STUDY REGARDING THE INFLUENCE OF WATER TEMPERATURE ON HORMONAL STIMULATION OF FEMALES FROM *POLYODON SPATHULA* BREED FOR MATURATION OF SEXUAL PRODUCTS

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Abstract

The aim of the current paper was to establish and to optimize the doses of Nerestin 5A hormonal synthesis mixture utilised for hormonal bio-stimulation of females belonging to Polyodon spathula breed, function of water temperature. The current research were carried out on three females' batches, of same age (13 years) and reared in the same environmental conditions in an aquaculture farm from Iaşi County. From all females from reproduction batch were gathered roes using biopsy method and was calculated polarization coefficient of their nucleolus. In this way were chosen 3 individuals from each batch, all of them having polarization coefficient between 0.05-0.07. Experiment started when water temperature reached the value of 13°C, when were injected the females from first batch, after that at 15°C second batch and at 17°C for the third one. Injecting was realised in two rounds: first 20% from dose and second, after 12 hours, 80%. The utilised doses were of 0.12, 0.2 and 0.3 ml/kg female body. Maturation percent was of 100% at all those three females' batches differing, function of water temperature, time for maturation. So, at the same administrated dose with the increasing of water temperature is observed a decreasing of maturation time. Roes' quantity which was gathered was between 73-79 g/kg female body.

Key words: *Polyodon spathula,* artificial reproduction, Nerestin 5A, sturgeons

INTRODUCTION

Polyodon spathula breed belongs to Polyodontidae family, this one being the sole representative of the family in North America [5]. Sturgeons, from which belongs also polyodon, are considered living fossils, existing from Early Jurassic [1]. Very good growing rhythm, specific of nutrition (zoo-plankton), meat and roes quality were factors for acclimatization of breed in Romania, the general idea being that this breed to replace bighead carp (*Arystichtys nobilis*), which have the same nutrition spectrum but is inferior regarding meat quality [2] [7] [9].

Assuring of quality youth and from own resources in an aquaculture farm is a condition for realisation of an integrated management [6]. Fishes reproduction or multiplication is an ensemble of physiological processes which includes: gametogenesis, deposition and fecundation of roes, embryonic development and post-embryonic development through which is assured formation of new generation and breed perpetuation [8].

Determination of hormonal mixture represents the second great step in realization of artificial reproduction after selection of breeders and finding of females capable for reproduction through determination of nucleolus polarization coefficient.

MATERIAL AND METHOD

The studied biological material was represented by 49 breeders individuals from which were selected 9 females which were reared in the same environmental conditions at Production and Aquaculture Research Farm SC Acvares SRL, Ţigănaşi, Iaşi. Those 9 females were selected from a batch of 49 breeders with age of 13 years. In each year, breeders were reared in a pond with 30 ha

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The manuscript was received: 28.09.2015
Accepted for publication: 17.11.2015

and during winter were placed in a basin with 1 ha area. At the beginning of March when water temperature reached the value 8°C, breeders were separated on sexes and females were weighted and were established polarization coefficient for roes' nucleolus using biopsy method. In according with this method a probe is made by effectuating an incision in the abdominal wall utilising bistouries through which is introduced a special probe for gathering some roes [4]. Those ones are boiled and are sectioned on longitudinal axis, animal pole, vegetal pole. The resulted halves are examined at microscope determining roes' diameter and the distance from nucleolus' edge to vitelline membrane. Polarization coefficient is calculated by diving the distance from nucleolus to membrane. The values of 0.05-0.07 are considered optimal for staring of ovulation [2].

To reach the proposed goals, biological material which was studied in 2015 was divided in three experimental batches, each of them with 3 females, respectively LF1, LF2, LF3.When water temperature reached the value of 11°C females were moved inside the reproduction station and were place from some basins of 4.5 m^3. In each basin were placed 3 females. Basins were continuously supplied with water at a flow of 18 l/minute. Supply was realised from a decanter basin through a PVC pipe with a diameter of 50 mm. Water flow was adjusted so the dissolved oxygen from water not to be under 5 mg/l. When water reached at temperature of 13°C females from batch LF1 were injected with the first doses. The doses are 0.12, 0.2 and 0.3 ml/kg female body. The utilised hormone, Nerestin 5A, is a Russian synthesis product variant A being created especially for sturgeons. The utilised hormone was produced in 2014. Injections were made intramuscular with a plastic material of syringe 1 cm^3, at 7-9 cm in front of ventral fins and at 2-3 cm from median ventral line on either flanks [3]. Injection of second batch LF2 was realised when water temperature reached the value of 15°C and for batch LF3 when water temperature was 17°C. After injection was tracked the time for gonads' maturation, physiological state of breeders, reaction of females under influence of reproduction hormone.

RESULTS AND DISCUSSIONS

To establish and to optimize the doses of hormonal mixture Nerestin 5A were utilised 9 individual female breeders from breed *Polyodon spathula.* Biometric indexes of the females from each batch and values of polarization coefficient are presented in table 1.

Table 1 Biometrical indexes for studied females

Specification	LF1		LF2		LF3	
	$\bar{x} \pm s_{\bar{x}}$	V%	$\bar{x} \pm s_{\bar{x}}$	V%	$\bar{x} \pm s_{\bar{x}}$	V%
Weight of females (kg/ex)	15.83	3.86	14.03	8.32	14.16	14.14
Total length (cm)	135.67	2.36	131.33	4.95	133.00	4.51
Polarization coefficient of roes' nucleolus	0.05	20.38	0.06	16.67	0.06	18.23

After determination of polarization coefficient for roes' nucleolus, its mean value was 0.05 for batch LF1 and 0.06 for the other two batches, so, all the females are considered to be able for reproduction and optimal for ovulation starting. Variability inside batch LF1 was lower regarding weight and total length and higher related to polarization coefficient. Inside batch LF2 variability was lower.

Physical-chemical water parameters, during our research, are presented in table 2.

Table 2 Physical-chemical parameters of water during hormonal stimulation period

Date	pH	^{0}dGH	NH_4^+ mg/l	NO_{3-} mg/l	NO_2^- mg/l	Ca2+ mg/l	Mg2+ mg/l
28.04	7.2	8.29	0.06	0	0.64	36.4	14.2
7.05	7.5	7.98	0.05	0	0.62	35.1	12.3
11.05	7.4	8.14	0.07	0	0.56	33.5	11.8

During whole experimental period water pH didn't varied much being between 7.2-7.5, water hardness was between 7.98-8.29 German degrees, ammonium varied between 0.05-0.07 mg/l, nitrogen amount was 0 and nitrites amount varied between 0.56-0.64 mg/l, calcium amount varied between 33.5-36.4 mg/l and the magnesium one between 11.8-14.2 mg/l. All those values are in according with the limits cited in literature regarding physical-chemical parameters of water utilised in artificial reproduction of sturgeons.

During experiments water temperature varied very low during stimulation for all those three batches (figure 1). Supply with water was realized from an external basin, water temperature in this basin varied day-night with 3-5 degrees, and from this basin water flows into a decanter basin situated inside reproduction station.

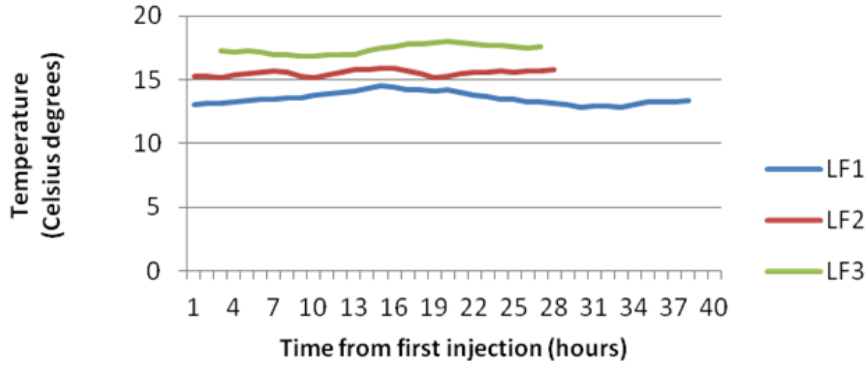

Figure 1 Variation of water temperature during experiments

Concentration of dissolved oxygen in water varied during experiments from a maximum value of 9.3 mg/l to a minimum value of 5.3 mg/l. At populating moment concentration in dissolved oxygen in the basins from all three batches varied around the value of 9 mg/l. During experiments dissolved oxygen decreased till the value of 5.3 mg/l even if water floe was increased. To keep the value for dissolved oxygen was utilised an ACO 006 type compressor.

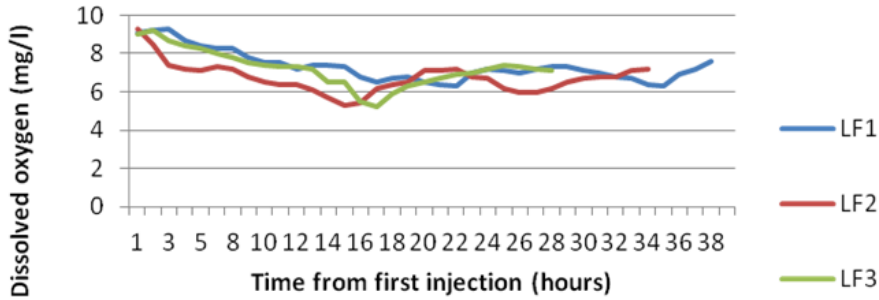

Figure 2 Variation of dissolved oxygen during research

When water temperature reached the value of 13°C, first injections were made. Function of females' weight from this first batch we established the total dose and were administrated 20% of it (table 3). After 12 hours from first dose was effectuated the second injection with the rest of 80% from dose. Necessary time for maturation was 38 hours for the first female stimulated with 0.12 ml/kg body, 38 hours for females stimulated with 0.2 ml/kg body and 34 hours for female stimulated with 0.3 ml/kg body.

Table 3 Hormonal doses administrated at first females' batch, when water temperature reached the value of 13°C

Batch and female number	Female weight (kg)	Administrated dose (ml/kg female body)	Total dose (ml/female)	1st dose (20% from total dose) (ml/kg female body)	2nd dose (80% from total dose) (ml/kg female body)	Necessary time for maturation (hours)
LF1, F1	16.5	0.12	1.98	0.39	1.59	38
LF1, F2	15.7	0.2	3.13	0.62	2.51	38
LF1, F3	15.3	0.3	4.58	0.91	3.67	34

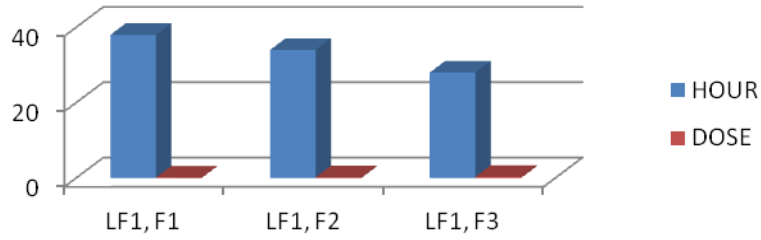

Figure 3 Necessary time for maturation function of hormonal dose utilised for batch LF1

When water temperature reached the value of 15°C were injected the females from the second batch. Like at the first batch, function of females' weight was established the total dose and was administrated 20% from it (table 4). After 12 hours from first dose was effectuated the second injection with the rest of 80% from dose. Necessary time for maturation was 30 hours for the first female stimulated with 0.12 ml/kg body, 29 hours for females stimulated with 0.2 ml/kg body and 24 hours for female stimulated with 0.3 ml/kg body.

Table 4 Hormonal doses administrated at second females' batch, when water temperature reached the value of 15°C

Batch and female number	Female weight (kg)	Administrated dose (ml/kg female body)	Total dose (ml/female)	1st dose (20% from total dose) (ml/kg female body)	2nd dose (80% from total dose) (ml/kg female body)	Necessary time for maturation (hours)
LF2, F1	13.8	0.12	1.65	0.33	1.32	30
LF2, F2	13	0.2	2.60	0.52	2.08	29
LF2, F3	15.3	0.3	4.58	0.91	3.67	24

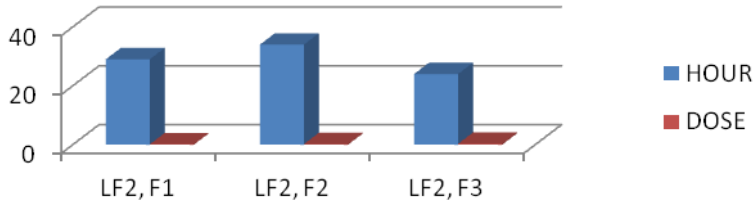

Figure 4 Necessary time for maturation function of hormonal dose utilised for batch LF2

When water temperature reached the value of 17°C were injected the females from the third batch. Like at previous batches, function of females' weight was established the total dose and was administrated 20% from it (table 5). After 12 hours from first dose was effectuated the second injection with the rest of 80% from dose. Necessary time for maturation was 26 hours for the first female stimulated with 0.12 ml/kg body, 22 hours for females stimulated with 0.2 ml/kg body and 22 hours for female stimulated with 0.3 ml/kg body.

Table 5 Hormonal doses administrated at third females' batch, when water temperature reached the value of 17°C

Batch and female number	Female weight (kg)	Administrated dose (ml/kg female body)	Total dose (ml/female)	1st dose (20% from total dose) (ml/kg female body)	2nd dose (80% from total dose) (ml/kg female body)	Necessary time for maturation (hours)
LF3, F1	12.1	0.12	1.45	0.29	1.16	26
LF3, F2	14.3	0.2	2.85	0.57	2.28	22
LF3, F3	16.1	0.3	4.82	0.96	3.86	22

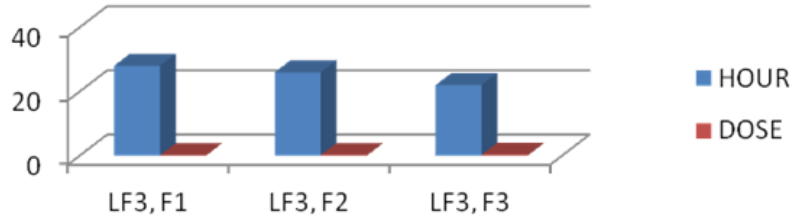

Figure 5 Necessary time for maturation function of hormonal dose utilised for batch LF3

Table 6 Statistical interpretation regarding necessary time for maturation, maturation percentage and gathered roes' yield function of the administrated doses between those three studied batches

Specification	Batch	*n*	$\overline{x} \pm s_{\overline{x}}$	*V%*	*Min.(%)*	*Max.(%)*
Necessary time for maturation (hours)	LF1	3	36.66	6.3	34	38
	LF2	3	27.66	11.66	24	30
	LF3	3	23.33	9.3	22	26
	Statistical interpretation	LF1 vs. LF2 vs. LF3 = ***; F(14.3130) > Fα(3.4668)				
Maturation percentage (%)	LF1	3	100	0	100	100
	LF2	3	100	0	100	100
	LF3	3	100	0	100	100
	Statistical interpretation	LF1 vs. LF2 vs. LF3 = n.s.; F(0.0000) <Fα(5.1432)				
Roes' gathered yield (g)	LF1	3	77	2,6	75	79
	LF2	3	75	3.53	73	78
	LF3	3	76.66	2.72	75	79
	Statistical interpretation	LF1 vs. LF2 vs. LF3 = n.s.; F(0.6739) <Fα(5.1432)				

From statistical point of view exist distinct significant differences regarding necessary time for maturation function of water temperature, so, at temperature of 13°C being necessary 36.66 hours, at temperature of 15°C, 27.66 hours and at temperature of 17°C, 23.33 hours. Maturation percent was 100% at those three females' batches, and weren't observed statistical differences between them. Roes' gathered quantity wasn't influenced by water temperature neither by the utilised hormonal dose.

Inside batches could be observed that utilised hormonal doses don't have a significant influence on necessary time for roes' maturation, maturation process and nor on roes' gathered quantity, being possible to use a smaller quantity of hormone for reaching the same goal.

CONCLUSIONS

The utilised hormone (Nerestin 5A) had very good results in hormonal stimulation of females belonging to *Polyodon spathula* breed for all those three values of water temperature. Water temperature influence the necessary maturation time, increasing being linear, so, for the same administrated dose at the same time with water temperature increasing could be noticed a decreasing of necessary time for maturation. Hormonal doses utilised correlated with water temperature didn't influence roes' gathered quantity.

Hormone optimal dose was 0.12 ml/kg female body.

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