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## ARTIFICIAL SPAWNING OF EURASIAN PERCH, *PERCA FLUVIATILIS* L. USING OVOPEL

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ABSTRACT. Spermiation and ovulation in the Eurasian perch *Perca fluviatilis* L. were induced with Ovopel, pellets containing GnRH, and then compared with hCG and CPE. Males from the control group produced a significantly smaller volume of milt (average about 17 ml kg BW<sup>-1</sup>) than those from the hormonally-treated groups (over 24 ml kg BW<sup>-1</sup>). Spermatozoa motility was similar in both the treated and untreated males, but the fertilization ability varied significantly between individual males. Over 90% of the treated females ovulated. Ovulation in the control group was noted in a limited number of females. Mean embryo survival to the eyed-egg-stage was over 50% in the treated groups, in contrast to 7.5% in the control group. The poor quality of perch gametes might result from high water temperature oscillations before the natural spawning season. The quality of eggs, expressed as survival to the eyed-egg-stage, decreased with latency time.

Key words: PERCH, ARTIFICIAL SPAWNING, HORMONAL TREATMENT, OVOPEL, CPE, hCG

## INTRODUCTION

Rapid advances in Eurasian perch (*Perca fluviatilis* L.) aquaculture have been observed over the last few years (Kestemont et al. 1996, Mélard et al. 1996). All new breeding technologies usually begin with methods of obtaining gametes under controlled conditions (Skrzypczak et al. 1998, Szczerbowski 1998). 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 (Flajshans and Gondor 1989, Kucharczyk et al. 1997). 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 Eurasian and yellow perch (*Perca flavescens* (Mitchill)) females. Human chorionic gonadotropin (hCG) with common carp (*Cyprinus carpio* L.) pituitary extract (CPE)

were tested by Kayes and Calbert (1979) and Kucharczyk et al. (1996b). LH-RHa with a dopamine antagonist (pimozide) was tested on yellow perch by Dabrowski et al. (1994). Similar studies were conducted with GnRH analogue (Kouril and Linhart 1997, Kouril et al. 1997) and FSH+LH (follicle stimulating hormone + luteinizing hormone) with dopamine antagonists (Kucharczyk et al. 1998a). 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 eyed-egg-stage, varied widely and the improvement of spawning techniques is required.

This paper presents the results of studies concerning the artificial spawning of Eurasian perch after treatment with Ovopel and compares the results to those obtained after injecting acetone-dried carp pituitary extract with the addition of hCG, which worked very well in perch (Kucharczyk et al. 1996b). Ovopel is a GnRH analogue-containing pellet. One Ovopel pellet (average weight - 25 mg) contains a mammalian GnRH analogue (D-Ala<sup>6</sup>, Pro<sup>9</sup>Net-mGnRH at a dose of 18-20 µg) and metoclopramide (a dose of 8-10 mg) (Horvath et al. 1997). Ovopel has been successfully tested in several domestic and wild species, i.e. common carp, tench, *Tinca tinca* (L.), grass carp, *Ctenopharyngodon idella* (Valenciennes), silver carp, *Hypophthalmichthys molitrix* (Valenciennes) (Horvath et al. 1997) and burbot, *Lota lota* (L.) (Kucharczyk et al. 1998c). The second aim of this study was to investigate the effect of Ovopel on the individual fertilization ability of males treated with it.

## MATERIAL AND METHODS

### BROODSTOCK COLLECTION

Perch spawners were obtained in early April 1999 from Kortowskie Lake in Olsztyn, Poland. The water temperature was 8.5°C when the catches were made. 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 had to have started spermiating. The selected males and females were kept in separate 1000 l tanks in a hatchery where the temperature (10-11°C) and photoperiod LD 14:10, were controlled. All spawners were similar in size (200-300 g), and the mean body weight in the experimental groups ranged from 240 to 255 g.

## CHECKING OOCYTES MATURATION

All fish were individually marked using floy tags, weighed and oocytes were taken from females using the method described by Kujawa and Kucharczyk (1996). Before marking the perch individuals, the spawners were anesthetized with a 0.2 ml l<sup>-1</sup> dose of 2-phenoxyethanol. The oocytes were sampled *in vivo* and placed in Serra's solution to clarify the cytoplasm. After 5 minutes, the position of the oocyte nucleus was determined using a four-stage scale:

- stage 1 - germinal vesicle in a central position
- stage 2 - early germinal vesicle migration (less than half of the radius)
- stage 3 - late germinal vesicle migration (more than half of the radius)
- stage 4 - periphery germinal vesicle or germinal vesicle breakdown (GVBD)

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

## HORMONAL TREATMENT

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

1. hCG (Biogonadyl, Biomed Lublin, Poland) - human chorionic gonadotropin with CPE (Argent, USA) - carp pituitary extract (Kucharczyk et al. 1996b);
2. Ovopel pellets: one pellet contains mammalian GnRH analogue (D-Ala<sup>6</sup>, Pro<sup>9</sup>Net-mGnRH at a dose of 18-20 µg) and metoclopramide (a dose of 8-10 mg) (Horvath et al. 1997);
3. 0.9% NaCl sterile solution (control group).

All doses are given in Table 1. The number of females and males in each group were 12 and 7, respectively. One day after checking female maturation, the fish were treated with respective hormonal injections. All hormones were homogenized or dis-

TABLE 1

Hormonal treatment applied to induce artificial spawning in perch - time between priming and resolving injections was 24 h

Group	Male	Female	
	Hormonal dose	Priming dose	Resolving dose
1	2.0 mg CPE	1000 IU hCG 0.4 mg CPE	3.6 mg CPE
2	1/2 Ovopel pellet	1/10 Ovopel pellet	1 Ovopel pellet
3 (control)	+	+	+

+ - injections from 0.9% NaCl

solved in sterile NaCl solution (0.9%). Injections were administered intramuscularly in the dorsal area of the body.

Ten additional males (average wet body weight  $115 \text{ g} \pm 11$ ) were stimulated by Ovopel (one dose = 1 pellet  $\text{kg BW}^{-1}$ ) with a single injection at the same time as the last female injection.

### MANIPULATIONS WITH BREEDERS

All manipulations were carried out by the method described in Kucharczyk et al. 1996b. After the last injection, the genital pore of each female was sewn up to prevent spontaneous egg dropping. At the same time, the water temperature was raised to  $13^{\circ}\text{C}$ .

Checking the females was begun 12 h after the last injection. During the next 10 h, the females were checked every hour and then every 2-3 h. After 48 hours, oocyte samples were taken from the few females in which ovulation had not been observed. Milt was collected with plastic syringes and kept at a temperature of  $4^{\circ}\text{C}$  until further treatment. Spermatozoa motility was estimated under a light microscope ( $\times 500$ ) within 30 min after sperm collection. The activating media was a 0.5% NaCl solution. Stripped eggs were fertilized using the dry method (Dabrowski et al. 1994) with sperm pooled from all males from the same group. Two replicates of 400-500 eggs of each fish were incubated in special incubation chambers (Kucharczyk et al. 1996a) at a temperature oscillating between  $13^{\circ}\text{C}$  (beginning of incubation) and  $16^{\circ}\text{C}$  (hatching time); this has been found to be optimal for perch embryonic development (Kokurewicz 1971). Eggs were incubated up to the eyed-egg-stage (embryo eyes were pigmented).

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 (spermiating success, latency and incubation success) were analysed with Duncan's multiple range test ( $P < 0.05$ ). The relationships between embryo survival to the eyed-egg-stage and latency time were calculated using regression analysis.

### RESULTS

Males from the control group produced a significantly smaller volume of milt ( $17.2 \text{ ml kg BW}^{-1}$ ) than those from the treated groups (more than  $24 \text{ ml kg BW}^{-1}$ ) (Table 2).

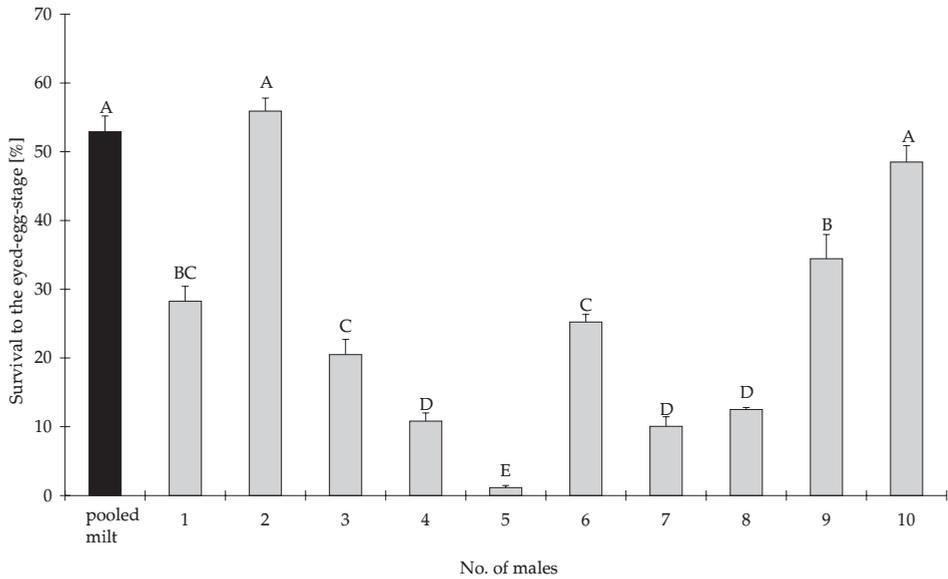


Fig. 1. Embryo survival (2 replicates/treatment) in groups fertilized by milt collected from ten separated males. "Pooled" is the control group (milt from ten males). SE is shown as vertical bars. Groups marked with the same letter did not differ statistically ( $P > 0.05$ ).

The motility of spermatozoa in all samples of perch sperm ranged from 70 to 90%. There were no statistical differences between the spermatozoa motility of sperm collected from different groups. Significant differences in the fertilization ability of sperm collected from individual males was noted (Fig. 1). The maximum survival rates to the eyed-egg-stage were observed in eggs which had been fertilized with milt from males numbers 2 and 10. This data did not differ significantly from the result obtained in the control group where eggs were fertilized with pooled milt from ten males.

The percentage of ovulated females from the treated groups was over 90%. Ovulation in fish from the control group was noted in a limited number of females (Table 2). One egg strand obtained from a female from group 2 was fragmented. The latency in the treated groups was 18.1 and 23.0 hours in groups 1 and 2, respectively. When latency was calculated without late ovulated females (1 female in group 1 and 2 females in group 2), the above values were much lower at 15.9 and 18.5 hours (Table 2). The mean embryo survival to the eyed-egg-stage was over 50% in the treated groups; this is in contrast to the control group where it was 7.5%. When these values were calculated without late-ovulated females, the average survival in both experimental groups was over 65%. The biological quality of eggs, expressed as survival to the eyed-egg-stage, decreased with time after the last injection (Fig. 2).

TABLE 2

Results obtained after artificial spawning of perch

	Group 1 (hCG + CPE)			Group 2 (Ovopel)			Group 3 (Control)		
	Mean	Range	SE	Mean	Range	SE	Mean	Range	SE
Volume of milt [ml kg BW <sup>-1</sup> ]	24.1*	19.5-28.5	1.1	24.2*	19.4-30.4	1.2	17.2	15.2-20.8	0.7
Percentage of ovulated females	92	-	-	92	-	-	17	-	-
Latency [h]									
all females	18.1*	14.0-40.0	2.2	23.0*	18.0-44.0	9.1	42.0	40.0-44.0	1.4
without late-ovulated	15.9	14.0-20.0	0.7	18.5	18.0-19.0	0.1	-	-	-
Embryo survival [%]									
all females	53.3*	6.1-78.9	6.8	53.4*	0.1-82.3	8.6	7.5	6.7-8.4	0.6
without late-ovulated	65.3*	39.8-78.9	3.5	65.8*	48.6-82.3	4.6	-	-	-
Spawner survival [%]	95	-	-	90	-	-	95	-	-

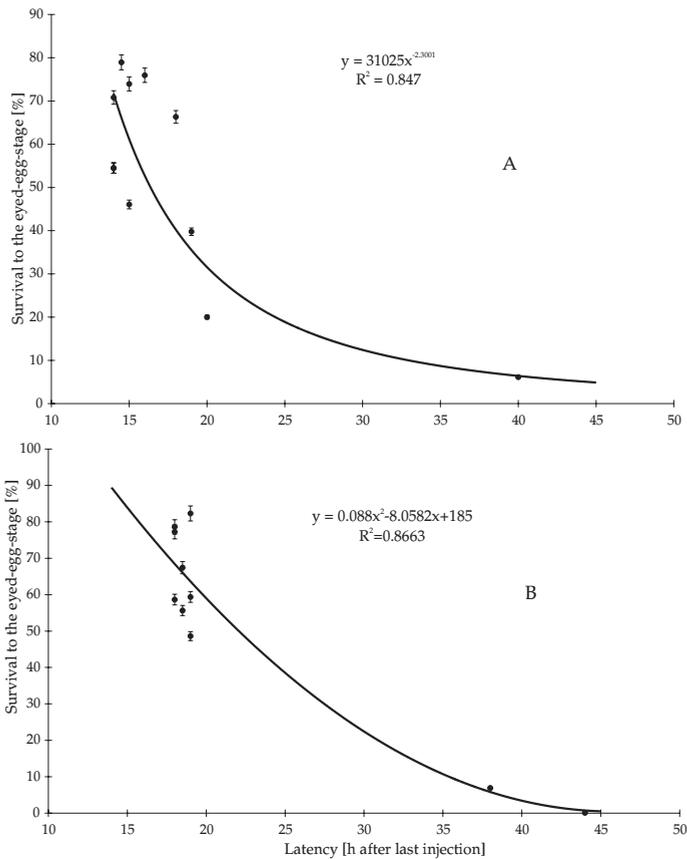
\* - marked groups did not differ statistically ( $P > 0.05$ )

Fig. 2. Relationship between Eurasian perch embryo survival to the eyed-egg-stage (2 replications/treatment) and time of ovulation (latency) (A: group 1; B: group 2). SE is shown as vertical bars. N = 11 in both treatment groups.

Migrations of the nucleus in oocytes were observed in non-ovulated females. Oocytes in the females from treated groups were in stage 4, whereas in fish from the control group only slight migrations were observed (the oocytes were usually in stage 3).

The survival of the spawners before and after spawning was very good. The total mortality in all groups was lower than 10%.

## DISCUSSION

All males had just begun spermiating at the moment of catch. In the present experiment, the hormonal treatment resulted in a significantly higher production of milt. This was in contrast to our earlier works with Eurasian perch (Kucharczyk et al. 1996b, Kucharczyk et al. 1998a) where hormonally induced males gave more milt than those from the control group, but these differences were not statistically significant. Dabrowski et al. (1994) reported that yellow perch, *P. flavescens* males treated with LH-RHa gave relatively more milt in February and March than those from the control group. However, during the natural spawning season in April, there were no differences in the amount of milt obtained.

The initial observations of the individual fertilization ability of Eurasian perch males showed high variability. This might depend on spermatozoa motility, as well as spermatozoa concentration (not examined). Data concerning the characteristics of perch semen are very limited (Piironen and Hyvärinen 1983, Lahnsteirner et al. 1995). For this reason, further investigations on the biochemical indicators of perch sperm quality, especially in comparison to the fertilization ability, are still needed.

There were no differences in the numbers of ovulated females from either of the treated groups. The high percentages of ovulated females in the treated groups and the slight degree of ovulation in the control group were similar to earlier data obtained for yellow and Eurasian perch (Dabrowski et al. 1994, Kucharczyk et al. 1996b, Kucharczyk et al. 1998a), and they were better than data reported by Kouril and Linhart (1997) and Kouril et al. (1997). In the present experiment, we used a lower dose (20-22 µg) of GnRH per 1 kg BW than those recommended by Dabrowski et al. (1994) (100 µg - single injection or 110 µg - double injection; in both cases with 10 mg of pimozide) and Kouril et al. (1997) (125 µg - single injection). The relatively good results (percentage of ovulated females, high synchronization, good embryo survival) obtained in the present experiment after treatment with a lower dose of hormones, in comparison to other data, may result from the fact that, firstly, lower doses

of GnRH were not tested, and, secondly, that no dopamine antagonist was used. On the other hand, we used a GnRH analogue with metoclopramide, which, in some fish species, worked much better than pimizide or domperidon. This was confirmed not only with Eurasian perch (Kucharczyk et al. 1998a), but also with common carp (Drori et al. 1994, Yaron 1995, Kulikovskiy et al. 1996).

The latency time calculated for all the ovulated females between the last injection and the initial egg release in perch females treated with hCG and CPE was 18.1 h; in fish treated with Ovopel it was longer at 23.0 h. These figures were much higher than those obtained in earlier experiments (Kucharczyk et al. 1996b, Kucharczyk et al. 1998a). However, when latency was calculated without the few late-ovulated females, the data were very similar to those noted earlier. The differences in latency in females treated with CPE and GnRH have been reported in many papers. Drori et al. (1994) and Yaron (1995) suggested that latency was always shorter in fish treated with carp pituitary extract than in fish treated with other hormones and drugs. This may be explained by the fact that GnRH release from the pituitary and the ovarian response to the release of hormones is a sequential process, while in fish injected with carp pituitary extract, the ovarian response to the exogenous GtH was a single process (Drori et al. 1994).

Ovulation in females from the experimental groups was synchronous. The short time between the first and last ovulation, observed in all the experiments cited, may be the result of the high level of synchronous oocyte maturation. Latency in perch and yellow perch is varied; females from both species began ovulating from between 12-14 or 18 h (Kayes and Calbert 1979, Kucharczyk et al. 1996b, Kucharczyk et al. 1998a) to more than four days (Dabrowski et al. 1994, Kouril and Linhart 1997, Kouril et al. 1997, Kucharczyk et al. 1998b) after resolving the injection. The survival of perch spawners throughout the experiment was high. Dabrowski et al. (1994) and Kouril et al. (1997) reported some problems with spawner survival, especially in females. The similar size of the perch spawners used may have also resulted in synchronous spawning. This observation was made under controlled conditions (Kucharczyk et al. 1998a) and in the natural environment (Gillet et al. 1995).

The oocyte samples taken from non-ovulated females from the treated groups showed that oocyte maturation (nuclear migration) was usually at stage 3-4. Glubokov et al. (1991) reported that bream, *Abramis brama* (L.) oocytes matured after stimulation with LH-RH analogue. A similar situation was noted by Kucharczyk et al. (1996b and 1998a) for perch treated with lower doses of hormones. The percentage

of embryo survival to the eyed-egg-stage in both experimental groups was much lower than data of perch embryo survival reported in other papers (Dabrowski et al. 1994, Kucharczyk et al. 1996b, Kouril et al. 1997, Kucharczyk et al. 1998a). However, eggs obtained a longer time after injection showed decreased fertility (Fig. 2). A similar situation was observed for many cyprinid fish (Kozłowski 1994). Very low embryo survival was noted when eggs were obtained from untreated females. We think that the problem with the biological quality of perch gametes in the present work (spermatozoa and oocytes) might have been influenced by the high water temperature oscillations before the natural spawning season in 1999. In Kortowskie Lake, from which the breeders were collected, perch usually spawn in late April and early May, but in 1999 the water temperature increased rapidly in mid-March. At this time, the perch were migrating to their spawning grounds and the males were spermiating. In the following few days, the temperature rapidly decreased and the perch spawners left their spawning site. Kayes and Calbert (1979) and Dabrowski et al. (1996) suggested that specific, modified photothermal conditions may have a modulatory influence on spawning. Dabrowski et al. (1996) reported that low embryo survival was reported when fish in captivity were exposed to manipulated photothermal conditions. Długosz (1983) reported asynchronous oocyte growth in perch from an artificially heated lake.

The present work shows that Ovopel may be used for inducing spawning in Eurasian perch, even when photothermal conditions are changed. This is clearly shown when the data from the experimental groups are compared with those from the control group.

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## STRESZCZENIE

### SZTUCZNY ROZRÓD OKONIA, *PERCA FLUVIATILIS* L., PRZY UŻYCIU OVOPELU

Celem prowadzonych badań było określenie wpływu stosowania stymulacji hormonalnych na wyniki sztucznego rozrodu okonia. Tarlaki okonia podzielono na trzy grupy (po 12 samic i 7 samców w każdej). Samice z grupy pierwszej otrzymały dwie dawki hormonu (domięśniowo 1000 IU hCG i domięśniowo 0,4 mg CPE, a po 24 h – 3,6 mg CPE), z grupy II – domięśniowo dwie dawki Ovopelu (1/10 tabletki, a po 24 h 1 tabletkę). Samce z grupy I iniekowano 2,0 mg CPE, a z grupy II 1/2 tabletki Ovopelu. Wszystkie ryby z grupy III (grupa kontrolna) poddano iniekcji 0,9% roztworem NaCl.

Samce z grupy kontrolnej produkowały znacznie mniejszą ilość mleczka (średnio 17 ml kg<sup>-1</sup>) w porównaniu z samcami poddanymi iniekcji hormonalnej, które produkowały średnio 24 ml mleczka w przeliczeniu na kilogram masy ciała. Ruchliwość plemników w nasieniu była podobna u wszystkich badanych grup, jednak zdolność do zapłodnienia była istotnie różna pomiędzy samcami.

Wśród samic poddanych iniekcji hormonalnej owulowało ponad 90%, podczas gdy tylko niewielki odsetek samic (17%) z grupy kontrolnej przystąpił do tarła. Średnia przeżywalność embrionów do stadium zaoczkowania w grupach I i II wyniosła 50%, natomiast w grupie kontrolnej jedynie 7,5%. Słaba jakość gamet mogła być wynikiem wysokich skoków temperatury wody przed naturalnym sezonem rozrodczym okonia.

Badania wykazały, że Ovopel może być wykorzystywany w celu wywoływania sztucznego tarła okonia, nawet w przypadku zmian temperatury i warunków świetlnych.

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