

# Artificial propagation of European catfish (*Silurus glanis*): application of a single dose of pellets containing D-Ala<sup>6</sup>, Pro<sup>9</sup>NEt-mGnRH and dopamine inhibitor metoclopramide to stimulate ovulation in females of different body weight

E. BRZUSKA

Institute of Ichthyobiology and Aquaculture, Polish Academy of Sciences, Gołysz, Poland

**ABSTRACT:** Effects of reproduction of European catfish females of different body weight (4.60–11.00 kg) were investigated in two experiments after ovulation stimulation with two doses of carp pituitary (0.4 + 3.6 mg/kg body weight) or a single dose of Ovopel – a preparation containing mammalian GnRH analogue D-Ala<sup>6</sup>, Pro<sup>9</sup>NEt-mGnRH and dopamine receptor antagonist, metoclopramide (1 pellet/kg body weight). It was found in both experiments that all the lighter females spawned after Ovopel treatment while the percentage of spawning females of higher body weight treated with this ovulation stimulator was lower. No statistically significant effect of the experiment was observed with respect to traits characterising the weight of eggs or their quality. The effect of applied ovulation stimulator was statistically significant ( $P \leq 0.01$ ) for the percentage of live embryos after 56-h incubation, however the eggs obtained after Ovopel treatment were characterised by higher quality. The body weight of the females significantly ( $P \leq 0.05$ ) affected the weight of obtained eggs, though expressed in grams only, and the percentage of live embryos after 56-h incubation ( $P \leq 0.01$ ). The interaction between the ovulation stimulator and the body weight of females did not affect the traits determining the weight of eggs or the percentage of live embryos after 24- and 48-h incubation. However, it was statistically significant ( $P \leq 0.01$ ) for the percentage of live embryos after 56-h incubation.

**Keywords:** European catfish; artificial propagation; carp pituitary; single dose of Ovopel

In the course of long-term studies on the effects of controlled reproduction of females of various fish species, conducted in the Gołysz Institute of Ichthyobiology and Aquaculture of the Polish Academy of Sciences (Brzuska and Adamek, 1997; Brzuska *et al.*, 1998; Brzuska, 1999a,b; Brzuska and Grzywaczewski, 1999; Brzuska, 2000a,b; 2001a,b, 2002) experiments are also carried out with European catfish (Brzuska and Adamek, 1999; Brzuska, 2001c). The results reported by different authors (Epler *et al.*, 1986; Kouřil *et al.*, 1987, 1996; Epler and Bieniarz, 1989; Linhart *et al.*, 1997) and the results of tests conducted in Gołysz (Brzuska and Adamek, 1999; Brzuska,

2001c) show that like in the case of numerous fish species of economic importance ovulation in the females of European catfish can also be stimulated using synthetic stimulators and give interesting results. Quite a small number of data in the literature concerning stimulated reproduction of this valuable fish species justifies further studies within the scope of this problem.

Ovopel (Horváth and Szabó, 1996; Horváth *et al.*, 1997) was found effective in the stimulation of ovulation in European catfish not only in the natural season of spawning (Kłodzińska and Okoniewski, 1998; Brzuska, 2001c) but also outside it (Ulikowski, 2001). Good effects

on reproduction of numerous fish species after Ovopel treatment (see review in Brzuska, 2001c) and numerous favourable traits of this stimulator (Kłodzińska and Okoniewski, 1998; Kucharczyk and Szabó, 1998) encourage further experiments with its use.

The application of a single dose of Ovopel gave interesting results in the reproduction of African catfish (*Clarias gariepinus*) (Brzuska *et al.*, 1998, 2000; Brzuska, 2002), instigating new studies on the ovulation stimulation in European catfish without a priming dose of this preparation. In the case of controlled spawning the treatment with one dose of this stimulator limits the necessary manipulations that are a source of stress to fish. The high costs connected with the reproduction of thermophilous fish in the conditions of hatcheries could also be reduced. These important aspects were taken into consideration in the project of the present investigation.

The aim of the presented investigation was to determine the possibility of obtaining eggs from European catfish treated only with one dose of Ovopel and whether the effects of reproduction after Ovopel application differed from those observed after traditional double hypophysation. The results of previous investigation conducted on this species showed that the spawning stimulated with carp pituitary or Ovopel applied in two doses (as recommended by Horváth *et al.*, 1997) was significantly affected by the weight of females used for reproduction (Brzuska, 2001c). Therefore in the present study it was attempted to determine the dependence between the body weight of females used for reproduction and the effects of spawning

after the stimulation of ovulation with a single dose of Ovopel or carp pituitary.

## MATERIAL AND METHODS

The data constituting the material of the study were derived from two experiments carried out in the season of natural spawning of this species, i.e. in June. The pooled data from the experiments conducted in the same conditions allowed to increase the number of investigated females. It was also attempted to determine whether the results obtained in one experiment significantly differed from those recorded in the other. In both experiments directly after the fish were taken from ponds, females were selected out of a larger population on the basis of external signs of maturity (Horoszewicz, 1971). They were transferred to a hatchery and divided into two groups. In both experiments a half of each group was composed of individuals of lower body weight and the other half of those of higher body weight (Table 1). The fish were placed in tanks (2.5 m<sup>3</sup> in volume) at the hatchery, two individuals in one tank (the one of lower and the other of higher body weight) in water at 22–23°C. After one day adaptation period ovulation stimulation began, the fish in group I being treated with carp pituitary homogenate and those in group II with Ovopel. The preparation Ovopel contains the mammalian GnRH analogue (D-Ala<sup>6</sup>, Pro<sup>9</sup>NEt-mGnRH) and metoclopramide – a water-soluble blocker of dopamine receptors (Horváth and Szabó, 1996; Horváth *et al.*, 1997). The applied doses of the two stimulators of ovulation are given in Table 1. Both

Table 1. Numbers and average weight of females used in the investigation in group I and II of the experiments, the applied ovulation stimulators and their doses ( $\bar{x}$  = arithmetical mean; SD = standard deviation)

Experiment	Group	No. of females <i>n</i> = 24	Lighter females $\bar{x} \pm \text{SD}$	Heavier females $\bar{x} \pm \text{SD}$	Ovulation stimulator	Doses/kg body weight of females
1	I	6	5.30 ± 0.89	8.90 ± 1.11	carp pituitary	0.4 mg and 3.6 mg after 12 h
	II	8	6.05 ± 0.33	10.20 ± 0.93	Ovopel	1 pellet
2	I	4	6.30 ± 0.99	9.05 ± 0.35	carp pituitary	0.4 mg and 3.6 mg after 12 h
	II	6	6.47 ± 0.76	10.07 ± 0.83	Ovopel	1 pellet

the pituitary and Ovopel were applied to fish in the form of intraperitoneal injections.

A slight manual pressure on the abdomen of females (Littak and Okoniewski, 1975; Brzuska and Adamek, 1999; Brzuska, 2001c) was carried out to check ovulation. In both experiments the control

was started within 10 hrs after the second dose of pituitary and 10 hrs after Ovopel treatment. The checking was continued every hour during the successive six hours. Eggs were taken from each female separately, weighed and fertilized with mixed milt of controlled quality, taken from macerated testes

Table 2. Statistical characteristics of the data

Variable	Descriptive statistics					
	<i>n</i>	$\bar{x}$	$\bar{s}$	min	max	SD
Weight of females (kg)						
group I lighter females	5	5.70	0.43	4.60	7.00	0.97
heavier females	5	8.96	0.36	7.80	10.00	0.80
group II lighter females	7	6.23	0.21	5.60	7.00	0.54
heavier females	7	10.14	0.31	9.30	11.00	0.82
Weight of eggs (g)						
group I lighter females	4	535.00	125.12	265.00	805.00	250.23
heavier females	5	941.25	37.33	875.00	1 020.00	74.65
group II lighter females	7	655.00	99.30	255.00	920.00	262.71
heavier females	5	790.00	151.92	350.00	1 250.00	339.71
Weight of eggs (% of female body weight)						
group I lighter females	4	8.74	2.39	2.92	13.60	4.77
heavier females	5	9.29	0.66	8.31	11.20	1.33
group II lighter females	7	10.35	1.62	4.25	16.12	4.29
heavier females	5	7.67	1.29	3.68	11.30	2.89
Live embryos after 24-h incubation						
group I lighter females	4	85.75	5.95	68.00	93.00	11.89
heavier females	5	89.50	1.94	84.00	93.00	3.87
group II lighter females	7	92.57	1.04	90.00	98.00	2.76
heavier females	5	90.80	1.16	88.00	94.00	2.59
Live embryos after 48-h incubation						
group I lighter females	4	81.25	5.86	65.00	93.00	11.73
heavier females	5	83.50	1.32	80.00	86.00	2.65
group II lighter females heavier females	7	84.85	1.20	82.00	91.00	3.18
heavier females	5	80.60	1.78	76.00	87.00	3.97
Live embryos after 56-h incubation						
group I lighter females	4	61.75	3.33	54.00	70.00	6.65
heavier females	5	62.00	3.67	56.00	72.00	7.35
group II lighter females	7	81.71	0.94	79.00	85.00	2.50
heavier females	5	62.20	4.10	50.00	70.00	9.18

$\bar{x}$  = arithmetical mean;  $\bar{s}$  = standard error of the mean; SD = standard deviation

of several killed males. The males used for reproduction had been hypophysectomized with carp pituitary homogenate applied intramuscularly at a dose of 3.6 mg/kg body weight.

The incubation of fertilized eggs obtained in the two experiments was conducted in a Weiss glass for each female separately in water at 22–23°C. After 24, 48, and 56 hrs of egg incubation the percentage of live embryos was calculated using the method given by Brzuska and Adamek (1999). After the hatching of larvae the percentage of deformed individuals was also calculated for each female separately.

The statistical characterisation of data was presented in Table 2. The data obtained from the two experiments were subjected to analysis of variance using the least-squares method (Harvey, 1960, 1987). The aim of analysis was to estimate the effect of the main classification factors (experiment, ovulation stimulator, and female body weight) on the investigated traits. The investigated traits were: weight of eggs expressed in grams and in percentage of female body weight and percentage of live embryos after 24-, 48-, and 56-hour incubation. Analysis of variance was carried out according to a linear model also including the interaction between the ovulation stimulator (group) and the female body weight.

Analysis of variance was conducted according to the following linear model:

$$Y_{ijkl} = \mu + a_i + g_j + c_k + (gc)_{ij} + e_{ijkl}$$

where:  $\mu$  = overall mean

$a_i$  = effect of an experiment ( $i = 1 \dots 2$ )

$g_j$  = effect of an ovulation stimulator  
( $j = 1 \dots 2$ )

$c_k$  = effect of the size of the female  
( $k = 1 \dots 2$ )

$(gc)_{ij}$  = interaction between the ovulation stimulator and the size of the female

$e_{ijkl}$  = random error connected with observation  $l$

The significance of the effect of an experiment, group connected with an ovulation stimulator, body weight of the females, and the interaction between the ovulation stimulator and size of the female on the investigated traits was checked using the  $F$ -test. Analysis of the data allowed to estimate constants and least-squares means illustrating the values of the individual traits investigated within the main effects determined. The constants and

least-squares means are given in Table 3. The values of the least-squares means for the investigated interaction are presented in graphic form in Figure 1.

## RESULTS

### Percentage of females ovulating after hormonal stimulation

In experiment 1, 66.67% of lighter and 100% of heavier fish spawned after the pituitary treatment. After the application of Ovopel all the fish of lower body weight and 75% of heavier females spawned (Figure 1).

In experiment 2, eggs were obtained from all the fish, both of lower and higher body weight, treated with pituitary and from 100% of lighter fish treated with Ovopel. After Ovopel application eggs were obtained from 66.67% of the females of higher body weight (Figure 2).

### Ovulation time

The time between the second hypophysectomy and ovulation was 11 h for fish from group I in the first and second experiment. After Ovopel application all the females that passed ovulation in the two experiments yielded eggs within 15 h after the stimulator was injected.

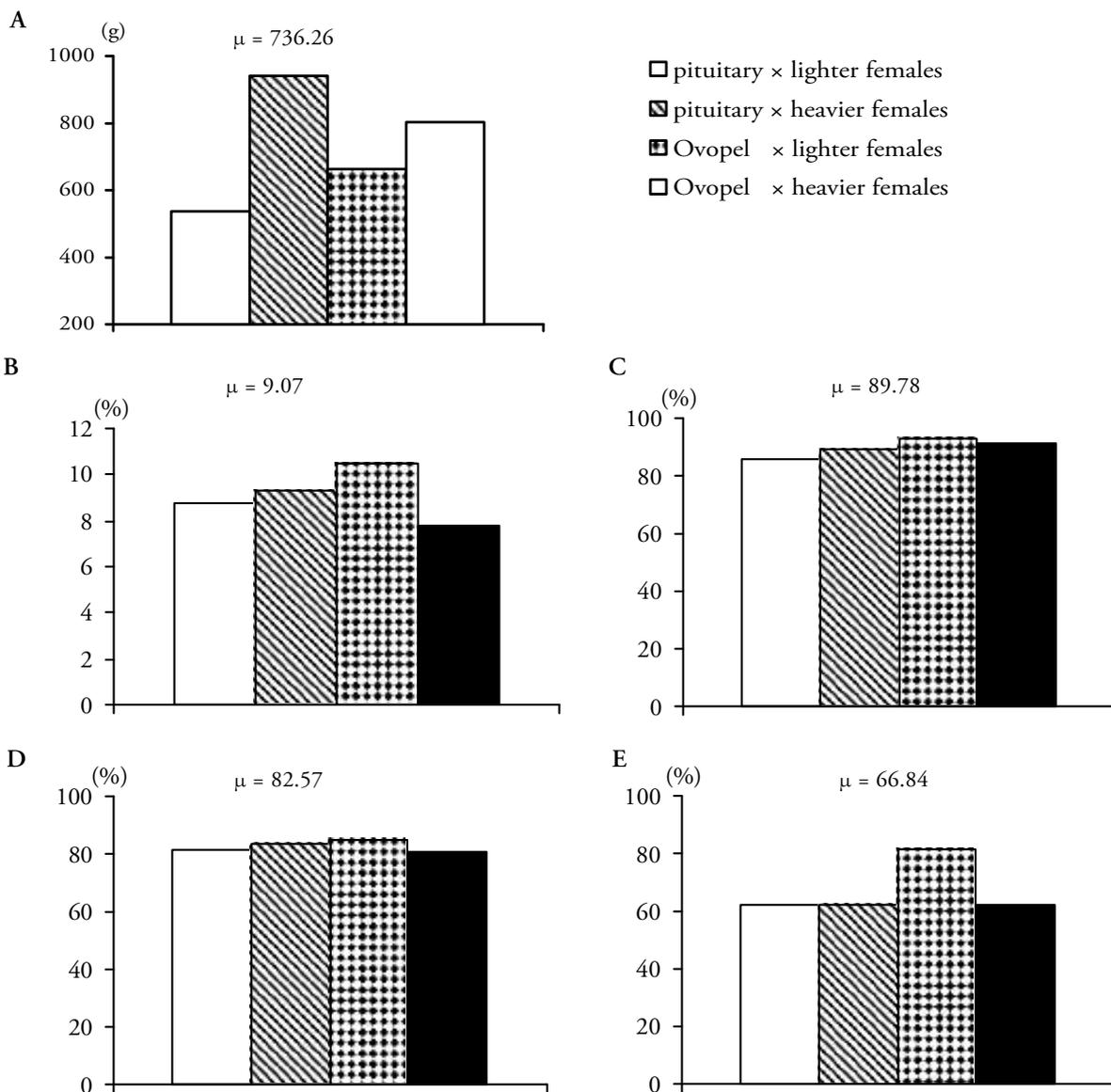
### Effect of experiment on the weight and quality of eggs

The results of analysis of variance and the  $F$ -test did not show any significant effect of the experiment on the traits determining the weight or quality of obtained eggs (Table 4). It should be mentioned, however, that with respect to the least-squares means determining the weight of eggs (both in grams and in percentage of female body weight) the obtained values show a higher weight of eggs recorded in experiment 2. In this experiment the constant of the least squares for the weight of eggs in grams manifested a deviation of +69.44 from the overall mean. For the weight of eggs expressed as percentage of female body weight the value of the deviation was only +0.58 (Table 3). As the values of the least-squares means estimated for the percent-

Table 3. Constants (LSC) and least-square means (LSM) estimated for investigated traits

Classification factor	Weight of eggs (g)			Weight of eggs (% of female body weight)			Percentage of living embryos									
	$\mu = 736.26$			$\mu = 9.07$			$\mu = 89.78$			$\mu = 82.57$			$\mu = 66.84$			
	LSC	LSM	SE	LSC	LSM	SE	LSC	LSM	SE	LSC	LSM	SE	LSC	LSM	SE	
Experiment																
1	-69.44	666.82	79.09	-0.58	8.48	1.16	-1.44	88.34	1.78	-0.18	82.39	1.88	0.92	67.76	2.03	
2	69.44	805.70	86.03	0.58	9.65	1.26	1.44	91.22	1.94	0.18	82.74	2.05	-0.92	65.92	2.21	
Ovulation stimulator																
carp pituitary (group I)	1.86	738.13	90.62	-0.05	9.02	1.32	-2.15	87.63	2.04	-0.19	82.38	2.15	-4.96	61.88	2.33	
Ovopel (group II)	-1.86	734.40	75.69	0.05	9.11	1.11	2.15	91.93	1.71	0.19	82.76	1.80	4.96	71.80	1.95	
Body weight of fish																
lighter females	-136.30	599.96	80.43	0.52	9.59	1.18	-0.52	89.26	1.81	0.50	83.07	1.91	4.83	71.67	2.07	
heavier females	136.30	872.60	86.17	-0.52	8.54	1.26	0.52	90.29	1.94	-0.50	82.07	2.05	-4.83	62.01	2.22	

SE = standard error of least-squares means;  $\mu$  = overall mean



A – weight of eggs in grams; B – weight of eggs as percentage of female body weight; C – percentage of live embryos within 24-h of incubation; D – percentage of live embryos within 48-h of incubation; E – percentage of live embryos within 56-h of incubation

Figure 1. Least-squares means for the interaction of ovulation stimulator and body weight of females ( $\mu$  is overall mean)

age of live embryos after 24-, 48-, and 56-hour incubation show, the quality of eggs was very similar in the two experiments (Table 3).

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

No statistically significant effect of the ovulation stimulator was observed with respect to any of the

two traits determining the weight of obtained eggs or percentage of egg fertilization after 24- and 48-hour incubation. The effect of the ovulation stimulator was only significant ( $P \leq 0.01$ ) with respect to egg quality after 56-hour incubation (Table 4). As the values of the least-square means for this trait show, eggs obtained after Ovopel treatment were of much higher quality than those obtained after hypophysation (the respective values being 71.8 and 61.9; Table 3).

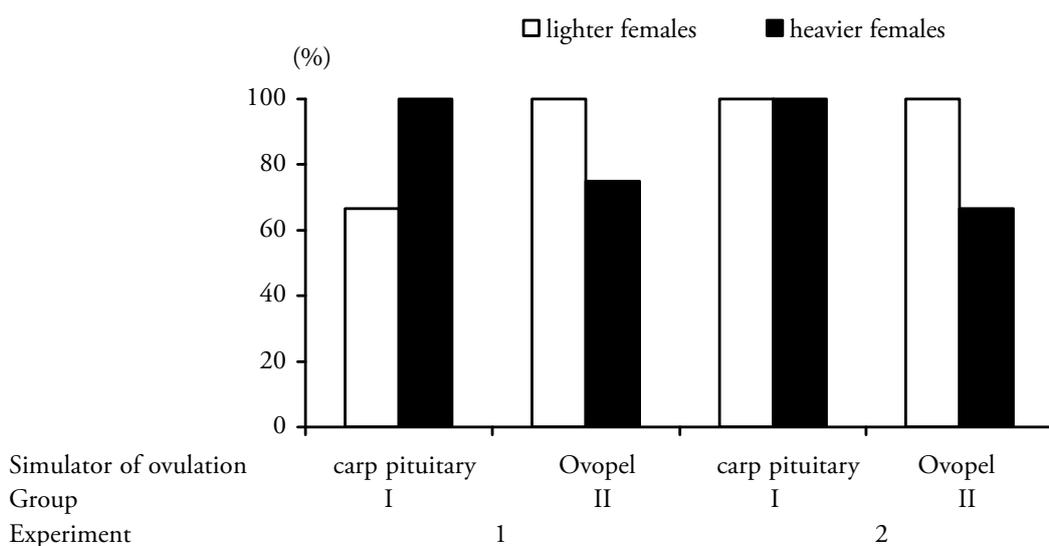


Figure 2. Percentage of females ovulating after hormonal stimulation

### Effect of body weight of females used for reproduction on the weight and quality of eggs

The statistically significant ( $P \leq 0.05$ ) effect of female body weight on the weight of eggs expressed in grams was evidenced (Table 4). The least-squares mean for this trait estimated for females of lower body weight had a lower value, i.e. 599.96 g, while for heavier females it was 872.60 g. When the weight of eggs was expressed as the percentage of the body weight of spawners, the values of the least-squares means did not significantly differ for lighter and heavier females (Tables 3 and 4). Neither was the effect of the body weight of spawners statistically significant with respect to the percentage of live embryos after 24- and 48-h incubation.

However, a statistically significant ( $P \leq 0.01$ ) effect of this classification factor on the percentage of live embryos was recorded after 56-h incubation (Table 4). As the least-square means estimated for this trait show, lighter females yielded eggs of about 10% higher quality (Table 3).

### Interaction

Analysis of variance and the  $F$ -test showed that the interaction between the ovulation stimulator and the body weight of females had no statistically significant effect on the weight of eggs presented either in grams or in percentage of female body weight (Table 4). The values of the investigated interaction for the weight of eggs expressed in

Table 4. Results of the  $F$ -test

Classification factor	Weight of eggs (g)	Weight of eggs (% of female body weight)	Percentage of living embryos (hours of incubation)		
			24	48	56
Experiment	—	—	—	—	—
Ovulation stimulator	—	—	—	—	**
Body weight of females	*	—	—	—	**
Interaction between ovulation stimulator and female body weight	—	—	—	—	**

\* $P \leq 0.05$ ; \*\* $P \leq 0.01$

Table 5. Correlation between the traits of lighter females (above the diagonal) and heavier females (under the diagonal)

Variables	Weight of females (kg)	Weight of eggs (g)	Weight of eggs as percentage of female body weight	Percentage of living embryos (hours of incubation)		
				24	48	56
	1	2	3	4	5	6
1		0.45	0.26	0.62*	0.29	0.58
2	0.25		0.95*	0.41	0.34	0.44
3	0.09	0.93*		0.47	0.50	0.45
4	0.14	-0.16	0.04		0.85*	0.66*
5	0.06	0.24	0.26	0.46		0.52
6	0.53	0.01	-0.16	-0.22	0.31	

\*correlation significant at  $P \leq 0.05$

grams show that the highest weight of eggs was found after hypophysation of females of the higher weight and the lowest after the pituitary treatment of lighter fish (Figure 1A). After Ovopel treatment a higher weight of eggs was obtained from heavier females, but only if it was expressed in grams (Figure 1A). In calculating the weight of eggs as the percentage of female body weight, the value of the interaction obtained for this trait was higher for lighter females in comparison with heavier ones (the respective values being 10.44 and 7.79; Figure 1B). The least-squares means for this trait showed that for lighter females treated with Ovopel the interaction attained the highest value exceeding 10% (Figure 1B). No statistically significant effect of the investigated interaction on the percentage of live embryos after 24- or 48-h incubation of eggs was recorded (Table 4). The data presented in Figure 1C, however, distinctly show that already after 24-h incubation the best quality characterised eggs obtained from lighter females treated with Ovopel. The statistically significant ( $P \leq 0.01$ ) effect of the interaction was found for the percentage of live embryos after 56-h incubation (Table 4). The presented values of the least-squares means estimated for this trait show the highest quality of eggs (81.6% of live embryos after 56-h incubation) obtained from females of lower body weight stimulated with Ovopel in comparison with the remaining values investigated, which varied about 62% (Figure 1E).

#### Dependence between the investigated traits

The values of correlation coefficients between the investigated traits estimated for lighter and heavier females are given in Table 5. The correlation between the body weight of females and the remaining five traits investigated had higher values for lighter fish compared with heavier females. A statistically significant ( $P \leq 0.05$ ) correlation was found only between the body weight of lighter fish and the percentage of live embryos after 24-h incubation of eggs, the value of the coefficient being 0.62. In lighter fish a statistically significant ( $P \leq 0.05$ ) correlation was found between the percentage of live embryos after 24-h incubation and the percentage of live embryos after 48-h incubation and also between the percentage of live embryos after 24-h incubation and the percentage of live embryos after 56 h incubation of eggs (the respective values being 0.85 and 0.66). All the correlation coefficients estimated for lighter fish were characterised by positive values. In heavier females negative correlation values were recorded between the weight of eggs in grams and the percentage of live embryos after 24-h incubation, between the weight of eggs expressed as percentage of body weight of females and the percentage of live embryos after 56-h incubation, and between the percentage of live embryos after 24-h incubation and the percentage of live embryos after 56-h incubation.

## Occurrence of deformed larvae

The occurrence of deformed larvae not exceeding four individuals per 100 ones was observed after the application of the two ovulation stimulators. The body deformations were observed only among the larvae hatched from eggs obtained from heavier females.

## DISCUSSION

From the aspect of hatchery practice a very important result was obtained in the present investigation showing that in both experiments all the females spawning after Ovopel treatment yielded eggs at the same time, i.e. 15 h after injection of one dose of this stimulator in the amount of 1 pellet/kg. Synchronized ovulation in all the females after stimulation with one dose of Ovopel (1 pellet/kg) was also found in the investigation conducted on African catfish (*Clarias gariepinus*) (Brzuska *et al.*, 1998, 2000; Brzuska, 2001b). The same time of egg yielding by all the females of European catfish treated with one dose of Ovopel is important because after the application of two doses of Ovopel (1/5 + 1 pellet/kg female body weight) the spawning time was not the same for all the investigated females of this species (Brzuska, 2001c). It should be stressed that the above experiment was carried out in conditions corresponding with those of the present study. After the treatment with two doses most females yielded eggs within 11 h after the second Ovopel dose and the remaining ones two hours later (Brzuska, 2001c).

The varied time of egg yielding, disorganising the work of the hatchery, was also recorded by Brzuska and Adamek (1999) in European catfish females treated with des-Gly<sup>10</sup>, [D-Ala<sup>6</sup>] LHRH-Ethylamide + pimozone or with Ovaprim [(D-Arg<sup>6</sup>, Pro<sup>9</sup>-NEt) sGnRH + domperidone; Peter *et al.*, 1993]. In the case of the former stimulator the latency time ranged from 26 to 30 h while after Ovaprim treatment from 24 to 30 h. Neither did Kouřil *et al.* (1996) obtain the synchronised time of ovulation in all fish of the investigated groups treated with des-Gly<sup>10</sup>(D-Ala<sup>6</sup>) GnRH-Ethylamide (at the dose twice higher than that applied by Brzuska and Adamek, 1999) without the blocker of dopamine receptors or with Isofloxythepin, a dopamine inhibitor. The latency time for this stimulator, as reported by Kouřil *et al.* (1996), was also very long, ranging about 30 hours.

The latency time naturally depends on the temperature of water in which fish are kept (Drori *et al.*, 1994) although in all the experiments described above the temperature showed optimum values for the reproduction of European catfish, i.e. about 23°C. It should be mentioned as well that the synchronization of egg yielding by all the females stimulated with a given preparation is possible only if the degree of maturity of the females is similar at the time of treatment.

From the practical standpoint it seems worth stressing that in European catfish the ovulation stimulation with Ovopel at one or two doses makes the occurrence of ovulation probable after a much shorter time than after GnRH-a treatment with dopamine receptor blocker or without it. It was suggested by the results obtained by the following authors: Epler *et al.* (1986), Epler and Bieniarz (1989), Kouřil *et al.* (1987, 1996), Kłodzinska and Okoniewski (1998), Brzuska and Adamek (1999), Brzuska (2001c) and present data; Ulikowski (2001).

The results concerning the percentage of spawning fish in relation to all the individuals treated with stimulators show that the application of one Ovopel dose to lighter females led to ovulation in all of them in both experiments discussed in this paper. Heavier fish responded to the synthetic stimulator to a lower degree as expressed by the lower percentage of spawning females. The above observation corroborates the results of an earlier study carried out on the same fish species with two doses of Ovopel (1/5 + 1 pellet/kg) (Brzuska, 2001c). The comparison of results concerning the percentage of spawning females after one or two doses of Ovopel (Brzuska, 2001c) distinctly shows that a decrease in the amount of the stimulator by omitting the priming dose did not negatively affect this important trait determining the reproduction efficiency of spawners.

The data obtained from the present investigation clearly show that the results of reproduction depend on whether the ovulation stimulation was carried out on heavier or lighter females. The values of interaction between the ovulation stimulator and the weight of evaluated fish for the investigated traits illustrate the effects of reproduction separately for heavier and lighter females.

Analysis of the least-squares means for the investigated interaction shows that the weight of eggs expressed in grams did not depend on the ovulation stimulator applied but on the body weight of spawning females. Heavier females stimulated

either with pituitary or with Ovopel yielded eggs of higher weight in comparison with lighter fish. However, in the case of one-dose Ovopel treatment of lighter females the weight of eggs expressed as a percentage of female body weight was much higher than that obtained from heavier females.

The values of interaction between the ovulation stimulator and weight of females for the percentage of live embryos after 24-hour incubation were higher in fish treated with Ovopel irrespective of their higher or lower weight. After 56-hour incubation of eggs, however, these obtained from heavier fish were characterised by about 20% poorer quality than the eggs yielded by lighter females. It should be stressed that after a single-dose Ovopel treatment lighter fish yielded eggs of the best quality out of the four combinations investigated. The same observation was made when the ovulation stimulation was carried out with two doses of Ovopel (Brzuska, 2001c). In the investigation on African catfish (*Clarias gariepinus*) the application of a single dose of Ovopel also resulted in the yield of eggs of better quality in comparison with those obtained from heavier females (Brzuska, 2001b).

Another interesting observation also made on the basis of the present work shows that after 56-hour incubation the quality of eggs yielded by lighter or heavier hypophysed females did not differ. This information agrees with the results of studies conducted on African catfish (*Clarias gariepinus*) though the percentage of live embryos of this species was calculated only after 24-hour incubation (Brzuska, 2002).

In the discussed investigation containing the results of two separate experiments an important finding was that no eggs of very poor quality were yielded either by lighter or by heavier fish treated with Ovopel. This could be determined already after 24-hour incubation. The above observation is in agreement with data obtained after applying this ovulation stimulator though at a higher amount and at two doses (Brzuska, 2001c). In the case of Ovaprim application to females of this species 20% of them yielded eggs, which showed 0–20% of live embryos after 24-hour incubation (Brzuska and Adamek, 1999).

The problem of reproduction effects observed in lighter and heavier females is important from the aspect of practice. As the presented data show after ovulation stimulation with a single dose of Ovopel (greatly reducing the stress to fish and the labour-consuming operations necessary in controlled re-

production) better results were recorded in lighter females than in the heavier ones. Higher percentage of lighter females spawned and the obtained eggs were of better quality. It seems important that no deformed larvae hatched from eggs obtained from lighter fish. In analysing the obtained results there arises a question whether it is worthwhile to rear and reproduce spawners of body weight exceeding 7 kg. The results of earlier studies conducted on various fish species (Brzuska, 1987, 1991, 2001a; Brzuska and Adamek, 1997; Kłodzińska and Okoniewski, 1998; Brzuska *et al.*, 1998, 2000) stressed the problem, highly important in hatchery practice, of minimising doses of the applied ovulation stimulators. The results of former experiments and those obtained in the present work show that the investigations concerning reduced amounts of applied stimulators are fully justified. They should be continued and aim at the polyoptimization of reproduction effects (Brzuska, 1991). The attempts at reducing the high costs connected with controlled reproduction of European catfish (both by reducing the amounts of applied stimulators and selecting females of body weight not exceeding 7 kg) cannot be regarded as of no avail.

### Acknowledgements

The author thanks Mr J. Adamek and Mrs U. Czudek working in the Institute of Ichthyobiology and Aquaculture, Polish Academy of Sciences in Gołysz, for technical assistance in the experiments, and Mrs U. Karosek for careful technical preparation of the manuscript.

### REFERENCES

- Brzuska E. (1987): The use of the low doses of carp pituitary homogenate for inducing ovulation in carp (*Cyprinus carpio* L.) during late spring. In: Proceedings of Fifth Congress of European Ichthyologists. 12–16 August 1985, Stockholm, 273–275.
- Brzuska E. (1991): Estymacja efektów kontrolowanego tarła karpia (*Cyprinus carpio* L.) oraz próba ich polioptymalizacji. Zesz. Nauk. Akademii Rolniczej (Kraków), Rozprawa habilitacyjna, No. 153, 1–77.
- Brzuska E. (1999a): Stymulowanie owulacji u samic karpia pochodzenia polskiego i węgierskiego przysadką mózgową karpia, Ovopem oraz Daginem. Kom. Ryb., No. 4, 23–25.

- Brzuska E. (1999b): Artificial spawning of herbivorous fish; use of an LHRH-a to induce ovulation in grass carp, *Ctenopharyngodon idella* (Valenciennes) and silver carp, *Hypophthalmichthys molitrix* (Valenciennes). *Aquacult. Res.*, 30, 849–856.
- Brzuska E. (2000a): Artificial spawning of carp (*Cyprinus carpio* L.); differences between the effects on reproduction in females of Polish and Hungarian provenance treated with carp pituitary and (D-Ala<sup>6</sup>) GnRH ProNHEt (Kobarelin). *Aquacult. Res.*, 31, 457–465.
- Brzuska E. (2000b): Artificial spawning of the carp (*Cyprinus carpio* L.); differences between the effects of reproduction in females of Hungarian and Polish provenance treated with carp pituitary and Ovopel. In: Proc. of the 6th Int. Symp. on the Reproductive Physiology of Fish, 4–9 July 1999, Bergen, Norway, 452.
- Brzuska E. (2001a): Artificial spawning of carp (*Cyprinus carpio* L.): the use of Aquaspawn and carp pituitary to induce ovulation in females of Lithuanian line B. *Aquacult. Res.*, 32, 357–364.
- Brzuska E. (2001b): Próba wykazania zależności pomiędzy masą ciała samic a efektami rozrodu suma afrykańskiego (*Clarias gariepinus* Burchell 1822) po stymulowaniu owulacji przysadką karpia lub Ovopel. *Kom. Ryb.*, No. 1, 27–28.
- Brzuska E. (2001c): Artificial spawning of European catfish (*Silurus glanis* L.); differences between propagation results after stimulation of ovulation with carp pituitary and Ovopel. *Aquacult. Res.*, 32, 11–19.
- Brzuska E. (2002): Artificial spawning of African catfish, *Clarias gariepinus*: stimulation of ovulation using carp pituitary or Ovopel. *J. Appl. Aquacult.*, 12, 13–22.
- Brzuska E., Adamek J. (1997): Próba stymulowania owulacji u karpia *Cyprinus carpio* L. przy zastosowaniu Ovaprimu. *Kom. Ryb.*, No. 1, 19–21.
- Brzuska E., Adamek J. (1999): Artificial spawning of European catfish *Silurus glanis* L.; stimulation of ovulation using LHRH-a, Ovaprim and carp pituitary extract. *Aquacult. Res.*, 30, 59–64.
- Brzuska E., Grzywaczewski R. (1999): Artificial spawning of carp (*Cyprinus carpio* L.); differences between the effects on reproduction in females of Israeli strain Dor-70 and its crossbreed treated with carp pituitary and Ovopel. *Aquacult. Res.*, 30, 559–570.
- Brzuska E., Rzemieniecki A., Adamek J. (1998): Wyniki stymulowania owulacji u suma afrykańskiego *Clarias gariepinus* (Burchell 1822) przy zastosowaniu Ovopelu. *Kom. Ryb.*, No 4, 15–16.
- Brzuska E., Ráczkevi-Radics J., Radics F. (2000): Stimulation of ovulation in African catfish (*Clarias gariepinus* Burchell 1822) with carp pituitary, Ovopel and HCG (in Czech – with English summary). In: Czech Ichtyol. Symp., 10–12 April 2000, University of South Bohemia, Vodňany, Czech Republic, 16–19.
- Drori S., Ofir M., Levavi-Sivan B., Yaron Z. (1994): Spawning induction in common carp (*Cyprinus carpio*) using pituitary extract or GnRH superactive analogue combined with metoclopramide: analysis of hormone profile, progress of oocyte maturation and dependence on temperature. *Aquaculture*, 119, 393–405.
- Epler P., Bieniarz K., Sokołowska M., Popek W., Mikołajczyk T., Motyka K. (1986): Hormonalna stymulacja sztucznego tarła suma bez stosowania homogenatu przysadki mózgowej. *Gospod. Ryb.*, No. 4, 10–11.
- Epler P., Bieniarz K. (1989): Gonadal maturation and hormonal stimulation of spawning in wels (*Silurus glanis* L.). *Polish Arch. Hydrobiol.*, 36, 417–429.
- Harvey W.R. (1960): Least squares analysis of data with unequal subclass numbers. *Agricultural Research Service United States Department of Agriculture*, 8–20, 1–157.
- Harvey W.R. (1987): User's guide for LSMLMW PC-1 Version. Mixed Model Least-Squares and Maximum Likelihood Computer Program. Copyright W.R. Harvey, U.S.A. 1–59.
- Horoszewicz L. (1971): *Sum. Państwowe Wydawnictwo Rolnicze i Leśne, Warszawa*, 1–192.
- Horváth L., Szabó T. (1996): Hatchery testing of GnRH analogue-containing pellets on ovulation in different fish species. In: *Int. Conf. on Fish Reproduction '96*. 9–12 September 1996. České Budějovice, Czech Republic, Abstracts Book, 53.
- Horváth L., Szabó T., Burke J. (1997): Hatchery testing of GnRH analogue-containing pellets on ovulation in four cyprinid species. *Polish Arch. Hydrobiol.*, 44, 221–226.
- Kłodzińska H., Okoniewski Z. (1998): Ovopel – nowy preparat do stymulowania rozrodu u ryb. *Wylęgarnia 1997–1998. Publikacja IRŚ, Olsztyn*. 45–49.
- Kouřil J., Barth T., Hamáčková J. (1987): Striping the females of sheatfish (*Silurus glanis* L.) with LH-RH analogue induction (in Czech). *Práce VÚRH Vodňany*, 16, 62–68.
- Kouřil J., Hamáčková J., Linhart O., Barth T., Glubokov A.J., Haffray P. (1996): Induce ovulation of European catfish (*Silurus glanis* L.) by carp pituitary, GnRH analogue and/or dopamine inhibitor Isofloxythepin. *Živoč. Výr.*, 41, 205–207.
- Kucharczyk D., Szabó T. (1998): Ovopel – nowy preparat hormonalny do stymulacji rozrodu u ryb karpiovatých. *Karpiovaté ryby Reofilne*. In: *Krajowa Konferencja Hodowców i Producentów Karpiovatých Ryb Reofilnych*, 10–11 Luty 1998, Brwinów, 65–68.

- Linhart O., Billard R., Kouřil J., Hamáčková J. (1997): Artificial insemination and gamete management in European catfish *Silurus glanis* L. Polish Arch. Hydrobiol., 44, 9–23.
- Littak A., Okoniewski Z. (1975): Pierwsze doświadczenia nad rozrodem sumy w warunkach sztucznych. II. Próba rozrodu w ZGS-IRŚ w Żabieńcu. Gospod. Ryb., No. 11, 3–6.
- Peter R.E., Lin H.R., van der Kraak G., Little M. (1993): Releasing hormones, dopamine antagonists and induce spawning. In: Muir J.F., Roberts R.J. (eds.): Recent Advances in Aquaculture. Blackwell Scientific Publications Ltd, Oxford. 25–30.
- Ulikowski D. (2001): Pozasezonowy rozród suma europejskiego (*Silurus glanis* L.). Wylęgarnia 1999–2000. Publikacja IRŚ Olsztyn. 59–62.

Received: 02–07–01

Accepted after corrections: 03–04–06

## ABSTRAKT

### Umělý výtěr sumce velkého (*Silurus glanis*): jednorázové podání přípravku obsahujícího analog GnRH a dopaminergní inhibitor pro stimulaci ovulace jikernaček různé hmotnosti

Byly provedeny dva experimenty s dosažením ovulace jikernaček sumce velkého (o hmotnosti v rozpětí 4,60 až 11,00 kg) pomocí stimulace dvěma dávkami kapří hypofýzy (0,4 + 3,6 mg/kg hmotnosti jikernaček) a jednorázově podanou dávkou přípravku Ovopel (1 peleta/kg hmotnosti těla). V obou experimentech bylo zjištěno nižší procento ovulovaných jikernaček o vyšší hmotnosti těla při použití přípravku Ovopel. Nebyl zjištěn statisticky signifikantní vliv hmotnosti jikernaček na hmotnost jiker v gramech a jejich kvalitu. Byl zjištěn statisticky průkazný účinek ( $P \leq 0,01$ ) použitého přípravku k dosažení ovulace na procento živých embryí po 56 hodinách inkubace; jikry získané při použití přípravku Ovopel měly lepší kvalitu. Hmotnost jikernaček měla statisticky signifikantní vliv ( $P \leq 0,05$ ) nejen na hmotnost získaných jiker vyjádřenou v gramech, ale rovněž na procento živých embryí po 56 hodinách inkubace ( $P \leq 0,01$ ). Nebyl nalezen vliv stimulatorů ovulace a hmotnosti jikernaček na hmotnost vytřených jiker a procento živých embryí za 24 a 48 h inkubace. Byl ale zjištěn statisticky signifikantní ( $P \leq 0,01$ ) vliv na procento živých embryí po 56 h inkubace.

**Klíčová slova:** sumec velký; umělý výtěr; kapří hypofýza, jednorázová dávka Ovopelu

---

#### Corresponding Author

Doc. dr hab. ing. Elżbieta Brzuska, Polish Academy of Sciences, Institute of Ichthyobiology and Aquaculture, Gołysz, 43-520 Chybie, Poland  
Tel. +48 1033 33 8561551, fax +48 1033 33 8589292, e-mail: golysz@fish.com.pl; zigr@bb.onet.pl

---