

## THE INFLUENCE OF REARING CONDITIONS ON GROWTH, MEAT QUALITY AND MORTALITY OF *Acipenser ruthenus*

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### Abstract

To evaluate the growth of sterlet sturgeon (*Acipenser ruthenus*, Linnaeus 1758) in different rearing production systems (recirculating aquaculture system - RAS and earthen ponds system), the meat quality (biochemical profile), growth (allometric and Fulton coefficient) and mortality indices were determined. Sterlet sturgeon specimen were reared for 60 days in a RAS system, after which half of the biological material was transferred into earthen ponds, while the other half was further reared in the RAS system. The physico-chemical parameters of the technological water were monitored during the experimental period. The fish specimens were fed by using the same feed and the same feeding strategy. It was observed that the specimen reared in earthen ponds presented an isometric growth, while those reared in the RAS system had a positive allometric growth. The results registered after biochemical fish meat analysis highlighted that specimen reared in earthen ponds had a higher protein concentration compared to the ones reared in RAS system. As well, the survival rate of fish individuals was higher in the earthen ponds system.

**Key words:** allometry, aquaculture systems, Fulton, meat biochemistry.

### INTRODUCTION

Due to the lack of bones, with a palatable meat and being a source of black caviar, sturgeons are considered a high value product.

Sterlet (*Acipenser ruthenus*) belongs to the *Acipenseridae* family, is a species relatively small compared with other members of the family.

It is a species with rapid sexual maturation. In a controlled environment, sturgeons grow faster and reach sexual maturity earlier, compared to a natural environment (Chebanov & Billard, 2001).

Sterlet is an endangered species in the wild, due to dam construction across the rivers, over-fishing, pollution, and the degradation of the natural environment (Billard & Lecointre, 2001). All these factors are claimed to disturb the migratory routs, affecting the reproduction potential of the sterlet (Heidary et al., 2012). One alternative solution to this dramatic decline in stocks could be represented by aquaculture.

Advances in nutrition and the management of the production cycle could improve the economic efficiency of the aquaculture industry. (Hassaan et al., 2019)

This study aimed to present the differences (length-weight correlation, biochemical profile of the meat, survival rate) between sterlet reared under controlled conditions in a recirculating aquaculture system and sterlet reared in floating cages under natural environmental conditions, while also comparing our data with relevant data from specialised literature.

Based on the presented data, adjustments can be made in order to optimise the technological parameters for rearing sterlet, with the goal of obtaining high quality fish products, valuable for human consumption.

### MATERIALS AND METHODS

Sterlet sturgeon specimen were reared for 30 days in a RAS system, from fecundated roes, up to the size of 192±29 mm and 2.09±0.6 g. After those 30 days, half of the fish fry were

transferred into earthen ponds, while the other half were further reared in the RAS system for another 60 days, which represents the total experimental period.

In the RAS system, 900 fish fry were equally stocked in 3 experimental fiberglass tanks (1.4×1.4×0.4 m), coded T1 (tank 1), T2 (tank 2) and T3 (tank 3). Also, in the earthen ponds, 900 fish fry were equally stocked in 3 floating cages (4×4×2 m), coded FC1 (floating cage 1), FC2 (floating cage 2) and FC3 (floating cage 3). The frame is constructed from double HDPE  $\Phi = 200$  mm. A growth enclosure made of polyamide (PA) mesh was used to line the inside of the floating cage. The size of the 7 mm eye of the mesh was chosen both to prevent the escape of sterlet, as well as to prevent other species from entering the cage, which could have competed for food.

During the experimental period, in the RAS system, compressed air was used through an air stone in each tank, in order to continuously provide water aeration.

The sterlet was reared under natural photoperiod in the RAS system as similar as possible to the earthen ponds.

Throughout the experiment, water temperature and dissolved oxygen concentrations, were recorded daily, in both the experimental tanks and floating cages.

Weakly, the water parameters presented in Table 1 were monitored in the RAS system and the floating cages, using kits and the corresponding methods for analysis.

Ammonia was determined using Seignette salt and Nessler reagent, based on pH level. The values are presented as average  $\pm$  standard deviation.

Table 1. Average values for monitored water parameters during the experimental period

Parameter	Unit	Tank 1	Tank 2	Tank 3	Floating Cage 1	Floating Cage 2	Floating Cage 3
pH	upH	7.91±0.15	7.93±0.14	7.96±0.19	7.65±0.32	7.26±0.11	7.39±0.18
Organic matter	mg KMnO <sub>4</sub> /l	25.12±13.95	25.99±12.03	24.63±6.57	46.01±2.83	52.18±7.99	38.31±1.02
CCO-Mn	mg O <sub>2</sub> /l	6.35±3.53	6.28±2.90	7.49±1.49	19.23±1.18	18.14±1.10	17.16±1.85
Calcium Ca <sup>2+</sup>	mg/l	53.10±5.14	56.11±6.06	49.48±8.82	60.12±6.11	56.11±4.86	64.08±7.27
Magnesium Mg <sup>2+</sup>	mg/l	27.95±5.36	23.13±5.59	26.38±6.74	41.31±2.55	31.87±2.19	39.16±4.37
Ca <sup>2+</sup> /Mg <sup>2+</sup>	-	1.90	2.43	1.88	1.45	1.76	1.63
Total hardness	°D	12.63±2.95	12.72±1.71	12.73±0.81	17.9±1.88	14.02±1.97	12.9±1.63
Nitrites NO <sup>2-</sup>	mgN/l	0.03±0.06	0.02±0.07	0.01±0.06	0.01±0.01	0.01±0.01	0.01±0.01
Nitrates NO <sup>3-</sup>	mgN/l	2.01±1.52	2.32±1.40	2.46±1.74	0.86±0.18	0.31±0.15	0.35±0.18
Chlorides Cl <sup>-</sup>	mg/l	25.93±5.24	25.48±4.20	24.57±6.35	12.76±3.22	12.76±2.59	12.76±2.80
Ammonium NH <sup>4+</sup>	mgN/l	0.36±0.30	0.26±0.27	0.39±0.17	0.25±0.13	0.25±0.09	0.25±0.10
Ammonia NH <sub>3</sub>	mgN/l	0.01±0.01	0.01±0.01	0.02±0.01	0.01±0.01	0.01±0.01	0.01±0.01

Average  $\pm$  standard deviation

The phytoplankton and zooplankton profiles of the water were analysed in the earthen pond where the floating cages were placed, in order to assess the present species (Table 2).

Table 2. Phytoplankton and zooplankton present in the water of the earthen pond

	Unit	Value	Systemic group, %		
Phytoplankton			Chlorophyta	Cyanophyta	Bacillariophyta
Density	no./m <sup>3</sup>	1471.69	8	5.5	86.5
Biomass	g/m <sup>3</sup>	378.58	1.5	1.75	96.75
Zooplankton			Rotatoria	Copepoda	Cladocera
Density	no./m <sup>3</sup>	19	11	71.25	17.5
Biomass	g/m <sup>3</sup>	5.55	1.5	77.75	20.75

Fish feed was provided 3 times per day to the fish, using a commercial diet for this species, with a feeding rate of 2% from total fish

biomass. The same fodder and feeding rate were used for all the tanks and floating cages.

The weight (g) and length (cm) of 50 individual fish specimen, at the start and the end of the experiment, were recorded. The data is presented for each tank and floating cage as average  $\pm$  standard deviation.

Ten fish specimens were sampled from each experimental tank and floating cage in order to perform the proximate analysis for the meat. Water content, total lipids, crude protein, and ash were determined using standard methods AOAC (1995).

Fish were anesthetized before sampling by bathing for 5 minutes in a clove oil solution, in order to abide to law no. 43/2014 on the

protection of animals used for scientific purposes and Directive 2010/63/EU of the European Parliament and of the Council from 22<sup>nd</sup> September 2010 on the protection of animals used for scientific purposes.

The correlation between fish length and weight is described using the equation:

$$w = a \times l^b,$$

where:

w - fish weight, g;

l - fish length, cm;

a - constant equal to w when l = 1;

b - exponential/allometric coefficient.

When the rearing process of the fish has a harmonic balance between length and weight, the exponential coefficient “b” is considered equal to 3 (isometric growth). When  $b > 3$ , the fish growth is described as having a positive allometry, and at the opposite pole, when  $b < 3$  the growth of the fish has a negative allometry (the fish grows more in length than in weight) (Bulat, 2017)

Fulton coefficient (K, %), that represents the fish condition factor, was determined using the formula:

$$K (\%) = \text{Weight (g)} / \text{Standard body length (cm)}^3 \times 100$$

The survival rate was measured using the formula:

$$\text{Survival rate (\%)} = (N_t / N_0) \times 100,$$

where:

$N_t$  - final number of fish,

$N_0$  - initial number of fish.

The data was submitted to one-way classification variance analysis and ANOVA test was used, to highlight statistical differences. When differences were statistically significant ( $p \leq 0.05$ ), Tukey's post hoc test was applied to identify which group differs. The data registered in this study was compared with data from aquaculture and wild ecosystems that are published in referenced journals.

## RESULTS AND DISCUSSIONS

Water parameters had values, both in the experimental tanks and in the floating cages, that fell within the accepted range for sterlet at this stage of developmental growth (Table 1).

The pH registered slightly lower values in the floating cages, with a higher organic matter concentration in the floating cages. Nitrogen compounds had values within the limits approved by Order no. 161/2006 emitted by the Ministry of Environment and Water Management, regarding the ecological status and quality of surface waters (Order no. 161/2006).

The length-weight correlation, at the end of the experiment, in the tanks from RAS, is represented in Figure 1. It can be observed that the regression coefficient b is higher than 3 in all experimental tanks, which suggests that fish had a growth focused on weight gain. The highest allometric growth was achieved in tank 2, the differences between the experimental tanks T1 and T3 were not statistically significant ( $p > 0.05$ ).

In an experiment done by Prokeš et al. (2011), sterlet reared under experimental conditions reached a regression coefficient  $b = 3.4834$ , for fish of similar age and size.

Lee et al. (2014), after administering an experimental fish feed supplemented with garlic powder for 12 weeks, obtained sterlet fingerlings with average weight ranging between  $25.64 \pm 1.12$  and  $30.18 \pm 0.21$  g. These values are higher than those presented in the present paper, but considering the length of our experiment, it can be assessed that our sterlet specimens would reach similar sizes after an equal amount of time.

The Fulton's condition factor had values ranging between  $0.47 \pm 0.01$  (T2) and  $0.49 \pm 0.03$  (T1). These values are comparable to those obtained by Prokeš et al. (1996) for sterlet at 170.9 mm and 22.8 g (0.45-0.37).

In the floating cages, the length-weight correlation was characterised by a negative growth, the fish growing rather in length than in weight, as in the tanks from the recirculating aquaculture system. The regression coefficient b had values between 2.9887 and 2.9954, without significant differences between FC1 and FC2 ( $p > 0.05$ ), but FC3 had significant differences between the other two experimental variants ( $p < 0.05$ ).

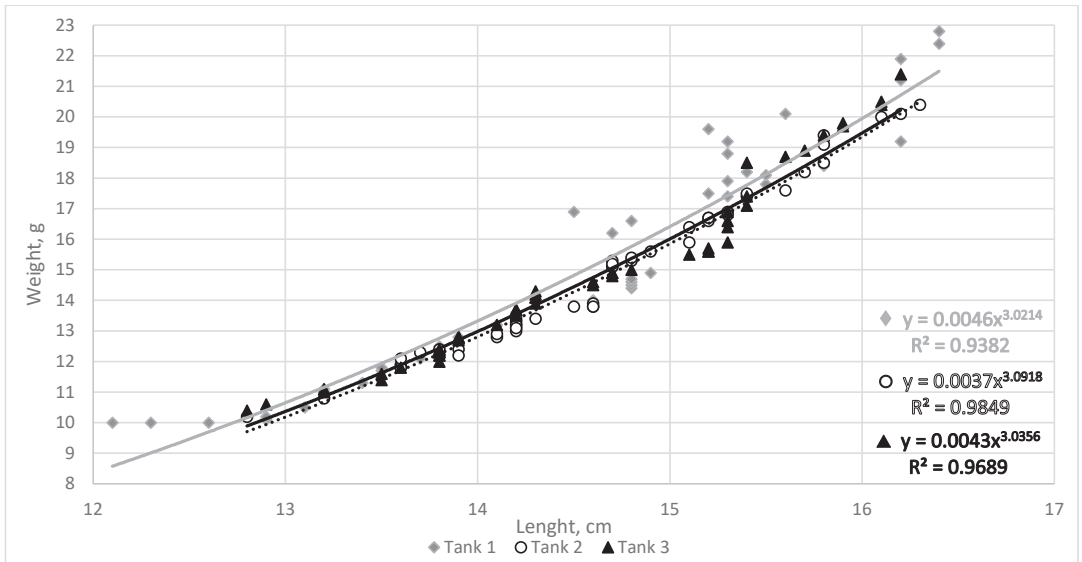


Figure 1. Length-weight correlation in sterlet reared in three tanks of a recirculating aquaculture system, for 60 days

In Figure 2, the length and weight are presented, as well as the correlation between these two parameters.

Rybnikár et al. (2011) observed a negative growth in sterlet reared from day 64 to day 72 after hatching, for fish of similar size.

The condition factor for sterlet reared in floating cages in our experiment, had values between  $0.53 \pm 0.03$  (FC2) and  $0.55 \pm 0.01$  (FC3), values comparable to what Rybnikár et al. (2011) measured in the paper mentioned above. In an experiment done by Wiszniewski et al. (2019), sterlet fed with feed supplemented with bromelain, had a final condition factor ( $0.42 \pm 0.03$ ) lower than the condition factor for the sterlet reared during experiment presented in this paper.

The average fish length in each tank is as follows:  $14.51 \pm 1.16$  cm (T1),  $14.64 \pm 0.80$  cm (T2) and  $14.58 \pm 0.89$  cm (T3). In the floating cages, the average length for sterlet was  $13.31 \pm 0.54$  cm in FC1,  $13.39 \pm 0.60$  cm in FC2

and  $13.32 \pm 0.63$  cm in FC3. These values did not have statistically significant differences between tanks or floating cages ( $p > 0.05$ ), but between experimental variants, the differences were significant ( $p < 0.05$ ).

Regarding the average weight of the sterlet fingerlings, in the tanks these values were:  $15.14 \pm 3.65$  g for T1,  $14.86 \pm 2.58$  g for T2 and  $14.86 \pm 2.85$  g for T3. There were significant differences between tank 1 and the other two tanks ( $p < 0.05$ ). Sterlet reared in the floating cages, measured an average weight of  $12.66 \pm 1.63$  g in FC1,  $12.80 \pm 1.96$  g in FC2 and  $13.19 \pm 1.92$  g in FC3. The differences between FC3 and the other two floating cages were statistically significant ( $p < 0.05$ ).

These differences in final average weight between tanks or floating cages can be explained by a lower fish density. The higher weight in both T1 and FC3, where there were significant differences, corresponds to lower final fish densities (Table 3).

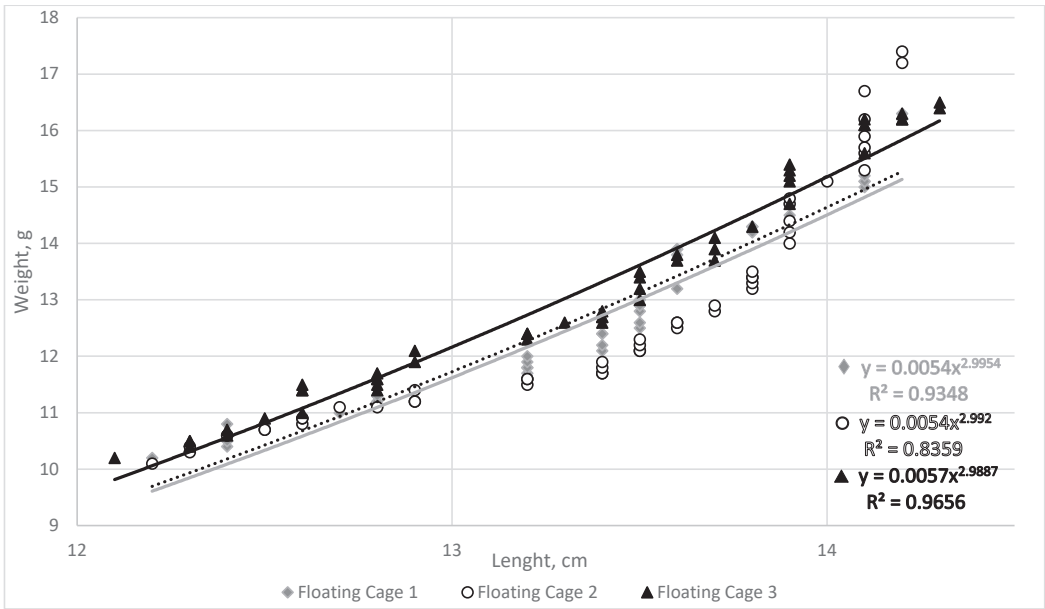


Figure 2. Length-weight correlation in sterlet reared in three floating cages from an earthen pond, for 60 days

In this experiment, the macronutrients from the meat of the fish were also determined.

At the start of the experiment, the average percentage of water in meat was  $92.89 \pm 0.73\%$ . After 60 days, the average water quantity reached values between  $78.02 \pm 1.18\%$  (T1) and  $78.41 \pm 0.53\%$  (T2) in the tanks from the recirculating aquaculture system, with significant differences between T1 and T2 ( $p < 0.05$ ).

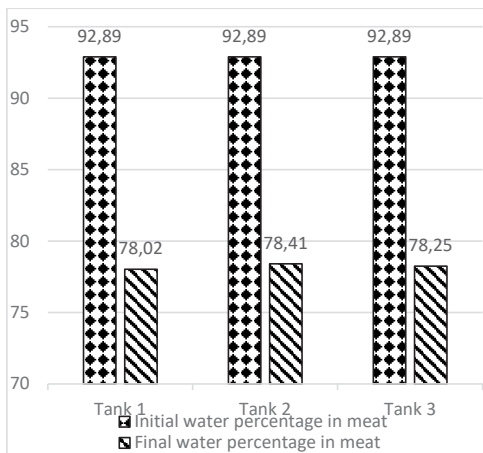


Figure 3. Water percentage in the meat of sterlet reared in tanks from a recirculating aquaculture system, at the start and end of the experiment

In the floating cages, after the same period, water represented between  $77.51 \pm 0.49\%$  (FC2) and  $77.72 \pm 0.12\%$  (FC3) in the meat of sterlet, values without significant differences ( $p > 0.05$ ).

Between the two experimental variants, the differences were statistically significant ( $p < 0.05$ ). Figures 3 and 4 present the graphical representation of these changes in water quantity from the meat of sterlet.

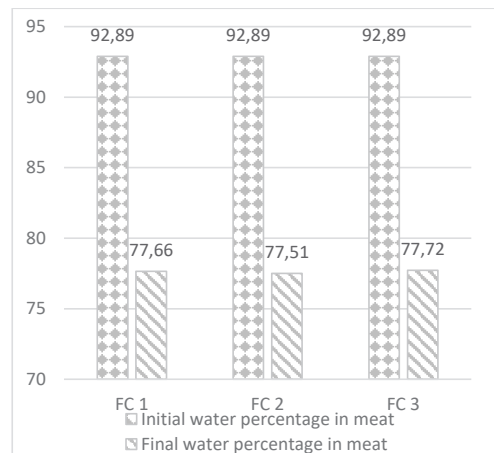


Figure 4. Water percentage in the meat of sterlet reared in floating cages from an earthen pond, at the start and end of the experiment

The initial protein quantity in meat, at the start of the experiment, represented  $5.82 \pm 0.92\%$ . At the end of the experiment, the highest protein percentage in the tanks, was measured in T1 ( $13.48 \pm 0.22\%$ ) and the lowest protein percentage in T2 ( $13.05 \pm 0.76\%$ ), with significant statistical differences between T1 and the other two tanks ( $p < 0.05$ ).

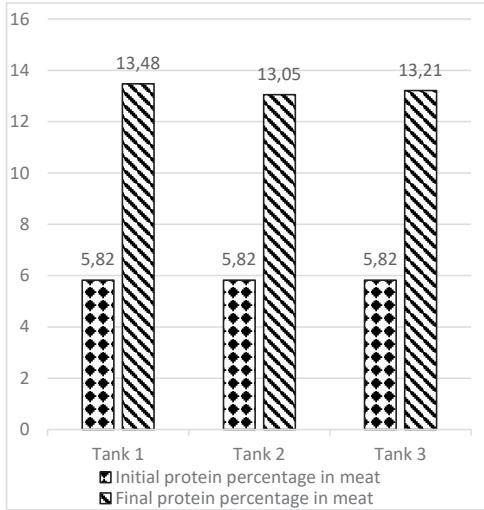


Figure 5. Protein percentage in the meat of sterlet reared in tanks from a recirculating aquaculture system, at the start and end of the experiment

Lipid percentage had the highest increase among the macronutrients. Starting with  $0.44 \pm 0.08\%$  lipids in the meat, sterlet accumulated, during the experimental period, up to 17 times more lipids. In the experimental tanks, lipids measured in meat varied between  $7.49 \pm 0.22\%$  (T2) and  $7.55 \pm 0.38\%$  (T1), without significant differences ( $p > 0.05$ ). In the floating cages, the values for lipids measured in meat, were between  $7.33 \pm 0.15\%$  (FC2) and  $7.47 \pm 0.09\%$  (FC3), with significant differences between FC2 and FC3 ( $p < 0.05$ ). The variation of lipids percentage in sterlet meat, is presented in Figures 7 and 8. Lipid content in fish meat from the tanks is different from that of fish reared in floating cages. This can be explained by the various

In the floating cages, the highest value was obtained in FC2 ( $14.05 \pm 0.5\%$ ) and the lowest protein percentage was measured in FC3 ( $13.72 \pm 0.83\%$ ). Between the experimental variants, the differences were statistically significant ( $p < 0.05$ ).

Figure 5 and 6 present the data measured for protein percentage in the meat of sterlet.

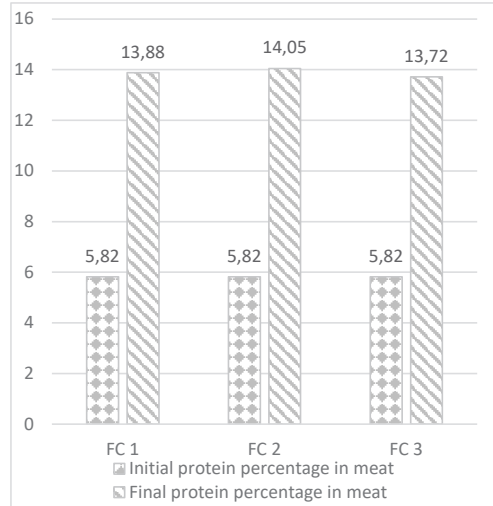


Figure 6. Protein percentage in the meat of sterlet reared in tanks from a recirculating aquaculture system, at the start and end of the experiment

behaviours that require various expenditures of energy, these factors cause an increased fat content in the tissue of sterlet (Ovissipour & Rasco, 2011; Ghomi et al., 2013).

The percentage of ash, presented in Figures 9 and 10, found in the meat of sterlet at the start of the experiment, had a value of  $0.68 \pm 0.02\%$ . At the end of the experiment, the ash represented  $0.83 \pm 0.01\%$  (T1 and T3) in the sterlet meat. The lowest average value was measured in T2 ( $0.81 \pm 0.01$ ). Values in the tanks had no significant statistical differences ( $p > 0.05$ ). The floating cages had an average value of ash, slightly higher than those obtained in tanks, with  $0.85 \pm 0.01\%$  in both FC1 and FC2, and the lowest value in this experimental variant was  $0.82 \pm 0.01\%$  in FC3.

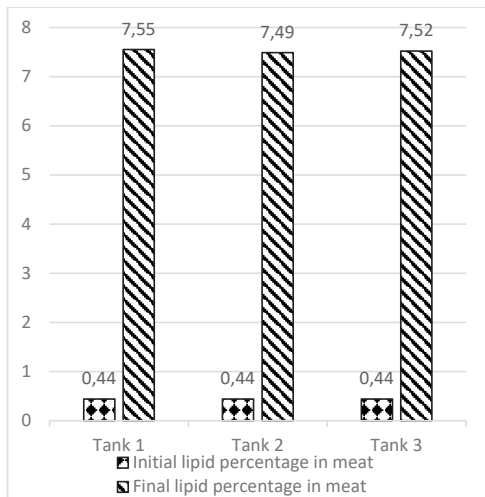


Figure 7. Lipid percentage in the meat of sterlet reared in tanks from a recirculating aquaculture system, at the start and end of the experiment

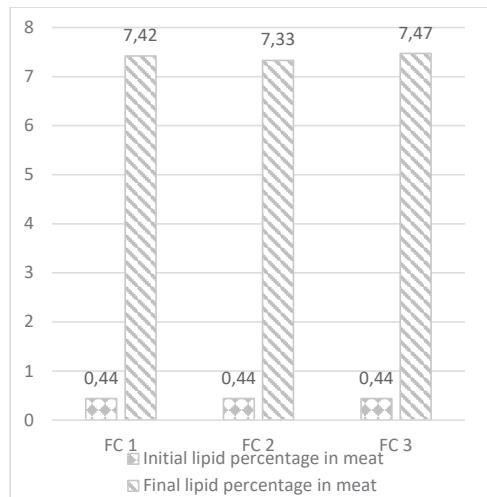


Figure 8. Lipid percentage in the meat of sterlet reared in tanks from a recirculating aquaculture system, at the start and end of the experiment

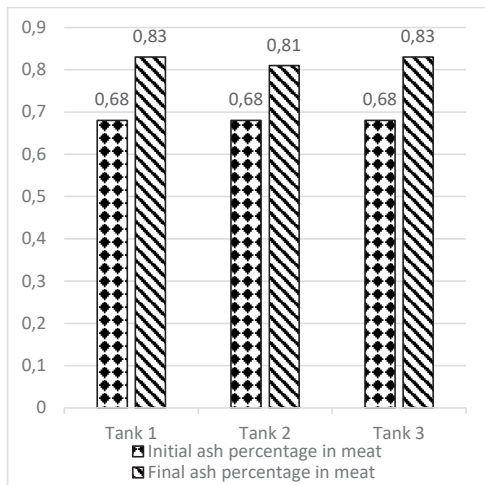


Figure 9. Ash percentage in the meat of sterlet reared in tanks from a recirculating aquaculture system, at the start and end of the experiment

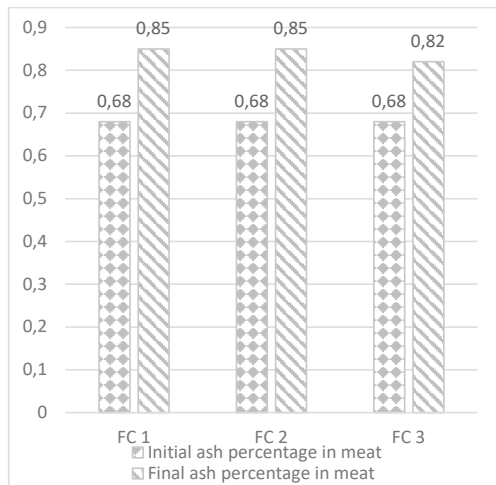


Figure 10. Ash percentage in the meat of sterlet reared in tanks from a recirculating aquaculture system, at the start and end of the experiment

In Figure 11, the survival rate of sterlet reared in tanks is represented as number of fish deaths per week, for each tank. Each tank was populated with 300 fingerlings, and at the end of the experiment there were 193 specimens in T1, 198 specimens in T2 and 199 specimens in T3. The survival rate for each tank was as follows: 64.33% in T1, 66% in T2 and 66.33% in T3.

For sterlet reared in floating cages, the survival rate was higher and reached 75.66% in FC1, 74.66% in FC2, and 72% in FC3. The final number of fingerlings that survived in the floating cages was: 227 specimens in FC1, 224 specimens in FC2, and 216 specimens in FC3. In Figure 12, the survival rate of sterlet reared in floating cages is represented as number of fish deaths per week, for each floating cage.

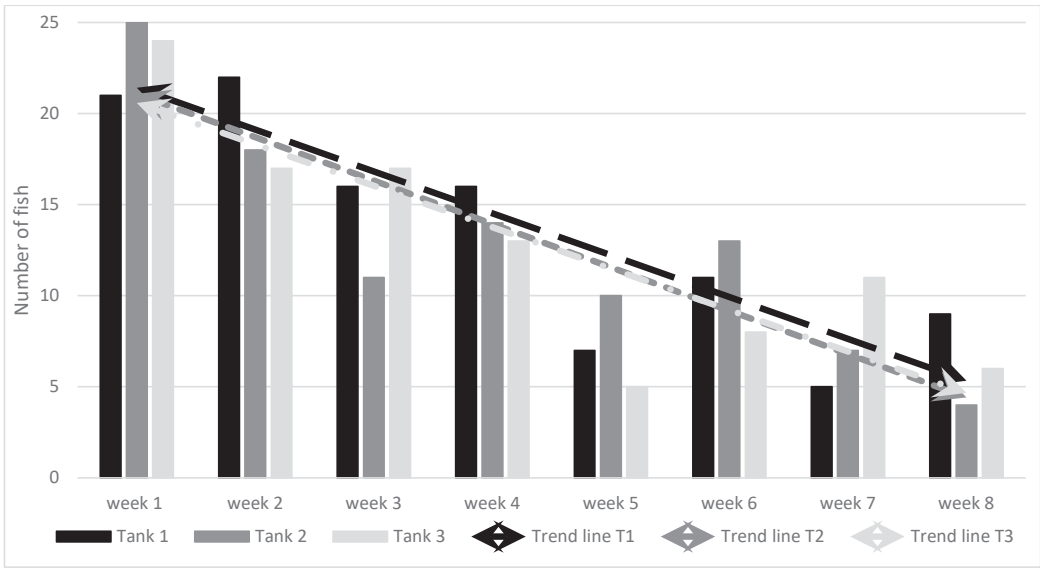


Figure 11. Number of fish deaths per week in tanks from recirculating aquaculture system, during the experimental period

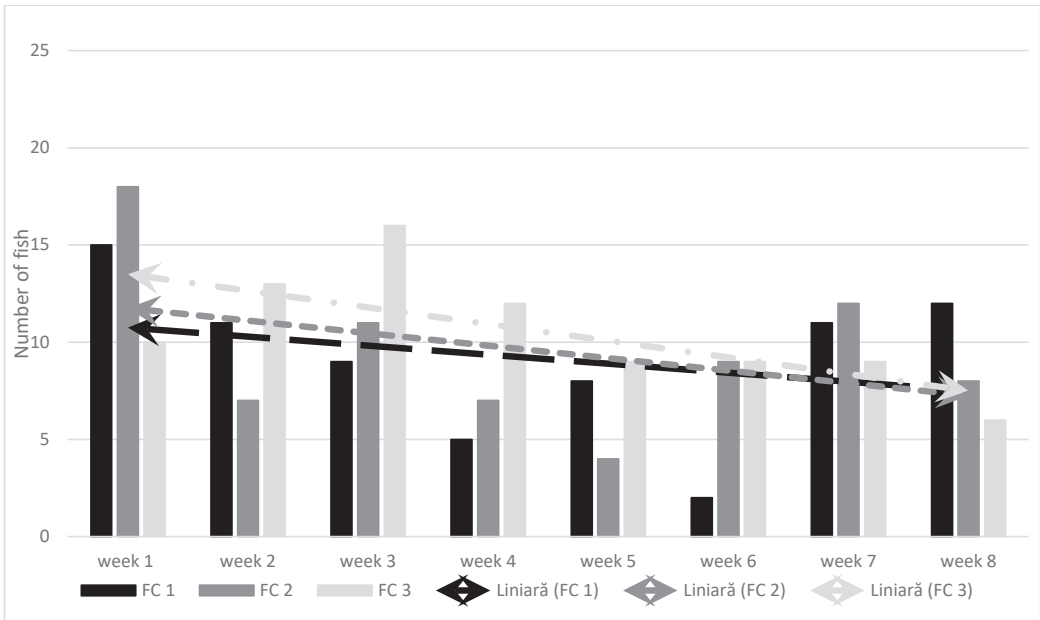


Figure 12. Number of fish deaths per week in floating cages from an earthen pond, during the experimental period

The differences in macronutrient accumulation and survival rate can be attributed to the influence of the rearing environment. The floating cages, being placed in an earthen pond, were subjected to the elements, while the tanks were part of a recirculating aquaculture system, a closed system rigorously monitored.

In the floating cages, sterlet fingerlings had, besides the administered fish feed, a natural source of nutrients represented by zooplankton organism such as Copepoda, Cladocera and Rotatoria. One of the most important elements in regard to the technology applied in intensive aquaculture, is represented by the administered



fish feed. Fish tissue is significantly shaped by feed quality, recipe and method of delivery. (Chipinov et al., 2012)

Therefore, sterlet reared under intense aquaculture conditions with just artificial feed leads to changes in the physiology and biochemistry in the bodies of the fish, compared to fish from natural environment. (Kireyev, 2011)

Stocking density also has an impact on the growth and survival of individuals, the floating cages having a much larger surface area and water volume. In Table 3, the stocking and final densities are presented as number of fish per unit of surface and unit of volume, for each experimental variant.

Table. 3 Fish densities in the rearing units during the experimental period

	Unit	Tank 1	Tank 2	Tank 3	Floating cage 1	Floating cage 2	Floating cage 3
Stocking density per surface	No. fish/ m <sup>2</sup>	153	153	153	18.8	18.8	18.8
	kg/m <sup>2</sup>	0.320	0.320	0.320	0.039	0.039	0.039
Stocking density per volume	No. fish/ m <sup>3</sup>	382.7	382.7	382.7	9.4	9.4	9.4
	kg/m <sup>3</sup>	0.800	0.800	0.800	0.020	0.020	0.020
Final density per surface	No. fish/ m <sup>2</sup>	98.5	101	101.5	14.2	14	13.5
	kg/m <sup>2</sup>	1.491	1.501	1.509	0.180	0.179	0.178
Final density per volume	No. fish/m <sup>3</sup>	246.2	252.6	253.8	7.1	7	6.75
	kg/m <sup>3</sup>	3.727	3.753	3.772	0.090	0.090	0.090

## CONCLUSIONS

Artificial rearing technologies of fish are dependent of the rearing systems and must consider the most optimal and economically efficient path to high production and safe-for-consumer fish products.

From our productional rearing results, sterlet reared in a recirculating system has a higher growth potential, with a better use of nutrients from the feed, but sterlet reared in earthen ponds has a better protein percentage with a higher survival rate.

Optimizing the fish feed and rearing conditions to increase the biological value of sterlet, will result in a better survival rate. Taking these measures, rearing sterlet could have a higher economic outcome with an improved nutritional value of the meat.

## ACKNOWLEDGEMENTS

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