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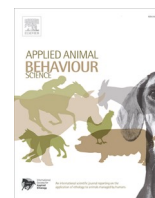
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## Evaluation of applying environmental enrichment to sterlets (*Acipenser ruthenus* L.) in early life stages

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### ABSTRACT

Traditional aquaculture farming conditions favour the development of maladaptive traits of reared fish. The hatchery releases are a crucial part of the population recovery action in sturgeon species. The manipulation of the environment could be one of the solutions to increase welfare and thus make the fish more adaptable to the changing post-release environment. The objective of our study was to compare the zootechnical parameters and behaviour of young sterlets *Acipenser ruthenus* reared in different culture conditions. Hatchery-like or bare (CTRL) and environmentally enriched (EE) conditions with small gravels were provided in duplicates in four aquariums. Following the rearing period, spatial exploration behaviour, swimming activity, stress coping, and boldness were analysed in different cross-maze tests. A bare maze was used with the absence of physical enrichment for the first test whereas for the second test, the environmental complexity was increased with the same type of gravel used in the rearing period. The fish from the EE conditions showed significantly lower mortality and greater survival ( $72.2 \pm 2.9\%$  vs.  $89.2 \pm 1.8\%$ ;  $P < 0.001$ ,  $\chi^2$  test) during the rearing period. Although they were significantly smaller throughout rearing, the EE fish did not have worse condition compared to the CTRL fish. The results also showed significantly better stress coping under different circumstances and elevated exploration behaviour assuming higher boldness in EE fish. In summary, the present study highlights the potential beneficial effects of using environmental enrichment on survival, behaviour and the overall welfare of juvenile sterlets.

### 1. Introduction

When habitat restoration is insufficient in conservation efforts, hatchery releases are considered the last resort to counter the effects of habitat degradation and over-fishing where *ex-situ* conservation has been recognized as a supplementary tool to save and maintain natural populations (Sousa-Santos et al., 2014; Mestanza-Ramón et al., 2020). Restocking programs have mainly focused on the impact of genetic processes on wild populations, however, the conditions of the rearing environments of fish for restocking purposes is garnering attention (Bergendahl et al., 2016; Carrera-García et al., 2016; Arechavala-Lopez et al., 2021). Due to the lifelong neural plasticity in brain neurogenesis

that occurs in fish, they can respond to changing social and environmental conditions. Namely, in a structured, stimulating environment fish can adapt their behaviour and physiology to cope with environmental challenges (Ebbesson and Braithwaite, 2012). Generally, farm environments are characterized by an absence of physical structure and sensory stimulation, relatively constant rearing conditions (Johnsson et al., 2014), and a lack of consideration for well-being in comparison to production, inevitably subjecting fish to several potential acute stressors until they reach the desired size for restocking. Consequently, artificial rearing environments may induce behavioural responses different from those considered normal in wild fish (Williams et al., 2009; Johnsson et al., 2014). Hatchery fish often perform poorly in the wild, choosing

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unfavourable habitats to settle, being unable to forage efficiently, having weak social skills and lacking of antipredator skills (Roberts et al., 2014). After release, individuals experience unpredictable and variable environments to which they are not accustomed, and consequently, acute and chronic stressors may suppress neural plasticity, leading to disease susceptibility and post-release mortality (Zhang et al., 2020). Many enrichment strategies have been proposed to cope with these issues and can reduce the prevalence of abnormal behaviour and stress (Arechavala-Lopez et al., 2021). Environmental enrichment is considered a strategy to increase fish welfare and naturalize the behaviour of released fish (Näslund and Johnsson, 2016). Physical enrichment generally aims to increase the heterogeneity and complexity of the rearing environment to provide the animal with more variable experiences (Näslund and Johnsson, 2016). Another relevant aspect of adding physical structure is modifications of fish activity, promoting exploratory behaviour, increasing swimming activity, resulting in higher ventilatory activity, ensuring the supply of oxygen and contributing to avoidance of oxidative stress at the cellular level, enhancing brain development and cognitive performance (Salvanes et al., 2013; Arechavala-Lopez et al., 2020). It might also contribute to reaching better growth parameters and survivorship of fish and subsequently improve their overall welfare and fitness-related traits (Carrera-García et al., 2016; Lee et al., 2018).

Different species and life stages need special consideration with respect to their natural history and preferences including light, water flow, and substrate (Arechavala-Lopez et al., 2020). The substrate may play an important role in the life histories of many riverine fish species, particularly during early life stages. Considering the dynamic ontogeny of *Acipenseridae* species, their substrate preference also changes rapidly during early development (Falahatkar and Shakoorian, 2011). Several studies have attempted to evaluate the effect of gravel rearing on sturgeons (Gessner et al., 2009; McAdam, 2011; Boucher et al., 2014), and the benefits of its application have been reported. In the last few decades, the importance of the recruitment of sturgeons has increased due to their worldwide population decline, which is mainly a result of anthropogenic activities (Bloesch et al., 2006; Lenhardt et al., 2006; Katopodis et al., 2019). The sterlet *Acipenser ruthenus* is a benthic, potamodromous, freshwater sturgeon species that usually inhabits large rivers and their tributaries (Peterson et al., 2006). Considering its conservation and socio-economic significance, the sterlet is artificially produced in 15 countries. However, the success of stock enhancement programs remains questionable (Mikheev et al., 2022). Despite the restocking measures, the sterlet is considered vulnerable by the IUCN (Kubala et al., 2021).

The response of an animal to an unfamiliar stimulus is essential for its survival, fitness and reproduction (Ferrari et al., 2015). The aim of the current study was therefore to evaluate if gravel substrate application as environmental enrichment affects growth, survival and other zootechnical parameters in young sterlets. Further, this study assessed the behavioural responses of fish using a maze apparatus (Colchen et al., 2017; Hope et al., 2019; Benvenuti et al., 2021) associated with the rearing condition with an emphasis on anxiety-like behaviours and boldness-exploration behavioural syndrome, and determined the correspondence between the behavioural variables.

## 2. Materials and methods

### 2.1. Experimental fish

The methods applied in this experiment were approved by the Institutional Animal Welfare Committee of the Hungarian University of Agricultural and Life Sciences Szent István Campus (license no. MATE-SZIC/7-1/2021). Fish management was accomplished by the regulation of the Animal Ethical Panel of the Research Center for Fisheries and Aquaculture established based on state law (10/1999.I.27). Fish specimens used in the trial were obtained via seasonal artificial propagation

of pond-reared sterlet breeders of the Research Center for Fisheries and Aquaculture, MATE AKI HAKI, Szarvas, Hungary in early April 2021. Broodstock handling and artificial propagation were carried out according to Ljubobratović et al. (2022). Fertilized eggs were incubated in Zug jars in a flow-through hatchery system at  $16.3 \pm 0.2$  °C. Six days after fertilization, the larvae hatched, which lasted for 24 hours. The swimming larvae were transported into the experimental system for pre-rearing for 23 days. On the day of transportation, the individual weight ( $10.74 \pm 1.0$  mg) and the individual total length ( $10.52 \pm 0.4$  mm) were measured for 31 larvae.

### 2.2. Experimental system and trial set

Three weeks before hatching, four experimental aquaria (100 cm × 40 cm × 40 cm, rearing volume of 150 L) were set up in a thermally controlled room with a natural photoperiod enabled through windows. The aquarium system consisted of an external filter (Oase FiltoSmart Thermo 100, Hörstel, Germany) that was formed for biological water treatment prior to fish stocking. A flow rate of 600 L per hour and additional aeration were supplied by an aerator compressor via two bubble stones per aquarium. The inflow pipes were positioned vertically at the same corner of the aquaria with the outflow enabling water circulation in the entire rearing unit.

Two sets of rearing conditions were tested in duplicated aquaria: control (CTRL) and enriched (EE). In EE units, 0.5–2 cm size gravels were stocked over the entire bottom, while in CTRL units bare aquaria remained. Upon hatching, a batch of larvae was stocked in EE or CTRL tanks where they were hand-fed with *Chironomus sp.* four times a day with 50% of the body weight. At 23 days-post-hatching (DPH) the trial was initiated and the larvae from each group were merged into a separate container with 272 randomly collected fish stocked per aquaria of the respective treatment. The trial set was conducted to accustom the fish to different rearing environments.

At the onset of the trial, 31 larvae per aquarium were measured for individual weight (CTRL =  $0.08 \pm 0.0$  g; EE =  $0.07 \pm 0.0$  g) and individual total length (CTRL =  $27.4 \pm 0.9$  mm; EE =  $25.8 \pm 0.1$  mm). Trials lasted for 44 days until the beginning of the behavioural testing that was performed from 68 DPH onwards. Fish were hand-fed bloodworms *Chironomus sp.* four times per day in four equal batches with 50% of the body weight of fish between 24 and 28 DPH. From 29 DPH until the end of the trial Larviva ProWean (pellet size 300 and 500 µm; crude composition protein 58%, lipid 12%, ash 11.1%, and cellulose 0.5%, Biomar®, France) and Coppens Advance (pellet size 500–800 µm; crude composition protein 56%, lipid 15%, ash 11.3%, and fibre 0.3%, Alltech Coppens®, The Netherlands) with 3% of the total biomass and supplemented with 10% of the biomass frozen bloodworms. Chemical water quality parameters of ammonium-nitrogen, nitrite-nitrogen and nitrate-nitrogen content were assessed twice per week and averaged in the CTRL treatment:  $0.41 \pm 0.4$  mg L<sup>-1</sup>;  $0.12 \pm 0.0$  mg L<sup>-1</sup> and  $2.95 \pm 0.9$  mg L<sup>-1</sup>, and in the EE treatment:  $0.22 \pm 0.1$  mg L<sup>-1</sup>;  $0.05 \pm 0.0$  mg L<sup>-1</sup> and  $2.43 \pm 0.7$  mg L<sup>-1</sup>. The water oxygen saturation ( $92.2 \pm 3.8\%$  and  $92.9 \pm 3.6\%$ ); the water temperature ( $18.7 \pm 1.1$  and  $18.8 \pm 1.1$ ) and the pH ( $8.1 \pm 0.1$  and  $8.2 \pm 0.1$ ) were recorded daily in CTRL and EE aquaria respectively. Significant differences were observed in the level of nitrite-nitrogen ( $P < 0.001$ ) and nitrate-nitrogen ( $P = 0.043$ ) being higher in the CTRL groups; however all the parameters were within the adequate range for sterlet juvenile rearing (Rónyai and Feledi, 2012; Kozłowski et al., 2014; Mihoc et al., 2021). Throughout the trial three water changes with a rate of 25% change per day were conducted. Aquaria were cleaned and mortalities were siphoned, counted and recorded twice per day, every morning and evening. Tank walls, sponges of external filters and pipes were cleaned twice per week.

Random subsamples ( $n=31$ ) were collected from each aquarium and were measured for weight and total length. In total, five samplings (23, 30, 37, 51, and 64 DPH) were performed throughout the rearing trial. Likewise, sample size measurements were made weekly before and after

the rearing trial. The sampled fish were euthanized with anesthetization in 2-phenoxyethanol diluted in water with a concentration of 2 mL/L before the measurements. At the end of the trial, all fish were counted. Considering the detected mortality as well as the number of survived fish, cannibalism was evaluated as a ratio of missing fish in the total number of stocked fish.

### 2.3. Test apparatus and testing protocol

The behavioural tests were carried out in a cross-maze made of 5 mm thin transparent plexiglass. The design of the maze was based on previous studies (Gould, 2010; Braida et al., 2014; Colchen et al., 2017) and has been slightly adapted and enlarged for the size of the juvenile sturgeon. During the test, the walls of the maze were covered. The maze consisted of a square start box (40×40 cm) which serves as an acclimatization zone and three smaller cross-shaped reward boxes. Toward both the right and left boxes called challenge boxes (CB), two 8-cm non-removable walls were placed challenging the fish to approach these reward boxes. The size of the maze was 154 cm long and 114 cm wide at its widest point and 10 cm high. The maze was virtually divided into ten compartments (Fig. 1). Two different mazes were used: a bare maze without any structures added, – Plain-maze test, at 84 DPH, and another one with small gravels (1–2 cm) on the bottom, – Gravel-maze test, at 91 DPH.

When the smaller group reached a body weight of at least 4 g (84 DPH), testing of the fish was initiated. In total 26 fish (13 fish per treatment) were randomly chosen from both treatments to engage in the first behavioural testing, and this operation was repeated in the second behavioural test as well. Individuals were gently removed from the

group of naïve fish using a large opaque plastic box to prevent causing further stress to the fish and transported directly to the maze apparatus located 2 m from the rearing unit. Each fish was run in a single session that lasted 10 minutes. The fish were then measured and returned to a separate, identical aquarium containing experienced fish that were not tested further. Between each session, the maze was emptied and refilled with aerated, temperature-matched freshwater due to the excretion of conspecific chemical cues. The maze was filled to a depth of 9 cm. A camera (SJCAM SJ4000 WI-FI, Shenzhen Zhencheng Technology Ltd., China) was mounted 1 m above the maze apparatus, and video recordings were made of each run.

To induce a searching behaviour, bloodworms (*Chironomus* sp.) were spread all over the area of the maze at the same location (three pieces in all the boxes and one piece in the centre of each zone) for each run.

### 2.4. Behaviour variables and zootechnical parameters

Relevant behaviours exhibited by the fish were defined by visual observation and encoded as behaviour variables. The entire video recordings of every individual were analysed. To assess boldness and exploration, the number of visits and time spent in different zones were measured: latency time to emerge from the start box (LES [s]) (Mazué et al., 2015; Naderi et al., 2016; Colchen et al., 2017) and the number of visits to challenge boxes (CB) of the left and right arms based on the idea of ‘number of arms visited’ in the study of Pasquet et al. (2016). To assess activity and exploration, we added the: total number of visited zones in ten minutes (TNVZ) (Pasquet et al., 2016; Colchen et al., 2017; Alnes et al., 2021). We assess stress as the time anxiety-like behaviour was exhibited such as duration of erratic movements and unusual

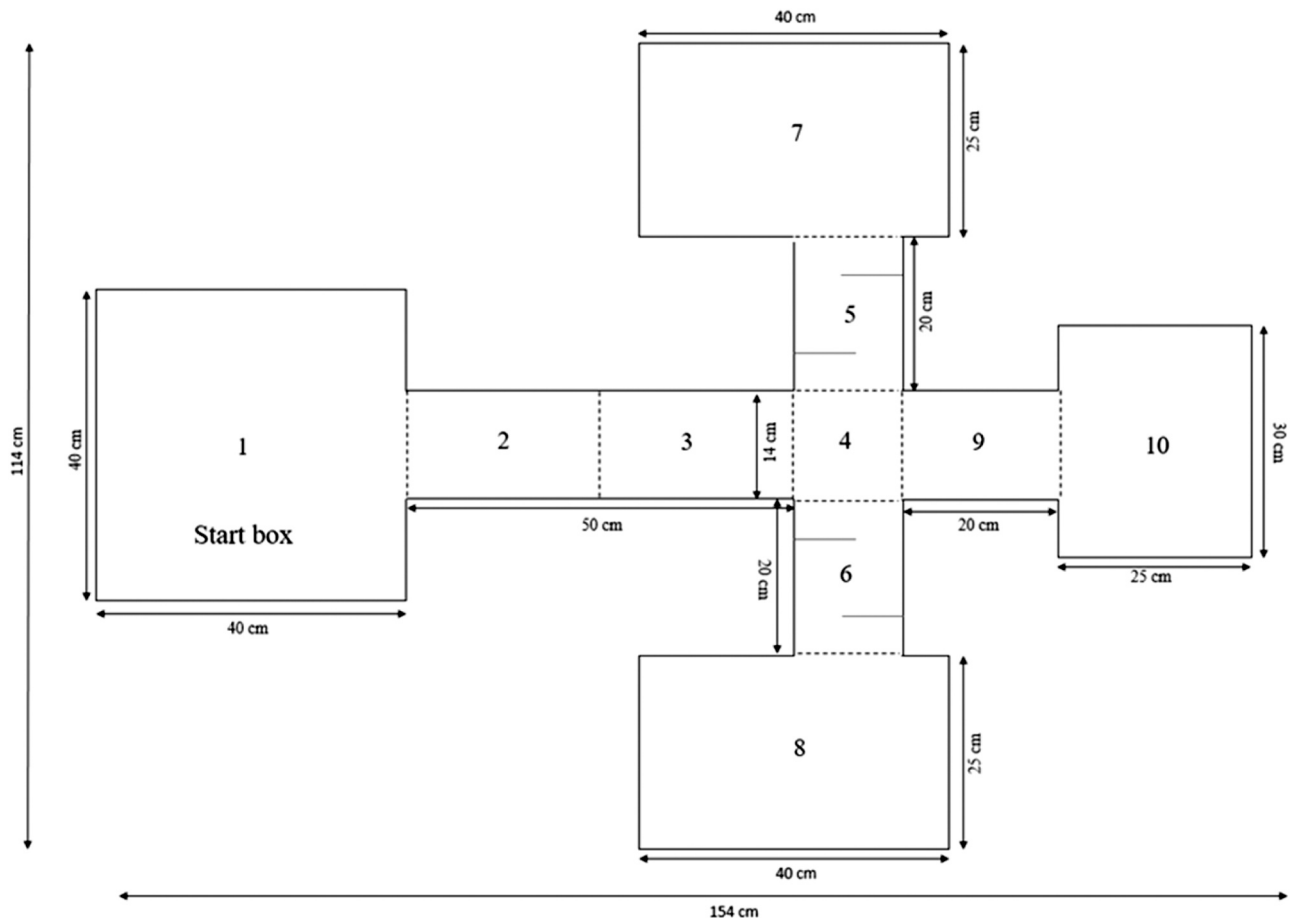


Fig. 1. Cross maze design and sizes with the number of compartments for the Plain-maze and Gravel-maze tests conducted in sterlet *Acipenser ruthenus* juveniles (used on 84 and 91 days post-hatching, respectively).

swimming patterns (S – stress [s]) (Cachat et al., 2010) and the duration of time spent immobile (F – freezing [s]) (Cachat et al., 2010; Pasquet et al., 2016; Alnes et al., 2021) (Table 1). Based on the assessed data, the following zootechnical parameters were analysed: initial and final body weight ( $BW_{i-f}$ , g), initial and final total length ( $TL_{i-f}$ , mm), coefficient of variation in initial and final body weight ( $CV_{BW_{i-f}}$ , %), total length ( $CV_{TL_{i-f}}$ , %), initial and final condition factor ( $CF_{i-f}$ ), survival (%), mortality (%), fish biomass gain (FBG,  $g\ l^{-1}$ ), specific growth rate (SGR,  $\% day^{-1}$ ) and cannibalism (%).

## 2.5. Statistical analyses

The Shapiro-Wilk test was used to test the normality of the distribution of some of the measured zootechnical (BW, TL, CF, Mortality) and all the behavioural variables (LES, CB, TNVZ, S, F). The F test was used for the analysis of variances. In the case of normal distributions and corresponding variances, a two-sample t-test was performed to examine the means ( $CF_i$ ,  $TL_i$ ,  $BW_8$ , F, TNVZ, nitrate-nitrogen) of each treatment to be compared, otherwise, the Mann-Whitney non-parametric test was used ( $BW_i$ ,  $BW_f$ ,  $TL_f$ ,  $CF_f$ ,  $BW_{15-91}$ , Mortality, LES, S, F, CB, ammonium-nitrogen, nitrite-nitrogen). A chi-square analysis was performed to compare the ratio of survival and cannibalism. Confidence interval overlapping (CI) was used to analyse the coefficient of variation in BW and TL, FBG and SGR. Multiple Spearman correlations were performed between behavioural and zootechnical variables ( $BW_{84,91}$ ) to find significant correlations. The data are presented as mean  $\pm$  standard deviation and differences were considered significant at  $P \leq 0.05$ . All statistical analyses were performed in the “R” (R Development Core Team, 2013) software environment.

## 3. Results

### 3.1. Growth and survival

The body weights of fish differed at every sampling point during the experimental period and were significantly less in the EE group including the  $BW_i$  ( $P < 0.001$ ) and  $BW_f$  ( $P < 0.001$ ) (Table 2, Fig. 2). Significant differences were also found in  $TL_i$  ( $27.4 \pm 0.9$  mm vs.  $25.8 \pm 0.1$  mm;  $P = 0.043$ ) and  $TL_f$  ( $59.1 \pm 5.9$  mm vs.  $47.2 \pm 1.3$  mm;  $P = 0.001$ ). Likewise, the two experimental groups significantly differed in the coefficient of variation in final body weight ( $79.0 \pm 10\%$  vs.  $36.0 \pm 10\%$ ; CI, no overlap) which was higher in the CTRL group. Finally, significantly higher survival ( $72.2 \pm 2.9\%$  vs.  $89.2 \pm 1.8\%$ ;  $P < 0.001$ ,  $\chi^2$  test) and lower detected mortality ( $19.3 \pm 3.4\%$  vs.  $5.3 \pm 1.3\%$ ;  $P < 0.001$ ) were noticed in the EE group (Table 2). Although, the statistical difference in cannibalism was not significant ( $8.46 \pm 0.5\%$  vs.  $5.51 \pm 0.5\%$ ;  $P = 0.178$ ,  $\chi^2$  test), more potential cannibals were found in the

**Table 1**

Description of the behavioural variables of interest analyzed during the Plain- and Gravel-maze test in juvenile sterlet *Acipenser ruthenus* reared under bare (CTRL) and enriched (EE) conditions.

Behaviour variables		Description
LES	Latency time	The time the fish spent in the start box before leaving it with the whole body.
TNVZ	Total number of visited zones	The number of zone changes during the 10-minute period of observation.
CB	Challenge boxes	The number of entries in the less accessible left and right reward boxes which the individual could access by passing two opaque walls.
S	Stress-related swimming behaviours	This is the total time that the fish exhibited abnormal swimming behaviour such as erratic movements (sudden, sharp, rapid change of position) and rapid circular swimming in a small radius.
F	Freezing-time	The total time the fish spent without any displacement during the 10-minute test period.

**Table 2**

Effect of environmental enrichment on zootechnical parameters in juvenile sterlet *Acipenser ruthenus* during the experimental period (23–64 days post-hatch, DPH) in bare (CTRL) and enriched (EE) environmental conditions.

Parameters	CTRL	EE
$BW_i$ (g)	$0.08 \pm 0.0^a$	$0.07 \pm 0.0^b$
$CV_{BW_i}$	$30.0 \pm 2.1$	$21.9 \pm 4.4$
$TL_i$ (mm)	$27.4 \pm 0.9^a$	$25.8 \pm 0.1^b$
$CV_{TL_i}$	$10.0 \pm 0.0$	$7.0 \pm 0.0$
$BW_f$ (g)	$0.9 \pm 0.3^a$	$0.6 \pm 0.1^b$
$CV_{BW_f}$ (%)	$56.5 \pm 7.3^a$	$44.9 \pm 0.7^b$
$TL_f$ (mm)	$59.1 \pm 5.9^a$	$47.2 \pm 1.3^b$
$CV_{TL_f}$ (%)	$22.0 \pm 0.0$	$13.0 \pm 0.0$
$CF_i$	$0.4 \pm 0.0$	$0.4 \pm 0.0$
$CF_f$	$0.5 \pm 0.0$	$0.5 \pm 0.0$
FBG ( $g\ l^{-1}$ )	$1.0 \pm 0.4$	$0.8 \pm 0.2$
SGR ( $\% day^{-1}$ )	$8.2 \pm 1.1$	$7.4 \pm 0.7$
Mortality (%)	$19.3 \pm 3.4^a$	$5.3 \pm 1.3^b$
Survival (%)	$72.2 \pm 2.9^a$	$89.2 \pm 1.8^b$
Cannibalism (%)	$8.5 \pm 0.5$	$5.5 \pm 0.5$

$BW_{i-f}$ , body weight at 23 DPH and final at 64 DPH;  $CV_{BW_{i-f}}$ , coefficient of variation at 23 DPH and final at 64 DPH;  $TL_{i-f}$ , total length at 23 DPH and final at 64 DPH;  $CV_{TL_{i-f}}$ , coefficient of variation in total length at 23 DPH and final at 64 DPH;  $CF_{i-f}$ , condition factor at 23 DPH and final at 64 DPH; FBG, fish biomass gain; SGR, specific growth rate.

Significant differences are marked with different letters in the superscript.

### CTRL group (Fig. 4).

### 3.2. Behavioural tests

During the Plain-maze test, the EE group spent significantly less time showing stress-related behaviour ( $165.2 \pm 144.1$  s vs.  $55.4 \pm 102.6$  s;  $P = 0.015$ ). The challenge boxes were visited significantly less often by the individuals from the CTRL group compared with EE fish ( $0.8 \pm 1.1$  vs.  $3.2 \pm 3.4$ ;  $P = 0.014$ ). Finally, the body weights of the individuals involved in the first test were significantly higher in the CTRL group (Fig. 3). Like the Plain-maze test, significance was also found in the Gravel-maze test in the duration of stress-related behaviour with shorter periods in the EE group ( $275.8 \pm 197.4$  s vs.  $96.1 \pm 118.3$  s;  $P = 0.024$ ) while there were no significant differences in the other variables (Table 3).

### 3.3. Correlations between variables

Correlations between all behavioural variables were examined separately for different maze tests and treatment groups (Table 4). Fish that were placed in different test environments than their rearing environment revealed more correlation between variables.

During the Plain-maze test, fish from the CTRL group that took a longer time to emerge from the start box yielded a lower total number of visited zones, and moreover, the total number of visited zones positively correlated with body weight ( $r_s = 0.59$ ;  $P = 0.036$ ). In the EE group, individuals visited challenge reward boxes (CB) more often as was shown by the significant positive correlation between the total number of visited zones and the visits to challenge boxes. Like the CTRL fish, bodyweight in EE fish was positively correlated with the total number of visited zones and negatively with freezing time (Table 4). The number of visited zones was also negatively correlated with freezing time, and stress-related swimming behaviour ( $r_s = -0.59$ ;  $P = 0.033$ ). Body weight was correlated with neither latency time to emerge from the start box nor stress-related behaviours.

Time to emerge from the start box was negatively correlated with body weight in CTRL fish in the Gravel-maze test. Likewise, in the CTRL group, fish that spent more time showing stress-related swimming behaviour showed a lower number of total visited zones ( $r_s = -0.660$ ;  $P = 0.020$ ) and more time spent in the start box. In line with this, the total number of visited zones was negatively correlated with freezing time



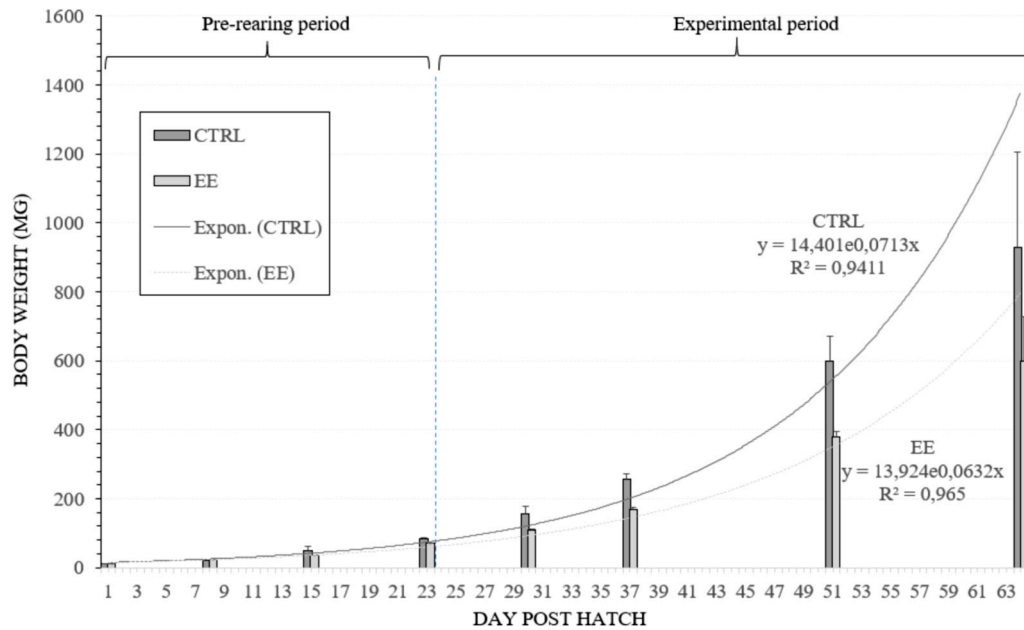


Fig. 2. Average body weight and exponential growth line of sterlet *Acipenser ruthenus* juveniles reared in bare (CTRL) and enriched (EE) environmental conditions during the pre-rearing (1–23 days post-hatching (DPH)) and experimental (23–64 DPH) periods. Significant differences occurred at every sampling point except the initial.

and latency time to emerge from the start box. In the EE-fish, body weight was also positively correlated with the total number of visited zones ( $r_s = 0.62$ ;  $P = 0.042$ ) (Table 4).

#### 4. Discussion

Hatcheries serve as key elements in sturgeon recovery plans. However, the success of hatchery-reared sturgeon in natural waters ultimately depends on the capabilities of adaptation. The stimulus-poor environment is considered a major contributor to developing weak fitness traits and a high mortality rate after release (Cámara-Ruiz et al., 2019). Our overall results reveal a strong relationship between overall sterlet performance and rearing environment. Fish of significantly lower weight exposed to spatial heterogeneity after a pre-rearing and rearing period without negatively influencing the condition factor implies that enriched-reared fish were perhaps forced to swim more searching for food among the gravels or the environmental enrichment made it harder for the fish to access the food, thus, showing higher swimming activity. These results agree with studies on juvenile North Sea cod *Gadus morhua* and zebrafish where fish reared with experiences of environmental variability were smaller than those under conventional ‘hatchery’ conditions (Braithwaite and Slavanes., 2005; Spence et al., 2011). Nevertheless, Boucher et al. (2018) found that the fish reared on gravel were significantly larger at every sampling time during their experiment conducted on larval white sturgeon *Acipenser transmontanus*. In the present study, mortality was significantly lower in EE groups, respectively, indicating that the gravels might serve as a shelter from possible cannibalistic behaviour for the fish during the experiment. Although not significant, cannibalism type II where the smaller sibling was consumed whole (Hecht and Appelbaum, 1988; Smith and Reay, 1991; Baras and Jobling, 2002) was higher in the CTRL group, indicating that the cannibalistic behaviour might be the reason for higher detected mortality in CTRL groups. Similar to our outcome, higher survival rate was obtained in enriched groups in zebrafish and white sturgeon *A. transmontanus* larvae (Boucher et al., 2014; Lee et al., 2018). However, the study of Carrera-García et al. (2016) in European sturgeon *A. sturio* shows that fish in enriched conditions tended to grow better without differences in survival or mortality between the groups. Therefore, the species-specific response to environmentally enriched

rearing conditions seems to occur in sturgeons.

Differences between treatments showed higher stress-related swimming behaviour in individuals reared under conventional hatchery conditions. Although anxiety-like swimming behaviour was observed in both groups, the individuals of the EE-group showed less unusual swimming behavioural patterns including erratic and frustration-induced stereotypic movements (Mason et al., 2007). Environmental enrichment may reduce stress response, enhance stress coping and stimulate exploratory behaviour (Fox et al., 2006; Alnes et al., 2021). Due to the behavioural flexibility caused by structural enrichment, environmentally enriched-reared juvenile North Sea cod featured faster recovery from stressful experiences induced by novelty (Braithwaite and Slavanes., 2005). Fast recovery from a stressful experience might be beneficial in the wild in terms of competition for food and less risk to the health of the fish (Krause et al., 1998). The release event is also likely a stressful process for the fish. In *Salmonidae*, the reduction of antipredator behaviour generated by acute stress was observed for a while, so increased shelter-seeking behaviour as a result of environmental enrichment could be advantageous for the immediate survival of hatchery-reared fish (Aarestrup et al., 2005; Roberts et al., 2011; Näslund et al., 2013). In addition, structural enrichment was found to maintain lower basal cortisol levels in specimens reared under environmentally enriched conditions during rearing in general (Näslund et al., 2013; Rosengren et al., 2017; Zhang et al., 2020) and have likely greater resilience to stressful events. Thus, the cortisol level in Chinook salmon *Oncorhynchus tshawytscha* reared with structures was lower compared to barren-reared fish during a stress treatment and even returned to a baseline level by several hours post-stress while the control treatment could not fully recover (Cogliati et al., 2019). Although the stress behaviour seems to be reduced in EE fish, the physiology was not the subject of the present study and could be of interest for future studies on sterlets and other sturgeon species.

During the Plain-maze test, both challenge boxes were visited by the EE fish more often. Exploratory behaviour can be increased by structural enrichment and reduce the neophobic response to the novelty (Brydges and Braithwaite, 2009; Tatamoto et al., 2021; Gatto et al., 2022). In addition, environmental enrichment might contribute to the development of bold phenotype related to genes involved in stress hormone production in zebrafish (Oswald et al., 2012). Likewise, in convict

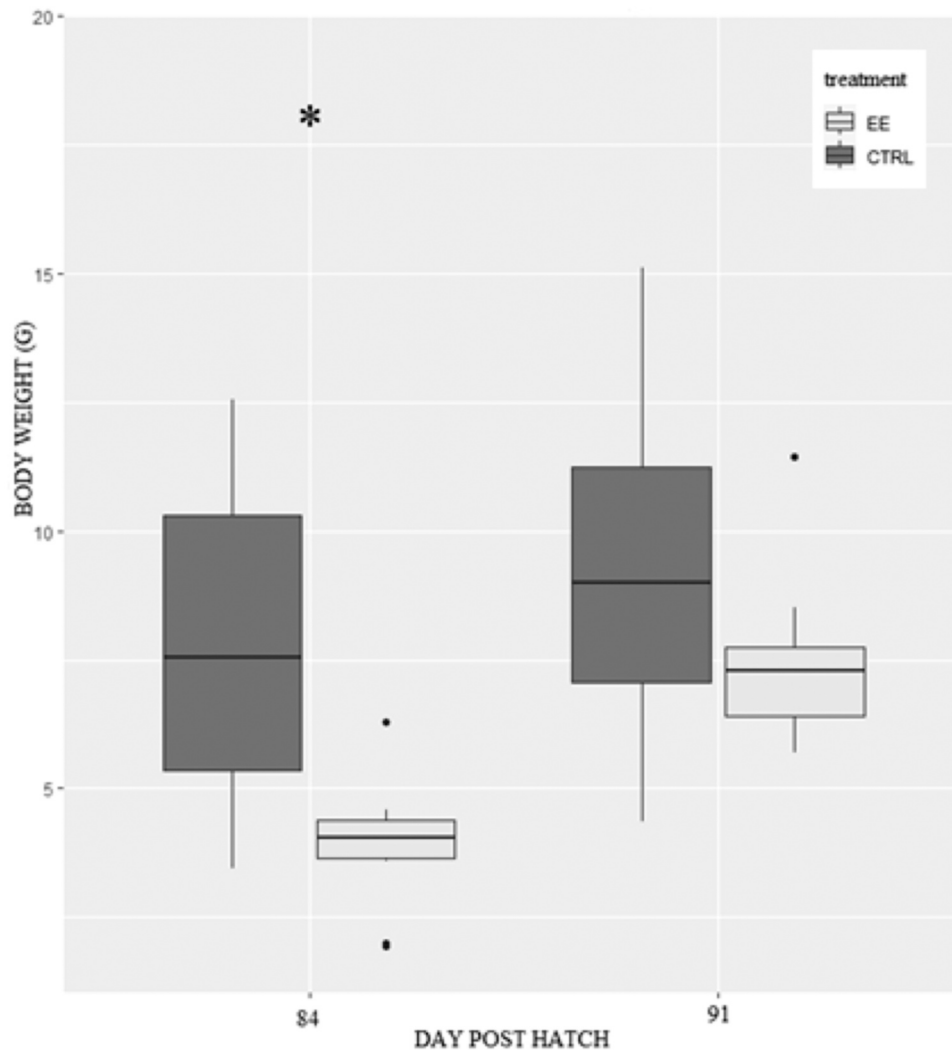


Fig. 3. Average body weight of sterlets *Acipenser ruthenus* juveniles reared in bare (CTRL) and enriched (EE) environmental conditions during the Plain-maze test (84 days post-hatching [DPH]) and Gravel-maze test period (91 DPH). Significant differences are marked with \* in the superscript.

cichlids *Amatitlania siquia*, bolder individuals explored more than timid ones (Mazué et al., 2015). Boldness and exploratory activity commonly form a behavioural syndrome in fish (Wisenden et al., 2011). Another explanation for more willingness to explore the novel plain environment can be related to elevated shelter-seeking behaviour (Braithwaite and Slavanes., 2005). In our study, the enriched-reared sterlets were accustomed to the use of interstitial space provided by the gravel substrate (Kynard et al., 2013). The utilization of enrichment is proved by the smaller body size and better survival of the fish at the end of the rearing period (Gessner et al., 2009). This hypothesis can be supported by the fact that we did not find any significant differences between the groups in spatial movement in the gravel-enriched maze, suggesting that the EE fish under partially familiar conditions did not show outstanding exploration behaviour which may indicate higher shelter-seeking. Slavanes et al. (2007) reported a great ability of varying the group behaviour of enriched-reared juvenile cod with respect to the use of space in contrast to barren-reared fish due to behavioural flexibility. On the other hand, no differences between the groups were noticed in either test for LES which is a commonly used proxy of boldness and exploratory behaviour (Colchen et al., 2017; Alnes et al., 2021). Some studies suggest that latency time is an indicator of motivation to engage in the test and explore the novelty rather than boldness and exploration (Bergendahl et al., 2016; Carbia and Brown, 2019). However, in our study, CTRL fish that left the start zone earlier showed less stress-related swimming

behaviour. The exact reason for the motivation of fish to explore a novel area is usually unclear and complex to determine due to its context-dependency and species specificity (Braithwaite and Slavanes., 2005). Different results are reported depending on the species and the form of environmental enrichment in terms of latency time before leaving the start zone. Juvenile cod reared in a structured-unstable environment but without predation risk left the start box more quickly to explore the novel area than barren-reared conspecifics (Braithwaite and Slavanes., 2005). Roberts et al. (2011) found that juvenile Atlantic salmon *Salmo salar* from enriched environments with threat of predation took a longer time to leave the acclimatization zone and chose a less risky strategy than hatchery-reared fish. Also, enriched-reared intertidal gobies *Bathygobius cocosensis* spent more time before leaving the start box than fish from a simple sand treatment but had better performance in spatial learning (Carbia and Brown, 2019). However, the enriched-reared mahseer *Tor putitora* and rainbow trout *Oncorhynchus mykiss* exited the start box significantly sooner than barren-reared conspecifics (Bergendahl et al., 2016; Ullah et al., 2017). Providing the suitable enrichment strategy in fish during rearing might be beneficial regarding risk-taking behaviour, as juvenile steelhead showed the ability to modify and adapt behaviour to a changed environmental circumstance developing their survival skills (Lee and Berejikan, 2008). Thus, sterlet juveniles from the EE group are considered more motivated to explore the novel environment than the 'hatchery-like-reared'

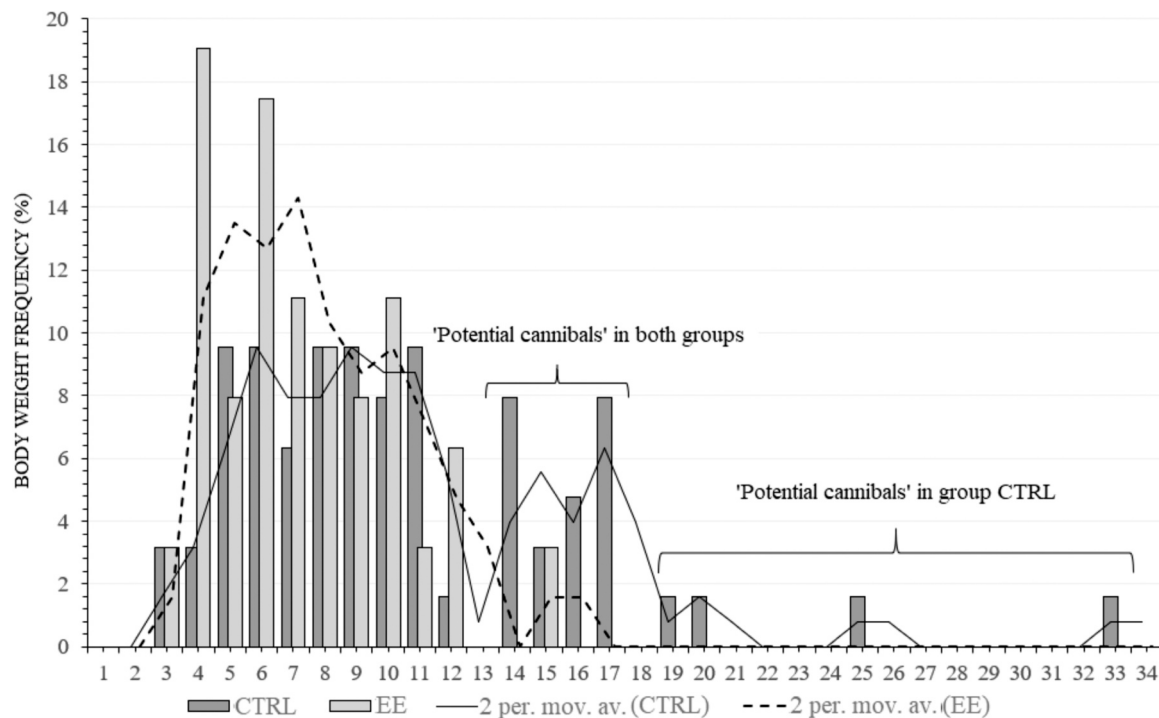


Fig. 4. Histogram-diagram of the last sampling of the sterlet *Acipenser ruthenus* juveniles reared in bare (n=31, CTRL) and environmental enriched (n=31, EE) conditions based on the final body weight ( $BW_t$ , at 64 days post-hatching) assuming the potential cannibals in both groups.

Table 3

Behavioural variables and body weights at 84 and 91 days post-hatch (DPH) sterlets *Acipenser ruthenus* reared in either bare (CTRL) or enriched (EE) environment involved in Plain-maze test performed in bare maze and in Gravel-maze test performed in gravel-enriched maze, respectively.

Variables	Plain-maze test			Gravel-maze test		
	CTRL	EE	p-value	CTRL	EE	p-value
LES (s)	93.0 ± 107.7	49.5 ± 60.2	0.095	216.5 ± 234.1	105.5 ± 78.6	0.411
F time (s)	14.1 ± 35.8	47.2 ± 92.4	0.139	30.5 ± 64.6	2.2 ± 8.0	0.117
S (s)	165.2 ± 144.1 <sup>a</sup>	55.4 ± 102.6 <sup>b</sup>	0.015	275.8 ± 197.4 <sup>a</sup>	96.1 ± 118.3 <sup>b</sup>	0.024
TNVZ	37.2 ± 23.0	35.5 ± 22.2	0.875	13.3 ± 12.3	19.5 ± 10.9	0.184
CB	0.8 ± 1.1 <sup>a</sup>	3.2 ± 3.4 <sup>b</sup>	0.014	0.7 ± 0.9	0.7 ± 0.8	0.822
BW <sub>84,92</sub> (g)	7.7 ± 3.1 <sup>a</sup>	3.9 ± 1.1 <sup>b</sup>	0.004	10.1 ± 4.4	7.4 ± 1.5	0.072

LES, Latency time to Emerge from Start box; F-time, Freezing time; S, Stress-related swimming behaviour; TNVZ, Total Number of Visited Zones; CB, Challenge Boxes; BW<sub>84,92</sub>, Body Weight at 84 DPH and 92 DPH. Significant differences are marked with different letters in the superscript.

conspecifics which could be favourable upon restocking.

Considering individual weight, CTRL individuals were significantly larger than EE fish during the Plain-maze test, but not in the Gravel-maze test. In both tests and treatments we found that body weight was positively correlated with the total number of visited zones, which was a good proxy for activity. According to the study of Millot et al. (2009), bolder personalities and better environmental adaptations were found in bigger fish as a result of hatchery selection compared to hatchery-reared conspecifics of wild-caught parents. Moreover, bigger fish are considered more reckless in rainbow trout (Johnsson, 1993). Similar patterns are visible in the current study through correlations within the treatment groups associated with the two tests performed. Although, the fish

from the EE group were significantly smaller no difference was found in activity compared to the CTRL group. Our results also agree with the study of Braitwhaite and Salvanes et al. (2005) where juvenile North Sea cod were bolder after rearing under spatial heterogeneity despite their smaller body size than traditionally reared bigger cod. Carbia and Brown (2019) found that smaller intertidal gobies were bolder or more motivated compared to the bigger individuals, but not only from the more complex environment. In the present study, size had a greater impact on behaviour within the treatments while between the two treatment groups, behaviour was more influenced by environmental enrichment over the body size of the sterlet.

## 5. Conclusions

In conclusion, our findings suggest that exposing sterlets to a heterogeneous environment during early life rearing promotes stress coping and might enhance exploration behaviour. These traits can be vital for immediate survival and long-term post-release success in natural waters. Decreased stress response, equal condition factor, better survival and lower mortality in fish from enriched backgrounds suggest that the application of suitable environmental enrichment might increase the overall welfare in juvenile sterlets.

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## CRediT authorship contribution statement

**Georgina Fazekas:** Conceptualization, Methodology, Investigation, Formal analysis, Writing – original draft. **Tamás Müller:** Writing – review & editing, Formal analysis, Visualization. **Jelena Stanivuk:** Writing – review & editing, Investigation. **Dorottya Lilla Fazekas:** Writing – review & editing, Methodology, Resources. **Jenő Káldy:** Writing – review & editing. **Flórián Tóth:** Formal analysis, Writing –



**Table 4**

Significant Spearman's correlations (Rho) between variables of Plain-maze and Gravel-maze tests in both group of juvenile sterlet *Acipenser ruthenus* reared under bare (CTRL, 84 days post-hatching (DPH)) and enriched (EE, 91 DPH) conditions.

Plain-maze test				EE			
CTRL				EE			
variable 1	variable 2	Spearman's rho	p-value	variable 1	variable 2	Spearman's rho	p-value
BW	TNVZ	0.59	0.036	BW	TNVZ	0.64	0.017
LES	TNVZ	-0.58	0.040	CB	TNVZ	0.68	0.011
				TNVZ	F	-0.59	0.033
				TNVZ	S	-0.59	0.033
				BW	F	-0.07	0.010
Gravel-maze test				EE			
CTRL				EE			
variable 1	variable 2	Spearman's rho	p-value	variable 1	variable 2	Spearman's rho	p-value
BW	TNVZ	0.56	0.047	BW	TNVZ	0.62	0.025
LES	S	0.58	0.038				
BW	CB	0.64	0.018				
TNVZ	F	-0.58	0.036				
TNVZ	S	-0.71	0.007				
LES	TNVZ	-0.56	0.048				
LES	BW	-0.77	0.002				

LES: Latency time to Emerge from Start box; F: Freezing time; S: Stress related swimming behaviour; TNVZ: Total Number of Visited Zones; CB: Challenge Boxes; BW: Body Weight.

review & editing. **József Bürgés:** Investigation. **Tatiana Colchen:** Investigation, Validation, Writing – review & editing. **Norbert Vass:** Investigation. **Uroš Ljubobratović:** Conceptualization, Supervision, Writing – review & editing.

#### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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#### Contributions

Each author declares substantial contributions through the following:

- (1) the conception and design of the study, or acquisition of data, or analysis and interpretation of data,
- (2) drafting the article or revising it critically for important intellectual content,

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