

Researches Regarding Artificial Reproduction Technologies to *Polyodon spathula* from C.C.D.P. Nucet

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Abstract. Artificial reproduction of *Polyodon spathula* (Walbaum 1792) species is final step for successfully acclimatization to a specifically environment conditions. After reproduction samples formation, those are injected with carp hypophysis, sturgeon hypophysis, LHR Ha, Nerestin 5A and HCG-Chorulon. The roe sampling was made by MIST method and for semen was used specifically syringes. After fecundation and dissolution, 1.6 kg roe were incubation in special incubators. During the incubation was determinate the embryonic percent and was applied the specific antibacterial and antifungicidal treatments. After eclosion, hatchlings were parked 10 days in Nucet incubators and 250 l carafes. The best results for gonads stimulation were obtained at Nerestin (95-100%) and LHR Ha (80-100%). The experience was made in 6 years, 2002-2007 period. Consonant with obtained results in 6 years we appreciate that acclimatization of *Polyodon spathula* (Walbaum 1792) species was a success. Was demonstrated that can realized artificial reproduction, obtain viable products and were perfected the main steps for technological process.

Keywords: *Polyodon spathula*, gonads, hormones, artificial reproduction

INTRODUCTION

Polyodon spathula species have some biological characteristics like: quickly growth rhythm, vegetarian diet, high adaptability to live and growth in captivity and different environmental conditions, moreover quality of meat and roe make this species to enter in attention of aquaculture specialist from entire world.

In Romania, the interest for introduction and acclimatization *Poyodon spathula* species existed from middle of '80 years, the first hatchlings lot was come in country in 1992, when was initiated acclimatization and introduction to culture for this species.

During 1992-1999 period, C.C.D.P. Nucet imported 2000-10000 hatchlings/year. Between years 1992 and 2002, were realized researches concerning growing to different ages in pond conditions, gonads development and feed of different ages, behavior and growth rhythm in different technological variants of *Polyodon spathula*.

First artificial reproduction was realized in 2002 and during 2002-2007 period were developed frequent experiences based on were elaborated and underlied artificial reproduction technologies and after-embryonic development technologies to *Polyodon spathula*, in Romania ecological and aquaculture technologies conditions. Material dedicated to population obtained by artificial reproduction (beginning to 2002 year) exists in many fish farms, today in all our country.

MATERIALS AND METHODS

For elaborate and underlie the artificial reproduction technologies for *Polyodon spathula* (Walbaum 1792), during 2002-2007 period were realized 5-6 artificial reproduction experiences in each year which have like technical and scientific objectives the following: establish the optimum moment to hormonal stimulation taking to account climatic conditions from Nucet, establish the optimum temperature interval for reproduction realization; establish females matured stage when hormonal stimulation have an optimal effect; establish the optimum schedule to *Polyodon spathula* females stimulation; comparative testing of commercial hormones substances; establish the sampling methods for semen products.

To March beginning, from single out reproduce lots, were confined 50-100 individual reproductive which are introduced in before-maturation basins, separated by sexes (Tab. 1). The exemplars number introduced in before-maturation basins was bigger than individual's reproductive number because some females can give small roe quantities or no.

Tab. 1

The individual reproductive situation of *Polyodon spathula* selected for artificial reproduction during 2002-2007 period (BP 2)

R. no.	Year	Females			Males			Total			
		Ex.	Kg	g/ex	Ex.	Kg	g/ex	Ex.	Ex/ha	Kg	Kg/ha
1	2002	25	311	12145	25	219	8765	50	100	530	1060
2	2003	35	435	12437	35	309	8847	70	140	744	1488
3	2004	42	527	12563	35	312	8923	77	154	839	1676
4	2005	44	557	12674	42	375	8942	86	172	932	1864
5	2006	47	608	12936	45	410	9126	92	184	1018	2036
6	2007	61	828	13577	45	417	9275	107	214	1245	2490

Depended to temperature evolution, in each year were realized 5-6 experiences set. In each experience were 4-5 females and 4-5 males used to reproduction.

For maturation stimulation and ovulation to *Polyodon spathula* species were used both origin animal hormones (carp hypophysis, sturgeon hypophysis) and synthesis hormones (HCG–Chorulon, LHRH, Nerestin 5 A). The doses establishment and administrations were based on American's literature dates (Graham et al., 1986; Shelton et al., 1997; Mims, 2001) și experiența cercetătorilor ruși (Vinogradov et al., 1987; Vedrasco et al., 2001) (Tab. 2).

Tab. 2

Exogenous hormonal dosed used to artificial reproduction to *Polyodon spathula* species

Hormone used	Females (total dose)	Males
Carp hypophysis (mg/ body kg)	2,0 – 6,5	2,0
Sturgeon hypophysis (mg/ body kg)	6,0 – 8,0	3,0 – 4,0
LHRH (mg / body kg)	0,1	0,05
Nerestin 5A (ml / body kg)	0,12 – 0,30	0,1
HCG – Chorulon (UI / body kg)	500 – 1100	300-500

The administrate hormones quantity, shooting and stimulation optimal moment conduits were realized by biometric characteristics (individual weight, especially), polarization index value and water temperature evolution (Tab. 3).

Tab. 3

Average values of main biometric and biological indexes of reproductive lots used to reproduction during 2002-2007 period

R. no.	Year	Females				Males		
		W (g)	Ls (cm)	C (cm)	Polarizing index	W (kg)	Ls (cm)	C (cm)
1	2002	12145	144	56	0,06	8765	134	42
2	2003	12437	144	57	0,07	8847	133	42
3	2004	12563	145	55	0,05	8923	135	43
4	2005	12674	145	56	0,07	8942	135	43
5	2006	12936	144	56	0,05	9126	136	44
6	2007	13577	146	58	0,04	9275	136	43

Note: W – weight; Ls –standard length; C – body circumference

After protocol realization and individual's reproductive injection were determinate: roe quantity (total, /♀, /kg), fecundation percent, hatching percent, number of hatchlings and their survival percent, also.

RESULTS AND DISCUSSION

The artificial reproduction of *Polyodon spathula* species was tried still '60 years of last century (Purkett, 1961, 1963 a, b; Needham, 1965), but a real artificial reproduction technologies was made by American researchers to beginning of '80 years (Russel, 1982).

The experience results made in 6 years to C.C.D.P. Nucet confirms dates to specialty literature (Vinogradov et al., 1987; Alexandrovna, 1989; Vedralco et al., 2001) that is to say hormonal stimulation is realized in optimal conditions for 0.05-0.07 polarization index value.

Corroborating the dates regarding the polarization index, obtained to *Polyodon spathula*'s artificial reproduction during 2002-2007 period with existing dates from specialist literature (Alexandrovna, 1989), was elaborated the hormones stimulating optimal schedule according to polarization index (Tab. 4).

Tab. 4

The optimal schedule of hormone stimulation to *Polyodon spathula*' females (Walbaum 1792) (Costache and Vedralco, 2002-2007)

Polarization index	Optimizing measures
0,30-0,35	The ovocytes are in IV mature incomplete stage The roe are immature The females are inapt to ovulate by hormonal stimulation
0,15-0,20	The ovocytes are in IV mature complete stage The females are apt to ovulate by hormonal stimulation administrate in 3 doses, with interval aggrandizement between injections
0,05-0,07	The ovocytes are in IV mature complete stage Optimal moment for reproduction Hormonal stimulation in 2 doses; time interval between preparatory and conclusive dose is 12 hours
0,01-0,02	The ovocytes are in IV mature complete stage The first symptoms of resorption process appear The females are apt to ovulate Hormonal stimulation by single injection with hormone dose reduction
Nucleus is blasted	Over mature ovocytes The females are inapt to artificial reproduction

The main influence factor about *Polyodon spathula*'s artificial reproduction was water temperature in incubation station.

The artificial reproduction experiences were initiated when the water temperature was established to 10⁰C. The artificial reproduction experience results indicate than optimal period of *Polyodon spathula*'s reproduction at Nucet condition is from March ending - April beginning to April last decade. From last climatically condition point of view, exist a concentrate trend of optimal period to 10-15 days interval and for this reason it necessary to assure parking and incubation capacities in such a way as to process bigger lots. Contrariwise appear the danger to loose the optimal period of roe's suction.

The days-degrees number accumulated between two successive reproductions is about 5000. In last 3 years, as global heat consequence, this number is about 5300 (Tab. 5).

Tab. 5

The days-degrees number accumulated till start artificial reproduction studies during 2002-2007 period

R. no.	Year	Days-degrees	Date
1	2002	5093,17	April, 10
2	2003	4894,95	April, 7
3	2004	5142,72	March, 30
4	2005	5047,30	April, 4
5	2006	5376,59	April, 3
6	2007	5303,81	April, 5

Females mature period was 43-52 hours at 8.5-10⁰C and 28-32 hours at 16.5-18.5⁰C (Tab. 6).

During first experience were recorded temperature values from 8.5-10⁰C followed by suddenly heating this has led to "condensation" of reproduction optimal period and a part of females goes to over mature and ovocytes resorption stage.

On the other hand, procurement of viable roe at 8.5⁰C (first lot) and their relative successfully incubation at 9-12⁰C is an absolute novelty according to literature dates.

Tab. 6

Polyodon spathula 's females mature period based on temperature

R. no.	Water temperature (°C)	Mature period (hours)			
		Nerestin	LHRHa	HCG	Sturgeon hypophysis
1	8.5 – 10,5	43	46	52	44
2	10,5 – 12,5	38	40	44	41
3	12,5- 14,5	36	38	41	37
4	14,5 – 16,5	33	35	36	35
5	16,5 – 18,5	28	30	32	32

Incubation process period and hatching percent are also influenced by water temperature from incubation station. The best results were obtained during 12-18⁰C interval; the lots incubate to higher temperature were given a negative evolution, massive mortality after 72 incubation hours. This phenomenon is partial given by roe and semen qualities, because in specialist literature are some cases about embryogenesis disturb at above 18⁰C temperature.

First lot at which incubation start at 8.5⁰C and run 10 days was observed a powerful disparting at embryonic development stages; a low synchronize of hatching – are necessary 72 hours to complete hatching.

This fact have impact about after embryonic development, was obtained a heterogenic hatchling lot which have a large size difference between saplings from same female and with negative impact about hatchlings development (cannibalism is favored).

At lots incubated in normal temperature interval (12-18⁰C) the hatching synchronize was better obtaining homogeny lots and this reflected in hatchlings ulterior development.

The five hormones tested results (LHRHa, Nerestin 5A, carp hypophysis, sturgeon hypophysis and HCG) confirm literature dates concerning LHRHa, respectively the efficiency Nerestin 5A (which is probably a derivate of LHRH) use (Tab. 7).

Tab. 7

The main technological indexes obtained to *Polyodon spathula* artificial reproduction during 2002-2007 periods

Year	Used hormone	Maturate percent (%)	Roe total (kg)	Roe/ maturate ♀ (g)	Roe/ maturate ♀ kg (g)	Fecundation percent (%)	Hatching percent (%)	5-6 days old hatchlings/ maturate ♀ (thousands)	5-6 days old hatchlings/ maturate ♀ kg (thousands)	Survival percent (%)
2002	Nerestin	100	14,8	987	81	81,2	75,1	62,5	5,17	91,3
	LHRHa	100	4,2	840	69	78,3	75,4	50,0	4,11	88,5
2003	Nerestin	95,0	18,9	994	80	84,6	75,8	64,7	5,22	90,7
	LHRHa	87,5	6,4	914	73	75,5	73,2	52,3	4,21	89,4
	Sturgeon hypophisi s	75 0	3,16	790	85	71,3	68,4	48,9	3,93	75,6
	HCG	25,0	0,4	40	33	27,2	31,3	2,0	0,17	47,4
2004	Nerestin	95,0	20,3	1068	84	83,9	75,6	73,7	5,87	93,1
	LHRHa	87,5	6,1	871	69	76,4	74,1	51,8	4,12	87,6
	Sturgeon hypophisi s	83,3	4,21	842	67	70,8	66,7	37,0	2,94	76,3
	HCG	33,3	0,9	45	37	25,9	29,8	2,0	0,16	45,1
2005	Nerestin	100	19,4	970	76	82,8	76,1	68,5	5,40	94,3
	LHRHa	80,0	7,3	912	72	74,6	72,3	50,0	3,94	85,4
2006	Nerestin	90,0	19,5	1083	84	83,4	72,7	70,5	5,45	92,6
	LHRHa	95,0	8,4	933	72	72,3	70,1	46,5	3,59	81,2
2007	Nerestin	90,0	19,8	1042	77	86,7	74,3	72,4	5,76	93,9
	LHRHa	90,0	8,5	944	69	75,8	73,6	50,9	3,75	84,7

Regarding use carp hypophysis which is tested efficacy in 2002, the react was negative because have obtained roe from neither female. The experiences weren't continued because the carp hypophysis isn't available on romaine market.

In table 7 is observed that at female's hormone stimulating with LHRHa (during 2002-2007 period) females maturing percent is between 87.5% and 100%; was obtained an average roe quantity of 840-944 g/roe/female and 69-73 g roe/ maturate female kg, with a fecundation percent of 72.3-78.3%. According to water temperature the incubation was between 6 and 12 days and hatching percents were between 70.1 and 75.1%.

At hormonal stimulation by Nerestin 5A, tested during 2002-2007 periods, females maturate percent was 90-100%; was obtained a quantity of 970-1083 g roe/female, 77-84 g roe/ maturate female kg, respectively. Fecundation percent was 81.2-86.7% and hatching percent was 72.7-76.1%.

Individual's reproductive of *Polyodon spathula* stimulating experiences by hypophysis from other sturgeon species were developed in 2003 and 2004. The females maturate percent was 75-83%; was obtained a quantity of 790-842 g roe/maturate female and 85-86 g roe/maturate female kg. Fecundation percent was 70.8-71.3% and hatching percent was 66.7-68.4%. Though the results were positive, the experiences weren't continued because the sturgeon hypophysis is difficult to procure.

Human corionic gonadotropine testing HCG at *Polyodon spathula*'s female hormones stimulation had small results. Were realized two experience sets (in 2003 and 2004). In 2003 were used 4 females and in 2004, 6 females. The females injected in 2003, a single one given 400 g roe. The fecundation percent was 27.2% and hatching percent was 31.3%

In 2004 of the 6 females injected only two given roe in diminished quantity (450g/female). The fecundation percent was 25.9% and hatching percent was 29.8%

The *Polyodon spathula*'s female hormones stimulation by HCG-Chorulon weren't repeated because had small results but also high price of this hormone and his unavailability. Comparative analyzes of biotechnological indexes regarding *Polyodon spathula*'s artificial reproduction process during 2002-2007 period, it can observed that all hormones tested had positive results and reliable, excepted carp hypophysis and Chorulon. Hormone selection will be determinate by price/quality ratio (LHRHa it's more expensive) and also market availability.

CONCLUSIONS

Artificial reproductive experiences realized during 2002-2007 period goes to following conclusions:

- *Polyodon spathula* species can be artificial reproduced starting to 8-8.5⁰C when is possible to make hormonal stimulation and roe sampling;
- the longer interval of embryonic development (10 days) is a reason for inadvisable the roe incubation at temperature under 12⁰C ;
- the optimal temperature interval for artificial reproduction is between 12 and 16⁰C when incubation period run 6-7 days and hatching is relative sinchrone.
- all hormones tested had positive results and reliable, excepted carp hypophysis and Chorulon. Hormone selection will be determinate by price/quality ratio (LHRHa it's more expensive) and also market availability.
- is necessary to assure thermostated of water used to incubation, at least for 0.5 l/s flow (for 5 incubating basins). During the *Polyodon spathula* reproduction period the sudden thermal variations are a common phenomenon and they can compromise for total the incubation process.

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