POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., Vol 27(4): 433–452, Y. 2012

EMPIRICAL AND PREDICTIVE EFFECTIVENESS OF STAGES IN CONTROLLED REPRODUCTION OF AFRICAN CATFISH *CLARIAS GARIEPINUS* BURCHELL 1822 – THE EFFECT OF FEMALE BODY WEIGHT

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K e y w o r d s: controlled reproduction, ovulation stimulation, Ovopel, female body weight, *Clarias gariepinus*.

A b s t r a c t

The results of stages in controlled reproduction of African catfish *C. gariepinus* were investigated in females in six classes of body weight (I: >0 ≤1.5 kg; II: >1.5 kg ≤2.5 kg; III: >2.5 kg ≤3.5 kg; IV: >3.5 kg ≤4.5 kg; V: >4.5 kg ≤6.5 kg; VI: >6.5 kg), their ovulation being stimulated with Ovopel (1 pellet kg^{-1}). The accepted classification significantly ($P \leq 0.01$) differentiated the weight of obtained eggs expressed both in grams and in percentage of female body weight. The highest weight of eggs was obtained from the heaviest fish (class VI) and the lowest from these of the lowest weight (class I) (1191.8 g and 218.7 g respectively) while in these two classes the obtained highest weight of eggs was expressed as the percentage of female body weight (14.21% and 20.19% respectively). The class of body weight significantly ($P \leq 0.01$) determined also the two investigated traits which described the quality of eggs. The lowest value of the least squares means for the percentage of fertilization and of live embryos was noted for the heaviest fish (class VI) (64.75% and 54.63% respectively) and the highest for the fish of the lowest weight, i.e., classes I and II (91.67% and 88.17%, 92.47% and 86.33%). In general the highest effectiveness of reproduction characterized the class of females whose body weight exceeded 4.5 kg but did not rise above 6.5 kg. In order to predict the weight of eggs [g], percentage of fertilization [12 h] and percentage of live embryos [24 h] within the investigated classes the regression equation was introduced using the weight of females as an independent variable. For each class regression equations were also derived in which the weight of females and weight of eggs were accepted as independent variables while the percentages of fertilization and of live embryos were used as dependent variables. The value of empirical data concerning the weight of eggs [g], fertilization percentage [12 h] and percentage of live embryos [24 h] were the basis for deriving a square function which permitted spatial prediction of the distribution of points of these variables on a plane – for each class of female body weight separately.

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EMPIRYCZNE I PREDYKCYJNE EFEKTY ETAPÓW KONTROLOWANEGO ROZRODU SUMA AFRYKAŃSKIEGO *CLARIAS GARIEPINUS* BURCHELL 1822 – WPŁYW MASY CIAŁA SAMIC

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Słowa kluczowe: kontrolowany rozród, stymulowanie owulacji, Ovopel, masa ciała samic, *Clarias gariepinus*.

Abstrakt

Badano wyniki etapów kontrolowanego rozrodu samic suma afrykańskiego *Clarias gariepinus* należących do sześciu klas masy ciała (I: >0 ≤1,5 kg; II: >1,5 kg ≤2,5 kg; III: >2,5 kg ≤3,5 kg; IV: >3,5 kg ≤4,5 kg; V: >4,5 kg ≤6,5 kg; VI: >6,5 kg), u których stymulowano owulację Ovopelem (1 pellet kg^{-1}). Przyjęta klasyfikacja różnicowała istotnie (P≤0,01) masę ikry wyrażoną tak w gramach, jak i w procencie masy ciała ryb. Najwyższą masę jaj pozyskano od ryb najcięższych – klasa VI, a najniższą od najlżejszych – klasa I (odpowiednio 1191,8 g i 218,7 g), przy czym w tych dwóch klasach uzyskano najwyższą masę jaj wyrażoną jako procent masy ciała samic (odpowiednio 14,21% i 20,19%). Klasa masy ciała determinowała istotnie (P≤0,01) również dwie badane cechy określające jakość pozyskanej ikry. Najniższą wartość średniej najmniejszych kwadratów dla procentu zapłodnienia i żywych zarodków odnotowano dla ryb najcięższych (klasa VI) – odpowiednio 64,75% i 54,63%, a najwyższą dla ryb najlżejszych (klasa I i II) – odpowiednio 91,67% i 88,17% oraz 92,47% i 86,33%. Najwyższą efektywnością rozrodu charakteryzowała się klasa samic, których masa ciała przekraczała 4,5 kg, ale nie była wyższa niż 6,5 kg. W celu predykcji masy ikry [g], procentu zapłodnienia (12 godz.) oraz procentu żywych zarodków [24 godz.] w obrębie badanych klas wyprowadzono równania regresji, wykorzystując masę samic jako zmienną niezależną. Dla każdej klasy wyprowadzono również równania regresji, w których jako zmienne niezależne przyjęto masę samicy i masę ikry, a jako zmienne zależne – procent zapłodnienia i żywych zarodków. Wartości danych empirycznych odnośnie do masy jaj [g], procentu zapłodnienia (12 godz.) oraz procentu żywych zarodków [24 godz.] stanowiły podstawę do wyprowadzenia funkcji kwadratowej pozwalającej na przestrzenną predykcję rozkładu punktów tych zmiennych na płaszczyźnie – oddzielnie dla każdej klasy masy ciała samic.

Introduction

In numerous fish species the dependence of the effects of controlled reproduction on the body weight of spawning females seems to present a serious problem in hatchery practice and in general in the fishery. The results of numerous studies show that heavier females (most often older), usually yield eggs of a higher mass compared with that obtained from females of a lower one (KAMLER and MALCZEWSKI 1982, BRZUSKA 1991, 2001a,b, 2010, CEJKO 2007). The diameter and weight of eggs obtained from females of a high body weight also most frequently exceed the diameter and weight of eggs yielded by light females (ZONOVA 1973, HULATA and MOAV 1974, KAMLER and MALCZEWSKI 1982, KAMLER and KATO 1983, KORWIN-KOSSAKOWSKI 1989, BRZUSKA 1997, ADAMEK et al. 2011). The known fact is that older spawners not always exceed the body weight of younger fish. In hatchery practice the age of spawners is not always known hence in selecting fish for controlled reproduction the easiest criterion is their body weight. However, it is worth noting that the maintenance of heavy spawners is very expensive on account of higher costs of feeding, service, and the heating and utilization of water. Besides, it necessitates a wider space in tanks where the heavy spawners are kept. The heavy females are treated with large amounts of expensive ovulation stimulators whose doses are calculated per 1 kg body weight of spawners. Compared with lighter females, those of the higher body weight are to a greater degree exposed to mechanical damage during all the treatments and manipulations connected with the preparation and performance of reproduction under controlled conditions.

In the case of *Clarias gariepinus*, like of other stenothermal fish species, the opinions concerning the use of females of a higher body weight for controlled reproduction are not unequivocal. ADAMEK (2001) suggests that the best effects of reproduction are obtained from females two – three years of age and the body weight of about 4 kg.

In the research carried out in our Institute attempts were made to show if in the effects of reproduction of this fish species differences could be found depending on the body weight of females whose ovulation was stimulated, among other preparations, with CPH (BRZUSKA 2001a, 2002), Ovopel (BRZUSKA 2001a, 2002), Aquaspawn (BRZUSKA 2003a), or Lecirelin (BRZUSKA et al. 2004). It should be noted that the experiments were carried out in the course of spawning of a small number of females divided only in two groups of body weight.

The aim of the investigation described in the presented paper was to compare the effects of reproduction of *Clarias gariepinus* females in six classes of body weight, stimulated with the most frequently used ovulation stimulator i.e., Ovopel. Model considerations were also carried out concerning the prediction of the effectiveness of reproduction within the investigated body weight classes of females.

Materials and Methods

The data used as the material for calculations were derived from nine controlled spawning carried out in the Institute of Ichthyobiology and Aquaculture of the Polish Academy of Sciences at Gołysz. 71 females

(0.91–9.40 kg) reared in the Institute were used for propagation. For each spawning females were selected from a greater population of spawners on the basis of the external signs of maturity (soft and large abdomen). Groups of two fish each were placed in tanks 2.5 m^3 in water at 24–25°C. The ovulation stimulation was carried out with one peritoneal dose (1 pellet kg^{-1}) of Ovopel (BRZUSKA et al. 1998, 2000), prepared according to the procedure given by HORVÁTH et al., 1997. The concentrations of D-Ala^6,Pro^{9}NEt-mGnRH-a and metoclopramide were 18–20 μg/pellet and 8–10 mg/pellet.

The fish were checked for ovulation by a gentle pressing of the abdomen (DE LEEUW et al. 1985, GOOS et al. 1987). Eggs obtained from each female separately were weighed and fertilized with pooled milt from macerated testes of three – four killed males (INYANG and HETTIARACHCHI 1994), which were not treated with Ovopel before. The incubation of fertilized eggs from each females separately was conducted in a Weiss glass (7L) at 24–25°C. After 12 h incubation, the percentage of fertilized eggs and after 24 h incubation the percentage of living embryos were calculated for individual females. Statistical characteristics of the data are given in Table 1.

Since the spawning was carried out in different years the multiplier corrections were estimated for a year and using them every observation was corrected (MILLER et al. 1966).

The following classification of females was carried out, their body weight being taken into consideration. Class I: >0 ≤1.5 kg; class II: >1.5 kg ≤2.5 kg; class III: >2.5 kg ≤3.5 kg; class IV: >3.5 kg ≤4.5 kg; class V: >4.5 ≤6.5 kg; class VI: >6.5 kg.

For the classified data analysis of variance was carried out using the least-squares method (HARVEY 1987) in order to estimate the effect of the class of female body weight on the investigated traits. The investigated traits describing the effects of stages in reproduction were the weight of eggs in grams, weight of eggs as percentage of female body weight, the percentage of fertilization after 12 h incubation, and the percentage of living embryos after 24 h incubation.

Analysis of variance was carried out according to the following linear model:

$$Y_{ij} = \mu + k_i + e_{ij}$$

where:

μ – the overall mean,

k_i – the effect of class of female body weight (i =1...6),

e_{ij} – the random error associated with observation j.

The analysis allowed to estimate the constants and means of the least squares which showed the values of the investigated traits within six classes of female body weight. The values of constants and least squares means are given in Table 2.

Table 1

Statistical characteristics of female body weight, weight of obtained eggs expressed in grams and in percentage of female body weight, fertilization percentage (12 h) and percentage of living embryos (24 h) in six classes of female body weight (I–VI)

Investigated traits	Descriptive statistics			
	n	$\bar{x}$	SD	S_e
Weight of females [kg]				
I	9	1.19	0.19	0.06
II	16	2.03	0.32	0.08
III	17	3.06	0.31	0.07
IV	12	3.95	0.27	0.07
V	7	5.43	0.43	0.16
VI	10	8.15	0.85	0.28
Weight of eggs [g]				
I	7	227.57	50.69	19.16
II	16	232.81	103.38	25.85
III	17	368.29	117.57	28.51
IV	12	489.92	181.50	52.40
V	7	495.33	239.92	97.95
VI	9	1163.78	516.09	172.03
Weight of eggs [% of female body weight]				
I	7	20.04	5.07	1.92
II	16	11.39	4.37	1.09
III	17	11.90	3.20	0.78
IV	12	12.33	4.14	1.19
V	7	9.15	4.27	1.74
VI	9	13.95	5.12	1.71
Fertilized eggs after 12 h incubation [%]				
I	6	91.67	4.50	1.84
II	15	92.47	4.36	1.12
III	16	77.41	20.25	4.91
IV	11	76.18	20.86	6.29
V	7	90.17	5.60	2.29
VI	8	66.88	11.00	3.89
Live embryos after 24 h incubation [%]				
I	6	88.17	6.88	2.80
II	15	86.33	8.76	2.26
III	16	71.59	22.13	5.37
IV	11	68.45	19.47	5.87
V	7	82.50	10.43	4.26
VI	8	56.50	7.69	2.72

$\bar{x}$ – arithmetical mean; SD – standard deviation; S_e – standard error of the mean

Table 2

Constants (LSC) and least squares means (LSM) for investigated reproduction traits within six classes of female body weight and results of F-test

Classes of female body weight	Weight of eggs [g] μ= 497.33				Weight of eggs [% of female body weight] μ= 13.19				Percentage of fertilized eggs after 12 h incubation μ= 83.62				Percentage of living embryos after 24 h incubation μ= 76.80			
	LSC	LSM	$S\bar{e}$	F**	LSC	LSM	$S\bar{e}$	F**	LSC	LSM	$S\bar{e}$	F**	LSC	LSM	$S\bar{e}$	F**
I	–278.66	218.67	97.37	–	6.99	20.19	1.73	–	8.05	91.67	4.02	–	11.36	88.17	4.43	–
II	–258.33	239.00	61.58	–	–1.39	11.80	1.09	–	8.85	92.47	2.55	–	9.53	86.33	2.80	–
III	–141.01	356.31	59.63	–	–1.72	11.48	1.06	–	–2.75	80.88	2.47	–	–1.11	75.69	2.71	–
IV	–14.43	482.90	75.42	–	–0.87	12.32	1.34	–	–1.82	81.80	3.12	–	–3.30	73.50	3.43	–
V	–1.99	495.33	97.37	–	–4.04	9.15	1.73	–	6.55	90.17	4.03	–	5.70	82.50	4.43	–
VI	694.42	1191.75	84.33	–	1.02	14.21	1.50	–	–18.87	64.75	3.49	–	–22.17	54.63	3.83	–

μ – overall means; $S\bar{e}$ – standard error of least squares means (**$P \leq 0.01$).

The significance of the main classification factor on the investigated traits was tested using the F-test (Table 2) and the Duncan's multiple range test was used for the analysing the significance of differences between the means of the six classes of female body weight (Table 3). For each body weight class the percentage of females in which the ovulation occurred (Figure 1) and also the phenotypic correlations between the investigated traits were calculated (Table 4).

Table 3

Significance of differences between the means of class female body weight for the weight of eggs (expressed in grams and in percentage of fish body weight), percentage of egg fertilization (12 h) and for percentage of living embryos (24 h) (Results of Duncan's test; $^{*}P\leq0.05$; $^{**}P\leq0.01$)

Class of female body weight	Weight of eggs in grams						Class of female body weight	Weight of eggs [in % of female body weight]					
	I	II	III	IV	V	VI		V	III	II	IV	VI	I
I	1	–	*	*	*	**	V	1	–	–	–	*	**
II		1	*	**	*	**	III		1	–	–	–	**
III			1	*	*	**	II			1	–	*	**
IV				1	–	**	IV				1	–	**
V					1	**	VI					1	**

Class of female body weight	percentage of fertilized eggs after 12 h incubation of eggs						class of female body weight	percentage of living embryos after 24 h incubation of eggs					
	VI	IV	III	V	II	I		VI	IV	III	V	II	I
VI	1	**	**	**	**	**	VI	1	**	**	**	**	**
IV		1	–	**	**	**	IV		1	–	**	**	**
III			1	**	**	**	III			1	*	**	**
V				1	–	–	V				1	*	*
II					1	–	II					1	–

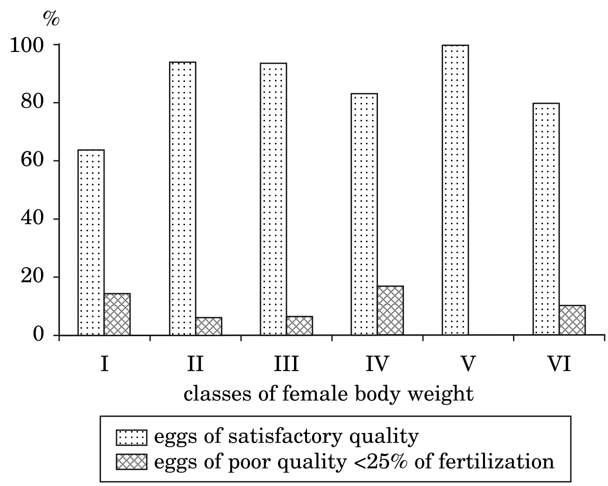


Fig. 1. Percentage of ovulating females after hormonal stimulation

Table 4

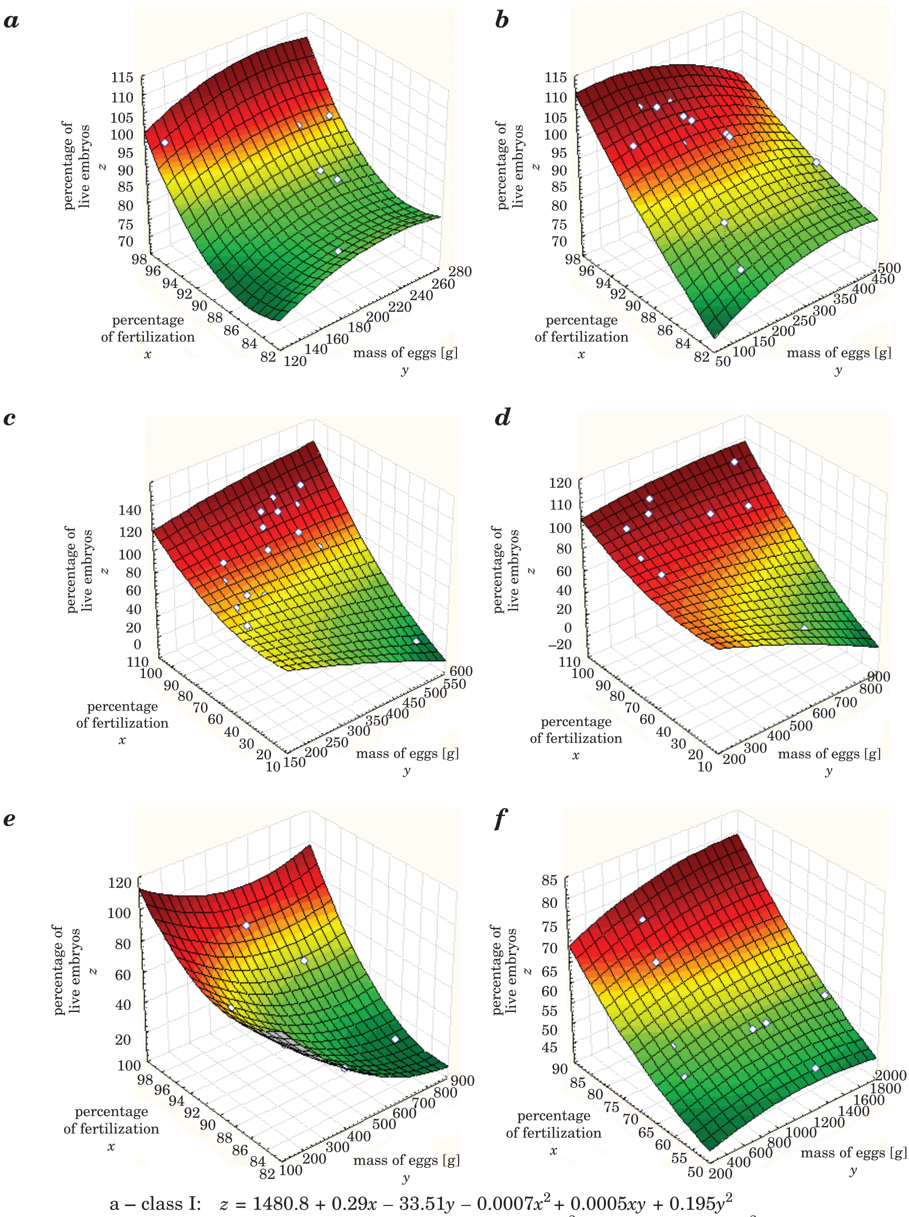
Correlation between the body weight of females, weight of eggs (expressed in grams and in percentage of female body weight), percentage of fertilized eggs (12 h) and percentage of living embryos (24 h) within six classes of female body weight (I–VI)

Investigation traits	Classes of female body weight	Body weight of females [kg] 1)	Weight of eggs [g] 2)	Weight of eggs [% of female body weight] 3)	Percentage of fertilized eggs after 12 h incubation 4)	Percentage of living embryos after 24 h incubation 5)
1	I	x	0.49	–0.81*	0.82*	0.80
	II	x	0.57*	0.21	–0.07	–0.13
	III	x	0.72*	0.50*	0.49*	0.49*
	IV	x	0.51	0.37	–0.44	–0.49
	V	x	0.17	–0.04	–0.48	–0.58
	VI	x	0.84*	0.73*	–0.83	–0.72*
2	I		x	0.90*	–0.14	–0.25
	II		x	0.91*	–0.09	–0.12
	III		x	0.96*	0.20	0.09
	IV		x	0.99*	–0.03	–0.08
	V		x	0.98*	0.13	–0.55
	VI		x	0.98*	–0.58	–0.48
3	I			x	–0.47	–0.54
	II			x	–0.06	–0.05
	III			x	0.06	–0.08
	IV			x	0.05	0.02
	V			x	0.24	–0.42
	VI			x	–0.46	–0.37
4	I				x	0.96*
	II				x	0.87*
	III				x	0.97*
	IV				x	0.96*
	V				x	0.73
	VI				x	0.96*

* Correlation significant at $P \leq 0.05$

The female body weight being used as an independent variable, regression equations were worked out within the investigated classes, the weight of eggs, fertilization percentage (12 h) and percentage of living embryos (24 h) being used as dependent variables. For each class of female body weight regression equations were derived, the female body weight but also the weight of obtained eggs being accepted as independent variables and the percentage of fertilization (12 h) and percentage of live embryos (24 h) as a dependent variable. The percentage of the explained variability was characterized by the determination index (R^2) as the adequacy of the equation matching the real system.

The values of empirical data concerning such variables as the weight of eggs expressed in grams, percentage of fertilization, and percentage of living embryos (24 h) were the basis of deriving a square function which allowed



a – class I: $z = 1480.8 + 0.29x - 33.51y - 0.0007x^2 + 0.0005xy + 0.195y^2$

b – class II: $z = 60.55 + 0.29\mathrm{x} - 1.80y - 8.6818\mathrm{E} - 5x^2 - 0.0027xy + 0.022y^2$

c – class III: $z = 77.55 - 0.12x - 0.64y - 4.4664\mathrm{E} - 5x^2 + 0.0019xy + 0.0066y^2$

d – class IV: $z = 72.18 - 0.079x - 0.51\mathrm{y} - 2.9134\mathrm{E} - 5x^2 + 0{,}0013xy + 0.0057y^2$

e – class V: $z = 1366.07 - 0.27x - 28.36y + 5.0823\mathrm{E} - 5x^2 + 0.0021xy + 0.16y^2$

f – class VI: $z = 47.46 + 0.003x - 0.53y - 2{,}8148\mathrm{E} - 6x^2 + 0.0001xy + 0.0083y^2$

Fig. 2. Surface charts for weight of eggs in grams (y), percentage of fertilization (x) and percentage of live embryos after 24 hours incubation (z) fitted by the square function

to predict the distribution of points of these variables on a plane – for each class of female body weight separately (Figure 2).

Results

Percentage of females ovulating after hormonal stimulation

In class I ovulation occurred in 77.80% of females, however, in 14.3% of fish treated with Ovopel the obtained eggs were of a very poor quality (Figure 1). In classes II and III respectively 94.12% and 93.75% of fish yielded eggs of satisfactory quality, while only 5.88% and 6.25% of poor quality (Figure 1).

The highest percentage of fish giving eggs of a low fertilization percentage was noted in class IV (16.67%; Figure 1) while in this class eggs of satisfactory quality were yielded by 83.33% of females (Figure 1). In class V eggs of good quality were obtained from all the fish after the Ovopel treatment (Figure 1). In the class of fish of the highest body weight (VI) ovulation occurred in 90% of females and eggs of satisfactory quality were obtained from 80% of fish treated ith the ovulation stimulator (Figure 1).

Effect of the female body weight class on the weight and quality of the eggs

A statistically significant effect of the main classification factor was found for the weight of eggs expressed both in grams and in percentage of female body weight ($P \leq 0.01$, $P \leq 0.01$; Table 2). The highest weight of eggs expressed in grams was obtained in class VI and the lowest one in class I (1191.75 g and 218.67 g); the difference between the means for these groups being 973.08 (Table 2). The least squares mean for class VI deviated from the overall mean for this trait by as many as +694.42 g (Table 2). It is worth noting that the means for successive classes of female body weight were characterized by an ascending tendency (Table 2).

The least squares means for the weight of eggs expressed in the percentage of female body weight show that the highest value of this trait was found in classes I and VI, and the lowest one in class V (20.19%, 14.21% and 9.15% respectively). In groups II, III, and IV the means had a similar value (within the range of 11.48% and 12.32%), all three means deviated in minus from the overall mean (Table 2).

The class of female body weight also significantly differentiated the investigated traits which characterized the quality of eggs ($P \leq 0.01$, $P \leq 0.01$; Table 2).

The lowest value of the least squares mean for the fertilization percentage was noted in class VI and the highest in classes I and II (64.75%, 91.67% and 92.47% respectively; Table 2). It should be stressed that the mean for class VI deviated from the overall mean by as many as –18.87%. The means for classes III and IV also deviated in minus from the overall mean for this trait, however, by ~2% only (Table 2). The least squares means which characterized the percentage of live embryos after 24 h incubation showed the highest values for classes I and II, attaining 88.17% in class I and 86.33% in class II (Table 2). The lowest least square mean of 54.63% for this trait was noted in class VI. This value deviated from the overall mean for this trait by as many as -22.13% (Table 2).

The results of the Duncan's multiple range test given in Table 3 show the significance of differences between the means of the investigated traits calculated for the different classes of the female body weight. The results of this test showed that differences between means characterizing weight of eggs in grams of all the investigated classes are significant except for the difference between classes I and II and classes IV and V (Table 3). Differences between the means characterizing the weight of eggs expressed in percentage of female body weight were statistically significant for class I and all the remaining classes of female body weight and for classes V and VI as well as classes II and VI (Table 3). The mean fertilization percentage significantly differed between class VI and all the remaining investigated classes, between class IV and V, II, I, as well as between class III and V, II, I. (Table 3). Differences between all means determining the percentage of live embryos were statistically significant except for the difference between classes IV and III as well as classes II and I (Table 3).

Dependences between the investigated traits

The body weight of females which responded with ovulation to the hormonal stimulation was positively correlated with the weight of obtained eggs (expressed in grams) in all the classes of female body weight. The highest statistically significant ($P \leq 0.05$) value of the index of correlation between these traits was found for classes VI, III and II (+0.84; +0.72; and +0.57 respectively) and the lowest for class V (+0.17; Table 4). The statistically significant positive correlation between the female body weight and the percentage of egg fertilization was found for body weight classes I and III, the highest value of the correlation coefficient being noted in the case of class I (Table 4). The female body weight also was positively correlated with the percentage of live embryos after 24 h incubation only in classes I and III (+0.80 and +0.49

respectively; Table 4). The index of correlation between these traits had the lowest value (–0.13) in the group of fish in body weight class II (Table 4). Correlation between the weight of eggs expressed in grams and the weight of eggs expressed in percentage of female body weight was positive in all the investigated classes, the value of the correlation index varying in the range of +0.90 to +0.98 (Table 4). The weight of eggs expressed in grams was positively correlated with the fertilization percentage only in the group of fish in body weight classes III and V, however, the value of the correlation coefficient for these classes was low (+0.20 and +0.13 respectively; Table 4). Correlation between the weight of eggs expressed in grams and the percentage of live embryos after 24h incubation was negative in all the classes of body weight of females, except for class III (Table 4). A positive statistically significant correlation between the fertilization percentage and percentage of live embryos after 24 h incubation was noted for all investigated classes of females except for group V (Table 4).

Regression predictions

In the regression equations given in Table 5 the dependent variables were: the weight of eggs expressed in grams, the percentage of fertilization after 12 h and that of live embryos after 24 h incubation; the independent variable was the female body weight. Analysis of values of the determination index (R^2) given with the equations where a dependent variable was the weight of eggs, showed that the highest R^2 was found for class VI, a slightly lower for class III and the lowest for class I (0.64, 0.52 and 0.01 respectively; Table 5). If the dependent variable was the fertilization percentage and the percentage of live

Table 5

Regresion predictions and determination indices (R^2) estimated within six classes of female body weight for the weight of eggs [g], percentage of fertilized eggs after 12 h incubation and the percentage of living embryos after 24 h incubation

Classes of body weight	Equations of regression	R^2	Equations of regression	R^2	Equations of regression	R^2
I	$y_1 = 235.74 - 7.01x_1$	0.01	$y_2 = 67.82 + 21.38x_1$	0.66	$y_3 = 52.43 + 32.05x_1$	0.64
II	$y_1 = -89.05 + 161.33x_1$	0.28	$y_2 = 94.33 - 0.93x_1$	0.01	$y_3 = 93.42 - 3.53x_1$	0.02
III	$y_1 = -461.41 + 271.51x_1$	0.52	$y_2 = -19.94 + 31.86x_1$	0.24	$y_3 = 33.98 + 34.55x_1$	0.24
IV	$y_1 = -823.24 + 332.72x_1$	0.24	$y_2 = 204.62 - 32.62x_1$	0.19	$y_3 = 202.61 - 34.07x_1$	0.24
V	$y_1 = -8.5 + 89.84x_1$	0.03	$y_2 = 121.23 - 5.73x_1$	0.22	$y_3 = 152.87 - 12.98x_1$	0.34
VI	$y_1 = -2779.0 + 483.85x_1$	0.64	$y_2 = 148.93 - 10.03x_1$	0.63	$y_3 = 106.32 - 6.08x_1$	0.52

Dependent variables: y_1 – weight of eggs [g]; y_2, percentage of fertilized eggs after 12 h incubation; y_3 – percentage of living embryos after 24 h incubation, independent variable: x_1 – body weight of females [kg] embryos, the R^2 obtained the highest value in class I (0.66 and 0.64 respectively), however, high values of this index were also found in class VI (0.63 and 0.52 respectively; Table 5). The lowest R^2 values were noted for class II of female body weight (0.01 and 0.02; Table 5).

The regression equations were given in Table 6, where the dependent variables were the percentages of fertilization and of live embryos, the independent variables being the weight of females as well as the weight of obtained eggs. The values of the determination index (given with the equations whose dependent variable was the percentage of egg fertilization) were found for class I and class VI (0.75 and 0.73 respectively) while the lowest value was noted for class II (0.01; Table 6). The values of this index for classes III, IV and V were similar (0.30; 0.24; 0.27; Table 6). In the case of the percentage of live embryos after 24 h incubation being the dependent variable, the highest value of the determination index was also found in classes I, VI and V (0.67, 0.56 and 0.55) and the lowest one in class II (0.02) – Table 6.

Table 6

Regression predictions and determination indices (R^2) estimated within six classes of female body weight for the percentage of fertilized eggs after 12 h incubation and percentage of living embryos after 24 h incubation

Classes of body weight	Equations of regression	R^2	Equations of regression	R^2
I	$y_2 = 55.94 + 25.83x_1 + 0.032x_2$	0.75	$y_3 = 42.50 + 35.76x_1 + 0.026x_2$	0.67
II	$y_2 = 93.88 - 0.29x_1 - 0.004x_2$	0.01	$y_3 = 92.71 - 2.52x_1 - 0.006x_2$	0.02
III	$y_2 = -46.92 + 47.73x_1 - 0.06x_2$	0.30	$y_3 = -82.79 + 63.26x_1 - 0.11x_2$	0.39
IV	$y_2 = 229.34 - 42.46x_1 + 0.03x_2$	0.24	$y_3 = 223.53 - 42.41x_1 + 0.02x_2$	0.28
V	$y_2 = 121.19 - 6.19x_1 + 0.005x_2$	0.27	$y_3 = 153.04 - 11.18x_1 - 0.02x_2$	0.55
VI	$y_2 = 170.96 - 14.03x_1 + 0.009x_2$	0.73	$y_3 = 122.01 - 8.94x_1 + 0.006x_2$	0.56

Dependent variables: y_2 – percentage of fertilized eggs after 12 h incubation; y_3 – percentage of living embryos after 24 h incubation, independent variables: x_1 – weight of female [kg]; x_2 – weight of eggs [g]

Distribution of values of the analysed traits in a special system

By analysing the shape of spacially arranged planes, the value of absolute term and values of variables X and Y, we could see that they were distinctly different for the investigated class of female body weight for the analysed arrangement (Table 7, Figure 2). The value of the multiple correlation coefficient allows to state that the best fit of the distribution of points to the plane is obtained for class II and the worse one to class VI.

Table 7
Main components of the square equation used for derivation of surface charts (R – mutiple correlation coefficient)

Arrangement of variables	Class of female body weight	Absolute term	X	Y	R
A	I	1480.80	0.29	–33.51	0.86
	II	60.55	0.29	–1.80	0.97
	III	70.55	–0.12	–0.64	0.89
	IV	72.18	–0.079	–0.51	0.88
	V	1366.07	–0.27	–28.36	0.85
	VI	47.46	0.003	–0.53	0.69

A – a set of variables: mass of eggs [g], percentage of fertilization [12 h], percentage of live embryos after 24 h incubation

Discussion

The obtained results showed that in the class of fish of the body weight exceeded 4.5 kg but did not rise above 6.5 kg ovulation occurred in 100% of females treated with Ovopel. It is worth stressing that of all the investigated classes in the above class only the obtained eggs were characterized by the satisfactory quality. In classes II (body weight of females >1.5 ≤2.5 kg) and III (body weight of females >2.5 ≤3.5 kg) ovulation also occurred in all the females, however, in both classes a low percentage of females yielded eggs of poor quality. In the class of fish of the least weight, i.e., whose body weight did not exceed 1.5 kg, ovulation was noted in the lowest percentage of females. In this class a high percentage (reaching 14.3%) of fish yielded eggs of poor quality. The highest percentage (16.67%) of fish yielding eggs with the fertilization percentage below 25% characterized the class of females of the body weight exceeding >3.5 ≤4.5 kg. It can be noted, in general, that in the classes of females of the least and the highest weight the percentage of fish ovulating after Ovopel treatment reached the lowest value.

In the Institute of Ichthyobiology and Aquaculture at Gołysz it was already attempted in previous studies to show dependences between the body weight of *Clarias gariepinus* females and reproduction effectiveness of this fish species after ovulation stimulation not only with Ovopel but also with carp pituitary homogenate (CPH). The results of the investigation showed that after the Ovopel stimulation females of the mean body weight of 3.80 kg as well as these of the mean body weight of 8.74 kg spawned in 100%. In the case of ovulation stimulation with carp pituitary homogenate eggs were obtained from only 67% of heavier fish while the ovulation occurred in all the fish of a lower body weight (BRZUSKA 2001a). The results of studies on the European catfish

Silurus glanis allowed to note that irrespective of the applied stimulator (CPH or Ovopel) females of the lower body weight (~5 kg) spawned in a higher percentage compared with heavier females (~12 kg) (BRZUSKA 2001b).

It was found on the basis of the presented investigation that the highest weight of eggs was given by the heaviest fish, i.e., the females of the body weight of 6.5 kg and above 6.5 kg, however, only in the case when the weight of eggs was expressed in grams. When the weight of eggs was expressed in the percentage of female body weight the highest value of the least squares mean for this trait was noted for the class of females of the least body weight, i.e., for fish whose body weight did not exceed 1.5 kg. A similar dependence was observed in the investigation of carp *Cyprinus carpio* carried out by CEJKO (2007). The results of the above investigation showed that in the class of the heaviest females the highest weight of eggs was obtained and in the class of the fish of the least body weight – the lowest, however, only in the case of expressing it in grams. The observation which was worth stressing showed that this dependence did not only occur after Ovopel stimulation of ovulation but also after the application of CPH (CEJKO 2007). Analysis of values of the least squares means which characterized the weight of eggs expressed in grams, calculated for the investigated classes of body weight of *Clarias gariepinus* females showed that these values had an ascending tendency in the successive classes of fish body weight. The same tendency was observed in the case of the carp irrespective of the preparation (Ovopel or CPH) used in ovulation stimulation (CEJKO 2007).

The results of studies of *Clarias gariepinus* carried out in the Institute of Ichthyobiology and Aquaculture at Gołysz in previous years showed that from females of the mean body weight of 8.9 ± 0.7 kg the weight of obtained eggs after Ovopel treatment was significantly higher compared with the weight of eggs obtained from females of the mean body weight of 3.8 ± 0.4 kg, but only in the case of the weight of eggs expressed in grams (BRZUSKA 2002). This observation confirmed the results obtained in the investigation of *Clarias gariepinus* in which the data were subjected to analysis of variance according to the linear model with the interaction between the ovulation stimulator and the body weight of females included (BRZUSKA 2001a).

Analysis of the results of controlled reproduction in this fish species in conditions of ovulation stimulation with CPH and also Aquaspawn preparation (containing GnRH-a and domperidon) shows that the fish of a mean body weight of 6.96 ± 0.72 kg released eggs of a higher weight (by 133 g) compared with the weight of eggs obtained from females of a lower mean body weight (4.89 ± 0.49 kg) (BRZUSKA 2003a). The results of an experiment with ovulation stimulators CPH and [D-Tle^{6},ProNHEt^{9}]mGnRH (Lecirelin; brand name Supergestran) also show that irrespective of the applied preparation from females of the higher body weight (3.91 ± 0.23 kg) the weight of eggs expressed in grams was distinctly higher than that from fish of the lower weight (2.63 ± 0.36 kg). It is worth stressing that the interaction between the ovulation stimulator and the body weight of females was statistically significant both in the case of the weight of eggs expressed in grams and in percentage of the body weight of fish used in the reproduction (BRZUSKA et al. 2004).

In was found in an investigation carried out in our Institute with European catfish *Silurus glanis* females of the mean body weight of 5.67 kg and 10.00 kg that both after hypophysation and after one dose of Ovopel a higher weight of eggs expressed in grams was obtained from heavier fish. In the case of presenting the weight of eggs as percentage of body weight of females after ovulation stimulation with Ovopel the value of the least square means for this trait was higher for fish of a lower body weight (BRZUSKA 2000).

In the case of ovulation stimulation with CPH in females of European catfish *Silurus glanis* of the mean body weight of 5.0 ± 0.4 kg and 7.00 ± 0.6 kg BRZUSKA and ADAMEK (2002) did not find statistical differences in the weight of obtained eggs.

An attempt was undertaken by BRZUSKA (2001b) to find dependences between the body weight of European catfish *Silurus glanis* females and the weight of obtained eggs; the mean body weight of the investigated females was 5.20 kg or 11.80 kg. The results of this study show similar mean values of the weight of eggs expressed in grams obtained from fish after hypophysation of higher or lower body weight, while after the application of Ovopel fish of a higher body weight released eggs of a considerably higher weight.

The effect of the body weight of carp *Cyprinus carpio* females on traits determining the weight of obtained eggs was investigated by CEJKO (2007). This author showed that the class of weight of female body significantly determined the weight of eggs in grams. The lowest weight of eggs was found for females of the lowest body weight (above 3 kg but not exceeding 5 kg) and the highest for females of the body weight exceeding 11 kg, irrespective of the applied ovulation stimulator (CPH or Ovopel).

The results of studies reported in the presented paper which concern the quality of eggs from females divided into six classes of body weight, show that the highest percentage of fertilization characterized eggs from females of the body weight not exceeding 2.5 kg, i.e., females of classes I and II. It should be distinctly stressed that during the next 12 h of incubation the quality of eggs from females of these classes did not deteriorate and the percentage of live embryos was high, exceeding 86%, in the two classes. A sufficiently good quality of eggs given by females of a low body weight (below 2.5 kg) was also noted in earlier studies on the stimulation of African catfish with Dagin (BRZUSKA 2011) and also with human chorionic gonadotropin – Biogonadyl (BRZUSKA et al. 2000).

The lowest quality expressed as the percentage of fertilization and live embryos after 24 h incubation characterized eggs from females of the highest body weight, i. e, exceeding 6.5 kg. The mean fertilization percentage of eggs from females of this body weight class did not reach 65%, while the mean percentage of live embryos was as low as 54%. High mean fertilization percentages in females of class V, i.e., whose body weight exceeded 4.5 kg but did not rise above 6.5 kg, should be stressed as well as the high mean percentage (82%) of live embryos after 24 h incubation calculated for fish of this class.

The results of the investigation of *C. gariepinus* females divided into two classes of body weight ($\bar{x}$ = 4.89 ± 0.49 and $\bar{x}$ = 6.96 ± 0.72), carried out by BRZUSKA (2003a), showed that irrespective of the applied ovulation stimulator (CPH or Aquaspawn) the quality of eggs obtained from fish of higher or lower body weight was similar after both 12 h and 24 h as well as after 28 h incubation. It was found in the experiment with CPH or Lecirelin as ovulation stimulators that irrespective of the applied preparation the percentage of live embryos (after 24 h and 28 h incubation) developing in eggs obtained from females of the higher body weight ($\bar{x}$ = 3.91 ± 0.23 kg) exceeded the percentage of live embryos developing in eggs obtained from females of the lower weight ($\bar{x}$ = 2.53 ± 0.36 kg) (BRZUSKA et al., 2004). After 28 h incubation the quality of eggs yielded by females of the lower body weight ($\bar{x}$ = 3.8 ± 0.40 kg) stimulated with Ovopel was also higher compared with the quality of eggs obtained from females of the higher mean body weight ($\bar{x}$ = 8.9 ± 0.7 kg) as was found by BRZUSKA (2002) in *C. gariepinus*.

An interesting dependence between the body weight of females, the fertilization percentage and the percentage of live embryos was found in this fish species after the application of two most frequently used ovulation stimulators, i.e., CPH and Ovopel. In the case of hypophysation the quality of eggs from females of a higher and lower body weight was very similar while after the treatment with Ovopel the quality of eggs from heavier fish was much poorer (BRZUSKA 2001a).

In an investigation of European catfish *Silurus glanis* the females were divided into two groups of a different mean body weight ($\bar{x}$ = 5.67 kg and $\bar{x}$ = 10.00 kg); the results showed that after the ovulation stimulation either with CPH or Ovopel eggs of a much poorer quality were obtained from heavier fish both after 24 and 48 and also after 56 h incubation (BRZUSKA 2001b). However, the results of three experiments carried out with this fish species showed that from females of the lower body weight eggs of a better quality were obtained only after the treatment with Ovopel (BRZUSKA 2003b).

The problem of dependence of the quality of eggs obtained from carp *Cyprinus carpio* females of different body weight were studied by CEJKO

(2007). The results reported by the above author contain information that eggs yielded by the heaviest females (body weight above 11 kg) both after hypophysation and Ovopel injection were characterized by the lowest percentage of live embryos after 24 h incubation while a much poorer quality was observed for eggs from hypophysed fish.

In summing-up the results of investigation reported in the presented paper it is possible to state that the highest effectiveness of reproduction characterized the class of females whose body weight exceeded 4.5 kg but did not rise above 6.5 kg. From 100% of females of this class eggs were obtained and their quality was satisfactory both after 12 and 24 h incubation. In this class fish yielded eggs of a mean weight of 495.3 g., this value approximating the general mean of the whole set for this trait. For this class of female body weight the prediction of the percentage of live embryos after 24 h incubation of eggs can be regarded as satisfactory if in the regression equation the independent variables were both the female body weight and the weight of eggs.

The poorest results of the conducted reproduction were obtained for the class of fish of the lowest body weight. In this class the lowest percentage of females spawned and as many as 14.3% of spawning fish gave eggs characterized with the fertilization percentage below 25. In this class of females the lowest mean weight of eggs expressed in grams, deviating from the general mean by as many as –278.7 g was obtained. The prediction of both the percentage of fertilization (12 h) and that of live embryos (24 h) can be evaluated as satisfactory in this class both if in the regression equation the independent variable was only the female body weight and if two independent variables, i.e., the female body weight and the weight of eggs were accepted.

Of the females in the sixth class of body weight, i.e., these of the highest weight, 80% yielded eggs whose quality could be regarded as satisfactory. In this class the highest mean weight of eggs was recorded, however, their quality (expressed as the percentage of fertilization and percentage of live embryos) considerably deviated in minus from the mean for the whole set for these two traits. For this class of fish body weight the prediction of the weight of eggs can be regarded as satisfactory. The prediction of the fertilization percentage and of live embryos can also be regarded as satisfactory both if in the equation the female body weight was taken as an independent variable and if the independent variables were the female body weight and the weight of eggs.

Translated by Elżbieta Wróblewska

Accepted for print 8.11.2012

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