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# RESEARCH ON SPERM QUALITY OF NORTH AMERICAN STURGEON SPECIES *POLYODON SPATHULA*

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***Abstract***
*The paper presents research on sperm quality and affecting factors in male of North American sturgeon species Polyodon spathula recently acclimated in Romania. Quality assessment was performed by microscopic and macroscopic analysis of sperm from a total of 5 males used in artificial spawning experiments in 2012. Microscopic analysis was to determine the concentration of sperm by haemocytometer method, sperm shaped and size determination using electronic microscopy and identification of specific staining, motility determination using dark field microscopy, testing the viability of sperm after sperm activation. Sperm quality parameters were interpreted by correlation with following factors: hormonal stimulation, the spawning season, temperature, extenders properties, short or long term storage of sperm, the biology of spawning (age, weight, length), health and wellness condition.*

***Key words****: Polyodon spathula, spawning, sperm quality*

## INTRODUCTION

Quantity and quality of sperm affect decisively the success of artificial spawning.
Milt quality is a measure of the ability of sperm to successfully fertilise an egg which such ability mostly depends on qualitative parameters of milt i.e. composition of seminal fluid, milt volume, sperm density and sperm motility.[6]
Making artificial spawning without the control of sperm quality can have a negative effect on its biotechnological indicators. [1]
It is necessary to understand the importance of theoretical and especially the dissimilar practical significance of quality control as it is accepted the quality control need of agricultural seeds.
On this context, inside of S.C.D.P Nucet were initiated research work to determine the sperm quality of *Polyodon spathula* sturgeon species by making some current spermiogram and reveal the correlations between environmental factors and fecundate capacity of sperm.

## MATERIAL AND METHOD

The spawning experiments accomplished during the years 2002-2010, revealed that paddlefish males can yield sperm a period of 5-6 days consecutively, due to hormonal stimulation of spermiation.
In the first part of breeding season, paddlefish males give a small amount of sperm and therefore require hormonal stimulation of spermiation.
Hormonal stimulation of breeders was accomplish by receiving Nerestin 5A synthetic hormone in a single dose of 0,1 mg/kg of body weight, on 4 of the 5 males used (M1 – M4). A male – wasn't stimulated being used as control in our experiments.
The semen was collected successively for 5 days, starting from the moment of hormonal induction.
Males were characterized biometrically, and the quality of sperm from each male was assessed by macroscopic and microscopic analysis.

Each male was contention, beat out from tank and well drain off. Sperm sampling, (Photo 1) was accomplished with 20 ml plastic siring, dried, provided to end with a cannula made by a 10 cm infusion tube. By this sampling technique can be obtain a sensibly amount of semen, without the risk of being contaminated by faeces or water.

Photo 1. Sperm sampling on *P. spathula*

**Macroscopic analysis** was to make the following determinations:
-Volume (measuring cylinder, expressed in ml);
-Color;
-Density or consistency;
-Impurities;
-Smell.
**Microscopic analyses** consisted of:
-Determination of spermatozoa concentration – spermatozoa number/ml (haemocytometer method);
It is based on the principle similar to the appreciation of figurative elements from blood, using Goreaev, Thoma, Burker etc. like counting chambers, and Potain dropper.
The sperm is aspirated in the Potain up to 0.5 division and is filled with a solution of 3 % sodium chloride up to 101 division.
Concentration of spermatozoa on sperm/mm$^3$ is calculated as:

$$C = \frac{N.I.S.D.}{n}$$

where:
N – number of spermatozoa counted in 5 big quadrates;
I – height or depth of chamber (1/10 mm);
S – surface of a square (1/400 mm$^2$);
D – amount of sperm dilution;
n – number of squares in which the sperm were counted.
-Determination of shape and size of *P. spathula* spermatozoa by electronic microscopy (Optika microscope) and determination of specific staining to measure the main components – head and tail – Photo 2)

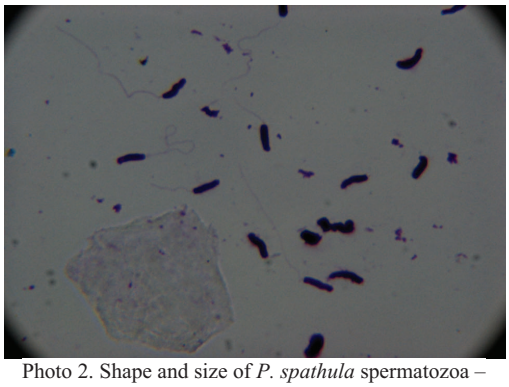

Photo 2. Shape and size of *P. spathula* spermatozoa – May-Grumwald-Giemsa dyeing

- Determination of sperm motility was performed using a simple microscope (ML-4 IOR tip). Optical combination suitable for this control is ocular 10X with lens 20X or 40 X. Was used a grid blade (Burker). The smear was read extemporaneously, according to conventional method known in the practice of artificial spawning after sperm activation in technological water and normal saline solution.

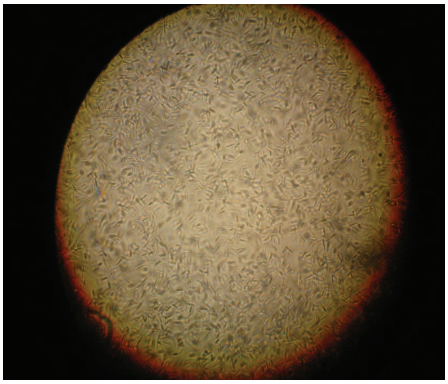

Photo 3. Microscopic examination of *P. spathula* sperm

- The way of spermatozoa movement: straight, cuff, riding, etc.
- Testing spermatozoa viability after sperm activation – examining the preparation

found between blade and slide;
Semen has been collected for five days, every 24 hours.
Sperm concentration was evaluated as billions / ml of semen.
Semen was collected in weighing bottles with lid. By the macro and microscopic sperm analysis was made the usual semen analysis with qualitative and quantitative indicators that can give us important information about the status of breeders used in the process of spawn.
The main biometric characteristics of males are presented in Table 1. After hormonal induction, the male breeders were stoked in canvas tanks specially made for this species and located into the hatchery.
Tank capacity is 4000 l of water; water flow is 15 l / min., dissolved oxygen - 9,0 mg $O_2$/l, water temperature 13 – 14 $^0$C.
During the experimental period breeders were monitoring continuously, and water temperature was measured and registered hourly.

## RESULTS AND DISCUSSIONS

Males used in artificial spawning experiments, in 2012, were aged 9 and 10 years, average weight between 9325 and 13200 g and standard length between 120.0 and 146.0 cm.

Table 1. Biometric characteristics of *P. spathula* males

| Male | Age (years) | Weight (g) | Length (cm) |
|---|---|---|---|
| M1 | 10 | 13200 | 146 |
| M2 | 10 | 13180 | 143 |
| M3 | 9 | 10260 | 136 |
| M4 | 9 | 10710 | 134 |
| M5 | 9 | 9325 | 120 |

The literature mentions that the volume of sperm released by sturgeon males is sometimes large (e.g. 25–200 ml for sevruga, *Acipenser stellatus*, 25–500 ml for Russian sturgeon,*Acipenser guldenstaedti,* and 800–1000 ml for kaluga, *Huso dauricus*) [2] .
In sturgeons the concentration of spermatozoa was usually reported as low compared to teleost fish. [5] ; [2]
In artificial propagation, the amount of sperm obtained per male is limited in paddlefish and in white sturgeon (*Acipenser transmontanus*), especially in the first period of the reproductive season, and therefore spermiation must be stimulated. [4]
The total amount of sperm per male during of the 5 days of sperm sampling was significantly bigger after injection with Nerestin 5A 0.1 mg/kg towards the uninjected male (Table 2).

Table 2. Evolution of paddlefish sperm after hormonal induction with Nerestin 5A

| Male | Volume of sperm harvested after n days (ml) | | | | | |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | Total |
| M1 | 8.0 | 96.7 | 117 | 105.8 | 111.8 | 432.2 |
| M2 | 8.4 | 96.0 | 110.0 | 98.8 | 95.0 | 408.2 |
| M3 | 8.5 | 103.8 | 138.1 | 108.2 | 121.0 | 479,6 |
| M4 | 10.1 | 96.0 | 107.2 | 102.2 | 91,1 | 396,5 |
| M5 | 3.5 | 15.5 | 31.0 | 16.5 | 4.1 | 67.1 |

The sperm was obtained at 00: 35 h (19 April 2012). (Table 3).
The amount of sperm harvested from one hormonally induced male was between 91.1 ml and 138.1 ml.
Sperm volume increased from dai 2 to day 5. It was found that the amount of sperm harvested from the 9 years old males was higher than that collected from the 10 years old males.
Data from literature shows that the volume of sperm collected from paddlefish during the experiment was similar to that of the sturgeon and Russian sturgeon. Also the concentration of paddlefish spermatozoa was similar to that of the sterlet or Persian sturgeon, but lower than that of the Russian sturgeon, sevruga and beluga. [2;5].
Semen color varies from milky white to dirty white with gray tint (Photo 3, Table 3).

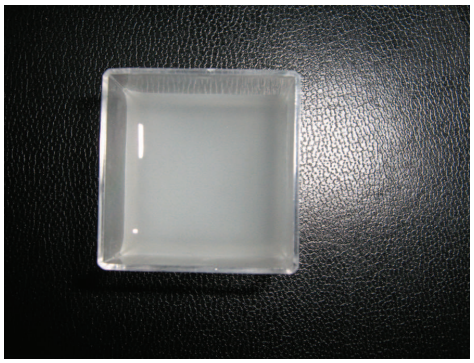

Photo 3. Macroscopic examination of *P. spathula* sperm

Consistency is aqueous (Table 3), and the smell is distinctive.

Table 3. Evolution of paddlefish sperm number hormonal induction with Nerestin 5A

| Male | Number of sperm harvested after n days ($\times 10^9$) | | | | | |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | Total |
| M1 | 14.1 | 46.3 | 49.4 | 25.2 | 9.1 | 144.1 |
| M2 | 9.3 | 44.7 | 36.3 | 22.3 | 5.6 | 118.2 |
| M3 | 9.6 | 42.8 | 43.9 | 29.0 | 9.6 | 134.9 |
| M4 | 11.2 | 13.4 | 12.3 | 5.6 | 4.5 | 47.0 |
| M5 | 10.3 | 2.0 | 9.3 | 2.0 | 1.0 | 14.3 |

The microscopic analysis shows that the total number of sperm per male ranged from 4.5 and 49.4 x $10^9$.

Total number of sperm per male was higher on day 2 (46.3 x $10^9$ per male), the highest in day 3 (49.4 x $10^9$ per male), and then easily lower in day 4 (29.0 x $10^9$ per male) and day 5 (9.6 x $10^9$ per male).

It was fond that although semen volume remains high on the day 4 and 5, sperm concentration decreases.

Fish sperm concentration, which is an important parameter of artificial reproductive management is very variable and depends on the species, individual fish size and season. [3]

Sperm motility was regularly observed during the 5 days in each experimental variant (Table 3). The percentage of motile sperm was not significantly different between males during the harvesting of sperm this ranging from 80 to 95 %.

Survival times of fresh spermatozoa after activation with water and saline solution varied in large limits (Table 4, Table 5).

Regarding the viability percentage, found that it was higher after sperm activation with saline solution than activation with technological water.

Studying sperm movement was observed that they have clear forward movement in the first 5 minutes from activation. After 8 minutes, only 20 – 30 % still has forward movement, others have weaker movements, of oscillation. It was observed that sperm are still motile al approx. 20 minutes of activation.

From studies on semen stored at room temperature, it was found that sperm are mobile even after there activation with saline solution at 4 hours after harvesting.

Table 4. Usual semen analysis on *P. spathula*

| Date/ hour Male | Temperature on harvest $^0$C | | Survival time after activation (minutes, seconds) | | Volume ml/fish | Concentration nr. of sperm billion/ml | Motility % | Consistency | Impurities | Color |
|---|---|---|---|---|---|---|---|---|---|---|
| | Air | Water | Water | Saline solution | | | | | | |
| Male sperm sampling on 19.04.2012, starting at $00^{35}$ | | | | | | | | | | |
| Mascul 1 | 10 | 13 | 1'42" | 6' -8 ' | 96.7 | 46,3 | 95 | Aqueous | No | Dirty white-gray tint |
| Mascul 2 | 10 | 13 | 1'40" | 2'30" | 96.0 | 44,7 | 85 | Aqueous | No | Milky white |
| Mascul 3 | 10 | 13 | 1'10" | 1'30" | 103.8 | 42,8 | 85 | Aqueous | Urine | Milky white |
| Mascul 4 | 10 | 13 | 45" | 3'30" | 46.0 | 13,4 | 90 | Aqueous | No | Milky white |
| Mascul 5 | 10 | 13 | 25" | 1'10" | 15.5 | 2 | 80 | Aqueous | Urine | White-gray |
| Sperm analyses of male 1, date 21.04.2012 | | | | | | | | | | |
| Male 1 | 11.5 | 13.5 | 1'48" | 8'56" | 111.8 | 9.1 | | Aqueous | No | |

Table 5. Data on *P. spathula* sperm viability after activation

| Data | Temperature at harvest °C | | Storage temperature $^0$C | No. hours from harvest | Motility % | Survival time after activation – minutes, seconds | | Observations |
|---|---|---|---|---|---|---|---|---|
| | Air | Water | | -ore- | | water | saline solution | |
| 19.04.2012, from $00^{35}$ | | | | | | | | |
| Male 1 | 10 | 13 | 20,0 | 2 | 90-100 | 1'42" -2 ' | 6' -8 ' | Sperm from one male |
| | 10 | 13 | 20,0 | 24 | - | - | | Agglutinated sperm has not been activated |
| | 10 | 13 | 4,0-8,0 | 24 | 80- 50 | 1'20" - 1'45" | 6' | |
| | 10 | 13 | 4,0-8,0 | 48 | 2-3 | - | 10" | |
| Male 2 | 10 | | 20,0 | 2,5 | - | - | - | Semen has not been activated |
| Male 3 | 10 | 13 | 20,0 | 2,5 | 70-25 | 1'35" | 5' | Sperm has not been activated, agglutination |
| Male 4 | 10 | 13 | 20,0 | 2,0 | 10-13 | 35" | 35" | |
| Male 5 | 10 | 13 | 20,0 | 1 | 100 | 1'25" | 6'45" | |
| | 10 | 13 | 20,0 | 4 | 70 - 90 | 55" | 3'35" | |
| | 10 | 13 | 20,0 | 24 | - | - | - | Sperm has been agglutinated |
| | 10 | 13 | 4,0-8,0 | 24 | 50 | 50" -55" | 2'35" | |
| | 10 | 13 | 4,0-8,0 | 48 | - | - | - | Sperm has not been activated |
| Analyses of male 1 sperm, date 21.04.2012 | | | | | | | | |
| Male 1 | | | 2.0 -4.0 | - | 90-100 | 1'30" - 1'48" | 6'56" -7' | |
| | | | 2.0-4.0 | 24 | 90 | 1'30" | 5' | |
| | | | 2.0-4.0 | 48 | 70- 40 | 1'10" | 5' | |
| | | | 2.0-4.0 | 72 | 50- 30 | 45" | 2'30" | |
| | | | 2.0-4.0 | 96 | 5-10 | - | 1'20" | |

## CONCLUSIONS

To increase the volume of semen in the spawning season the male hormonal stimulation is required. Although sperm volume is high even at four, five days after stimulation, the number of sperm decreases.

Qualitative parameters of milt (i.e. seminal fluid composition, spermatozoa motility and sperm production) could be influence by

several factors including biological characteristics of brooders (age, weight and length), rearing conditions of brooders (temperature, photoperiod, nourishment, and animal welfare and health), artificial induction of spawning, spawning season (repeated milt collection and spermiation time) and post stripping factors (chemical properties of diluents and short-term and longterm storage of milt).

The analyses results were materialized in achievement of usual semen analysis with quality indicators of semen. Understanding of the factors that affect sperm quality could be useful for adjustment and efficient management of them.


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