MAGYAR AGRÁR- ÉS
ÉLETTUDOMÁNYI EGYETEM

Hungarian University of Agriculture and Life Sciences

Investigating the effects of environmental factors and feeding strategies on the development and behaviour of Russian sturgeon (*Acipenser gueldenstaedtii*) and sterlet (*Acipenser ruthenus*) during their early life stages.

Doctoral (PhD) thesis

GEORGINA LEA FAZEKAS

Gödöllő
2025

Doctoral School

Name:	Doctoral School of Agricultural and Food Sciences
Discipline:	Animal reproduction sciences
Head:	Prof. Dr. Kovács Melinda University professor, PhD
Supervisors:	Dr. Uroš Rade Ljubobratović Senior research fellow, PhD Hungarian University of Agriculture and Life Sciences, MATE Institute of Aquaculture and Environmental Safety Research Center for Fisheries and Aquaculture
	Prof. Dr. Müller Tamás University professor, PhD Hungarian University of Agriculture and Life Sciences, MATE Institute of Aquaculture and Environmental Safety Department of Fishery Research and Development

....

Approval of the head of the doctoral school

....	....
Approval of the supervisor	Approval of the supervisor

Contents

1. Introduction and objectives

Aquculture is the most dynamically developing sector of global food production, playing a major role in sustainable protein production and reducing fishing pressure on natural fiah populations (Boyd et al., 2022). There is an increasing focus. ont he controlled breeding of endangered and economically significant species, particularly those whose populations have declined significantly doue to anthropogenic impacts and environmental changes (Elhetawy et al., 2023).

The sturgeons (Acipenseridae) are of significant economic, biological and ecological importance. Consequently, concerted global efforts are being made to ensure the sustainable exploitation of their stocks. Concurrently, owing to their heightened sensitivity during the early life stages of life, it is crucial to optimise their rearing conditions.

The majority of populations of sturgeons have either disappeared or declined to a critical point, therefore, breeding and conservation programs have been initiated for the Russian sturgeons (*Acipenser gueldenstaedtii*) and sterlet (*A. ruthenus*), among other species. The Russian sturgeon has become a strategically improtant species primarily as a result of conservation breeding and reintroduction programmes, while the sterlet has become important due to intensive aquaculture and fishing activities. Nevertheless, the success of sturgeon farming is dependent upon the refinement of environmental and technological parameters applied during the developmental stages.

The objective of this study was to ascertain the impact of varying stocking densities on the growth and survival rates of sterlet from hatching to the conclusion of the weaning period. Furthermore, present study aimed to determine the effect of gravel substrate as a form of environmental enrichment on the growth, survival, and ultimately behavioural indicators of sterlet fry through the use of a maze apparatuses with a particular emphasis in the examintaion of anxiety-like behaviour and exploratory behaviour. The objective of the present study was to determine the most optimal feeding strategy in terms of production and restocking during the rearing of russian sturgeon larvae and fry. The startegy was to be combined with the technique of environmental enrichment in the rearing units. The present study also aimed to examine the effects of these factors on the production and behavioural indicators of Russian sturgeon, through the use of a maze device.

2. Material and methods

2.1. The experimental fish

The live stock were used during the present study is obtained from artificial propagation of the broodstock of the MATE Research Center for Fisheries and Aquculture, Szarvas.

2.2. Examination of stocking density on the growth and survival of sterlet larvae (A. ruthenus) – experimental system and experimental settings

The propagation of sterlet broodstocks (12 females, 12 males) took place in April of 2020. The fertilized eggs were incubated at 16,6 ± 0,3 °C. After hatching, fish (10,08 ± 1,5 mg and (9,53 ± 0,5 mm) were randomly chosen and transported to the experimental system. The trial involved the examination of three distinct stocking densities withhin a toal of six rearing tanks, each with a capacity of 250 litres and comprising two replicates. The experiment spanned a duration of 30 days. The selected rearing densities were: low stocking density was set at 5 larvae/liter, the medium and high stocking density were at 10 and 20 larvae/liter, respectively. This resulted in 1250, 2500, and 5000 larvae being randomly distributed within each rearing unit. During the sampling process (n =5; on days post hatching 1, 8, 13, 23 and 30), the total body length and individual body weight of 31 randomly selected larvae per tank were also determined. At the termination of the experiment, the final body weight and body length were ascertained, along with their coefficient of variation (CV_{BW}, %; CV_{TL}, %), the condition factor (K), the survival rate (S, %), the mortality rate (%), the specific growth rate (SGR, %/day), and the biomass yield (FBG, g/l). The dissolved oxygen saturation of the water (101.1% ± 1.22%) and the water temperature (18.1 ± 0.4°C) were recorded on a daily basis. The chemical water quality parameters - ammonium nitrogen (0.11 ± 0.02 mg/l), nitrite nitrogen (0.02 ± 0.01 mg/l), nitrate nitrogen (2.79 ± 1.99 mg/l) content and pH (8.33 ± 0.13) – were examined on a twice-weekly basis.

2.2.1. The feeding schedule of sterlet larvae

Under the given rearing conditions, the newly hatched sterlet larve fed exlusively on endogenous sources for a period of approximately seven days. From the 8th day after hatching, freshly hatched *Artemia* sp. nauplius and finely chopped frozen *Chironomus* sp. larvae were offered to the larvae in the amount of 100 % of the total biomass. The larvae were offered eight times a day initially, and six times a day until they reached 12 days of age. At day 13, a co-feeding regime was initiated, comprising frozen Chironomus larvae and commercial feed (Aller Futura Ex 0 gr, 0.3-0.6 mm and Aller Infa 0.4 mm). Concurrently, the proportion of natural food was methodically reduced in favor of dry feed. The duration of co-feedong period lasted 11 days (13–24 days post-hatching (DPH)). During the initial four day of co-feeding period, the fish were provided with inert feed equivalent to 5 % of their body weight, as well as 50 % Chironomus larvae. Thereafter, the quantity of of dry feed was augmented to 10 % of body weight, whilst the the amount of bloodworms was diminished 5 % per day. Commencing on the 17 DPH, a mechanical feeder was utilised to deliver dry feed to the rearing tanks at 18-hour feeding intervals (from 9 a.m. to 3 a.m.). From day 25 onwarsd, the fish were fed exclusively on dry feed, with the quantity of feed corresponding to 7 % of their body weight. The feeding interval remained unchanged at 18 hours.

One-way ANOVA was utilised to detect statistical differences. The conditions for the analysis of variance were met for all the parameters that were examined. Pearson's correlation coefficient was performed to assess the relationship between stocking density and the parameters of interest. In the case of significant significant differences, Tukey's post-hoc test was employed to further analysis.

2.3. Effect of environmental enrichment on the growth, survival and behavioural parameters of sterlet – experimental system and experimental settings

The trial was conducted in the spring of 2021. Five male and six female broodstock were utilised for the purpose of reproduction. The fertilized eggs were incubated at a water temperature of 16.3 ± 0.2 °C. Four aquariums were specifically designed for experimental settings (100 cm x 40 cm x 40 cm; 150 liters of volume). The photoperiod was maintained naturally throughout the experiment, ensuring approximately 14 hours pf daylight and 10 hours of darkness (14 L:10D). A seperate external filter with a capacity of 600 liter/hours was installed for each aquarium. The free-swimming larvae were transferred tot he experimental aquarium system for 23 day-period of pre-rearing. At the time of transfer, the individual weight (10.74 ± 1.0 mg) and total length (10.52 ± 0.4 mm) of 31 randomly selected larvae were measured. Two different rearing condition were tested: a control environment without any sturctures (CTRL) and an enriched environment (EE). The bottom of the EE tanks was covered with a 5 cm layer of cleaned gravel (white gravel 8-16 mm diameter in size, SZAT P-4), while the CTRL units provided a traditional aquaculture conditions without substrate. Each group was replicated twice. During the pre-rearing stage, the sterlet larvae were stocked at a density of 6.6 larvae/liter. The chemical water quality parameters were the subject of two weekly tests. In the CTRL treatment, the levels of ammonium nitrogen, nitrite nitrogen, and nitrate nitrogen were recorded as 0.41 ± 0.4 mg/l, 0.12 ± 0.0 mg/l, and 2.95 ± 0.9 mg/l, respectively. In the EE treatment, the levels were recorded as 0.22 ± 0.1 mg/l, 0.05 ± 0.0 mg/l, and 2.43 ± 0.7 mg/l, respectively. The mean oxygen saturation levels were in the CTRL were 92.2 ± 3.8% and 92.9 ± 3.6% in the EE treatments. The water temperature was recorded as 18.7 ± 1.1 and 18.8 ± 1.1°C in the CTRL and EE groups, respectively, and the pH was determined to be 8.1 ± 0.1 and 8.2 ± 0.1. In order to maintain water quality, three partial water changes were performed on a daily basis, with 25 % of the total water volume being replaced. The aquariums were cleaned twice a day. Furthermore, the walls, external filter sponges and pipes were subjected to a twice-weekly cleaning regime. The number of dead fish were counted and recorded.

Following the pre-rearing stage, on day 23 DPH, the stocks reared under identical conditions were mixed, and 272 randomly selected fish were transferred back to the appropriate experimental aquariums. The initial body weight in the CTRL group was recorded as 0.08 ± 0.0 g, and 0.07 ± 0.0 g in the EE group, while the total body length was measured at 27.4 ± 0.9 mm (CTRL) and 25.8 ± 0.1 mm

(EE). The experiment continued for a further 44 days. During the trial, control measurements were performed on five occasions (on days 23, 30, 37, 51, and 64) to ascertain body weight and total body length. For the purpose of the measurements, 31 fish were selected at random and anaesthetised in a 0.4 ml/l aqueous solution of 2-phenoxyethanol prior to measurement. At the end of the trial, the initial and final body weight and total body length of the fish were measured, as well as their coefficient of variation (CVBWi-f, %; CVTLi-f, %). The initital and final condition factor (Ki-f), mortality (%) and survival (%) rates, fish biomass gain (FBG, g/l), specific growth rate (SGR, %/day), and cannibalism rate (%) were also determined.

2.3.1. Description of feeding practices for sterlet reared in different conditions

During the pre-rearing period, the fish were fed *Artemia* and chopped *Chironomus* larvae in quantities corresponding to 50-50 % of their body weight six times a day until the 16th day after hatching. Thererafter, the fry were fed exclusively chopped *Chironomus* larvae at rate of 50 % of their biomass four times daily. During the axperimental phase, on days 24-28 after hatching, the fish continued to be fed frozen bloodworms in amount of 50 % of their body weight. From day 29 until the conclusion of the experiemnt, two types of dry feed were administered tot he fish: Larviva ProWean and Coppens Advance, while gradually reducing the amount of frozen *Chironomus* to 10 %. The dry feed constituted 3% of the total biomass, while the frozen mosquito larvae accounted for 10%.

2.3.2. Effect of environmental enrichment ont he behaviour of sterlet

2.3.2.1. Presentation of the maze and test protocol

The behavioural tests were carried-out in a 5 mm-thick, transparent plexiglass cross-shaped maze. The structure comprised a square 40 x 40 cm start zone, which functioned as an acclimatisation zone, and three smaller reward zones located at the end of corridors. The corridors leading to the right and left zones (CB), two 8-cm non-removable walls were placed challenging the fish to approach these reward boxes. The walls of the maze was 10 cm height, 154 cm long and 114 cm wide at its widest point. The maze was virtually divided into 10 compartments, facilitating video analysis (Figure 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. The tests were conducted over two consecutive weeks. 13 fish were selected for each treatment in the behavioural tests. The fish were placed in the start zone and allowed to explore the maze for ten minutes. After the tests, the individual body weight were measured. All the water were replaced between each session. All tests were video recorded. To induce a searching behaviour, *Chironomus* larvae were spread all over the maze are of the same location: three pieces in all boxes and one piece in the centre of each zone.

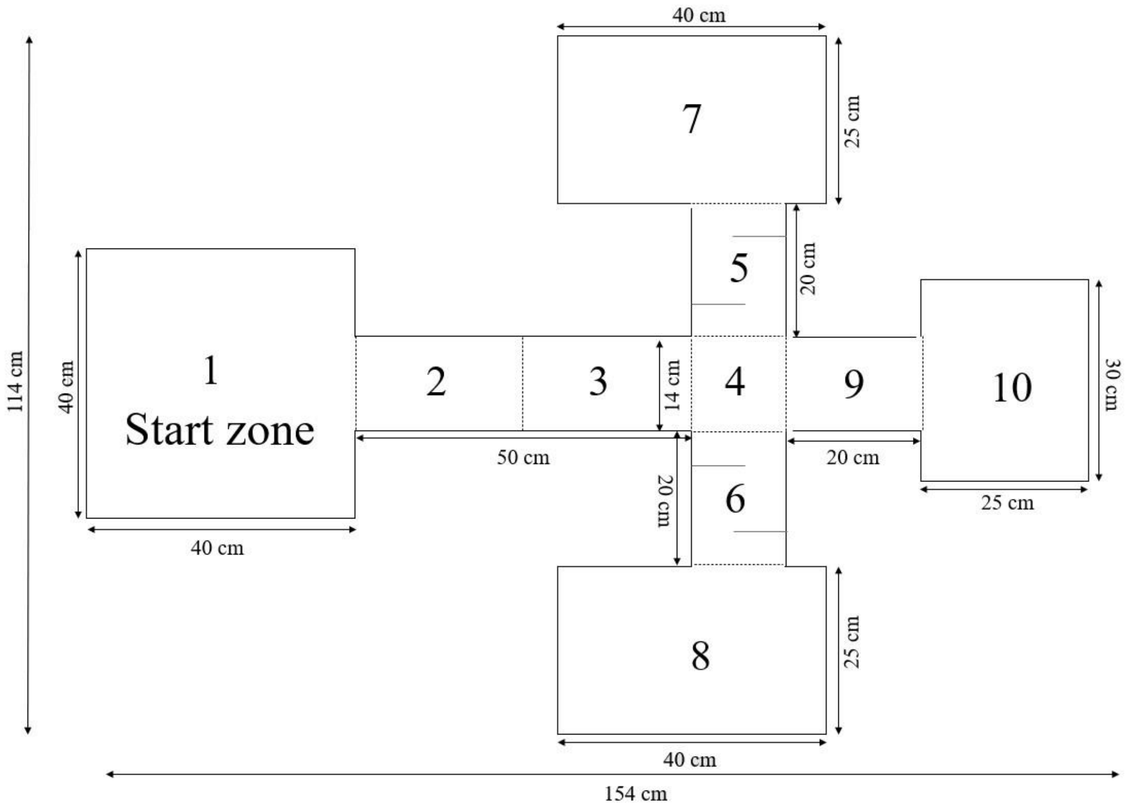

Figure 1. The design and a size of the cross maze used for the Plain-maze test and Gravel-maze tests on sterlet (*A. ruthenus*) fry (84 and 91 DPH), as well as the number of compartments.

2.3.2.2. Presentation of the behavioural variables examined in the case of sterlet fry

The ethological indicators of the fish were determined through visual observation and coded as predefined behavioural variables. In order to assess the level of „boldness" and „exploratory behaviour", the number of visits to each zone was measured, as was the time spent in each zone. The following variables were examined: latency time to leave tje start zone (LES [s]), number of visits to challenging zones on the left and right reward zones (CB), total number of visited zones in ten minutes (TNVZ), stress-related swimming bahvaiors („unpredictable movements" and „unusual swimming patterns", S – stress [s]), and „time spent immobile" (F – freezing [s]).

All statistical analyses were performed using the software „R" (R Development Core Team, 2013). In instances of normal distribution and equal variances, a two-sample t-test was employed to analyse the means of treatments under comparison. Conversely, The Mann-Whitney nonparametric test was utilised. Chi-square analysis and confidence interval (CI) overlap were performed where appropriate, and differences were considered to be significant at the $p \leq 0.05$ level.

2.4. The effect of different feeding strategies on the growth, survival and behaviour of Russian sturgeon – experimental system and experimental settings

Induced reproduction of Russian sturgeon was performed in 2021 – Trial 1 (Rónyai et al., 2009) using two males and three females, and using two males and two females in 2022 – Trial 2 (Ljubobratović et al., 2022). The egges were incubated at a water temperature of 16.7 ± 0.3 °C (2021) and 15.6 ± 0.2 °C (2022). On the first day after hatching (DPH), the larvae were transported tot he experimental larval rearing system, which consisted of eight tanks with volume of 250 liters each. The photoperiod (16L:8D) was provided by LED lamps positioned above each tank. The larval rearing system employed in both years was consistent, with a stocking density of 2500 larvae per tank. The dissolved oxygen content and temperature of the water were measured and recorded on a daily basis. The chemical water quality parameters – ammonium nitrogen, nitrite nitrogen, nitrate nitrogen, and total suspended solids – were measured twice a week in each tank separately. During the course of the experiment, the tanks were subjected to a daily cleaning procedures, and dead larvae was enumerated and noticed.

2.4.1. Experimental design and factors of interest in Trial 1

The fixed factors examined were: *Artemia*-enrichment (E/N; enriched or not enriched), use of frozent *Artemia* (F/L; frozen or live nauplii), use of frozen *Chironomus* (EChi/NChi; with or without *Chironomus*), and co-feeding (Co/W; co-feeding from the onset of exogenous feeding or weaning one week later). The experimental combinations in the Trial 1 were as follows:

1. Frozen, non-enriched *Artemia*, no *Chironomus* with weaning from 20 DPH (F-N-NChi-W)
2. Live, enriched *Artemia*, no *Chironomus* with weaning from 20 DPH (L-E-NChi-W)
3. Frozen, non-enriched *Artemia,* early *Chironomus* with Co-feeding method (F-N-EChi-Co)
4. Frozen, enriched *Artemia*, no *Chironomus* with Co-feeding method (F-E-NChi-Co)
5. Live, non-enriched *Artemia*, no *Chironomus* with Co-feeding method (L-N-NChi-Co)
6. Frozen, enriched *Artemia*, early *Chironomus* with weaning from 20 DPH (F-E-EChi-W)
7. Live, enriched *Artemia*, early *Chironomus* with Co-feeding method (L-E-EChi-Co)
8. Live, non-enriched *Artemia*, early *Chironomous* with weaning from 20 DPH (L-N-EChi-W)

The first feeding experiment lasted 36 days. The temperatures of the tanks were maintained at 16.9 ± 0.05 °C. The feed combintaions corresponding to the experimental setup was offered to the larvae ont he day following 11th after hatching. Subsequently, the fish were fed six times manually in six equal portions by live or frozen *Artemia* at a rate of 50% of the body weight of fish in groups fed *Artemia* only; 25%-25% of the body weight of fish fed *Artemia* and *Chironomus*; 12.5%-12.5%-2.5% of the body weight of fish fed *Artemia*, *Chironomus* and starter feed Coppens Advance and 25% -2,5% of the body weight of fish fed *Artemia* and co-fed starter feed Coppens Advance until 20 DPH. The co-feeding diets were offered from the onset of exogenous feeding in replacing 50 % of the natural food. The amount of live and inert feed was adjusted to the actual biomass of the tanks during the trial. The weaning was conducted between 20 and 23 DPH in groups not receiving *Chironomus*. Within the weaning period, the amount of live and frozen *Artemia* was gradually (33% daily) decreased while the quantity of inert diet was increased to 7 % of fish biomass and introduced to the fish by the automatic feeder for 15 hours a day. In groups fed *Chironomus* along with *Artemia,* frozen *Chironomus* was applied a week more upon weaning.

2.4.2. Experimental design and factors of interest in Trial 2

The following factors were determined as fixed factors: *Artemia*-enrichment: enriched (E) on non-enriched (N); use of *Chironomus* from the onset of exogenous feeind at 11 DPH (early *Chironomus* – EChi) or one week later, from 19 DPH (late *Chironomus* – LChi); time of weaning: early (19 DPH – W) or late (26 DPH – LW); application of environmental enrichment: environmentally enriched (EE) or bare (CTRL). Experimental combinations in Trial 2:

1. Enriched *Artemia* with early *Chironomus* and early weaning in the conventional environment (E-EChi-W-CTRL)
2. Non-enriched *Artemia* with late *Chironomus* and late weaning in a gravel-enriched environment (N-LChi-LW-EE)
3. Enriched *Artemia* with later *Chironomus* and early weaning in a gravel-enriched environment (E-LChi-W-EE)
4. Enriched *Artemia* with early *Chironomus* and late weaning in a gravel-enriched environment (E-EChi-LW-EE)
5. Non-enriched *Artemia* with early *Chironomus* and late weaning in the conventional environment (N-EChi-LW-CTRL)
6. Enriched *Artemia* with later *Chironomus* and late weaning in the conventional environment (E-LChi-LW-CTRL)
7. Non-enriched *Artemia* with later *Chironomus* and early weaning in the conventional environment (N-LChi-W-CTRL)
8. Non-enriched *Artemia* early *Chironomus* and early weaning in a gravel-enriched environment (N-EChi-W-EE).

The second feeding trial was conducted over a 40 days. Subsequently, the fish were transferred to eight circular nursery rearing tanks, each with a volume of 1 m^3. A layer of small gravel (light gravel, 6-16 mm, SZAT P-4) was placed at the bottom of the larval rearing tanks. The temperature of the tanks were maintained at 16,9 ± 0 °C until 67 DPH, and then gradually increased to 19,3 ± 0 °C prior to the behavioural testing (73 DPH). From the commencement of exogenous feeding (11 DPH), the larvae were provided with live, unenriched or enriched *Artemia* in quantities corresponding to 50% of the fish body weight (LChi-group), or a combination of *Artemia* and frozen *Chironomus sp.* in a 25–25% ratio (EChi-group) between days 13 and 18 after hatching. As of day 18, the half of the life food in the LChi groups was substituted with *Chironomus*, and the fish were fed accordingly until the conclusion of the feeding period. The fish were fed five times in equal portions. From the 19th day after hatching, gradual feed training was initiated in half of the teratments (the 'early' weaned group, W-group), which entailed replacing 5% of the natural feed with commercial feed daily. Following a period of seven days, the dry feed feeding ration reached 7% of the total biomass. Thereafter, the fish were fed Coppens Advance and Start Premium diet using an automatic feeder. The weaning was initiated one week later in the LW-group, on 26 DPH, in accordance with the same protocol applied in the W-goups. On the day of 40, fish were transferred to 1 m^3 nursery system for a further one month of rearing under traditional aquaculture conditions. The tanks werer equpped with automatic feeders.

In both experiments, five control measurements were performed in the larval rearing system and four additional control measurements were undertaken in the nursery system in 2022. The total body length and body weight of 31 fish per treatment were measured. The following zootechnical parameters were analysed in both experiment: final body weight (BW_f, g), 36 DPH in 2021; 40 and 66 DPH in 2022; final total body length (TL_f, mm), 36 DPH in 2021; 40 and 66 DPH in 2022; coefficient of variaton of final body weight and length (CV_{BWf}, %; CV_{TLf}, %) 36 DPH in 2021; 40 and 66 DPH in 2022; final condition factor (K_f), (lásd 3.2.1. fejezet), 36 DPH in 2021; 40 and 66 DPH in 2022; mortality (%), 36 DPH in 2021; 40 and 66 DPH in 2022; survival (%), 36 DPH in 2021; 40 and 66 DPH in 2022; fish biomass gain (FBG, g/l), 40 and 66 DPH in 2022; specific growth rate (SGR, %/nap), 36 DPH in 2021; 40 and 66 DPH in 2022; cannibalism rate (%), 36 DPH in 2021; 40 and 66 DPH in 2022.

2.4.2.1. The procedure of behavioural testing and the behavioural variables of interest

The behavioural test were conducted in the same cross maze and utilised the same test protocol as employed in my previous research with sterlet. Two different maze types were used in the study: a bare maze devoid of physical structures (Plain-maze test), and a maze with a bottom covered with small pebbles (1-2 cm) (Gravel-maze test). Twenty-four hours prior tot he commencement of the test, five fish (10,6±3,4 g, 72-84 DPH) were transported from each group to eight separate aquariums in the bahvioural testing room for two consecutive weeks. The experimental design involved the individual testing of 19 fish per treatment, with each fish being tested only once. *Chironomus sp***.** were placed in the same locations of maze during the Plain-maze test. Fish individual body weight ere measured after the testing. During the test period, the following behavioural variables were recorded: food intake during the 10-minute test period (FI); latency time to leave the start zone (LES); total number of visited zones (TNVZ); time spent freezing (FT) (lásd 2.3.2.2. fejezet 'F'); stress-related swimming behaviour (S); number of visits of start zone (VS); number of visits of challenge zones (VC) (lásd 2.3.2.2. fejezet 'CB'); and distance travelled in the maze (DT).

In order to measure the distance travelled (DT) by individual fish, a unique software called Aegear was developed. The software is capable of tracking fish movements in visually complex aquatic environments (Ljubobratović, 2023). In contrast to existing tools sich as idtracker.ai, which rely on a uniform background for accurate segmentation (Romero-Ferrero et al., 2019), Aegear is specifically designed to operate on heterogenous surfaces such as gravel bottoms, where background textures interfere with standard tracking algorithms.

The fractional factorial model comprised 8 (2^4–1) factorial combinations (Gunst et al., 2009). The experimental combinations were selected using the Planor package available in the R software (Kobilinsky et al., 2012). The homogeneity of variance were tested by Levene-test, then Tukey post-hoc tesztet or Kruskal-Wallis test were performed. The primary effects and interactions were analysed in both experiments usind analysis if variance (ANOVA) with a general linear model (GLM). The model was based on type IV. fractional factorial design (2^4–1) which was implemented in SPSS Statistics software. The ranking of the combinations was achieved through the calculation of a total score for each experimental setting, the score being determined on an ordinal scale derived from the cumulative value of the total z-values (El Kertaoui et al., 2019). At the conclusion of Trial 1 (36 DPH) and Trial 2 (66 DPH), five output variables were selected for the ultimate ranking as indicators of culture performance: final body weight (BW_f); final total length (TL_f); coefficient of variation of final body weight (CV_{BWf}); coefficient of variation of final total length (CV_{TLf}); survival rate (%).

3. Results

3.1. The effect of stocking density on the growth and survival rate of sterlet

A single statistically significant difference was identified between the groups in fish biomass gain (FBG) (ANOVA: $F_{2,3}$=94.008; p=0.002; Figure 2). All treatments involving hogher stocking densities resulted in significantly higher FBG (ANOVA, Tukey post-hoc test; p<0.05). Pearson's correlation analysis revealed a highly significant correlation between FBG and stocking density (r=0.98; p<0.001, Figure 2.), while no correlation was identified for the other parameters examined (p>0.05, Table 1).

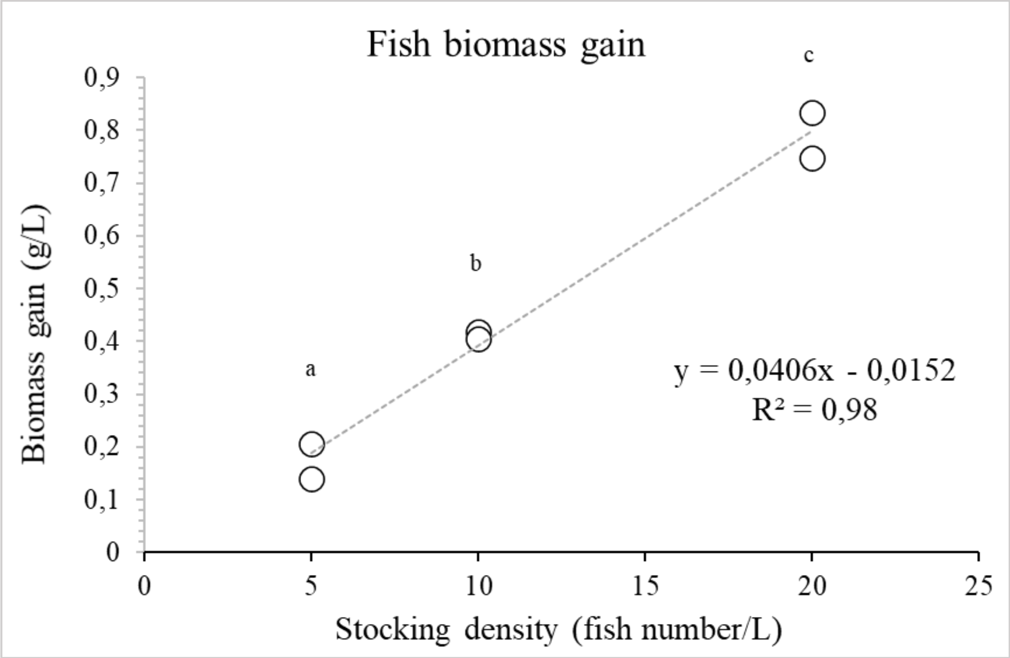

Figure 2. Representation of fish biomass growth as a function of three different stocking densities of sterlet at the end of the 30-day experiment. Significantly different treatments (one-way ANOVA, $p<0.05$) are marked with different letters in the superscript.

Table 1. Effect of three different stocking densities on growth and survival (means ± SD) in 30 days post-hatch sterlet larvae.

Parameter	L	M	H	Pearson's *R* érték	p-érték
BW$_f$ (mg)	91.7 ± 44.3	95.3 ± 43.6	96.1 ± 44.7	0.428	0.398
CV$_{BWf}$ (%)	70.0 ±0.3	29.2 ±0.0	58.6 ±0.2	-0.064	0.905
TL$_f$ (mm)	26.5 ±3.6	26.9 ±3.5	26.7 ±3.8	0.088	0.869
CV$_{TLf}$ (%)	14.7 ±0.0	11.2 ±0.0	14.9 ±0.0	0.215	0.682
K$_f$	0.5 ±0.1	0.5 ±0.1	0.5 ±0.1	0.777	0.690
Survival (%)	51.0 ± 10.8	51.8 ±3.5	47.6 ±2.3	-0.296	0.569
FBG (g/l)	0.2 ±0.0 ^{a}	0.4 ±0.0 ^{b}	0.8 ±0.1 ^{c}	0.990	0.000
SGR (%/ day)	7.4 ±0.3	7.5 ±0.1	7.5 ±0.0	0.433	0.391

Significantly different treatments (one-way ANOVA, $p<0.05$) are marked with different letters in the superscript. Relationship of each parameter with stocking density described with Pearson correlation coefficient *R* and its respective *p* – value. Abbreviations: BW$_f$, final body weight; K$_f$, final condition factor; CV$_{BWf}$, coefficient of variation in final body weight; CV$_{TLf}$; coefficient of variation in final tail length; FBG, fish biomass gain; S, survival; SGR, specific growth rate; TL_f, final total length, L – low stocking density: 5 larvae/L; M - medium stocking density: 10 larvae/L; H – high stocking density: 20 larvae/L.

In accordance with the findings of the Tukey post-hoc test, the L groups showed a tendency towards better growth during the first three weeks, however significant differences visible only at the second sampling point (Figure 3). At the end of the trial, no statistically significant differences ($p>0.05$) were observed between the groups with respect to the stocking density.

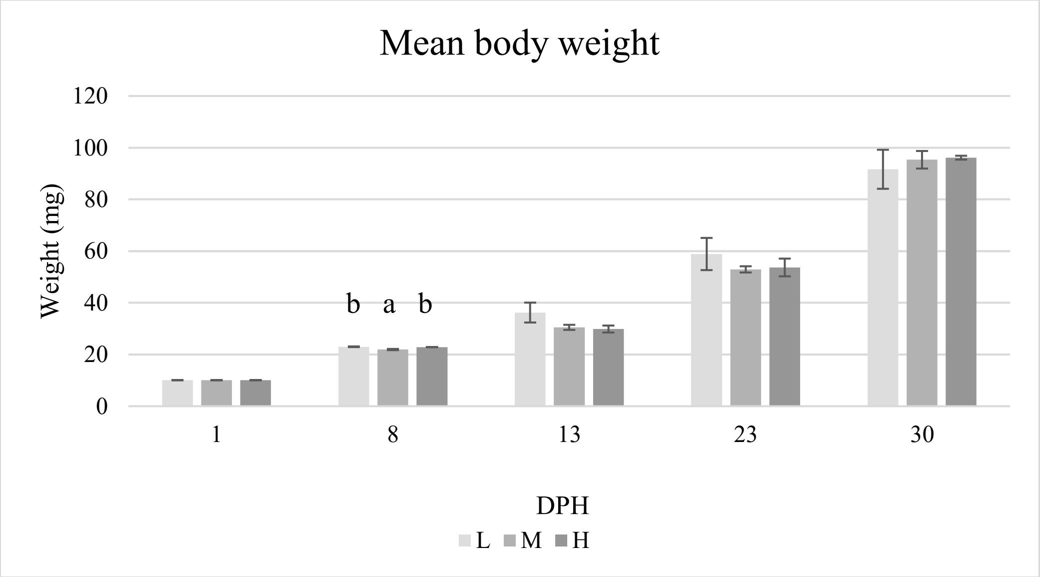

Figure 3. Mean body weight of sterlet in three different stocking densities over a 30-day trial period. Significantly different treatments (one-way ANOVA, $p<0.05$) are marked with different letters in the superscript. Abbreviations: L – low stocking density: 5 larvae/L; M - medium stocking density: 10 larvae/L; H – high stocking density: 20 larvae/L.

An examination of daily mortality revealed that the highest peaks were observed during the transition to exogenous feeding (9 DPH), and in the period following weaning (27 DPH). The cumulative mortality of different feeding periods showed a significant higher mortality appeared in the highest stocking density group (H) (one-way ANOVA, $F_{2,3}=15{,}524$; $p=0{,}026$; Pearson's ($r=0{,}95$, $p=0{,}004$; Table 2).

Table 2. Percentage of cumulative mortality (means ± SD) for feeding phases at three different stocking densities of sterlet larvae.

Feeding phase	DPH	L	M	H	Pearson's *R* value	p-value
endogenous feeding	1-7	2,36± 2,2	0,82± 0,5	0,98± 0,2	-0,361	0,482
exogenous feeding	8-12	5,16± 1,3^{a}	7,94± 1,3^{a}	12,77± 1,3^{b}	0,95	0,004
weaning	13-23	19,68± 1,9	16,24± 1,2	20,01± 0,3	0,235	0,654
post-weaning	24-30	16,48± 5,3	17,4 ± 2,3	12,76± 2,9	-0,523	0,287

Significantly different treatments (one-way ANOVA, $p<0.05$) are marked with different letters in the superscript. Abbreviations: L – low stocking density: 5 larvae/L; M - medium stocking density: 10 larvae/L; H – high stocking density: 20 larvae/L. Relationship of each parameter with stocking density described with Pearson correlation coefficient *R* and its respective p – value. Abbreviations: L – low stocking density: 5 larvae/L; M - medium stocking density: 10 larvae/L; H – high stocking density: 20 larvae/L.

3.2. The effect of environmental enrichment on the production and behavioural indicators of sterlet

3.2.1. The effect of environmental enrichment on the growth and survival of sterlet

The body weigth of the fish was significantly lower in the environmentally enriched (EE) group (Mann-Whitney non-parametric test; $p<0.05$). (Table 3; Figure 4). A significant difference in the initial total body length TL$_i$ (27,4 ± 0,9 mm vs. 25,8 ± 0,1 mm; two-sample T-test; $p<0,05$) and a final total body length TL$_f$ (59,1 ± 5,9 mm vs. 47,2 ± 1,3 mm; Mann-Whitney non-parametric test; $p<0,05$) was identified. A significant disparity was observed in the coefficient of variation of final body weight (79,0 ± 10% vs. 36,0 ± 10%; CI: no overlap) with the CTRL group demonstrating a higher value. Higher survival (72,2 ± 2,9% vs. 89,2 ± 1,8%; χ^2-test; $p<0,001$) and lower mortality rate (19,3 ± 3,4% vs. 5,3 ± 1,3%; Mann-Whitney non-parametric test; $p<0,001$) was found in the EE group (Table 3). Altough the difference in the frequency of cannibalism did not reach statistical significance (8,46 ± 0,5% vs. 5,51 ± 0,5%; χ^2-teszt; $p>0,05$), there were more potential cannibals in the conventionally reared (CTRL) group.

Table 3. 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. Significant differences are marked with different letter in the superscript.

Parameters	Abbreviations	Control group (CTRL)	Environmentally enriched group (EE)
Initial body weight	BW_i (g)	0,08 ± 0,0^{b}	0,07 ± 0,0^{a}
Coefficient of variation of initial body weight	CV_{BWi} (%)	30,0 ± 2,1	21,9 ± 4,4
Initial total body length	TL_i (mm)	27,4 ± 0,9^{b}	25,8 ± 0,1^{a}
Coefficient of variation of initial total body length	CV_{TLi} (%)	10,0 ± 0,0	7,0 ± 0,0
Final body weight	BW_f (g)	0,9 ± 0,3^{b}	0,6 ± 0,1^{a}
Coefficient of variation of final body weight	CV_{BWf} (%)	56,5 ± 7,3^{b}	44,9 ± 0,7^{a}
Final total body length	TL_f (mm)	59,1 ± 5,9^{b}	47,2 ± 1,3^{a}
Coefficient of variation of final total body length	CV_{TLf} (%)	22,0 ± 0,0	13,0 ± 0,0
Initial condition factor	K_i	0,4 ± 0,0	0,4 ± 0,0
Final condition factor	K_f	0,5 ± 0,0	0,5 ± 0,0
Fish biomass gain	FBG (g/L)	1,0 ± 0,4	0,8 ± 0,2
Specific growth rate	SGR (%/day)	8,2 ± 1,1	7,4 ± 0,7
Mortality (%)		19,3 ± 3,4^{b}	5,3 ± 1,3^{a}
Survival (%)		72,2 ± 2,9^{a}	89,2 ± 1,8^{b}
Cannibalism (%)		8,5 ± 0,5	5,5 ± 0,5

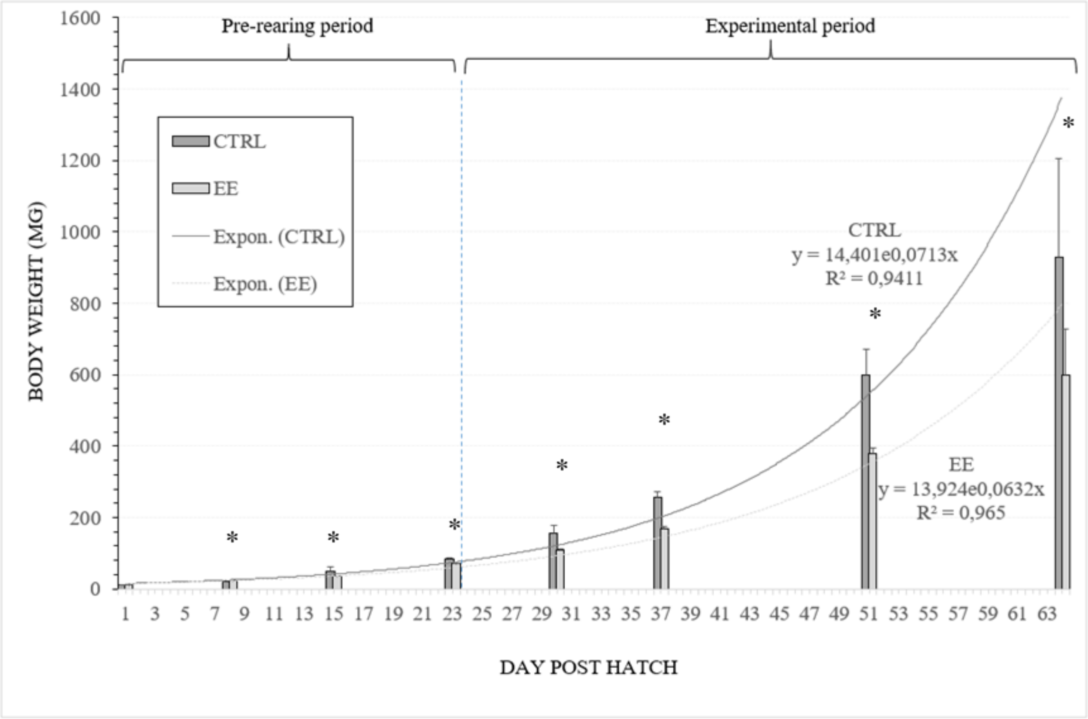

Figure 4. Average body weight and exponential growth line of sterlet *Acipenser ruthenus* larvae and juveniles reared in bare (CTRL) and enriched (EE) environmental conditions during the pre-rearing (1-23 days post-hatching (DPH)) and experimental (23-64 DPH) periods. Significant differences occurred at every sampling point except the initial.

3.2.2. The effect of environemntal enrichment ont he behaviour of sterlet

In the Plain-maze test, fish from EE group exhibited a significantly reduced stress-related behaviour (165,2 ± 144,1 s vs. 55,4 ± 102,6 s; Mann-Whitney non-parametric test; $p<0,05$). The CTRL fish visited the challenging zone significantly less often than those from the EE group (0,8 ± 1,1 vs. 3,2 ± 3,4; Mann-Whitney non-parametric test; $p<0,05$). The body weight of fish engaged in the Plain-maze test was higher in the CTRL group (Mann-Whitney non-parametric test; $p<0,05$). Likewise in the Plain-maze test, the stress-related swimming behaviour was reduced in the Gravel-maze test, with a shorter periods observed in the group raised in enriched conditions (275,8 ± 197,4 s vs. 96,1 ± 118,3 s; Mann-Whitney non-parametric test; $p<0,05$). No significant differences were found for the other variables examined (Table 4).

Table 4. Results of the Plain-maze test and Gravel-maze test performed on sterlet (*A. ruthenus*) juveniles. Significant differences are marked with different letter in the superscript.

Variable (description)	Plain-maze test		Gravel-maze test	
	Control group (CTRL)	Environmentally enriched group (EE)	Control group (CTRL)	Environmentally enriched group (EE)
LES (s) – latency time to leave the start zone	93,0±107,7	49,5±60,2	216,5±234,1	105,5±78,6
FT (s) – time spent freezing	14,1±35,8	47,2±92,4	30,5±64,6	2,2±8,0
S (s) – stress-related swimming behaviour	165,2±144,1^{a}	55,4±102,6^{b}	275,8±197,4^{a}	96,1±118,3^{b}
TNVZ – total number of visited zones	37,2±23,0	35,5±22,2	13,3±12,3	19,5±10,9
CB – number of visits of challenge zones	0,8±1,1^{a}	3,2±3,4^{b}	0,7±0,9	0,7±0,8
BW$_{84,92}$ (g) – body weight 84 and 92 DPH	7.7±3.1^{a}	3.9±1.1^{b}	10.1±4.4	7.4±1.5

Correlations between behavioural variables

Correlations among behavioural variables were examined separately for each maze test type and treatment group. In fish exposed to a test environment differing from their rearing conditions, several behavioural variables showed significant interrelationships. During the Plain-maze test, CTRL fish that required more time to emerge from the start zone (LES) explored fewer zones. Furthermore, the TNVZ showed a positive correlations with body weight (rs=0,59; p<0,05). In the EE group, fish tended to visit the challenge zones more frequently, as indicated by a significant positive relationship between the TNVZ and the number of visits of challenge zones. Similar tot he CTRL group, BW in EE fish positively correlated with the TNVZ and negatively with freezing time. The TNVZ was also negatively associated with both freezing time and stress-related swimming behaviour (S) (rs= −0,59; p<0,05).

In the gravel maze test, body weight was negatively correlated with LES in CTRL fish. Likewise, within the CTRL group, fish displaying more S, visited fewer zones in total (rs= −0,660; p<0,05) ans spent more time in the start boksz. Consistent with this, the TNVZ was negatively correlated with both freezing time and LES. Among EE fish, BW was again positively correlated with the TNVZ (rs= 0,62; p<0,05).

3.3. Effect of different feeding strategies on the growth, survival, and behaviour of Russian sturgeon

3.3.1. Effect of different feeding strategies on the growth and survival of Russian sturgeon in 2021

The effect of different combinations on growth, survival and condition factor are shown in Table 5. The findings of the general linear model, indicate that the factor of time had a significant influence on mortality during the period of the experiment. However, no statistically significant effect was detected on growth parameters. The utilisation of frozen *Artemia* (F) and co-feeding (Co) exhibited significant impacts on growth parameters onward, but yet did not affect mortality. Furthermore, the early introduction of Chironomus and F exhibited a considerable impact on growth parameters. Nevertheless, the F also had significant effect on mortality over time. The body weight (BW) and total length (TL) were found to be elevated in the EChi-group, while the use of frozen *Artemia* led to stunted growth at all sampling points (Kruskal-Wallis-teszt, p<0,005). Moreover, the interaction between co-feeding and time was not statistically significant, although it did approach the significance threshold in terms of body weight development (GLM, $F_{(2,705)}$=2.812; p=0,061), resulting in significant differences at every sampling time (Kruskal-Wallis-teszt, p<0,05) (Figure 5). The findings demonstrated that the mortality rate in group F exceeded that of the group fed live *Artemia* (L) at the second sampling time (29 DPH, Kruskal-Wallis-teszt, p<0,05). Moreover, higher mortality rate was observed in the Co-groups at the initial sampling point (19 DPH, Kruskall-Wallis-teszt, p<0,05) in comparison to the W groups. The EChi factor indicates a decrease in mortality, resulting in lower mortality values at the beginning and the end of the trial in the gropus consuming *Chironomus* from exogenous feeding (19 és 36 DPH, Kruskal-Wallis-teszt, p<0,05) (Figure 6).

Table 5. The results of the culture performance of Russian sturgeon after the larviculture phase (36 DPH) in 2021. The results are described as mean ± standard deviation. DPH, day post hatching.

Combinations/ Parameters	Abbreviation	1	2	3	4	5	6	7	8
Artemia enrichment	E		+		+		+	+	
application of *Chironomus*	EChi			+			+	+	+
frozen *Artemia*	F	+		+	+		+		
co-feeding	Co			+	+	+		+	
final body weight (g) at 36 day post-hatching	BW_f (g) 36 DPH	0,07 ± 0,0	0,13 ± 0,1	0,09 ± 0,0	0,06 ± 0,0	0,12 ± 0,0	0,10 ± 0,0	0,12 ± 0,0	0,17 ± 0,1
final total length (mm) at 36 day post-hatching	TL_f (mm) 36 DPH	24,52 ± 2,1	30,02 ± 3,9	27,47 ± 4,3	23,34 ± 2,3	29,37 ± 3,1	27,40 ± 2,4	29,40 ± 3,7	32,48 ± 3,9
coefficient of variation of final body weight (%)	CV_{BWf} (%)	26,41	37,91	38,68	33,06	30,12	25,64	35,85	31,07
coefficient of variation of final body length (%)	CV_{TLf} (%)	8,37	13,05	15,73	9,75	10,59	8,60	12,58	11,85
condition factor at 36 day post-hatching	K 36 DPH	0,49 ± 0,1	0,48 ± 0,1	0,46 ± 0,1	0,44 ± 0,1	0,46 ± 0,1	0,50 ± 0,1	0,47 ± 0,1	0,50 ± 0,1
specific growth rate at 36 day post-hatching	SGR 36 DPH	3,71	5,41	4,42	3,06	5,10	4,71	5,18	6,13
mortality (%)	Mortality s (%)	89,50	74,82	90,88	93,85	79,11	74,55	74,79	64,81
survival (%)	Survival (%)	5,85	19,05	8,92	2,94	17,02	24,51	21,77	30,52
cannibalism (%)	Cannibalism (%)	5,76	6,84	1,29	4,40	4,87	1,85	4,46	5,44
standard value	Z SCORE	-0,89	-0,21	-4,44	-3,89	1,54	2,94	0,18	4,76
total score ranking	Total score ranking	6	5	7	8	3	2	4	1

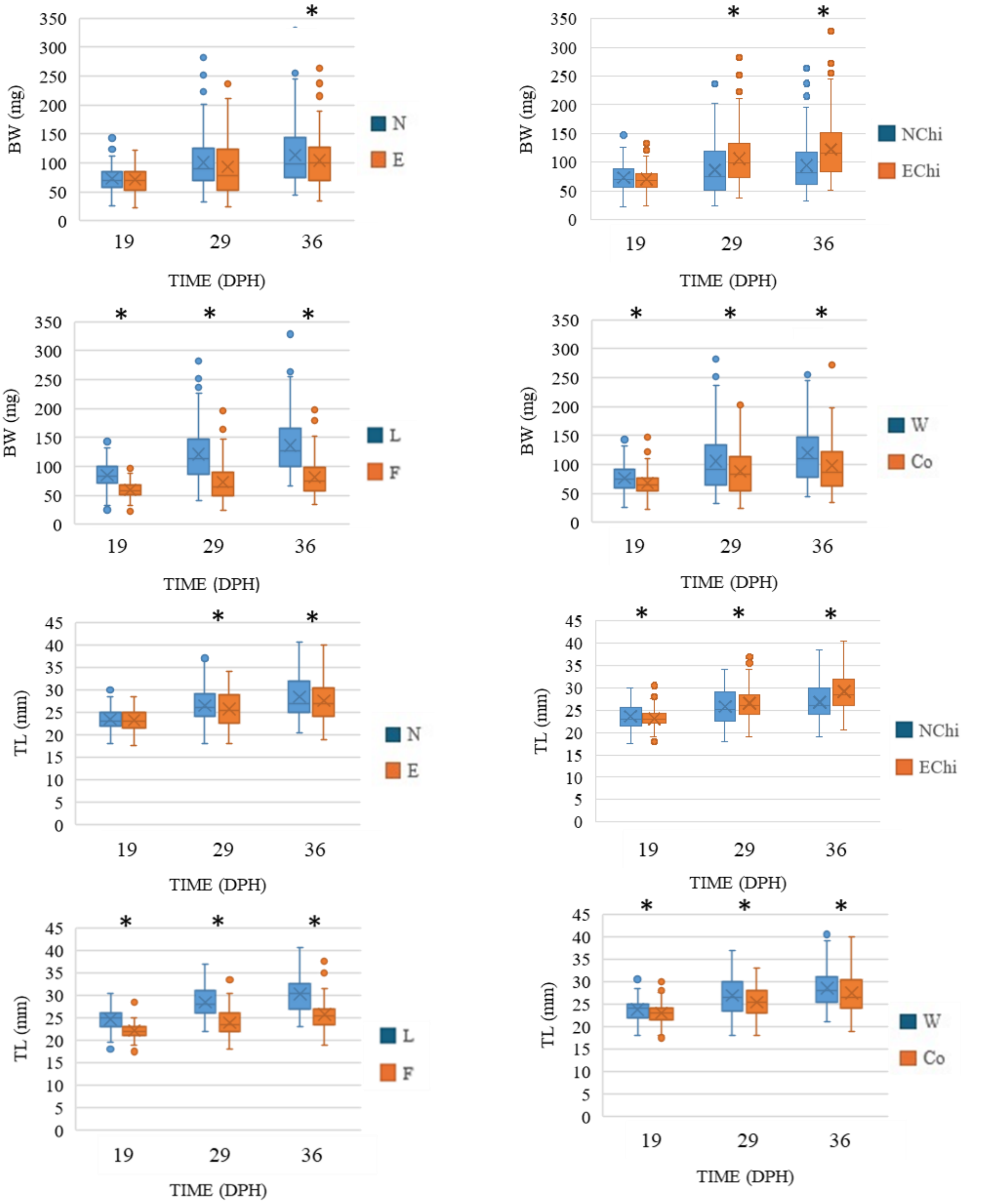

Figure 5. The alteration in growth of Russian sturgeon as a function of fixed factors at sampling points in Trial 1. Significant differences are marked with an asterisk in the superscript. Abbreviations: E, enriched *Artemia*; N, non-enriched *Artemia*; EChi, *Chironomus* feeding from the onset of exogenous feeding; NChi, non-*Chironomus*; F, frozen *Artemia*; L, live *Artemia*; Co, co-feeding; W, weaning.

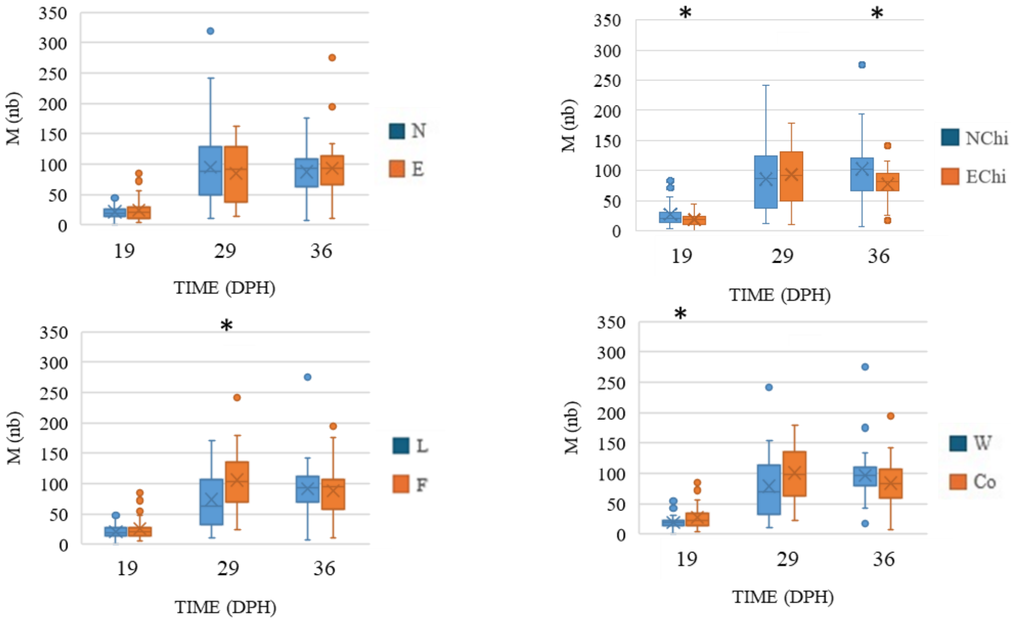

Figure 6. The alteration in mortality of Russian sturgeon as a function of fixed factors at sampling points in Trial 1. Significant differences are marked with an asterisk in the superscript. Abbreviations: E, enriched *Artemia*; N, non-enriched *Artemia*; EChi, *Chironomus* feeding from the onset of exogenous feeding; NChi, non-*Chironomus*; F, frozen *Artemia*; L, live *Artemia*; Co, co-feeding; W, weaning; BW, body weight; TL, total length.

3.3.2. The effect of different feeding strategy ont he growth and survival of Russian sturgeon in 2022

The effects of different treatment combinations on growth, survival and condition factor are shown in Table 6. The results of the GLM statistical analysis indicate that time as a factor has a significant influence on BW and TL during larviculture. However, the main effects themselves do not have significant effect on growth and mortality rates. The introduction of *Chironomus* (early – EChi / late – LChi) and the commencement date of weaning (early – W / late – LW) – as primary factors – also exerted a significant influence on BW and TL values over time, while the timing of weaning has a significant effect on mortality in addition to growth indicators. From the onset of exogenous feeding, the BW and TL values of EChi-groups were higher (on days 19, 26 and 39 after hatching (DPH); Kruskal-Wallis test, $p<0.005$) compared to the group that consumed *Chironomus* one week later (LChi). Meanwhile, the ’early’ fed group (W) exhibited lower BW compared to the results of the later fed group (LW) (26 and 33 DPH, Kruskal-Wallis test, $p<0.005$). However, this difference was no longer measurable at the conclusion of the larviculture phase (39 DPH). Concurrently, there was an increase in mortality rates in group W during the larval rearing phase (33 and 39 DPH; Kruskal-Wallis teszt, $p<0,05$). In the EE group, a significantly lower mortality rate on day 33 was recorded in comparison to the CTRL group (Kruskal-Wallis test, $p<0,05$), but no effect of EE was detectable on body size.

Considering the entire experimental period – encompassing both larval and fry rearing – the time factor persisted in exerting a considerable influence on growth indicators. In addition to the timing of *Chironomus* introduction, EE also had a significant effect on BW and TL. The early introduction of Chironomus from the onset of exogenus feeding led to an increase in body size growth at the end of the nursury phase (BW 60, 66 DPH, TL, 66 DPH;, Kruskal-Wallis test, p<0,05). However the EE had negative effect on BW, causing differences at 46, 53 and 66 DPH, as well as on TL at 66 DPH (p<0,05). The timing of weaning had a significant effect on the alteration of mortality throughout the entire rearing period. Lower mortality occured in the W group at the beginning of the nursery phase (46 DPH, Kruskal-Wallis test, p<0,05) (Figure 7, Figure 8).

Table 6a. The results of the culture performance of Russian sturgeon after the larviculture phase (40 and 66 DPH) in 2022. The results are described as mean ± standard deviation. DPH, day post hatching.

Combinations/ Parameters	Abbreviation	1	2	3	4	5	6	7	8
Artemia enrichment	E	+		+	+		+		
early Chi introduction (*Chironomus* larvae)	EChi	+			+	+			+
environmental enrichment	EE		+	+	+				+
weaning (early)	W	+		+				+	+
final body weight (g) at 40 day post-hatching	BW$_f$ 40 DPH (g)	0,30 ± 0,2	0,22 ± 0,1	0,33 ± 0,2	0,30 ± 0,1	0,37 ± 0,2	0,32 ± 0,1	0,27 ± 0,1	0,38 ± 0,2
final body weight (g) at 66 day post-hatching	BW$_f$ 66 DPH (g)	6,36 ± 2,8	3,60 ± 1,8	4,05 ± 2,2	4,93 ± 2,1	5,59 ± 2,5	5,13 ± 2,7	4,67 ± 2,6	5,39 ± 2,9
final total length (cm) at 40 day post-hatching	TL$_f$ 40 DPH (cm)	3,68 ± 0,6	3,40 ± 0,5	3,73 ± 0,8	3,76 ± 0,5	3,95 ± 0,6	3,72 ± 0,5	3,45 ± 0,6	3,85 ± 0,6
final total length (cm) at 66 day post-hatching	TL$_f$ 66 DPH (cm)	10,05 ± 1,8	8,12 ± 1,6	8,46 ± 1,7	8,97 ± 1,5	9,52 ± 1,6	9,28 ± 2,1	8,90 ± 1,9	9,27 ± 2,1
coefficient of variation of final body weight (%) at 40 day post-hatching	CV$_{BWf}$ 40 DPH (%)	54,24	55,92	64,87	43,94	52,90	42,13	48,23	44,74
coefficient of variation of final total length (%) at 40 day post-hatching	CV$_{TLf}$ 40 DPH (%)	16,60	15,88	21,49	14,17	15,68	14,68	17,02	16,28
coefficient of variation of final body weight (%) at 66 day post-hatching	CV$_{BWf}$ 66 DPH (%)	44,35	49,43	54,24	41,60	44,92	53,20	55,52	53,63
coefficient of variation of final total length (%) at 66 day post-hatching	CV$_{TLf}$ 66 DPH (%)	17,72	19,47	20,29	17,13	17,26	23,11	21,19	22,24

Table 6b. The results of the culture performance of Russian sturgeon after the larviculture phase (40 and 66 DPH) in 2022. The results are described as mean ± standard deviation. DPH, day post hatching.

Combinations/ Parameters	Abbreviation	1	2	3	4	5	6	7	8
Artemia enrichment	E	+		+	+		+		
early Chi introduction (*Chironomus* larvae)	EChi	+			+	+			+
environmental enrichment	EE		+	+	+				+
weaning (early)	W	+		+				+	+
condition factor at 66 day post-hatching	K 66 DPH	0,58 ± 0,1	0,63 ± 0,1	0,61 ± 0,1	0,64 ± 0,1	0,62 ± 0,1	0,59 ± 0,1	0,62 ± 0,1	0,63 ± 0,1
condition factor at 40 day post-hatching	K 40 DPH	0,55 ± 0,1	0,52 ± 0,1	0,56 ± 0,1	0,53 ± 0,1	0,55 ± 0,1	0,62 ± 0,3	0,61 ± 0,1	0,63 ± 0,1
specific growth rate at 40 day post-hatching	SGR 40 DPH	7,33	6,59	7,57	7,31	7,84	7,51	7,07	7,89
specific growth rate at 66 day post-hatching	SGR 66 DPH	9,07	8,21	8,38	8,68	8,87	8,74	8,60	8,82
fish biomass gain at 40 day post-hatching	FBG 40 DPH	1,10	0,91	1,12	1,45	2,25	1,55	0,82	1,40
fish biomass gain at 66 day post-hatching	FBG 66 DPH	3,49	1,51	1,93	3,10	3,87	3,10	2,21	2,84
mortality (%) at 66 day post-hatching	M 66 DPH (%)	61,82	76,12	61,62	60,47	60,63	61,17	61,59	63,72
survival (%) at 40 day	SR 40 DPH (%)	44,44	50,36	40,84	57,33	69,19	55,94	38,12	43,91
survival (%) at 66 day	SR 66 DPH (%)	25,27	19,55	22,20	29,00	31,92	27,94	21,99	24,29
cannibalism (%)	Cannibalism (%)	12,91	4,33	16,18	10,53	7,45	10,89	16,42	11,99
standard value at 66 day post-hatching	Z SCORE 66 DPH	3,81	-8,02	-8,23	6,09	9,51	1,95	-6,32	1,21
global score-ranking at 66 day post-hatching	Global score-ranking 66 DPH	3	7	8	2	1	4	6	5

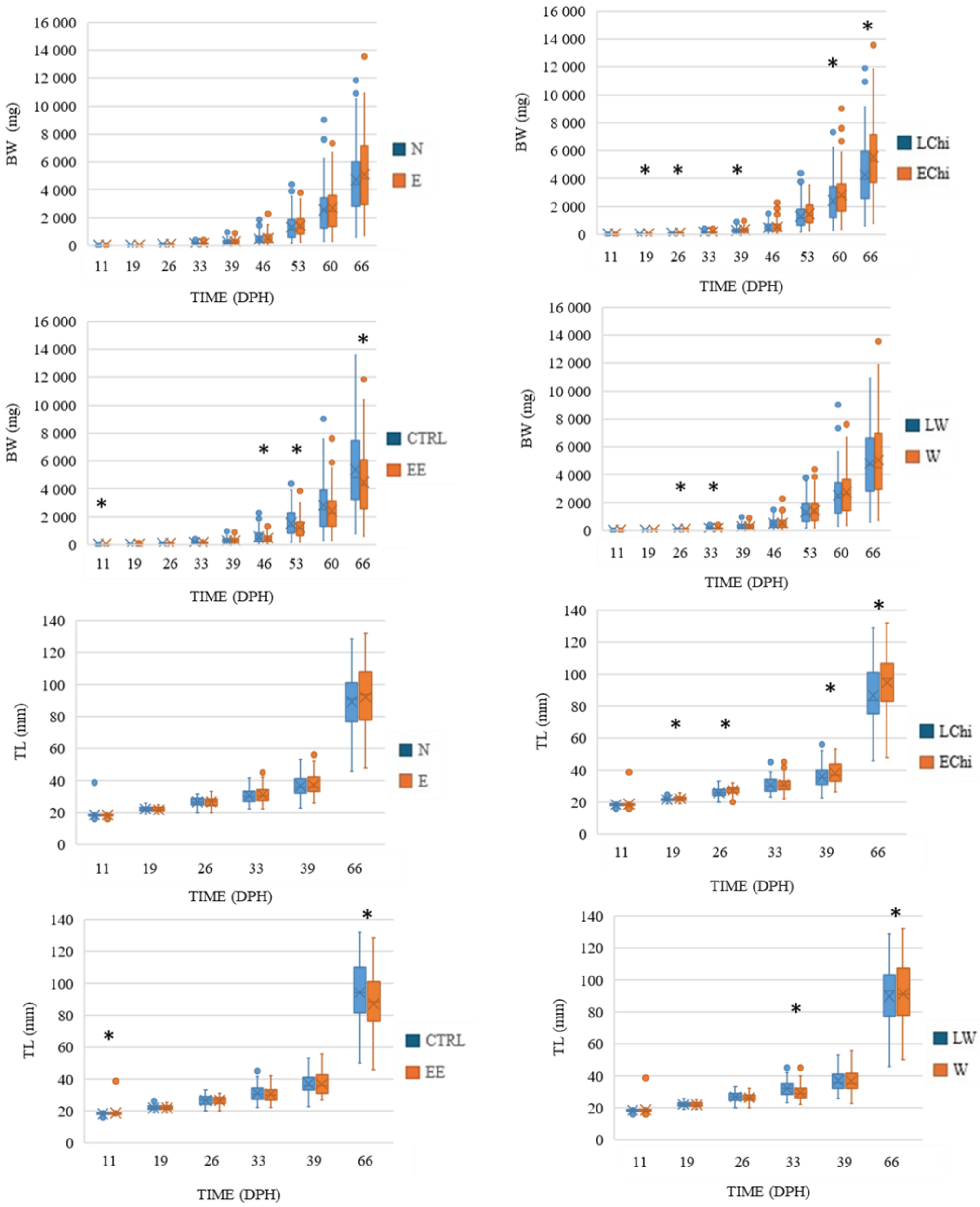

Figure 7. The alteration in growth of Russian sturgeon (*Acipenser gueldenstaedtii*) as a function of fixed factors at sampling points in Trial 2. Significant differences are marked with an asterisk in the superscript. Abbreviations: E, enriched *Artemia*; N, non-enriched *Artemia*; EChi, *Chironomus* feeding from the onset of exogenous feeding; LChi, *Chironomus* feeding one week later; W, 'early' weaning; LW, late weaning; D, dry feed; EE, environmental enrichment; CTRL, conventional environment; BW, body weight; TL, total length.

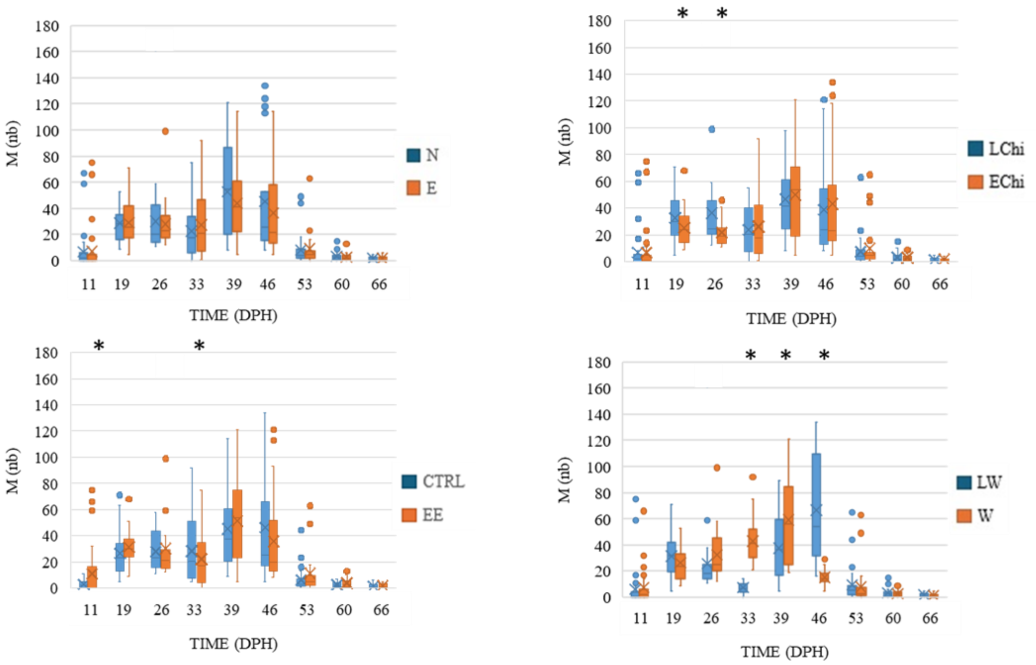

Figure 8. The alteration in mortality of Russian sturgeon (*Acipenser gueldenstaedtii*) as a function of fixed factors at sampling points in Trial 2. Significant differences are marked with an asterisk in the superscript. Abbreviations: E, enriched *Artemia*; N, non-enriched *Artemia*; EChi, *Chironomus* feeding from the onset of exogenous feeding; LChi, *Chironomus* feeding one week later; W, 'early' weaning; LW, late weaning; D, dry feed; EE, environmental enrichment; CTRL, conventional environment; M, mortality.

3.3.3. Effect of different feeding strategies ont he behaviour of Russian sturgeon in 2022

The fixed factors had no significant effect on feed intake during the Plain-maze test, however the impact of EChi factors tended to show towards significance (ANOVA/GLM, Kruskal-Wallis teszt, p=0,052) with higher feed intake in groups consumed *Chironomus* one week later (LChi). The LES was significantly lower (Kruskal-Wallis teszt, p<0,05), while the TNVZ were significantly higher (GLM, Tukey post-hoc teszt, p<0,05) in EChi-groups during the Plain-maze test. This disparity was not evident during the Gravel-maze test.

The fish reared in hatchery conditions took longer distance in the Plain-maze compared to the EE fish (Kruskal-Wallis teszt, p<0,05) but not during the Gravel-maze test. However, no significant differences were observed in the TNVZ values, although it is considered a good proxy for activity. The freezing time and stress-related swimming behaviour were not affected by the fix factors, altough the fish from the W-group tended to show less time freezing (Kruskal-Wallis teszt, p=0,052) than individuals from LW-group. Artemia enrichment (E) resulted in significantly higher body weight in the groups consuming enriched Artemia, as evidenced by the results of the Gravel-maze test. In both the the Plain-maze and Gravel-maze tests, the number of returns tot he start zone (VS) was found to be significantly correlated with the EChi fixed factor. Fish that were provided with Chironomus from the exogenous feeding regime exhibited higher frequency of return tot he initial. location compared to those in the other groups (Plain-maze test: Kruskal-Wallis test, p<0,05; Gravel-maze test: GLM, Tukey post-hoc test, p<0,05). The fixed factors did not have a significant effect on either the number of visits to challenging zones (VC) or S (Figure 9).

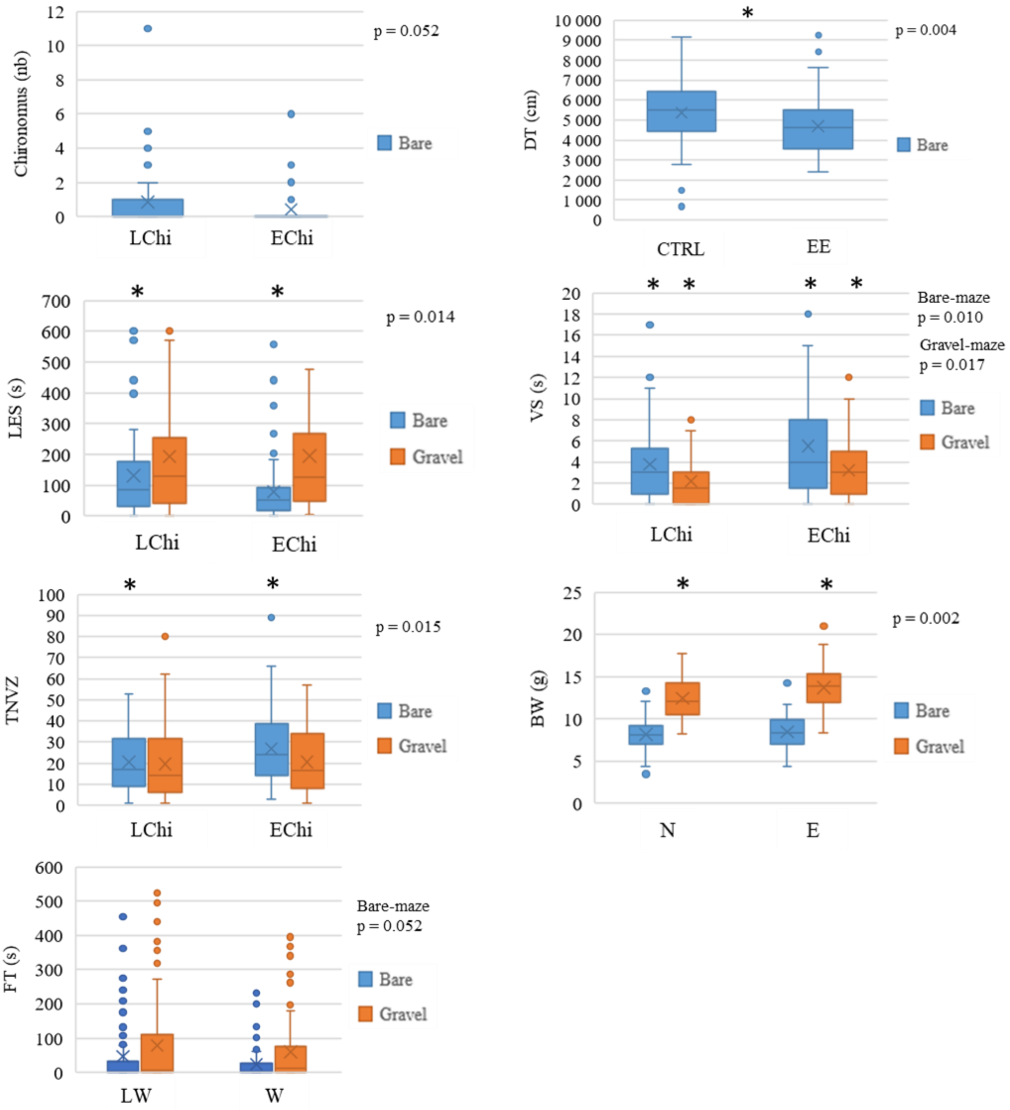

Figure 9. Diagram of behavioural indicators for Russian sturgeon at the conclusion of Trial 2. Significant differences are marked with an asterisk in the superscript. Abbreviations: E, enriched *Artemia*; N, non-enriched *Artemia*; EChi, *Chironomus* feeding from the onset of exogenous feeding; LChi, *Chironomus* feeding one week later; W, 'early' weaning; LW, late weaning; EE, environmental enrichment; CTRL, conventional environment; DT, distance travelled; LES, latency time to emerge from start zone; VS, number of visits of the start zone; TNVZ, total number of visited zones; BW, body weight; FT, time spent freezing; Bare, bare/plain-maze test; Gravel, gravel-maze test.

4. Conclusions and recommendations

4.1. The effect of stocking density on growth and survival rate of sterlet

The findings of the experiment demonstrated that the distinct stocking densities applied had no discernible impact ont he growth of the fry. It is evident that elevated stocking densities can engender deleterious effects on water quality, as well as the physiological condition and welfare of farmed animals. These effects can be manifested in performance and behaviour. Numerous studies have indicated that higher stocking densities may act as a chronic stressor over time (Ellis et al., 2002; Montero et al., 1999), and result in a decline in growth rates as fish density increases in sturgeon species (Mohseni et al., 2000; Mohler et al., 2000; Oprea & Oprea, 2009; Ni et al., 2016; Naderi et al., 2017). In the present study, such an adverse effect was only observed during the initial period of exogenous feeding (Figure 3). However, later on, even at a stocking density of 20 larvae/liter, no growth retardation was found. Consequently, this stocking density can be considered appropriate for the initial rearing of sterlet larvae from hatching until they reach an average body weight of 100 mg.

Stocking density may also influence larval viability or survival. In several species, density did not limit the survival of yolcsac-stage larvae during the endogenous feeding stage (Bauman et al., 2015; Mohseni et al., 2000), buta t the beginning of exogenous feeding phase, the mortality rate increased in groups with higher stocking densities (Mohseni et al., 2000). In the present study, a robust correlation was found between density and mortality during this life stage, particularly when comparing the highest and two lower density groups (Table 2). This phenomenon may be explained by the reduction in available space, which can lead to increased competition for food and aggressive behaviour (Mohler et al., 2000; Mohseni et al., 2000; Oprea & Oprea, 2009).

At the end of the experiment, a decreasing mortality trend was observed in the group initially showed weaker survival. This finding suggests that sterlet larvae possess the capacity to adapt to more intensive farming conditions, similar to some other fish species that can develope adaptive physiological responses even under chronic stress (Pickering & Stewart, 1984; Ruane et al., 2002).

In the present study, higher stocking density was associated with greater biomass growth. In the case of sterlet, higher densities may be justified in order to increase biomass production. According to previous data, the stocking density of sterlet larvae in practice usually ranges between 2.5 and 17 larvae/liter (Feledi & Rónyai, 2013b; Laczynska et al., 2020; Lundova et al., 2018; Rybnikár et al., 2011), however, the present study suggests that, under the right conditions, even higher densities can be safely applied. From the economic perspective, this can also be adventageous, as it has the potential to enhance the capacity utilisation of hatchery and rearing systems. Consequently, it can lead to an improvement in the economic efficiency of production.

In summary, under the conditions studied, stocking density did not have a detrimental effect on growth or survival, and a density of 20 larvae/liter can be safely used for the innitial rearing of sterlet larvae until they reach an average body weight of 100 mg. A significant avenue for future research could be the investigation of the adaptability of sterlet larvae and fry to chronic stressors encountered in intensive farming systems. Additionally, there is a need to explore the long-term implications of these processes in relation to economic exploitation and reintroduction for conservation objectives.

4.2. Effect of environmental enrichment (EE) on the growth and behaviour of sterlet

The success of sturgeon restocking is primarily determined by the adaptability of the fish, which can be significantly influenced by the stimulus-poor environment of hatcheries (Cámara-Ruiz et al., 2019). The comprehensive results obtained demonstrate a close relationship between the growth performance of sturgeon and the rearing environment. Fish exposed to spatial heterogeneity (EE-group) exhibited a significantly lower body weight, yet comparable condition values tot he control group (CTRL, low-stimulus rearing environment). This finding indicates that the heterogenous envrionment may have impeded their feeding, leading to an increase in swimming activity during the pre-rearing and experimental periods. These results are consistent with those obtained in studies conducted on other fish species (Braithwaite & Salvanes, 2005; Spence et al., 2011), while in a study by Boucher et al. (2018), white sturgeon (*A. transmontanus*) larvae raised on gravel substrate were significantly larger than their conventionally reared counterparts. In the present study, the mortality rate was found to be significantly lower in the EE-groups. This suggests that the gravel provided a refuge for the fish, thereby preventing them from engaging in cannibalistic behaviour during the experiment (Näslund & Johnsson, 2016). However, the response to physical environmental enrichment may be species specific in sturgeons (Boucher et al., 2014; Carrera-García et al., 2016).

Although anxiety-like swimming behaviour was observed in both groups during the test, individuals in the EE-group demonstrated a reduced incidence of unusual swimming patterns, including ”unpredictable” and ”frustration-induced” stereotypical movements (Mason et al., 2007). This is presumably the fact that environmental enrichment has been shown to reduce stress responses, enhance stress coping abilities, and stimulate exploratory behaviour (Fox et al., 2006; Alnes et al., 2021). Such traits may be adventageous in the wild (Krause et al., 1998).

Fish raised in enriched environments visited the challenge zones more frequently in the Plain-maze test. Exploratory behaviour can be enhanced by physical enrichment, and concominantly, neophobia, fear of nnovelty or novelty avoidance responses can be reduced (Brydges & Braithwaite, 2009; Tatemoto et al., 2021; Gatto et al., 2022). Another explanation for the greater willingness to explore a novel environment (such as a bare maze) is related to increased shelter seeking behaviour (Braithwaite & Salvanes, 2005), which was not observed in a heterogenous environment (Kynard et a., 2013). Conversely, the latency time to leave the start zone, a frequently employed metric of boldness and exploratory behaviour (Colchen et al., 2017; Alnes et al., 2021), exhibited no discernible variation between the groups in any of the conducted tests. According to several studies, latency time is a more significant indicator of an individual’s motivation to engage in the test and explore novelty, as opposed to their boldness or exploratory tendency (Bergendahl et al., 2016; Carbia & Brown, 2019).

In the present study, CTRL individuals that exited the start zone earlier demonstrated a reduced tendency to engage in stress-related behaviours. The precise motivation behind fish exploration of novel environments in context- and species dependent, and consequently, frequently challenging to ascertain with absolute certainty (Braithwaite & Salvanes, 2005; Roberts et al., 2011; Carbia & Brown, 2019). It has been hypothesised that the implementation of an appropriate EE strategy duriing the rearing period may have a beneficial effect ont he development of risk-taking behaviour (Lee & Berejikian, 2008). Thus, the fish from EE-group exhibit a greater propensity to explore new environments than their conventionally reared counterparts, which may be advantageous during reintroduction into natural waters.

In the Plain-maze test, CTRL individuals were significantly larger in body weight compared to EE fish, however, this difference was not apparent in the Gravel-maze test. In both tests and treatments, body weight exhibited a positive correlation with the number of zone changes, which is a reliable indicator of activity. According to Millot et al. (2009), larger fish may possess a more adventurous personality and superior environmental adaptability in comparison to hatchery offspring of wild-caught parents, a consequence of hatchery selection. In our study, analogous patterns ware observed based on correlations within the two maze tests and treatment groups. However, despite the fish in the EE group exhibiting a significant decrease in size, there was no observed difference in their activity levels when compared to the CTRL group. The finding of this study aare consistent with those reported by Braithwaite and Salvanes (2005), who found that fish raised in a heterogenous environment exhibited greater boldness than those reaised in traditional environment. According to our findings, body size had a greater effect on behaviour within treatments, while environmental enrichment rather than body size influenced behaviour between treatments.

In summary, the results of this study suggest that exposing fish to a heterogenous environment during the early stage of life may promote resilience to stress and enhance exploratory behaviour. These traits may be vital for immediate survival and long-term success in natural waters following release. The reduced stress response, similar condition factor, improved survival and lower mortality rates of fish from enriched rearing conditions provide evidence that the use of appropriate environmetal enrichment can increase the overall welfare of sturgeon fry.

4.3. The effect of combining different feeding strategies and environmental enrichment on the zootechnical parameters and behaviour of Russian sturgeon

The reduced distance travelled by fish raised in enriched environments, while the number of zone changes remained similar, can be explained by the diminished swimming speed of fish which is associated with heightened exploratory behaviour (Pasquet et al., 2016). This may iindicate a reduced fear response or a higher sense of safety (Amichaud et al., 2024). Increasing the physical heterogeneity of the environment has been demonstrated to encourage natural behaviour and reduce fear response in fish (Collymore et al., 2015; Brunet et al., 2022). The results from behavioural testing show that an appropriate environmental enrichment strategy, implemented at the optimal time can result in long-term impacts on fish rearing.

The body size of the Russian sturgeon was influenced by the heterogenous rearing environment during the endogenous feeding and fry phase, but not during the larval rearing phase. At the end of the endogenous feeding phase, the EE fish has greater body weight and length; however, this was accompanied by higher mortality rates in comparison to the groups reared without gravel substrate. The increasing in size resulting from the EE combinations can be explained by greater efficiency in the absorption of endogenous resources (Boucher et al., 2014). However, the poor swimming ability of the fish may have impeded the movement of the larvae in some individuals during this developmental phase, leading to higher mortality in the EE group. The provision of EE has been shown to have a positive effect on survival rate of fish from the onset of exogenous feeding. EE has been demonstrated to mitigate aggression and stress in fish in the rearing unit, thereby enhancing the capacity of animals to cope with stress and increasing the survival rate of fish (Näslund & Johnsson, 2016; Arechavala-Lopez et al., 2022; Zhang et al., 2024).

Subsequently, the impact of EE on growth manifested itself exclusively during conventionally nursery phase (46–66 DPH). In the absence of gravel substrate, smaller body sizes were observed in the EE combinations. The size disparity observed during the rearing of juvenile fish may be attributable to the fish being relocated from the heterogeneous environment to which they had become accustomed during larval rearing. This event inevitably stressful for the fish, even though their access to food was now unhindered in the new environment. It has been previously reported hat the stress can have substantial effect on fish growth, as it results in the reallocation of energy expenditure from growth processes to the physiological processes of coping with stress (McCormick et al., 1998; Sadoul & Vijayan, 2016). By the fifth week post-transport, the size difference had been eliminated.

The effect of EE may vary depending ont he stage of life and the duration of its application (Amichaud et al., 2024). In the present trial, the positive effect of EE on growth could not be fully demonstrated due to the transition to conventional hatchery conditions during the nursery phase. It is therefore recommended that further research be conducted in order to explore the effects of long-term environmental enrichment on the development and stress responses of Russian sturgeon.

The use of frozen *Artemia* also had a detrimental effect on the growth and survival rates of Russian sturgeon larvae and fry. This finding is consisted with previous studies conducted on other sturgeon species (Mohler et al., 2000; Valentine et al., 2017; Piotrowska et al., 2021), which may be due to the fish's preference for actively moving prey (Efatpanah et al., 2024) and the fact that the nutrient content of frozen *Artemia* had a negative effect on feeding efficiency (Grabner et al., 1981; Sharma & Chakrabarti, 2009).

On the other hand, the present study was only able to demonstrate the effect of consuming live *Artemia* enriched with essential fatty acids and vitamins in the long term through the higher body weights of fish fed enriched *Artemia* (79–84 DPH, $p<0.005$). A comparable outcome was witnessed in lake sturgeon (A. *fulvescens*) (Yoon et al., 2022). It is well established that early nutrition can have a significant impact on later nutrient metabolism (Hales & Barker, 1992; Gluckman & Hanson, 2004), and it can be assumed that the critical window for intestinal development in fish coincides with the onset of exogenous feeding. The availability of fatty acids can have a long-term effect on metabolism, growth and the developoment of nervous system (Sargent et al., 1999a, 1999b; Kabaran & Besler, 2015). This, in turn, can indirectly impact digestive functions by contributing tot he development of the digestive nervous system (Heuckeroth & Schäfer, 2016). In contrast to our findings, previous research has reported that enriched Artemia has been shown to have a beneficial effect on the short-term growth of other sturgeon species Jalali et al., 2008; Hafezieh et al., 2009; Kamaszewski et al., 2014a). Consequently, *Artemia* enrichment is recommended, given its documented, long-term positive effect on the growth of Russian sturgeon, while the use its frozen form should be omitted.

The results of the present study suggest that fish consuming Chironomus from the initiation of feeding (EChi) were more motivated to explore the novel environment, but less interested in food. It is widely accepted that decline in food intake is a characteristic behavioural response of fish to stress (Schreck et al., 1997; Bonga, 1997). However, the results of the experiment suggest that EChi fish probably devoted their energy to exploration rather than feeding during the test. The role of exploratory behaviour may extend beyond the pursuit of food; it may also be driven by curiosity, the search for an escape route, or the need to find a familiar environment (Suarez & Gallup, 1985). This behaviour has been observed to be self-reinforcing by several authors, as it engenders understanding of the predictability of the environment and the development of a sense of control over it (Westerath et al., 2009). This may offer a potential explanation for the repeated return of fish to the acclimatisation zone, which could have provided them with a sense of safety and /or control over the environment. This, in consequence, may have induced a positive affective state in the fish, thereby enhancing their spatial learning ability (Brunet et al., 2022). Nevertheless, the impact of early *Chironomus* application on subsequent behaviour still remained unclear. The nutritional status of fish during the initial stages of ontogenesis plays a pivotal role in determining their long-term performance; however, it has been shown that the provision of nutrients at a later stage cannot compensate for deficiences experienced during early development (Fuiman & Ojanguran, 2011). The high essential nutrient content of Chironomus (Bogut et al., 2007, Kamler et al., 2008) combined with the nutrients present in the Artemia has been hypothesised to enhance the development of the larvae's nervous system and have a long-term effect on fish behaviour (Sargent et al., 1999a). Consistent with our finding, the beneficial effect of providing fish with essential fatty acids during early life stages on behaviour has been evidenced in other fish species (Lund et al., 2014). The beneficial effects of feeding frozen Chironomus from the onset of exogenous feeding on growth were observed in both experiments and were also evident in the long term in Trial 2. In comparison with zooplankton, the energy value of zoobenthos is able to satisfy the caloric requirements of sturgeon larvae, as well as due to its accessibility, it emerges as a more favourable nutrient source during feeding (Williot et al., 2005; Ruban, 2020). The utilisation of frozen *Chironomus* in concominant with live zooplankton is advocated from the initiation of exogenous feeding for a period of two to three weeks during the rearing of Russian sturgeon, owing to the short- and long-term developmental benefits.

From the onset of exogenous feeding, the practice of combining natiral and inert (non-living, chemically relatively unchanged) micronutrients, called as co-feeding, is considered a good option for promoting successful feeding of fish larvae (Halpati et al., 2024; Marinho et al., 2024). In the Trial 1 (2021), the body size of the groups co-fed natural end dry diet was consistently smaller that that of the groups weaned traditionally throughout the experiment. Additionally, the mortality rate was also higher in the co-fed groups before weaning. The practice of co-feeding has been demonstrated to facilitate the digestion of commerciaé feed (Ballagh et al., 2010). The reduced growth observed in the groups co-fed can be attributed tot he suboptimal utilization of inert feed in comparison to live or frozen natural feed, a phenomenon that has bee previously documented in Atlantic and Persian sturgeon (Piotrowska et al., 2013; Agh et al., 2013). However, several studies have reported the successful use of co-feeding regime in other sturgeon species, even immediately after the onset of exogenous feeding (Agh et al., 2013; Asgari et al., 2014; Lee et al., 2022). In consideration of the findings of this study, it can be concluded that the practice of co-feeding is not recommended for Russian sturgeon.

In the Trial 2 (2022), the Russian sturgeons were provided with a diet of natural food for a period of one to two weeks before the process of weaning started. The ’early’ weaned groups exhibited a smaller body size and higher mortality and lower survival rates (41,8 ± 2,9 % vs. 58,2 ± 7,9 %) than the late weaned group at the end of larviculture phase. The findings of this study provide further evidence that the consumption of natural food is beneficial to the early life stages of sturgeon (Memiş et al., 2009; Valentine et al., 2017; Lee et al., 2022).

The mortality rate peaked approximately three weeks after the onset of the weaning process. In the LW group, this sensitive period coincided with the week following transport, which resulted in increased mortality in both groups. However, this increase was more pronounced in the LW group. This finding indicates that the stress experienced by the fish could have been mitigated if transportation had been scheduled a week later. This observation can be of particular relevance to the context of hatchery practices.

Fish in grou W displayed a reduced tendency to remain motionless during the Plain-maze test. This behavioural manifestation has been associated with anxiety, which in frequently occurs with elevated stress levels (Egan et al., 2009; Cachat et al., 2010). As demonstrated in previous studies, the weaning process exerts a significant influence ont he behaviour of pikeperch raised in ponds (Molnár et al., 2018). In the present study, fish that weaned earlier exhibited increased stress tolerance, reduced anxiety levels in the maze and demonstrated greater competitive behaviour during weaning. The underlying reason may be attributable to species-specific factors or divergent life history characteristics, given that the larvae in our study were reared under continuous intensive RAS conditions.

With regard to the timing of weaning, three of the four optimal combinations were allocated to the later weaned groups. Consequently, when determinining the timing of weaning, it is advisable to strike a balance between the desired culture performance, larval rearing capacity and labour costs associated with maintaining natural food sources.

Following a comprehensive evaluation of the zootechnical parameters, the most effective treatment among the combinations tested was to provide the fish with a diet of *Chironomus* in combination with live, non-enriched *Artemia* commencing from the exogenous feeding. The gradual weaning process was initiated after a period of two weeks exclusive natural feeding. The results of the present study demonstrated that the application of *Artemia* enriched by essential fatty acids and vitamins in combination with *Chironomus* for a two to three weeks resulting in long-term growth benefits. The use of Chironomus from the onset os exogenous feeding is highly recommended. The environmental enrichment has been proven to yield developmental benefits during rearing for reintroduction, particularly with regard to enhancing stress coping. Conversely, the loss of envrionmental enrichment may have led to chronic stress. Additionally, enhanced environmental perception resulting from the environmental heterogeneity and high quality feeding can also improve cognitive functions. Accordingly, hatcheries are advised to use environmental enrichment in the rearing units during larviculture and to follow the highest standard feeding protocols.

5. Summary of new scientific findings

- In terms of larval development, the application of a higher stocking density (20 larvae L^{-1}) up to a mean bodyweight of 100 mg is considered appropriate for sterlet larviculture.
- The implementation of environmental enrichment in the rearing unit presents a trade-off between the cultivation of more advanced cognitive abilities (i.e. exploratory abilities, more ffctice stress-coping mechanisms), the enhancement of animal welfare status (i.e. the stimulation of species-specific behaviour, the reduction of chronic stress), and the rapid size growth in sterlet. Nevertheless, these characteristics are advantageous in case of restocking of natural waters.
- The environmental complexity modulates exploratory behaviour in Russian sturgeon juveniles, while its elimination leads to chronic stress, hindering the capacity of fish for rapid adaptation to the novel environment.
- The use of frozen *Artemia* result in a significant reduction in the growth of the Russian sturgeon's body size, thus being considered ill-advised.
- The provision of natural feeding from the initiation of exogenous feeding of Russian sturgeon, with a particular emphasis on the utilisation of benthic organism, in combination with environmental heterogeneity, results in a more efficacious assessment and perception of the surrounding environment, thereby enhancing the overall welfare of the fish. The incorporation of *Chironomus* and *Artemia* from the onset of exogenous feeding for a minimum of two weeks yields long-term growth and behavioural benefits for Russian sturgeon.

6. References

Agh, N., Noori, F., Irani, A., Van Stappen, G., Sorgeloos, P., 2013. Fine tuning of feeding practices for hatchery produced Persian sturgeon, *Acipenser persicus* and Beluga sturgeon, *Huso huso*. Aquacult. Res. 44, 335–344. https://doi.org/10.1111/j.1365-2109.2011.03031.x

Alnes, I.B., Jensen, K.H., Skorping, A., Salvanes, A.G.V., 2021. Ontogenetic change in behavioral responses to structural enrichment from fry to parr in juvenile Atlantic Salmon (*Salmo salar* L.). Front. in Vet. Sci. 8, 638888. https://doi.org/10.3389/fvets.2021.638888

Amichaud, O., Lafond, T., Fazekas, G.L., Kleiber, A., Kerneis, T., Batard, A., Goardon., L., Labbé, L., Lambert, S., Milla, S., Colson, V., 2024. Air bubble curtain improves the welfare of captive rainbow trout fry and fingerlings. Aquaculture, 586, 740828. https://doi.org/10.1016/j.aquaculture.2024.740828

Arechavala-Lopez, P., Cabrera-Álvarez, M.J., Maia, C.M., Saraiva, J.L., 2022. Environmental enrichment in fish aquaculture: A review of fundamental and practical aspects. A review of fundamental and practical aspects. Rev. Aquac. 14(2), 704–728. https://doi.org/10.1111/raq.12620

Asgari, R., Rafiee, G., Eagderi, S., Shahrooz, R., Poorbagher, H., Agh, N., Gisbert, E., 2014. Ontogeny of the digestive system in hatchery produced Beluga (*Huso huso* Linnaeus, 1758); a comparative study between Beluga and genus *Acipenser*. Aquacult. Nutr. 20(6), 595–608. https://doi.org/10.1111/anu.12113

Ballagh, D.A., Fielder, D.S., Pankhurst, P.M., 2010. Weaning requirements of larval mulloway, *Argyrosomus japonicus*. Aquacult. Res. 41(10), e493–e504. https://doi.org/10.1111/j.1365-2109.2010.02519.x

Bauman, J.M., Baker, E.A., Marsh, T.L., Scribner, K.T., 2015. Effects of rearing density on total length and survival of lake sturgeon free embryos. N. Am. J. Aquacul. 77, 444–448. https://doi.org/10.1080/15222 055.2015.1037475

Beitinger, T.L., 1990. Behavioral reactions for the assessment of stress in fishes. J. Gt. Lakes Res. 16(4), 495–528. https://doi.org/10.1016/S0380-1330(90)71443-8

Bergendahl, I. A., Salvanes, A. G. V., Braithwaite, V. A., 2016. Determining the effects of duration and recency of exposure to environmental enrichment. Appl. Animal Behav. Sci. 176, 163–169. https://doi.org/10.1016/j.applanim.2015.11.002

Bogut, I.E., Has-Schon, Z., Adamek, V., Rajković, V., Rajković., 2007. *Chironumus plumosus* larvae—a suitable nutrient for freshwater farmed fish. Poljopr., 13, 159–162. https://hrcak.srce.hr/16128

Bonga, S.E.W., 1997. The stress response in fish. Physiol. Rev. 77(3), 591–625. https://doi.org/10.1152/physrev.1997.77.3.591

Boucher, M.A., Baker, D.W., Brauner, C.J., Shrimpton, J.M., 2018. The effect of substrate rearing on growth, aerobic scope and physiology of larval white sturgeon *Acipenser transmontanus*. J. Fish. Biol. 92, 1731–1746. https://doi.org/10.1111/jfb.13611

Boucher, M.A., McAdam, S.O., Shrimpton, J. M., 2014. The effect of temperature and substrate on the growth, development and survival of larval white sturgeon. Aquaculture, 430, 139–148. https://doi.org/10.1016/j.aquaculture.2014.03.011

Boyd, C.E., McNevin, A.A., Davis, R.P., 2022. The contribution of fisheries and aquaculture to the global protein supply. Food Secur. 14(3), 805-827. https://doi.org/10.1007/s12571-021-01246-9

Braithwaite, V.A., Slavanes, A.G.V., 2005. Environmental variability in the early rearing environment generates behaviourally flexible cod: implications for rehabilitating wild population. P. Roy. Soc. 272, 1107–1113. https://doi.org/10.1098/rspb.2005.3062

Brunet, V., Kleiber, A., Patinote, A., Sudan, P.L., Duret, C., Gourmelen, G., Moreau, E., Fournel., C., Pineau, L., Calvez, S., Milla, S., Colson, V., 2022. Positive welfare effects of physical enrichments from the nature-, functions-and feeling-based approaches in farmed rainbow trout (*Oncorhynchus mykiss*). Aquaculture, 550, 737825. https://doi.org/10.1016/j.aquaculture.2021.737825

Brydges, N.M., Braithwaite, V.A., 2009. Does environmental enrichment affect the behaviour of fish commonly used in laboratory work? Appl. Anim. Behav. Sci. 118, 137–143. https://doi.org/10.1016/j.applanim.2009.02.017

Cachat, J., Stewart, A., Grossman, L., Gaikwad, S., Kadri, F., Chung, K.M., Wu, N., Wong, K., Roy, S., Suciu, C., Goodspeed, J., Elegante, M., Bartels, B., Elkhayat, S., Tien, D., Tan, J., Denmark, A., Gilder, T., Kyzar, E., DiLeo, J., Frank, K., Chang, K., Utterback, E., Hart P., Kalueff, A.V., 2010. Measuring behavioral and endocrine responses to novelty stress in adult zebrafish. Nat. Protoc. 5(11), 1786–1799. https://doi.org/10.1038/nprot.2010.140

Cámara-Ruiz, M., Santo, C., E., Gessner, J., Wuertz, S., 2019. How to improve foraging efficiency for restocking measures of Juvenile Baltic sturgeon (Acipenser oxyrinchus). Aquaculture, 502, 12–17. https://doi.org/10.1016/j.aquaculture.2018.12.021

Carbia, P.S., Brown, C., 2019. Environmental enrichment influences spatial learning ability in captive-reared intertidal gobies (*Bathygobius* *cocosensis*). Anim. Cogn. 22, 89–98. https://doi.org/10.1007/s10071-018-1225-8

Carrera-García, E., Rochard, E., Acolas, M.L., 2016. European sturgeon (*Acipenser sturio* L.) young of the year performance in different rearing environments –study within a stocking program. Environ. Biol. Fish. 99, 887–901. https://doi.org/10.1007/s10641-016-0531-8

Colchen, T., Faux, E., Teletchea, F., Pasquet, A., 2017. Is personality of young fish consistent through different behavioural tests? Appl. Anim. Behav. Sci. 194, 127–134. https://doi.org/10.1016/j.applanim.2017.05.012

Collymore, C., Tolwani, R.J., Rasmussen, S., 2015. The behavioral effects of single housing and environmental enrichment on adult zebrafish (*Danio rerio*). J. Am. Assoc. for Lab. Anim. Sci. 54(3), 280–285.

Efatpanah, I., Falahatkar, B., Sajjadi, M.M., Monsef Shokri, M., 2024. The Effect of Feeding with Chironomid and Artemia on Fatty Acids and Amino Acids Profiles in Persian Sturgeon (*Acipenser persicus*) Larvae. Aquacult. Nutr., 2024(1), 6975546. https://doi.org/10.1155/2024/6975546

Egan, R.J., Bergner, C.L., Hart, P.C., Cachat, J.M., Canavello, P.R., Elegante, M. F., Elkhayat, S.I., Bartels, B.K., Tien, A.K., Tien, D.H., Mohnot, S., Beeson, E., Glasgow, E., Amri, H., Zukowska, Z., Kalueff, A.V., 2009. Understanding behavioral and physiological phenotypes of stress and anxiety in zebrafish. Behav. Brain Res., 205(1), 38-44. https://doi.org/10.1016/j.bbr.2009.06.022

El Kertaoui, N., Lund, I., Assogba, H., Domínguez, D., Izquierdo, M.S., Baekelandt, S., Cornet, V., Mandiki, S.N.M., Montero, D., Kestemont, P., 2019. Key nutritional factors and interactions during larval development of pikeperch (*Sander lucioperca*). Sci. Rep. 9(1), 7074. https://doi.org/10.1038/s41598-019-43491-1

Elhetawy, A.I., Vasilyeva, L.M., Sudakova, N., Abdel-Rahim, M.M., 2023. Sturgeon aquaculture potentiality in Egypt in view of the global development of aquaculture and fisheries conservation techniques: an overview and outlook. Aquat. Sci. Eng. 38(4), 222-231. https://doi.org/10.26650/ASE20231277641

Ellis, T., North, B., Scott, A.P., Bromage, N.R., Porter, M., Gadd, D., 2002. The relationships between stocking density and welfare in farmed rainbow trout. J. Fish Biol. 61, 493-531. https://doi.org/10.1111/j.1095-8649.2002.tb00893.x

Feledi, T., Rónyai, A., 2013b. Preliminary results on siberian sterlet fry rearing and their comparison with some production performance parameters of “european” sterlet. Turkish J. Fish. Aquat. Sci. 13, 551–553. https://doi.org/10.4194/1303-2712-v13_3_20

Fox, C., Merali, Z., Harrison, C., 2006. Therapeutic and protective effect of environmental enrichment against psychogenic and neurogenic stress. Brain Behav. Res. 175, 1–8. https://doi.org/10.1016/j.bbr.2006.08.016

Fuiman, L.A., Ojanguran, A.F., 2011. Fatty acid content of eggs determines antipredator performance of fish larvae. J. Exp. Mar. Biol. Ecol. 497(2), 155–165. https://doi.org/10.1016/j.jembe.2011.06.004

Gatto, E., Bruzzone, M., Dal Maschio, M., Dadda, M., 2022. Effects of environmental enrichment on recognition memory in zebrafish larvae. Appl. Anim. Behav. Sci. 247, 105552. https://doi.org/10.1016/j.applanim.2022.105552

Gluckman, P.D., Hanson, M.A., 2004. The developmental origins of the metabolic syndrome. Trends Endocrinol. Metab. 15, 183–187. https://doi.org/10.1016/j.tem.2004.03.002.

Grabner, M., Wieser, W., Lackner, R., 1981. The suitability of frozen and freeze-dried zooplankton as food for fish larvae: a biochemical test program. Aquaculture, 26, 85–94. https://doi.org/10.1016/0044-8486(81)90112-5

Gunst, R.F., Mason, R.L., 2009. Fractional factorial design. Wiley Interdiscip. Rev.: Comput. Stat. 1(2), 234–244. https://doi.org/10.1002/wics.27

Hafezieh, M., Kamarudin, M.S., Bin Saad, C.R., Abd Sattar, M.K., Agh, N. and Hosseinpoar, H. 2009. Effect of enriched *Artemia urmiana* on growth, survival and composition of larval Persian sturgeon. Turk. J. Fisheries and Aquat. Sci. 9, 201–207. https://doi.org/10.4194/trjfas.2009.0212

Hales, C.N., Barker, D.J.P., 1992. Type 2 (non-insulin-dependent) diabetes mellitus: the thrifty phenotype hypothesis. Diabetol. 42, 1215–1222. https://doi.org/10.1093/ije/dyt133.

Halpati, R.P., Sukham, M., Pailan, G.H., Dasgupta, S., Sahoo, S., Malik, M.A., Sathees, M., Bhusare, S., Patekar, P., Marbaniang, B., Chandegara, A., 2024. Optimized co-feeding strategy of Anabas testudineus (Bloch 1792) larvae with enriched Moina micrura and egg custard-based inert diet; effects on growth, survival, and physio-metabolic responses. Aquacult. Int., 32, 7007–7029. https://doi.org/10.1007/s10499-024-01499-z

Heuckeroth, R.O., Schäfer, K.H., 2016. Gene-environment interactions and the enteric nervous system: Neural plasticity and Hirschsprung disease prevention. Dev. Biol. 417, 188–197. https://doi.org/10.1016/j.ydbio.2016.03.017.

Jalali, M.A., Hosseini, S.A., Imanpour, M.R., 2008. Effect of vitamin E and highly unsaturated fatty acid-enriched *Artemia urmiana* on growth performance, survival and stress resistance of Beluga (*Huso huso*) larvae. Aquacult. Res. 39, 1286-1291. https://doi.org/10.1111/j.1365-2109.2008.01992.x

Kabaran, S., Besler, T.T., 2015. Do fatty acids affect fetal programming? J. Health Popul. Nutr. 33, 1–9. https://doi.org/10.1186/s41043-015-0018-9.

Kamaszewski, M., Ostaszewska, T., Prusińska, M., Kolman, R., Chojnacki, M., Zabytyvskij, J., Jankowska, B., Kasprzak, R., 2014a. Effects of *Artemia* sp. enrichment with essential fatty acids on functional and morphological aspects of the digestive system in *Acipenser gueldenstaedti*i larvae. Turk. J. Fish. and Aquat. Sci. 14(4), 929-938. https://doi.org/10.4194/1303-2712-v14_4_12

Kamler, E., Wolnicki, J., Kamiński, R., Sikorska, J., 2008. Fatty acid composition, growth and morphological deformities in juvenile cyprinid, *Scardinius erythrophthalmus* fed formulated diet supplemented with natural food. Aquaculture, 278(1), 69–76. https://doi.org/10.1016/j.aquaculture.2008.03.012

Kobilinsky, A., Bouvier, A., Monod, H., 2012. PLANOR: an R package for the automatic generation of regular fractional factorial designs. R package version, 1–5.

Krause, J., Loader, S.P., McDermott, J., Ruxton, G.D., 1998 Refuge use by fish as a function of body length related metabolic expenditure and predation risks. Proc. R. Soc. B 265, 2373–2379. https://doi.org/10.1098/rspb.1998.0586

Kynard, B., Parker, E., Kynard, B., Horgan, M., 2013. Behavioural response of Kootenai white sturgeon (Acipenser transmontanus, Richardson, 1836) early life stages to gravel, pebble, and rubble substrates: guidelines for rearing substrate size. J. Appl. Icht. 29, 951–957. https://doi.org/10.1111/jai.12279

Laczynska, B., Siddique, M.A.M., Ziomek, E., Shelton, W.L., Fopp-Bayat, D., 2020. Early weaning effects on survival, growth, and histopathology of larval sterlet *Acipenser ruthenus*. N. Am. J. Aquacult., 82(2), 181–189. https://doi.org/10.1002/naaq.10141

Lee, J.S.F., Berejikan, B.A., 2008. Effects of the rearing environment on average behavior and behavioural variation in steelhead. J. Fish Biol. 70, 1736–1749. https://doi.org/10.1111/j.1095-8649.2008.01848.x

Lee, S., Zhai, S., Deng, D.F., Li, Y., Blaufuss, P.C., Eggold, B.T., Binkowski, F., 2022. Feeding strategies for adapting lake sturgeon (*Acipenser fulvescens*) larvae to formulated diets at early life stages. Animals, 12(22), 3128. https://doi.org/10.3390/ani12223128

Ljubobratović, R., 2023. Aegear: Computer vision toolkit for analyzing animal motion in complex aquatic environments. Retrieved from https://github.com/ljubobratovicrelja/aegear

Ljubobratović, U., Bogár, K., Káldy, J., Fazekas, G., Vass, N., Feledi, T., Kovács, G., 2022. Optimizing the gonadoliberin dosage and evaluating the egg quality in the preseason and seasonal artificial reproduction of pond-reared sterlet *Acipenser ruthenus*. Anim. Reprod. Sci. 247, 107097. https://doi.org/10.1016/j.anireprosci.2022.107097

Lund, I., Höglund, E., Ebbesson, L.O., Skov, P.V., 2014. Dietary LC-PUFA deficiency early in ontogeny induces behavioural changes in pike perch (*Sander lucioperca*) larvae and fry. Aquaculture, 432, 453–461. https://doi.org/10.1016/j.aquaculture.2014.05.039

Lundova, K., Kouril, J., Sampels, S., Matousek, J., Stejskal, V., 2018. Growth, survival rate and fatty acid composition of sterlet *(Acipenser ruthenus*) larvae fed fatty acid-enriched *artemia nauplii*. Aquacult. Res. 49, 3309–3318. https://doi.org/10.1111/are.13794

Marinho, Y.F., Oliveira, C.Y.B., Mendes, L.E.M., Santos, I.R.A., Dias, J.A.R., Ândrade, M., Lopes, Y.V.A., Azevedo, J.W.J., Lourenço, C.B., Moura, R.S.T., Ottoni, F.P., 2024. Co-feeding using live food and feed as first feeding for the small catfish *Trachelyopterus galeatus* (Linnaeus 1766). Arq. Bras. de Med. Vet. e Zootec. 76(2), 323–332. https://doi.org/10.1590/1678-4162-13060

Martins, C.I., Galhardo, L., Noble, C., Damsgård, B., Spedicato, M.T., Zupa, W., Beauchaud, M., Kulczykowska, E., Massabuau, J., Carter, T., Planellas, S.R., Kristiansen, T., 2012. Behavioural indicators of welfare in farmed fish. Fish Physiol. and Biochem. 38, 17–41. https://doi.org/10.1007/s10695-011-9518-8

Mason, G., Clubb, R., Latham, N., Vickery, S., 2007. Why and how shuld we use environmental enrichment to tackle stereotypic behavior? Appl. Anim. Behav. 102, 163–188. https://doi.org/10.1016/j.applanim.2006.05.041

McCormick, S.D., Shrimpton, J.M., Sloan, K.E., O'Dea, M.F., Carey, J.B., Moriyama, S., Björnsson, B.Th., 1998. Repeated acute stress reduces growth rate of Atlantic salmon parr and alters plasma growth hormone, insulin-like growth factor 1 and cortisol. Aquaculture 168, 221–235. https://doi.org/10.1016/S0044-8486(98)00351-2

Memiş, D., Ercan, E., Çelikkale, M. S., Timur, M., & Zarkua, Z., 2009. Growth and survival rate of Russian sturgeon (*Acipenser gueldenstaedtii*) larvae from fertilized eggs to artificial feeding. Turk. J. Fish. Aquat. Sci. 9(1).

Millot, S., Bégout, M.L., Chatain, B., 2009. Exploration behavior and flight response toward a stimulus in three sea bass strains (*Dicentrarchus labrax* L.). Appl. Anim. Behav. Sci. 119, 108–114. https://doi.org/10.1016/j.applanim.2009.03.009

Mohler, J.W., King, M.K., Farrell, P.R., 2000. Growth and survival of first-feeding and fingerling Atlantic sturgeon under culture conditions. N. Am. J. Aquacult., 62(3), 174-183. https://doi.org/10.1577/15488454(2000)062<0174:GASOFF>2.3.CO;2

Mohseni, M., Pourkazemi, M., Amiri, B.M., Kazemi, R., Foshkhomi, M.R., Kaladkova, L. N., 2000. A study on the effects of stocking density of eggs and larvae on the survival and frequency of morphological deformities in Persian sturgeon, great sturgeon and stellate sturgeon. Iran. J. Fish. Sci. 2, 75–90.

Molnár, T., Csuvár, A., Benedek, I., Molnár, M., Kabai, P., 2018. Domestication affects exploratory behaviour of pikeperch (*Sander lucioperca* L.) during the transition to pelleted food. PLoS One, 13(5), e0196118. https://doi.org/10.1371/journal.pone.0196118

Montero, D., Izquierdo, M.S., Tort, L., Robaina, L., Vergara, J.M., 1999. High stocking density produces crowding stress altering some physiological and biochemical parameters in gilthead seabream, *Sparus aurata*, juveniles. Fish Physiol. and Biochem. 20, 53–60. https://doi.org/10.1023/A:1007719928905

Naderi, M., Jafaryan, H., & Jafaryan, S., 2017. Effect of different stocking densities on haematological parameters and growth performance of great sturgeon (*Huso huso* Linnaeus, 1758) juveniles. Iran. J. Aquat. Anim. Health, 3, 1–10. https://doi.org/10.29252/ijaah.3.2.1

Näslund, J., Johnsson, J.I., 2016. Environmental enrichment for fish in captive environments: effects of physical structures and substrates. Fish Fish 17, 1–30. https://doi.org/10.1111/faf.12088

Ni, M., Wen, H., Li, J., Chi, M., Bu, Y., Ren, Y., Zhang, M., Song, Z., & Ding, H., 2016. Effect of stocking density on mortality, growth and physiology of juvenile Amur sturgeon (*Acipenser schrenkii*). Aquac. Res. 47, 1596–1604. https://doi.org/10.1111/are.12620

Oprea, D., Oprea, L., 2009. The effect of density on bester (*H. huso* × *a. ruthenus*) larvae reared in a superintensive system. Lucrări Ştiinţ. Ser. Zooteh. 52, 655–660.

Pasquet, A., Sebastian, A., Begout, M.L., LeDore, Y., Teletchea, F., Fontaine, P., 2016. First insight into personality traits in Northern pike (*Esox lucius*) larvae: a basis for behavioural studies of early life stages. Environ. Biol. Fish. 99, 105–115. https://doi.org/10.1007/s10641-015-0459-4

Pickering, A.D., Stewart, A., 1984. Acclimation of the interrenal tissue of the brown trout, *Salmo trutta* L., to chronic crowding stress. J. Fish Biol. 24, 731–740. https://doi.org/10.1111/j.1095-8649.1984.tb048 44.x

Piotrowska, I., Szczepkowska, B., Kozłowski, M., 2021. Influence of the size and form of Artemia sp. nauplii on the growth and survival of Atlantic sturgeon (*Acipenser oxyrinchus* Mitchill) larvae. Fish. & Aquatic Life, Arch. Pol. Fish. 29(2), 69–79. https://doi.org/10.2478/aopf-2021-0009

Piotrowska, I., Szczepkowska, B., Kozłowski, M., Wunderlich, K., Szczepkowski, M., 2013. Results of the larviculture of Atlantic sturgeon (*Acipenser oxyrinchus*) fed different types of diets. Fish. & Aquatic Life, Arch. Pol. Fish. 21(1), 53–61. https://doi.org/10.2478/aopf-2013-0006

Roberts, L.J., Taylor, J., Garcia de Leaniz, C., 2011. Environmental enrichment reduces maladaptive risk-taking behavior in salmon reared for conservation. Biol. Conserv. 144, 1972–1979. https://doi.org/10.1016/j.biocon.2011.04.017

Romero-Ferrero, F., Bergomi, M.G., Hinz, R.C., Heras, F.J.H., de Polavieja, G.G., 2019. idtracker.ai: tracking all individuals in small or large collectives of unmarked animals. Nat. Method. 16(2), 179–182.

Rónyai, A., 2009. Effect of different synthetic gonadotrop-releasing hormone analogues and their combinations with an anti-dopaminergic compound on the reproduction performance of sterlet (*Acipenser ruthenus* L.). Aquacult. Res. 40(3), 315-321. https://doi.org/10.1111/j.1365-2109.2008.02094.x

Ruane, N.M., Carballo, E.C., Komen, J., 2002. Increased stocking density influences the acute physiological stress response of common carp *Cyprinus carpio* (L.). Aquacult. Res. 33(10), 777-784. https://doi.org/10.1046/j.1365-2109.2002.00717.x

Ruban, G.I., 2020. Exogenous feeding in the early life stages of sturgeon (*Acipenseridae*). Inland Water Biol. 13(4), 613-619. https://doi.org/10.1134/S1995082920040094

Rybnikár, J., Prokeš, M., Mareš, J., Cileček, M., 2011. Early development and growth of sterlet (*Acipenser ruthenus*) in The Czech Republic. Acta Univ. Agric. et Silvic. Mendel. Brun. 59, 217–226. https://doi.org/10.11118/actaun201159050217

Sadoul, B., Vijayan, M.M., 2016. Stress and growth. In: Schreck, C.B., Tort, L., Farrell, A.P., Brauner, C.J. (Eds.), Fish Physiology (Vol. 35, pp. 167-205). Academic Press. https://doi.org/10.1016/B978-0-12-802728-8.00005-9

Sargent, J.R., Bell, G., McEvoy, L., Tocher, D., Estevez, A., 1999a. Recent developments in the essential fatty acid nutrition of fish. Aquaculture, 177, 191–199. https://doi.org/10.1016/S0044-8486(99)00083-6

Sargent, J.R., McEvoy, L.A., Estevez, A., Bell, M., Henderson, J., Tocher, D., 1999b. Lipid nutrition of marine fish during early development: current status and future directions. Aquaculture, 179, 217–229. https://doi.org/10.1016/S0044-8486(99)00191-X

Schreck, C.B., Olla, B.L., Davis, M.W., 1997. Behavioral responses to stress. In: Randy, H. (Eds.), Seminar Series-Society for Experimental Biology, Cambridge University Press. 62(1) pp. 145-170).

Sharma, J.G., Chakrabarti, R., 2009. Comparative growth performance and proteolytic enzyme activity of Indian major carp larvae, fed with live food and refridgerated-plankton food. Indian J. Anim. Sci. 79, 1185–1188.

Spence, R., Magurran, A.E., Smith, C., 2011. Spatial cognition in zebrafish: the role of strain and rearing environment. Anim. Cogn. 14, 607–12. https://doi.org/10.1007/s10071-011-0391-8

Suarez, S.D., Gallup Jr, G.G., 1985. Open-field behaviour in chickens: A replication revisited. Behav. Process. 10(4), 333–340. https://doi.org/10.1016/0376-6357(85)90034-8

Tatemoto, P., Valença-Silva, G., Queiroz, M.R., Broom, D.M., 2021. Living with low environmental complexity increases fear indicators in Nile tilapia. Anim. Behav. 174, 169–174. https://doi.org/10.1016/j.anbehav.2021.02.006

Valentine, S.A., Bauman, J.M., Scribner, K.T., 2017. Effects of alternative food types on body size and survival of hatchery-reared Lake Sturgeon larvae. N. Am. J. Aquacult. 79(4), 275–282. https://doi.org/10.1080/15222055.2017.1330788

Westerath, S.H., Laister, S., Winckler, C., Knierim, U., 2009. Exploration as an indicator of good welfare in beef bulls: an attempt to develop a test for on-farm assessment. Appl. Anim. Behav. Sci. 116(2-4), 126–133. https://doi.org/10.1016/j.applanim.2008.08.012

Williot, P., Brun, R., Rouault, T., Pelard, M., Mercier, D., 2005. Attempts at larval rearing of the endangered western European sturgeon, *Acipenser sturio* (Acipenseridae), in France. Cybium, 29(4), 381–387.

Yoon, G.R., Amjad, H., Weinrauch, A.M., Laluk, A., Suh, M., Anderson, W.G., 2022. Long-term effects of EPA and DHA enriched diets on digestive enzyme activity, aerobic scope, growth and survival in age-0 Lake Sturgeon (*Acipenser fulvescens*). Aquaculture, 552, 737972. https://doi.org/10.1016/j.aquaculture.2022.737972

Zhang, Z., He, Y., Wang, J., Zheng, Y., Mo, J., Zhang, X., Liu, W., 2024. A Global Synthesis of Environmental Enrichment Effect on Fish Stress. Fish and Fish. 0, 1–24. https://doi.org/10.1111/faf.12870

7. Publications

Doctoral School of Agricultural and Food Sciences

Georgina Lea Fazekas

LIST OF PUBLICATIONS

List of publications related to the thesis (2)

1. **Fazekas, G**., Káldy, J., Kovács, Gy., Müller, T., Ljubobratovic, U., 2022. The effect of stocking density on sterlet *Acipenser ruthenus* (Linnaeusm 1758) larvae in the recirculating aquaculture system. Journal of Applied Ichthyology, 00:1–8. http://doi.org/10.1111/jai.14341
   IF: 0,7

2. **Fazekas, G**., Müller, T., Stanivuk, J., Fazekas, D. L., Káldy, J., Tóth, F., Bürgés, J., Colchen, T., Vass, N., Ljubobratović, U., 2023. Evaluation of applying environmental enrichment to sterlets (*Acipenser ruthenus* L.) in early life stages. Applied Animal Behaviour Science, 268, 106090. https://doi.org/10.1016/j.applanim.2023.106090
   IF: 2,0

List of other publications

Foreign language scientific articles in international journals (17)

3. Káldy, J., Mozsár, A., Fazekas, Gy., Farkas, M., Fazekas, D.L., **Fazekas, G.L**., Goda, K., Gyöngy, Zs., Kovács, B., Semmens, K., Bercsényi, M., Molnár, M., Patakiné Várkonyi, E., 2020. Hybridization of Russian Sturgeon (Acipenser gueldenstaedtii, Brandt and Ratzeberg, 1833) and American Paddlefish (Polyodon spathula, Walbaum 1792) and Evaluation of Their Progeny. GENES 11:753 https://doi.org/10.3390/genes11070753

4. **Fazekas, G**., Vass, V., Demény, F., Tóth, F., Ljubobratović, U., 2021. The effect of different surface cleaning devices on the success of swim bladder inflation in pikeperch (Sander lucioperca L.) larvae. North American Journal of Aquaculture, 83(2), 78–82. https://doi.org/10.1002/naaq.10172

5. Ljubobratović, U., **Fazekas, G**., Koljukaj, A., Ristović, T., Vass, V., Ardó, L., Stanisavljević, N., Vukotić, G., Pešić, M., Milinčić, D., Kostić, A., Lukić, J., 2021. Pike-perch larvae growth in response to administration of lactobacilli-enriched inert feed during first feeding. Aquaculture 542:736901. https://doi.org/10.1016/j.aquaculture.2021.736901

6. Káldy, J., Patakiné Várkonyi, E., **Fazekas, G.L**., Nagy, Z., Sándor, Z.J., Bogár, K., Kovács, G., Molnár, M., Lázár, B., Goda, K., Gyöngy, Z., Ritter, Z., Nánási, P.,Jr., Horváth, Á., Ljubobratović, U., 2021. Effects of Hydrostatic Pressure Treatment of Newly Fertilized Eggs on the Ploidy Level and Karyotype of

Pikeperch Sander lucioperca (Linnaeus, 1758). LIFE, 11:1296. https://doi.org/10.3390/life11121296

7. Özgür, M.E., Erdoğan, S., Rašković, B., **Fazekas, G**., Ljubobratović, U., 2021. Mid-autumn spermiation in outdoor-cultured pikeperch (*Sander lucioperca*) using different gonadoliberin application strategies. Aquaculture Reports 21:100891. https://doi.org/10.1016/j.aqrep.2021.100891

8. Ljubobratović, U., Bogár, K., Káldy, J., **Fazekas, G**., Vass, N., Feledi, T., Kovács, Gy., 2022. Optimizing the gonadolibering dosage and evaluation the egg quality in the preseason and seasonal artificial reproduction of pond-reared sterlet Acipenser ruthenus. Animal Reproduction Science, 247:107097. https://doi.org/10.1016/j.anireprosci.2022.107097

9. Ljubobratović U., **Fazekas G**., Nagy Z., Kovács Gy., Tóth F., Fehér D., Zarski D. (2022). Fish with larger pre-seasonal oocytes yields lower egg quality in season – A case study of outdoor – cultured domesticated Pikeperch (Sander lucioperca). Animal Reproduction Science, 238:106936. https://doi.org/10.1016/j.anireprosci.2022.106936

10. Ljubobratović U., Kitanović N., Milla, S., Marinović Z., **Fazekas, G**., Stanivuk, J., Nagy Z., Horváth, Á., 2023. Predicting population's oocyte maturation competence and evaluating individual's latency time using in vitro oocyte maturation in pikeperch (*Sander lucioperca*). Aquaculture, 562:738851. 10.1016/j.aquaculture.2022.738851

11. Bogár, K., Stanivuk, J., Géczi, A., **Fazekas, G.L**., Kovács, B., Lázár, B., Molnár, M., Ardó, L., Ljubobratovic, U., Kovács, Gy., Péter, D., Várkonyi, E., Káldy, J., 2024. Investigation of Sexes and Fertility Potential of Female Russian Sturgeon (*Acipenser gueldenstaedtii*) and Male American Paddlefish (*Polyodon spathula*) Hybrids. Life, 14(7), 818. https://doi.org/10.3390/life14070818

12. Stanivuk, J., Berzi-Nagy, L., Gyalog, G., Ardó, L., Vitál, Z., Plavša, N., **Fazekas, G.L**., Horváth, Á., Ljubobratović, U., 2024. The rank of intensification factors strength in intensive pond production of common carp (*Cyprinus carpio* L.). Aquaculture, 583, 740584. https://doi.org/10.1016/j.aquaculture.2024.740584

13. Ljuboratović, U., Rašković, B., Horváth, Á., **Fazekas, G**., Markelić, M., Ristović, T., Nagy, Z., Stanivuk, J., Rocha, E., Bürgés, J., Milla, S., 2024. Effect of sex isolation on the reproduction of pikeperch (*Sander lucioperca* L.) submitted to the cycle shift from outdoor to fully controlled conditions. *Aquaculture*, 741903. https://doi.org/10.1016/j.aquaculture.2024.741903

14. Amichaud, O., Lafond, T., **Fazekas, G.L**., Kleiber, A., Kerneis, T., Batard, A., Goardon, L., Labbé, L., Lambert, S., Milla, S., Colson, V., 2024. Air bubble

curtain improves the welfare of captive rainbow trout fry and fingerlings. Aquaculture, 586, 740828. https://doi.org/10.1016/j.aquaculture.2024.740828

15. Ljubobratović, U., Ardó, L., **Fazekas, G**., Horváth, Z., Lukić, J., 2024. Effect of thermal management on vitellogenesis and maturation in indoor-reared pikeperch (Sander lucioperca). Czech Journal of Animal Science, 69(1), 18-28. https://doi.org/10.17221/136/2023-CJAS

16. Káldy, J., **Fazekas, G**., Kovács, B., Molnár, M., Lázár, B., Pálinkás-Bodzsár, N., Ljubobratović, U., Fazekas, G., Kovács, G., Várkonyi, E., 2024. Unidirectional hybridization between American paddlefish *Polyodon spathula (Walbaum, 1792*) and sterlet *Acipenser ruthenus (Linnaeus, 1758*). PeerJ 12:e16717 https://doi.org/10.7717/peerj.16717

17. Stanivuk, J., Marinović, Z., Kitanović, N., Mozsár, A., Káldy, J., Várkonyi, E., Molnár, M., Müller, T., **Fazekas, G**., Kovács, B., Bogár, K., Nagy, Z., Fazekas, D., Horváth, Á., Ljubobratović, U., 2025. Evaluation of biotechnology approaches–Polyploidization and hybridization–For improvement of pikeperch (*Sander lucioperca*) larviculture and juvenile on-grow with special regard to morphological traits and gonadal development. Aquaculture, 742938. https://doi.org/10.1016/j.aquaculture.2025.742938

18. Ljubobratović, U., Kitanović, N., Milla, S., Marinović, Z., Stanivuk, J., **Fazekas, G**., Vass, N., Nagy, Z., Horváth, Á., 2025. The development of oocyte maturation competence, egg, oil globule and larval size are population-dependent in pikeperch (Sander lucioperca). Aquaculture, 742932. https://doi.org/10.1016/j.aquaculture.2025.742932

19. Ljubobratović, U., Rašković, B., Horváth, Á., **Fazekas, G**., Markelić, M., Ristović, T., Nagy, Z., Stanivuk, J., Rocha, E., Bürgés, J., Milla, S., 2025. Effect of sex isolation on the reproduction of pikeperch (*Sander lucioperca* L.) submitted to the cycle shift from outdoor to fully controlled conditions. Aquaculture, 596, 741903. https://doi.org/10.1016/j.aquaculture.2024.741903

Other journal articles in hungarian (1)

20. Káldy, J., Mozsár, A., Fazekas, Gy., Farkas, M., Fazekas, D.L., **Fazekas, G.L**., Goda, K., Gyöngy, Zs., Kovács, B., Ken, Semmens et al. 2020. A vágótok (Acipenser gueldenstaedtii, Brandt and Ratzeberg, 1833) és az amerikai lapátorrú tok (Polyodon spathula, Walbaum 1792) hibridizációja és az utódaik jellemzése. HALÁSZAT 113. évf. 3, 105–106.

Conference abstracts

Hungarian abstracts (10)

1. Káldy, J., Mozsár, A., Fazekas, Gy., Farkas, M., Fazekas, D.L., **Fazekas, G.L**., Goda, K., Gyöngy, Zs., Kovács, B., Semmens, K., Bercsényi, M., Molnár, M., Patakiné Várkonyi, E., 2020. Az első sikeres hibridizáció a vágótok (*Acipenser gueldenstaedtii*) és az amerikai lapátorrú tok (*Polyodon spathula*) között, mely életképes utódokat eredményezett. HALÁSZATFEJLESZTÉS, 37, 36–40. https://haki.naik.hu/sites/default/files/uploads/2020-10/halaszatfejlesztes_37.pdf

2. **Fazekas, G**., Káldy, J., Kovács, Gy., Ljubobratović, U., 2020. Kecsegelárva (*Acipenser ruthenus* L.) megmaradásának és növekedésének vizsgálata eltérő telepítési sűrűségek függvényében. HALÁSZATFEJLESZTÉS, 37, 18–20. https://haki.naik.hu/sites/default/files/uploads/2020-10/halaszatfejlesztes_37.pdf

3. Ljubobratović, U., Nagy, Z., Nagy, G., Lévai, F., **Fazekas, G**., 2020. A fekete sügér (*Micropterus salmoides*) potenciális szerepe a tógazdasági haltermelés intenzifikálásában. HALÁSZATFEJLESZTÉS, 37, 12–13. https://haki.naik.hu/sites/default/files/uploads/2020-10/halaszatfejlesztes_37.pdf

4. Ljubobratović, U., Kitanović, N., Marinović, Z., Vass, N., **Fazekas G**., Stanivuk J., Nagy Z., Horváth, Á., 2022. Háziasított süllő anyahal mesterséges hormon stimulációra kész állapotánal meghatározása; HALÁSZATFEJLESZTÉS, 39, 48–52. https://press.mater.uni-mate.hu/51/1/Hal%C3%A1szatfejleszt%C3%A9s_39_2022m%C3%A1jus.pdf

5. Stanivuk, J., Káldy, J., Várkonyi, E., Molnár, M., **Fazekas, G.L**., Horváth, Á., Ljubobratović, U., 2023. A triploid fogassüllő (*Sander lucioperca*) lárvanevelése. HALÁSZATFEJLESZTÉS, 40, 54–58.

6. **Fazekas, G.**, Michaud, O., Lafond, T., Kleiber, A., Kerneis, T., Batard, A., Goardon, L., Labbé, L., Lambert, S., Milla, S., Ljubobratović, U., Colson, C., 2024. Buborékfüggöny hatása a tenyésztett pisztrángok viselkedésére. HALÁSZATFEJLESZTÉS, 41, 121.

7. Káldy, J., Bogár, K., Stanivuk, J., Géczi, A., **Fazekas, G.L.**, Kovács, B., Molnár, M., Ardó, L., Ljubobratović, U., Kovács, Gy., Péter, D., Lázár, B., Várkonyi, E., 2024. A vágótok (♀) (*Acipenser gueldenstaedtii*) és az amerikai lapátorrú tok (♂) (*Polyodon spathula*) hibridek nemének meghatározása és korai gonádfejlődésének vizsgálata. HALÁSZATFEJLESZTÉS, 41, 94–100.

8. Stanivuk, J., Ljubobratović, U., Káldy, J., Varkonyi, E., Molnár, M., **Fazekas, G.**, Kitanović, N., Lefler, K., Horváth, Á., Zoran, M., 2024. Az interspecifikus hibridizáció és a triploidizáció együttes hatása a fogassüllő (*Sander lucioperca*) és a fogassüllő (♀) x kősüllő (♂) (*Sander volgensis*) hibridek korai ivarfejlődésére. HALÁSZATFEJLESZTÉS, 41, 89–93.

9. Fazekas, G., Müller, T., Berzi-Nagy, L., Ljubobratović, R., Stanivuk, J., Fazekas, D.L., Káldy, J., Vass, N., Ljubobratović, U., 2025. Befolyásolja-e a takarmányozási stratégia és a környezetgazdagítás a vágótok ivadékok környezetükhöz való viszonyát? – Lárvanevelési gyakorlatok optimális faktoriális kombinációjának keresése. HALÁSZATFEJLESZTÉS, 234, 25–32.

10. Vass, N., Fazekas, G., Nagy, Z., Stanivuk, J., Géczi, A., Ljubobratović, U., 2025. Két akvakultúrában jelentős fészkelő halfaj ívási viselkedésének értékelése mesterséges körülmények között (előzetes eredmények). HALÁSZATFEJLESZTÉS, 234, 87–93.

International abstracts (9)

1. Stanivuk, J., Horváth, Á., Marinović, Z., **Fazekas, G.**, Káldy, J., Ljubobratović, U., 2022. Hybridization and triploidization as tools to improve larviculture in pikeperch (*Sander lucioperca*); In Aquculture Europe 2022: Innovative solutions in a changing world - Abstracts (pp. 1241–1242). European Aquaculture Society. https://aquaeas.org/Program/PaperDetail/39829

2. **Fazekas, G**., Stanivuk, J., Fazekas, D., Ljubobratović, U., 2022. Evaluation of applying environmental enrichment on sterlet Acipenser ruthenus in early life stages. In Aquculture Europe 2022: Innovative solutions in a changing world - Abstracts (pp. 419–220). European Aquaculture Society. https://aquaeas.org/Program/PaperDetail/39624

3. Ljubobratović, U., Kitanovic, N., Milla, Sylvian, Marinovic, Z., Fazekas, G., Stanivuk, J., Zarski, D., Horváth, Á., 2022. Predicting the egg quality based ont he oocyte diameter and in vitro maturation in domesticated pikeperch (*Sander lucioperca*). In A. Ciereszko & D. Zarski (Eds.), 8th International Workshop on the Biology of Fish Gametes - Book of Abstracts (p. 30). Gdansk, Lengyelország.

4. Stanivuk, J., Marinovic, Z., Kitanović, N., **Fazekas, G.L.**, Várkonyi, E., Molnár, M., Káldy, J., Ljubobratović, U., Horváth, Á., 2023. Triploidization as a fertility reduction tool in pikeperch (*Sander lucioperca*) and volga pikeperch (*Sander volgensis*) hybrid production. In Balanced Diversity in Aquaculture Development: Aquaculture Europe 2023 - Abstract Book (pp. 1408–1409). European Aquaculture Society. https://eposters.blob.core.windows.net/eas-eposters/AE2023AbstractBook.pdf

5. Nagy, Z., Ljubobratović, U., Biró, J., **Fazekas, G**., 2023. Effect of different rearing environments on the water quality and on the production traits of hybrid stripped bass (*Morone saxatilis x Morone chrysops*) in Hungary. In Balanced Diversity in Aquaculture Development: Aquaculture Europe 2023 - Abstract Book (p. 990). European Aquaculture Society. https://eposters.blob.core.windows.net/eas-eposters/AE2023AbstractBook.pdf

6. Ljubobratović, U., Kitanović, N., Stavinuk, S., Marinović, M., **Fazekas, G**., Nagy, Z., Horváth, Á., 2023. Predictability of latency time using the in vitro oocyte maturation in pikeperch (*Sander lucioperca*). In Balanced Diversity in Aquaculture Development: Aquaculture Europe 2023 - Abstract Book (p. 801). European Aquaculture Society. https://eposters.blob.core.windows.net/eas-eposters/AE2023AbstractBook.pdf

7. Stanivuk, J., Berzi-Nagy, L., Gyalog, G., Vitál, V., Fazekas, G.L., Ljubobratović, U., 2024. Water quality in intensive Common carp (*Cyprinus carpio* L.) pond production. In FAO Proceedings of the Sixth International Carp Conference (p. 36). Food and agriculture Organization of the United Nations. https://openknowledge.fao.org/server/api/core/bitstreams/adc4d2af-ca24-42e0-bb2e-42916ec7e9c6/content

8. Vass, N., **Fazekas, G**., Nagy, Z., Stanivuk, J., Géczi, A., Ljubobratović, U., 2025. Spawning behaviour of two commercially important nesting perciformes. In Aquaculture Europe 2025: Aquaculture for everyone – Abstract Book (pp. 1851–1852). European Aquaculture Society. https://eposters.blob.core.windows.net/eas-eposters/AE2025AbstractBook.pdf

9. Ljubobratović, U., Kitanovic, N., **Fazekas, G**., Stanivuk, J., Marinovic, Z., Vass, N., Nagy, Z., Horváth, Á., 2025. Different scenarios of photothermal management and its impact ont he out-of-season egg quality in pikeperch (*Sander lucioperca*)? In Aquaculture Europe 2025: Aquaculture for everyone – Abstract Book (pp. 989–990). European Aquaculture Society. https://eposters.blob.core.windows.net/eas-eposters/AE2025AbstractBook.pdf