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ARTIFICIAL SPAWNING OF IDE (*LEUCISCUS IDUS*) UNDER CONTROLLED CONDITIONS

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ABSTRACT

The present paper describes the results of induction spermiation and ovulation of ide (*Leuciscus idus* L.) during and out of spawning season using CPE with addition of hCG, hCG and ovopel - GnRH containing pellets. Males from control group produced significantly smaller volume of milt (season) than those from hormonally treated

groups. Significant differences in spermatozoa motility was noted between treated and control males. The percent of ovulated females from groups treated with CPE and ovopel was 100%. Fish from control groups and treated with hCG did not ovulate. The total spawners mortality was quite high, especially in fish treated with ovopel. Generally, all recorded parameters in out of spawning season were lower than those obtained during artificial propagation in natural spawning time.

Key words: ide, artificial propagation, hormonal treatment, spawners mortality.

INTRODUCTION

The one of the most important problem in cyprinid aquaculture is obtaining good quality gametes [6, 15]. For this reason hormonal treatment is used for stimulating of gametes maturation in commercial cyprinid production. One of the most commonly applied spawning agents is carp pituitary extract (CPE) [19, 22], in same cases with addition of human chorionic gonadotropin (hCG) [9]. The good results in induced ovulation in cyprinid fish were also obtained after hormonal stimulation from synthetic analogue of gonadotropin releasing hormone (GnRH), frequently with strong dopamine antagonists [2, 21]. Last time Horvath et al. [6] proposed a new technique of stimulation ovulation in commercial cyprinids using GnRH analogue-containing pellets (ovopel). One ovopel pellet (average weight about 25 mg) contains a mammalian GnRH analogue (D-Ala^6, Pro^{9}Net-mGnRH at dose 18-20 μg) and dopamine antagonist: metoclopramide (dose 8-10 mg).

The present above problem of artificial spawning is much better visible in case of wild cyprinids, mostly captured from natural populations. Since many cyprinid wild stocks become extinct, there is a need to fast develop techniques of controlled propagation of these fish. Generally, the papers presented methods of artificial spawning of wild cyprinids are very limited, as well as data about reproductive biology of wild cyprinid and on hatchery techniques [1, 11, 16]. Some of presented methods of artificial reproduction are finished on the moment of ovulation [5]. Our research on wild cyprinids showed that the very important problem was also the biological quality of gametes [10]. On the other hand the development of controlled reproduction of wild cyprinids is still needed as an integral component of ongoing conservation efforts [20].

Ide (*Leuciscus idus*) is wild European cyprinid which inhabits river and lake ecosystems. From about twenty years the caughts of ide in Poland systematically decreases. In some dam lakes and part of rivers the ide populations are extinct due to environmental pollution and excessive sport fishing. On the other hand ide is also an important component of pond cyprinids aquaculture.

There is a need to develop techniques of controlled propagation in ide culture. Source data are available on reproductive biology and on hatchery techniques of this species [3, 12]. Till now, no attempts to obtain gametes without spawning season were carried out. The purpose of this study was to develop an efficient method of artificial propagation of ide with using hormonal stimulation.

MATERIALS AND METHODS

Broodstock collection

Ide spawners were obtained in Pierzchaly Dam Reservoir (North Poland) and Dgal Experimental Hatchery near Gizycko. After catching fish were transported to the Hatchery in

Olsztyn. To reproduction the selected spawners were kept in separate 1000-L tanks with controlled temperature and photoperiod [14].

Checking the oocytes maturation

All fish were individually marked using floy tags, weighted and oocytes were taken from females using the method described by Kujawa and Kucharczyk [13]. Oocytes were sampled *in vivo* and placed in Serra's solution for clarification of the cytoplasm. After 5 minutes, the position of oocyte nucleus was determined using a 4-stages scale:

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

For further experiment only those females were used which oocyte maturation was between 2-3 and 3 division, i.e. is the best moment for hormonal stimulation in cyprinids [7].

Artificial propagation during spawning season

Fish were divided into four groups: three experimental and control one. After five days of acclimation to the temperature of 15 °C, the fish were treated with respective hormonal injections of common carp pituitary (Argent, USA) extract with the addition of hCG, hCG alone (Biomed, Poland) or ovopel (Table 1). All spawning agents were prepared with 0.9% NaCl: pituitary extract was homogenizated, hCG dissolved and ovopel pellets were pulverised in a mortar and that dissolved. Injections of hCG were intramuscular in the dorsal area of the body [19]. Injections of pituitary [9] and ovopel [6] extracts were intraperitoneal at the base of the pelvic fin. Before manipulations fish were anaesthetised with 2-phenoxyethanol (0.5 mg l^{-1}). Time intervals between respective injections are 24 hrs.

Table 1. The doses of hormones applied in artificial spawning of ide (*Leuciscus idus* L.) during spawning season

Group	Males	Females	
	hormonal dose	priming dose	resolving dose
1	2.0 mg CPE	1000 IU hCG	3.6 mg CPE
		0.4 mg CPE	
2	1000 IU hCG	500 IU hCG	2000 IU hCG
3	1/2 ovopel pellet	1/10 ovopel pellet	1 ovopel pellet
Control	+	+	+

+ - injections from 0.9% NaCl.

Artificial propagation before spawning season

Spawners were caught in October in Pierzchaly Dam Reservoir. Fish were transported to the hatchery and kept in 1000-L tanks with controlled temperature and photoperiod. The changes in water temperature and photoperiod was shown at Fig. 1. Fish were divided into three

groups (Table 2). The artificial spawning was made during method described in part of this paper: “artificial propagation during spawning season”.

Figure 1. Changes in temperature and photoperiod during out of season spawning on ide (*Leuciscus idus* L.). An arrow indicated the moment of applying hormonal stimulation

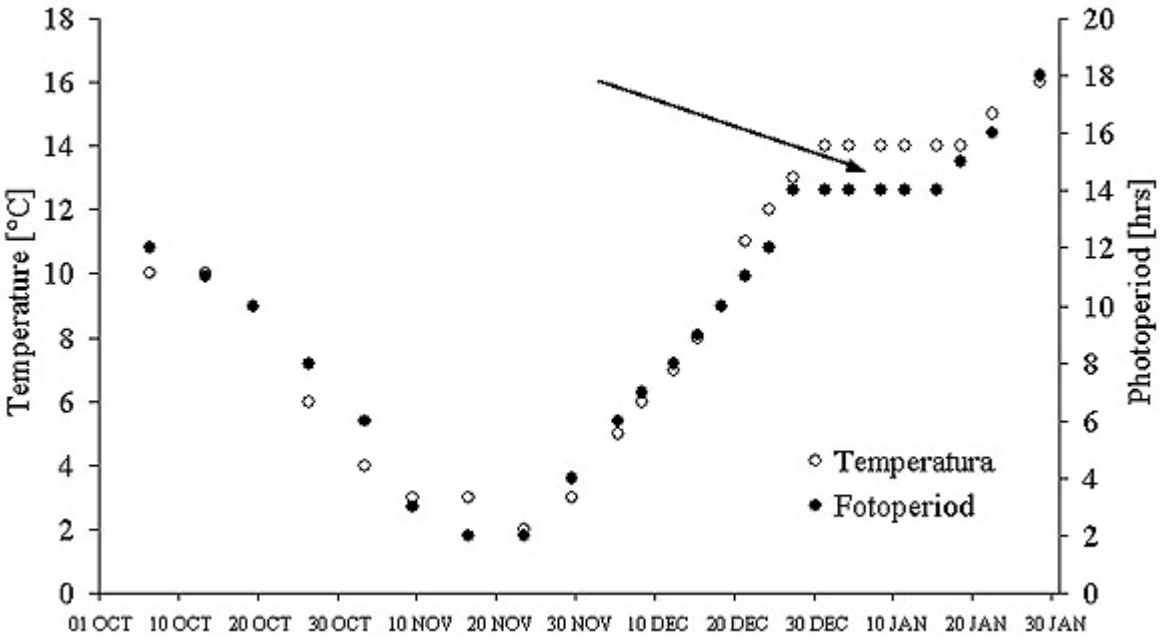

Table 2. The doses of hormones applied in artificial spawning (out of season) of ide (*Leuciscus idus* L.)

Group	Males	Females	
	hormonal dose	priming dose	resolving dose
1	2.0 mg CPE	1000 IU hCG	3.6 mg CPE
		0.4 mg CPE	
2	1/2 ovopel pellet	1/10 ovopel pellet	1 ovopel pellet
Control	+	+	+

+ - injections from 0.9% NaCl.

Collection of gametes and incubation

Ripe gamete donors were anaesthetised in a solution of 2-phenoxyethanol (0.2 ml per 1l). Milt was collected with plastic syringes and kept at 4 °C until further treatment. Within 30 min after collection spermatozoa motility was estimated subjectively under a microscope (x 500) in 0.5% solution of NaCl; such solution ensured intensive motility of spermatozoa [8].

Females were checked every one hour between 24 and 40 hours after resolving injections. Eggs were stripped into a plastic vessel. Eggs were fertilized using “dry method” [9]. For eggs fertilization only those sperm were taken which showed the motility of more than 70% of spermatozoa. Two egg samples (250-300 eggs each) from each females were mixed with 0.5 ml of pooled milt sample. Eggs were incubated at temperature of 14-15°C.

Milt from males induced to spermiate was collected 36 hrs after hormonal stimulation. All spawners were kept one weeks after end of experiment to observe their survival.

Statistical analysis

Statistical differences between groups (spermiation success and incubation success) were analysed with Duncan's multiple range test ($P < 0.05$) [17].

RESULTS

Artificial propagation during spawning season

All males from control and treated groups were spermiated (Table 3). The significant differences were observed in quantity of obtained milt. The highest volume of sperm was produced by males stimulated with CPE and ovopel in contrast to fish from control group. Males treated with hCG gave similarly volume milt to those from control, but with much higher spermatozoa motility. Milt from all hormonally stimulated groups shown average spermatozoa motility over 70%.

Table 3. The results (± SD) obtained in artificial spawning of ide *Leuciscus idus* L.)

Group of fish	Control	1.	2.	3.
Nos. of males	5	6	6	6
Spermation success [%]	100	100	100	100
Quantity of milt [ml·kg^{-1}]	2.1 ± 0.3^{b}	4.3 ± 0.4^{a}	2.2 ± 0.5^{b}	4.4 ± 0.5^{a}
Spermatozoa motility [%]	52 ± 21^{b}	76 ± 9^{a}	77 ± 8^{a}	75 ± 11^{a}
Males mortality [%]	0	17	0	34
Nos. of females	8	8	7	8
Percentage of ovulation	0	100	0	100
Oocyte maturation in non-ovulated females	slightly	-	yes	-
The latency time [hrs]	-	30-32	-	36-40
Embryos survival [%]	-	65.9 ± 9.7^{b}	-	79.3 ± 8.6^{a}
Females mortality [%]	0	12	14	25

Data market this the same letter did not differ statistically. Groups are described in Table 1.

Ovulation was observed only in two treated groups (CPE and ovopel) (Table 3). The latency time was over 30 hrs, but fish treated with CPE ovulated earlier. The embryo survival (to the eyed-egg-stage) was higher in group stimulated with ovopel. Oocyte maturation was observed in fish treated with hCG, in contrast to those from control one.

The mortality of spawners was low, except group where fish were treated by ovopel.

Artificial propagation before spawning season

All males used in this experiment were spermiated (Table 4). Fish treated with CPE produced highest volume of milt. Males from both treated groups gave sperm with higher spermatozoa motility than those from control one.

Table 4. The results (± SD) obtained in artificial spawning of ide (*Leuciscus idus* L.)

Group of fish	Control	1.	2.
Nos. of males	4	4	4
Spermation success [%]	100	100	100
Quantity of milt [ml·kg^{-1}]	1.1 ± 0.2^{b}	3.3 ± 0.5^{a}	1.8 ± 0.4^{b}
Spermatozoa motility [%]	41 ± 11^{b}	66 ± 12^{a}	67 ± 11^{a}
Males mortality [%]	0	25	50
Nos. of females	6	6	6
Percentage of ovulation	0	100	100
Oocyte maturation in non-ovulated females	slightly	-	-
The latency time [hrs]	-	30-33	36-42
Embryos survival [%]	-	62.8 ± 10.5	63.6 ± 9.4
Females mortality [%]	17	34	50

Data market this the same letter did not differ statistically. Groups are described in Table 2.

All females from treated groups were ovulated (Table 4). The latency time was over 30 hrs. The average embryo survival to the eyed-egg-stage was over 60%. The highest mortality was noted in group 3 (ovopel).

All recorded parameters were lower than those obtained during artificial propagation in spawning season.

DISCUSSION

In many papers concerning artificial spawning of cyprinids, problem of spermiation is in out side of aim study [2, 4, 4, 6, 19]. Only a limited number of works describes this problem [9, 18]. From hatchery practise of work with cyprinids, especially from wild stocks or populations, it is good known that one of the main problem is not ovulation, but small volume of sampled milt, in many cases with low spermatozoa concentration and bad spermatozoa motility.

As in previous work [9, 18] in cyprinids males after hormonal stimulation produced significantly more milt than those from control with better spermatozoa motility. In gudgeon, (*Gobio gobio* L.), injection from LHRH with addition of pimozide did not provide to obtained more milt than from "control" males (dr. Patrick Kestemont, - personal communication). Generally, males during spawning season produce more milt with higher spermatozoa motility than those spawned out of season. Probably, this was a result of much longer keeping these fish in captivity (stress, feeding, etc.).

Two hormonal combination (CPE with hCG and ovopel) work well during and out of spawning season. Similarly, as in case of milt, better result were obtained during spawning season. Such phenomenon was reported by Kucharczyk et al. [10] for common bream.

Quality of ide gametes obtained in present work after hormonal stimulation was relatively high, especially in comparison to previous papers about artificial propagation of this species

[3]. Probably, good quality of gametes might be also correlated with water temperature of keeping spawners and incubated eggs.

The differences in latency in females treated with CPE and GnRH (ovopel) were reported in many papers. Drori et al., [4] and Yaron, [21] suggested that latency was always shorter in fish treated with carp pituitary extract than in fish treated with other hormones and drugs. It may be explained by fact that GnRH release from the pituitary and the ovarian response to the released for hormones is a sequential process, while in fish injected with carp pituitary extract, ovarian response to the exogenous GtH was a single process [4].

The main problem observed during this study was survival of spawners, especially fish treated with ovopel. These hormonal combination (mammalian GnRH with metoclopramide) provide very good results, but the mortality problem shown that further studies are still needed.

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