POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., Vol 26(3): 259–269, Y. 2011

EFFECT OF DIFFERENT HORMONAL TREATMENTS ON SPAWNING EFFECTIVENESS AND ECONOMIC PROFITABILITY IN WILD NASE, *CHONDROSTOMA NASUS* (L.), UNDER CONTROLLED CONDITIONS

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K e y w o r d s: hormonal stimulation, GnRH analogue, economic profitability, cyprinids, reproduction.

A b s t r a c t

This study determined the effect of hormonal stimulation of the wild female nase, *Chondrostoma nasus* (L.), on its basic reproduction indices (percentage of ovulations, latency time, embryo survival) and the economic profitability of its use. Two commercial preparations were used in the experiment: Ovopel and Ovaprim. They were used separately (group 1 and 2 for Ovopel and Ovaprim, respectively) and in combination (group 3), where Ovopel was given in initial and Ovaprim in resolving injection. The study found a high effectiveness of all the hormonal treatments applied (ovulation rate 90–100%, latency time 36 h, embryo survival rate 78.6–81.2%) ($P>0.05$), which may be evidence of the greater susceptibility of the nase to stimulation with the less active mammalian analogue of GnRH as compared to other species of rheophilic cyprinids. In consequence, the lowest cost of hormonal stimulation (0.59 EUR per 10,000 viable embryos) was achieved with Ovopel. Using the hormonal agents in combination (in group 3) reduced the cost of stimulation by 0.17 EUR as compared to Ovaprim (group 2), where the cost was the highest (1.57 EUR per 10,000 viable embryos). The results presented in this study are providing useful information for fish breeders who manage wild populations of the nase and other species of rheophilic cyprinids.

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WPŁYW RÓŻNYCH PREPARATÓW HORMONALNYCH NA EFEKTYWNOŚĆ ROZRODU I EKONOMICZNĄ OPŁACALNOŚĆ ICH STOSOWANIA U ŚWINKI, *CHONDROSTOMA NASUS* (L.), W WARUNKACH KONTROLOWANYCH

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S ł o w a k l u c z o w e: stymulacja hormonalna, analog GnRH, efektywność ekonomiczna, ryby karpiowate, rozród.

A b s t r a k t

Celem pracy było określenie wpływu rodzaju stymulacji hormonalnej dzikich samic świnki, *Chondrostoma nasus* (L.), na podstawowe wskaźniki rozrodowe (odsetek owulacji, czas latencji, przeżywalność embrionów) oraz ekonomiczną opłacalność ich stosowania. Zastosowano dwa komercyjne preparaty: Ovopel (zawierający ssaczy analog gonadoliberyny [GnRH]) i Ovaprim (zawierający łososiowy analog GnRH). Były one stosowane osobno (grupa 1 – Ovopel i grupa 2 – Ovaprim, odpowiednio) oraz w kombinacji (grupa 3), gdzie Ovopel był podany w iniekcji wstępnej, a Ovaprim w wyzwalającej. Uzyskane wyniki wskazują na wysoką efektywność wszystkich zastosowanych wariantów hormonalnych (odsetek owulacji 90–100%, czas latencji – 36 h, przeżywalność embrionów średnio – 78.6–81.2%) (P>0.05). Może to świadczyć o tym, że świnka jest podatniejsza na stymulację mniej aktywnym ssaczym analogiem GnRH w porównaniu z innymi gatunkami karpiowatych ryb reofilnych. Dzięki temu najniższy koszt stymulacji hormonalnej (0.59 EUR na 10000 sztuk żywotnych embrionów) uzyskano po zastosowaniu Ovopelu. Zastosowanie kombinacji środków hormonalnych (w grupie 3) obniżyło koszt stymulacji o 0,17 EUR w porównaniu z Ovaprimem (grupa 2), gdzie koszt stymulacji był najwyższy (1.57 EUR na 10000 sztuk żywotnych embrionów). Wyniki przedstawione w pracy mogą być bardzo przydatne dla hodowców zarządzających dzikimi populacjami świnki oraz innymi gatunkami karpiowatych ryb reofilnych.

Introduction

Transformations of running waters due to melioration works and dams built on rivers have brought about degradation of natural habitats of many endemic fish species. This applies to both spawning grounds and specific habitats for offspring growth. In consequence, it has reduced the size of many populations (PENCZAK and KRUK 2000, SCHIEMER et al. 2003). Rheophilic cyprinids, which require access to various habitats during their lifetime, are especially exposed to such threats (MANN 1996, PENCZAK et al. 1998). This also applies to species which used to form large and stable populations, such as the nase, *Chondrostoma nasus* (L.) (LUSK and HALACKA 1995, PENAZ 1996, KECKEIS et al. 1997). As a consequence, recent years have seen a considerable increase in scientific research into the production of stocking material of rheophilic cyprinids for restoration purposes under controlled conditions

(KAMLER et al. 1998, WOLNICKI and MYSZKOWSKI 1999, SZABO et al. 2002, SPURNY et al. 2004, KREJSZEFF et al. 2008, TARGOŃSKA et al. 2010). This, in turn, has enabled the development of fish restoration pilot projects by river managers which has improved the quality of many populations. However, adequate abundance of many populations still may only be maintained by supporting natural recruitment through restocking (COWX 1994, PHILIPPART 1995, KUCHARCZYK 2002, PONCIN and PHILIPPART 2002).

Production of larvae and broodstock under controlled conditions enables the production of high quality stocking material (KWIATKOWSKI et al. 2008, KUJAWA et al. 2010). Proper planning may help to effectively manage production outcome (KUPREN et al. 2008, TURKOWSKI et al. 2008). However, controlled reproduction, which directly affects the effectiveness of further procedures, is the basis of success (KREJSZEFF et al. 2008, ŻARSKI et al. 2011). Its effectiveness, in turn, depends on the stock origin (KREJSZEFF et al. 2010a, KUJAWA et al. 2011), thermal manipulations (TARGOŃSKA et al. 2010, ŻARSKI et al. 2010) and the type of the hormonal preparation used (ŻARSKI et al. 2009). The latter factor is an essential condition for producing high quality gametes in wild cyprinid populations (KREJSZEFF et al. 2009).

The effectiveness of controlled reproduction of some wild cyprinids, such as the barbel, *Barbus barbus* (L.), and the chub, *Leuciscus cephalus* (L.), is very low (KREJSZEFF et al. 2008, 2010a) compared to domesticated fish (KREJSZEFF et al. 2009, TARGOŃSKA and KUCHARCZYK 2011). Hence, fish produced in captivity are frequently the basis of a broodstock (KREJSZEFF et al. 2009, 2010a). This allows fish farmers to lower production costs and reduce the interference in the natural spawning course because it is during the reproduction season that spawners are most frequently caught (KUCHARCZYK et al. 2008, TARGOŃSKA et al. 2008). However, it is very important in a restoration programme to manage the gene pool properly in order to maintain a low level of inbreeding (HARADA et al. 1998). Therefore, the reproduction of wild fish is still a very important element of stocking material production. On the other hand, an increase in its effectiveness makes it possible to reduce the intensity of the exploitation of wild spawners (KUCHARCZYK et al. 2008) and considerably lower production costs. This largely applies to economic profitability which arises from using different kinds of hormonal preparations to stimulate final oocyte maturation (FOM) and to synchronise ovulation (HAKUĆ-BŁAŻOWSKA et al. 2009, 2010). It should be stressed that data on the controlled reproduction of the nase is scarce (TARGOŃSKA et al. 2008).

The aim of the study was to determine the effectiveness of the controlled reproduction of the nase with the use of two commercial hormonal preparations (Ovopel and Ovaprim) and their combination, taking into account its economic profitability.

Materials and Methods

Broodstock management

Nase spawners (40 females, 15 males) were caught in autumn (October) 2009 with the use of electrofishing in the Oder river near Opole (in the south-west of Poland). After being caught, the fish were transferred to the flow-through earthen pond in the Paliwoda Fish Farm (PZW Opole), where they were kept through winter. In the early spring, when the water temperature in the pond reached 9°C, the fish were caught and transported to the hatchery of the Knieja Fishery Farm (near Częstochowa, south-east of Poland) where they were put into 1,000 l tanks with controllable thermal and light conditions (KUJAWA et al. 1999). The males and females were kept separately. The water temperature in the tanks was 9°C. After another 24 h, the FOM stimulation procedure started. All the manipulations were preceded by anaesthetising the fish in 2-phenoxyethanol solution (0.5 ml l^{-1}).

Stimulation of FOM and the spawning procedure

Before the hormonal stimulation, oocyte samples were taken from the females with a catheter. They were subsequently put into clarifying solution (ethanol 70%, 36% formaldehyde and 95% acetic acid at the proportion of 6:3:1). The position of the germinal vesicle was determined after 3–5 minutes; it was used as the basis for determination of the oocyte maturity stage according to the classification described by BRZUSKA and BIENIARZ (1977). All the females were in the 2/3 stage of maturity, which was regarded as the best moment for carrying out hormonal stimulation (KUCHARCZYK 2002).

The fish were randomly divided into four groups: three experimental ones (1–3) and a control one. Group 1st comprised fish stimulated with Ovopel (Unic-Trade, Hungary) (a preparation containing 18–20 μg of mammalian analogue of gonadoliberine (GnRH) [(D-Ala^6, Pro^9-Net)-mGnRH] and 8–10 mg dopamine antagonist – metoclopramide) (HORVATH et al. 1997), group 2nd – ones stimulated with Ovaprim (Syndel, Canada) (a complex containing 20 μg of salmon analogue of GnRH [(D-Arg^6, Pro^9-Net) sGnRH] and 10 mg dopamine antagonist – domperidone) (PETER et al. 1993); fish in group 3rd were stimulated with a combination of those preparations, which was described as very effective in the reproduction of rheophilic cyprinids (ŻARSKI et al. 2009). The doses of the hormonal preparations used were the smallest doses which were effective in the reproduction of rheophilic cy-

prinids (e.g. KUCHARCZYK et al. 2008). These doses are shown in Table 1. Injections in each group of fish were made twice in a 12-hour interval. After the first (initial) injection (when fish in group 2 were given a placebo with 0.9% NaCl, the same as in the control group), the water temperature was raised to 11°C. After the second (resolving) injection, the temperature was raised to 12.5°C and then to 13.5°C after another 24 hours. The males were not stimulated. Ovulation control was started after 36 hours from the second injection. To that end, a fish was anaesthetised (2-phenoxyethanol, 0.5 ml l^{-1}), dried gently and the possibility of obtaining single eggs was checked by gently pressing its abdomen. When ovulation was certified, eggs were collected to separate dry plastic containers. Subsequently, three egg samples were collected from each female (100–150 eggs in each) and placed on separate Petri dishes. Samples were fertilised with 0.05 ml of sperm mixture (from at least 5 males). Eggs were incubated in closed water circulation at 14°C, as described by KREJSZEFF et al. (2010b). The embryo survival rate was determined at the eyed-egg stage. Ovulation control was carried out until 42 hours after the second injection. After that time, egg samples were taken with a catheter from the females which did not ovulate and the maturity stage was determined or the occurrence of egg atresia was checked. The ovulation rate and the latency time from the second injection was recorded.

Table 1

Kinds and the doses of spawning agents used for induction of final oocyte maturation in nase, *Chondrostoma nasus* (L.)

Specification	Initial injection	Resolving injection
–	Spawning agent (dose)	Spawning agent (dose)
Control group	NaCl (0.5 ml kg^{-1})	NaCl (0.5 ml kg^{-1})
Group 1	Ovopel (0.2 pellet kg^{-1})	Ovopel (1 pellet kg^{-1})
Group 2	NaCl (0.5 ml kg^{-1})	Ovaprim (0.5 ml kg^{-1})
Group 3	Ovopel (0.2 pellet kg^{-1})	Ovaprim (0.5 ml kg^{-1})

Calculation of economic profitability

The economic profitability of hormonal stimulation was determined based on ovulation rate, relative fecundity of fish, embryo survival rate and the price of the hormonal preparations. The cost of a hormonal injection for each 10,000 viable embryos obtained as a result was taken as the measure of the economic profitability of the procedure; it was calculated according to the following formula:

$$EPHS = ([100\ CHS\ (n\ s^{-1})]\ RF^{-1})\ (N_o\ N_1^{-1})^{-1}$$

where:
EPHS – economic profitability of hormonal stimulation (EUR),
CHS – the total cost of hormonal stimulation per 1 kg of a female (EUR),
n – the number of embryos for which the cost is calculated (here: 10,000),
s – embryo survival rate (%),
RF – relative fecundity (eggs kg^{-1}),
N_1 – total number of females in a group,
N_o – number of ovulating females in a group.

The actual prices of hormonal preparations in 2010, converted to euro (EUR), were taken for calculations. Conversion ratio used for calculation (from USD to EUR) was 1.35. The cost of purchase of 1 granule of Ovopel amounted to 0.4 EUR, while the cost of 1 ml of Ovaprim was 2.37 EUR.

Data analysis and statistic

All fish were weighted before first hormonal injection and prior to spawning (BW). Similarly weight of the stripped eggs (EGW) was estimated. The data were used to calculate the pseudo-gonado-somatic index expressed as percentage of the stripped eggs to the weight of the female prior to spawning (PGSI=[100 EGW] BW^{-1}). Relative fecundity (RF) was calculated on the basis of three subsamples of eggs taken from each female, where all eggs were counted and weighted within an accuracy of 0.001 g.

Statistical differences between groups were determined by an analysis of variance (ANOVA). Before the analysis, all percentage data were subjected to arc-sin transformation. The statistical analysis was performed with Statistica 9.0 (StatSoft, Inc.) software.

Results

Hormonal stimulation of the nase was necessary to induce FOM and ovulation because no eggs were obtained from any female in the control group (Table 2). After 42 hours from second injection, the oocyte maturity stage was the same as at the beginning of the experiment (stage 2/3). Ovulation was observed in at least 90% of females in the experimental groups. After 42 hours from the last injection, egg atresia was observed in non-ovulating females in groups 1 and 2. The latency time was identical in all the groups – 36 h. PGSI was similar ($P>0.05$) in all the groups

and it ranged from 10.7 to 22.1% BW. The average *RF* in all the experimental groups was also similar ($P>0.05$) and ranged from 6781 to 15 188 eggs kg^{-1}. No statistical differences between the groups ($P>0.05$) were found in the rate of embryo survival to the eyed-egg stage.

Table 2
Results obtained during induced spawning of nase, *Chondrostoma nasus*, after application of different spawning agents

Specification	Control	Group 1 (Ovopel)	Group 2 (Ovaprim)	Group 3 (Ovopel/Ovaprim)
Number of females	10	10	10	10
Initial weight of females [g]	366.8 ± 52.4	366.5 ± 57.4	341.7 ± 54.6	370.2 ± 43.3
Ovulation rate [%]	0	90	90	100
Latency time [h]	–	36	36	36
PGSI [% of BW]	–	18.5 ± 2.1	16.2 ± 3.2	18.6 ± 1.9
Relative fecundity [eggs kg^{-1}]	–	11 506 ± 2199	10 494 ± 2398	11 139 ± 1741
Embryos survival [%]	–	78.6 ± 5.6	79.8 ± 5.2	81.2 ± 4.6

Data presented as mean ± SD. No statistical differences were found between treatment groups ($P>0.05$)

An analysis of economic profitability has shown that the lowest cost of producing 10,000 viable embryos (0.59 EUR on average) was achieved when a double injection of Ovopel was applied (group 1). The other treatments required a higher cost, which amounted to 1.57 EUR in stimulation with Ovaprim, and 1.40 EUR in stimulation with a combination of hormonal preparations (Figure 1).

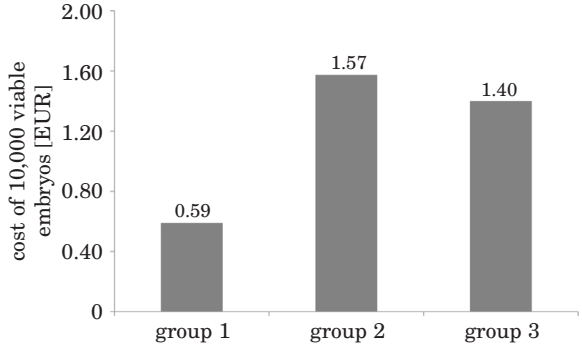

Fig. 1. Cost of 10,000 viable embryos of nase obtained after different hormonal stimulations. Females were injected with Ovopel, Ovaprim and combination of Ovopel and Ovaprim in groups 1, 2 and 3, respectively (for doses see Table 1)

Discussion

Gonadotropins contained in carp pituitary extract (CPE) have been used with success in the reproduction of cyprinids (YARON et al. 2009). However, GnRH analogues administered together with dopamine antagonist (e.g. Ovopel and Ovaprim) proved more effective in a number of cases (see KUCHARCZYK et al. 2008); they affect the pituitary gland and stimulate FOM with endogenous gonadotropins (YARON 1995, ZOHAR and MYLONAS 2001). Analogues of GnRH have also proven effective in rheophilic cyprinids (KREJSZEFF et al. 2008, TARGOŃSKA et al. 2010). There have been reports lately of very good results achieved when a combination of two preparations was used containing different GhRH analogues, where the initial dose of Ovopel improved the effectiveness of Ovaprim administered in a resolving dose (ŻARSKI et al. 2009).

The results of the nase reproduction achieved in this experiment are highly satisfactory when compared to the findings of other authors. SZABO et al. (2002) achieved comparable results (83% of ovulations, 83.5% embryo survival) after administering 20 μg of mammalian analogue of GnRH with 10 mg of dopmeridone in a single dose. The other treatments tested by the authors (CPE at 3 and 5 mg kg^{-1}) were much less effective (67% ovulation rate in both groups, 69.1 and 74.7% embryo survival rate for the CPE dose of 3 and 5 mg kg^{-1}, respectively). ŻARSKI et al. (2008) used the same hormonal preparations which were used in the present experiment and also achieved good results (89 and 100% ovulations following stimulation with Ovopel and Ovaprim, respectively). However, the RF observed in this experiment was more than three times lower than that reported by ŻARSKI et al. (2008) (40200 and 36800 eggs kg^{-1} for Ovopel and Ovaprim, respectively), which may have resulted both from the varied female size (those authors used female fish of more than 500 g) and population-related factors. However, more data are needed to examine the issue in more detail. Moreover, the latency time was also different (from 51 to 57 h), which was probably a result of thermal fluctuations, observed by ŻARSKI et al. (2008), when fish were kept under natural thermal conditions. It must be emphasised that the latency time observed in this study was exactly the same in all groups and the spawning was highly synchronised. Synchronisation of ovulation was possible only owing to the temperature control applied in the experiment. This is of great importance in commercial production, where the amount of labour (which grows as ovulation becomes less and less synchronised) needed to carry out the operation directly affects its cost effectiveness. However, the result is surprising considering the fact that Ovaprim was four times more effective in FOM stimulation in other rheophilic cyprinids, such as the asp, *Aspius aspius* (L.), the ide, *Leuciscus idus* (L.), and the dace, *Leuciscus leuciscus* (L.), inducing ovulation of the high quality eggs in a much

larger number of female fish than was the case with Ovopel (ŻARSKI et al. 2008, 2009, TARGOŃSKA et al. 2010). This resulted from the fact that the form of salmon analogue of GnRH is much closer to the natural form of salmon GnRH, which is a native form occurring in cyprinids (PODHOREC and KOURIL 2009). Therefore, the results suggest that the nase is much more susceptible to stimulation with less active mammalian analogue of GnRH as compared with other rheophilic cyprinids. However, the effect of using different dopamine antagonists contained in the preparations cannot be rejected.

The economic profitability of hormonal stimulation has not drawn much scientific attention to date (HAKUĆ-BŁAŻOWSKA et al. 2009, 2010), despite the fact that it is a factor which largely affects the type of hormonal preparations used in commercial hatcheries. It has been reported that the highest economic profitability in stimulating rheophilic cyprinids is achieved with Ovopel and Ovaprim. The cost of producing 1,000 viable embryos of the ide was exactly the same when either of the preparations was used. However, the relative cost of using Ovaprim in asp reproduction was lower by 30% (HAKUĆ-BŁAŻOWSKA et al. 2009), although Ovaprim itself was more expensive. This was caused by a much higher percentage of ovulations and embryo survival rate following the use of Ovaprim. On the other hand, the cost of stimulation with Ovaprim of cultured barbel was twice as high as when Ovopel was used (HAKUĆ-BŁAŻOWSKA et al. 2010). This resulted from the fact that the result of the reproduction was similar following the use of each of the preparations. A similar relationship was observed in this experiment: the result of the reproduction in each of the hormonal treatments was very good, hence the economic profitability was better in the group where the cheaper preparation – Ovopel – was used. It is noteworthy that administering a combination of hormonal agents resulted in higher ovulation rate which, in turn, reduced the cost of hormonal stimulation as compared to a single Ovaprim injection. However, it has to be pointed out that administration of hormonal preparation in single dose may positively affect economic effectiveness of single Ovaprim dosage using due to limitation of labor costs.

The results achieved in this study show that reproduction of wild nase spawners may be successfully carried out under controlled conditions. The very good spawning result achieved in each treatment of hormonal stimulation indicates that reproduction can be successfully carried out even on farms which have not done so with the nase, but which have experience with other cyprinids. In the view of the obtained results, one can recommend Ovopel stimulation of FOM and ovulation in the nase due to its high economic profitability.

Translated by JOANNA JENSEN

Accepted for print 16.05.2011

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