The application of [D-Tle^6,ProNHEt^9]mGnRH (Lecirelin) with the dopaminergic inhibitor metoclopramide to stimulate ovulation in African catfish (*Clarias gariepinus*)

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ABSTRACT: The results of reproduction were tested in females of the African catfish (*Clarias gariepinus* Burchell 1822) after stimulation of ovulation with carp pituitary (4 mg/kg body weight) or with Lecirelin (15 μg/kg) and metoclopramide (10 mg/kg). After administering the synthetic substance eggs were obtained from all females while in the group treated with pituitary homogenate 7 out of 8 hypophysed females spawned. The applied spawning agent did not significantly influence the weight of eggs expressed in grams, but in the case of females treated with carp pituitary homogenate a significantly higher weight of eggs expressed as the percentage of body weight of fish was recorded. The applied stimulators of ovulation did not affect any trait reflecting the quality of eggs. Females used as an experimental material belonged to two categories in respect of body weight: lighter females with average body weight of 2.63 ± 0.36 kg and heavier females with average body weight of 3.91 ± 0.48 kg. It was proved that the weight of eggs expressed either in grams or as a percentage of a female's weight was significantly related to the body weight of a female ($P \leq 0.01$ and $P \leq 0.05$, respectively), as well as the percentage of fertilised eggs and the percentage of living embryos after 28 hours of incubation ($P \leq 0.05$ and $P \leq 0.05$, respectively). The interaction between the stimulator of ovulation and the female body weight was significant only for traits reflecting the weight of obtained eggs ($P \leq 0.05$ and $P \leq 0.01$).

Keywords: *Clarias gariepinus*; carp pituitary homogenate; Lecirelin; induced ovulation; artificial propagation

The paper is a successive contribution in the series presenting the results of studies on the effects of the reproduction of African catfish (*Clarias gariepinus*) after ovulation stimulation with various substances, conducted in the Institute of Ichthyobiology and Aquaculture, Gołysz, Polish Academy of Sciences. In earlier experiments the tested stimulating substances were as follows: pituitary of the bream (*Abramis brama* L.) (Brzuska *et al*., 1998a), human chorionic gonadotropin (Brzuska *et al*., 1998b, 2000), Ovopel (Brzuska *et al*., 1998c, 2000; Brzuska, 2001a, 2002), des Gly^{10}[D-Ala^6] LHRH Ethylamide (Brzuska *et al*., 1998b, 1999) and Aquaspawn (Brzuska, 2003a). In all experiments the control was carp pituitary administered in one dose of 4 mg/kg female body weight (Adamek, 1995).

In the presented experiment the synthetic peptide [D-Tle^6,ProNHEt^9]mGnRH (Lecirelin) was evaluated as a potential spawning agent. This analogue with a brand name Supergestran was synthetized in the Czech Republic. It has been used for the stimulation of ovulation in livestock since 1983 (Flegel *et al*., 1983) and is registered for domestic animals in European Agency for the Evaluation of Medical Products, Veterinary Medicine Evaluation Unit (Barth *et al*., 2002a). Since the registration of this analogue it has been designed for common use in the stimulation of fish reproduction (one of the

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conditions required for its registration is the explanation of its fate in the animal). Metabolic products of [D-Tle^6,ProNHEt^9]mGnRH have been studied in various tissues of the carp (*Cyprinus carpio* L.) and tench (*Tinca tinca* L.). The results of tests proved that Lecirelin was totally disintegrated within the liver tissue, this allowing for practical application of the biologically active peptide (Barth *et al*., 2002b,c).

The results of testing the effectiveness of Lecirelin in ovulation stimulation in various fish species obtained so far seem to be very important. It was successfully used by Kouřil *et al*. (1986, 2003) for the induction of spawning in tench (*Tinca tinca* L.). The first attempts to stimulate ovulation with [D-Tle^6,ProNHEt^9]mGnRH in carp (*Cyprinus carpio* L.) were undertaken by Kouřil *et al*. (1992b). The results of controlled reproduction after stimulation of ovulation with Lecirelin in rudd (*Scardinius erythrophthalmus* L.) were reported by Hamáčková *et al*. (2001), in ide (*Leuciscus idus* L.) by Kouřil *et al*. (2002), and in genetically distant lines of common carp (*Cyprinus carpio* L.) by Brzuska (2003b).

The aim of research described in the present paper was to prove the effectiveness of reproduction in African catfish (*Clarias gariepinus*) – a species important in European aquaculture, after ovulation stimulation with Lecirelin, compared with the results of reproduction after carp pituitary homogenate treatment. A possible effect of females' body weight on the results of controlled propagation was also studied.

MATERIAL AND METHODS

The experiment was conducted in the Institute of Ichthyology and Aquaculture, Gołysz, using 16 female African catfish (*Clarias gariepinus*) with body weights ranging between 1.95 and 4.5 kg. The spawners bred from fry produced in the Institute were divided into two groups of 8 fish each. In both groups a half of the individuals belonged to the class of lighter fish (average body weight for group I was 2.56 ± 0.45 kg, and for group II 2.70 ± 0.28 kg), while the remaining 50% of fish were heavier (average body weight for group I was 3.71 ± 0.28 kg, and for group II 4.10 ± 0.18 kg). The tested females were placed in 8 tanks (volume of 2.5 m^3), 2 fishes – one lighter and one heavier in each. The temperature of water during the experiment was maintained within 24–25°C. Stimulation of ovulation was conducted by administering carp pituitary homogenate to females from group I, and Lecirelin to females from group II. Metoclopramide easily dissolved in physiological saline was used in group II as a blocker of dopamine receptors. Lecirelin was supplied by the Institute of Organic Chemistry and Biochemistry, Prague, Czech Academy of Sciences; metoclopramide was purchased from Sigma. The doses of applied substances are given in Table 1. The control of ovulation was initiated 10 hours after the application of spawning agents and was repeated every hour during the next 3 hours. The fish were checked for ovulation by gently pressing the abdomen.

Eggs yielded by stripping fish were weighed and fertilised separately for each female with mixed milt, taken from macerated testes of three killed males. Eggs from females were incubated in a separate Weiss glass, in water at 24–25°C. After 12 hours of incubation the percentage of fertilised eggs was calculated, and after 24 and 28 hours of incubation the percentage of living embryos was recorded. After the hatching of larvae the correctness of their development was observed and the percentage of deformed individuals was calculated. The obtained data (their statistics are given in Table 2) were subjected to analysis of variance using the least-squares method to prove whether the stimulator of ovulation and the size of females affected the tested traits, i.e. weight of eggs in grams, weight of eggs as a percentage of female's body weight, percentage of fertilised eggs, and percentage of living embryos

Table 1. Substances used in the experiment and their doses, method of application and the number of females in groups

Group	Number of females and range of body weights (kg)		Substances	Dose*
I	4 (1.95–2.90)	4 (3.20–4.50)	carp pituitary	4 mg (*i.p.*)
II	4 (2.30–2.90)	4 (3.65–4.50)	Lecirelin	15 μg (*i.p.*)
Σ	8	8	metoclopramide	10 mg (*i.p.*)

*dose per 1 kg of female body weight; *i.p.* = intraperitoneally

Table 2. Statistical characteristics of the data

Variable	Descriptive statistics					
	n	$\bar{x}$	$\bar{s}$	min	max	S.D.
Weight of females (kg)						
Group I	8	3.14	0.27	1.95	4.50	0.77
Group II	8	3.40	0.26	2.30	4.50	0.81
Weight of eggs (g)						
Group I	7	477.10	35.08	347.40	598.10	92.82
Group II	8	437.96	51.71	269.10	689.90	146.26
Weight of eggs (% of female body weight)						
Group I	7	15.67	1.12	12.35	19.20	2.97
Group II	8	12.44	0.64	10.54	16.23	1.82
Fertilised eggs after 12-h incubation (%)						
Group I	7	83.05	3.54	70.66	93.00	9.35
Group II	8	89.78	2.15	81.00	97.00	6.08
Living embryos after 24 h incubation (%)						
Group I	7	63.45	3.92	54.00	82.00	10.38
Group II	8	68.02	3.17	56.23	83.00	8.98
Living embryos after 28 h incubation (%)						
Group I	7	58.22	4.99	42.00	80.00	13.19
Group II	8	57.50	3.84	40.00	71.00	10.87

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

after 24 and 28 hours of eggs incubation. In the linear model an interaction between the stimulator of ovulation and body weight of females was taken into account. Analysis of variance was performed according to the following linear model:

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

where: μ = overall mean
g_i = effect of an ovulation stimulator (i = 1…2)
p_j = effect of the size of the female (j = 1…2)
$(gp)_{ij}$ = interaction between the ovulation stimulator and the size of the female
e_{ijk} = random error connected with observation k

The significance of main classification factors on the tested traits was checked using the *F*-test. The estimated constants and least-squares means reflecting the values of particular traits within the tested main classification factors are given in Table 3. The values of the least-squares means for the interaction between the stimulator of ovulation and female size for each studied trait are presented in Figure 1 (A,B,C,D,E). The correlations between the investigated traits were computed for group I and group II separately (Table 5).

RESULTS

Ovulation time

In spawning females from group I ovulation took place in 12 hours after the treatment with carp pituitary homogenate. In group II synchronisation of ovulation was also observed in all tested individuals, however the ovulation took place one hour later.

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

Classification factor	Weight of eggs (g)			Weight of eggs (% of female body weight)			Percentage of fertilisation			Percentage of living embryos					
							after 12 h incubation			after 24 h incubation			after 28 h incubation		
	μ = 455.27			μ = 14.23			μ = 86.69			μ = 66.01			μ = 58.30		
	LSC	LSM	SE	LSC	LSM	SE	LSC	LSM	SE	LSC	LSM	SE	LSC	LSM	SE
Ovulation stimulator															
Carp pituitary (group I)	17.30	472.57	32.19	1.79	16.01	0.66	–3.08	83.61	2.74	–2.01	64.00	3.78	0.80	59.10	4.19
Lecirelin (group II)	–17.30	437.96	29.81	–1.79	12.44	0.61	3.08	89.78	2.54	2.01	68.03	3.50	–8.80	57.50	3.90
Body weight of fish															
Lighter females	–76.16	379.11	32.19	0.85	15.08	0.66	3.82	90.51	2.74	2.94	68.96	3.78	6.06	64.36	4.19
Heavier females	76.16	531.43	29.81	–0.85	13.38	0.61	–3.82	82.87	2.54	–2.94	63.07	3.50	–6.06	52.24	3.90

SE = standard error of least-squares means; μ = overall mean

Percentage of females ovulating after hormonal stimulation

In group I, 87.50% of hypophysed fish spawned, and in group II eggs were obtained from 100% females treated with Lecirelin.

The effect of ovulation stimulators on the weight and quality of eggs

The difference between means for the weight of eggs expressed in grams for females belonging to groups I and II was not significant (Tables 3 and 4). However, the effect of applied spawning agent on the weight of eggs expressed as a percentage of female body weight was statistically significant ($P \leq 0.01$). For hypophysed females the value of the least-squares mean for this trait was higher in comparison with the value of the least-squares mean estimated for fish treated with Lecirelin (16.01% and 12.44%, respectively). The applied stimulator of ovulation did not significantly affect any of the three traits reflecting the quality of eggs (Table 4). However, the values of least-squares means characterizing the percentage of fertilised eggs and the percentage of living embryos after 24 hours of incubation of eggs yielded by females from group II were higher than in the case of females from group I (89.78 and 83.61; 68.03 and 64.00, respectively; Table 3). The values of least-squares means for the percentage of living embryos after 28 hours of incubation were similar for both groups: 59.10% for group I and 57.50% for group II (Table 3).

The effect of the body weight of females used for reproduction on the weight and quality of eggs

The means of the weight of eggs (expressed both in grams and as a percentage of fish body weight) calculated for lighter and heavier females were significantly different ($P \leq 0.01$ and $P \leq 0.05$, respectively; Table 4). The values of least-squares means estimated for the weight of eggs expressed in grams indicate that the higher weight of eggs was obtained from heavier females in comparison with those from lighter individuals (531.43 and 379.11, respectively; Table 3). However, considering the values of least-squares means for the weight of eggs expressed as a percentage of female body weight, a higher weight

Table 4. Results of *F*-test

Classification factor	Weight of eggs (g)	Weight of eggs (% of female body weight)	Percentage of fertilised eggs	Percentage of living embryos	
			incubation		
			12 h	24 h	28 h
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 investigated traits of females treated with carp pituitary homogenate – group I (above the diagonal) and Lecirelin – group II (under the diagonal)

Traits	Weight of females (kg)	Weight of eggs (g)	Weight of eggs as % of female body weight	Percentage of fertilised eggs	Percentage of living embryos	
				after 12 h incubation	after 24 h incubation	after 28 h incubation
	1	2	3	4	5	6
1		0.72	–0.76*	–0.14	–0.29	–0.44
2	0.90*		–0.12	–0.04	0.07	–0.11
3	0.45	0.73*		0.12	0.33	0.38
4	–0.52	–0.76*	–0.70		0.64	0.65
5	–0.12	–0.46	–0.68	0.67		0.97*
6	–0.35	–0.62	–0.59	0.84*	0.84*	

*correlation significant at $P \leq 0.05$

of eggs was obtained from lighter fish (Table 3). The values of least-squares means for all three tested traits characterizing the quality of eggs were higher in lighter fish and significant differences were found for the percentage of fertilised eggs as well as for the percentage of living embryos after 28 hours of egg incubation (Tables 3 and 4).

Interaction

Statistical significance of the studied interaction was demonstrated for the weight of eggs expressed in grams and as a percentage of the female body weight ($P \leq 0.05$ and $P \leq 0.01$, respectively). Considering the values of least-squares means for the interaction and the weight of eggs in grams, the highest weight of spawn was obtained after the treatment of heavier females with Lecirelin while the lowest after its application to lighter fish (558.60 and 317.40, respectively; Figure 1A). After the application of pituitary homogenate, the weight of eggs yielded by heavier and lighter females was similar (respective values: 440.91 g and 504.30 g; Figure 1A). There was no significant effect of the interaction on traits determining the quality of eggs (Table 4). It is however important to point out that after 28 hours of incubation the eggs produced by lighter fish were of much higher quality, no matter whether they were obtained as a result of pituitary homogenate or Lecirelin treatment (Figure 1E).

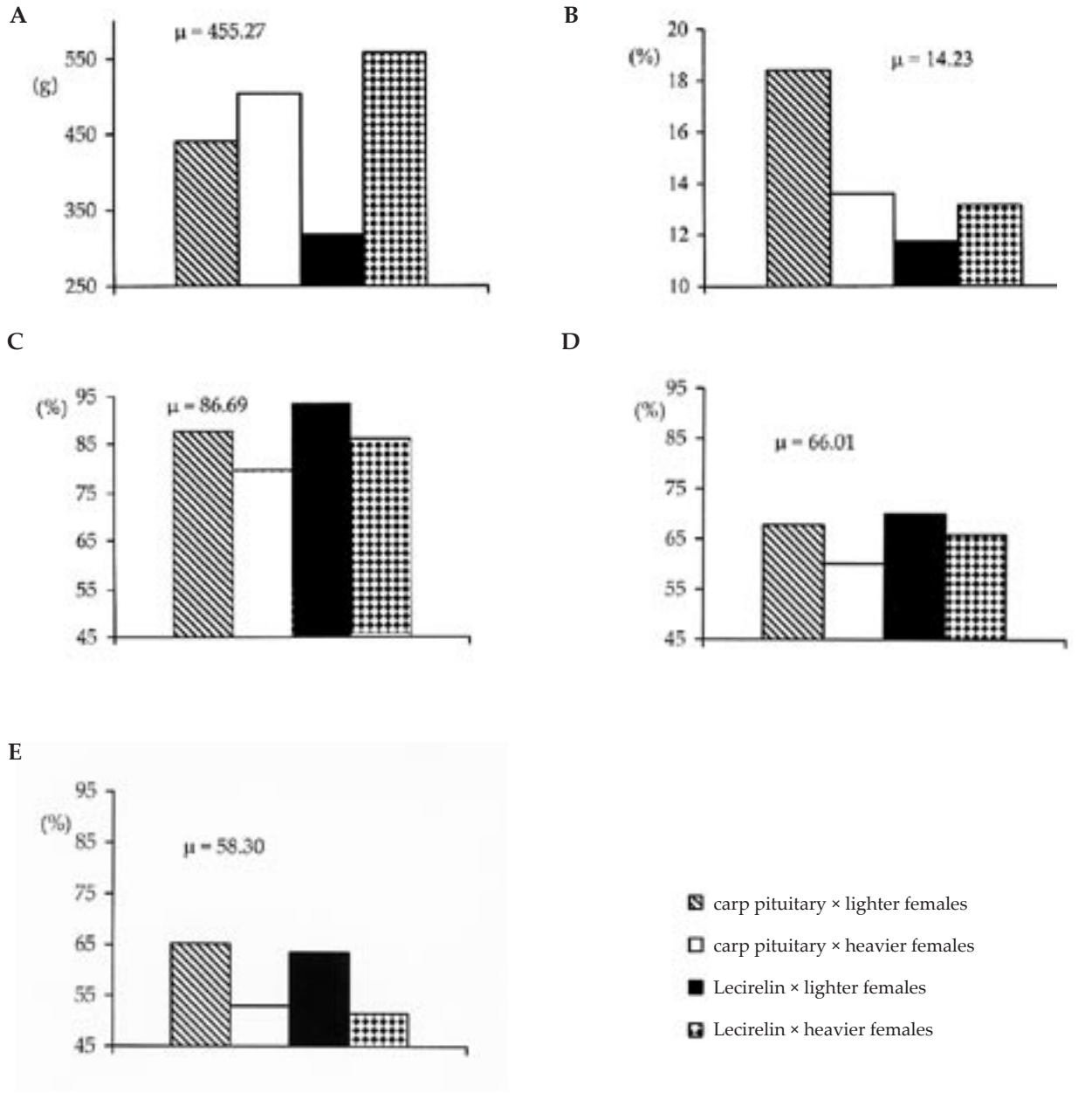

Figure 1. The least-squares means for the interaction of ovulation stimulator and body weight of females (μ = theoretical general mean)

A – weight of eggs in grams; B – weight of eggs as a percentage of female body weight; C – percentage of fertilisation after 12 h of incubation; D – percentage of living embryos after 24 h of incubation; E – percentage of living embryos after 28 h of incubation

Relations between the investigated traits

In group I the body weight of females was positively correlated with the biomass of eggs expressed in grams but negatively with all the remaining tested traits. Statistically significant ($P \leq 0.05$) was only the correlation between the body weight of females and the weight of eggs expressed as a percentage of female body weight (Table 5). The coefficient of correlation between the percentage of fertilised eggs

and the percentage of living embryos after 24 and 28 hours of incubation had similar positive values (+0.64 and +0.65, respectively; Table 5). In this group the highest and statistically significant ($P \leq 0.05$) correlation was found between the percentages of living embryos after 24 hours and 28 hours of incubation (Table 5).

In group II the body weight of females was positively correlated with both traits characterizing the weight of eggs. However, the value of the coefficient of correlation between the body weight of females and the weight of eggs expressed in grams was twice higher and statistically significant ($P \leq 0.05$), compared with the coefficient of correlation between the body weight of females and the weight of obtained eggs expressed as a percentage of fish body weight. The percentage of fertilised eggs from females of this group was positively correlated with the two remaining traits characterizing the quality of eggs. But a statistically significant ($P \leq 0.05$) correlation was found only between the percentage of fertilised eggs and the percentage of living embryos after 28 hours of incubation (Table 5). In this group, the value of correlation coefficient between the percentages of living embryos after 24 hours and 28 hours of egg incubation was +0.84, i.e. lower than in group I, where its value was as high as +0.97 (Table 5).

Occurrence of deformed larvae

In both tested groups the incidence of deformed larvae amounted maximally to 12%. However, the percentage of deformed individuals was not influenced by either the substance used for treatment or the body weight of females.

DISCUSSION

On the basis of data from the described experiment it is possible to state that the results of reproduction of African catfish (*Clarias gariepinus*) after stimulation of ovulation with [D-Tle^6, ProNHEt^9]mGnRH (Lecirelin) can be considered as satisfactory. After the administration of this analogue 100% of treated fish spawned and the synchronisation of ovulation occurred in all females. The induction of ovulation in this fish species with [D-Ala^6, ProNHEt]GnRH did not lead to spawning in all females. After the administration of this analogue in the dose of 20 μg per kg body weight 30% of fish stripped while in the group where it was applied together with dopaminergic inhibitor – isophloxythepin (respective doses of 20 μg/kg and 4 mg/kg) 50% of females spawned (Kouřil *et al*., 1992a).

After the application of Lecirelin to carp females of two different breeding lines two spawning periods were recorded for each line (Brzuska, 2003b). The lack of synchronisation of ovulation after stimulation with [D-Tle^6, ProNHEt^9]GnRH was demonstrated for tench (*Tinca tinca* L.) (Kouřil *et al*., 1986), for rudd (*Scardinius erythrophthalmus* L.) (Hamáčková *et al*., 2001), and for ide (*Leuciscus idus* L.) (Kouřil *et al*., 2002).

The mean weight of eggs, expressed in grams, obtained from females of the African catfish (*Clarias gariepinus*) after stimulation of ovulation with Lecirelin was comparable with the mean weight of eggs produced by hypophysed females. The quality of eggs, expressed as the percentage of fertilised eggs and percentage of living embryos (after 24 and 28 hours of incubation), produced by fish treated with Lecirelin, did not differ significantly from the quality of eggs obtained from fish treated with carp pituitary homogenate. It has to be pointed out that in the group where females were treated with Lecirelin, no increase in the percentage of deformed larvae was found, compared with the group where stimulation of reproduction was performed using the carp pituitary homogenate. After the application of another synthetic mammalian analogue, i.e. desGly^{10}, [D-Ala^6]LHRH – ethylamide in this fish species, a higher percentage of deformed larvae was found, compared with groups where spawning agents of natural origin (i.e. carp pituitary or human chorionic gonadotropin) were administered to females (Brzuska *et al*., 1998b).

The results of research presented in this paper clearly indicate that the body weight of females used for reproduction is a factor which significantly affects the weight of eggs, expressed either in grams or as percentage of fish body weight. The body weight of females also significantly affected two traits characterizing the quality of eggs, i.e. the percentage of fertilisation and the percentage of living embryos after 28 hours of egg incubation. It should be mentioned that the higher weight of eggs was obtained from heavier females but only if expressed in grams. An important fact is that the quality of eggs produced by heavier females was much lower than that of eggs obtained from lighter fish.

In analysing the values of interaction between the body weight of females and the spawning agent it is possible to state that after treatment with carp pituitary homogenate the weight of eggs (expressed in grams) from heavier and lighter fish was similar. However, after the application of Lecirelin, the weight of eggs from heavier fish was much higher than the weight of eggs produced by lighter females. These data are in compliance with the results of research carried out on the European catfish (*Silurus glanis* L.). Nevertheless, it is necessary to mention that in studies on the European catfish (*Silurus glanis* L.) (Brzuska, 2001b) one group was stimulated with carp pituitary homogenate while the other with Ovopel (Horvath *et al*., 1997). In tests on African catfish (*Clarias gariepinus*), in both treatments: with carp pituitary homogenate and Ovopel, heavier females produced eggs of higher weight than lighter fish (Brzuska, 2001b). It is important to point out that in the tests where ovulation in African catfish (*Clarias gariepinus*) was stimulated with carp pituitary or a single dose of Ovopel (1 pellet/kg), the quality of eggs produced by lighter and heavier females was similar after the administration of pituitary, but after the treatment with Ovopel it was much higher in lighter fish. In the present experiment eggs of much higher quality were produced by lighter females after the application of pituitary homogenate or Lecirelin.

Data obtained in earlier studies on African catfish (*Clarias gariepinus*) (Brzuska, 2001a, 2002, 2003a) as well as data resulting from the present experiment allow to conclude that the body weight of females stimulated for controlled spawning is not irrelevant to the efficiency of reproduction. The problem of relationship between the body weight of females and the effects of controlled reproduction was analysed for carp (*Cyprinus carpio* L.) by Brzuska (1991) and perch (*Perca fluviatilis* L.) by Kouřil and Linhart (1997). The dependence of reproduction effects on the body weight of females treated with various spawning agents seems to be an interesting research issue. The results of such studies can lead to practical solutions that would result in the possible highest yield of full quality hatch and minimise costs connected with controlled reproduction. The results of the described experiment justify the continuation of studies on this interesting fish species. Testing the effects of various doses of Lecirelin upon fish of different body weight seems to be a proper direction for further research.

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ABSTRAKT

Podání [D-Tle^{6},ProNHEt^{9}]mGnRH (Lecirelin) a dopaminergního inhibitoru metoclopramid ke stimulaci ovulace sumečka afrického (*Clarias gariepinus*)

Byly testovány výsledky reprodukce jikernaček sumečka afrického (*Clarias gariepinus* Burchell 1822) po stimulaci ovulace pomocí kapří hypofýzy (4 mg/kg hmotnosti těla) nebo Lecirelinu (15 μg/kg hmotnosti těla) spolu s metoclopramidem (10 mg/kg). Po podání synteticky vyrobených přípravků byly získány jikry od všech jikernaček, po hypofyzaci se vytřelo 7 z 8 injikovaných jikernaček. Druh injikovaného přípravku neměl signifikantní vliv na hmotnost vytřených jiker v gramech, ale byla zaznamenána statisticky vysoce průkazně vyšší relativní hmotnost vytřených jiker vztažená k hmotnosti těla u jikernaček injikovaných hypofýzou. Aplikované preparáty neměly vliv na kvalitu získaných jiker. Použité jikernačky jako experimentální materiál náležely ke dvěma velikostním skupinám; lehčí jikernačky měly průměrnou hmotnost 2,63 ± 0,36 kg, těžší jikernačky 3,91 ± 0,48 kg. Jako nevhodné se ukázalo v jednom ze dvou případů hodnocení parametru závislosti hmotnosti vytřených jiker v gramech na hmotnosti jikernaček, jelikož byla zjištěna signifikantní závislost hmotnosti vytřených jiker v gramech i jejich hmotnosti, vyjádřené v procentech, na hmotnosti jikernaček ($P \leq 0,01$ a $P \leq 0,05$), jakož i procenta oplozených jiker a procenta živých embryí za 28 h od začátku inkubace ($P \leq 0,01$ a $P \leq 0,05$). Interakce mezi způsobem stimulace ovulace a hmotností jikernaček byla signifikantní jen při hodnocení vlivu na hmotnost vytřených jiker ($P \leq 0,01$ a $P \leq 0,05$).

Klíčová slova: *Clarias gariepinus*; kapří hypofýza; Lecirelin; stimulace ovulace; umělý výtěr

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