Ex situ protection of the European mudminnow (*Umbra krameri* Walbaum, 1792): spawning substrate preference, larvae rearing under controlled conditions

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Short title: Ex-situ protection of the European mudminnow

Abstract

Captive breeding programs of endangered fish species such as European mudminnow *Umbra krameri* are essential for its population restoration. To improve the captive spawning and larvae rearing under controlled conditions, two experiments were carried out. In the first trial spawning substrate preference was tested where five different kinds of artificial surface in triplication were provided for mudminnow pairs (1. sand, 2. artificial plant, 3. gravel, 4. sand + artificial plant, 5 gravel + artificial plant). All fish preferred the gravel + artificial plant combination which indicates, that this kind 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.

Keywords: *Umbridae*, captive breeding, endemic species, conservation, larvae diet

INTRODUCTION

European mudminnow (*Umbra krameri*) is endemic species of the middle and lower Danube and Dniester Rivers' 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 is categorised as “vulnerable A2c” by the International Union for Conservation of Nature due to an estimated 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 mainly responsible 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, 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) maintenance of the genetic diversity of the species by -2026511753 *ex situ* breeding program is required.

The 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 in controlled condition has been very limited (Demény et al., 2014), although developing the propagation technique and mass larvae rearing under controlled conditions for stocking into natural waters would be 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.

Rearing of larvae of endangered fish bred for species conservation purposes must however meet special criteria, warranting species integrity and high survival rate of these valuable larvae. Consequently, such larvae should preferably be reared in monospecific, intensive larvicultures, 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.

MATERIAL 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 (12th April 2014).

Trial 1. Substrate preference test

Fish were introduced into a 2 m^3 plastic tank equipped with triplicates of 5 types of inorganic (artificial) spawning substrate, settled in plastic trays (ERZ 12,5+D, Ø=291 mm) on the bottom: gravel (EURO-PET, 1-2 mm), sand (EURO-PET, 0.3-0.6 mm), artificial plant (Raschel-net (green), gravel+ artificial plant, sand + artificial plant.

Trial 2. Larvae rearing in controlled conditions

Rearing conditions

At the beginning of the exogenous feeding, larvae were stocked in 9 × 2 litres containers 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; NO_2–N <0,1ppm; NO_3-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 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 faeces 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 -2026511545 *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 every week and 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 stress likely to be induced by catching and handling of individuals of the strictly protected species. Each group was weighed as a whole and average body weight was calculated from 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 was used to compare the effects of treatment on growth and Kruskal-Wallis test for comparing mortality among the groups.

RESULTS

Trial 1. Captive breeding (Artificial spawning substrate)

Fertilised eggs were discovered on April 26th (3rd day after introduction) on gravel + artificial water plant substrate. Water temperature was 10° C at the time of finding (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 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 29th of April at gravel + water plant habitat (water temperature 12 °C). In this case, eggs were guarded by the spawning female (body length of 68 mm), colour of eggs was pale yellow. After 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 happen 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 growth rate diminished following the transition from *Artemia* to dry feed on day 5 or day 10, treatment groups A5P16 and A10P11, respectively. By the end of the experiment all 3 treatment groups significantly differed from each other (for statistics see *Table 2).*

Final body weight of fish fed exclusively with *Artemia* was substantially higher than the two dry feed treatment groups which did not differ from each other significantly.

Mortality by the 5th day of the experiment reached 13% on average. During the first 5 days fish in all 9 tanks were treated equally, 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.

DISCUSSION

Habitat preference of mudminnow for spawning has been disputed. Bohlen (1995) considered mudminnow as a phytophill species. However, in several cases spawning was observed on sandy or gravel bottom (Craciun et al. 1997), thus the species can be characterised as psammophilous (Botta 1981) or phytolithophilous (Kováč 1995, 1997). Craciun 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 from different inorganic substrates to evaluate whether any of the presented pattern is preferred and could spawning 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 plant 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 which were indicated by the hatching rates slightly over 95% in both case. 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 were based on our experiences gained with rearing pike (*Esox lucius*) (Kucska et al. 2005), a species with similar feeding behaviour during early ontogenesis and belonging to the same taxonomic order (Esociformes). 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 that handicapped larvae when re-introduced into the natural habitat.

Following the feeding trial, 400 juveniles of *U. krameri* from gropus of *Artemia*, A10P11 and 70 individuals, which did not take part in 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 case of the mudminnow, the value of a single individual is about US$ 400, thus interestingly, the reintroduced mudminnow of the present experiment represent a conservation value of about US$ 160,000. The current study is 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 environment using inorganic substrate for spawning, and it was concluded that European mudminnow larvae adapt poorly to commercial dry foods, and thus if large larvae of good fitness are needed (i.e. for stockings to natural habitats), then they should be reared on live food diet.

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Authors' contributions

All authors contributed in this paper. Conceived and designed the experiments: BK, PK, TM. Performed the experiments: BK,PK, JH, LV, DV, MMT, ST, BU, DZ, TM. Analyzed the data: BK, PK, JH, MMT, TM. Contributed reagents/materials/analysis tools: BK,PK, JH, LV, DV, MMT, ST, BU, DZ, TM. Wrote the paper: BK, PK, TM.

Conflict of interest disclosure

None

References

[1] *Bajomi, B., Tatár, S., Tóth, B., Demény, F., Müllerné-Trenovszki, M., Urbányi, B., Müller, T.* (2013). Captive-breeding, re-introduction and supplementation of the European mudminnow in Hungary, In: *Global Re-introduction Perspectives: 2013. Further case studies from around the globe* (Ed.: Soorae, P.S.), 15-20. Gland, Switzerland: IUCN/ SSC Re-introduction Specialist Group and Abu Dhabi, UAE: Environment Agency-Abu Dhabi. xiv + 282 pp.

[2] *Balon, E. K.* (1967). Ryby Slovenska. Obzor, Bratislava, **95**, 155-156.

[3] *Bănărescu, P.* (1964). Fauna Republicii Populare Romîne. Volumul XIII. Pisces – Osteichthyes, (Ed. Acad. R.P.R.) Bucharest

[4] *Bohlen, J.* (1995). Laboratory studies on the reproduction of the European mudminnow, *Umbra krameri* Walbaum, 1972.- *Ann Naturhist Mus Wien* **97B**, 502–507.

[5] *Botta I.* (1981). Adatok a lápi póc (*Umbra krameri* WALBAUM) szaporodásbiológiájához. *Halászat* **74(2),** 44-45. (in Hungarian)

[6] *Crăciun, N., Ionaşcu, A., Hanganu, D.* (1997). Cercetari de etologie la ţiganuş (*Umbra krameri).* Analele Ştiinţifice ale Institutului Delta Dunării, Tulcea, 185-194. (in Romanian)

[7] *Demény, F., Müllerné, T. M., Sokoray-Varga, S., Hegyi, Á., Urbányi, B., Żarski, D., Ács B., Miljanović, B., Specziár, A., Müller T* (2012). Relative efficiencies of *Artemia* nauplii, dry food and mixed food diets in intensive rearing of larval Crucian carp (*Carassius carassius* L.). *Turk. J. Fish. Aquat. Sci.* **12,** 691-698.

[8] *Demény, F.,Müllerné-Trenovszki, M., Tatár, S., Sipos, S., Urbányi, B., Kucska, B., Müller, T (2014).* Effect of feeding frequencies on the growth of the European mudminnow larvae (*Umbra krameri* WALBAUM, 1792) reared in controlled conditions. *Bulg. J. . Agricult. Scien.* **20(3),** *688-692.*

[9] *Freyhof, J.* (2011). *Umbra krameri*. In: IUCN 2012. IUCN Red List of Threatened Species. Version 2012.2. <www.iucnredlist.org > . Downloaded on 20 April 2015.

[10] *Kovác, V.* (1995). Reproductive behaviour and early development of the European mudminnow, *Umbra krameri*. *Folia Zool.* **44(1),** 57-80.

[11] *Kovác, V., Hensel, K., Černy, J., Otahelova, H.* (1996). Ex-situ Protection of *Umbra krameri* (Final report). Biodiversity Protection Project. FNS IE CU, Bratislava, 34.

[12] *Kovác, V.* (1997). Exeperience with captive breeding of the European mudminnow, *Umbra krameri* Walbaum, and why it may be in danger of extinction. *Aqua. Sci. Conserv.* **1,** 45-51.

[13] *Kucska, B., Müller, T., Sári, J., Bódis, M. Bercsényi, M.* (2005). Successful growth of pike fingerlings (*Esox lucius*, L.) on pellet at artificial condition. *Aquaculture*

[14] *Müller, T., Balovan, B., Tatár, S., Müllerné-Trenovszki, M., Urbányi, B., Demény, F*. (2011). Propagation and rearing of European mudminnow (*Umbra krameri*) in the interest of natural stock maintenance. *Pisces Hungarici* **5**, 15-20. (in Hungarian with English abstract)

[15] *Pekárik, L., Hajdó, L., Kosco, J.* (2014). Identifying the key habitat characteristics of threatened European mudminnow (*Umbra krameri*,Walbaum 1792). Fundam. Appl. Limnol. **184(2)**, 151-159.

[16] *Tatár, S., Bajomi, Bt., Balován, B., Tóth, B.,Sallai, Z., Demény, F.., Urbányi, B., Müller, T.* (2012). Habitat reconstruction for marshland fish species. természetvédelmi Közlemények **18**, 487-498. (in Hungarian with English abstract)

[17] *Tatár, S., Sallai, Z., Demény, F., Urbányi, B., Tóth, B., Müller, T.* (2010). European Mudminnow Conservation Programme. Halászat **103(2)**, 70-75. (in Hungarian with English abstract)

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

♀	68	62	61				
♂	60	56	53	51	58	65	66

Table 2. Summarised results of *U. krameri* larvae reared during the experiment (mean ± SD, different letters indicate significant differences at P ≤ 0.05 level, * group statistics).

	Artemia	**A10P11**	**A5P16**
Initial length (mm)		7.2 ± 0.28	
Final length (mm)	15.1 ± 1.39 ^{a}	12.8 ± 1.31 ^{b}	11.4 ± 1.32 ^{c}
***Final bodyweight (mg)**	37.7 ± 10.00 ^{a}	18.1 ± 1.20 ^{b}	13.7 ± 4.00 ^{b}
***Survival rate (%)**	80.0 ± 6.60 ^{a}	$^{\&}$5.0 ± 2.50 ^{ab}	69.2 ± 5.20 ^{b}

Fig 1. Spawning substrates in the preference test

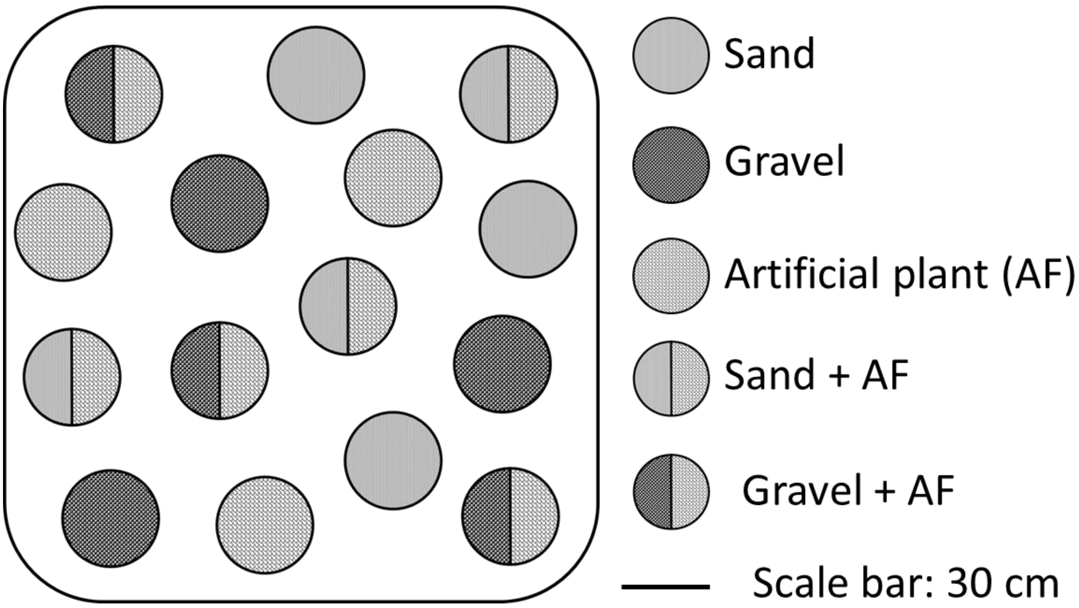

Fig 2. Eggs on the gravel

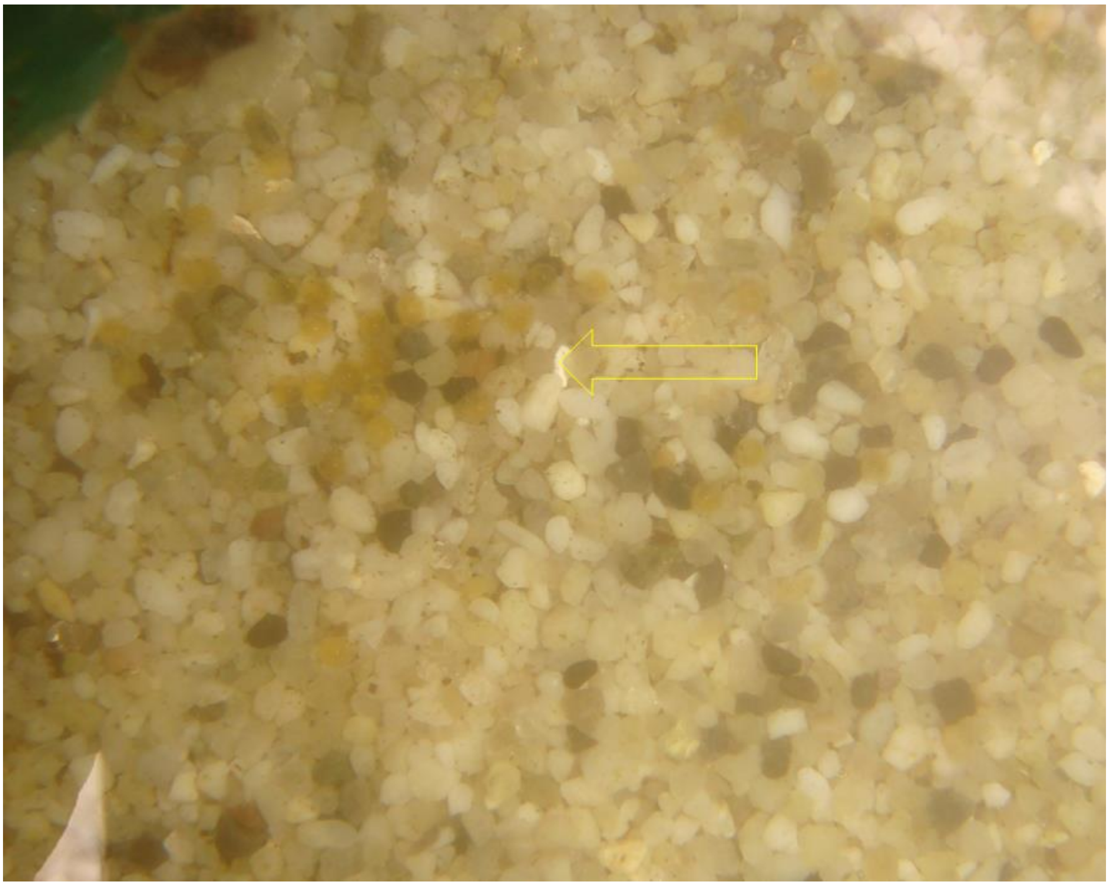

Fig 3. Growth as mean ± SD length of fish in the three treatment groups (Group *Artemia* fed with *Artemia* exclusively, group A10P11 fed with *Artemia* for 10 days than with dry feed for 11 days and group A5P16 fed with *Artemia* for 5 than with dry feed for 16 days)

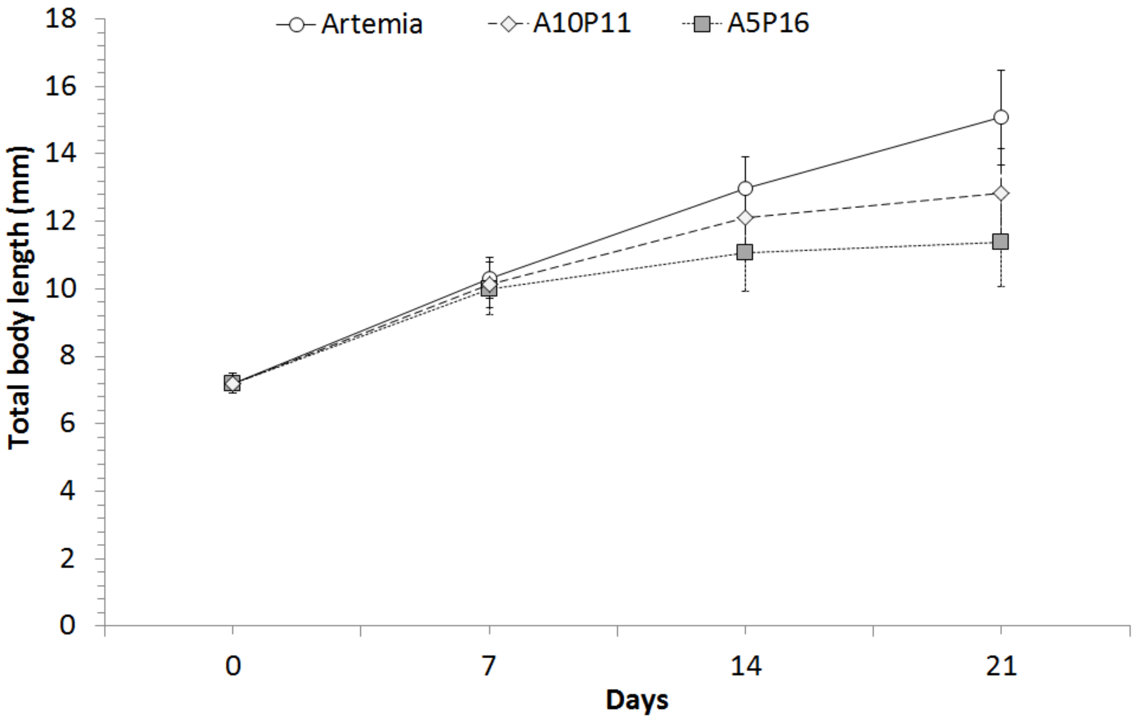

Fig 4. Mortality rate of the experimental groups