

ARTIFICIAL REPRODUCTION OF THE IDE *LEUCISCUS IDUS* (L.) BRED UNDER CONTROLLED CONDITIONS

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Key words: ide, hormonal stimulation, GnRHa, farm fish school.

Abstract

The aim of the study was to compare the effectiveness of hormonal stimulation of the female ide (*Leuciscus idus* L.), following the application of two commercial preparations concerning different GnRHa combined with dopamine inhibitors: Ovopel and Ovaprim. Ide spawners were bred under controlled conditions. The examined parameters included the percentage of ovulating females, time of ovulation and the rate of embryo survival to the eyed-egg stage. The growth and survival rates for larvae produced by farm and wild fish were compared. Stimulation with Ovopel resulted in a shorter latency time (36), whereas stimulation with Ovaprim resulted in a higher percentage of live embryos at the eyed-egg stage (79.7%). Eggs was obtained from 20% of females in the control group, whereas in treated groups it was at level 90–100%. No differences were found between the growth rate of the ide larvae produced by the farm fish and that obtained from wild fish.

SZTUCZNY ROZRÓD JAZIA *LEUCISCUS IDUS* (L.) WYHODOWANEGO W WARUNKACH KONTROLOWANYCH

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Słowa kluczowe: jaz, stymulacja hormonalna, GnRHa, stado hodowlane.

Abstrakt

Celem pracy było porównanie efektywności stymulacji hormonalnej samic jazia (*Leuciscus idus* L.) po zastosowaniu dwóch różnych preparatów zawierających analog GnRH i inhibitor dopaminy (Ovopel i Ovaprim). Eksperyment przeprowadzono w warunkach kontrolowanych. W trakcie

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jego trwania notowano odsetek owulujących samic, czas owulacji oraz przeżywalność embrionów do stadium zaoczkowania. Porównano również tempo wzrostu i przeżywalność larw uzyskanych od ryb hodowlanych i ryb dzikich. Stymulacja Ovopolem poskutkowała krótszym okresem wystąpienia owulacji u samic (36), natomiast Ovaprimum – wyższym odsetkiem żywych embrionów w stadium zaoczkowania (79,7%). Od 20% samic z grupy kontrolnej uzyskano także ikrę, podczas gdy od ryb doświadczalnych od 90–100%. Nie stwierdzono różnic w tempie wzrostu larw jазia pozyskanych ze stada hodowlanego i od ryb dzikich.

Introduction

For many years, artificial reproduction of non-commercial cyprinids was outside the scope of interest of breeders and scientists (KREJSZEFF et al. 2008, ŻARSKI et al. 2009, TARGOŃSKA et al. 2010). However, an increase in the body of research into different aspects of reproduction biotechnology, such as obtaining gametes, their quality and effects of incubation (KUCHARCZYK et al. 1997a, b, c, 2005, SZABO et al. 2002, TARGOŃSKA et al. 2010, ŻARSKI et al. 2011), sperm biology (GLOGOWSKI et al. 1997, 1999, KOWALSKI et al. 2003, 2004, CEJKO et al. 2010), genomic manipulations (KUCHARCZYK et al. 1997d) and sperm cryopreservation (BABIĄK et al. 1998) has been observed in recent years. The results of reproduction are greatly affected by the origin of spawners and their breeding conditions (KREJSZEFF et al. 2009, 2010). Frequently, the origin of fish, spawner rearing technique and the degree of their domestication affect the economic profitability of reproduction (HAKUĆ-BŁAŻOWSKA et al. 2009, 2010).

Reproduction of freshwater fish is often impossible without any hormonal stimulation and this applies mainly to commercial fish, such as the carp, *Cyprinus carpio* L. (BRZUSKA 2000, 2005, KUCHARCZYK et al. 2008), the tench *Tinca tinca* L. (MAMCARZ et al. 2006, KUJAWA et al. 2011) or goldfish *Carassius auratus* L. (TARGOŃSKA, KUCHARCZYK 2011) and rheophilic cyprinids, such as the asp *Aspius aspius* L. (TARGOŃSKA et al. 2010), the ide *Leuciscus idus* L., the dace *Leuciscus leuciscus* L. (ŻARSKI et al. 2009), the nase *Chondrostoma nasus* L. (SZABO et al. 2002) and the chub *Leuciscus cephalus* L. (KREJSZEFF et al. 2008). Hormonal stimulation is not necessary to start reproduction in percidae and the burbot *Lota lota* L., but it positively affects spawning synchronisation and increases the percentage of fish which start reproduction (KUCHARCZYK et al. 1996, 1998, SZCZERBOWSKI et al. 2009, ŻARSKI et al. 2010). Only in some domesticated fish is it possible to obtain gametes without hormonal stimulation (KREJSZEFF et al. 2009, KUCHARCZYK et al. 2010). Examination of a hormonal agent for common use in fish reproduction takes into account its effect on quantitative parameters (the number of fish ready to spawn, working and relative fertility, volume of milt) as well as qualitative ones (post-spawn

mortality) for spawners and its products (spermatozoa motility, embryo survival rate). Convenience of use and simplicity of the procedure are also important. So, there is need a possibility to prepare new spawning protocols for domesticated stock of fish, especially when the fish stock was a 'live gene bank'. There is no data about artificial reproduction of ide bred under controlled conditions. Such stimulation in fish can be effected at the level of the hypothalamus, the pituitary gland or gonads. The method of reproduction used initially was based on introducing exogenous gonadotropins to the body (YARON 1995). Spawning success was frequently affected by the quality of pituitary homogenate and concentration of the hormones contained in it and, consequently, by the choice of the dose for injection. The necessity, both economic and practical, to improve the effectiveness of fish reproduction has forced breeders to seek more effective methods. Stimulation has been effected with human chorionic gonadotropin hCG, and synthetic analogues of GnRH (PETER 1993, KUCHARCZYK et al. 1996, 1998). However, due to the fact that secreting gonadotropin by the pituitary gland may be inhibited by dopamine in many fish species (PETER and YU 1997, MYLONAS, ZOHAR 2001), it has become necessary to administer dopamine antagonists (metoclopramide or domperidone) with the hormone (HORVATH et al. 1997, YARON et al. 2009), which has greatly complicated the injections, especially on a mass scale. It was a breakthrough in controlled reproduction when preparations acting on endogenous gonadotropins with the ready mixtures of GnRH analogues (mammalian or piscine) and dopamine antagonists appeared on the market.

The aim of this study was to compare the effectiveness of selected hormonal preparations in controlled reproduction of the ide, *Leuciscus idus* (L.) bred under controlled conditions.

Materials and Methods

Spawners breeding

Ide spawners with individual weight ranging from 123 to 170 g (220 fish) were reared from larvae. The spawners' parents (breeding generation F₁) had been brought to the Aquarium Hall of the Department of River and Lake Fisheries, University of Warmia and Mazury, Olsztyn from the Fishing Farm Knieja near Częstochowa, and their reproduction was carried out by the method described by ŻARSKI et al. (2009) with the use of Ovopel at total dose 1.2 pellet kg⁻¹. The larvae produced as a result were reared at the temperature of 25°C and were initially fed with *Artemis* nauplii followed by granulated feed. When the fish had reached the length of 4 cm, the water temperature was lowered to

14–15°C and maintained until the end of the rearing period. The fish were given mixed feed: granulated trout feed produced by Aller Aqua and frozen chironomids larvae in the proportion of 1:1. The daily feeding dose ranged from 1 to 1.5% of the fish biomass.

Fish handling

Before the experiment started, the water temperature was lowered to 10°C for 14 days. Before being transferred to the tanks, the fish were divided in groups according to their sex. The 1000 dm³ tanks in which the spawners were placed were fitted out with aerating equipment and devices for controlling water temperature and photoperiod (KUJAWA et al. 1999). The water temperature in the tanks was 10°C on the day when spawners were transferred into it. Hormonal injections were preceded by a several days of adaptation to the breeding conditions. The photoperiod was constant throughout the experiment and equal to 12 h (12 L : 12 D). Oocyte samples were taken from females using a catheter and the maturity stage was determined based on the position of the germinal vesicle according to the 4-degree scale (BRZUSKA 1979):

- stage 1 – the germinal vesicle is in the oocyte centre;
- stage 2 – the germinal vesicle is in less than half the oocyte radius;
- stage 3 – the germinal vesicle is outside half of the oocyte radius;
- stage 4 – the germinal vesicle is on the cell fringes or on the wane (germinal vesicle break-down – GVBD).

Hormonal stimulation

Hormonal stimulation was performed when the oocytes were at 2–2/3 maturity stage. Before the first injection, the fish were labelled using floy-tags and divided into groups according to the hormonal preparation used for stimulation. Fish which were given physiological saline (0.9% NaCl) were used as control. Hormonal stimulation of the fish in the study groups was performed with: Ovopel (Unic-Trade, Hungary) (contains mammalian analogue of GnRH [(D-Ala⁶, Pro⁹-Net)-mGnRH] and dopamine antagonist – metoclopramide) (HORVATH et al. 1997) and Ovaprim (Syndel, Canada) (a complex of the salmon analogue of GnRH [(D-Arg⁶, Pro⁹-Net) sGnRH] and domperidone – dopamine antagonist) (PETER et al. 1993). Injections in females were performed intraperitoneally, under the ventral fin, at the doses presented in Table 1. After the injection, the water temperature in the spawner tank was raised to 12°C for the next 12 hours. After 12 hours, the fish were subjected to another hormonal

injection. Injections in males were performed at the time of the second injection in females at the same doses as females. After the second injection, the water temperature was raised to 14°C. After 30 hours from the second injection ovulation control was started. The females in each group were checked for the next 16 hours every three-four hours.

Table 1
The hormonal agents used in stimulation of the ide reproduction per kg of the spawner's body weight.
The time between injections was 24 hours

Group	Control	Ovaprim	Ovopel
I injection	0.9% NaCl	0.1 cm ³	0.2 granule
II injection	0.9% NaCl	0.5 cm ³	1.0 granule

Gametes were obtained from spawners by delicate abdominal massage and pressing. Eggs from each experimental group were collected to separate plastic bowls. In order to determine the effect of the stimulant on the biological quality of gametes in each experimental group, 3 egg samples were taken in each of the groups (100–150 eggs), and subsequently incubated on Petri dishes in water at 16–18°C. The embryo survival rate was determined on the day when the spawn achieved the eyed-egg stage. All the manipulations on fish were preceded by anaesthetising them by immersion in 0.5 cm³ dm⁻³ solution of 2-phenoxyethanol (Sigma-Aldrich, Germany).

Larvae rearing

Larvae obtained from farm and wild fish, reproduced by the same method with the use of Ovaprim (ŻARSKI et al. 2009) (the spawners were caught in the Pisa river) were reared for 21 days in 20 dm³ tanks at the density of 50 fish dm⁻³. They were fed *ad libitum* 3 times a day with Artemis nauplii (groups *A*) or with Artemis nauplii for 12 days, followed by trout starter (groups *P*). The temperature of rearing was 25°C. Thirty fish were taken from each of the variants in weekly intervals and were anaesthetised in 2-phenoxyethanol solution (Sigma-Aldrich, Germany) at 0.4 cm³ dm⁻³. The fish were photographed and then returned to the tanks from which they were taken. The documentation thus accumulated was used to measure the total length of the larvae. The measurements were made within an accuracy of 0.01 mm (ProgRes® Capture Pro 2.5, Jenoptic, Germany). Larvae mortality and percentage of developmental deformities were also recorded during the rearing period. The experiment was performed in three replications.

Statistical analysis

The statistical differences between groups were analysed with the analysis of variance (ANOVA), and the Tukey's post hoc test was used after obtaining significant values ($P < 0.05$). Before the statistical analysis, the data expressed in percentage were subjected to arcsine transformation.

Results

90–100% of females in the study groups started the reproduction process, whereas only 20% of females in the control group ovulated (Table 2). The time of ovulation in the study was longer after Ovaprim was used as compared to the other study group and lasted 38–48 hours. Ovulation was observed after 36 hours in the fish stimulated with Ovopel. The females in the control group were ready to spawn after 36 to 50 hours. The rate of embryo survival at the eyed-egg stage in the Ovaprim group was significantly higher than that in the Ovopel group and was close to 80% (Table 2). The rate of embryo survival was the lowest in the control group, but it was not statistically different from the Ovopel group. No mortality among the spawners was found.

Table 2

The results of the ide reproduction under controlled conditions

Attribute/group	Control	Ovaprim	Ovopel
Number of males	10	10	10
Spermiation	100%	100%	100%
Number of females	20	20	20
Ovulation	20%	100%	90%
Latency time (h)	36–50	38–48	36
Rate of embryo survival to the eyed-egg stage (%)	46.5 ± 6.5 ^c	79.7 ± 2.1 ^a	67.3 ± 3.1 ^b
Spawner mortality (%)	0	0	0

The groups in lines with the same letter index are not statistically different

No differences in the final length of larvae from spawners of different origin were observed (Table 3). Nor were there any differences in the rate of embryo survival in groups A, whereas a higher survival rate was observed in fish in groups P obtained from farm spawners, compared to that observed in groups A.

Table 3

The results of ide rearing when larvae were obtained from spawners from different breeding systems

Fish school	Farm		Wild	
	<i>A</i>	<i>P</i>	<i>A</i>	<i>P</i>
Initial length	8.12 ± 0.24 ^a	8.12 ± 0.24 ^a	8.27 ± 0.27 ^a	8.27 ± 0.27 ^a
Final length	19.11 ± 0.87 ^a	19.08 ± 1.02 ^a	19.05 ± 1.12 ^a	19.01 ± 1.26 ^a
Survival	98.8 ± 0.83 ^a	98.4 ± 1.05 ^a	98.6 ± 1.00 ^a	92.4 ± 1.34 ^b
Deformations	0	0.1 ± 0.1	0	0.1 ± 0.1

The groups in lines with the same letter index are not statistically different

Discussion

With the growing demand for fry-stocking material of many fish species, it is becoming necessary to improve the techniques of stimulation and reproduction control, and to develop such methods from scratch for some species. It is particularly important when it applies to endangered species (PHILIPART 1995). Also, when spawners from wild, farm or even domesticated fish schools can be used for reproduction, it is important to develop proper procedures of hormonal stimulation (KREJSZEFF et al. 2009, 2010). Developing detailed reproduction biotechniques makes it possible to implement restoration programmes and to carry out the relevant research (BABIÁK et al. 1998, TARGOŃSKA et al. 2010). A number of hormonal preparations have become available during the past several years, which can be used in controlled fish reproduction (YARON 1995, BRZUSKA 2000, 2005, SZABO et al. 2002). However, this has required many studies into determining the optimal conditions for the procedure as well as the type of the preparations to be used and its dosage. This study confirms earlier reports on the effectiveness of the hormonal preparations in reproduction of rheophilic cyprinids, used in order to induce ovulation in fish, including the ide (ŻARSKI et al. 2009). A positive effect of stimulation with such agents has been observed in the nase (SZABO et al. 2002), the chub (KREJSZEFF et al. 2008) and the asp (TARGOŃSKA et al. 2010). A high percentage of ovulations observed in the study groups in this experiment (90–100%) was close to the results of reproduction of the ide living in natural conditions and farm fish during the reproduction period (KREJSZEFF et al. 2009, ŻARSKI et al. 2009). Ovulation observed in the fish in the control group is proof of the progressive domestication and likely increasing resistance of fish to stress (KREJSZEFF et al. 2009).

Different latency time after injection of hormonal agents has been previously reported (YARON 1995, BRZUSKA 2000, 2005, KREJSZEFF et al. 2009). This

frequently applies to differences of body reaction to carp pituitary homogenate (CPH) as compared to preparations which contain GnRH analogues (KUCHARCZYK et al. 2005). Two different analogues of GnRH have been used in this experiment, whose action is the same and which contain different dopamine antagonists. Application of Ovaprim in the present paper elongated latency time in comparison to females which was stimulated with Ovopel. A different reaction of the body, which manifested itself in the time of ovulation, and which was the result of injections of different GnRH analogues, has been observed, for example, in the carp (BRZUSKA 2000). A similar reaction in the ide was reported by ŻARSKI et al. (2009), who observed the highest synchronisation of ovulation following administration of Ovopel (36 h) as opposed to the group stimulated with Ovaprim, in which ovulation was observed between 36 and 44 hours after the hormone-releasing injection. A longer time of latency following the use of Ovaprim as compared to Ovopel has been observed in the asp (TARGOŃSKA et al. 2010). One of the main parameters of effectiveness of controlled reproduction with hormonal stimulation is the biological quality of gametes, expressed as the percentage of live embryos at the eyed-egg stage. This study has revealed a higher embryo survival rate after using Ovaprim as compared to Ovopel. A similar relationship has been reported by JAMRÓZ et al. (2008) for the ide and ŻARSKI et al. (2009) for the ide and the dace.

The growth rate of the ide larvae, observed in this study, is similar to that observed by other authors (KWIATKOWSKI et al. 2008, ŻARSKI et al. 2008). The absence of differences between the growth rate of ide larvae from different environments shows the full usability of ide spawners bred under controlled conditions as material for reproduction. This provides the possibility of keeping fish schools under controlled conditions as live gene pools.

The results have shown that much better results can be achieved in controlled reproduction of the ide with Ovaprim. This is indicated by better quality of gametes, expressed in this experiment as survival of embryos to the eyed-egg stage. Better synchronisation of ovulation after using the preparation can be achieved by making the first (initiating) injection of Ovopel. However, both the hormonal agents can be successfully used in controlled reproduction of fish from a school bred under controlled conditions. Although the larvae produced were slightly smaller than those obtained from wild fish, no negative effect of the fish origin on the results of initial larvae growth rate was observed. This means that fish schools bred under controlled conditions can be used as live genome pools of valuable populations or species.

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