

## Influence of temperature conditions on the mobile fish hatchery efficiency


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**Abstract:** The demand for angling native fish species and their conservation value are steadily increasing nowadays. Mobile fish hatchery developed by the Department of Aquaculture at MATE AKI allows for immediate on-site propagation after capturing the broodstock. Several experiments have demonstrated the usefulness of the system, but there are certain aspects changing dynamically such as temperature conditions due to the small size of system and environmental exposure, which require further investigation. In this study, applicability of mobile hatchery was compared with a closed recirculation hatchery system. Model species used in the experiments was the chub (*Squalius cephalus* L.). Results show the difference in daily heat input and average temperature between differently positioned Zuger jars in mobile hatchery; however, it does not affect hatching rate, larval mortality rate or body length of freshly hatched larvae. The closed recirculation system had a higher proportion of deformed larvae than the mobile hatchery; in addition, the hatching rate was positive in all Zuger jars. Based on statistical analysis, no statistically significant difference was detected in body length between Zuger. Body length of freshly hatched larvae in the closed recirculation system was significantly smaller than in case of groups incubated in Zuger jars 1 ( $P < 0.05$ ) and 2 ( $P < 0.05$ ). Results show that water temperature of mobile hatchery is affected by the temperature outside, but hatchery units provide optimal temperature for developing eggs even at low air temperatures.

**Keywords:** mobile hatchery, temperature, embryonic development, incubation

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### Introduction

The management of natural waters in Hungary has changed significantly over the past 15 years. While commercial fishing in natural waters used to be common, Law CII on Fisheries, which came into force on 1 September 2013, links the primary use of waters to angling and the development of angling tourism (Ferincz & Staszny, 2020). At the same time, it can be observed that natural fish populations have been reduced in many areas due to overexploitation, loss of spawning grounds and water pollution (Keijzer et al., 2024; Sallai & Juhász, 2020). It has led to the need to rebuild and continu-

ously replenish fish stocks in many areas of our natural waters (Daupagne et al., 2021; Weiperth et al., 2021). However, restocking is a complex and often costly process (Mickiewicz & Wołos, 2012). On the one hand, it has been proven that the population of large fish of a size that can be caught by anglers can be associated with serious risks, for instance, inter- and intraspecific competition (Simonović et al., 2014), reduction of the genetic stock of populations (Hargrove et al., 2022), and loss of natural spawning populations. On the other hand, the positive impact of these programmes on usable yields is also questionable (Simonović et

al., 2014). Therefore, research that had been conducted on this topic so far shows that the best means to conserve natural populations is the restoration of natural habitats and spawning grounds (Manfrin et al., 2019). If it is not possible for economic or other reasons, efforts should be made to reduce the potential risks of stocking. It is required that the widest possible range of broodstock from natural habitats be used and the youngest possible age class be released (Araki, 2008). The aim of stocking programmes is to ensure that artificial propagation only helps the species through critical points that cause the greatest losses and which, if eliminated, do not cause significant genetic selection (Klütsch et al., 2019). Such critical points causing high losses may be the lack of spawning habitat (Gao et al., 2016), the inadequate incubation environment of eggs (Crane & Farrell, 2013) or the presence of a starter food (Meira et al., 2022; Skaramuca et al., 1994).

Mobile fish hatchery developed by the Department of Aquaculture at MATE AKI can offer a solution to all these problems. This device allows for immediate on-site propagation of broodstock after capture in natural waters in a way that is as humane as possible for the broodstock. Rapid on-site propagation can maximise the number of broodstock used, and this in turn reduces the risk of genetic narrowing caused by stocking. A mobile hatchery provides opportunity to incubate eggs in situ and to release the fry quickly and efficiently while eliminating mortality due to transport (Csorbai & Urbányi, 2020; Hekli, 2022; Ketut Suwetja et al., 2017).

Usability of the new system has already been tested by the developers in a number of field trials, which have focused on the system's load-bearing capacity. In order to determine the practical usability, it was important to determine how many eggs and larvae the mobile hatchery could incubate. In the first experiment, the system was fed with 350 g of 54% protein fry feed to model the nitro-

gen load of fry rearing. Nitrogen content of the fish feed was 30 g per day. Total ammonium nitrogen (TAN) was measured at  $0.3 \pm 0.2$  mg/L, while nitrite was measured at  $0.1 \pm 0.05$  mg/L (Csorbai & Urbányi, 2020). These values provide sufficient conditions for the incubation of most fish species (Tilak et al., 2002). Following theoretical investigations, two model fish species (African catfish *Clarias gariepinus* Burchell 1822, and common carp *Cyprinus carpio* L.) and three target fish species (ide *Leuciscus idus* L., chub *Squalius cephalus* L., tench *Tinca tinca*) have shown that the hatchery can be used in practice (Csorbai, 2021).

There are, however, certain dynamically changing aspects, such as temperature parameters, which are key to long-term applicability due to the small size of the system and its exposure to the environment, and which merit further investigation. Water temperature is the most important environmental parameter that influences egg development and embryonic development period (Avakul & Jutagate, 2015), hatching success, growth and survival of newly hatched larvae (Kucharczyk et al., 1997; Roessig et al., 2004). Previous experiments have shown that chub larvae are more sensitive to extreme conditions during incubation (Kupren et al., 2008), but also tolerate increasing temperature variation and temperature fluctuations (i.e. incubation at non-constant temperatures) under optimal conditions. Although the incubation time is shorter in both increasing and fluctuating temperature incubations, no difference in larval deformation rate and hatching percentage can be detected (Kupren et al., 2011). Literature also shows that temperature plays a crucial role in the embryonic development of fish and its rate; therefore, the exploration of this parameter is crucial when developing a breeding technique for a fish species.

For the investigation of the influence of temperature conditions on the mobile fish hatch-

ery efficiency the chub as a model species was chosen, as this species is becoming increasingly popular among anglers, but its natural populations have been decreasing in many areas, so that its establishment has become necessary (Weiperth et al., 2021) and the reference data to accurately evaluate the results were available (Krejszefc et al., 2008; Nagy et al., 2023).

## Materials and Methods

The broodstock was collected in the Ipolytölgyes section of river Ipoly using an electric fishing machine (Samus 725M). Research licence no. HaGF/154/2021 was at disposal to use the electric fishing machine. The temperature of the river was 14 °C. The 12 female and 31 male chubs were captured and transported to Gödöllő and placed into the mobile fish hatchery which was set to the MATE AKI Aquaculture Department. Ovopel AUV® was used to induce ovulation and stimulate spermiation. The priming dose (0.1 Ovopel/1 kg of broodstock) was followed by the final dose (0.9 Ovopel/1 kg of broodstock) after 24 hours. After mixing the eggs and milt, system water was used for fertilization. After that, Woynárovich's solution (40 g table salt and 30 g urea dissolved in 10 L system water) was used to prevent eggs from sticking together. The final removal of stickiness was done with tannin solution (5 g tannin, 10 L system water). The batches of eggs were mixed and then divided into four equal parts to obtain 4 × 620 mL of swollen egg batches. One batch was placed in a 7-L Zuger jar in the recirculation system of the Department of Aquaculture at MATE AKI and the remaining three batches of eggs were placed in three different Zuger jars of the mobile fish hatchery numbered serially. Number 1 was the Zuger jar closest to the entrance, number 2 was the middle one and number 3 was the Zuger jar furthest from the door. The mobile fish hatch-

ery system is based on a basin with a 1.2 m<sup>3</sup> capacity, which is suitable for temporary housing of broodstock and short-term rearing of the fry. Mechanical filtration of water in the system is provided by a sponge filter downstream of the basin. Water from the basin flows through three filter sponges, each of which is 50 mm thick, then reaches the UV filter where four 25 W UV tubes ensure the elimination of unwanted bacteria, viruses and fungi. The pump (Grundfos Alpha 1) is located after the UV filter and transfers system water to a 0.288 m<sup>3</sup> biological filter tank containing 0.144 m<sup>3</sup> of biomedica fill. The K1 biomedica has a useful surface area of 836 m<sup>2</sup>/m<sup>3</sup>. Water flows by gravity from the bio-filtration reactor to the Zuger jars or rearing units. Five 8 L Zuger jars (SDK Sp. z o. o.) can be installed for the hatchery at the same time, which can be easily exchanged during the rearing phase for five larval rearing jars of 100 L (Csorbai & Urbányi, 2020). Temperature stability of the system is ensured by two 250 W aquarium heaters (Eheim Jäger 250) controlled by a thermostatic socket (Sygonix TX3; hysteresis 0.5 °C). Mechanical filtration of the Zuger jar in the control unit was also provided by a sponge filter. It contained a 40-W UV filter and a 150-L biological filter. Temperature and dissolved oxygen content in the system were continuously monitored (probe type WTW FDO-IQ) and the measured data were fed into a computer. When the dissolved oxygen level reached the critical lower limit, the system notified the operators on telephone. Moreover, when the system reached the lower or upper limit of the set desired temperature, the computer reacted by controlling the system accordingly and in case the water temperature reached the lower limit, heating was provided by a 500 W (Eheim Jäger 500) aquarium heater. In cases when the water temperature during the measurement was higher than the desired upper limit, cooling was provided automatically by a 375 W Aqua Medic Titan Chiller 1500.

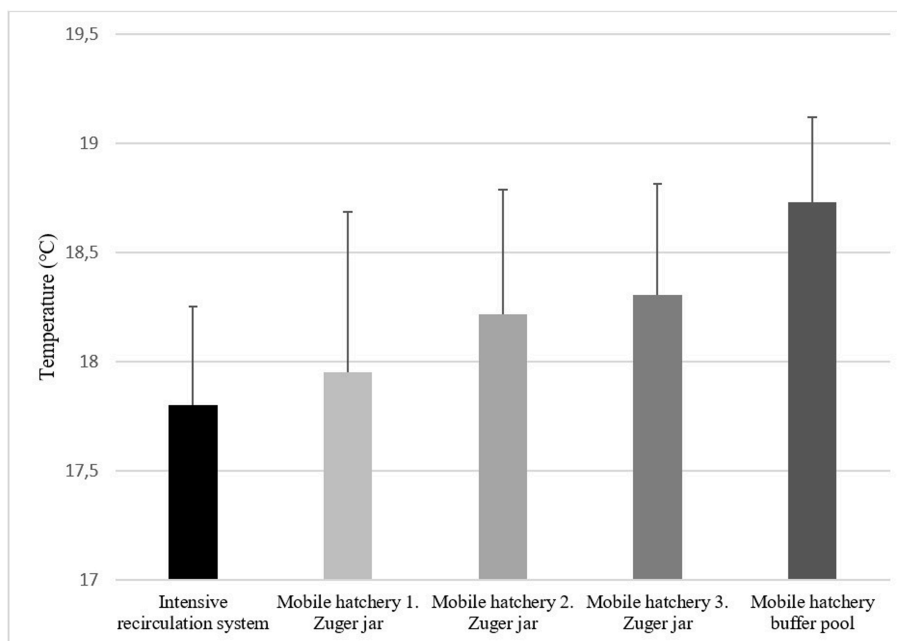


Figure 1: Evolution of average water temperatures during incubation.

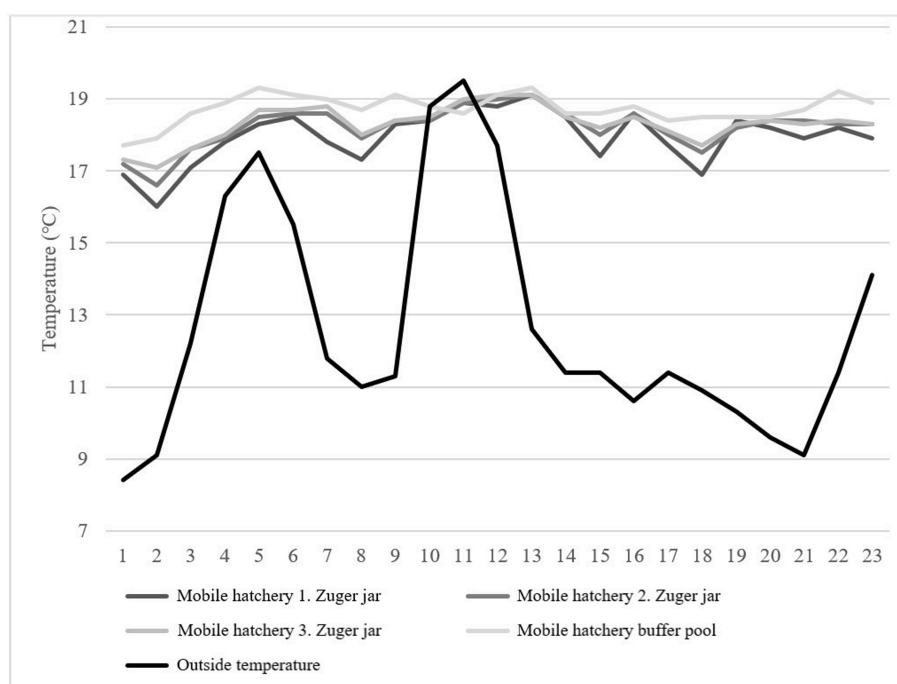


Figure 2: Comparison of the water temperature values measured in the mobile fish hatchery and the outside ambient temperature.

In the indoor recirculation system, the replenishment water volume was constantly 18 L/hour, which means a daily water change rate of 20%. In the mobile fish hatchery,

water was changed once a day, also at a rate of 20%. In both cases, the make-up water was mains water at the temperature of 16 °C. Water change was not only to wash

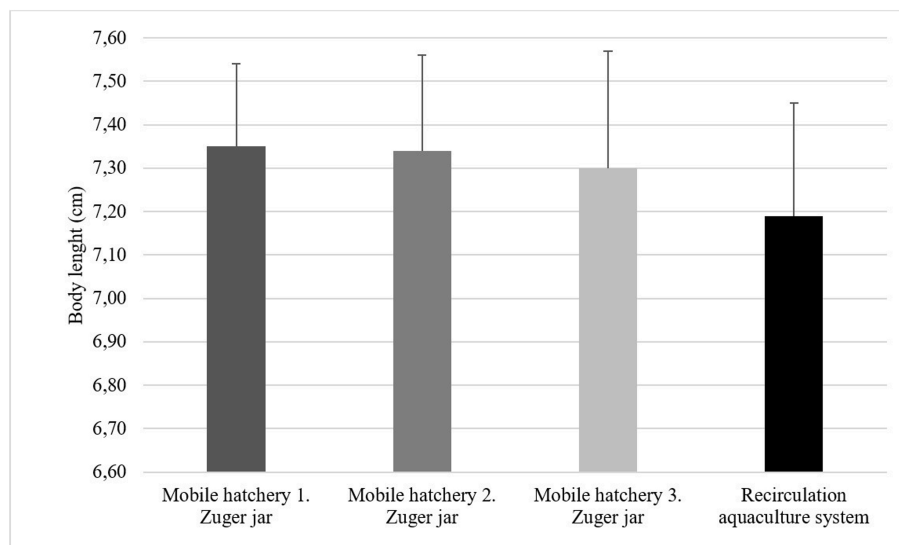


Figure 3: Body length (mean $\pm$ SD) of freshly hatched larvae for groups incubated in different units. Groups with different letters proved to be significantly different by ANOVA ( $P < 0.05$ ).

out unwanted nitrogen forms but also to reduce temperature, so it was carried out in the early afternoon. The desired temperature during incubation was 18 °C in both systems. Temperature measurements in the mobile fish incubator and in the indoor recirculation system were carried out every 4 hours at 0:00, 4:00, 8:00, 12:00, 16:00 and 20:00 throughout the incubation period. Measurements were conducted in the four experimental Zuger jars and in the brood fish holding tank of mobile fish hatchery located in the middle of the water body. A Hach HQ 2200 manual measuring device was used for the measurements. For comparison with external air temperature parameters, data of the Hungarian Meteorological Service in Aszód (Station No. 44214), the nearest station to Gödöllő were used.

Hatching rate was determined at the moment of hatching from 150 to 200 eggs using a Leica EZ4E microscope. At the time of hatching, 2  $\times$  25 eggs were taken from each Zuger jar and after, the properly positioned fish were photographed with a Leica M205 FA microscope and a DFC7000 T camera. The

50 larvae per each Zuger jar in the images were measured using ImageJ (Developed by Wayne Rasband, version 1.52). The fish photographed were reviewed one by one and deformations were noted.

Results of body length measurements were compared using one-point analysis of variance, with 95% significance level for each difference in all cases and GraphPad Prism 4.0 Statistical software was used for statistical analysis.

## Results

Average water temperature in the Zuger jar connected to the closed recirculation system was 17.8  $\pm$  0.45 °C during the incubation of eggs. The mean temperature measured and standard deviation of water temperatures measured were the lowest in the Zuger jar mentioned. The average temperature of Zuger jar number 1 in mobile fish hatchery was 18.0  $\pm$  0.73 °C and the same value of number 2 was 18.2  $\pm$  0.57 °C, while in case of number 3, it was 18.3  $\pm$  0.51 °C. In all cases, the standard deviation values of

water temperatures measured in the Zuger jars in mobile fish incubator were higher than the standard deviation of incubator parameters measured in the closed recirculation system. It was observed that the average temperature of Zuger jar no. 1 in mobile fish hatchery is the lowest and its standard deviation is the highest; in addition, the average temperature increases while the standard deviation decreases as the distance from the door increases (Fig. 1). The average water temperature of the 1.2 m<sup>3</sup> fish holding pool in mobile fish hatchery was found to be the warmest, namely 18.7 °C during the experiment. Standard deviation of the measured results was the lowest in this case ( $\pm 0.39$  °C). The fact that the temperature of pool is more stable than regarding any of the incubators is probably due to the fact that the large volume of water has a much smaller specific surface area and is, therefore, less exposed to environmental influences than small (8-L) Zuger jars.

Air temperature data of the Hungarian Meteorological Service were also compared with the data of mobile fish hatchery. The diagram shows that although water temperature change follows the trend of air temperature change, the daily heat input remains adequate even in colder temperatures and water temperature did not drop below the critical 16 °C regarding any of the Zuger jars during the incubation period (Fig. 2).

The calculated hatching rate in the mobile fish hatchery was very similar in all three Zuger jars. The Zuger jar located closest to the door had a hatching rate of 67.1%, the Zuger jar in the middle had 67.5%, and the hatchery jar furthest from the door had 66.2%. A lower hatching rate of 63.3% was counted in the closed recirculation system. The average total body length of hatching larvae incubated in Zuger jar 1 of mobile hatchery was  $7.35 \pm 0.19$  mm. The same value for Zuger jar 2 was  $7.34 \pm 0.22$  mm and for Zuger jar 3 was  $7.30 \pm 0.27$  mm. Never-

theless, the average body length of the closed recirculation hatched larvae was  $7.19 \pm 0.26$  mm. Based on statistical evaluation, no statistically verifiable difference in hatching larval body length was detected between Zuger jars 1, 2 and 3. However, the body length of freshly hatched larvae in closed recirculation system was significantly smaller than in case of groups incubated in Zuger jars 1 ( $P < 0.05$ ) and 2 ( $P < 0.05$ ) (Fig. 3).

Differences were also observed in larval deformation: the proportion of malformed and weakly hatched larvae was 12% in Zuger jar 1, 10% in Zuger jar 2 and 14% in Zuger jar 3 of mobile hatchery. For larvae hatched in the closed recirculation system, this rate was 22%.

## Discussion

SI In the experiment, temperature conditions in the mobile hatchery and their effect could be determined during the egg incubation period. Results show that water temperature in mobile hatchery is affected by the outside temperature, yet hatchery units provide adequate temperatures for developing eggs even at low air temperatures (Kupren et al., 2008). It was observed that there was a difference in daily heat input and average temperature between the differently located Zuger jars, but it did not affect hatching rate or body length of newly hatched larvae. In the experiment, mobile hatchery was compared with a closed recirculation system having a lower daily heat input. There was no significant difference in hatching rate, but the percentage of deformed larvae was lower in mobile hatchery. There was a statistically verifiable ( $P < 0.05$ ) difference in mean body length of larvae hatched in the closed recirculation system and the mobile hatchery. The difference was significantly higher only for Zuger jars 1 and 2 with higher heat input and the Zuger jar attached to the closed system. Besides all, results were found to be simi-

lar to those of Kupren et al. (2011). It was also found that the chub is well tolerant to changes in temperature conditions during the incubation period. While similarly to the Polish research group, no difference was found in hatching rate; however, the body length of freshly hatched larvae was statistically verifiably longer in Zuger jars with higher temperature fluctuations. Larger body size at hatching also affects initial feeding and growth, which may have a positive effect on individuals throughout their development. Based on the results of this research and those cited in literature review, it can be concluded that mobile hatcheries can offer a sustainable op-

tion for enhancing natural fish stocks and their temperature conditions are as expected.

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