THE CORRELATION BETWEEN THE POLARIZATION INDEX AND THE DEGREE OF OOCYTE MATURATION IN THE SIBERIAN STURGEON *ACIPENSER BAERII* (J.F. BRANDT, 1869)

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Abstract

The need to discover new food sources due to the continuous growth of the world's population is a challenge for today's aquaculture, which is looking for new non-native fish species with high productivity, destined to be grown in recirculating systems. Siberian sturgeon in the culture systems is artificially reproduced by injecting gonadotropic hormone, dosed according to the degree of oocyte maturation which is determined with increased precision by using the method of biopsic puncture.

Depending on the migration of the germinal vesicle inside the fish egg and the water temperature, the degree of oocyte maturation can be determined to achieve a hormonal injection scheme for obtaining the maximum amount of collected eggs for each female. This experiment indicates how to correlate the polarization index with the maturation time of females at different water temperatures, for the elaboration of the injection scheme with different percentage doses of hormones, in order to obtain the maximum number of eggs during the artificial breeding of Siberian sturgeon.

Key words: Siberian sturgeon, polarization index, hormonal stimulation

INTRODUCTION

Sturgeon culture has developed significantly in recent decades both internationally and nationally. Due to the growing market demand determined by the increasing consumption of sturgeon meat and caviar [3], aquaculture is facing the need to raise and reproduce sturgeon species with high productivity.

For the reproduction of sturgeons in captivity , in the absence of the natural and seasonal hypothalamic stimuli that influence the migration periods (water temperature, presence of substrate for spawning, etc.), for artificial reproduction it is necessary to assess the maturation of the female gonad in order to establish the hormonal injection scheme.

In cultured sturgeons, the ovulation is influenced by water temperature, the photoperiod, the stress induced by handling or the transport of broodstock, the protocols for inducing maturation by exogenous hormonal intake, the genetics of the broodstock batch, etc

The Siberian sturgeon *Acipenser baerii* (J.F. Brandt, 1869) is one of the species of domesticated sturgeon, widely raised in recirculating aquaculture systems, whose growth parameters in captivity are better than in the natural environment and reach age of sexual maturation earlier, at 6 years for male and 7 years for female, in the temperate climates [1]. For artificial reproduction, a correct assessment of the stage of individual maturation of the specimens is necessary ever since the selection of the broodstock batch and subsequently it is necessary to determine the degree of oocyte maturation [2] on which the evaluation of the optimal amount of injectable hormone is based and influences the quantity and quality of the eggs obtained per female [9].

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This experiment aims to demonstrate how the use of the polarization coefficient of oocytes influences the hormonal injection scheme of females and determines the amount of eggs obtained from the artificial reproduction of Siberian sturgeon [8].

The degree of oocyte maturation is determined by the polarization coefficient according to which, the total amount of gonadotropic hormone per kilogram of female body is calculated the injectable hormone quantity administered in two doses and their administration interval, correlated with the physiological stage of maturation for each tested female. Failure to correlate the degree of polarization with the need for hormonal addition [10] may determine the lack of ovulation, or when it is present but oocyte maturation is not appropriate, can lead to low egg fertility or very low embryonic survival.

The most accurate and least expensive method for assessing the degree of oocyte maturation according to which the amount of injectable hormone for each female is calculated is the biopsy puncture method [4]. This method consists in extracting several eggs from the ovaries of the females with the help of a cannula. The eggs extracted from the puncture can be examined by assessing their size and appearance and after boiling and sectioning, the distance from the position of the germinal vesicle, in the process of its migration through the cytoplasm to the animal pole, is measured and related to the diameter of the egg, resulting a subunitary ratio which represents the polarization index of the fish egg [7].

MATERIAL AND METHOD

The experiment took place within the recirculating system of the Research-Development Institute for Aquatic Ecology, Fisheries and Aquaculture Galati between 11-18.02.2021 and the recirculating system Sturgeon Farm Dâmbovița County between 02-09.03.2021, on the species Siberian sturgeon *Acipenser baerii* (J.F. Brandt, 1869).

Tests were performed at different water temperatures depending on the location and the recirculating system, at I.C.D.E.A.P.A. Galați, the maturation of the females took place indoors, the females were matured in pools at a temperature of 19^0C for 2-7 days, and at Sturgeon Farm the maturation of the females in recirculating system was achieved at 16^0C water temperatures for 4-7 days. The fish broodstock was divided into three lots, a batch of four mature Siberian sturgeon females were used to calculate the polarization index based on which the individual injection strategy with hormone extract was established (used in the experiments of artificial reproduction of Siberian sturgeon in Galați) and four females which consisted of two batches with two females each, according to the value of the polarization coefficient, were injected with different doses of hormones which were administered at different time intervals in the Sturgeon Farm batch (Table 1). To calculate the mean polarization index, an average of four oocytes were taken for each biopsy per each female.

The materials used for the gonad biopsy puncture consists of a cannula, Petri dishes, a razor blade, scalpel, a container and a heat source for boiling fish eggs and Betadine for disinfection [5]. The sturgeon females were previously anesthetized with a dose of 0.1 ml x l^{-1} of clove oil (eugenol 4-allyl-2-mathoxyphenol -C10H12O2).

To obtain a sample of eggs, a small incision (6 mm), was made at approximately 3 to 5 ventral scutes anterior from the pelvic fin, using a scalpel and with the cannula was extracted a few eggs.

The measurements made to calculate the polarization index were performed on the stereomicroscope Olympus SZ 61 equipped with a SC 50 video camera.

Hormonal stimulation was performed with Nerestin A in total doses of 0.2125 ml hormone extract / kg body, for N1, N2, N3, N4 and to which was added a standard quantity of 10% from the total amount of the calculated hormone and 0,24 ml hormone extract / kg body (with 10% inclusiv) for N5, N6, N7, N8 [8]. The eggs were collected by repeated abdominal massage every two hours, weighed in portions and the final egg mass was reported in table no.2. Statistical data processing was performed using the Microsoft Excel program.

RESULTS AND DISCUSSIONS

The polarization index for N1, N2, N3, N4 females reproduced in the I.C.D.E.A.P.A. Galați at the water temperature of 19^0C is presented in photographs 1, 2, 3, 4, and the polarization index for the females N5, N6, N7, N8 reproduced within the Sturgeon Farm Dâmbovița recirculating system at the water temperature of 16^0C is represented in the photographs 5, 6, 7, 8.

Photo 1-N1, $c_{p.}$=0,18 (t=19^0C)

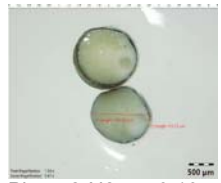

Photo 2-N2, $c_{p.}$=0,10 (t=19^0C)

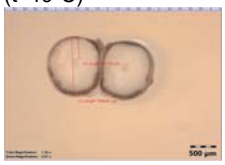

Photo 3-N3, $c_{p.}$= 0,36 (t=19^0C)

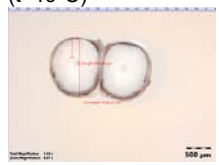

Photo 4-N4, c_p= 0,34 (t_r=19^0C)

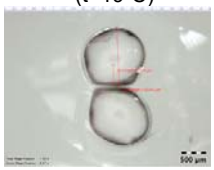

Photo 5-N5, $c_{p.}$=0,35 (t=16^0C)

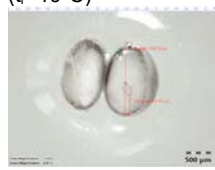

Photo 6-N6, $c_{p.}$=0,28 (t=16^0C)

Photo 7-N7, $c_{p.}$=0,24 (t=16^0C)

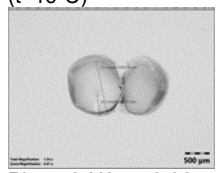

Photo 8-N8, $_{p.}$=0,36 (t=16^0C)

Notification: the polarization coefficient used as a reference was chosen the one with the closest value to the average.

The broodstock females reached the degree of oocyte maturation stage IV c, with a polarization index c = 0.05-0.07 are suitable for reproduction [9], the onset of the ovulation process is optimal, and the obtaining of eggs has maximum values. Females with a higher polarization index are immature and were reevaluated after a period of time from two to seven days, depending on the polarization coefficient obtained and the water temperature.

According on the degree of oocyte maturation, females N3, N4, N5 and N8 in stage IV a were reevaluated after seven days of maturation, females N6 and N7 in stage IV b, due to the water temperature of 16^0C, required four more days of maturation for achieving the polarization index of 0.08. Females N1 and N2 were injected after two days of maturation in water of 19^0C temperature.

Injections for the hormonal stimulation were performed at 24 h intervals for both experiments [10]. The table below shows the body mass and total dose of hormone established for each female, the injection scheme for the percentage distribution of hormones in the two injections and the amount of eggs obtained from each female at different water temperatures.

Table no. 1 The relationship between body weight, hormonal stimulation and the amount of collected eggs

No.	B. W. (kg)	Pol. Ind.	Hormon total dose (ml)	I1 (%)	I2 (%)	T. (^{0}C)	Collected eggs / female (kg)
N1	6.6	0.18	1.54	20	80	19	0.85±0.2
N2	6.6	0.10	1.54	20	80	19	0.87±0.2
N3	8.3	0.36	1.58	20	80	19	0.70±0.3
N4	6.7	0.34	1.26	20	80	19	0.53±0.2
N5	8.3	0.35	2	40	60	16	0.99±0.2
N6	13.6	0.28	3.3	40	60	16	1.18±0.4
N7	6.4	0.24	1.54	20	80	16	0.64±0.2
N8	7.3	0.36	1.75	20	80	16	0.58±0.3

Note: N= Siberian sturgeon; I1-the first injection; I2-the second injection

From the data presented in table no. 2 it is observed that the degree of oocyte maturation and the total dose of injectable hormone vary inversely with water temperatures. Analyzing the amount of hormone administered in the two injections, performed at an interval of 24 hours, it is observed that from females N5 (c = 0.35) and N6 (c=0.28) a higher amount of eggs was obtained due to the splitting of the total injectable dose in two doses of 40%

and 60%, respectively, compared to females N7 (c = 0.24) and N8 (c = 0.36) in which the two doses of injectable hormone were 20% and 80%, at water with 16^{0}C temperature.

For females N1 (c = 0.18), N2 (c = 0.1), N3 (c = 0.36), N4 (c = 0.34), the total dose for hormonal stimulation was divided into 20% for the first injection and 80% for the second one, under 19^{0}C water temperature conditions (chart no1).

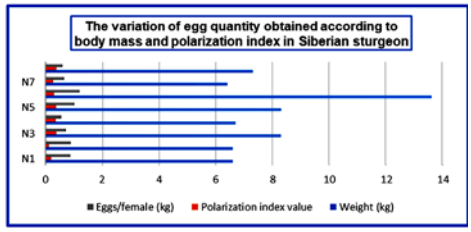

Chart no 1 Variation of eggs obtained according to body mass and polarization index in Siberian Sturgeon

The percentage ratio of the number of eggs harvested from each female to the individual mass varies from 7.95% for N8 to 13.2% for N2, according to Table 2.

Table no. 2 The percent of collected eggs per female

Broodstock specimens	Body weight (kg)	Collected eggs/female (kg)	Collected eggs /body weight (%)
N1	6.6	0.858	13
N2	6.6	0.871	**13.20**
N3	8.3	0.703	8.47
N4	6.7	0.536	**8.00**
N5	8.31	0.997	12.00
N6	13.61	1.184	8.70
N7	6.41	0.641	10.00
N8	7.31	0.581	7.95

The maximum percentage of eggs obtained, in relation to the body mass of the broodstock is 13.20% for the female N2 and the minimum of 8% for the female N4 at a water temperature of 19^{0}C in the variant of injection with a ratio I1: I2 of 20%: 80%. There is also the female N4 with a very good percentage - 12% obtained at a water temperature of 16^{0}C, due to the modification of the injection scheme (depending on the lower water temperature and the initial polarization index of c = 0.34) by the dose ratio I1: I2 of 40%: 60%.

CONCLUSIONS

1-After performing the biopsy puncture in Siberian sturgeon females and calculating the individual polarization index, its value must be directly proportional to the variation of maturation time and the value of water temperature, to obtain good ovulation results.

2-The individual hormonal stimulation scheme presents values that vary directly proportional to the polarization coefficient and the water temperature.

3-The amount of eggs collected and the efficiency of artificial reproduction of Siberian sturgeon is inversely dependent on the polarization coefficient and directly proportional to the maturation temperature of the broodstock, being strictly dependent on the precision of the hormonal stimulation scheme, which is based on the correct assessment of oocyte maturation.

4-It is recommended to resume the experiments with the use of an increased number of broodstock to provide sufficient data for a statistical analysis for increasing the predictability on the hormonal stimulation scheme acording to the polarization index value, but especially related to the amount of eggs obtained in artificial reproduction of Siberian sturgeon.

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