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RESEARCH ON THE USE OF DIFFERENT HORMONAL SUBSTANCES TO STIMULATE MATURATION AND OVULATION IN PERCH (*PERCA FLUVIATILIS* L.)

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Abstract

Diversification of production in fish culture by introducing valuable species for which there is demand and tradition for consumption, is one of the main directions of development of aquaculture. Perch (Perca fluviatilis L.), a valuable autochthonous species, is recognized for the quality of meat but mainly for the satisfaction offered in sport fishing. A crucial step for the introduction and expansion of perch in aquaculture, is to obtain biological material for stocking through artificial spawning. The paper presents results of experiments for artificial spawning of perch performed between March 20 and April 1, 2015. Were used a total of 120 broodstock collection in Arcesti and Ionesti reservoirs on the river Olt in the fall of 2014. For maturation and ovulation induction was used: carp pituitary extract - CPE, human chorionic gonadotropin - Chorulon HCG, GnRH or analogues, sometimes combined with dopamine antagonists - Ovopel. Technological parameters obtained from artificial spawning were within the following ranges: the percentage of maturation of females 34 -73%; embryo rate was 64 -85%, hatching rate 23 -65%. The best results were obtained when using HCG Chorulon as a stimulating agent for maturation and ovulation.

Key words*: artificial spawning, hatching rate, incubation, maturation, ovulation.*

INTRODUCTION

Diversification of production in fish culture by introducing valuable species for which there is demand and tradition for consumption, is one of the main directions of development of aquaculture. Perch (*Perca fluviatilis* L.), a valuable autochthonous species, is recognized for the quality of meat but mainly for the satisfaction offered in sport fishing. Controlled reproduction is the most reliable method for obtaining a high number of perch larvae. Perch spawners from both wild and cultured stocks spawn easily in captivity. However, the spawning period is a long process that lasts for more than two weeks (Kucharczyk D., et al. 1996a). This is very inconvenient for starting incubation and rearing, and it requires more elaborate facilities. For these reasons, a method to synchronize perch spawning is still needed. Many kinds of hormonal treatments have been used to stimulate ovulation in perch females. Human chorionic gonadotropin (hCG) with common carp (*Cyprinus carpio* L.) pituitary extract (CPE) were tested by Kucharczyk et al. in 1996 (Kucharczyk et al., 1996b). Independent of temperature, spawner size and gonad maturity, or the type of hormonal stimulation applied, synchronized ovulation was observed to a lesser or greater degree in all of the above experiments. Nevertheless, the biological quality of the eggs, expressed as the percentage of egg survival to the larvae, varied widely and the improvement of spawning techniques is required.

The knowledge of physiological processes in fish has facilitated the use of substances that stimulate maturation and reproduction (Dabrowski et al., 1996). Obtaining the maturity can be stimulated in percids (perch, pikeperch) using carp pituitary extract (CPE), human chorionic gonadotropin (hCG) and luteinizing hormone-releasing hormone (LH-RH) or super-active analogs (LH-RHa), sometimes with dopamine antagonists (Ronyai, 2007).

This paper presents results of experiments of artificial spawning of perch obtained from the Fisheries Research and Development Station of Nucet, between March 20 and April 1, 2015.

MATERIALS AND METHODS

Artificial spawning was achieved by proceeding sequentially through the following steps: broodstock collection, females selection, the stage determination of maturation of gonads, application of hormonal treatment, eggs ripening, harvesting sexual products, eggs incubation and hatching.

For maturation and ovulation induction was used: carp pituitary extrac – CPE, human chorionic gonadotropin - Chorulon HCG, GnRH or analogues, sometimes combined with dopamine antagonists – Ovopel.

Broodstock collection

Were used a total of 120 breeders captured in Arcesti and Ionesti reservoirs on the river Olt in the fall of 2014. Fish were parked in winter in pools of the ground the size of 3 x 25 m. There was fishing in March, at 6-7°C.

Fish were selected according to the following criteria: the belly of the females had to be fully distended, bulging and soft and resilient to the touch; the males have started the spermiation process.

Males and females selected were kept apart in the fall with volume of 2.000 litters hatchery. All breeders (Figure 1) were close in height, weighing between 180-275 g.

Figure 1. Breeders: male and female of perch

Determining the maturation stage of oocytes

Oocyte maturity was determined by using biopsy techniques. In this technique eggs (oocytes) are taken from the ovary, cleared with a prepared solution (e.g. Serra's solution), and viewed under a microscope.

There are many different methods of sampling oocytes from fish ovary. One of them is taking sample using a catheter. This method was tested many times in perch (Kucharczyk et al., 1996a), as well as in many other fish species, especially in cyprinids.

The main evaluation criteria of perch oocytes maturity stages, like other *Teleostei* fishes, are the location of germinal vesicle (GV) and additionally coalescence of the oil droplets.

Oocytes classified according the above-mentioned criteria are divided into four stages:

1. Oocytes in I (first) maturity stage have GV in the central position
2. Oocytes classified as II (second) maturity stage have shifted GV less than a half radius.
3. Oocytes classified as maturity stage III have positioned GV on the periphery, near the oocyte membrane.
4. Oocytes without visible GV, i.e. in which the process of GV breakdown (GVBD) has begun or GV is present near the zone, should be classified as maturity stage IV.

Only females whose oocyte maturation was between stage 2-3 and 3 were used for further investigations.

Hormonal treatment

For maturation and ovulation induction was used: carp pituitary extract – CPE, human chorionic gonadotropin - Chorulon HCG, GnRH or analogues, sometimes combined with dopamine antagonists – Ovopel .

The fish were divided into four groups that were injected with the following:

1. Extract from pituitary glands, i.e. carp (CPE) (Ronyai, 2007);
2. Chorionic gonadotropins human HCG;
3. Ovopel pellets. One Ovopel pellet (average weight about 25 mg) contains a mammalian GnRH analogue (D-Ala6, Pro9Net-mGnRH at dose 18-20 µg) and dopamine antagonist: metoclopramide (dose 8-10 mg) (Kucharczyk et al., 1996a);
4. 0.9% NaCl sterile solution (control group)

All spawning agents are usually prepared with 0.9% NaCl. The doses are presented in Table 1. The number of females and males in each group were 20 and 10, respectively. Injections were administered intramuscularly in the dorsal area of the body.

The range of total doses of chosen spawning agents should be as follows:

- Chorionic gonadotropins (200 – 1000 IU/kg BW) (Ronyai, 2007);
- CPE (1.0 – 3.5 mg / kg BW) (Horvath et al., 1997; Kouril et al., 1997);
- Ovopel (1.2 – 2.0 pellets / kg BW) (Ronyai, 2007).

If two injections are planned, the initial dose should be 20 – 50% in the case of chorionic gonadotropins and 10 – 20% in the case of other spawning agents.

Table 1. Hormonal treatment applied to induce artificial spawning in perch

Specifications	Female		Male
	Preparatory dose	Decisive dose	Single dose
CPE	0.5 mg/kg BW	2.0 mg/kg BW	1.0 mg/kg BW
hCG	200 IU/kg BW	1000 IU/kg BW	500 IU/kg BW
Ovopel	1/10 Ovopel pellet	1.0 Ovopel pellet	½ Ovopel pellet
Control	injections from 0.9% NaCI		

Manipulations with breeders

Females of perch were screened at about 12 hours after the last injection. If the female perch is ready to give eggs usually begin to fall spontaneously genital pore.

Before stripping the genitors must be clean and dry with a soft towel. They should not be allowed to mix with water gametes because they are eliminated. The fish is held with one hand around the tail fin and the other is a slight pressure to the abdomen. If ovulation has occurred, a stream of eggs will appear. Where there is a flow of eggs, abdomen should be massaged from front to back to remove all the eggs.

The eggs are usually collected in a small plastic container. Artificial eggs stripped wait, usually for adding sperm. Sperm collection was performed using plastic syringes.

The sperm from each male was collected separately and added over eggs. After fertilization, the eggs were incubated pasted on nytal frames in Nucet incubators.

Fish from all the groups were kept for an additional ten days after the end of all the experiments in order to observe their survival.

Statistical analysis

Statistical differences between groups were analysed with Duncan's multiple range test ($P < 0.05$). The relationships between embryo rate to the hatching rate were calculated using regression analysis.

RESULTS AND DISCUSSIONS

Results of different spawning agents in perch reproduction under controlled conditions are presented in Table 2.

Table. 2. Efficiency of different spawning agents for artificial spawning of perch

Specifications	Control (0.9% NaCl)	CPE	hCG	Ovopel
No. of females	20	20	20	20
Ovulation (%)	34	52	73	67
Embryo rate (%)	64.1± 3.2B	74± 4.3C	85± 2.1 A	82± 3.8 A
Hatching rate %	23.1± 1.2B	50 ± 3.3C	65± 1.1 A	62± 3.1 A

The percentage of females ovulate in the treated groups was between 34 and 67% highest value recorded when using Ovopel, and the smallest group of fish in the control group. Ovulation occurred after about 17 - 23 hours after the last injection.

Females stimulated agencies reproductive hCG and Ovopel yielded eggs after 17 and 19 hours at 15^0C.

The rate of embryo was located between the limits of 64% obtained in fish in the control group and 85% for fish stimulated hormone hCG. Elevated this technological indicator was obtained and the use Ovopel.

Hatching rate was 23% in the control group and 65% in fish stimulated with hCG. The reproductive survival, before and after

spawning, was very good. The mortality in all groups was less than 10%.

All males have started the spermiation process at the moment of catch. In the present experiment, the hormonal treatment resulted in a significantly higher production of milt.

Ovulation in females from the experimental groups was synchronous. The short time between the first and last ovulation, observed in all the experiments, may be the result of the high level of synchronous oocyte maturation. The survival of perch spawners throughout the experiment was high (Dabrowski et al., 1994) and (Gillet et al., 1995) reported some problems with spawner survival, especially the females. The similar size of the perch spawners used may have also resulted in synchronous spawning.

CONCLUSIONS

1. Controlled reproduction is the most reliable method for obtaining a high number of perch larvae.
2. Perch spawners of wild and cultured stocks spawn easily in captivity.
3. The main factors that influence the amount and quality of perch artificial spawning are: gametes; temperature, photoperiod and physiological status of the parents.
4. The use of different types of hormonal treatments can lead to getting quality material gametes and species for aquaculture development.
5. Reproduction of agents used in our experiments the best results were obtained when hCG and Ovopel.
6. The doses of spawning agents depend on many factors: time of spawning (season, out-of-season) maturation stage of fish gonads and type of hormone.

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