POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., Vol 26(2): 151–157, Y. 2011

OPTIMIZATION OF ARTIFICIAL REPRODUCTION OF ASP, *ASPIUS ASPIUS* (L.) UNDER CONTROLLED CONDITIONS

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K e y w o r d s: asp, artificial reproduction, wild cyprinids, Ovopel, Ovaprim.

A b s t r a c t

Artificial reproduction of asp under controlled conditions was done using two different spawning agents based on GnRH analogues and dopamine antagonists (Ovopel and Ovaprim). Fish in the Ovopel and Ovaprim and combined treatment groups were treated with a dose equivalent to 1.2 pellets (0.2 and 1.0), 0.5 cm^3 liquid (0.1 and 0.4) and 0.2 pellets and 0.4 cm^3 liquid per kg of body weight respectively. The highest percentage of ovulation (100%) and embryo-survival to the eyed-egg--stage (81.3%) was recorded after the application of a combination of Ovopel and Ovaprim in comparison with other groups. Fish from the control group did not ovulate. The latency time was shorter in the groups where Ovopel and Ovopel with Ovaprim was applied (40) than in Ovaprim group (42–44 hrs). The obtained results indicates that combination of Ovopel with Ovaprim might be successfully used for artificial reproduction of asp.

OPTYMALIZACJA KONTROLOWANEGO ROZRODU BOLENIA, *ASPIUS ASPIUS* (L.) W WARUNKACH KONTROLOWANYCH

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S ł o w a k l u c z o w e: boleń, rozród kontrolowany, ryby karpiowate, Ovaprim, Ovopel.

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Abstrakt

Kontrolowany rozród bolenia przeprowadzono z zastosowaniem dwóch różnych preparatów opartych na analogach GnRH i inhibitorach dopaminy (Ovopel i Ovaprim). U ryb z grupy kontrolnej nie stwierdzono owulacji. Najwyższy odsetek owulacji (100%) oraz przeżywalność embrionów do stadium zaoczkowania (81.3%) zaobserwowano po zastosowaniu kombinacji Ovopelu i Ovaprimu. Krótszy czas owulacji odnotowano w grupach, w których aplikowano Ovopel oraz Ovopel z Ovaprimem (40 godzin) niż w grupie, w której zastosowano tylko Ovaprim (42–44 godziny). Uzyskane wyniki wskazują, że Ovopel w kombinacji z Ovaprimem mogą być z powodzeniem używane w kontrolowanym rozrodzie bolenia.

Introduction

Cyprinid aquaculture has been developing rapidly in the last two decades. The list of cultured species was still increasing and there are two main topics of production: for human consumption and for restocking. In Europe, such species as ide *Leuciscus idus* (KREJSZEFF et al. 2009), dace *Leuciscus leuciscus* (ŻARSKI et al. 2009), chub (KREJSZEFF et al. 2008, 2010) and asp *Aspius aspius* (TARGOŃSKA et al. 2010) are cultured in closed systems and ponds. Every year, millions of larvae and juveniles are put into lakes and rivers (WOJDA 2004). Quickly increasing the economic effectiveness of such kind of production is of critical importance (HAKUĆ-BŁAŻOWSKA 2009, 2010). But in all cases, the artificial reproduction has been the basis of production.

The reproduction of asp under controlled conditions was only possible after applying hormonal stimulation (TARGOŃSKA et al. 2008, 2010, FALAHATKAR et al. 2010). To date, different spawning agents have been tested in artificial reproduction of asp: carp pituitary homogenate (CPH) and commercial products containing GnRH analogues combined with dopamine inhibitors: Ovopel and Ovaprim. Obtaining results showed that hormonal stimulation was important not only in the case of females but also in the case of males (CEJKO et al. 2008). Recently, the combination of Ovopel and Ovaprim was found to have a high impact on artificial reproduction in wild cyprinids (ŻARSKI et al. 2009).

The aim of this study was to compare the spawning effectiveness of Ovopel, Ovaprim and their combination in artificial reproduction of asp.

Material and Methods

The spawners were collected during the winter (end of February) from Lake Mosąg (Olsztyn district). River Łyna are flowing through the Lake Mosąg (Dam Reservoir). Selected spawners were transported to the hatchery of the Department of Lake and River Fisheries, University of Warmia and Mazury in

Olsztyn. The size of spawners ranged from 1.7 to 2.4 kg for males and from 1.9 to 3.6 kg for females. The selected males and females (without visible damages) were kept in separate 1000 L tanks in the hatchery with controlled temperature and photoperiod (12 L : 12 D) (KUJAWA et al. 1999). The maximum fish load in the tanks was 25 kg m^{-3}. The dissolved oxygen level was maintained above 6 ppm.

All fish were individually marked using floy tags and weighed. Oocytes from females were taken *in vivo* by catheterization and placed in Serra's solution (6:3:1, 70% ethanol, 40% formaldehyde and 99.5% acetic acid) for five minutes. After clarification of the cytoplasm, the position of the germinal vesicle was determined according to a four-stage scale described by BRZUSKA (1979) for common carp. All females had oocytes in 2nd maturation stage.

Fish were divided into four groups: a control group and three experimental ones. Number of females in each groups was presented in Table 1. In the hatchery, the temperature of water in fish tanks was gradually raised from 8 to 10°C. After 2–3 days of acclimation at 10°C, the fish from the experimental groups were treated with two commercial products: Ovopel (Unic-trade, Hungary) or Ovaprim (Syndel, Canada). One pellet of Ovopel typically contained 18-20 μg mammalian LHRH analogue (mLHRHa) [D-Ala^6 Pro^9Net--mLHRH] and 8–10 mg of the dopamine antagonist metoclopramide (HORVATH et al. 1997). Ovaprim contains 20 μg of salmon LHRH analogue (sLHRHa) [D-$Arg^6$$Pro^9$Net-sLH-RH] and 10 mg of the dopamine antagonist domperidon in 1 cm^3 propylene glycol (PETER et al. 1993). Ovopel pellets were pulverized in a mortar and then dissolved in saline (0.25 cm^3 0.9% NaCl per one pellet). Ovaprim is ready to use in a liquid form. Hormones were applied intraperitoneally in double injections at the base of the ventral fin. The time between injections was 24 hours. Fish from the control group were injected with a sterile saline solution (0.5 cm^3 kg^{-1}). Fish in the Ovopel and Ovaprim and combined treatment groups were treated with a dose equivalent to 1.2 pellets (0.2 and 1.0) , 0.5 cm^3 liquid (0.1 and 0.4) and 0.2 pellets and 0.4 cm^3 liquid per kg of body weight respectively. The males were not hormonally stimulated. The average weight of females is presented in Table 1. Before injection, fish were anaesthetized with 2-phenoxyethanol (0.5 cm^3 dm^{-3}) (Sigma-Aldrich, Germany). After hormonal treatment, the water temperature was raised to 11°C and to 12°C after a further 24 hours (TARGOŃSKA et al., 2008). Milt from males was collected using plastic 1 cm^3 syringes and kept at 4°C. Females were checked every 2–4 hours between 36 and 48 hours post injection. Eggs were stripped into a plastic vessel and were fertilized using the "dry method". Only those samples of milt which showed a motility of more than 70% of spermatozoa were used for fertilization. Three egg samples (100–150 eggs each) from each female were mixed with 0.05 mL of pooled milt

taken from at least three males. Eggs were incubated at 12°C on Petri dishes in a closed-water system and survival to the eyed-egg stage was observed. Oocyte samples from non-ovulated females were taken after the experiment and their maturity stage was recorded.

Table 1

Results (mean ± SD) of artificial reproduction of asp under controlled conditions

Parameter/groups	Control	Ovopel	Ovaprim	Ovopel/ Ovaprim
Number of females	4	4	4	4
Mean weight (mean ± SD) of females [kg]	2.3 ± 0.2^{a}	2.4 ± 0.3^{a}	2.3 ± 0.3^{a}	2.2 ± 0.4^{a}
Ovulation [%]	0	50	75	100
Oocyte-maturity-stage after stimulation in non-ovulated females	2/3	3/4	3/4	–
Range of latency time [hrs] (mean ± SD)	–	40	42–44 (43.3 ± 1.2)	40
Fecundity [eggs kg^{-1}]	–	$50124 + 2121^{b}$	$53172 + 2592^{ab}$	$54199 + 1524^{a}$
Embryo survival to the eyed-egg-stage [%]	–	46.5 ± 2.9^{c}	74.2 ± 3.1^{b}	81.3 ± 1.8^{a}

Data in the same row marked with different letters were significantly different ($P < 0.05$).

The control groups did not spawn and, therefore, no control data was included in the statistical analysis. All the data expressed as percent were subjected to arcsine transformation before being analysed statistically. The data for the fecundity were analysed by Kruskal-Wallis non-parametric test ($\alpha = 0.05$). The other data (mean weight of females and embryo survival to the eyed-egg-stage) were analysed by ANOVA. Where the analysis revealed statistically significant differences, a *post-hoc* Duncan test ($\alpha = 0.05$) was carried out.

Results and Discussion

The cultured and wild cyprinids need the application of hormonal agents for final gamete maturation (BABIAK et al. 1998, BRZUSKA 2005), especially in the case of females (KUCHARCZYK et al. 1997, 2005, KREJSZEFF et al. 2008, 2010, PODHOREC, KOURIL 2009, TARGOŃSKA, KUCHARCZYK 2011). Without hormonal stimulation, only slight maturation of oocytes (germinal vesicle migration) was usually observed (KUCHARCZYK et al. 2005, TARGOŃSKA et al. 2010) – Table 1. In the present study only sligthy oocytes maturation was noted in fish from control groups. Only in some cases of domesticated stock of cyprinids is it

possible to obtain eggs without hormonal stimulation under controlled conditions (KREJSZEFF et al. 2009, KUJAWA et al. 2011).

Ovulation rates in the present study were between 50 and 100%, independent of the type of hormonal stimulation (Table 1). This was similar to data obtained for asp by TARGOŃSKA et al. (2010). In wild cyprinids, the ovulation rates were different, e.g. for ide and dace they were usually between 90% and 100% (KREJSZEFF et al. 2009, ŻARSKI et al. 2009) but for chub (30–70%) they were much lower than for asp (KREJSZEFF et al. 2008, 2010). The differences in the fecundity between groups were small: the highest fecundity was noted after the application combined from Ovopel and Ovaprim and the smallest after Ovopel injections. In this case, the fecundity was higher than that described by ŚLIWIŃSKI (1998) for asp and similar to the data presented by TARGOŃSKA et al. (2008). But it is significant that ŚLIWIŃSKI (1998) worked on young and small females (average weight over 1 kg) cultured in carp ponds.

Latency time in the present study for asp females was between 40 and 44 hours after the resolving injection (Table 1). Exactly the same data was noted for this species by TARGOŃSKA et al. (2010) after the application of Ovopel and Ovaprim in single doses. Generally, the latency time was correlated with the type of applied hormonal stimulation, water temperature regimes and fish species. In wild, reophilic cyprinids, such as dace, ide or asp, the latency time was noted from 30 to 50 hrs. For asp, which were usually reproduced at a water temperature of 12–13°C, the latency was usually over 40 hours (TARGOŃSKA et al. 2008). Asp embryos survival rate to the eyed-egg-stage was the highest after the application of Ovopel and Ovaprim: 81.3% (Table 1). Lower survival was noted in Ovarpim group (74.2%) and the lowest in the Ovopel group (46.5%). Similar data was recorded by ŻARSKI et al. (2009) for dace and ide. It also resulted in the highest recorded asp embryo survival (TARGOŃSKA et al. 2008).

The data in the present study showed that the application of two commercial products, Ovopel and Ovaprim, influenced the artificial spawning effectiveness of asp under controlled conditions. This hormonal combination affected the highest spawning effectiveness in comparison to Ovopel and Ovaprim applied alone.

Translated by JOANNA JENSEN

Accepted for print 1.04.2011.

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