# Artificial propagation of African catfish (*Clarias gariepinus*): the application of a single dose of pellets containing D-Ala$^6$,Pro$^9$NEt-mGnRH and dopamine inhibitor metoclopramide

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**ABSTRACT**: The effects on reproduction of African catfish (*Clarias gariepinus* Burchell 1822) were investigated in three experiments conducted under controlled conditions, carp pituitary (at the dose of 4 mg/kg body weight) or Ovopel – a preparation that contains a mammalian GnRH analogue D-Ala$^6$,Pro$^9$NEt-mGnRH (1 pellet/kg body weight) and dopamine receptor antagonist metoclopramide (10 mg/kg) being used as ovulation stimulators. The application of Ovopel induced the statistically significantly ($P \leq 0.01$) higher weight of eggs per kg female body weight and the statistically significantly ($P \leq 0.05$) higher quality of eggs after 24 h incubation in comparison with the effects of hypophysation. No effect of the experiment on the weight or quality of obtained eggs was determined while the interaction between the experiment and the ovulation stimulator was statistically significant ($P \leq 0.05$) with respect to the percentage of egg fertilization. Statistically significant ($P \leq 0.05$) correlation was found between the percentage of egg fertilization and that of living embryos, the determined correlation coefficient being higher after the application of Ovopel than after the carp pituitary homogenate.

**Keywords**: *Clarias gariepinus*; stimulation of ovulation; hypophysation; single dose of Ovopel; artificial propagation

The satisfactory results of ovulation stimulation with Ovopel observed in various fish species (see a review in Brzuska, 2001) and numerous merits of this preparation (i.e. the possibility of precise dosing without weighing the stimulator, the simple method of its preparation for the treatment of fish, the elimination of an additional injection of dopamine receptor blocker, and the possibility of repeated application after a short interval if no ovulation occurs) distinctly show its high value. Ovopel contains a mammalian GnRH analogue, D-Ala$^6$,Pro$^9$NEt-mGnRH-a and water-soluble dopamine receptor antagonist – metoclopramide. The concentrations of D-Ala$^6$,Pro$^9$NEt-mGnRH and metoclopramide are 18–20 μg/pellet and 8–10 mg per pellet, respectively (Horváth *et al*., 1997). Since the preparation was developed for Cyprinidae and the induced reproduction of this family is more effective when the ovulation stimulation is performed with two doses of carp pituitary, Horváth *et al*. (1997) recommended applying two doses of Ovopel.

The results of studies conducted on European catfish (*Silurus glanis* L.) show that in the case of Ovopel the two doses (i.e. 1/5 pellet/kg body weight as the priming dose and 1 pellet/kg as the resolving dose) are not necessary if the stimulation is carried out during the season of natural spawning and the spawners are in a good reproductive condition. With the application of one dose of Ovopel (1 pellet/kg body weight) the results of reproduction were satisfactory (Brzuska, 2003).

The effects of ovulation stimulated in African catfish (*Clarias gariepinus*) with two doses (1/5 + 1 pellet/kg) or one dose (1 pellet/kg) of Ovopel show that the differences in the weight of eggs and their quality (expressed by fertilization percentage and by the percentage of living embryos after 24-hour incubation) between the groups treated with the above doses were statistically insignificant (Brzuska *et al*., 1998). The treatment either with one Ovopel dose or with two doses did not affect the percentage of fish yielding eggs (in rela-

tion to all the fish after hormonal stimulation) or the latent period.

In the investigation conducted by Tan-Fermin *et al*. (1997) on Asian catfish *Clarias macrocephalus* Gunther (a tropical freshwater fish of the order Siluriformes) only one dose (0.05 μg/g body weight) of D-Ala$^6$,Pro$^9$LHRH-ethylamide was used as an ovulation stimulator. However, Legendre *et al*. (2000) reported that both in experimental reproduction and in fry production in Indonesian fry production units of Asian catfish *Pangasius hypophthalmus* (the most common cultured pangasiid catfish throughout Southeast Asia) two injections of Ovaprim, a preparation containing sGnRH-a (D-Arg$^6$,Trp$^7$,Leu$^8$,Pro$^9$Net) and domperidone, were applied.

In conducting controlled fish reproduction particular attention should be paid to the reduction of stress threatening the spawners during numerous manipulations accompanying it. Among other factors hormonal injections are responsible for the stress. The reduction of their number seems justified if it does not negatively affect the reproduction results. The African catfish (*Clarias gariepinus*) – a valuable species with a well-grounded position in European aquaculture (Huisman and Richter, 1987) is highly sensitive to stress. Therefore it is advisable to reduce the number of manipulations associated with ovulation stimulation in this species.

The aim of the presented investigation was to find out if any differences can be determined in the effects of reproduction stimulated with carp pituitary homogenate or one dose of Ovopel. The effects of experiment on the results of spawning controlled by the above stimulators were also investigated.

## MATERIAL AND METHODS

The data used as a basis of calculations were obtained from three experiments conducted under controlled conditions in water at 24–25°C. The experiments included 41 females of the average body weight of 3.18 (±2.54) kg. In each experiment the fish were divided into two groups; in group I ovulation was stimulated with carp pituitary homogenate and in group II with Ovopel. The number of females in groups I and II and the applied doses of two stimulators injected intraperitoneally in each experiment are given in Table 1. The injected solution of Ovopel was prepared according to Horvath *et al*. (1997).

The checking of ovulation started 10 h after the injection of each stimulator and continued at one hour intervals during the next 4 hours. After stripping the eggs were weighed and fertilized with mixed milt from macerated testes of three killed males. The milt production was not hormonally stimulated. The milt was 100× thinned in 0.9% NaCl. The eggs were fertilized in portions of about 200 g, 2–5 ml of milt being applied at the concentration given above. Then eggs were washed with a water solution of tannin (7–10 g/l) for 20 s to remove stickiness (Adamek, 2003).

In each of three experiments the incubation of fertilized eggs from each female separately was conducted in a Weiss glass in water at 24–25°C. After 12-hour incubation the fertilization percentage and after 24 h the percentage of living embryos were calculated for each fish. A sample of eggs was taken from the central part of the Weiss glass; 100 eggs from the sample were placed on a Petri dish and ob-

Table 1. Number of females in the experiments, substances and doses used for ovulation stimulation, percentage of spawning females after hormonal treatment and latency time (defined as the time interval between the injection and stripping; Richter *et al*., 1987b)

| Experiment | Group | No. of females | Ovulation stimulator | Dose per kg body weight | Percentage of spawning females after hormonal treatment | | Latency time |
|---|---|---|---|---|---|---|---|
| | | | | | full ovulation | partial ovulation | |
| A | I | 6 | carp pituitary | 4 mg | 83.33 | – | 11 |
| | II | 10 | Ovopel | 1 pellet | 100 | – | 13 |
| B | I | 4 | carp pituitary | 4 mg | 100 | – | 11 |
| | II | 5 | Ovopel | 1 pellet | 100 | – | 13 |
| C | I | 8 | carp pituitary | 4 mg | 87.50 | 12.50 | 12 |
| | II | 8 | Ovopel | 1 pellet | 75.00 | – | 14 |

served under a binocular at 3× magnification. Dull and unfertilized eggs were separated from transparent living ones. Three measurements were carried out and mean percentage of fertilization and living embryos were separately calculated for each of the investigated females.

The data obtained in the experiments were subjected to analysis of variance using the least-squares method (Harvey, 1960, 1987) to estimate the effect of the experiment and of the ovulation stimulator on the investigated traits. The evaluated traits included the weight of eggs per kg of female body weight, the fertilization percentage after 12-hour incubation, and the percentage of living embryos after 24-hour incubation. Analysis of variance of the data obtained in experiments was conducted using the following linear model:

$$Y_{ijk} = \alpha + g_i + p_j + (gp)_{ij} + bW_{ijk} + e_{ijk}$$

where: $\alpha$ = the theoretical general mean with the assumption that $W_{ijk} = 0$
$g_i$ = the effect of an experiment ($i = 1, \ldots 3$)
$p_j$ = the effect of ovulation stimulator ($j = 1, \ldots 2$)
$(gp)_{ij}$ = the interaction between the experiment and the ovulation stimulator
$b$ = the regression on the body weight of a female
$W_{ijk}$ = the body weight of a female
$e_{ijk}$ = the random error associated with observation $k$

The significance of the main effects on the investigated traits was evaluated by *F*-test. Analysis of the data allowed to evaluate the constants and means of the least squares characterizing the effects of reproduction associated with the given experiment and ovulation stimulator. The constants and the least-squares means are given in Table 2. The least-squares means estimated for the interaction between the experiment and the ovulation stimulator for the investigated traits are given in the graphic form (Figure 1).

The phenotypic correlation was calculated between the following traits: weight of eggs per kg of female body weight, percentage of fertilized eggs and percentage of living embryos. The calculation was conducted separately for females treated with carp pituitary homogenate or with Ovopel. The values of correlation coefficients are given in Table 3.

## RESULTS

### Percentage of females yielding eggs

In the group of hypophysed fish (group I) of experiment A eggs were obtained from 83.33% of females and in experiment B from 100% of females (Table 1). In experiment C after pituitary homogenate treatment also 100% of females spawned, however in 87.50% of the fish full ovulation and

Table 2. Constants (LSC) and least-squares means (LSM) estimated for investigated traits characterizing the reproduction effects of experiments A, B, C and groups (SE, standard error of the least-squares means; α, theoretical general mean)

| Classification factor | Weight of eggs in grams per 1 kg female body weight | | | Percentage of fertilized eggs after 12 h incubation | | | Percentage of living embryos after 24 h incubation | | |
|---|---|---|---|---|---|---|---|---|---|
| α | | 118.65 | | | 89.34 | | | 71.55 | |
| | LSC | LSM | SE | LSC | LSM | SE | LSC | LSM | SE |
| Experiment | | | | | | | | | |
| A | 7.31 | 125.99 | 12.76 | –15.40 | 73.93 | 2.92 | –9.31 | 62.23 | 3.60 |
| B | –22.23 | 96.42 | 33.05 | 0.93 | 90.28 | 7.57 | –1.17 | 70.38 | 9.33 |
| C | 14.91 | 133.57 | 87.64 | 14.47 | 98.82 | 20.08 | 10.49 | 82.04 | 24.74 |
| Ovulation stimulators | | | | | | | | | |
| carp pituitary (group I) | –27.13 | 91.52 | 40.64 | –1.78 | 87.56 | 9.31 | –3.78 | 67.76 | 11.47 |
| Ovopel (group II) | 27.13 | 145.79 | 38.57 | 1.78 | 91.13 | 8.83 | 3.78 | 75.34 | 10.88 |
| Regression/ body weight | –12.67 | –12.67 | 18.01 | 0.08 | 0.08 | 4.12 | –2.29 | –2.29 | 5.08 |

in 12.50% partial ovulation (< 30 g of eggs) were recorded (Table 1).

After Ovopel treatment (group II) in experiment A and experiment B eggs were obtained from all the treated females. In experiment C 75% of Ovopel treated females spawned (Table 1) with one female yielding valueless eggs whose fertilization reached only 3%. The data obtained from this female were disregarded in the calculations.

## Latency time

After Ovopel treatment (group II) in all the experiments the latency time was longer by two hours than after hypophysation; in experiments A and B it took 13 hours and in experiment C 14 hours (Table 1).

## Effect of the experiment on the weight and quality of eggs

No statistically significant effect of the experiment on the weight of eggs was determined. However, if the values of the least-squares means for the weight of eggs are considered, the highest weight of eggs was found in experiment C and the lowest in experiment B (Table 2). No statistically significant effect of the experiment on the quality of eggs was recorded either after 12 or 24 hours of incubation. The values of the least-squares means for traits characterizing the quality of eggs were estimated for the individual experiments, showing the highest quality of eggs in experiment C, slightly poorer one in experiment B, and the poorest in experiment A (Table 2).

## Effect of ovulation stimulators on the weight and quality of eggs

A statistically significant ($P \leq 0.01$) effect of the ovulation stimulator on the weight of eggs was found. The value of the least-square means estimated for the weight of eggs per kg of female body weight distinctly shows that eggs of higher weight were from fish of group II, i.e. from females treated with Ovopel (Table 2). The effect of the ovulation stimulator was statistically insignificant with respect to the percentage of fertilized eggs, being significant ($P \leq 0.05$) in respect of living embryos. The least-squares means estimated for the percentage of living embryos show that as compared with eggs from fish treated with carp pituitary homoge-

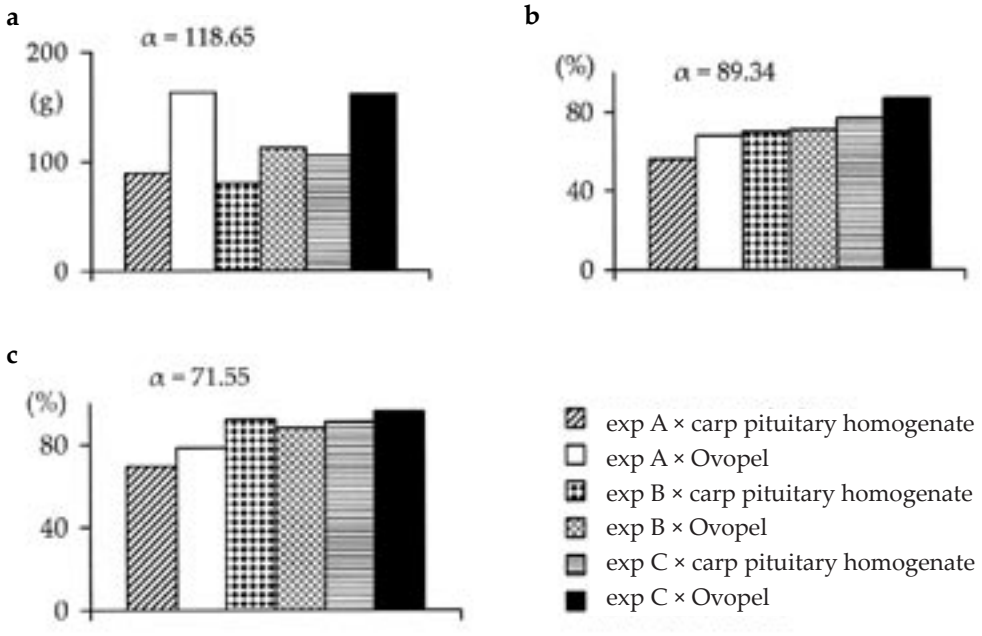


Figure 1. The least-squares means for the interaction of experiment and ovulation stimulator (α is the theoretical general mean)

a – weight of eggs in grams per kg of female body weight; b – percentage fertilization after 12 h of incubation; c – percentage of living embryos after 24 h of incubation

Table 3. Correlations between the investigated traits of females treated with carp pituitary (above diagonal) or Ovopel (below diagonal)

| Investigated traits | Weight of eggs in g per kg female body weight | Percentage of fertilization after 12 h incubation | Percentage of living embryos after 24 h incubation |
|---|---|---|---|
| | 1 | 2 | 3 |
| 1 | | 0.53* | 0.63* |
| 2 | 0.29 | | 0.85* |
| 3 | 0.47 | 0.94* | |

*correlation significant at $P \leq 0.05$

nate the eggs of better quality were from females of group II, i.e. those treated with Ovopel (Table 2).

### Interaction between the experiment and the ovulation stimulator

The investigated interaction was statistically insignificant for the weight of eggs. The least-squares means describing the value of this interaction for the weight of eggs per kg of female body weight show that the highest weight of eggs was found in the case of females treated with Ovopel in experiment A and C (Figure 1a). The lowest weight of eggs was from hypophysed females in experiment B (Figure 1a). Statistical significance ($P \leq 0.05$) of this interaction was determined for the percentage of fertilized eggs. The eggs of the best quality were obtained from fish treated with Ovopel in experiment C and of the poorest one from hypophysed females in experiment A (Figure 1b). This tendency was maintained during the next 12 hours of egg incubation (Figure 1c) though the investigated interaction for the percentage of living embryos was statistically insignificant.

### Correlations between the investigated traits

The weight of eggs recorded in the two investigated groups was positively correlated with both the percentage of egg fertilization and the percentage of living embryos (Table 3). In the group of fish treated with carp pituitary homogenate the values of correlation between the weight of eggs and fertilization percentage and between the weight of eggs and percentage of living embryos were higher and statistically significant in comparison with the same values for the group treated with Ovopel (Table 3). The coefficient of correlation between the percentage of egg fertilization and percentage of living embryos showed higher value in the group of fish stimulated with Ovopel (Table 3).

## DISCUSSION

From the aspect of hatchery practice the application of Ovopel at only one dose seems justified in the investigated fish species on account of the occurrence of synchronized ovulation in all the fish within each of the three experiments conducted. In the case of ovulation stimulation with GnRH-a analogues the prolonged time of spawning of different females is a drawback of using these substances. Numerous authors described it in various fish species (Peter *et al*., 1988; Makeyeva *et al*., 1996; Brzuska and Adamek, 1999; Brzuska and Grzywaczewski, 1999; Brzuska, 2000).

Kouřil *et al*. (1992) treated African catfish (*Clarias gariepinus*) females with (D-Ala$^6$)GnRH ProNHEt at the dose of 20 μg/kg body weight (with isofloxythepin, the dopaminergic inhibitor at the dose of 4 mg per kg), finding that not all stimulated fish spawned at the same time. The time of ovulation varied from 12 to 16 hours after the hormonal treatment. A considerable differentiation, reaching 8 hours, was also recorded in the time of egg yielding by females stimulated with various doses of D-Ala$^6$, Pro$^9$-LHRH-ethylamide applied with pimozide. Tan-Fermin and Emata (1993) described these results in Asian catfish (*Clarias macrocephalus*).

De Leeuw *et al*. (1985) reported the latency time of 12.3 h equal for all the females of African catfish (*Clarias gariepinus*) in an experiment with des Gly$^{10}$(D-Ala$^6$)LHRH-ethylamide and pimozide as

dopamine receptor antagonist. Also in females of this species treated with desGly$^{10}$(D-Ala$^{6}$)LHRH-ethylamide and pimozide the ovulation occurred at the same time, i.e. 16 hours after the injection as given by Richter *et al*. (1987a).

In European catfish (*Silurus glanis* L.) the application of one Ovopel dose equal to that applied in the presented investigation (i.e. 1 pellet/kg female body weight) induced the egg yielding by all the females at the same time (Brzuska, 2003).

The data obtained in the discussed study show that in all three experiments the results of reproduction of *Clarias gariepinus* females were better after one dose of Ovopel than after hypophysation. This was documented in detail if the values of the least-squares means for the interaction between the ovulation stimulator and the experiment were taken into consideration. In all three experiments the higher weight of eggs per kg of female body weight was recorded after Ovopel than after carp pituitary homogenate treatment. The quality of eggs (expressed as the percentage of living embryos after 24-hour incubation) obtained from the investigated females treated with Ovopel was higher than that obtained from hypophysed fish. It should be noted that within the discussed experiments no partial ovulation took place in any of the Ovopel treated females.

In recapitulating the results obtained in the presented study it can be concluded that in African catfish (*Clarias gariepinus*) the stimulation of ovulation by one dose of Ovopel not only resulted in successful reproduction but also reduced the stress to fish and the costs of the stimulation. An important point is that the application of one Ovopel dose reduces the labour consumption of controlled reproduction. The value of the correlation coefficient between the percentage of egg fertilization and the percentage of living embryos after 24-h incubation should also be stressed. After Ovopel treatment this coefficient was higher in relation to its value calculated for the females after carp pituitary homogenate application.

The positive properties of Ovopel presented in the Introduction, its reasonable price and satisfactory reproduction results allow to recommend this preparation for controlled hatchery reproduction of this species. The recommendation is also supported by the absence of pimozide in the composition of Ovopel. It is known that in African catfish (*Clarias gariepinus*) the application of GnRH-a without a blocker of dopamine receptors induced the ovulation in a very low percentage of females (Goos *et al*., 1987; Brzuska, unpubl. data). On the other hand, pimozide, which gives satisfactory results if combined with GnRH-a (De Leeuw *et al*., 1985; Brzuska *et al*., 1999), cannot be used in fish farming as a preparation not available commercially for this purpose (Van Oordt and Goos, 1987).

It is also worth noting that in spite of numerous studies on the ovulation stimulation in this species with various preparations (Hogendoorn, 1979; De Leeuw *et al*., 1985; Richter *et al*., 1985; Richter *et al*., 1987a,b; Goos *et al*., 1987; Kouřil *et al*., 1992; Inyang and Hettiarachchi, 1994; Brzuska *et al*., 1999; Brzuska, 2002) none of them is used in practice on a larger scale. Hence it seems that further studies on ovulation stimulation with Ovopel can be recommended. The results would allow to optimize reproduction effects in this interesting species.

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**ABSTRAKT**

**Umělý výtěr sumečka afrického (*Clarias gariepinus*): jednorázové podání přípravku obsahujícího analog GnRH a dopaminergní inhibitor metoclopramid**

Byl sledován vliv použití kapří hypofýzy (v dávce 4 mg/kg tělesné hmotnosti) a přípravku Ovopel, obsahujícího analog savčího GnRH D-Ala$^{6}$,Pro$^{9}$Net-mGnRH (1 peleta/kg tělesné hmotnosti) a metoclopramid – blokátor dopaminového receptoru (10 mg/kg), jako stimulátorů ovulace při reprodukci sumečka afrického (*Clarias gariepinus* Burchell 1822) ve třech pokusech v řízených podmínkách prostředí. Ve srovnání s účinky podání hypofýzy vedla aplikace Ovopelu ke statisticky významně vyšší produkci hmotnosti jiker přepočtené na 1 kg tělesné hmotnosti jikernaček a ke statisticky významně vyšší kvalitě jiker po 24 hodinách inkubace. Nebyl zaznamenán vliv pokusu na hmotnost ani kvalitu jiker, zatímco interakce mezi pokusem a stimulátorem ovulace byla statisticky významná ($P \leq 0,05$), pokud se jedná o procento oplození jiker. Byla nalezena statisticky významná korelace ($P \leq 0,05$) mezi procentem oplozenosti jiker a procentním podílem živých embryí, když úroveň zjištěného korelačního koeficientu byla vyšší po aplikaci Ovopelu ve srovnání s podáním homogenátu kapří hypofýzy

**Klíčová slova**: *Clarias gariepinus*; stimulace ovulace; podání hypofýzy; jednorázová dávka Ovopelu; umělý chov ryb

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