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PROOF

Artificial Reproduction of Different Dace, *Leuciscus leuciscus* (L.) Populations as a Method for Biodiversity Preservation

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Abstract

This study presents the results of inducing spermiation and ovulation of dace (*Leuciscus leuciscus* L.) spawners collected from different parts in of Poland. Artificial reproduction was performed using carp pituitary homogenate (CPH), human chorionic gonadotropin (hCG), agents containing a GnRHa with a dopamine inhibitor supplement (Ovopel and Ovaprim, GnRHa combined with metoclopramide) and GnRHa alone conducted during the spawning season. Males from the control group produced similar volumes of semen and sperm motility as fish after hormonal preparation application. Stimulation of ovulation by manipulation of environmental factors as well as by GnRHa alone did not trigger ovulation within 70 hrs following administration of a resolving injection, but did cause GV migration. Applying GnRHa with the dopamine inhibitors or CPH produced 80-100 % ovulation in females. The differences in reproduction between fish populations were reflected in ovulation synchronization, which was highly variable among populations or embryo survival. The fish stimulated with CPH ovulated several hours earlier than those injected with GnRHa combined with a dopamine inhibitor. The data obtained in the present paper could be applied to the production of the rheophilic cyprinid stocking process and, consequently, could form an important tool in the conservation of endangered or over-exploited finfish populations.

Keywords: Artificial reproduction; rheophilic cyprinids; wild populations; CPH; GnRHa; hCG

Introduction

Dace, *Leuciscus leuciscus* (L.), is one of the rheophilic cyprinids, which include, among others, vimba bream, *Vimba vimba* (L.), barbel, *Barbus barbus* (L.) which are naturally tied, associated with, or restricted to, running waters. Dace exist in large areas of Europe and Asia, mainly populating the upper and middle parts of rivers. The dace reproduction season is between February and May, when the water temperature ranges from 5°C to 12°C (Mann, 1996). In recent years, several populations of this species have declined or disappeared (Augustyn, 2004; Penczak *et al.*, 2004). In the Red Book, dace is listed as LC (least concern), but no new data about this species population in the wild has been published. The data from the Warta drainage system showed that the population of this species over the past 30 years has dramatically decreased (Penczak and Kruk, 2000; Penczak *et al.*, 2004). In the case of Warmia and Mazury (north-east part of Poland), a collection of the spawners of this species is practically unavailable. The last study of the fish population in this area of the river was made in the 1960s.

The main causes of this situation are pollution of the aquatic environment, regulation of river flow and excessive fishing (Penczak *et al.*, 2004). As rheophilic cyprinids are not economically significant in aquaculture, their reproduction has not been sufficiently studied earlier and there is a lack of information on their reproductive biology (Kucharczyk *et al.*, 2008) or presented data are incomplete (Cejko *et al.* 2012, Kowalski *et al.* 2012). The dace in many parts of Europe is also a sport fish. However, increasing attention is now being paid to conservation of biological diversity through rebuilding populations of endangered species and their populations. These activities require effective protocols for artificial spawning and initial rearing of stocking material concerning different aspects of these biotechnological procedures (Kowalski *et al.*, 2003; Augustyn, 2004; Kucharczyk *et al.*, 2008; 2014; Targońska and Kucharczyk, 2011; Targońska *et al.*, 2011a, Demény *et al.*, 2012; Żarski, 2012; Nowosad *et al.*, 2014; Kujawa *et al.* 2015).

The genus Leuciscus is represented in European ichthyofauna by three species: dace, chub *Leuciscus*

cephalus (L.) and ide *Leuciscus idus*, but the number of publications on the artificial reproduction of each of them varies significantly. Knowledge of ide reproduction under controlled conditions is particularly advanced (Targońska-Dietrich *et al.*, 2004; Krejszeff *et al.*, 2009; Cejko *et al.*, 2010, Cieśla 2014). A smaller number of studies have been carried out on the artificial reproduction of chub (Calta, 2000; Krejszeff *et al.*, 2008; Krejszeff *et al.*, 2010) and, in the case of dace, there are no studies directly devoted to this issue, especially focused for wild populations (Kucharczyk *et al.*, 2008). There are single studies concerning dace male spermiating and quality of spermatozoa after reproduction under controlled conditions (Cejko *et al.*, 2012, Kowalski *et al.* 2012) but without data concerning female ovulation and biological quality of eggs.

Low quality of gametes and low viability of offspring are the result of improperly prepared protocols (Kucharczyk *et al.*, 2008; Targońska *et al.* 2008). This is caused, among other factors, by thermal regimes during the pre-spawning period (Targońska *et al.*, 2010, 2011b), time and methods undertaken to handle the spawners, spawning agent used (Brzuska, 2006; Yaron *et al.*, 2009, Cejko *et al.* 2012), differences among populations (Targońska-Dietrich *et al.*, 2004; Krejszeff *et al.*, 2010) and domestication level (Krejszeff *et al.*, 2009, Cieśla 2014). Thus, it is very important to re-examine the effectiveness of the proposed methods on different species and populations to improve the knowledge of fish reproduction. In the case of dace, there are some data published mainly focusing of male spermiating and spermatozoa quality (Cejko *et al.* 2012, Kowalski *et al.* 2012) or influence of temperature on final oocyte maturation (FOM) and ovulation in females (Nowosad *et al.* 2014). No study was published concerning influence of different hormonal stimulation of females reproduction and for eventual differences between population during artificial reproduction under controlled conditions.

This study is aimed at optimizing dace artificial reproduction using different spawning agents and considering population differences with essential focusing on females reproduction parameters and biological quality of eggs.

Materials and Methods

Brood-Stock Handling and Management

The work was conducted over several spawning seasons. Dace spawners were obtained from various areas of Poland using traps. For experiment 1, fish were collected in the Lake Gim (population P2). For experiment 2, spawners were sampled in the Marózka River (P1), Lake Gim (P2), the Rospuda River (P3) and the Grabia River (P4) in autumn (Figure 1). Some spawners of P2, were also caught in early spring. For experiment 3, the fish were from the Marózka River (P1) and for the experiment 4 from population P1 and P3.

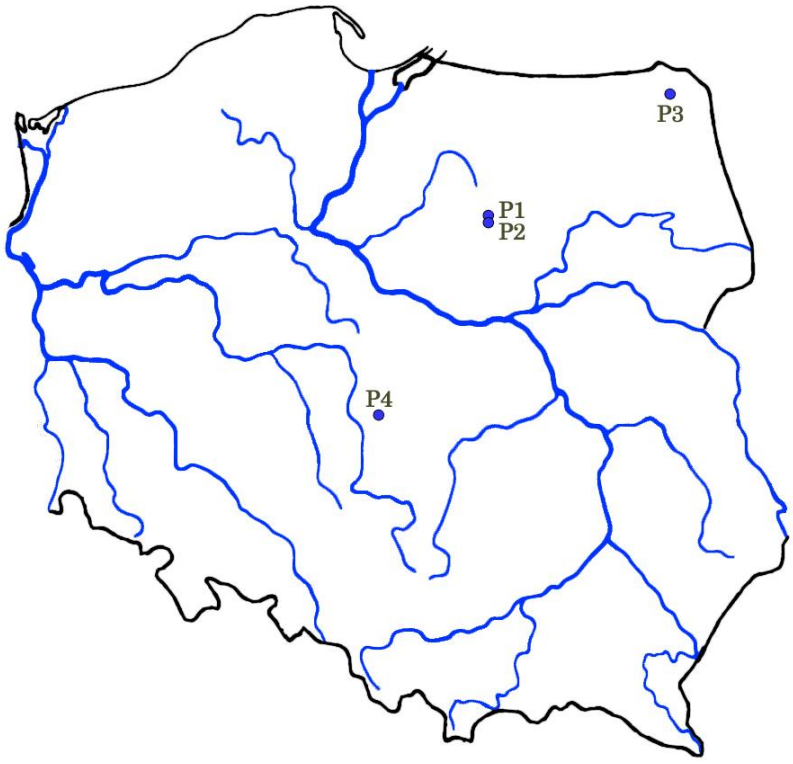

Figure 1. Sampling sites for dace (*Leuciscus leuciscus*) used in the present study; – Marózka river (P1),, Lake Gim (P2),, – Rospuda river (P3) and Grabia river (P4).

After capturing, all individuals were transported and kept separately (both separately in sex and populations) in ponds at the Czarci Jar Fish Farm until mid-February. They were transported to the hatchery when the water temperature reached 6°C. All fish were individually marked using floy-tags, weighed (individual weight 0.1 - 0.3 kg) and the oocytes were obtained by means of a catheter and placed in Serra's solution for clarification of the cytoplasm. After 5 minutes, the position of the oocyte nucleus was determined using a 4–stage scale:

- germinal vesicle in the central position (CGV, stage 1)
- early migration of germinal vesicle (less than half of the radius – MGV, stage 2)
- late migration of the germinal vesicle (more than half of the radius – PGV, stage 3)
- peripheral germinal vesicle or germinal vesicle breakdown (GVBD, stage 4) (Brzuska, 1979)

Further experiments were done on females with oocytes at stages 2-3 (Krejszeff *et al.*, 2008; Krejszeff *et al.*, 2009) and spawners from both sexes are without visible damages. Marked females and males were separated by sex and each population was placed in 1,000 L aerated basins with a water temperature from 6-10°C (Kujawa *et al.*, 1999) for 5 days. During all manipulations, the spawners were anaesthetized by 2 – phenoxyethanol (Sigma Aldrich, Germany) in a tank (30 L) at a concentration of 0.4 $mL \cdot L^{-1}$.

Hormonal Stimulation

Four separate experiments were conducted in the present study. In all experiments, spawning agents were injected intraperitoneally under the ventral fin. In the first and second experiments, two different doses of hormonal agents were used in all hormonal treatments, excluding Ovaprim. The time between the initial and resolving injections was 24 h. The temperature was increased to 12˚C at the time of the second injection.

Experiment 1:

In experiment 1, females from P2 (collected in early spring) were tested for the suitability of four different stimulators:

(a) common carp pituitary homogenate (CPH) (Argent, USA),

(b) human chorionic gonadotropin (hCG) (Biomed, Poland),

(c) Ovopel (Unic-trade, Hungary)

(d) Ovaprim (Syndel, Canada).

One Ovopel pellet (average weight about 25 mg) contains 18-20 µg of mammalian GnRH analogue (D-Ala^6, Pro^9NetPro^{9}NEt-mGnRHa) and 8-10 mg of a dopamine antagonist (metoclopramide) (Horvath *et al.*, 1997). Ovaprim is a liquid, ready-to-use preparation containing 20 µg of salmon GnRH analogue [D-Arg^6 , Pro^9 Net]-sGnRH and 10 mg of dopamine antagonists (domperidon– domperidone) (Peter *et al.*, 1993) in 1 ml. All spawning agents (except Ovaprim) were prepared with 0.9% NaCl. The pituitary was homogenized, hCG was dissolved and Ovopel pellets were pulverized in a mortar and then dissolved. The exact type of hormones, doses, numbers of individuals in groups and numbers of injections are presented in Table 1.

Experiment 2:

During the experiment 2, two stimulators (CPH and Ovopel) were used to compare the effects of artificial reproduction of dace originating from different populations (P1, P2, P3, P4). The doses of spawning agents, procedure and thermal regime were the same as those used in experiment 1 (Table 1). The number of males ranged between 18 to 24 and the

Table 1. The dose of applied hormones, sperm quality and quantity, ovulation rate, latency period and survival of embryo in dace (*Leuciscusleuciscus*) collected from population P2 (Gim Lake) after using different hormonal treatments

Fish group	Control	CPH	Ovopel	hCG	Ovaprim
First injection (kg^{-1})	0.9% NaCl	0.4 mg	0.2 pellet	200 IU	-
Second injection (kg^{-1})	0.9% NaCl	3.6 mg	1.0 pellet	1000 IU	0.5 mL
Spermiation rate	10/10	10/10	10/10	10/10	10/10
Sperm quantity* (mL)	0.6 ± 0.1^a	0.7 ± 0.1^a	0.7 ± 0.1^a	0.6 ± 01^a	0.7 ± 0.1^a
Sperm motility* (%)	62 ± 9^a	71 ± 8^a	70 ± 7^a	67 ± 8^a	74 ± 7^a
Mortality in males	1/10	2/10	2/10	2/10	1/10
Ovulation rate	0/20	20/20	20/20	20/20	20/20
Oocyte maturation in non-ovulated females	Slightly (2-3)	-	-	3-4	-
Latency time (h)	-	30-32	32-36	-	34-40
Embryo survival to the eyed-egg-stage* (%)	-	90.2 ± 4.6^a	92.1 ± 4.4^a	-	92.2 ± 3.3^a
Mortality in females	1/20	1/20	1/20	3/20	3/20

*Data marked with the same letter did not differ statistically ($P < 0.05$). Time between injections was 24 hrs. Males obtain half dose applied to females.

The values are mean ± SD. The number of females was N = 20, males N = 10.

number of females was between 17 and 23.

Experiment 3:

In experiment 3, the suitability of OVA-RH (synthetic salmon GnRHa – Syndel, Canada) was tested alone or in combination with different doses of metoclopramide (MET). In addition, Ovopel was also used as a positive control group. The fish for this experiment were from the P1 population (Marózka River). All hormones and drugs were applied in one injection to protect spawners against mortality. The thermal regimes and all manipulations with fish were as described above for experiment 1.

Experiment 4:

In experiment 4, the selected combination of OVA-RH with MET, Ovaprim and Ovopel was tested for two populations (P1 and P3). All hormones and drugs were applied in one injection to protect the spawners against mortality. The thermal regimes and all manipulation with fish were as described above for experiment 1.

Obtaining the Gametes and Incubation

The milt from males was collected 48 h after hormonal stimulation using syringes (Cejko *et al.* 2012), and kept at 4°C and in vials which were kept chilled at 4°C until examination. The total volume of obtained milt was recorded and the motility of spermatozoa was determined in a 0.5 % solution of NaCl under a microscope (magnification 500x) within 30 min of collection (Glogowski *et al.*, 1997).

Ovulation was examined every 4 h, between 24-48 h after the second injection. The oocytes extracted from each female were placed separately into plastic bowls and then about 100 oocytes from each female were placed on separate Petri dishes for fertilization test. Only sperm samples with a milt motility higher than 60% were used to fertilize the oocytes at a volume of 0.05 mL per each eggs sample. After mixing the gametes 10 mL of hatchery water was add for activation. Samples of fertilized oocytes in Petri dishes were incubated in water with very low ammonia concentration (Nowosad *et al.*, 2013) in basins placed in a recirculating system at 12°C (± 0.1) which was found to be the optimal temperature for dace embryo development (Kupren *et al.*, 2008). The survival of embryos was counted at the eyed-egg-stage (live embryos x 100%/all eggs).

After the experiment, the spawners and the offspring were returned to the natural environment.

Statistical Analysis

The data expressed as percentages were log-transformed before calculations. An ANOVA test was used to test the effect of population (sampling site) on the milt quantity and quality of the variants as well as on embryo survival. The significance of differences between groups was estimated using a *post-hoc* Duncan's multiple range test with a significant level of $\alpha = 0.05$.

Results

Neither volume of semen obtained (0.6-0.7 $mL \cdot kg^{-1}$) nor sperm motility (ca. 60-70 %) increases in hormonally-stimulated fish from the Lake Gim (P2) collected in spring were compared to that of the controls. The measured parameters also did not differ among hormonally-stimulated groups (Table 1). The group of fish maturing spontaneously (controls) was not ready to release their gametes 48 hours after the last injection, similar to the fish treated with hCG. The earliest ovulation was observed in the CPH group (after 30-32 h), while the longest latency period was observed in the Ovaprim group (36 – 40 h). The survival rate of embryos did not significantly differ among the hormonally-treated group and was close to 90% (Table 1).

In the second experiment, no significant differences in milt quality or quantity between populations were recorded among different populations (sampling sites) (Table 2). The spermiation in all groups was 100 % regardless of the hormonal treatment. Ovulation was around 80 % in females from the Rospuda River (P3) and the Grabia River (P4), stimulated using CPH and was lower than females from P1 regardless of their origin (Table 2). A significant difference was observed in terms of latency period; either from the effects of hormonal treatment or the effect of population (sampling site). The fish stimulated with CPH ovulated 3-4 h earlier than females treated with Ovopel. Similar to the first of the experiments, no significant differences were observed in the quantity or quality of milt obtained from males stimulated with CPH or Ovopel. Neither the population nor the hormonal treatment significantly changed the survival rate, which was up to 70 % (Table 2).

In experiment 3, there were no ovulation and no differences between treatment of OVA-RH alone and the control group (Table 3). The quantity of produced milt in these groups was at the same level and was also much lower than in other treated groups. The addition of MET to OVA-RH resulted in higher milt production and ovulation. The combination of 10 µg of OVA-RH and 5-10 mg of MET produced ovulation at the level of 40-60%. The combination of 10 µg of OVA-RH and 20 mg of MET led to 100% ovulation, similar to Ovopel treatment (also with 20 mg MET). In all treatments with OVA-RH, the embryo survival was higher than in Ovopel treatment and fish stimulated with a lower dose of MET ovulated slightly later (Table 3).

Table 2. Sperm quality and quantity, ovulation rate, latency period and survival of embryo in dace (*Leuciscus leuciscus*) collected from different sampling sites (population) after using different hormonal treatment at two doses

Hormone	Ovopel (0.2+1.0 pellet kg^{-1})				CPH (0.4 + 3,6 mg kg^{-1})			
Group/Population	P1 (Marózka River)	P2 (Gim Lake)	P3 (Rospuda River)	P4 (Grabia River)	P1 (Marózka River)	P2 (Gim Lake)	P3 (Rospuda River)	P4 (Grabia River)
Numbers of males	24	21	22	18	23	22	18	22
Spermiation rate	24/24	21/21	22/22	18/18	23/23	22/22	18/18	22/22
Sperm quantity* (mL)	0.5 ± 0.1 [a]	0.6 ± 0.1 [a]	0.6 ± 0.1 [a]	0.5 ± 0.1 [a]	0.7 ± 0.1 [a]	0.6 ± 0.1 [a]	0.6 ± 0.1 [a]	0.7 ± 0.1 [a]
Sperm motility* (%)	65 ± 6 [a]	67 ± 7 [a]	62 ± 6 [a]	63 ± 6 [a]	63 ± 7 [a]	59 ± 11 [a]	67 ± 2 [a]	63 ± 4 [a]
Mortality of males	2/24	3/21	0/22	2/18	0/23	0/22	4/18	8/22
Numbers of females	21	17	21	21	23	23	21	20
Ovulation rate	21/21	17/17	21/21	21/21	23/23	23/23	17/21	16/20
Oocyte maturity in non-ovulated females	-	-	-	-	-	-	Yes	Yes [1]
Latency time (h)	32-36	30-34	34-38	36-42	30-32	29-32	31-34	32-37
Embryo survival to the eyed-egg-stage* (%)	73.2 ± 5.4 [a]	69.2 ± 3.5 [a]	72.1 ± 4.5 [a]	69.7 ± 5.8 [a]	74.3 ± 4.6 [a]	72.1 ± 4.7 [a]	69.2 ± 6.2 [a]	66.1 ± 8.2 [a]
Mortality of females	1/21	2/17	3/21	4/21	2/23	3/23	5/21	8/20

*Data marked with the same letter did not differ statistically ($P < 0.05$). Time between injections was 24 hrs. Males received half the dose applied to females.
[1]- resorbtion of oocytes
The values are mean ± SD.

Table 3. Dose of applied hormones, sperm quality and quantity, ovulation rate, latency period and survival of embryo in dace (*Leuciscusleuciscus*) collected from population P1 (Marózka River) after using different hormonal treatments

	Control	OVA-RH	OVA-RH + MET	OVA-RH + MET	OVA-RH + MET	OVOPEL
Dose of hormones for females (in one injection)	-	10µg	10µg + 5 mg	10µg + 10 mg	10µg + 20 mg	2 granule
Spermiation rate	10/10	10/10	10/10	10/10	10/10	10/10
Sperm quantity*(mL)	0.4 ± 0.1[a]	0.4 ± 0.1[a]	0.5 ± 0.1[a]	0.5 ± 0.1[a]	0.6 ± 0.1[a]	0.5 ± 0.1[a]
Sperm motility* (%)	66% ± 9[a]	68% ± 9[a]	74% ± 11[a]	77% ± 12[a]	78% ± 13[a]	73% ± 10[a]
Ovulation rate	0/10	0/10	4/10	6/10	10/10	10/10
Latency time (h)	-	-	48	46	44	44
Embryo survival to the eyed-egg-stage* (%)	-	-	78.3 ± 3.4[a]	77.2 ± 3.3[a]	79.4 ± 3.2[a]	71.1 ± 2.9[b]

*Data marked with the same letter did not differ statistically ($P < 0.05$). No mortality in spawners was observed. Males received half the dose applied to females.
The values are mean ± SD. Number of females was N = 10, males N = 10.

In experiment 4, in which Ovopel, Ovaprim and a combination of 10 µg of OVA-RH and 20 mg of MET were tested on fish from two populations (P1-from Marózka River and P3-from Rospuda River), there were no differences, excluding embryo survival. Fish stimulated with Ovopel produced lower egg quality. Such a situation was noted in both populations, but was especially noted for population P3-from Rospuda River (Table 4). In the experiments 3 and 4, the latency times were much longer than in the earlier experiment, but all hormones were applied in one injection.

Discussion

This study showed that spawning agents such CPH, Ovopel, Ovaprim, OVA-RH with MET might be successfully used in dace artificial reproduction. hCG did not involve females ovulating within 70 hrs of resolving injection or manipulation of photo-thermal conditions. There were differences in ovulation rate and egg quality (in the present study measured as embryo survival) between different hormonal treatments and fish origin (population). There were no differences in spermiation rate, sperm quantity or motility between treatments. Females stimulated with one injection ovulated 10-16 hrs later than fish females stimulated with two injections.

The data published to date present a clear disproportion between the number of publications on the characteristics of gametes originating from females and males stimulated under controlled conditions. The majority focused on the quality of oocytes (Horváth *et al.*, 1997; Szabó *et al.*, 2002; Kouril *et al.*, 2008; Kucharczyk *et al.*, 2008; Krejszeff *et al.*, 2009; Krejszeff *et al.*, 2010), while only a few studies focused on the milt or both gametes (Takashima *et al.*, 1984; Kucharczyk *et al.*, 1997a; Kucharczyk *et al.*, 2005; Krejszeff *et al.*, 2008; Targońska *et al.*, 2010). Regarding the issues presented in this paper, it is interesting that other authors found that hormonal stimulation increased

Table 4. Sperm quality and quantity, ovulation rate, latency period and survival of embryo in dace (*Leuciscus leuciscus*) collected from different sampling sites (population) after using different hormonal treatments

	P1 (Marózka River)			P3 (Rospuda River)		
	OVOPEL	OVAPRIM	OVA-RH + MET	OVOPEL	OVAPRIM	OVA-RH + MET
Dose of hormones (in one injection) kg^{-1}	2 pellets	0.5 mL	10µg + 20 mg	2 pellets	0.5 mL	10µg + 20 mg
Spermiation rate	10/10	10/10	10/10	10/10	10/10	10/10
Sperm quantity* (mL)	0.5 ± 0.1^{a}	0.7 ± 0.1^{a}	0.6 ± 0.1^{a}	0.5 ± 0.1^{a}	0.7 ± 0.1^{a}	0.6 ± 0.1^{a}
Sperm motility* (%)	74 ± 9^{a}	78 ± 10^{a}	77 ± 9^{a}	71 ± 11^{a}	82 ± 12^{a}	79 ± 9^{a}
Ovulation rate	10/10	10/10	10/10	10/10	10/10	10/10
Latency time (h)	44	46	44	44	44-46	44
Embryo survival to the eyed-egg-stage* (%)	70.2 ± 3.2^{b}	80.2 ± 2.8^{a}	77.3 ± 3.2^{a}	59.5 ± 4.5^{c}	81.5 ± 3.2^{a}	79.2 ± 3.1^{a}

*Data marked with the same letter did not differ statistically ($P < 0.05$). No mortality in spawners was observed
The values are mean ± SD. Number of females was N = 10, males N = 10.

both the semen quantity and quality of the studied species (Takashima *et al.*, 1984; Lahnsteiner *et al.*, 2004; Cejko *et al.*, 2010). However, in common gudgeon *Gobio gobio* (L.), hormonal stimulation did not influence the volume of the obtained milt (Kestemont, 1989).The dace is similar to *G. gobio*, also in size, in that milt volume and sperm motility were not affected by hormonal treatment. These parameters were usually similar in milt quantity and quality regardless of the hormonal treatment applied or the sampling site.

The present study showed that reproduction performances were more affected in dace females according to sampling sites (population). Dace females from the control group did not ovulate. At the same time, despite maintaining them under favorable environmental conditions, only a low percentage of oocyte maturation (expressed as the germinal vesicle migration) was observed and the females stimulated with hCG and OVA-RH alone did not spawn. The low effects of hormonal stimulation of these heterologous hormones have already been reported in some cyprinids which spawn only once during the season, such as common bream *Abramis brama* (L.) (Kucharczyk *et al.*, 1997a; 1997b, Kucharczyk *et al.*, 2005), ide (Krejszeff *et al.*, 2009; Targońska-Dietrich *et al.*, 2004; Jamróz *et al.*, 2008) chub (Krejszeff *et al.*, 2008; Krejszeff *et al.*, 2010) or common carp (Yaron *et al.* 2009). However, hCG has been reported to be effective in other species, mostly cyprinids with batch spawning (Kucharczyk *et al.*, 1997c; Krejszeff *et al.*, 2010) and Percids (Kucharczyk *et al.*, 1996; Ronyai, 2007). The positive effect of subsequent stimulation with Ovopel in females previously treated with hCG indicates that the failure is not due to an inadequate state of the fish ovary, but rather a lack of response of this species to hCG and OVA-RH. In the present study, high spawning efficacy of artificial spawning was obtained in the CPH group in terms of the percentage of ovulation rate and the survival rate of embryos. This hormone has been successfully applied to a considerable number of finfish reproduction projects in the past several decades (Peňáz *et al.*, 1983). Commercial GnRHa products which contain dopamine inhibitors (such as Ovopel, Ovaprim and OVA-RH with MET) have also been introduced to fish farms. Successful reproduction using these hormones has been reported in other rheophilic cyprinids such as asp *Aspius aspius* (L.) (Kujawa *et al.*, 1997; Targońska *et al.*, 2008), chub (Krejszeff *et al.*, 2008, Krejszeff *et al.*, 2010) or ide (Jamróz *et al.*, 2008). The lowest percentage of ovulation stimulated by CPH was observed in fish population from the Grabia River (P4) and from Rospuda River (P3), which might be related to fish stress due to the long transportation period to the hatchery.

In the present study, differences in reproductive parameters were observed between CPH and GnRHa containing dopamine inhibitor preparations. Fish stimulated with CPH ovulated a few hours earlier than those of the Ovopel and Ovaprim groups, which is probably associated with the different level of action of these hormones on the hypothalamic-pituitary-gonadal axis in the fish (Yaron, 1995; Mylonas *et al.*, 1997). It has been widely observed that CPH (containing gonadotropins) influences the gonads directly and causes oocyte maturation (Kucharczyk et. al 1997a, Yaron *et al.*, 2009), while Ovopel, Ovaprim and OVA-RH (combined with MET) are synthetic peptides affecting the gonads through the pituitary gland, which releases gonadotropins and causes gamete maturation (Brzuska, 2006; Jamróz *et al.*, 2008; Krejszeff *et al.*, 2008). In the present study, the latency period was lowest in fish from Marózka River (P1) and was the longest in fish from Grabia River (P4). On the other hand, if a single injection was applied, the latency was later than if the same hormones were applied in two injections. This suggests the need to reduce the number of injections during dace reproduction. This knowledge would be very useful, as the preservation of biodiversity is a very important part of science, especially where many suitable biotopes for this species have been modified and human expansion is still ongoing (Penczak *et al.*, 2004; Bolland *et al.*, 2008).

Conclusions

The present study showed that dace females need hormonal stimulation to induce final egg maturation and ovulation under controlled conditions. Stimulation by environmental factors (control group) as well as hCG and GnRHa alone did not induce ovulation. Males from the control group produced similar volumes of milt, but with lower sperm motility compared to other fish. Better results were obtained by applying Ovaprim (containing GnRHa and combined dopamine inhibitors), OVA-RH with MET and CPH. The differences in ovulation synchronization were high between different populations. The data obtained in the present paper could be applied to the production of rheophilic cyprinid stocking material from local population better manage wild stocks, and, consequently, could form an important tool in the conservation of endangered or over-exploited finfish populations in the wild.

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