PRELIMINARY RESULTS REGARDING THE POST-LARVAL STAGE FOR THE SPECIES *ACIPENSER BAERII* (J. F. BRANDT, 1869) IN RECIRCULATING AQUACULTURE SYSTEM

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Abstract

The survival and growth of Siberian sturgeon in the post-larval stages, juvenile, growing and up to marketable size is of great importance both for sturgeon species conservation programs and for commercial purposes. Given the diversification of production systems there is a concern for continuous improvement of incubation technologies that allow the production of high-quality Siberian sturgeon larvae. Although larval growth of Siberian sturgeons can be considered more basic compared to other sturgeon species such as sterlet, there is a need to focus on optimizing larval feeding to maximize their survival, given that this phase of breeding technology has a considerable impact on economic profitability. The post-larval stage of Acipenser baerii is the most critical period in which significant losses are recorded and, as a result, special attention is paid to the applied technology. In this sense, the adaptation to the conditions of intensive growth in the recirculating system, as well as the optimization of the administered feeding regime led to the increase of survival rate to 73,50%. The average individual weight of Acipenser baerii at the beginning of exogenous feeding was between 0,0054 – 0,0069 g and the maximum weight at the end of the experimental period (after 30 days) was 1,05 – 1,20 g. Experiments on the intensive breeding and adaptation of Siberian sturgeon in the post-larval stage may be feasible, if adequate food and environmental conditions corresponding eco-physiological requirements are provided.

Key words: post-larval stage, *Acipenser baerii*, RAS

INTRODUCTION

Siberian sturgeon, *Acipenser baerii* is an anadromous migratory species, living in deep parts of large rivers, spawning over gravel or sand substrates with moderate to swift current [18]. It can tolerate low temperatures, lives in freshwater and consumes a wide variety of food types (Holcik 1989; Koksal et al. 2000), which make it a suitable species for aquaculture [15], [19]. In recent years, two new sturgeon species have been introduced into freshwater aquaculture, namely the American sturgeon (paddlefish-*Polyodon spathula*) and the Siberian sturgeon (*Acipenser baerii*), highly adaptable species, suitable for aquaculture. Wild sturgeon populations are facing a decline and the most important legal instruments for the protection of wild sturgeons are legislative protection acts (Habitats Directive 92/43, Annex II, 1992) and national fishing bans (prohibition orders). In Romania and Bulgaria a moratorium banning sturgeon fishing is in effect until 2021 [8].

The Siberian sturgeon inhabits rivers flowing northwards from Siberia. The migratory form of the Siberian sturgeon spends most of its life in the middle and lower part of Siberian Rivers, and when migrating into the Arctic Ocean bays, it enters a brackish environment [28], [17], [25].

In aquaculture, stocking density is a key factor in determining the profitability and economic sustainability of a company [23] and farmers often tend to increase it to favor productivity [11], causing, eventually, chronic stress to the animal [24]. Embryonic development in teleosts is profoundly

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influenced by the environmental conditions that determine the rate of myogenesis, the distribution of the number and size of muscle fibers, and gene expression [16]. During the larval phases, the plasticity of fish muscles in response to the environment is usually not reversible due to the rapid rate of ontogenetic changes in this developmental period [16]. The growth potential of the larvae could be affected by modifications on the proliferative capacity of myogenic cells. Both the maximum size and the growth rate are, indeed, related to the number of muscle fibers in young fish [29].

Farming fish requires knowledge of fast muscle ontogeny and functional anatomy. In the present, the Siberian sturgeon has been included in the International Union for Conservation of Nature [12] red list for endangered species, this means it is considered at risk of extinction in the near future. Siberian sturgeon is an endangered species, which began to undergo a sharp decline in the 1930s (years in which the demand was particularly higher) and continues to decline nowadays. The success of Siberian sturgeon farming is due to the great robustness, the reduced oxygen demand in the juvenile phase, and the high quality of caviar and meat, or secondary value products as the skin [10]. However, the early larval stages still present relevant mortality, and few studies have been performed concerning the effects of the environmental factors. Understanding the mechanisms that underlie the development and growth of muscle and the state of stress in the early life stages is, therefore, essential in order to identify optimal strategies for rearing and conservation of Siberian sturgeon [1].

The purpose of this experiment is to adapt the larvae and fry of Siberian sturgeon to the conditions of the recirculating aquaculture system. Given that the post-larval stage of *Acipenser baerii* is the most sensitive period in which significant losses are recorded and as a result, special attention is paid to the post-larval technology that will be applied, especially to the control of water physico-chemical parameters and the feeding scheme used. Thus, the current case aims at the artificial reproduction of the species *Acipenser baerii* for the first time in I.C.D.E.A.P.A. Galați.

MATERIAL AND METHODS

The biological material is represented by *Acipenser baerii* (J. F. Brandt, 1869) larvae resulted from the artificial reproduction within the Research Pilot Station of I.C.D.E.A.P.A. Galați. After hatching, siberian sturgeon pre-larvae were taken and transferred to the 6 rearing units of the recirculating aquaculture system, with 5447 larvae/unit, totaling at 32.680 larvae. The stocking density of the biological material in the 6 growth units was of 20 larvae/l with a water flow rate between 7-15 l/min depending on water temperatures (15,5-20,7 ^{0}C). This stocking density was maintained in the first 10 experimental days, after that the biological material was redistributed in the 6 rearing units, with 2.047 specimens/unit and a stocking density of 7 larvae/l. The total number of 10 day old larvae of *Acipenser baerii* was 12.282. Although, the literature recommends, for example, for starry sturgeon, 5 larvae/l with an average dissolved oxygen content of 8,9 mg/l. Throughout the experimental period, a higher concentration of dissolved oxygen was maintained to ensure the specific physiological requirements of the post-larval stage, recording short-term oscillations.

The recirculating technological system of aquaculture used, consists in: a) system for collecting the supply water from the urban network provided with a supply tap; b) tank for dechlorination and refreshment of technological water in a percentage of 20% in 24 h to replace losses resuled from the removal of residual solids from the settling tank as well as the water used for washing the filters; c) settling tank with mechanical and biological filtration types, by gravitational separation of sediments and the action of biological filter with bactobolts in order to pre-filter the technological water with the load of organic substances from the system; d) pumping group; e) mechanism for water conditioning composed of mechanical filter with sand bed, filter with activated carbon bed and UV sterilization system; f) buffer tank which supplies water to the rearing units; g) technological water evacuation system with the role of transporting, through pipes, the wastewater to the settling tank for the separation of sedimented solids, digestive metabolic residues and unconsumed food.

The used recirculating system scheme can be found in figure 1.

The experimental rearing units are represented by 2 circular rearing pools and 6 square rearing tanks. The circular rearing pools have a diameter of Ø 4,4 m and the optimal volume of water – 12,1 m^3 and Ø 3,6 m respectively with the optimal volume of water – 8,1 m^3 while the other 6 tanks each have a side of 1,4 m and the maximum volume of water – 0,6 m^3.

The optimal volume of water used in the 6 growth units was initially 0.3 m^3, then gradually increased according to technological requirements.

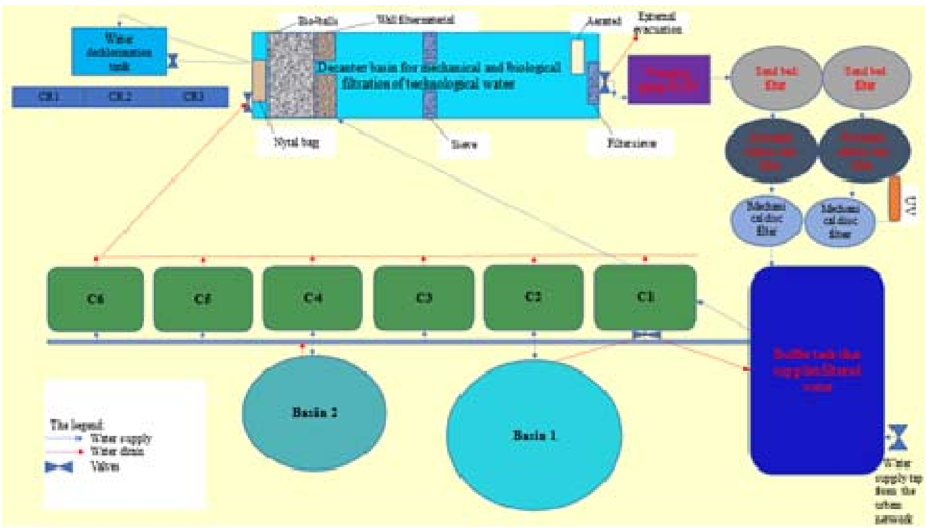

Fig. 1 Scheme of the recirculating aquaculture system.

The feeding scheme of Siberian sturgeon larvae, was according to the recommended technology for post-larval stage rearing. Thus, feeding takes place 4 days after hatching when natural food, represented by *Artemia salina*, was administered. This food was distributed at an interval of 3 hours, *ad libitum*. In the first days of feeding, an attempt was made to supplement it with mosquito larvae and *Chironomide*, which was not widely accepted for consumption.

After the mentioned experiments, natural food represented by the oligochete *Tubifex sp.,* collected from the natural environment and treated beforehand, was administered. In the first days, the food administered was exclusively live or frozen natural food, then the transition to mixed feeding was made, with a mixture of *Tubifex sp.* and fodder *Microstarter Nori* obtained through a special technology- micronization and marumerization having a high nutritional value, similar to natural food. Subsequently, a larger amount of feed was gradually administered until the end of the post larval period, when the food administered was exclusively artificial granulated food. The frequency of meal administration both in the case of mixed feeding and in exclusively fodder feeding was 6 meals / day every 4 hours. The biochemical composition of the *Microstarter Nori* indicates a protein content of 65% and 13% lipids, also contains: squid flour, fish meal, fish oil, lecithin, fish gelatin, krill flour, microalgae, without synthetic additives. These components are natural, obtained from fresh sources with high nutritional properties that remain active even after processing. The fodder also contains natural vitamins and minerals, without synthetic additives. This feed is especially recommended for the first feeding related to the larval stage in order to obtain a high survival rate, a quick, uniform development of the brood. Being microcapsulated to protect the nutrients, the fodder lasts a long time in the water (to be consumed by the larvae) and also avoids the deterioration of the quality of the culture water.

During the post-larval stage of the studied species, special attention was paid to the daily monitoring of water quality parameters, as well as their maintenance within optimal physiological limits for the species *Acipenser baerii*. The assessment of water quality in the culture system was performed by daily monitoring of temperature, oxygen and pH using portable oxygen meter HACH HQD Field Case 58258-00, respectively by periodic determination of nitrates, nitrites, ammonium, chlorine and dissolved organic matter by the colorimetric method with the DR 2800 spectrophotometer, the values thus determined were compared with those optimally admissible for the crop species.

RESULTS AND DISCUSSIONS

The early development of sturgeon larvae is accompanied by very complex changes from a morphological point of view. It is known that during this period, the growth of different parts of the body is characterized by allometric mathematical models [21]. Also, changes in body shape are directly related to the completion of the functions of various organs, such as breathing, feeding and swimming [27], [26]. The characterization of morphogenesis processes and the development of growth models can lead to a better understanding of the early stages of development and offer new perspectives in understanding the biological, behavioral and ecological aspects of the species [9]. During the post-larval of Siberian sturgeon, the culture water must comply with the optimal values corresponding to the eco-physiological requirements of the species *Acipenser baerii*, especially at this stage, when any modification of the organic matter, ammonia, nitrites, hydrogen sulfide, etc. leads to mass mortality of larvae [22].

During the endogenous feeding stage, stress plays an important role and stressing rearing conditions may cause mortality and impaired growth [2], [3]. The environmental conditions experienced during early life stages can have an influence on traits during later ontogenetic stages [5] and may, therefore, have an impact on the growth performance in aquaculture. The major stressors in aquaculture are stocking density, temperature and low dissolved oxygen.

During the experiment, the physico-chemical parameters represented by temperature and dissolved oxygen were monitored daily at an interval of 2 hours, and the pH, nitrogen compounds, organic matter, etc. were determined periodically. Thus, the temperature registered values between 15,5 – 21,1 ^{O}C and an average of 19,3 ± 0,86 ^{O}C, as being technologically optimal for the studied crop species. The values recorded during the experimental period related to the post-larval of Siberian sturgeon are shown in Figure 2.

The quality of water in a recirculating culture system is determined by its concentration in dissolved oxygen, unionized ammoniacal nitrogen, nitrites and carbon dioxide. The level of nitrate concentration, pH and alkalinity are also important parameters for assessing water quality [4]. During the embryonic development period, the value of dissolved oxygen must not fall below 5 mg O_2/l. If this value is lower then unwanted phenomena such as larvae cease active feeding, growth depression occurs, and significant mortality often happens [22].

During the experimental period, the dissolved oxygen recorded values between 5,07-10,15 mg/l, with an average value of 7,5 ± 0,75 mg/l. The recorded values of dissolved oxygen in the experimental period are shown in Figure 3. The pH values in the intensive system were on average 8,38 ± 0,12 pH units..

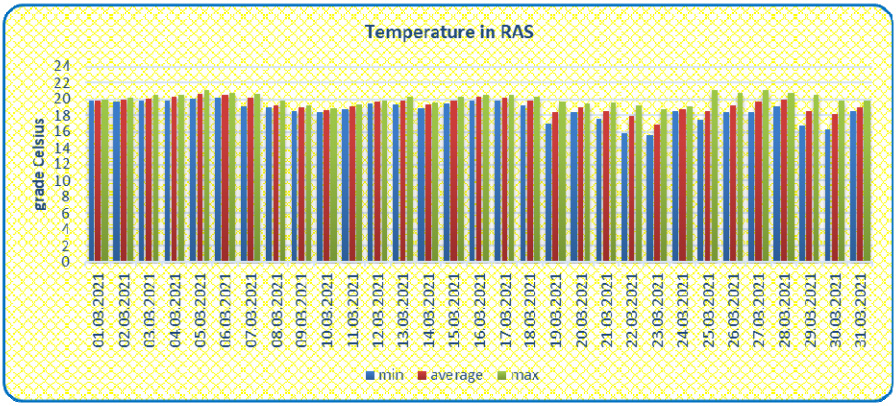

Fig. 2 Temperature variation in RAS.

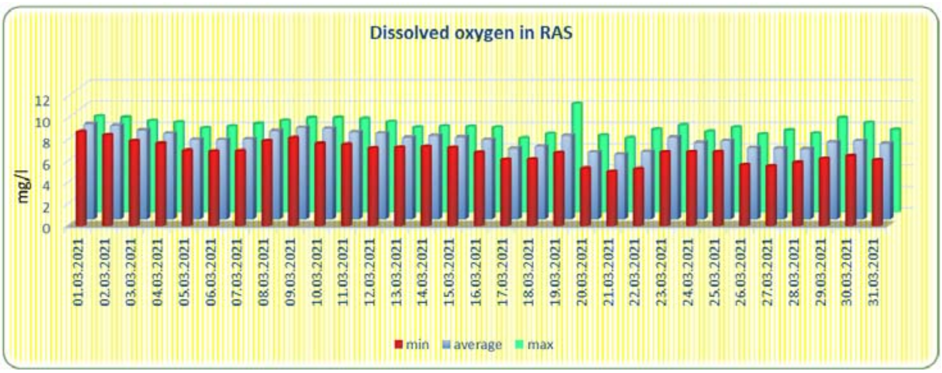

Fig. 3 Dissolved oxygen variation in RAS

Nitrites ($N\text{-}NO2^-$) recorded during the experimental period remained in the range of 0,02-0,03 mg/l, and nitrates ($N\text{-}NO3^-$) indicated values between 2,68-6,07 mg/l. During the experimental period, the values of ammonium ion (NH_4^+) ranges 0,01-0,03 mg/l. All recorded values are shown in Figure 4.

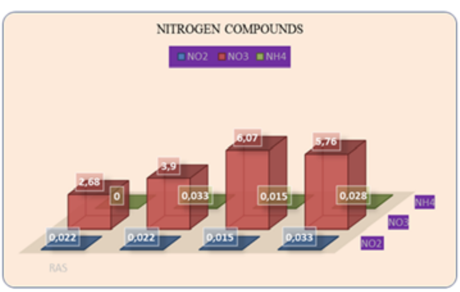

Fig. 4 Nitrogen compounds in RAS

The larval stage involves two phases, the first period that lasts from hatching until the larvae begin active swimming in the horizontal plane and the second period starting with active swimming and it ends by obtaining the planned biotechnological indices (1-2 g/ex). In the first period, special attention is paid to observing the phenomenon of negative phototropism, and the second period of postembryonic stage requires the application of technology related to larval period when there is a transition from vertical swimming to horizontal swimming and to feeding exclusively artificially. This technology involves increased vigilance with regard to the feeding operation, which is considered to be the most critical of the entire operational flow. Thus, the applied feeding regime consisted in the administration, in the first phase, of live food (the first 10 days), followed by mixed feeding which involves mixing natural food with artificial food (gradually increasing the proportion of artificial food), then adapting to feeding exclusively with fodder by calculating rations according to biomass.

Regarding the post-larval stage of *Acipenser baerii*, it was taken into account that not all individuals adapt to artificial food and then they appear in individual differentiation of size and weight that require close monitoring and the need to sort biological material. At the same time, in this stage the manifestation of the cannibalism phenomenon can be observed, due to the insufficiency of the food or the inaccessibility to it, in order to ensure the nutritional requirements. The sorting by size classes was performed and for the small dimensions larvae natural food was still administered in order to recover the lost intake. Table 1 shows the size classes in growth units in which it was distributed according to the average weight.

Table 1 Size classes of *Acipenser baerii* larvae

Size classes			
Acipenser baerii	**Class I in tanks C1 and C2**	**Class II in tanks C3 and C4**	**Class III in tanks C5 and C6**
Weight range [g]	0,2542 – 0,4813	0,4814 – 0,6502	0,6503– 0,9196
Length range [mm]	22-27	28-32	33-38

In this context, several authors have attempted to evaluate whether long-term growth and survival of Siberian sturgeon were influenced by the administration of live feeds at first feeding and determine if overfeeding is beneficial at early developmental stages. Special attention may be taken for not overfeeding larvae, since an excess of feed may deteriorate water quality and exacerbate larval mortality by encouraging protozoan growth, bacterial contamination, as well as increase the likelihood of gill infection. However, under nourishing fish especially at these early developmental stages introduces risks of starvation and cannibalism. Feeding rate, water temperature and fish size are among the three most important factors affecting the growth of fish, and thus determining the optimal feeding rate is important to the success of any aquaculture operation. This is particularly true for larval fish because they are very susceptible to over- and underfeeding, both resulting in increased incidences of disease and mortality due to improper larval nutrition or water quality.

Mortality during endogenous feeding was less than 1 – 1,5% and was observed especially in larvae with morphological deformities. However, after the population in the tanks, the larvae of *Acipenser baerii,* swam dispersed in the mass of water, then left on the bottom of the rearing tanks forming the so-called "cloud" or "swarm" specific to the phenomenon of removing the pigment plug which is the physiological critical moment, when significant losses are found (figure 5).

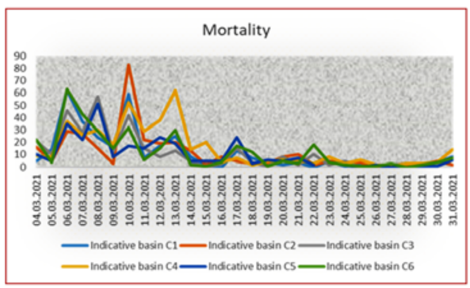

Fig. 5 Mortality registered in the post-larval stage

At the onset of exogenous feeding, the mortality indicated a significant increase in all experimental rearing units and accounted for 22%. The losses were also due to the adaptation to exogenous feeding, as well as to the occurrence of the cannibalism phenomenon in a small proportion (2-3%). Mortality decreased substantially after switching to mixed exogenous feeding, remaining constant in the case of exclusively artificial feeding.

Morphogenesis and differentiation are more intense during the pre-larval than the larval and early juvenile stages of development. Characteristically, during the pre-larval period, embryonic adaptations and functions are replaced by definitive ones, such as gill respiration, exogenous feeding and active swimming. Such modifications involved dramatic alterations in the relationship of the developing fish with the environment that were reflected in morphological and morphometric changes. Formulated diet can be successfully used in the early stages of larval development of Russian sturgeon with higher mortality than feeding larvae with natural live diet or mixed diet in the transition to exogenous feeding period which is critical for sturgeon development [7]. In principle, artificial fodder are formulated to fulfill the nutritional prerequisite of fish with the extra advantages of less cost, suitable feeding and easy for storage [14]. Moreover, previous studies revealed that incorporation of artificial diets in the early life stage of some species of sturgeon including *Acipenser guldenstaedti* and *Acipenser baerii* have suggested that these diets could be successfully utilized for the intensive commercial farming of sturgeon [7], [20].

CONCLUSIONS

Transition to exogenous feeding marks the completion of the pre-larval phase of development and the onset of the larval stage, and is associated with changes in respiration, metabolism, growth rate and survival of sturgeon larvae. Regarding the adaptation to the conditions of intensive rearing in the recirculating system and the optimization of the administered feeding scheme led to the achievement of a survival of the biological material of 73.50%. The average individual weight of *Acipenser baerii* at the beginning of exogenous feeding was between 0,0054 – 0,0069g and, respectively, the maximum weight at the end of the experimental period (after 40 days) was between 1,05 – 1,20 g. The ability to control the physico-chemical parameters of water and the optimization of the applied feeding scheme that ensures an optimal growth value for the post-larval stage, it is an obvious advantage of recirculating aquaculture systems and is considered essential for their profitability.

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