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The Effect of Stocking Density on Growth Performance and Hematological Profile of Stellate Sturgeon (*A. stellatus*, Pallas, 1771) Fingerlings Reared in an Industrial "Flow-through" Aquaculture System

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Abstract. The most studied among sturgeon native species, when we speak about growth performance in intensive aquaculture systems is stellate sturgeon (*Acipenser stellatus*, Pallas1771). The level of intensity of an industrial growth system was give by its stocking density maximum level, which generally defines the quantity of biomass per unit of volume.

The present paper aims to assess, in general terms, the production potential of industrial flow-through aquaculture systems for stellate sturgeon fingerlings growth and especially to determine a certain stocking density that maintains a positive correlation between growth rate and physiological state of biological material.

The biological material consists in a number of 1200 stellate sturgeon fingerlings, with an average weight of 5,52g±0.06 g, that were divided into six growth units by three stocking densities, in replicate: 2,78 kg/m^3 (V_1), 3,66 kg/m^3 (V_2) and 4,59 kg/m^3 (V_3). At the end of 45 days trial, the biological material was evaluated physiologically and in terms of growth. At the end of the experiment, the growth performance indicators registered insignificant values ($p>0.05$), higher in case of the experimental variant with lower stocking density. This variant also presents a better-feed conversion ratio. The hematological profile remains constant for the three experimental variants. The only indicator that is significantly influenced ($p<0.05$) by stocking density is the hematocrit and implicitly the mean corpuscular volume. A higher stocking density for stellate sturgeon fingerlings growth in an industrial flow-through aquaculture system did not significantly influenced neither the biological material growth nor its physiological state but decreased the survival rate.

Keywords: stocking densities, *Acipenser stellatus*, flow-through system

INTRODUCTION

It is clear that sturgeon aquaculture can help in the conservation of wild populations, declined through restocking and by providing a consistent supply without exploiting wild population (Memis *et al.*, 2008)**.** Rearing sturgeon in intensive aquaculture systems requires a good knowledge of the limiting factors that influence growth and development of a certain species. Stocking density is one of the important factors that affect rearing results and it have being confirmed that this factor plays a key role in the rearing of sturgeon (Mohler *et al.*, 2000). Thus, it has been demonstrated that high stocking density may suppress fish growth due to the deteriorating water quality, social behavior and the alteration in metabolic rate induced by crowding stress associated with stocking densities (Ellis *et al.*, 2002; Lupatsch *et al.*, 2010; Papoutsoglou *et al.*, 2006; Tolussi *et al.*, 2010).

The stocking density expressed as the level of rearing systems intensity and often was defined as total biomass per unit of volume (Cristea *et al.*, 2002). In case of sturgeons,

most authors (Fajfer, 1999; Szczepkowski *et al.*, 2010; Dapeng Li *et al.*, 2012) correlates stocking density with the surface (square meters) and not with the volume (cubic meters), the bentofag character of sturgeons serving as an argument of this approach.

The overall goal of this work is to determine the bio-productive potential of industrial flow-through systems for rearing stellate sturgeon fingerlings and the specific goal is to observe the effect of stocking density on growth performance and welfare of biological material.

MATERIALS AND METHODS

Experimental design. The experimental base of this work was represented by Horia sturgeon station, situated in Tulcea County. The 45 days experiment took place between 23.07.2012-05.09.2012. The biological material is reared in light green tronconic fiberglass tanks, with the following dimensions (130x120x40 cm). The amount of water available in each rearing unit was about 330 liters, with a flow rate of 6.7 l/min, which assures a total water exchange rate in about 45 minutes. Supply with water was made with continuous flow, by gravity, from the main tank of the station. The water is pumped from Horia Lake and it passes through a mechanical filter –Crystal filter with 1-1.5 mm silica particles and a filtration capacity of 20 m^3 and then goes through a suitable UV filter.

The biological material for this experiment consisted on total of 1200 stellate sturgeon fingerlings, 70 days old, with an average weight (±SD) of 5.52±0.06 g. The fingerlings were divided into six rearing units, applying three-stocking densities, in duplicate (Tab. 1*)*: 2.78 kg m^{-3} (V_1), 3.66 kg m^{-3} (V_2) and 4.59 kg m^{-3} (V_3).

Tab. 1

Initial technological parameters

Initial technological parameters / *Rearing unit*	V_1		V_2		V_3	
	B_1V_1	B_2V_1	B_1V_2	B_2V_2	B_1V_3	B_2V_3
Biomass (g)	838	832	1114	1082	1382	1376
Stocking density related to feeding surface (kg m^{-2})	0.95	0.94	2.16	2.13	2.91	2.77
Stocking density related to water volume (kg m^{-3})	2.79	2.77	3,71	3.60	4.60	4.58
Number of fingerlings	150	150	200	200	250	250
Average weight (g)	5.59	5.55	5.57	5.41	5.53	5.50

The feed was administrated 6 times per day. During the 45 experimental days, in accordance with the sturgeon rearing technology, several types of feed were used but at a constant feeding intensity of 4% BW. Thus, in relation with the type of feed used (*Tab. 2*), the experiment was divided into four stages:

- ✓ Stage 1- Nutra Pro 3 /2 ratio 1:1- 23.07.2012-31.07.2013 (8 days);
- ✓ Stage 2- Nutra Pro 2 -31.07.2012-16.08.2012 (17 days);
- ✓ Stage 3-Nutra Pro 0 – 16.08.2012-28.08.2012 (12 days);
- ✓ Stage 4-Nutra Pro 0/MPT ratio 3: 1-28.08.2012-05.09.2012 (8 days).

Tab. 2

The biochemical composition of administered feed

Composition / granulation	Nutra Pro 3	Nutra Pro 2	Nutra Pro 0	Nutra Pro MPT
Granulation (mm)	0.5-0.7	0.7-1.1	1.1-1.7	1.7
Crude Protein (%)	55	54	54	50
Crude fat (%)	16	18	18	20
Crude cellulose (%)	1	1	1	0.7
Crude ash (%)	10	10	10	9
Phosphorus (%)	1.4	1.4	1.4	1.3
Digestible energy (MJ / kg)	18.8	19.4	19.4	19.7
Vitamin A (IU)	14000	14000	14000	12000
Vitamin D3 (IU)	2300	2300	2300	1800
Vitamin E (mg)	250	250	250	180
Vitamin C (mg)	500	500	500	500
Lysine (%)	3.5	3.5	3.5	-
Methionine (%)	1.5	1.5	1.5	-
Cystine (%)	0.7	0.7	0.7	-

The main physical-chemical parameters (temperature and dissolved oxygen) were daily monitored with a portable multiparameter EXTECH 407 510.The nitrogen compounds ($N\text{-}NO_3^-$, $N\text{-}NO_2^-$, $N\text{-}NH_4^+$) and pH were monitored weekly in the research laboratory of Aquaculture, Environmental Science and Cadastre Department–Faculty of Food Science and Engineering "Dunarea de Jos" University of Galati. The pH was measured with pH meter model WTW 340 and nitrogen compounds were determined by Spectroquant Nova 400 spectrophotometer, with Merk compatible kits.

Growth performance indicators. At the end of the experiment, the entire experimental group was weighed, prompting a series of technological indicators (daily growth rate, feed conversion factor, specific growth rate, protein efficiency), that were calculated using the following formula:

- biomass (weight) gain (BW)=final biomass (Wt)-initial biomass (W0)(g);
- feed conversion ratio (FCR)=total amount of feed distributed (F)/biomass (weight) gain (BW)(g/g)
- specific growth rate (SGR)=100 x (ln Wt-ln W0)/t (% BW/day),
- protein retention efficiency (PER)=biomass gain (BW)/feed protein (P)(g)

Also 7-10 fingerlings per experimental variant were measured and the results were graphically represented by Power regression correlating length and weight and determining the "b" coefficient from the growth equation W=a*Lb where W-fish weight (g), L=total length (cm). For better characterize the experimental groups condition state, the final weight variation coefficient for each experimental variant was also calculated (CV=stdev x 100/M, where stdev-the standard deviation of final individual weight, M-arithmetic mean of individual final weight) and Fullton coefficient ($F=M/Lt^b$ where M–average final weight, Lt-final total length average, b-allometric coefficient, experimentally determined).

Hematology analysis. At the end of the experiment, about 0,5-0,8 ml of blood was taken by caudal puncture, from 25 fingerlings (7-10 fingerlings per each rearing unit). After sampling, a part of the blood was placed in heparinized Eppendorf tubes in advance and the other part in unheparinized tubes. Using the routine methodology of fish hematology (Blaxhall and Daisley, 1973; Svobodova, 2001), hematological indices were measured and analyzed. The number of erythrocytes (RBCc $x10^6/\mu L$) was determined by counting the erythrocytes from all 5 squares of Neubauer hematocytometer, using Vulpian as a contrast solution. The hematocrit (Ht%) was analyzed by Hettich Haematockit 210, for 5 minutes at

12,000 rotations/minute, in duplicate. The hemoglobin concentration in blood (Hb g/dl) was quantitatively determined by colorimetric method with Drabkin reagent and spectrophotometer Spectrocord 210 Analytikjena, at a 540 nm wavelength.

For biochemical indices measurements (glucose and serum proteins), unheparinized blood was used. Thus, in order to obtain blood serum, the blood was centrifuged without anticoagulant, for 10 minutes, at 3500 rotations/min. Serum glucose was colorimetric dosed with o-toluidine and it was determined at the wavelength λ=635 nm. Serum proteins were spectrophotometrically determined by biuret test, at a wavelength λ=546 nm. After determining the hematological indices, erythrocyte constants were calculated using standard formulas (mean corpuscular volume-MCV, mean erythrocyte hemoglobin-MCH, mean erythrocyte hemoglobin concentration-MCHC).

Statistical data analysis. Growth indicators were statistically analyzed using SPSS Statistics 17 from which the following tests were used: descriptive statistics, one-way Anova test ($p<0.05$). For statistical analysis of hematological indicators and derived erythrocyte constants, Microsoft Excel 2010 statistical calculation was used, from which the following statistical tests were applied: descriptive statistics, Single Factor ANOVA test ($p<0.05$).

RESULTS AND DISCUSSIONS

Water quality. A decrease in fish growth is often associated with a poor technological water quality that can be due to an agglomeration ambient. In the present experiment, the main physicochemical parameters did not exceed the permissible limit for growth and development of stellate sturgeon fingerlings. Therefore, the temperature recorded specific values for warm season (fig. 1), with a minimum of 22.7°C and a maximum of 25.7°C. To maintain an optimum range for temperature, sometimes14-17°C water coming from deep drilling was added. Dissolved oxygen recorded minimum values of 5.78 mg/l, not exceeding the minimum permissible saturation range between 50%-70%, favorable for feeding sturgeons fingerlings (Jobling, 1995).

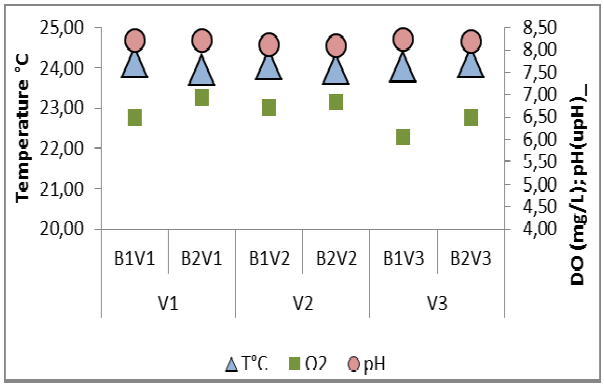

Fig. 1. The temperature, dissolved oxygen and pH variation

Regarding pH, it had created normocapnic conditions (normal values of CO_2 in the blood), ranging in alkaline part, from 7.15 to 8.63 upH. Crocker and Cech (1996) reported for *A. transmontanus* juveniles a high exposure to hypercapnia (increased CO_2 in the blood) under a slightly acidic pH (below 7), but did not affect growth.

The nitrites and ammonia, nitrogen compounds, which describe the dynamics of metabolic and nitrification products, have registered allowable values (significantly higher in nitrite case) for technological water and showed no significant differences between variants throughout the experimental period ($p<0.05$).

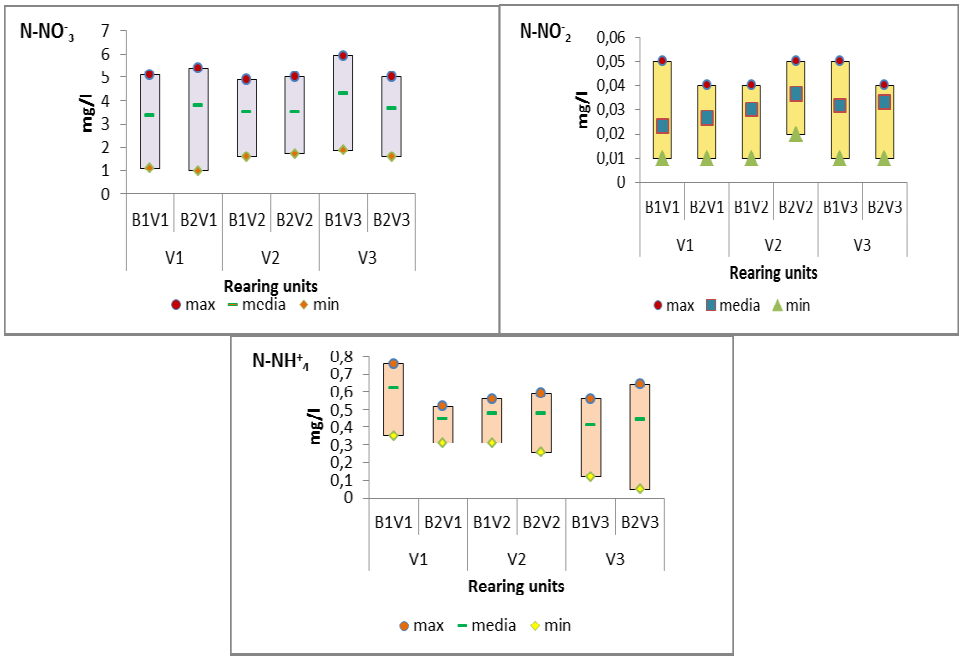

Fig. 2. The nitrogen compounds dynamics

Growth performance. At the end of the experiment, the culture biomass increased by 85% at V_1, 79% at V_2 and 76% at V_3. Also, a final stocking density greater than 24 kg m^{-3} (the highest stocking density) at V_3 (*Fig. 5*) and a final average weight of 37.54±0.2 g at V_1 (lower stocking density) were observed. Thus, if related to experimental variants, it can be observed an inversely proportional relationship between stocking density and biomass gain (*Fig. 4*), at experimental stages (*Fig. 3*) where a irregular growth of culture biomass appears. Therefore, in the second experimental phase, it has been registered the best biomass gain for all three experimental variants, being significantly higher for V_1, so it can be said that the feed used for this stage, associated with feeding intensity applied, led to the best growth performance for stellate sturgeon fingerlings.

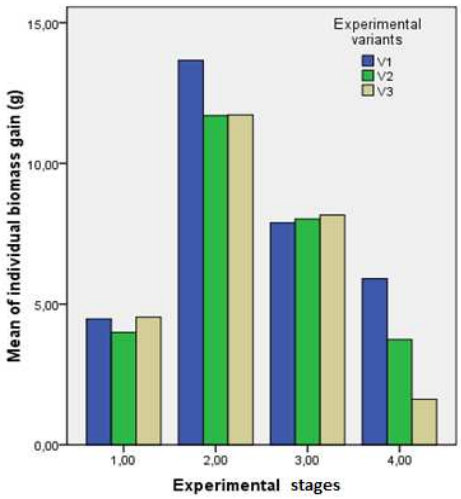

Fig. 3. Mean of IWG/stages

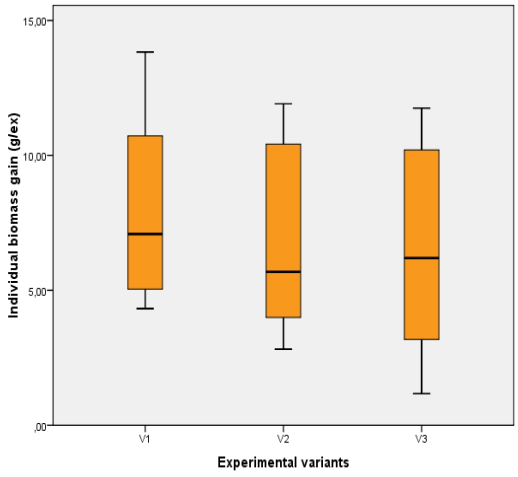

Fig. 4. IWG (mean values ±SD)/variants

In the case of first and last experimental stage, a lower individual biomass gain was recorded. Thus, if we can discuss about feed accommodation in the first experimental stage

(granulated feed), in the fourth stage, the differentiation between variants is certainly the result of the three different stocking densities. Similar results obtained Szczepkowski *et al.* (2010) at *A. oxyrinchus* species, pointed out significant differences ($p<0.05$) between the following densities: 1.27 kg m^{-2}, kg m^{-2} 2.49 and 3.80 kg m^{-2}.

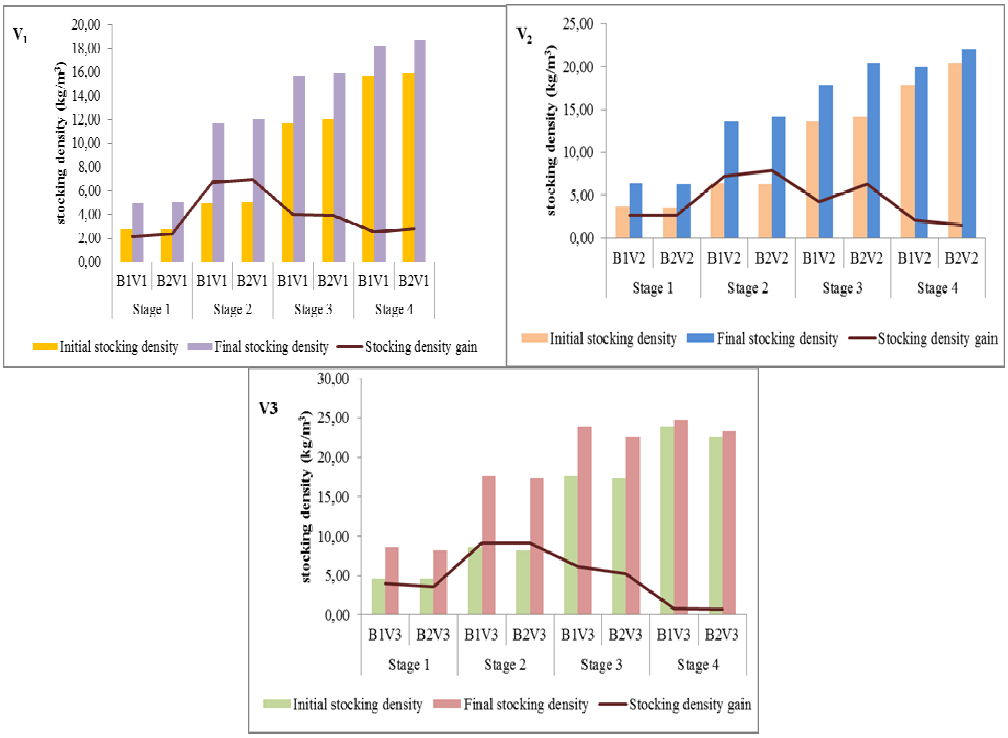

Fig. 5. Stocking density variation/experimental variants and stages

Like IWG, the density gain registered higher values in the second stage of the experiment, for all the experimental stages and lower values in the last stage (*Fig. 5*). It should be noted that both the highest and lowest density gain values were recorded in variant V_3, at the second and fourth stage. Differences between experimental variants of density gain, on each stage, are not statistically significant ($p>0.05$; $p=0.77$).

Regarding SGR values, it can be observed *(Fig. 6)* that higher values were found in the first experimental stage and, there are no statistically significant differences between experimental variants ($p>0.05$; $p=0.595$). These results are comparable to those of Szczepkowski et al. (2010) (in the case of Atlantic sturgeon), which showed statistically significant differences between the specific growth rate of the group reared at a density of 1.27 kg m^{-2} and the one reared at a 2.49 kg m^{-2} stocking density.

In addition, SGR values above 7%/day were reported by Zhu *et al.* (2006) for fingerlings of *A. sinensis* species, reared at temperatures between 19 and 22°C. The higher values from the first experimental stage do not respect the IWG trend that registered better values in the second experimental stage, which can be explained by the fact that second stage duration (17 days) is greater than the first (8 days).

The stocking density did not represent a decisive influence factor over the nutrient retention (PER, *Fig. 7*) and feed conversion (FCR, *Fig. 8*). As with SGR, for retention and feed conversion indicators, better values were observed in the first experimental stage and lowest in the fourth stage. Also, insignificant statistically differences were marked among the three experimental variants. FCR values obtained in this current experiment are also as recorded in similar studies (Mohler, 2000; Cristea *et al.*, 2003). Thus, from the analysis of

performance indicators we conclude that juvenile stellate sturgeon fingerlings showed a good adaptability to feed and that if it would be not consider as variable the changing of feed, imposed by the technological requirements, the idea that stocking density can influences the growth of biological material over time can be submitted.

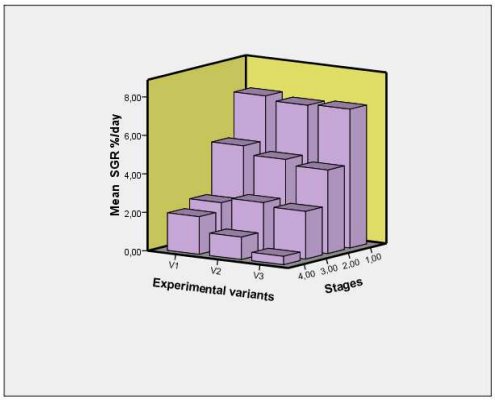

Fig. 6. Mean SGR/variants and stages

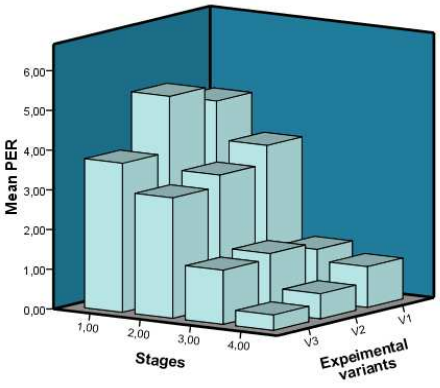

Fig. 7. Mean PER/ stages and variants

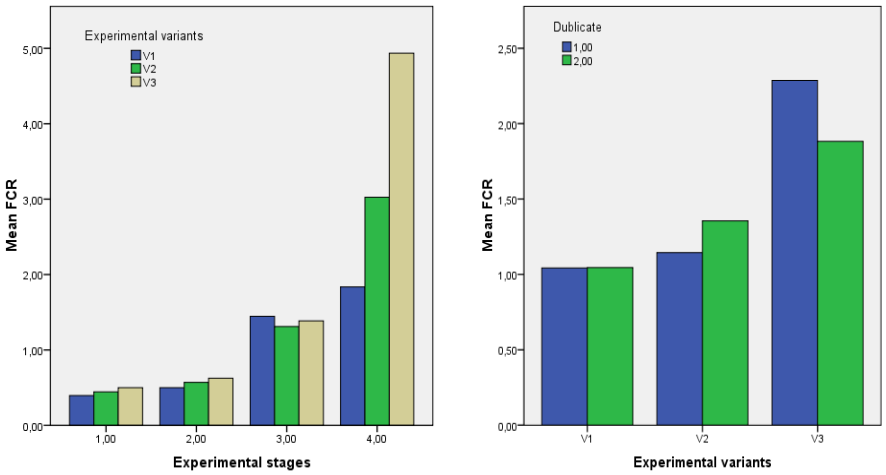

Fig. 8. Mean FCR/ stages and variants

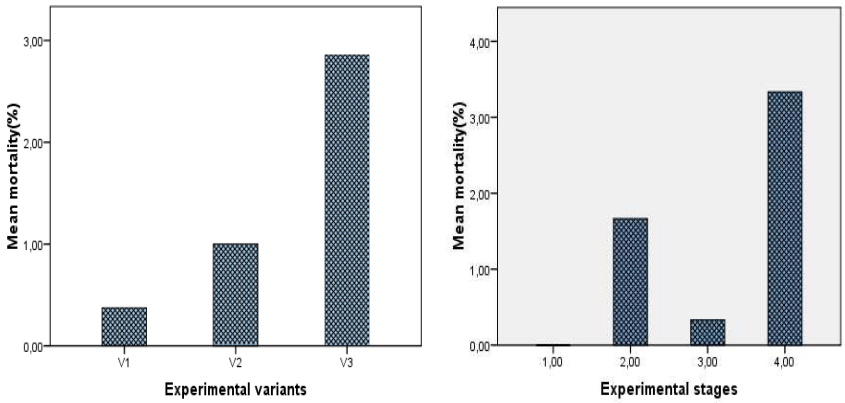

Fig. 9. Mean mortality/ variants and stages

If in case of growth performance indicators no significant differences are observed between the experimental variants, when it comes to mortality, significant differences

($p<0.05$; $p=0.04$) are found both between experimental variants and among experimental stages (*Fig. 9*). Thus, as can be seen in *Figure 9*, highest mortalities were recorded at highest stocking density variant -V_3 and also in the last stage of the experiment, indicating that stocking density represents in case of stellate sturgeons fingerlings a limiting factor.

Fish condition. A critical analysis of stellate sturgeon fingerlings physiological condition, grown under different stocking density, requires calculating for each experimental group, the relative robustness of the biological material (condition state). In this sense, a length (Lt) -mass (M) regression (*Fig. 10*) for a representative sample of each experimental variant was plotted. Also the feeding grade of biological material was evaluated (Williams, 2000) by using the profile index / condition factor F ($F=W*L^b$, where "b" is an allometric exponent that has been determined experimentally). At the end of this experiment we can see that the index profile (F) –respect the trend of growth performance indicators, with better values *(Tab. 3)* at the experimental variants with lower stocking densities (V_1, V_2).

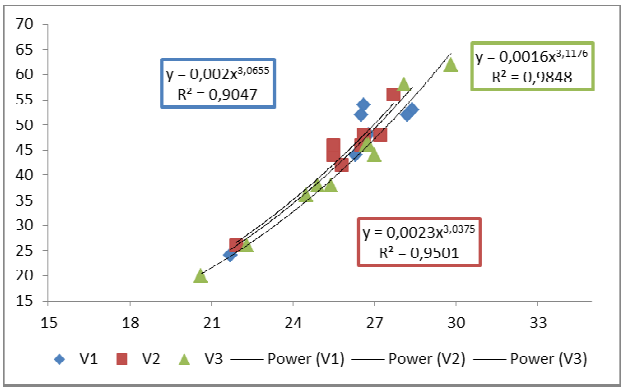

Fig. 10. Length-mass relation for a sample of stellate sturgeon fingerlings, maintained in different intensity conditions

For a better assessment of culture biomass the coefficient of variation was calculated, (*Tab. 3*) in terms of which we have seen an increase in heterogeneity more obvious at V_3, where biomass intensity was higher. But, one can not conclude that only stocking density represents a growth factor that induced the growth of biological material variability, which is due also to other factors that might include: the initial structure of the fish population, genetic variability, dynamic of physical-chemical water quality parameters or rearing units hydrodynamics.

Tab. 3

The evolution of the variation coefficient, allometric exponent and Fullton coefficient

Condition indicators	Experimental variant		
	V_1	V_2	V_3
Coefficient of variation (cv)	22.68	19.18	31.15
Allometric exponent ("b")	3.0655	3.0375	3.1176
Index profile (F)	0.0020	0.0022	0.0016

Welfare. The data obtained from hematological examination characterize the physiological state of culture biomass. The average of main hematological indicators for each variant and derivatives erythrocyte constants was shown in *Table 4*. Among the analyzed hematological indicators, only the hematocrit presented significant differences among values obtained between the experimental variants ($p<0.05$; $p=0.02$). At the same time, the constant

derived from hematocrit MCV showed significant differences between the experimental variants ($p<0,05$; $p=0,008$).

Tab. 4

Hematological indicators at the end of the experimental period

Experimental variant	Hematological indicators					
	Erythrocyte no. ($x10^6$)	H_t (%)	H_b (g/dL)	VEM (μm^3)	HEM (pg)	CHEM (g/dl)
V_1 2.78 kg m^{-3}	0.67±0.18	29.29±3.7	11.52±3.3	461.32±104	188.24±76.2	39.84±11.54
V_2 3.66 kg m^{-3}	0.70±0.10	25.75±3.7	10.12±2.4	378.69±98.5	145.94±34.7	40.24±11.28
V_3 4.60 kg m^{-3}	0.81±0.22	23.1±4.65	9.97±2.04	300.68±58.9	134.19±58.9	45.47±16.08

The average number of erythrocytes is maintained in the normal range for stellate sturgeon species, between 0.67 and $0.81x10^6/\mu L$ (Ghittino, 1983). The low number of red blood cells, compared to teleostean fish, can be explained by the lower systematic position of sturgeons. The hematocrit is an accurate indicator of stressful conditions or even indicates the installation of secondary stress (Biswas *et al.,* 2006) and the dehydration degree (Brânză, 1985). At current experiment, the hematocrit values decrease at the variant with bigger stocking density but they are situated in the optimal allowed range for fish, 22-40% (Docan *et al.,* 2011; Zolfaghari).

The concentration of hemoglobin presents high values for all experimental variants. After Nicula (2004) increases in hemoglobin concentration are due to physical or environmental stress. Mean corpuscular volume increases significantly with increasing stocking density, having higher values in V_1 case. Mean erythrocyte hemoglobin and mean erythrocyte hemoglobin concentration did not show significant differences between the variants

Tab. 5

Biochemical indicators at the end of the experimental period

Experimental variant	Biochemical indicators	
	TP (g/dl)	GLU (mg/dl)
V_1 2.78 kgm^{-3}	4.21±0.4	228.92±43.6
V_2 3.66 kg m^{-3}	4.29±0.3	228.91±23.2
V_3 4.60 kg/m^{-3}	3.95±0.2	195.85±30.6

Determinations of blood glucose and serum proteins are the most effective and least expensive stress evaluation (Patriche *et al.,* 2011). Thus, if in the case of serum proteins it could say that is within the optimum spacing for fish 3.5-5.5 g/dl, regarding serum glucose this presents a slight increase over the specific values of freshwater fish species (25-200 mg/dl). High glucose levels have being reported by Hung *et al.* (1993) for *A. transmontanus* and may be due to commercial administrated feed, with high carbohydrate content.

CONCLUSION

After the analysis of growth performance data, obtained for stellate sturgeon reared under different intensity conditions, it could said that the industrial flow trough system has a good rearing potential for sturgeons. Also, it has been highlighted that the water quality parameters from the system were not affected by the increase of stocking density biomass. Also, based on the performance indicators, it can be reinforce the idea that sturgeons are fish that have the ability to convert food effectively (Mims *et al.,* 2002; Memiş *et al.,* 2009). So,

an obvious argument for this experiment is the subunit values of FCR's, especially for versions with lower stocking density, in the early stages of the experiment. Although there were no statistical significant differences between experimental variants, both in terms of growth and feeding efficiency -nutrient retention, better values have been observed at variant V_1 (lower stocking density). Regarding the analysis of growth performance indicators by the imposed nutritional management stages, it could say that stocking density influences the growth of biological material over time. Although mortality was not high during the experiment, significant differences were both among the variants as well as among the experimental stages. Thus, it could say that for stellate sturgeon fingerlings, a high stocking density value may cause an increase of mortality over time. Respecting the trend of growth performance indicators, the values of the coefficients that characterize the condition state of experimental groups showed no significant differences between the experimental variants.

The data from hematological assessments showed that stocking density does not significantly influence the physiological state of stellate sturgeon fingerlings.

Stocking density of stellate sturgeon fingerlings, reared in an industrial flow-through aquaculture system, did not significantly affect the growth of biological material nor its physiological state, but decreases survival over time.

Therefore, the results of this experiment, combined with data from the literature (Blackburn and Clarke, 1990), converge to the idea that stocking density has no impact on growth performance or fish physiological condition while maintaining the quality of the culture environment at the production system optimal level.

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