COMPARATIVE STUDY ON THE USE OF VARIOUS HORMONAL PREPARATIONS (CARP PITUITARY, NERESTIN 6A) IN THE ARTIFICIAL REPRODUCTION OF *PERCA FLUVIATILIS*

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Abstract

The objective of this experiment was to test the use of hormonal preparations, which are commonly used in the artificial reproduction of various fish species, on Perca fluviatilis in order to develop and optimize an artificial reproductive technology under controlled conditions. The hormonal preparations used in the experiment were carp hypophysis and Nerestin 6A, and the biological material consisted of 20 females and 6 males.

The females were divided into two lots: (L1) carp hypophysis (4 mg / kg female body weight) and (L2) Nerestin 5A (0.25 ml / kg females body weight). Hormonal stimulation was done by intraperitoneal syringe administration for the carp hypophysis and intramuscular injection for Nerestin 5A, both in two divided doses as follows: 20% of the total dose in the first injection and 80% in the second.

Males did not require hormonal stimulation. The average body weight of females was 399.1 ± 9.66 g (L1) and 425.10 ± 25.77 (L2).

The average latency time between the first injection and the ovulation time was 121.43 ± 10.87 hours in L1 and 84.71 ± 6.12 hours in the case of L2. The mean value of the relative fecundity index was 209±66.5 $x10^3$ eggs/kg in L1 and 273.1±34.5 $x10^3$ eggs/kg in L2. The mean value of the absolute fecundity index was 87.2±23.4 $x10^3$ eggs/ female in L1 and 109±11.1 $x10^3$ eggs /female in L2. The fertility rate had average values of 87.80 ± 2.63 for group L1 and 91.14 ± 2.87 for L2. The survival of females during the experiment was 80% in the L1 group, 90% in the L2 group and 100% in the males. The hatching rate averaged 36% in the first group (L1) and 52% in the second group (L2). Significant statistical differences between the two groups were recorded for the amount of harvested eggs and latency time, these indicators being higher for the L2 group, while insignificant differences were recorded for the other indicators.

The results have shown that artificial reproduction is possible under controlled conditions and can be used to develop aquaculture of the species

Key words: artificial reproduction, perch, nerestin 5A, carp pituitary

INTRODUCTION

For a long period, perch (*Perca fluviatilis*) was considered an invasive species with low economic value. The cultivation of this species is in focus in the last years due to the good quality of the bone free white meat, very highly valued in developed countries.

These strengths led to study of different aquaculture system for growing the perch, in the countries like France, Belgium, Sweden, Denmark, the Czech Republic and Italy.

However, there are also some weaknesses, represented by the difficulty of scraping the scales and by the relatively small dimensions of the mature perches.

The reproduction of the perch in a controlled environment was intensively studied. It was tried the artificial reproduction using different hormones which stimulates sexual maturation but also a semi-artificial reproduction technology that supposed to make reproduction in a controlled environment, without hormonal stimulation [1], [4], [9], [14].

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Studies related to oocyte maturation stages have helped in standardize some doses of hormonal preparations and have made possible the ovulation synchronization [21], [22].

The hormonal preparations identified by undertaken studies so far are: carp pituitary, Ovopel, Ovaprim, Supergestran, Choluron, Gonazon [10], [14].

A very important stage in the application of artificial breeding technology to the perch is represented by the anesthesia of the breeders, the results being poor without[5].

Another important step in the development of this aquaculture species was the growth of larvae. Researchers have succeeded in feeding them with commercial feeds [6].

Various studies have shown that the species can be reproduced in the extra season, a monosex population can be obtained and the reproduction is also possible using steroid hormones [1], [13], [17].

MATERIAL AND METHOD

The aim of the study was testing hormonal preparations like carp pituitary and Nerestin 6A in the artificial reproduction of *Perca fluviatilis*.

The biological material consisted of 20 females and 6 European perch (*Perca fluviatilis*) 3 years old and an average weight of 412.10 ± 13.72 g / specimen for females and 107.5 ± 6.36 in males. They were divided into lots (L1 using carp pituitary, L2 using Nerestin 6A) each lot consisting of 10 females and 3 males. The breeders were raised under the same environmental conditions at the Research and Development Center for Aquaculture and Aquatic Ecology in land basins, as an additional species used in populations formulas where carp *(Cyprinus carpio)* was the dominant species.

When the water temperature in the land basins reached 9°C the breeders were caught and moved separately by gender into maturation tanks located within the artificial breeding station. Maturation tanks have a useful volume of 0.78 cubic meters and are part of the recirculating system of the Research-Development Center for Aquaculture and Aquatic Ecology, Ezareni, Iasi. The recirculating system consists of a mechanical filter with roller, biological `filter and UV filter, pumps, pipes, air blowers, technical oxygen supply system (in the case of adamege) and growth tanks.

After a period of 10 days of accommodation inside the artificial breeding station and at a time when the water temperature was on a rising trend of more than 14°C, the females were hormonally stimulated to maturate and harvest the sexual products. Before hormonal stimulation, in order to reduce the stress caused by the manipulation of the breeders, the females were anesthetized. Anesthesia was done using the solution of cloves oil [5]. It was composed of 1 part cloves oil and 10 parts ethyl alcohol (91%). The dose used was 4 ml of solution per 10 liters of water. The bathing time was 3 minutes, being enough for a female to be totally anesthetized. In group L1, the hormonal simulation was done with carp pituitary [18], the dose being 4 mg/kg female. Carp pituitary has undergone preparatory grinding and mixing with physiological saline to be administered with the syringe. The grinding of pituitary was made with a mortar and then mixed with saline serum 0.5 ml/4 mg and intraperitoneal administered with a syringe at the base of the pectoral fin. The Nerestin 6A product of Russian origin was used in the second lot, the dose being 0.25 ml / kg female body. Nerestin is a liquid product that does not require preparation prior to administration. Both hormonal products were administered in two doses, the first representing 20% of the total dose and the second remaining 80%

The main reproductive parameters determined were:

- Latency time (h) = it is the time from the moment of the first injection to ovulation.
- Ovulation (%) = number of ovulated females

Harvested eggs (g) = the quantity of eggs harvested

- Absolute fecundity index = the number of eggs in 1 g *ovary mass (kg) $*10^3$ (the ovary resulting from milking has been weighed, a sample of one gram has been taken, the eggs are counted in the sample and the result is multiplied by the total ovarian weight).

- Relative fecundity index = (Af index/females weight in g*10^3)

- Percentage of eggs (% of female BW) = is the percentage of ovary weight relative to the female weight.

- S (survival rate %) = Number of survive fish / number of fish at the beginning x100.

- Fertilization rate (%)

- Hatching rate (%) = (Hl / Te) * 100

Where H1 represents the number of hatched larvae and Te represents the total number of eggs after fertilization.

The reproductive parameters (Latency time, ovulation, harvested eggs, absolute fecundity, relative fecundity, percentage of eggs, survival rate, fertilization rate, hatching rate) were analyzed statistically by one-way analyses of variance, ANOVA($P \leq 0.05$) followed by the post-hoc Tukey's test.

RESULTS AND DISCUSSIONS

Throughout the experiment, the temperature was measured daily together with the dissolved oxygen in water and the pH. The mean values calculated over the whole period are highlighted in the following table:

Table 1 The main parameters of the water during the experiment

Analyzed parameters	Measure unit	Results	Allowed values in aquaculture
Temperature	°C	14.3	max. 28
pH	pH units	7.8	6.5 – 8.5
Dissolved oxygen (mg/l)	mg/l	12.19	min. 6
Conductivity	µS/cm^3	1204	1300
Nitrates (NO_3^-)	mg/l	14.3	30
Nitrites (NO_2^-)	mg/l	0.06	3
Ammoniac (NH_3^+)	mg/l	absent	0.3
Ammonium (NH_4^-)	mg/l	absent	3
Phosphor(total)	mg/l	0.01	0.1

The females had an average weight of 399.1 ± 9.66 g/ex in L1 and 425.10 ± 25.77 g/ex in L2, the doses of hormone used being highlighted in Table 2. Males did not required hormonal stimulation, the amount of harvested sperm being sufficient.

Table 2 Female weight and doses of hormonal preparation used

Female number	Weight (g)		Total dose	
	L1	L2	L1 (mg)	L2 (ml)
1	458	430	1.72	0.108
2	405	637	1.62	0.159
3	340	410	1.36	0.102
4	378	395	1.51	0.098
5	425	431	1.7	0.107
6	393	420	1.57	0.105
7	388	436	1.55	0.109
8	401	378	1.6	0.094
9	409	325	1.62	0.081
10	394	389	1.57	0.097

After hormonal simulation, the water parameters were in focus, the main ones (dissolved oxygen in water, temperature and pH) being measured twice a day. At this point, the target parameter was the latency between the first injection of females and the time they were ovulating. After 24 h from the first injection the females were checked hourly by gently massaging the abdomen of each female. When the first eggs appeared, the female was introduced into the anesthetic solution and then the eggs were harvested.

After harvesting the eggs from each female, the eggs were fertilized with sperm from the three males corresponding to each group.

It should be noted that the eggs of the species *Perca fluviatilis* have a particularity, is like a ribbon. Before fertilization of eggs from of each female, approximately 1 g was taken to determine the relative and absolute fecundity index. For this, the 1 g sample was weighed using the analytical balance and counted the eggs in that sample.

The fertilization was of the semi-wet type, the eggs and the sperm were harvested in dry recipients, after which the sperm was activated with water and poured over the eggs. They were mixed for 3 minutes after which they were rinsed two times in water and incubated. After rinsing with water, approximately 100 fertilized eggs were harvested, and were analyzed on a stereomicroscope to determine the percentage of fertilization.

Fertilized eggs were transferred for incubation in Nucet incubators. The detaching operation that is specifies in artificial fish reproduction technology was not necessary because the fertilized eggs were assembled in the ribbon form and could easily be spread so as not to form agglomerations.

The average latency for the L1 using the carp pituitary product was 121.43 hours ± 10.87. In group L2 using Nerestin 6A, the average latency time was 84.71±6.12 hours being smaller than the L1 group, thus significant differences ($p<0.05$) were recorded. Latency is usually influenced by the stage of ovarian development and temperature.

The ovulation percentage varied in the two lots and was 50% in the L1 group and 70% in the L2 lot. For this indicator statistically the differences are insignificant ($p>0.05$).

The amount of harvested eggs varied between mean values of 47.71 ± 12 g / female in group L1 and 86.86 ± 7.18 g / female, with significant differences ($p<0.05$).

The value of absolute fecundity index was high and varied between 87.2 ± 23.4 x 10^3 in the L1 group and 109 ± 11.1 x 10^3 in the second group, with no significant differences ($p<0.05$). Values are good and in the same range as the values reported by Voican for the Romanian perch [19]. These results fits the prolificity of perch females (*Perca fluviatilis*) between the values of 32930-151015 spawn / females. Percentage of relative fecundity index is averaged between 209±66.5x10^3 for lot L1 and 273.1±34.5x10^3 for lot L2. Statistical differences between them are insignificant ($p>0.05$).

The percentage of eggs in relation to the female body weight varied between the average values of 11.91±3.59 in the L1 group and 19.10±0.76 in the L2 group, the differences being statistically insignificant ($p>0.05$).

Table 3 Latency time (h), Ovulation (%), Harvested eggs (g), Absolute fecundity index (pcs.), Relative fecundity index (pcs.), Percentage eggs (% of female BW)

Lot	Latency time (h),	Ovulation (%)	Harvested eggs (g),	Absolute fecundity index (the number of eggs in 1 g *ovary mass (kg) *10^3(pcs.)	Relative fecundity index Af index /females weight in g*10^3 (pcs.)	Percentage eggs (% of female BW)
L1	121.43±10.47^a	50±16.67^a	47.71±12.18^a	87.2±23.4^a	209±66.5^a	11.91±3.59^a
L2	84.71±6.12^b	70±15.28^a	86.86±7.18^b	109±11.1^a	273.1±34.5^a	19.10±0.76^a

Note: Values (mean ± SE) in the same column not sharing a common superscript letter are significantly different ($p<0.05$)

Survival rate of females during the experiment was 80 % in L1 and 90 % in L2 with no significant differences between the two lots ($p>0.05$). In the case of males, survival rates was 100%. We assume that this results are owed to the fact that males were less manipulated (males were not stimulated with hormones) and to the fact that they were not injected.

Fertility rate was high in both lots (91.14±2.87 L1 and 87.80±2.63 L2) however differences were not statistically significant ($p>0.05$).

Hatching rate was 52±7.83 in L1 and 36±10.03 in L2, these results being considered as average according to the literature. The differences between the two lots regarding the hatching rate were statistically insignificant.

Table 4 S (survival rate, %) = Number of survive fish / number of fish at the beginning x100, Fertilization rate (%), Hatching rate (%)

Lot	S(survival rate, %) = Number of survive fish / number of fish at the beginning x100	Fertilization rate (%)	Hatching rate (%)
L1	80[a]	91.14±2.87[a]	52±7.83[a]
L2	90[a]	87.80±2.63[a]	36±10.03[a]

Note: Values (mean ± SE) in the same column not sharing a common superscript letter are significantly different (p<0.05)

DISCUSSIONS

Carp pituitary is widely used in fish reproduction from almost all species. It acts and stimulates the gonads in a similar way as a hormonal product hCG type in contrast to Nerestin 6A that contain GnRHa and acts and stimulates the brain. [22] Nerestin type hormonal products are made in Russia and several types are available (1, 1A, 1B, 2, 5, 5A, 5B, 6, 6A, 7A) depending on the species of fish.

The latency time recorded in this experiment had high values between 84-121 h. However, it is known that for *Perca fluviatilis* the latency time can vary a lot, from 18 to 120 hours [12].

An experiment where Ovaprin hormonal preparation was used in the perch reproduction, showed a value between 45-77 hours for latency time [11].

A comparative study using a hormonal preparation called Gonazon and carp pituitary showed that latency had an average of 79 hours for Gonazon and 92 hours for carp pituitary [16]. In our experiment there were statistically significant differences between the two lots, the use of the Russian Nerestin 6A preparation, resulting in a shorter time from the first injection to ovulation.

The ovulation rate of females was within the limits reported by other researchers. An experiment in which carp pituitary and Ovopel was tested, showed that 40-92% of females ovulate, the percentage being influenced by the average of development stage [11]. Similar results related to ovulation were recorded in the case of yellow perch (*Perca flavescens*) [3].

Significant statistical differences (p<0.05) were also recorded for the amount of harvested eggs, with better results in group II.

The percentage of absolute fertility had values above average, higher than those reported by Migaud or Křišťan [7], [9].

The percentage of relative fecundity also had high values, higher than those reported by Tonner or Křišťan [9], [18]. Statistically there were no differences in this index, the type of hormonal preparation used not having a noticeable influence.

The survival rates of the breeders were high (80-90% in females and 100% in males) similar to those recorded in other experiments [12], [18].

The fertilization percentages of over 87% indicate that the quality of the eggs and sperm was superior and are similar to those of other researchers' experiments [3], [9], [10], [11], [12], [18].

Hatching percentages were lower (36-52%) compared to other experiments where values were over 80% [9], [12], [18]. These results were mainly influenced by conditions during incubation and less by the type of hormonal preparation used.

CONCLUSIONS

Analyzing all the results, we can say that the artificial reproduction of *Perca fluviatilis* was successfully performed under controlled conditions, and the best results were given by Nerestin 6A. Significant statistical differences (p <0.05) were recorded for latency and the amount of harvested eggs. In addition to the two parameters where statistical differences have been found, it should be noted that Nerestin 6 A is in liquid form, ready-available, making it easier to use.

Nevertheless, it should beconsidered that Russian product availability can be decresed due to the limitation of exports from non-EU countries.

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