has been very limited (Demény et al., 2014), although developing a propagation technique and mass larvae rearing under controlled conditions for stocking of natural waters is important. In the usual artificial rearing paradigm, natural materials (roots of sedge, aerial roots of grey willow, bunches of moss) were presented to the mudminnow females for spawning. The first trial was based on our previous experiences and the aim was to improve the breeding method by experimenting with readily available and sterilizable artificial spawning materials. The rearing of the larvae of endangered fish bred for species conservation purposes must, however, meet special criteria, warranting species integrity and a high survival rate of these valuable larvae. Consequently, such larvae should preferably be reared in monospecific, intensive larval cultures, which works effectively if a feasible dry food-based feeding protocol is available (Demény et al., 2012). The aim of the second trial was to improve the larval rearing of mudminnow using different types of feeding.

MATERIALS AND METHODS

Breeding stock

Seven mature male and three female fish were captured by electrofishing in the 1st pond of Szada, Hungary (N 47° 37' 37.02", E 19° 17' 31.83") at a water temperature of 10°C (April 12, 2014).

Trial 1. Substrate preference test

Fish were introduced into a 2-m³ plastic tank equipped with triplicates of 5 types of inorganic (arti-

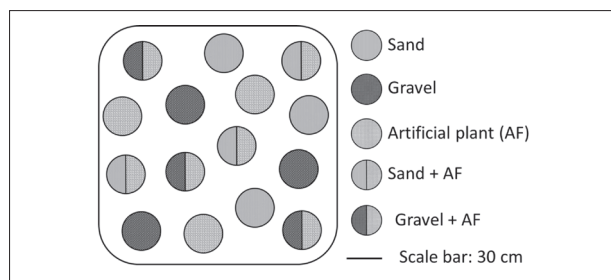


Fig 1. Spawning substrates in the preference test

ficial) spawning substrate, settled in plastic trays (ERZ 12.5+D, Ø=291 mm) on the bottom: gravel (Europet, 1-2 mm), sand (Europet, 0.3-0.6 mm), artificial plants (Raschel netting (green), gravel+ artificial plant, sand + artificial plant (Fig 1.).

Trial 2. Larvae rearing in controlled conditions

At the beginning of the exogenous feeding, larvae were stocked in 9 × 2-L containers with 40 larvae each. The containers were linked to a recirculation system (settling compartment, biological filter and sump) designed for zebrafish (*Danio rerio*). The system was run with aerated tap water (pH 8.3; dGH 13; TAN <0.1ppm; NO₂-N <0.1ppm; NO₃-N <1ppm). The dissolved oxygen was kept close to 100% saturation. The water temperature was 17.5±1°C; the flow rates were set to achieve a water exchange of 400% tank⁻¹ h⁻¹. During the experiment, a 14 h light/10 h dark cycle was used.

Experimental setup

Three tanks were randomly assigned into one of the three treatment groups so every treatment was applied in triplicate. All fish were fed 4 times a day for 21 days. The feces and feed residues were removed by siphoning prior to each feeding. All 3 treatment groups were fed initially with 300-400 *Artemia* nauplii per fish. The first group was fed exclusively with *Artemia* nauplii throughout the experiment (group *Artemia*). The second group was fed with *Artemia* for the first 10 days. From day 11 to day 13, fish were fed with *Artemia* and dry feed (Perla Larva Proactive,

Skretting, Italy) and during this 3-day transitional period, the amount of *Artemia* was gradually decreased. From day 14, fish were fed exclusively with dry feed (group A10P11). Treatment of the third group was similar except that the transitional phase started at day 6 and the dry-feed-only period started on day 9 (group A5P16).

Data collection and evaluation

Fish were photographed (Nikon D7000; macro lens 2.8/50) once a week and the total body length of each fish was determined by ImageJ 1.48 (National Institute of Health) analysis of the digital photographs. At the end of the experiment, fish were weighed in a non-invasive manner, eliminating any stress likely to be induced by catching and handling individuals of a strictly protected species. Each group was weighed as a whole and the average body weight was calculated from the group weight (Sartorius scale ± 0.01 g) and number of fish in the group. Statistical analyses were carried out with the SPSS 13.0 for Windows. One-way ANOVA followed by Tukey's test were used to compare the effects of treatment on growth, and the Kruskal-Wallis test for comparing mortality among the groups.

RESULTS

Trial 1. Captive breeding (Artificial spawning substrate)

Mature fish were standard length (Table 1). The fish were reared on an artificial substrate described in Fig. 1. Fertilized eggs were found on gravel in April 26 (3rd day after introduction) on the substrate of gravel + artificial water plant (Fig 2.). Water temperature was 10°C at the time of discovery (8:15 am), and increased to 12°C by noon. The first egg batch contained 194 eggs and the diameter of eggs were 1.8-1.9 mm (female body length was 61 mm). Eggs were transported in a hatchery tank and 185 larvae out of 194 eggs hatched (95.4 % hatching rate) during six days (water temperature 13°C). The second batch of eggs was discovered on April 29 in the gravel + water plant

Table 1. Standard length (mm) of the mudminnow broodstock.

♀	68	62	61				
♂	60	56	53	51	58	65	66

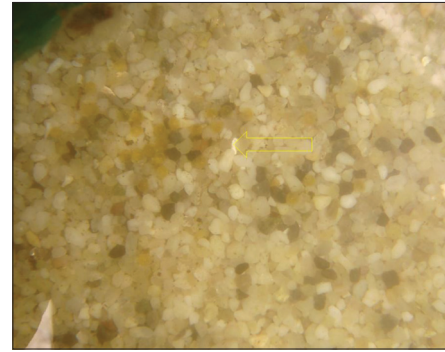


Fig 2. Eggs on the gravel. The arrow points to an egg.

habitat (water temperature 12°C). In this case, eggs were guarded by the spawning female (body length of 68 mm), the color of eggs was pale yellow. After the second spawning, 356 eggs were collected, of which 339 larvae hatched (95.2% hatching rate) over six days (eggs were in substrate for two days at 12°C) at 13°C. The third female with a male was seen on the third gravel + artificial plant substrate but spawning did not take place due to unknown causes.

Trial 2. Larvae rearing in controlled conditions

Treatment had a significant dose-dependent effect on the final length ($F=152.592$ $P < 0.05$) and weight of the fish ($F=12.264$ $P < 0.05$). There were no differences among the groups in body length by the end of the first week of the experiment; however, the growth rate diminished following the transition from *Artemia* to dry feed on day 5 or day 10 for treatment groups A5P16 and A10P11, respectively (Fig. 3.). By the end of the experiment, all 3 treatment groups significantly differed from each other (for statistics see Table 2). The final body weight of fish fed exclusively with *Artemia* was substantially higher than that of the two dry-feed treatment groups, which did not differ from each other significantly. Mortality by day 5 of the experiment reached 13% on average. During the first 5 days,

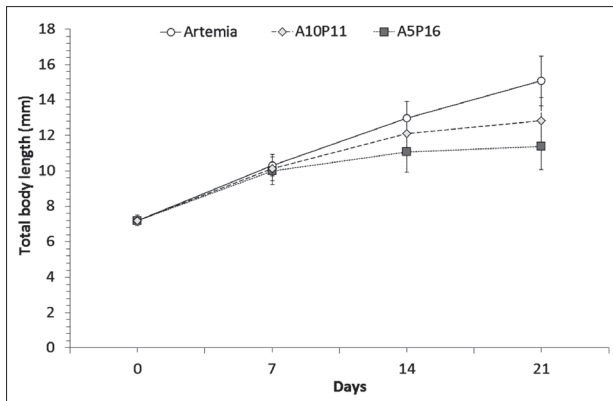


Fig 3. Growth expressed as means \pm SD of the length of fish in the three treatment groups. The experimental groups are as follows: **Artemia**: fed with *Artemia* exclusively; **A10P11**: fed with *Artemia* for 10 days than with dry feed for 11 days; **A5P16**: fed with *Artemia* for 5, followed by dry feed for 16 days).

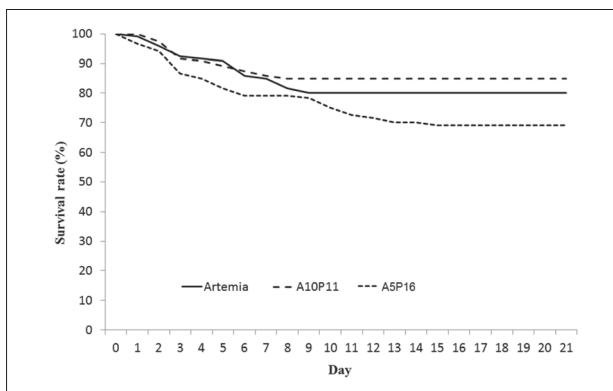


Fig 4. Mortality rates of the different experimental groups. The fish groups are as follows: **Artemia**: fed with *Artemia* exclusively; **A10P11**: fed with *Artemia* for 10 days than with dry feed for 11 days; **A5P16**: fed with *Artemia* for 5, followed by dry feed for 16 days).

Table 2. Summarized results of *U. krameri* larvae reared during the experiment (means \pm SD, different letters indicate significant differences at $P\leq 0.05$ level; * group statistics).

	Artemia	A10P11	A5P16
Initial length (mm)		7.2 \pm 0.28	
Final length (mm)	15.1 \pm 1.39 ^a	12.8 \pm 1.31 ^b	11.4 \pm 1.32 ^c
*Final bodyweight (mg)	37.7 \pm 10.00 ^a	18.1 \pm 1.20 ^b	13.7 \pm 4.00 ^b
*Survival rate (%)	80.0 \pm 6.60 ^{ab}	85.0 \pm 2.50 ^a	69.2 \pm 5.20 ^b

fish in all 9 tanks were treated equally, and therefore it is difficult to explain the significantly lower survival rate of group A5P16 before treatment. Later drops in survival in all three groups did not coincide with the conversion from *Artemia* to dry food (Fig. 4).

DISCUSSION

The habitat preference of mudminnow for spawning has been disputed. Bohlen (1995) considered mudminnow as a psammophil species. However, in several cases spawning was observed on sandy or gravel bottoms (Crăciun et al., 1997), thus the species can be characterized as psammophilous (Botta, 1981) or phytolithophilous (Kováč, 1995, 1997). Crăciun et al. (1997) observed spawning on sandy bottoms without any water plants present. Kovác (1995) found mudminnow nests as shallow pits in fine gravel hidden under vertical roots of *Salix cinerea*. According to observations of Hajdú (personal communication), when mudminnows were presented with plant material or inorganic (sand, gravel and stones) substrates in a seminatural environment, the fish spawned exclusively on the plant substrates (roots of *S. cinerea*, *Carex riparia*, bunch of *Vesicularia* sp.) and neglected the inorganic materials.

For *ex-situ* breeding, inorganic materials have several advantages over organic ones because they are readily available and easy to disinfect. As mudminnow prefer organic materials when present but accept inorganic ones when plant materials are not available, in the present experiment we forced the fish to choose between different inorganic substrates to evaluate whether any of the presented patterns are preferred and whether spawning could be successful under such conditions. All three females in this experiment chose the gravel substrate with plastic plants, and two of them successfully spawned on them. Although the limited number of subjects does not allow statistical analysis of preference, it is clear that gravel with artificial plants is a suitable spawning substrate for the mudminnow.

The number of eggs laid by the fish of body length 61 mm and 68 mm was 194 and 356, respectively,

which is typical for females of such size (Balon, 1967). The hatching success was high, indicated by hatching rates slightly over 95% in both cases. Overall, the first experiment suggests that gravel substrate with plastic plants is accepted by the fish and is suitable for the eggs to develop.

Feeding fish larvae with zooplankton in an intensive culture is expensive and unreliable. Therefore, our aim with the feeding experiment was to determine whether *Artemia* can be replaced with dry food. The determination of the two conversion periods was based on our experiences gained with rearing pike (*Esox lucius*) (Kucska et al., 2005), a species with similar feeding behavior during early ontogenesis and belonging to the same taxonomic order (Eso-ciformes). Despite such similarities, the conversion from *Artemia* to dry feed caused significant reduction in growth rate of mudminnow. Although mortality rate could not be linked conclusively to treatment, the smaller body size of larvae reared on dry food indicates that the nutritional value and/or the acceptance of that diet was not appropriate and it would probably diminish the survival rate of such handicapped larvae when reintroduced into the natural habitat.

Following the feeding trial, 400 juveniles of *Umbra krameri* from groups of *Artemia*, A10P11 and 70 individuals, which did not take part in the feeding experiment were introduced into ponds at the Pilot Demonstration Area, Szada, as part of local action plans (Freyhof, 2011; Tatár et al., 2012; Bajomi et al., 2013). In Hungary, a conservation value is assigned to every protected species. In the case of the mudminnow, the value of a single individual is about US\$ 400, and thus, interestingly, the reintroduced mudminnow of the present experiment represent a conservation value of about US\$ 160000. The current study was limited due to the small sample size, which could not be increased because the mudminnow is strictly protected.

CONCLUSION

Our experiments suggest that the endangered mudminnow can be bred in artificial environments using

inorganic substrate for spawning, and that European mudminnow larvae adapt poorly to commercial dry foods; thus, if large larvae of good fitness are required (i.e. for stocking natural habitats), they should then be reared on a live food diet.

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Conflict of interest disclosure: None

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