

**A COMPARISON OF THE ECONOMIC
EFFECTIVENESS OF VARIOUS SPAWNING AGENTS
FOR STIMULATING THE REPRODUCTION
OF THE CULTURED AND WILD FORMS
OF THE COMMON BARBEL *BARBUS BARBUS* (L.)**

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Key words: hormonal stimulation, economic effectiveness.

Abstract

The objective of this study was to evaluate the economic effectiveness of various hormonal products for stimulating the reproduction of the cultured and wild forms of the common barbel *Barbus barbus* (L.). Three hormonal products were analyzed: Ovopel, Ovaprim (cultured and wild fish) and hCG (cultured fish). The economic effectiveness of hormonal stimulants was analyzed in view of ovulation rates, the price of hormonal products and spawner mortality rates. The cost of hormonal injection per 1000 eggs and 1000 hatchlings was adopted as an indicator of economic effectiveness. The best results were reported for hormonal stimulation using synthetic GnRH analogues during induced spawning of the barbel in a group of cultured fish. The above product's effectiveness in the controlled spawning of cyprinids is demonstrated by high ovulation rates and high embryo survival rates.

**PORÓWNANIE OPŁACALNOŚCI ZASTOSOWANIA RÓŻNYCH PREPARATÓW
HORMONALNYCH DO STYMULACJI ROZRODU BRZANY *BARBUS BARBUS* (L.)
- FORMY HODOWLANEJ I DZIKIEJ**

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Słowa kluczowe: stymulacja hormonalna, opłacalność.

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Abstrakt

Celem badań przedstawionych w pracy była ocena opłacalności zastosowania różnych preparatów hormonalnych do stymulacji rozrodu brzany *Barbus barbus* (L.) zarówno jej formy hodowlanej, jak i dzikiej. Do badań przyjęto trzy środki hormonalne: Ovopel, Ovaprim (forma hodowlana i dzika) i hCG (forma hodowlana). Oceniono opłacalność zastosowania tych preparatów hormonalnych do stymulacji rozrodu brzany, biorąc pod uwagę płodność ryb, cenę preparatów hormonalnych i śmiertelność tarlaków. Jako miarę opłacalności przyjęto koszt iniekcji hormonalnej w przeliczeniu na uzyskane 1000 ziaren ikry i 1000 sztuk wylęgu. Najbardziej opłacalna okazała się stymulacja hormonalna za pomocą syntetycznych analogów GnRH w trakcie kontrolowanego rozrodu brzany w grupie ryb hodowlanych. Wynika to ze skuteczności tych środków hormonalnych w kontrolowanym rozrodzie ryb karpiowatych, o której świadczy wysoki odsetek owulacji, jak i wysoki odsetek przeżywalności zarodków.

Introduction

The progressing loss of habitats and the extinction of rheophilic fish of the family Cyprinidae in recent years calls for the urgent implementation of protection programs. There is an immense demand for adequately selected stocking material of those valuable fish species to support stock management in open waters (MEJZA et al. 1996, AUGUSTYN 2002, KUJAWA 2004, PENCZAK et al. 2004, WOJDA 2004, WOLNICKI 2005, BOLLAND et al. 2008). Two main methods are applied to produce spawners of rheophilic cyprinid fish. The first is artificial spawning in a controlled environment (KUCHARCZYK 2002, JAMRÓZ et al. 2008a,b, KREJSZEFF et al. 2009, 2010, ŻARSKI et al. 2008b, 2009, WOLNICKI, MYSZKOWSKI 1998, CIEŚLA et al. 2000b, MYSZKOWSKI et al. 2000), and the second involves artificial reproduction of caught wild spawners in a hatchery both with and without hormonal stimulation (KOURIL et al. 1988, CIEŚLA et al. 2000a, AUGUSTYN 2002). The production of stocking material based on the artificial spawning of rheophiles is gaining new significance as advances are made in the reproduction biotechnology, thus contributing to higher production in commercial aquaculture (KUPREN et al. 2008b, TURKOWSKI et al. 2008). The success of induced spawning is determined by the choice of the right hormonal preparation and its dose. Hormonal stimulation takes place at the level of the hypothalamus, the pituitary gland and the gonads (BIENIARZ and EPLER 1991, PETER and YU 1997, ZOHAR and MYLONAS 2001, PODHOREC and KOURIL 2009, YARON et al. 2009). Hormonal products which are most frequently used in the induced spawning of cyprinids include the homogenate of carp and bream pituitary glands (CIEŚLA 1998, KUCHARCZYK et al. 2008), human chorionic gonadotropin (hCG) (KUCHARCZYK et al. 1997a,b,c, 2005), gonadotropin-releasing hormone (GnRH) and its analogues, often in combination with a dopamine antagonist (KREJSZEFF et al. 2008, 2009, 2010, ŻARSKI et al. 2008b, 2009).

Artificial spawning and breeding has to deliver economic advantages. There is a scarcity of publications discussing the economic aspects of rearing the larvae of rheophilic cyprinid fish under controlled conditions (KUPREN et al. 2008b, TURKOWSKI et al. 2008). The cost of performing hormonal stimulations in the reproduction of rheophilic fish also receives weak coverage in the related literature (KŁODZIŃSKA and OKONIEWSKI 1998, HAKUĆ-BŁAŻOWSKA et al. 2009). The objective of this study was to evaluate the economic efficiency of various hormonal products for stimulating the reproduction of wild and cultured forms of the common barbel *Barbus barbus* (L.).

Materials and Methods

Spawners and fish manipulation

Barbel spawners were obtained from two sources. Cultured fish (F_3 generation) were acquired from the Czarci Jar Fish Farm near Olsztynek (NE Poland). The stock originated from fish caught in the Vistula Basin. It was kept in tanks with a capacity of 1000 dm³ and an environment control option (KUJAWA et al. 1999). The first group of wild fish (WF-1) was caught in the Narew River in Dębe in the fall, and it was transported to the Czarci Jar Fish Farm where it was kept in a flow-through earthen pond. Starting in the fall, both groups were kept in natural temperatures. The photoperiod of cultured fish was similar to the natural regime and it was controlled by a light clock. Both groups were fed the same diet comprising trout pellet and frozen chironomids in a weight ratio of 1:1 (1.5% fish biomass). In late spring when water temperature reached 15°C, both groups were kept under identical conditions in fiberglass tanks with a capacity of 1 m³. Fish density did not exceed 30 kg m⁻³. The third experimental group (WF-2) comprised fish caught in the Vistula Basin (dam on the Narew River in the village of Dębe) in the spring, immediately before the spawning season, when water temperature in the river reached 15°C. The fish were transported to the Czarci Jar Fish Farm where they were placed in tanks and kept under the same conditions as the remaining two groups.

Prior to any manipulation, fish were anesthetized in a bath of 0.5 cm³ dm⁻³ 2-phenoxyethanol solution (Sigma-Aldrich, Germany). Temperature in the tanks was 15°C on the day of spawner introduction. Hormonal injections were performed after a four-day adaptive period during which water temperature was gradually increased to 17°C. Fish from both hatchery groups were exposed to a constant photoperiod of 14 h (14 L : 10 D).

Experiments

The experiments were carried out within a single breeding season. The first experiment was performed on the group of cultured fish, and it verified the efficacy and economic effectiveness of selected hormonal products. Prior to the first injection, fish were marked and randomly divided into groups subject to the applied hormonal product. Hormonal stimulation was performed using three products: Ovopel ([D-Ala⁶, Pro⁹-Net]-mGnRH) (Unic-trade, Hungary) (homogenized in 0.9% NaCl solution and administered according to the method described by Horvth et al. 1997), Ovaprim ([D-Arg⁶, Pro⁹-Net]-sGnRH) (Syndel, Canada) (PETER et al. 1993) and hCG – human chorionic gonadotropin (Argent, USA). Saline solution injections (0.9% NaCl) were used in the control group. Hormonal product doses are presented in Table 1.

Table 1
Hormonal doses applied to stimulate the reproduction of the common barbel (cultured form) during the natural spawning season

Group	Males	Females	
	hormonal dose	preliminary dose	release dose
1 control	+	+	+
2	0.25 ml Ovaprim	0.1 ml Ovaprim	0.5 ml Ovaprim
3	1000 IU hCG	500 IU hCG	2000 IU hCG
4	0.5 Ovopel granules	0.1 Ovopel granules	1 Ovopel granule

+ 0.9% NaCl injections

Table 2
Hormonal doses (in terms of kg BW) applied to stimulate the reproduction of the barbel (wild and cultured forms) during the natural spawning season

	Cultured fish		Wild fish	
	1 st injection	2 nd injection	1 st injection	2 nd injection
Control	+	+	+	+
Ovopel [granule]	0.1	1.0	0.1	1.0
Ovaprim [ml]	0.1	0.5	0.1	0.5

+ 0.9% NaCl injections

The second experiment compared the efficacy and economic effectiveness of two hormonal stimulants in the induced spawning of wild (groups WF-1 and WF-2) and cultured fish (females). Similarly to the first experiment, fish were marked and divided into groups (subject to the applied hormonal stimulant) before the first injection. The stimulation was performed using two products:

Ovopel and Ovaprim. Saline solution injections (0.9% NaCl) were used in the control group. Hormonal product doses for cultured fish and wild fish are presented in Table 2.

hCG injections were administered intramuscularly under the dorsal fin. Ovaprim and Ovopel was injected intraperitoneally under the ventral fin. After the first injection, the temperature of water was increased over a period of 17 h to reach 18°C. The second hormonal injection was administered 12 h after the first injection. Males received a single injection at the time of the second manipulation in females. Next, temperature was raised to 19–20°C after the second treatment.

Gamete acquisition

Recognition of ovulation began 15 h after the second injection. Barbel females in every group were monitored every three hours in the course of successive 15 hours. Gametes were acquired from spawners by massaging and applying gentle pressure to the abdomen. Eggs were collected from each female into separate plastic bowls. To determine the effect of hormonal stimulants on the biological quality of gametes, the eggs from each female (three samples of 100–200 eggs each) were fertilized with mixed semen collected from five males. The samples were incubated separately in water at 19–20°C. Embryo survival rates were determined upon hatching. After the experiment, spawners were kept in the pond for another 14 days to determine whether experimental manipulation increased fish mortality rates.

The body weight of fish in each group and the reported results (including fertility and embryo survival rates) were evaluated by an analysis of variance (ANOVA) and Tukey's post-hoc test at a significance level of $\alpha = 0.05$. The values expressed in terms of percent were subjected to arcsine transformation before statistical analyses.

Economic effectiveness of hormonal stimulation

The economic effectiveness of hormonal products stimulating reproduction was analyzed in view of ovulation rates, the price of hormonal products and spawner mortality rates. The cost of hormonal injection per 1000 eggs and 1000 hatchlings was adopted as an indicator of economic effectiveness. Calculations were performed in view of the purchasing price of selected hormonal products, converted into Polish zloty in accordance with the exchange rate quoted on the day of purchasing Ovaprim and hCG stimulants. The cost of

purchasing 10000 IU hCG was USD 33, 10 ml Ovaprim – USD 25.50, 1 Ovopel granule – EUR 0.4. The cost of a single Ovopel, Ovaprim and hCG dose per kg BW of fish was calculated. At the next stage, the total cost of every hormonal product was computed based on the ovulation rates and spawner mortality rates in every group. Fixed cost components that were not affected by the type of the applied hormonal product (e.g. equipment depreciation, lighting, labor) were not accounted for in the calculations (HAKUĆ-BŁAŻOWSKA et al. 2009).

Results

Efficacy and economic effectiveness of hormonal stimulation in fish cultures

Hormonal stimulation clearly affected barbel spawning (Table 3). Ovulation rates in experimental fish groups were significantly higher (53–77%) than in the control group (30%). The highest percentage of ovulating females was noted following the administration of Ovaprim and Ovopel. The highest percentage of hatched embryos was reported in the group stimulated with Ovaprim. Embryo viability was higher in all experimental groups than in the control group. Hormonal stimulation and fish manipulation did not increase spawner mortality rates.

Table 3
The results of stimulating the reproduction of the common barbel (cultured form) with various hormonal products

Fish group	Control	Ovaprim	Ovopel	HCG
Number of males	12	11	12	12
Average body weight of males	108 + 12	110 + 14	114 + 21	109 + 18
Male mortality rate [%]	0	0	0	0
Number of females	10	13	15	15
Average body weight of females	128 + 22	133 + 25	127 + 32	129 + 30
Percentage of ovulating females	30	77	73	53
Fecundity [eggs/kg]	1078 + 221 ^b	1547 + 325 ^a	1654 + 322 ^a	1089 + 159 ^b
Hatching rate [%] (± SD)	66.3 + 3.4 ^a	83.2 + 4.1 ^a	77.2 + 3.0 ^b	75.0 + 2.9 ^b
Female mortality rate [%]	0	0	0	0

Data marked by the same letters in rows do not differ statistically

The costs of applying different hormonal products to stimulate barbel reproduction in a commercial farm varied significantly. The cost of one dose per kg BW of male fish reached: Ovaprim – PLN 1.66, Ovopel – PLN 0.72 and

hCG – PLN 8.25. As regards females, the cost of a combined product dose (preliminary dose and release dose) was: Ovaprim – PLN 3.82, Ovopel – PLN 1.58 and hCG – PLN 20.63. Total Ovaprim costs and total Ovopel costs differed more than two-fold, reaching PLN 6.60 and PLN 3.01, respectively, while total hCG costs were significantly higher at PLN 50.71.

The costs of hormonal stimulation of the barbel in terms of 1000 eggs and 1000 hatchlings are presented in Figure 1. The cost of hormonal stimulation in terms of 1000 eggs was the lowest for Ovopel and Ovaprim at PLN 1.74 and PLN 4.15, respectively. hCG (PLN 45.41 per 1000 eggs) proved to be the least cost-effective hormonal product. Similar differences in the profitability of different stimulants were produced by calculating hormonal injection costs per 1000 hatchlings.

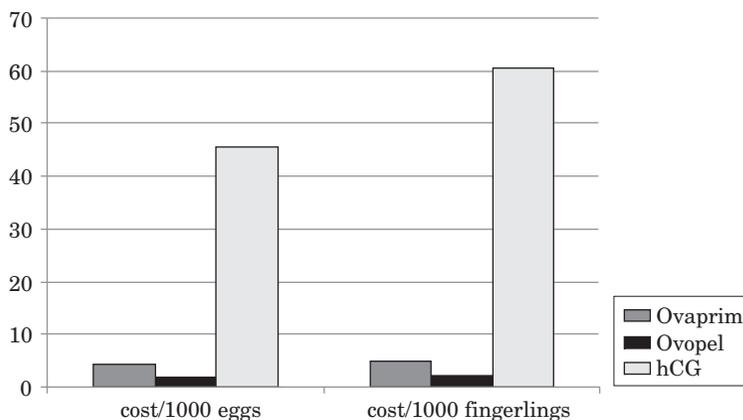


Fig. 1. The profitability of various hormonal products in the artificial spawning of cultured barbel

Efficacy and economic effectiveness of hormonal stimulation in cultured and wild fish

Hormonal stimulants had a considerable effect on the investigated reproduction parameters of the barbel, in particular its cultured form (Tables 4–6). In the control group, none of the wild-type females matured sexually, and mortality rates reached 50% in groups WF-1 and WF-2. The ovulation rate in the cultured group was 30%. A comparison of the reproductive performance of cultured fish and wild fish administered Ovopel showed much higher ovulation rates in the group of cultured fish (80%) than wild fish (0–13%) (Table 5). Significant differences in embryo hatching rates were not determined. The mortality of wild spawners was as high as 50% of the stock.

Table 4

The results of artificial spawning in the control group of wild and cultured fish

Fish group	Cultured	WF-1	WF-2
Number of females	10	6	6
Average body weight of females	0.324	3.874	3.587
Percentage of ovulating females	30%	0	0
Fecundity [eggs/kg]	1054 + 124	0	0
Hatching rate [%] (\pm SD)	64.2 + 3.8	–	–
Female mortality rate [%]	0	50	50

Table 5

The results of stimulating the reproduction of the common barbel (cultured and wild forms) with Ovopel

Fish group	Cultured	WF-1	WF-2
Number of females	10	8	8
Average body weight of females	0.254	3.245	3.687
Percentage of ovulating females	80	13	0
Fecundity [eggs/kg]	1654	1020	0
Hatching rate [%] (\pm SD)	79.3 + 63.6 ^a	80.2 + 3.0 ^a	0
Female mortality rate [%]	0	13	50

Data marked by the same letters in rows do not differ statistically

Table 6

The results of stimulating the reproduction of the common barbel (cultured and wild forms) with Ovaprim

Fish group	Cultured	WF-1	WF-2
Number of females	10	8	8
Average body weight of females	0.287	3.875	3.364
Percentage of ovulating females	90	25	13
Fecundity [eggs/kg]	1875	1645	1587
Hatching rate [%] (\pm SD)	82.2 + 3.3 ^b	81.1 + 3.7 ^b	89.2 + 1.2 ^a
Female mortality rate [%]	0	38	50

Data marked by the same letters in rows do not differ statistically

Similar results were noted following the administration of Ovaprim (Table 6). Ovulation rates were higher and embryo hatching rates were insignificantly higher than in the groups stimulated with Ovopel. Mortality rates of wild-type fish also reached 50%. In general, Ovaprim produced more satisfactory results than Ovopel.

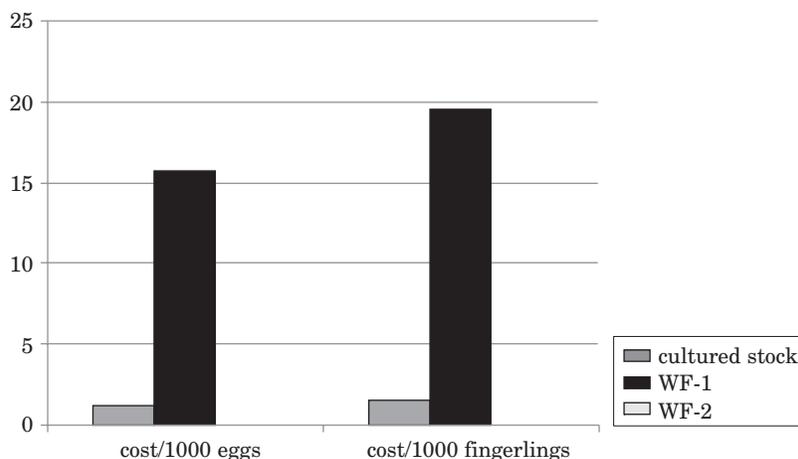


Fig. 2. The profitability of Ovopel in the artificial spawning of the common barbel: wild and cultured forms

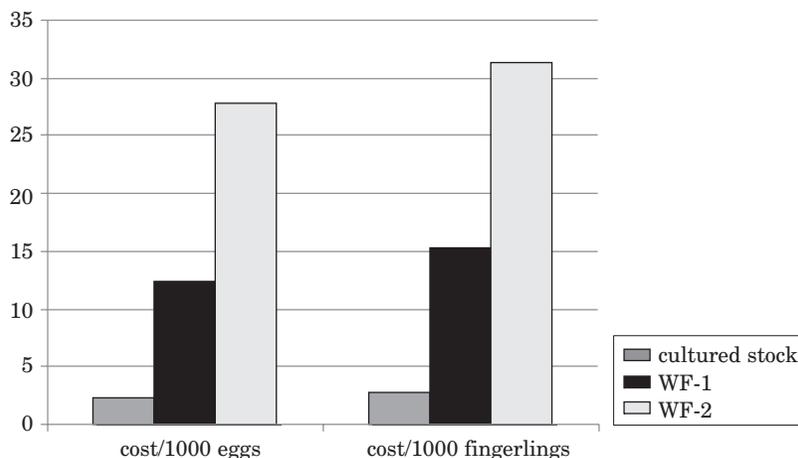


Fig. 3. The profitability of Ovaprims in the artificial spawning of the common barbel: wild and cultured forms

The costs of stimulating the reproduction of cultured and wild barbel with Ovopel varied significantly. The cost of a single Ovopel dose per kg BW of female fish reached an average of PLN 1.58 for both wild and cultured forms. The total cost of administering Ovopel was significantly higher in both wild fish groups (WF-1 – PLN 41.02 and WF-2 – PLN 46.60) than in cultured fish (PLN 4.01) which resulted from the fact that the body weight of cultured females was several times lower. When spawner mortality rates were taken into account, the cost of hormonal manipulation increased by PLN 13 (WF-1) and PLN 52 (WF-2).

The costs of Ovopel stimulation in cultured and wild fish (WF-1) per 1000 eggs and 1000 hatchlings are presented in Figure 2. Group WF-2 was not taken into account due to an absence of ovulating females. The cost of hormonal stimulation per 1000 eggs was significantly lower in cultured fish (PLN 1.20) than in wild fish where it reached PLN 15.69. Ovopel injections proved to be less profitable in wild fish than in cultured fish also in terms of 1000 hatchlings.

Discussion

The production of stocking under controlled conditions, including of rheophilic cyprinid fish, is becoming the predominant source of income for many fish farms in Poland (WOJDA 2004, TURKOWSKI et al. 2008). Stocking material is produced by artificial spawning. Under controlled conditions, selected species, such as the burbot, *Lota lota* L., or the perch, *Perca fluviatilis* (L.), may reproduce with or without hormonal stimulation (KUCHARCZYK et al. 1996, 1998c, SZCZERBOWSKI et al. 2009, ŻARSKI et al. 2010). Hormonal stimulation is not required in aquarium fish and domesticated fish species (KREJSZEFF et al. 2009, KUCHARCZYK et al. 2010). Yet in most cases, the production of high quality gametes and abundant stocking material may require hormonal stimulation (BRZUSKA et al. 2000, 2005, KUCHARCZYK et al. 2005, 2008, CEJKO et al. 2008, 2010, ŻARSKI et al. 2009).

In induced spawning, the selection of the appropriate hormonal stimulant determines ovulation success. There are scant data on the controlled reproduction of the common barbel (WOLNICKI and MYSZKOWSKI 1998, CEJKO et al. 2009, KAMIŃSKI et al. 2009). The effect of hCG on the artificial spawning of selected fish species has been described by KUCHARCZYK et al. (1998b). Except for a small number of species, hCG proved to be ineffective in most rheophilic cyprinid fish (KUCHARCZYK et al. 1997a,b). Satisfactory results were reported as regards the induced spawning of the rudd and the chub stimulated with hCG (KUCHARCZYK et al. 1997c, KREJSZEFF et al. 2010). Recent years have witnessed the growing popularity of synthetic GnRH analogues with the addition of dopamine inhibitors. Many Polish researchers reported highly satisfactory results in stimulating the reproduction of most rheophilic cyprinid species with the use of Ovopel (CIEŚLA 1998, KŁODZIŃSKA and OKONIEWSKI 1998, KUCHARCZYK et al. 1998, ŚLIWIŃSKI 1998, KUCHARCZYK et al. 1999, TARGOŃSKA-DIETRICH et al. 2004, JAMRÓZ et al. 2008b, KREJSZEFF et al. 2008, ŻARSKI et al. 2008b, HAKUĆ-BŁAŻOWSKA et al. 2009) as well as Ovaprim (KUCHARCZYK et al. 2007, JAMRÓZ et al. 2008a,b, ŻARSKI et al. 2008a,b, HAKUĆ-BŁAŻOWSKA et al. 2009).

The results of the present experiment point to the high effectiveness of Ovopel and Ovaprim in the induced spawning of farmed barbel fish. hCG was marked by lower efficacy in comparison with GnRH analogues. Hormonal stimulation with hCG produced ovulation rates of 53%, while the administration of Ovaprim and Ovopel induced ovulation in 77% and 73% of females, respectively. Other authors also noted the high effectiveness of Ovopel and Ovaprim in the artificial spawning of other rheophilic cyprinids (KUCHARCZYK 2002, TARGOŃSKA-DIETRICH et al. 2004, JAMRÓZ et al. 2008b, KREJSZEFF et al. 2008, 2009, ŻARSKI et al. 2008a, 2009, TARGOŃSKA et al. 2010). In comparison with the control group, the results of hormonal injections in wild barbel also validate the efficacy of Ovopel and Ovaprim in artificial spawning. GnRH analogues stimulated ovulation in a small percentage of females, but the absence of hormonal stimulation resulted in zero ovulation. The above findings suggest that GnRH analogues are highly effective in the induced spawning of the common barbel, and they point to the economic effectiveness of spawner breeding in fish farms. As suggested by a limited number of comparative studies, hormonal stimulation delivers more satisfactory results in cultured rather than in wild fish (TARGOŃSKA-DIETRICH et al. 2004, KREJSZEFF et al. 2009, 2010). Hormonal stimulation involving any of the analyzed spawning agents also contributed to higher embryo survival rates (in both cultured and wild fish groups) in comparison with the control group.

The dynamic growth of commercial fish stock production in pond and lake farms prompts research into the economic profitability of the process. To date, few studies investigating the problem have been published (KŁODZIŃSKA and OKONIEWSKI 1998, TLUSTY 2002, TURKOWSKI 2002, HAKUĆ-BŁAŻOWSKA et al. 2008, 2009, KUPREN et al. 2008b, TURKOWSKI et al. 2008). In cultured fish production was significantly more profitable with the involvement of GnRH synthetic analogues than hCG. The cost of a single dose of human chorionic gonadotropin was more than five-fold higher than the cost of a single Ovaprim dose and 13-fold higher than an Ovopel dose. The results of studies evaluating the economic effectiveness of hormonal stimulation in the ide *Leuciscus idus* (L.), the asp *Aspius aspius* (L.) (HAKUĆ-BŁAŻOWSKA et al. 2009) and the pikeperch *Stizostedion lucioperca* (L.) (HAKUĆ-BŁAŻOWSKA et al. 2008, KUPREN et al. 2008a) also pointed to the higher profitability of GnRH synthetic analogues in artificial spawning. However, smaller fertility of common barbell causes larger costs of hormonal stimulation in comparison to results of artificial reproduction of ide, or asp.

When the economic effectiveness of Ovopel and Ovaprim was compared in the artificial reproduction of cultured and wild barbel fish, more satisfactory results were reported for Ovopel. Yet the most significant differences in the overall cost of hormonal stimulation were noted when the same product was

used in cultured fish and wild fish. The cost of hormonal stimulation with Ovopel was ten-fold higher in wild-type female fish than in cultured fish. Taking into account spawner mortality rates, hormonal stimulation costs increased in the group of wild fish, compared with cultured fish. The above was attributed mainly to the average body weight of females (wild females were more than ten-times larger than cultured females) and spawner mortality rates (noted only in the group of wild fish). The differences in economic profitability should be leveled out when production costs are calculated in terms of 1000 eggs and 1000 hatchlings, yet the eggs yield of both wild fish groups did not reduce the difference in Ovopel's profitability. There were no sexually mature females in group WF-2, and the ovulation rate in group WF-1 reached 13%. Due to an absence of ovulating females, group WF-2 was not taken into account in cost calculations. The stimulation of cultured fish with Ovopel proved to be more profitable even in view of embryo survival rates in the studied groups.

Similar differences in economic effectiveness were reported in respect of Ovaprim. In view of the body weight of fish and spawner mortality rates, the overall cost of hormonal stimulation in wild fish groups exceeded simulation costs in cultured fish 15-fold. Ovaprim was also a less cost-effective stimulant in wild fish due to their significantly lower ovulation rates (in particular in group WF-2). The low profitability of hormonal treatments in wild-type fish resulted from the low effectiveness of induced spawning in this group of fish. As regards economic profitability, the most satisfactory results were reported in respect of GnRH synthetic analogues administered during the induced spawning of cultured fish.

Translated by JOANNA POPRAWKA

Accepted for print 23.06.2010

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