ABSTRACT

Sturgeons are one of the ancient fish breeds which populate the world's waters, are appreciated as being one of the most valuable fish breeds, due to their meat sensorial and nutritive qualities as well as due to the quality of roes (Bura, 2008, p. 9).

The highest demand for sturgeons is given not by meat and roes quality which have a remarkable taste but also by the many by-products, man understanding that those ones are a limited resource, restrain to a family, with majority of breeds on extinction, and some of them even disappeared.

Acclimatization of *Polyodon spathula* breed had the role to decrease the pressure given by excessive fishing of sturgeons' flocks from natural waters and to replace, in time, bighead in populating formulas, because it is considered to have a lower quality meat.

Introduction in culture of *Polyodon spathula* breed also aimed the utilisation of the whole natural trophic potential of a basin, increasing the fish quantity obtained on surface unit, assortment diversification of production, increasing of meat quality, increasing of economical efficiency and exploitation profitability.

The main aim of the current paper is to improve the artificial reproduction technology for *Polyodon spathula* breed, at the level of a fishery farm, by making different studies regarding the selection of breeders by different methods, optimization of the usage of reproduction hormones, short time preservation of seminal material, founding of new solutions for desizing, development of new technologies for larvae rearing which to lead to a superior survival rate and development of cultivation methods for live feed (cladocera and nematodes).

The novelty character is given by the fact that the whole breeder batch was reared into a domestic fishery farm, was tracked their development from the stage of 25 days juveniles till the one of mature breeder, artificial reproduction was realised in North-East of Moldavia, area with different climatic conditions face to SCDP Nucet, and breeders are coming from an acclimatised batch, originally from USA, which strengthens the statement that breed is well acclimatised in Romania.

The paper was structure in 4 series of experiments:

First series of experiments – Contributions regarding the knowledge of environmental characteristics and rearing technologies;

Second series of experiments – Contributions regarding selection of a breeding batch belonging to *Polyodon spathula* breed and optimal age for reproduction;

Third series of experiments – Contributions regarding stimulation of sexual elements maturity, gathering, fecundation and desizing of roes and their incubation;

Forth series of experiments – Contributions regarding post-embryo development of *Polyodon spathula* breed and methods for cultivation of cladocera (*Daphnia sp., Moina sp.*) and nematodes (*Enchytraeus buchholzi, Enchytraeus albidus*) utilised in feeding of *Polyodon spathula* sapling.

First series of experiments – Contributions regarding the knowledge of environmental characteristics and rearing technologies

Had in view to determine the hydrological and hydro-biological conditions of water and enlighten the rearing technologies for *Polyodon spathula* breed from the age of 25 day till the age of a mature individual, able-bodied for reproduction fact realised into a fishery farm during a 12 years period of time.

Water temperature in analysed period oscillated between minimal values obtained in January, 2.53ºC and maximum of 27.2ºC, obtained in July. Dissolved oxygen reached the minimum of 4.61 mg/l in August and maximum of 15.85 mg/l in January, in direct correlation with waters' temperature.

The values for the other parameters (pH, Ca, Cl, NO_3, NO_2, NH_4, PO_4) were in the limits from literature, placing the utilised water, in 2nd category of fishery waters, values being normal for these category.

Analysing the hydro-biology of water we conclude the zoo-benthonic mass reached the maximum level of development in July with a value of 23.82% from total gathered quantity in the studied period. Zoo-plankton biomass reached its maximum in May having a value of 38.8% related to total and regarding phytoplankton maximum was reached in March and October, corresponding with low water temperatures.

Analysing the hydro-biological results we conclude that the level of development are normal to superior, correlated with the data from literature, which finally leaded to a good development of biological material belonging to *Polyodon spathula* breed.

Determinations connected with rearing technologies relieved the fact that in 1st summer saplings reached a mean weight of 238.6 g/individual, survival rate being 71.4%, corresponding to a production of 334 kg/ha. In the 2nd summer, polyodon reached at a mean weight of 2562.5 g/individual, survival rate being 74.75% and production of 82 kg/ha. Rearing in the 3rd summer was realised in poly-culture, polyodon being supplementary breed. Rearing from age of 2 years till age of 12 years was realised also in poly-culture, the realised density at populating being 5-10 individual/ha. The realised mean weight was 13.780 kg/individual. The total mean length was 141.9 cm. Survival rate from age of two years till age of 12 years was of 50% being mainly influenced by predators.

Second series of experiments – Contributions regarding selection of a breeding batch belonging to *Polyodon spathula* breed and optimal age for reproduction.

The aim was to determine the maturity degree of gonads and optimal age for reproduction, determinations based on biopsy method and selection of a breeding batch, by visual analysis and also by utilisation of some indexes for corporal appreciation.

For realisation of those, batch was split by sexes, breeders being measured and weighted, were calculated different corporal indexes (profile index, thickness index, quality index, Fulton index), to enlighten both their maintenance state but also to discover the eventually differences between females batch face to males one, for an easier selection of them.

At the end of gravimetric and dimensional determinations for female batches, were recorded the following mean values: body mass 13.66 kg, total length (L) 137.55 cm, standard length (l) 120.81 cm, length of head (lc) 55.5 cm, length of caudal peduncle (lp) 16.74 cm, maximum height of body (H) 24.71 cm, maximum circumference of body (C) 62.07 cm, maximum thickness of body (G) 15.17 cm.

At the end of gravimetric and dimensional determinations for male batches, were recorded the following mean values: body mass 11.14 kg, total length (L) 132.84 cm, standard length (l) 115.32 cm, length of head (lc) 52.75 cm, length of caudal peduncle (lp) 17.52 cm, maximum height of body (H) 22.99 cm, maximum circumference of body (C) 57.62 cm, maximum thickness of body (G) 13.38 cm.

After calculating the corporal indexes and comparison between females and males batch the founded results were as follows:

Profile index had lower values for both batches (4.94 at female batch and 5.03 for male batch), which show that corporal format of the studied individuals; both males and females had a convex aspect.

Thickness index had the value of 61.85 at female batch and 58.28 at male batch. So could be observed that fattening degree and musculature thickness was more developed at females' batch.

Quality index had lower values and close between those two batches (1.95 for female batch and 2 for male batch), which show that musculature is well developed in both cases.

Fulton index had value of 0.77 at female batches and 0.74 at male batches. Those values show a good maintenance state of fishes.

Carnosity index presented values of 45.94 at female batch and 45.73 at male batch. Values are quite high, due to breed specific corporal conformation, rostrum being quite big related to the whole body. At the end of determination for main corporal indexes and comparative statistical analysis between those two batches (females and males) we could say that fishes had a good maintenance state and could be utilised for artificial reproduction.

Polarization coefficient of roes' nucleolus was calculated after application of biopsy through which were extracted some roes from all those 15 females.

The analysed females had different development stages for ovocytes, some of them being placed in the IV unaccomplished stage (will be used for reproduction in the next year), and some of them being already in the IV accomplished stage, and could be used for reproduction in the respective year.

After males' selection through analysis of sperm mobility, in according with Persov scale, was observed the fact that only one male had sperm with a very low mobility of spermatozoa (<25%), otherwise all males had 4 or 5 points on Persov scale, so the movement at almost all spermatozoa is rapidly and forwards, which categorizes them as males able for reproduction.

Third series of experiences - Contributions regarding stimulation of sexual elements maturity, gathering, fecundation and desizing of roes and their incubation

Aimed to develop the knowledge regarding different hormones utilised for stimulation of sexual elements maturity, gathering, fecundation, desizing and hatching in specific incubators for breed.

For determination of the most efficient hormone for reproduction, was made a comparative study between LHRH-A product, with an American provenance, and Nerestin 5A product, with a Russian provenance. Also in the frame of this goal, but in the next year of experiences, was realised a study regarding optimal utilization of Nerestin 5A hormonal product for stimulation of the breeders belonging to *Polyodon spathula* breed. Was also studied the possibility for a short time storage of seminal material gathered from *Polyodon spathula* breed, as well as the utilization of new substances for desizing (alcalase enzymes, *Bacillus licheniformes*).

After hormonal stimulation with LHRH-A product, from female batch, only two reached the maturity of sexual products, and from those were gathered roes. Males, from which sperm could be gathered, after injections with this product, were in number of 4.

After hormonal stimulation with Nerestin 5A product, all females reached the maturity of sexual products, from those were gathered roes which were suitable for fecundation. All males were able to produce sperm.

After comparison of the females' breeding batches LF1 vs. LF2, from the point of view of the necessary time for maturity, the differences between those two products were distinct significant. Regarding the number of mature females, after a comparison between those two batches result the fact the differences between them are significant. The quantity of gathered roes from mature females was 82 g/kg body, respectively 89 g/kg body.

After comparison between male' breeding batches, regarding the necessary time for sexual elements maturity, number of males which produce sperm and the gathered sperm quantities it was observed that the differences between them are insignificant. Gathered quantity of soft roes was 8.20 ml/kg body male at batch LM1 and 9.42 ml/kg body, in the case of batch LM2.

The obtained results regarding optimization of usage of hormonal product Nerestin 5A aimed to determine the optimal quantity of utilised hormone, proved the fact that at a water temperature of 13ºC, the necessary time for maturity is almost the same for all three batches, no matter of the utilised hormonal dose. At a water temperature of 15ºC the necessary time for maturity of females from second batch started to be different function of the utilised quantity of hormone, and at water temperature of 17ºC, the necessary time for maturity decreased at all those three batches. After statistical interpretation of the results could be observed that are differences distinct significant regarding necessary times for maturity between those three batches. All the females reached maturity being able to be milked for roes. Analysing the gathered roes quantity could say that differences between batches' means are insignificant, variability coefficient having low values (V% = 2.6 at batch LF1, V% = 3.53 at batch LF2 and V% = 2.72 at batch LF3), homogeneity inside batches being big. At the end of this analysis we could say that temperature influence the time in which females reach the maturity of sexual products, the increasing is linear, and didn't influence the quantity of gathered roes, hormone optimal quantity being 0.12 ml/kg body female, so, we can use a lower hormone quantity if the water temperature is higher.

Research regarding short time storage of *Polyodon spathula* sperm had the aim to initiate a storage protocol for sperm, at a constant temperature of 4ºC, for a certain period of time as long as it gets, in a certain way so sperm mobility and its qualities to be less influenced.

To reach this goal were utilised three storage methods, and at the end of analysis of the obtained results was established which one is the most efficient. Sperm was kept in three recipients, respectively a syringe, a pack bag in which together with sperm was introduced air and a pack bag in which together with sperm was introduced technical oxygen.

After 2 hours from placing at refrigeration the differences regarding mobility were insignificant. However was observed that the value of variability coefficient have a medium homogeneity, in case of first batch (L1), its value being V% = 11.91, which means that at some samples the mobility of spermatozoa being to decrease.

After 18 hours after storage in refrigeration conditions, at first batch the points associated with Persov scale continue to decrease, variability inside batch being medium, V% = 15.21, which means that sperm, from spermatozoa mobility point of view is almost uniform depreciated.

At batches L2 and L3 mobility points associated with Persov scale, started a light depreciation and between them aren't any statistical differences. Variability coefficient had mean values for both batches (L2 and L3), V% = 11.91. In according with statistical analysis resulted that between those three batches the differences are significant.

After 36 hours for storage at cold, variability coefficient at those three batches had mean values, V% = 15.97 for batch L1, V% = 10.54 for batch L2 and V% = 17.68 for batch L3. At the first batch continue to decrease the mobility of sperm, reaching at a mean of 2.8 points. For the other batches mobility kept values quite high (4.2 at L2 and 4 at L3), fact which allow the samples to be utilised for artificial reproduction. From statistical point of view, differences between those two batches are distinct significant, could be remarked that the values for first batch had a significant decreasing. Sperm gathered from those batch couldn't be utilised for artificial reproduction or it can be utilised but with weak results.

After 48 hours of cold storage could be observed that variability inside batches was higher for first batch (L1), where variability coefficient had the value V% = 38.03 and medium for batches L2 and L3, variability coefficient having the following values V% = 15.21 and V% = 11.77. After statistical comparison could it be observed that differences between those three batches are distinct significant. At first batch, mean value of points on Persov scale reached at 2.2, which means that sperm couldn't be utilised for artificial reproduction. Between batches L2 and L3 differences from statistical point of view are insignificant; both methods leading to preservation of sperm in interval 0-48 hours with a decreasing of its mobility with only 28 percent for batch L2 and 24 percent for batch L3. We could affirm that for interval 0-18 hours all the preservation methods lead to very good results regarding sperm mobility, and for interval 0-48 hours only storage of sperm in nylon sterile pack bags with addition of atmospherically air and technical oxygen are viable, without any differences between them.

All the other stages of artificial reproduction for *Polyodon spathula* breed (fecundation, incubation, hatching) had a normal evolution, with results very close to the limits cited by literature.

Forth series of experiences - Contributions regarding post-embryo development of breed and different methods for cultivation of live food

The aim was to enlighten the post-embryo development stage of breed, rearing of larvae in different densities, assuring of live food by cultivation of cladocera *Daphnia sp.* and *Moina sp.* as well as of nematodes *Enchytraeus buchholzi* and *Enchytraeus albidus*.

To realise those was made a comparative study regarding larvae rearing in an intensive system, in period 1-20 days and 20-40 days. Was experimented cultivation of cladocera in ground pools and was compared the efficiency of the method face to their harvesting from larger rearing ponds. In this study was tested a cultivation technology of nematodes *Enchytraeus buchholzi* and *Enchytraeus albidus* in plastic casseroles.

For that were effectuated two experiments. The first one supposed larvae rearing in period 1-20 days at densities of 1 larvae/l water (L1) and 5 larvae/l water (L2) and the second one presumed larvae rearing in period 20-40 days at 2 different densities respectively 200 individuals/m^3 water and 400 individuals/ m^3 water.

In case of the first experiment, the results showed that exist significant differences between batches L1 and L2 regarding the survival rate. Differences between the weights realised by larvae in those two batches are very significant, higher densities leading to lower weights and at comparison of total length could be observed that differences are very significant. The results are good in comparison with the ones from literature and optimal density was 1-3 individual/l. The weight of larvae at populating was almost equal for all the basins with a mean of 0.0011 g/individual and at the end of rearing period reached a mean weight of 0.184 g/individual for batch L1 and 0.133 g/individual for batch L2.

In case of the second experiment, the results enlighten the fact that are significant differences between batches L1 and L2 regarding survival rate and differences between corporal weights realised by those two batches are very significant, higher density leading to realization of lower values. Also at the comparison of total length realised by larvae from those two batches the differences were very significant.

Larvae weight at populating was almost equal for all basins, with a mean of 0.132 g/individual. At the end of rearing period the sapling reached a mean weight of 8.2 g/individual for batch L1 and 5.2 g/individual for batch L2.

Cultivation of cladocera *(Daphnia sp.* and *Moina sp.)*

The obtained results show the fact that mean of cladocera individuals per one litre of water was 1650 individuals for the basin in which cultivation was made (P2) and 1135 individuals in the rearing pond, between them there aren't significant differences.

Cultivation of nematodes was realised in plastic casseroles with an area of 200 cm^2. Quantity of worms which was gathered once per week was 3-8 g/casserole for breed *Enchytraeus albidus* and 1-6 g/ casserole/week for breed *Enchytraeus buchholzi.*

After realization of those experiments we could say the breed is well adapted to the conditions from the fishery farm which hosted the research, the development of sexual elements was realised normally, sexual maturity appeared earlier face to the data cited in literature, artificial reproduction was successfully realised and the aims were reached.