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## REPRODUCTION OF NASE, *CHONDROSTOMA NASUS* (L.), UNDER CONTROLLED CONDITIONS

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ABSTRACT. The aim of this work was to compare the effectiveness of the application of two hormonal preparations (Ovopel and Ovaprim) in the controlled reproduction of nase, *Chondrostoma nasus* (L.). The experiment was conducted under natural thermal and light conditions. Positive effects of hormonal stimulation were obtained in both experimental groups. No oocytes were obtained from any of the females in the control group. The most highly synchronized ovulation was noted in females that had received Ovaprim. The results of this study confirm reports that female nase must be stimulated hormonally during artificial reproduction in captivity. The results also indicate that Ovaprim is a more effective preparation than is Ovopel.

Keywords: NASE, *CHONDROSTOMA NASUS*, HORMONAL STIMULATION, OVOPEL, OVAPRIM, GnRH

## INTRODUCTION

Nase, *Chondrostoma nasus* (L.), is a rheophilic cyprinid that belongs to the lithophilic reproductive guild. Natural reproduction happens in riffle segments of rivers at water temperatures ranging from 8 and 12°C (Keckeis et al.1996b, Keckeis 2001). Nase is currently endangered species in many European countries. Until recently, nase belonged to a dominant fish species of the barbel zone in rivers (Witkowski 1992, Lusk and Halačka 1995, Peňáz 1996, Keckeis et al. 1997). The disappearance of this species is primarily a consequence of the regulation of flowing waters and the construction of barriers across them that has limited access to areas requisite for spawning and nursery habitats. Other significant threats include river pollution, as well as, more recently, the

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increasing intensity of climatic anomalies that impact the hydrological regimes of rivers (Peñáz 1996, Penczak and Kruk 2000, Schiemer et al. 2003). This is why interest is growing in the restoration of this species to European waters, and, consequently, efforts in the field of aquaculture are intensifying (Keckeis 2001, Schiemer et al. 2003). Evidence of this is research aimed at developing reproductive biotechnologies (Lahnsteiner et al. 2000, 2003, Szabó et al. 2002), and nursery techniques for nase (Keckeis et al. 1996a, Kamler et al. 1998, Wolnicki and Myszkowski 1999, Jaworski and Kamler 2002, Spurný et al. 2004, Sysa et al. 2006).

The stability of restoration programs is largely dependent on the production of stocking material under controlled conditions (Poncin and Philippart 2002), which is why the development of reproduction biotechnology has a fundamental impact on the effectiveness of any restoration efforts that are undertaken (Kucharczyk 2002). The reproduction of many fish species in hatcheries is impossible without the application of hormonal preparations. This refers to spawners originating from wild fish populations (Kucharczyk et al. 1997, Szabó et al. 2002, Heyrati et al. 2007, Krejszeff et al. 2008), as well as from those reared pond conditions (Brzuska 2003, Brzuska and Adamek 2008). The application of hormonal preparations influences ovulation synchronization variously in different fish species, even in those that are related. This is also why the choice of hormonal preparation and dosage has a substantial impact on the effectiveness of reproduction (Kucharczyk 2002). The aim of the current study was to compare controlled nase reproduction effectiveness when applying two different hormonal preparations under hatchery conditions.

## MATERIALS AND METHODS

The nase spawners were caught in the Skawa River (southern Poland) in two subsequent years. The experiment was conducted at the Field Station Department of Applied Ecology, University of Łódź (central Poland). The individuals were tagged with Visible Implant Elastomer (VIE, northwest Marine Technology, USA) to aid identification and were then held in ponds. In the following year, when the water temperature in the ponds reached 12°C the fish were moved to the hatchery and stocked into tanks with a volume of 1 m<sup>3</sup>. Males and females were held separately, and were not fed during their stay in the hatchery (10 days). The tanks were supplied with water from the Tresta

stream, a right-bank tributary of the Sulejowski Reservoir on the Pilica River. Neither water temperature nor photoperiod were regulated.

Females were divided randomly into three groups, and different hormonal stimulation variants were applied to each. Prior to hormonal stimulation, egg samples were collected in vivo with a catheter. These were fixed in Serra fluid and the maturity stage was determined. The fish with oocytes in stage IV maturity were injected with Ovaprim and Ovopel. Ovaprim (Syndel International Inc., Canada) is a liquid preparation containing salmon GnRH analogue (D-Arg<sup>6</sup>, Pro<sup>9</sup> Net-sGnRH) and domperidone, a dopamine antagonist. The manufacturer's recommended dose is 0.5 cm<sup>3</sup> per kg of spawner body weight. Ovopel (Unic-Trade, Hungary), a granulated product that has to be dissolved in a saline solution (0.9% NaCl) prior to administration, contains mammalian analogue GnRH (D-Ala<sup>6</sup>, Pro<sup>9</sup> Net-mGnRH) and the dopamine receptor antagonist metoclopramid (Horvath et al. 1997).

The males were not stimulated hormonally. The females were injected intraperitoneally under the left ventral fin. The control group females were injected with a saline solution (group I). The fish in each experimental group received one hormonal injection. The spawners from group II were stimulated with Ovopel at dose 1 pellet kg<sup>-1</sup> of body weight (BW). The females from group III were stimulated with Ovaprim at dose 0.5 cm<sup>3</sup> kg<sup>-1</sup> BW. The first check of female maturity was 21 h after injection, followed by subsequent checks every 5 h. When ovulation occurred, the eggs were stripped from the females by abdominal massage and placed in plastic containers. Oocyte samples were taken from each female (3 × 0.5 cm<sup>3</sup>), and then the eggs in each sample that had not swelled were counted (quantity not swelled eggs – NSE). This was how the relative working fecundity was determined and was expressed as the number of eggs per female body weight unit. The eggs from the pooled samples of each treatment group were fertilized with sperm obtained from 10 males. Following several rinses in clean water, the eggs were incubated in Weiss jars. During the experiment, female body weight (± 1 g) was determined prior to the first injection and just before egg stripping. Additionally, the water temperature in the tanks was checked at four-hour intervals (Fig. 1). All of the manipulations were performed after the fish had been anesthetized in a solution of 2-phenoxyethanol (Merck, Germany) at a concentration of 0.3 cm<sup>3</sup> dm<sup>-3</sup> and a mean exposure time of 4.0 ± 1.0 min.

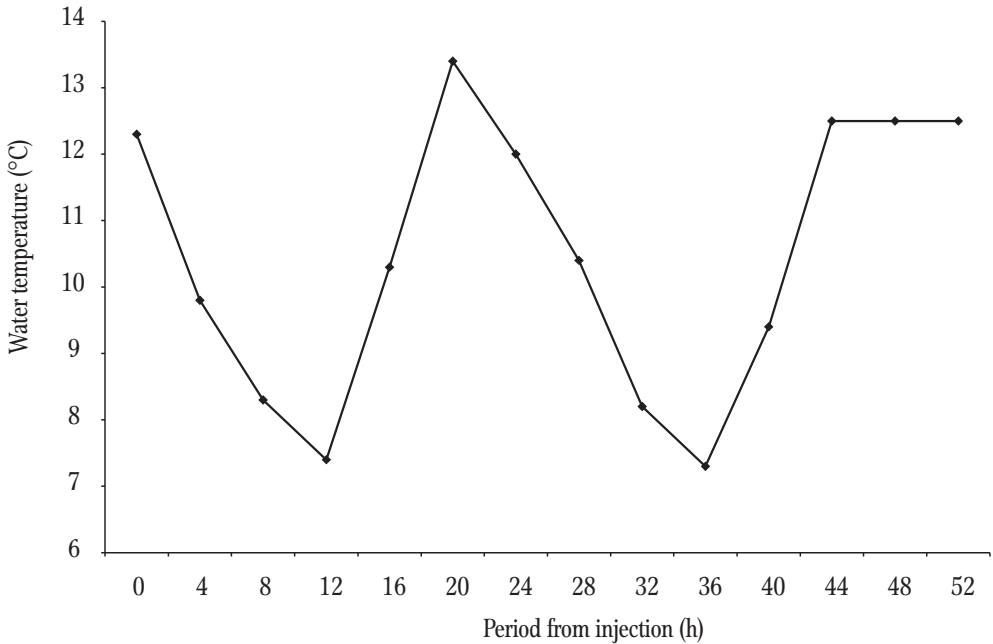


Fig. 1. Thermal regime of water during nase, *Chondrostoma nasus* reproduction under hatchery conditions.

The analysis of mean body weights and lengths of the females among the treatment groups was performed with ANOVA, and then Tukey's test ( $P < 0.05$ ). Increases in female body weight, relative working fecundity, and mean number of eggs that did not swell per volume unit were compared with the t-test.

## RESULTS

The mean female length and weight in the different treatments groups did not differ significantly statistically (ANOVA,  $P > 0.05$ ); however, female body size in group III was slightly larger than that in the remaining experimental groups. A statistically significant difference in body weight increase was noted between the fish injected with saline solution and hormonal preparations (Table 1). The greatest increase in body weight was confirmed in the females from group III (ANOVA,  $P < 0.05$ ).

TABLE 1

Results of controlled nase, *Chondrostoma nasus*, reproduction with the application of hormonal preparations

|   | Control                 | Ovopel                    | Ovaprim                 |
|---|-------------------------|---------------------------|-------------------------|
| Number of females                                     | 5                       | 9                         | 8                       |
| Ovulation (%)   | 0                       | 89                        | 100                     |
| Body weight (g)*                                      | 522 ± 136 <sup>a</sup>  | 522 ± 147 <sup>a</sup>    | 617 ± 175 <sup>a</sup>  |
| Total length (cm)*                                    | 37.7 ± 3.9 <sup>a</sup> | 37.6 ± 4.1 <sup>a</sup>   | 39.8 ± 4.1 <sup>a</sup> |
| Ovulation time (hours)                                | -                       | 51-75                     | 51                      |
| Increases in body weight (%)*                         | 0.5 ± 0.2 <sup>c</sup>  | 2.9 ± 1.2 <sup>b</sup>    | 4.1 ± 1.7 <sup>a</sup>  |
| Relative working fecundity (indiv. g <sup>-1</sup> )* | -                       | 40.2 ± 9.3 <sup>a</sup>   | 36.8 ± 4.3 <sup>a</sup> |
| NSE (indiv. 0.5 cm <sup>-3</sup> )*                   | -                       | 118.1 ± 14.5 <sup>a</sup> | 93.3 ± 7.9 <sup>b</sup> |
| Spawner survival (%)                                  | 100                     | 100                       | 87.5                    |

\*Values in the same row marked with the same letter index did not differ significantly statistically ( $P < 0.05$ )

No ovulation was noted in the females from group I. Hormonal stimulation induced ovulation in 89% of the females from group II and in 100% of those from group III. The period from injection to ovulation depended on the hormonal preparation used; eggs were stripped from 56% of the females in group II 51 h following injection, and from a subsequent 22% after 75 h. The last female that received Ovopel spawned spontaneously into the tank more than 75 h following injection. All of the females in group III ovulated 51 h following injection (Table 1). No statistically significant differences were noted in the relative working fecundity of the ovulating females (t-test,  $P > 0.05$ ), while the mean number of eggs that did not swell was higher in group II (t-test,  $P < 0.05$ ).

## DISCUSSION

The reproduction in captivity of fish caught in the wild during the spawning season does not always produce the anticipated results. This is linked to both spawning phenology, when the males and females can appear at the spawning grounds at different times, as well as to the risk of obtaining an inadequate number of spawners at the appropriate moment (Halačka and Lusk 1995, Kucharczyk 2002, Szabó et al. 2002). One solution might be to collect spawners over the course of a year and hold them until the following spawning season, as was done in the current experiment. When populations are not abundant, collecting a spawning brood may require more than one season.

Nase reproduction in captivity requires hormonal stimulation. To date, there have been no reports of obtaining oocytes from females without it. Similar observations have been made regarding other species of rheophilic cyprinids including ide, *Leuciscus idus* (L.), dace, *Leuciscus leuciscus* (L.) (Kucharczyk 2002), and chub, *Leuciscus cephalus* (L.) (Krejszeff et al. 2008). Szabó et al. (2002) reported that positive reproduction results with nase can be obtained with the application of either carp pituitary extract (CPE) or an analogue of gonadotropin-releasing hormone (GnRH). The latter produced positive results that were statistically better than those obtained with CPE, but only in combination with domperidone, a dopamine antagonist. This is why, in the current experiment, the effectiveness of preparations containing both GnRH analogue as well as dopamine antagonists (metoclopramid and domperidone) was tested. The best nase reproduction rates were obtained with the application of GnRH analogue and domperidone (Ovaprim).

The period from injection to ovulation during the current experiment was 11 h longer than that reported by Szabó et al. (2002), who maintained the water in the tanks at a constant temperature (12°C). Additionally, the period to ovulation was 24 h longer in group II. This was probably linked to the unstable thermal conditions of the water (from 7.4 to 13.4°C) while the fish were held in the hatchery. Both water temperature and photoperiod have a substantial influence on the reproductive cycles of fish, and the former becomes most influential in the final phases of the maturation of the gametes and during ovulation. Fluctuations in temperature can either accelerate or retard (depending on the species) the moment females are ready to spawn (Bromage et al. 2001, Davies and Bromage 2002, Anguis and Cañavate 2005). However, the synchronized ovulation achieved in group III suggests that the controlled reproduction of nase is substantially more effective with Ovaprim. This is especially noteworthy since the experiment was conducted in waters with a natural thermal regime. The second hormonal preparation (Ovopel), used widely for the controlled reproduction of nase, caused desynchronization in the final maturation of the gametes.

The smaller number of eggs that did not swell per volume unit in group III is evidence that the eggs obtained through artificial reproduction stimulated with Ovaprim are larger, although it cannot be ruled out that this was linked to female body size. The results of the current study confirm the necessity of stimulating female nase during artificial reproduction with gonadotropin analogues and dopamine inhibitors. Additionally, the study suggests that Ovaprim is more effective than Ovopel.

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## STRESZCZENIE

### ROZRÓD ŚWINKI, *CHONDROSTOMA NASUS* (L.) W WARUNKACH KONTROLOWANYCH

Celem pracy było porównanie efektywności działania dwóch środków hormonalnych: Ovopelu i Ovaprimu, w kontrolowanym rozrodzie świnki, *Chondrostoma nasus* (L.). Tarlaki złowiono poza naturalnym sezonem rozrodczym i do momentu zabiegu sztucznego rozrodu przetrzymywano w stawie. Eksperyment przeprowadzono w naturalnych warunkach termicznych i świetlnych. Przeprowadzono tylko jedną iniekcję hormonalną. W obu doświadczalnych grupach uzyskano pozytywny efekt stymulacji. W grupie kontrolnej nie pozyskano oocytów od żadnej samicy, a po zastosowaniu Ovaprimu uzyskano największą synchronizację owulacji samic. Uzyskane wyniki potwierdzają dotychczasowe doniesienia o konieczności stymulacji samic świnki w niewoli, w trakcie kontrolowanego rozrodu, przy użyciu analogu GnRH wraz z antagonistą dopaminy. Najbardziej do tego celu przydatny okazał się Ovaprim.